Message

From: Agarwal, Ilena [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=DA61841DC43F4A9F8BFD3223AD9C230D-AGARWAL, IL]
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Subject: Compilation 10/22/2018

E-15

High Plains Journal: Trump orders EPA to start year-round E15 regulatory process

AgPro: EPA Told To Initiate Process To Allow Summertime E15 Sales

Forbes: Can California Be The U.S. Auto Industry's New Best Friend?

Coal Ash

The Hill: Greens challenge Trump's rollback of coal ash standards

Regulations

The Hill: Supreme Court given another opportunity to rein in regulatory state

Breitbart: EPA Reverses Obama Era Last Minute Uranium Mining Regulations

Slate: Trump's Biggest Attempts to Roll Back Environmental Regulations Remain at the Starting Gate

Emissions

The Ecologist: Do we need to be concerned about asbestos again?

E&E News: Greens sue over EPA denial of cross-state ozone petitions

Earth.com: What happens when the EPA doesn't protect the environment?

E&E News: White House pressured EPA to favor industry over climate

PBS News: Why radiation protection experts are concerned over EPA proposal

The State- Chemical exposure news trickles out to residents

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E&E News: EPA's carbon rule sparks debate over cost-benefit analyses

The Hill: Supreme Court given another opportunity to rein in regulatory state

https://thehill.com/opinion/judiciary/412355-supreme-court-given-another-opportunity-to-rein-in-regulatory-state
BY MARK MILLER, OPINION CONTRIBUTOR — 10/22/18 08:00 AM EDT

Federal courts have taken welcome steps in recent years to rein in the runaway regulatory state. Too often executive agencies charged with applying the laws as written by Congress have done so in ways unsupported by the text of those laws. In response, the courts have stepped into the breach to right those violations of the separation of powers set out

by our Constitution. This week, the Supreme Court will be asked again to set things right, in a case called Marquette County Road Commission v. EPA.

It is a David v. Goliath story, but here David does not have a slingshot; instead, his weapons are legal papers, the Administrative Procedure Act and two important Supreme Court cases.

The facts are straightforward and, for the most part, not in dispute. In Marquette County, Michigan, the Road Commission wished to build a road extension — County Road 595 (CR 595) — as a shortcut for commercial truck traffic. The proposed 21-mile road would allow trucks to bypass busy city streets, thereby benefiting the health, safety and welfare of county residents.

To complete the road necessitated filling in 25 acres of wetlands, which required a permit under Section 404 of the Clean Water Act. States are authorized to approve a Section 404 permit if the application meets certain conditions. The EPA retains limited oversight authority when the state takes on this authority, which includes the ability to overrule the state's decision. If the EPA objects, the property owner must begin the permitting process anew with the U.S. Army Corps of Engineers.

That's what happened here. In 2011, the Road Commission submitted a permit application to the Michigan Department of Environmental Quality (MDEQ), the agency in charge of administering the federally approved permitting plan for the state. The Road Commission and MDEQ worked together to arrive at an approved plan for CR 595, and the MDEQ submitted the permit plan to the EPA.

But the EPA offered unsupported and vague objections to the permit application. The Road Commission attempted to remedy these objections, offering to protect and preserve 63 acres of wetlands for every acre of wetlands filled. But the EPA demanded more. Over nearly two years, no matter what the Road Commission offered, the EPA lodged objections.

Finally, after a September 2012 hearing on the objections and repeated rejections from the EPA, the MDEQ submitted a revised permit application, stating that it believed the application complied with all federal and state laws, and urging the EPA to withdraw its objections. The EPA refused and, in December 2012, lodged an entirely new set of objections unsupported by law.

The MDEQ and the Road Commission could not hope to comply with vague, unlawful objections, and under the Clean Water Act, after 30 days those objections resulted in a veto of the permit. Authority to approve CR 595 then transferred from the MDEQ to the Corps of Engineers, which would have required the Road Commission to start the entire permitting process from square one.

Instead, the Road Commission sought a federal court review of the EPA's veto of the road project. No one argues that the EPA cannot object to a state-approved Clean Water Act permit; all the Road Commission is saying is that the EPA cannot object for arbitrary and capricious reasons. The EPA says that it can. From the agency's perspective, its rejection of the state's plan for the road is unreviewable in court and cannot even be challenged in court.

In other words: the king will not be questioned by the serfs.

The Road Commission rejects that interpretation of the law. The EPA's veto of the road project is a final decision that has legal consequence. Seen properly, the Administrative Procedure Act allows for the veto to be reviewed in court to consider whether the EPA decision was arbitrary and capricious.

Two recent Supreme Court precedents, Sackett v. EPA and Army Corps of Engineers v. Hawkes, suggest the Road Commission is right. In those opinions, the high court held unanimously that final agency decisions in the Clean Water

Act context are reviewable in court. To hold otherwise, the court said, would allow for federal agency "strong-arming" of regulated parties.

The Road Commission knows exactly how strong-arming feels: they had a permit to build a road, but the EPA took away that permit and demanded the commission restart the permitting process, which can take years and costs hundreds of thousands of dollars. That's local taxpayer money down the drain, to anyone seeing the dispute through the lens of common sense.

The people of Marquette County and the state of Michigan know best how to protect their waters and provide for infrastructure, and Congress explicitly gave them that decision-making authority in the Clean Water Act. But EPA bureaucrats high-handedly overruled both Congress and the people of Michigan by nixing the state plan for CR 595. This case presents the Supreme Court with another chance to remind the EPA — and all federal agencies — that their job is to implement the will of Congress and the American people, not to ignore it.

Mark Miller is a senior attorney for Pacific Legal Foundation, which litigates nationwide to achieve court victories enforcing the Constitution's guarantee of individual liberty.

Forbes: Can California Be The U.S. Auto Industry's New Best Friend?

https://www.forbes.com/sites/energyinnovation/#6652a4f3738e

By Margo Oge- October 22, 2018

Margo Oge served the US EPA for over 32 years and was director of EPA's Office of Transportation and Air Quality from 1994-2012.

on fuel economy standards, California could be the auto industry's new best friend
Trump's fuel economy rollback will also diminish the global competitiveness of domestic car companies

A new report issued by the Intergovernmental Panel on Climate Change calls on nations to take "unprecedented" actions to cut their carbon emissions over the next decade to prevent a world of irreversible food shortages, life-threatening heat waves, droughts, coastal flooding and wildfires.

Sadly, it comes on the heels of the Trump administration's proposal to roll back the 2025 greenhouse gas (GHG) standards for cars and light trucks that were on track to double United States fuel economy and dramatically reduce U.S. carbon pollution - a move that could impose $450 billion in new costs on consumers.

President Donald Trump and Vice President Mike Pence meet with automobile industry leaders.THE WHITE HOUSE

But while loosening regulations may seem like an economic driver for the auto industry, rolling back fuel economy standards could cost domestic auto manufacturers their competitiveness in more than 40% of the U.S. auto market - and some of the biggest foreign auto markets. It may seem ironic to expect economic upside from stricter regulations, but on fuel economy standards, California could be the auto industry's new best friend .

Rolling back regulations could throw automaker sales in reverse

The current 2025 regulations were developed in 2012 jointly by the U.S. Environmental Protection Agency (EPA), which is responsible for reducing greenhouse gas emissions and the National Highway Traffic Safety Administration (NHTSA), which is responsible for setting fuel economy standards. The Economist characterized the standards as one of leading global policy actions to slow global warming.

Beyond increasing emissions, Trump's fuel economy rollback will also diminish the global competitiveness of domestic car companies , the very group Trump says he's trying to help. Automakers' best chance to avoid falling behind the rest of the world lies in a new partnership with California, which has led the nation with its stringent vehicle standards, and which 12 other states and Washington D.C. follow.

It Takes An Ecosystem: Transportation-Focused Executives Turn To Outside Providers, Customers, Suppliers And Competitors
This unusual situation began developing after the 2016 election. Around that time, the EPA and NHTSA jointly published a report confirming that compliance with the 2025 standards would cost even less than originally expected. But the auto industry asked the Trump administration for a review of the standards. He obliged and told both agencies to look at relaxing the existing standards.

What followed was a new low point for the EPA. While both the EPA and NHTSA signed off on the August 3rd proposal, the vast, technical emissions expertise of the EPA played virtually no role in the analysis. Acting EPA Administrator Andy Wheeler ignored his top career scientists and engineers, siding with the NHTSA analysis, and signed off.

Legal and economic consequences for domestic automakers

The result is a Trump administration proposal that is not only built on faulty analysis, but one that relaxes regulations beyond what some automakers expected. And it sets up serious legal and business consequences.

First, a 2007 Supreme Court ruling determined that EPA not only has the authority to set greenhouse gas emission standards but that it is obligated to do so - no matter what NHTSA does. The fact that NHTSA "sets mileage standards in no way licenses EPA to shirk its environmental responsibilities," wrote the court, "EPA has been charged with protecting the public's "health" and "welfare," 42 U. S. C. §7521(a)(1), a statutory obligation wholly independent of DOT's mandate to promote energy efficiency'.

Clearly, EPA leadership has completely abdicated the obligation created by this Supreme Court ruling. So California, along with Washington D.C. and 16 other states representing 43% of U.S. car sales, is suing the administration against any rollback of the existing standards. These states stand a reasonable chance of winning and automakers are looking at three to four years of regulatory uncertainty and protracted litigation.

Second, this proposal will push domestic automakers in one direction while the biggest markets in the world quickly head in another. By disincentivizing the development of clean technologies, U.S. automakers could fall back into the bad habits that nearly destroyed the industry in 2009 and cost taxpayers some $9.3 billion in bailouts.

After 2020, GHG standards for personal vehicles in the European Union and China - the world's other large markets - will diverge rapidly from U.S. standards. The EU recently voted to reduce greenhouse gas emissions from cars by 40% by 2030, and China is seriously considering banning gasoline-powered vehicles entirely.

Domestic automakers now face a dilemma: They can choose to live with years of regulatory uncertainty, or they can seek a compromise with the states suing them.

Finding friends in unlikely places

Mary Nichols, the chairwoman of the California Air Resources Board, is considering a formal public counter offer to the Trump administration's proposal. Last week she asked car manufacturers to present detailed information on their future product plans, and explain why they can't meet the existing standards and what type of relief they are looking for.

California's leadership can help car manufacturers find a solution to their current dilemma without the courts tying their hands. If the auto industry asks for reasonable flexibilities to meet the 2025 standards, California will listen. California and the auto industry should be able to find a constructive path out of the quagmire – but the next few months may offer the last opportunity to do so.

The clock is ticking as the comment period ends October 26th. If the auto industry is serious about a deal that will help avoid years of litigation and uncertainty, and also help achieve the unprecedented carbon emissions reductions needed to avoid the worst impacts of climate change, California can be its new best friend.

Margo Oge served as the director of EPA's Office of Transportation and Air Quality from 1994-2012 and is the author of "Driving the Future: Combating Climate Change with Cleaner, Smarter Cars."

Earth.com: What happens when the EPA doesn't protect the environment?

https://www.earth.com/news/epa-protect-environment/

By Kay Vandette- October 22, 2018

The Environmental Protection Agency (EPA) was established in 1970 in direct response to increased, widespread concern about the environment.

At the time when President Nixon first established the agency, photographers hired by the EPA documented the overwhelming pollution problems facing America at the time. The EPA was tasked with monitoring, regulating, and enforcing environmental protection.

But how well has the agency upheld those key cornerstones of sweeping environmental legislation, and what is the EPA's role when it comes to the balance between economic gains versus environmental protections?

The EPA has certainly made the news recently, and mostly because of concerns that the Trump Administration is working to roll back environmental protections set in place by the Obama Administration to help combat climate change.

Taking a closer look at the early beginnings of the EPA and its actions throughout different administrations can provide us with a better picture of the government environmental agency to see if it has stayed true to its original mission.

As you'll soon see, Trump's moves to rollback EPA policies and regulations is not the first time the agency has been on the chopping block for budget cuts and policy rollbacks.

A press release written by William D. Ruckelshaus, the first Administrator for the EPA, highlights not only why the EPA was formed but also what Ruckelshaus saw as the key mission of the agency.

The press release reads:

"It [the EPA] has no obligation to promote agriculture or commerce; only the critical obligation to protect and enhance the environment. It does not have a narrow charter to deal with only one aspect of a deteriorating environment; rather it has a broad responsibility for research, standard-setting, monitoring and enforcement with regard to five environmental hazards; air and water pollution, solid waste disposal, radiation, and pesticides."

Ruckelshaus was later chosen to the head the FBI as the acting director, and when Nixon ordered Ruckelshaus to fire the special prosecutor who was investigating Watergate, he refused and was consequently fired.

It wasn't long before the idyllic mission statement of the EPA was strongly contested.

The agency began receiving pushback from industries and businesses, which eventually forced the EPA to take economic issues and risks assessment into consideration, according to Adam Rome, an environmental historian who spoke with The Atlantic.

By all accounts, the EPA's first few years were successful, as the agency worked diligently to clean up America's pollution.

Those first few years of the EPA gave us the Clean Air Act and the Clean Water Act, legislation that has been instrumental in regulating water and air quality and limiting pollutants.

A few presidential terms later, Ronald Reagan was elected into office as the 40th President of the United States, marking the beginning of a particularly tumultuous chapter of the EPA's history.

When Reagan took office, the EPA was going through several scandals and, reminiscent of current events, then EPA Administrator Anne Gorsuch was forced to resign.

Gorsuch, the first female Administrator in the history of the EPA, seemed to do everything in her power to essentially gut the agency to a shell of its former self. Budget cuts, personnel changes, and gross mismanagement of the Superfund cleanup program were Gorsuch's legacy.

The Superfund Cleanup Program had been established by Congress to help clean up hazardous waste, and the program originally identified around 14,000 potential dumping grounds.

However, of these, only five had work done, and Gorsuch was cited for contempt of Congress for refusing to turn over subpoenaed documents on different sites, according to Newsweek.

In 1983, the Washington Post had this to say of Gorsuch's budget cuts:

"The cuts are so massive that they could mean a basic retreat on all the environmental programs of the past 10 years, according to agency sources and administration critics. At the same time, divisions between Administrator Anne M. Gorsuch and career agency staff over her approach to policy making have all but reached open warfare."

After Gorsuch left, Reagan, in a move to restore the public's faith and trust in the EPA, brought back William Ruckelshaus, according to a piece in the Conversation.

Even amidst Reagan's famed tax cuts, Ruckelshaus was able to implement environmental protections and steer the EPA out of its mired, scandalous past.

Under the direction of the Ruckelshaus, the EPA laid the groundwork for some crucial environmental and human health and safety measures including finding ways to protect the ozone layer and banning asbestos.

According to the Conversation, Ruckelshaus' second involvement with the agency proved that environmentalism still had a place and voice in a conservative, Republican administration, something that we could maybe learn from today.

Let's jump forward in time a couple of decades to the early 2000s, when climate change began picking up steam and with it, political discourse about the EPA's role in mitigating its impact.

In 2007, the Supreme Court ruled that the EPA had to act on previous findings (by the EPA itself) that greenhouse gas emissions were a form of pollution and as such endanger human health.

But today, Trump, Scott Pruitt, and now Andrew Wheeler are careful in their discourse on climate change to help push their own agendas without outright denying the impacts of climate change.

For example, the rolling back of coal pollution standards and allowing states to set their own coal plant emission limits chips away at greenhouse gas emission protections.

To the outside eye, and for many concerned environmental groups and politicians, these policies go in the face of the EPA's mission and what it was legally mandated to do, regulate CO_2 emissions which are a threat to public health and the environment.

Andrew Wheeler also recently disbanded EPA committee panels charged with reviewing air quality standards, particulate matter, and ozone levels.

Unbiased and thorough vetting of climate and pollution research is crucial for deciding policy, and by disbanding certain review committees, it could set a dangerous precedent for biased and uninformed policy.

The EPA, like any government agency, has not been without its problems and is also largely influenced by whichever administration currently holds office.

The agency can and will only ever be as good as both its administration and its administrator. When these facets act together in the service of private interests rather than the health and safety of the American public and environment, the original aim of the EPA is reduced to a punchline.

And unless more is done by Wheeler and the EPA to work with energy companies, limit emissions, protect the environment, and shift the focus to renewables, this chapter in the EPA's history could be looked at decades from now as a major low point of choosing to act against the well-being of the American people and the environment.

E&E News: White House pressured EPA to favor industry over climate

https://www.eenews.net/climatewire/2018/10/22/stories/1060103909

Maxine Joselow- Monday, October 22, 2018

White House officials urged EPA to make changes to an Obama-era methane rule that would maximize cost savings for the oil and gas industry while allowing the release of more planet-warming emissions, government documents show.

The exchange came this past spring and summer, as EPA was working to relax a 2016 rule stipulating how frequently oil companies must check for and repair methane leaks.

The White House Office of Information and Regulatory Affairs repeatedly pressured EPA to relax inspection requirements, according to hundreds of pages of documents posted last week on Regulations.gov and first reported by Bloomberg.

But some EPA officials worried that easing the requirements would allow more methane to enter the atmosphere. Methane is roughly 30 times more potent as a heat-trapping gas than carbon dioxide.

In May, EPA officials presented an interagency working group with a slideshow detailing their initial proposal for relaxing the Obama administration's New Source Performance Standards for new and modified oil and gas facilities.

The proposal would have saved the industry an estimated $246 million over six years.

In subsequent correspondence, OIRA officials pushed EPA to increase the projected cost savings to $484 million.

The OIRA officials argued that greater cost savings could be achieved through yearly compressor station inspections, rather than quarterly inspections.

Less frequent inspections were on the wish list of several oil and gas industry groups. Indeed, "monitoring frequency" was the first item on the agenda of a May meeting between Trump administration officials and representatives of the American Petroleum Institute, Chevron Corp., Royal Dutch Shell PLC and other oil companies.

EPA initially pushed back on OIRA's suggestions, noting that they could double the amount of methane entering the atmosphere.

"While Option 3 provides for the highest net benefits, it also provides the highest amount of forgone emission reductions," EPA said.

By July, EPA had begun implementing some of OIRA's suggested changes, the documents show.

In September, EPA released its proposed revisions to the Obama-era methane rule, which required industry to monitor wells on an annual basis and low-production ones every other year (Greenwire, Sept. 11).

The revelations come as the Trump administration touts the cost savings from its deregulatory agenda.

The White House on Wednesday unveiled its fall 2018 regulatory plan, which says that federal agencies achieved $23 billion in net regulatory cost savings in fiscal 2018. A senior administration official, in a conference call with reporters, described the past two years as an "amazing turnaround" (Greenwire, Oct. 17).

Amit Narang, regulatory policy advocate with Public Citizen, said the documents show that the White House is bent on helping industry at the expense of public health and the environment.

"These documents show very clearly that OIRA only had maximizing cost savings to the oil and gas industry in mind, and really wasn't concerned about the massive amount of methane emissions it would lead to," Narang said. "That will have an effect not only on climate change, but on the health of Americans."

EPA is now soliciting public comments on the methane proposal through Dec. 17. A final rule is expected next year.

Transparency

The documents underscore the need for transparency regarding communications between agencies and the White House regulatory affairs shop, sources said.

"This exchange demonstrates the importance of transparency in administrative policy development," Janet McCabe, acting EPA air chief under former President Obama, said in an email.

Not all federal agencies are required to publicize their communications with the White House. EPA is required to do so through a special provision in the 1990 amendments to the Clean Air Act. That's why the documents about the methane rule were posted online at Regulations.gov, where they were accessible to E&E News and other media outlets.

"All of this is coming out because of this really unique provision in the Clean Air Act," Narang said. "The provision says that if EPA is doing regulations under the Clean Air Act and OIRA is reviewing those regulations, all of those communications and changes need to be included in the docket."

James Goodwin, senior policy analyst with the Center for Progressive Reform, echoed those sentiments.

"This shows the importance of whoever included those provisions in the Clean Air Act amendments," Goodwin said. "And that's exactly what they had in mind, because agencies weren't disclosing stuff from OIRA review before that."

New legislation could affect the transparency of the OIRA review process.

Sen. Elizabeth Warren (D-Mass.) this summer introduced the "Anti-Corruption and Public Integrity Act," S. 3357.

Although the bill is largely focused on ethics in law enforcement, it also contains a small regulatory reform section aimed at increasing "disclosure of intergovernmental rule changes

High Plains Journal: Trump orders EPA to start year-round E15 regulatory process

http://www.hpj.com/ag_news/trump-orders-epa-to-start-year-round-e-regulatory-process/article_d3c73816-d2f4-11e8-8808-970923fa37c6.html

By Larry Dreiling October 22, 2018

President Donald Trump Oct. 9 ordered the Environmental Protection Agency to begin the regulatory process that would lead to year-round sales of E15, or 15 percent blend of ethanol in gasoline.

Currently, E15 sales are prohibited during summer months. Critics of the increased amounts of ethanol say higher amounts of ethanol blends will increase ozone pollution in warm weather.

EPA will have to move fast to accomplish Trump's goal, given the federal rule-making process by June 1, the usual regulatory review cut-off date.

Trump told reporters of his decision as he left the White House destined for Council Bluffs, Iowa, for a campaign rally to boost Republican candidates.

"So we're heading out to Iowa, where we have a big statement to make, as you know, on ethanol and for our farmers," Trump said as he prepared to board Marine One. "Likewise, we're taking care of our refineries and our refiners, and they've done a fantastic job. But we want to get more fuel into the system, and this is a great thing.

"But they're great for our farmers, and it was a promise that I made during the campaign. And, as you know, I keep my promises. So that's the way it is."

Later when asked about the petroleum industry's opposition to E15, Trump added, "Well, I want more industry. I want more energy. I want more, because I don't like $74. (Crude oil's price per barrel the day of the announcement.). It's up to $74. And if I have to do more, whether it's through ethanol or through another means, that's what I want. I want low prices. So I'm OK with it.

"You know, it's an amazing substance. You look at the Indy cars—they run 100 percent on ethanol. And you look at other certain forms of very modern energy, it's ethanol-based, and that meant a lot to me.

"But what it really means is we're helping our farmers and we're also going to be helping our refiners, and you know that too—because they're both very important. But the farmers have been so terrific, and they produce great product. So I think it's going to be great."

In a reference to those concerns that E15 causes ozone pollution in summer, Trump said, "And it has no impact—12 months. That was a misnomer. There is no negative impact. In fact, there are those that say, you do this and the air is cleaner. I agree with that. But they say, you go 12 months instead of eight—you go 12 months, and the air is actually cleaner."

Secretary of Agriculture Sonny Perdue joined Trump in the Oval Office for the announcement, Politico said, along with Republican Sens. Chuck Grassley and Joni Ernst of Iowa, Deb Fischer of Nebraska and John Thune of South Dakota, along with Rep. David Young, R-IA.

The article made no mention of Environmental Protection Agency Administrator Andrew Wheeler, who will be in charge of the rulemaking process of allowing the year-round sales of E15.

Perdue said in a statement, "This is another case of 'Promises Made, Promises Kept' for President Trump."

“Expanding the sale of E15 year-round is sound policy for a variety of reasons,” Perdue said. “Consumers will have more choices when they fill up at the pump, including environmentally friendly fuel with decreased emissions.“

“It is also an excellent way to use our high corn productivity and improved yields. Year-round sale of E15 will increase demand for corn, which is obviously good for growers. This has been a years-long fight and is another victory for our farm and rural economies. Along with E15 expansion, we also welcome much-needed reforms to the RIN market, which will also increase transparency.

“President Trump has again made it abundantly clear that he is unleashing the full potential of American energy production as we retake our rightful place as the world’s leader.”

Agriculture reacts

American Farm Bureau Federation President Zippy Duvall said in a statement, “I thank President Trump for his steadfast support of E15 expansion, while also acknowledging the close working relationship we’ve developed with Acting EPA Administrator Andrew Wheeler. I look forward to working with the EPA to see rulemaking and year-round E15 completed by the driving season of 2019.”

“AFBF applauds the president for his support of homegrown biofuels and upholding the campaign promises made to rural America.”

National Farmers Union President Roger Johnson said, “Allowing use of E15 gasoline year-round is an important step toward realizing a renewable energy future for transportation fuel sector, and we’re appreciative of the administration’s support for higher level blends of ethanol.At the same time, this ‘compromise’ does nothing to address the billions of gallons of ethanol demand that were lost as a result of the EPA’s RFS waiver handouts to oil refiners.

“Family farmers are in significant financial distress right now, and the administration’s surreptitious biofuel demand destruction has made matters worse. The bottom line here is that if the president wants to do right by his promises to support family farmers and American grown biofuels, his administration must support net increases in biofuel use. Even with an E15 waiver, family farmers are at a net loss in biofuel demand over the past two years.”

National Corn Growers Association President Lynn Chrisp thanked Trump and said that regulations requiring retailers to stop selling E15 in the summer are outdated.

“Earlier this year, the president correctly described this barrier as ‘unnecessary’ and ‘ridiculous’,” said Chrisp. “The president also faced pressure to fix this regulatory problem through a bad deal that would have been harmful to farmers. He made the right decision to move this common-sense regulatory relief on its own, and farmers are very grateful.”

Growth Energy CEO Emily Skor said, “We thank President Trump for delivering on his promise to rural America by lifting the summer restriction on E15 sales.”

“He answered the call from American farmers by removing the single most important barrier to growth in higher biofuel blends.

“This announcement is great news for farmers, biofuel workers, retailers and consumers everywhere who want to enjoy cleaner, more affordable options at the fuel pump. This is a critical step toward giving American motorists higher-octane options at a lower cost all year long.

"We sincerely appreciate President Trump's steadfast commitment to rural America. Nationwide E15 sales promise to drive demand for 2 billion bushels of American corn and help restore growth in rural communities hit hardest by the downturn in farm income."

Skor said Growth Energy expects that the oil industry and environmental groups will bring litigation against the year-round sales, but that she agrees with the Trump administration that EPA will be on firm ground when the cases reach the courts.

Renewable Fuels Association President and CEO Geoff Cooper said, "Securing fair market access for E15 and other higher blends has been our top regulatory priority for several years, and we are pleased that the first official step in this process is being taken. When markets are open and competitive, American consumers win."

Critics speak out

The American Petroleum Institute said the Trump administration's decision to allow for year-round sales of higher ethanol E15 blended gasoline "is a bad deal for consumers."

"Putting a fuel into the marketplace that the vast majority of cars on the road were not designed to use is not in the best interest of consumers," said API President and CEO Mike Sommers. "Vehicle compatibility tests have shown that high ethanol levels in gasoline can damage engines and fuel systems."

"EPA has previously stated that it does not have the legal authority to grant the E15 waiver, and we agree with that assessment," Sommers said. "The industry plans to aggressively pursue all available legal remedies against this waiver."

ActionAid USA said, "This is an attempt to buy off farmers with a giveaway to big ethanol. More corn ethanol isn't going to address the real challenges farmers are facing, or make up for the lack of a farm bill.

"It will continue to worsen the harm corn ethanol is doing to the environment. Our system of agriculture is broken. Instead of giving in to the demands of agribusiness and big corporations for more ethanol, we need to build a system that works for farmers, eaters and the planet."

Breitbart: EPA Reverses Obama Era Last Minute Uranium Mining Regulations

https://www.breitbart.com/politics/2018/10/20/epa-reverses-obama-era-last-minute-uranium-mining-regulations/

By Penny Starr- October 20, 2018

The United States Environmental Protection Agency (EPA) announced on Friday that it is withdrawing the pending rulemaking for uranium mining and thorium mill tailings.
The regulations were put in place on Jan. 19, 2017, just hours before Barack Obama left office and President Donald Trump's inauguration.

"In a rush to regulate during the waning hours of the previous administration, the agency proposed a regulation that would have imposed significant burdens on uranium miners and the communities they support," Acting Administrator Andrew Wheeler said in the press release announcing the decision.

Rep. John Barrasso (R-WY), chairman of the Senate Environment and Public Works Committee, praised the agency's move.

"Today's announcement is the right decision," Barrasso said. "The Nuclear Regulatory Commission – our nation's principal nuclear regulator – has said there is no health or safety justification for EPA's midnight rule."

"The NRC has regulated in situ uranium recovery for nearly 40 years," Barrasso said. "The agency has never found an instance of groundwater contamination that would be addressed by this rule."

"I'm glad the Environmental Protection Agency has acknowledged this reality," Barrasso said. "I applaud it for withdrawing this punishing and unnecessary regulation on America's uranium producers."

The proposed rule expressed concerns from federal partners, including the Nuclear Regulatory Commission (NRC) and stakeholders, about the agency's legal authority under the Uranium Mill Tailings Radiation Control Act of 1978.

"Today's action underscores that EPA believes existing regulatory structures are sufficient at this time to ensure the protection of human health and the environment at current uranium in-situ recovery (ISR) activities," the press release stated. 'Today's withdrawal has no impact on EPA's regulation of radiation."

PBS News: Why radiation protection experts are concerned over EPA proposal

https://www.pbs.org/newshour/nation/why-radiation-protection-experts-are-concerned-over-epa-proposal

By — Ferenc Dalnoki-Veress, Oct 20, 2018 1:49 PM EDT

The Takata Corporation sold defective air bag inflators that resulted in the death of 16 people in the United States and a massive recall of cars. While it was rare for the air bags to fail, the brutal consequences of this defective device in even minor collisions was easy to recognize. But the effects of low-dose ionizing radiation – high energy waves or particles that can strip electrons from atoms and physically damage cells and the DNA within – on people's health is much harder to see, and prove.

When the Associated Press reported that the Trump administration's Environmental Protection Agency solicited the advice of a controversial toxicologist, Edward Calabrese, to consider changes to how it regulates radiation, it sent shock waves through the radiation protection community. Calabrese is well known for his unconventional and outlying view that low-dose radiation is not dangerous.

I'm a physicist at the James Martin Center for Nonproliferation Studies at the Middlebury Institute of International Studies who focuses on risks of emerging technology. I am keenly aware of the danger of exaggerating the effect of ionizing radiation, which has led to a phobia of radiation and stigma toward those who suffered radiation exposure. However, underestimating these effects can be just as detrimental. And doing so may only be in the interest of certain stakeholders that have the ear of the current administration.

High-dose radiation kills; what do low doses do?

It is important to note that the health effects of high doses of radiation are well established. We all know about the horrific effects based on studies of the populations of Hiroshima and Nagasaki after the atomic bombs were dropped. Then there was also the recent case of Russian defector Alexander Litvenenko who quickly sickened and died 23 days after being poisoned with the radioactive isotope polonium-210 in 2006.

However, the effects of low doses of radiation are not well understood. Part of the reason is that these low doses are difficult to measure.

Current understanding of the health effect of radiation relies primarily on a decades-long study of the survivors of the Hiroshima and Nagasaki atomic bomb attacks. That population was exposed to a one-time large dose of radiation, with individual exposure dependent on where they were at the time of the explosion.

In those high-dose radiation studies, researchers found that there is a proportionate relationship between dose and effect. The way the EPA gauges the effect of low doses of radiation draws from these studies as well as studies following other incidents. The current guidelines for the EPA adhere to what is called the linear no-threshold (LNT) model, which implies that even low doses of radiation have an effect across a population. Some scientists dubbed it to be a "reverse lottery," where an unlucky few within a given population will get cancer during their lifetime due to their exposure to radiation.

There have been questions as to whether the LNT model is appropriate for measuring cancer risk from low doses of radiation. That's because when the radiation-induced cancer rate is low, and the sample size is small, there is more statistical uncertainty in the measurement. This allows more wiggle room in putting forward alternative dose-response models such as Calabrese's, which have little scientific backing but that promise financial benefits for regulated industries.

Overall, the general feeling in the radiation protection community is that for now until new research proves otherwise, the LNT model, because of the lack of understanding of the effect of low doses, is the prudent model to use to set protective limits.

Also, not being able to determine the effect of a low dose of radiation is a problem in measurement, not in the underlying linear threshold model. As doses of radiation decrease, fewer cases of radiation-induced cancers occur, making it more difficult to identify those specific cases.

This is especially true given that cancer is already a common occurrence, making it nearly impossible to disentangle radiation exposure from many other potential cancer risk factors. This is where the analogy with Takata air bags fails, because it is not possible to prove that a specific cancer death is due to ionizing radiation, but this does not make it any less real or significant.

Who profits if radiation guidelines change

The EPA issues guidance and sets regulations to "limit discharges of radioactive material affecting members of the public" associated with the nuclear energy industry. The EPA defines what radiation levels are acceptable for a protective cleanup of radioactive contamination at Superfund sites. It also provides guidance on the levels of radiation exposure that would trigger a mass evacuation. It is not surprising that certain stakeholders would welcome modifications in EPA assessment of low-dose radiation exposure given the high costs involved in preventing or cleaning up sites and in compensating victims of such exposure.

Recently, the National Council on Radiation Protection and Measurements (NCRP) – scientists who provide guidance and recommendations on radiation protection under a mandate from Congress – supported the LNT model. NCRP analyzed 29 epidemiological studies and found that the data was "broadly supportive" of the LNT model and that "no alternative dose-response relationship appears more pragmatic or prudent for radiation protection purposes."

In fact, the National Academies' Nuclear and Radiation Studies Board, the International Council on Radiation Protection, and other international bodies and regulators all use the LNT model for guidance and radiation protection.

From my perspective, as someone who has worked with radioactive sources, the EPA should be cognizant of the warning by the late Harvard sociologist Daniel Yankelovich that just because an effect can't be easily quantified does not mean it is not important or does not exist.

Green Car Report: Deadline for comments on EPA fuel-economy rollback is Tuesday

https://www.greencarreports.com/news/1119481_deadline-for-comments-on-epa-fuel-economy-rollback-is-tuesday

Eric C. Evarts- Oct 22, 2018

EPA Acting Administrator Andrew WheelerEPA Acting Administrator Andrew Wheeler

Readers who want to make their voices heard in objection to (or support of) the EPA proposal to freeze fuel economy standards have until tomorrow to get their comments in.

The EPA proposed in early August to undo Obama-era rules requiring cars to reach increasingly stringent fuel-economy targets through 2025. Called the Safer Affordable Fuel-Efficient Vehicles Rule, the proposal would freeze the standards at 2020 levels through 2026.

Although cars have already become more efficient, the upcoming years were expected to extend such efficiency gains to light trucks, such as pickups and large SUVs, which have captured an increasing number of American car sales in recent years.

Not only will the looser standards result in more emissions from gas (and diesel) cars and trucks produced from 2021 to 2026, the proposal seeks to strike down the California law that has resulted in the production of electric cars to be sold in the state and 12 others.

California and 16 other states have already sued the EPA and NHTSA over the proposal.

As with most federal regulations, the proposal requires a 60-day comment period before the executive branch can implement it. That 60 days expires on Tuesday (tomorrow.)

The EPA held three public hearings in September to gather public comments, but its website is still open to receive further comments until the deadline expires.

Public comments get heard, according to former EPA officials. Former head of the EPA's Office of Air Quality and Transportation, Margot Oge, told the New York Times in September that personal stories leave a lasting impression. The comments also become part of the legal record when a regulation is challenged in court—as this one already has been.

We know our readers love to weigh in on issues. This opportunity may be more effective than most.

The State- Chemical exposure news trickles out to residents

https://www.thestate.com/news/business/national-business/article220271165.html

BY DELLA HASSELLE AND NICK REIMANN- October 21, 2018 12:01 AM

It's been 15 years since Marcia Llewellyn left Norco, a community located next to an industrial complex long known for its distinctive odor, and a place that many residents said had become too polluted to live in.

"That's what I always remember about it — the smell," Llewellyn said as she stood inside her home on Union Street in Montz, a sleepy town along River Road on the east bank of St. Charles Parish. "You know the chemicals from the plants there had to be very strong, with a smell like that."

This month, Llewellyn was shocked to discover her dead-end street in the quiet neighborhood she calls home might carry even more significant health risks.

According to a recent report from the U.S. Environmental Protection Agency, residents in her census tract face the highest risk of breast or lymphoid cancers of anyplace in the country because of emissions of the carcinogen ethylene oxide. The chemical is produced in large amounts at the Union Carbide Corp. plant across the Mississippi River in Taft.

After the report came out, a spokeswoman for Dow Chemical Co., which owns Union Carbide, acknowledged that the company is one of the largest producers of ethylene oxide in the country but said it has "safely produced" it in St. Charles Parish since the company took ownership of the plant in 2001.

However, understanding of the dangers associated with the chemical has changed over time, according to the EPA. It was only categorized as a carcinogen in 2016.

Llewellyn, 58, said she wasn't aware of the EPA's latest National Air Toxics Assessment, which came out in August. And, she said, she didn't think much about chemical exposure when deciding on the best place to live with her sisters, nephews and a year-old baby.

"I mean, I had concerns, but they've always been in the back of my mind," she said.

Indeed, news of cancer risk from the airborne pollutant has been slow to infiltrate many neighborhoods in the River Parishes.

About 10 miles upriver, a small group of activists in St. John the Baptist Parish are more in-the-know about the EPA's latest assessment, which focused on ethylene oxide based on chemical emissions data from 2014.

That's because they mobilized several years ago, when the EPA identified areas around the Denka Performance Elastomer plant in LaPlace as the most at-risk in the U.S. from the "likely carcinogen" chloroprene, a chemical used to make synthetic rubber that has been produced at the plant for decades.

Elsewhere, however, many residents are dubious or even fatalistic when presented with the emissions data — an unsurprising response, environmentalists say, for people accustomed to living in the corridor between New Orleans and Baton Rouge that is known for its reliance on the petrochemical industry.

In areas of St. Charles Parish where the risk is high, many residents seem torn between acknowledging the findings of the air pollution study and defending the chemical companies that employ their friends and family members.

Just a handful of local and state elected officials are starting to question the safety of the air in neighborhoods and schools throughout St. Charles, St. John and Ascension parishes as they dig into the data provided by the EPA study.

Overall, the reaction in the River Parishes stands in stark contrast to the outrage that has erupted in Willowbrook, Illinois, where the Sterigenics International facility produces ethylene oxide, commonly used to sanitize medical equipment, fumigate spices, and make polyester and antifreeze.

There, citizen groups quickly organized, and the political pressure grew so furious that Illinois Gov. Bruce Rauner called for the plant to be shut down.

For his part, Louisiana Gov. John Bel Edwards declined to comment on the EPA study at a press conference earlier this month, saying he had not had a chance to catch up on the issue.

Other elected officials tasked with representing St. Charles and St. John parishes have been equally silent. Of 20 council members, school board members, state representatives and senators contacted about the emissions, only seven responded. All but two said they would have to study the issue further before taking any action.

Environmentalists Wilma Subra of the Louisiana Environmental Action Network and Anne Rolfes of the Louisiana Bucket Brigade called the response "lackluster."

"Shouldn't they have rolled up their sleeves and looked into it by now?" Rolfes asked. "And just imagine how it is for those who live across from these facilities. They get no response."

As for Llewellyn, she believes she shouldn't have to think about air pollution when choosing a place to live.

"Yes, it bothers me," she said. "I would like to live in a safe area. You go outside and breathe the air and you might get sick — that doesn't sit well."

In Louisiana, the Union Carbide Corp. plant is the largest producer of ethylene oxide and has been manufacturing the chemical since the late 1960s.

While the chemical is produced throughout the United States, the highest cancer risk from emissions is across the river from the plant in census tract 601, where Llewellyn lives.

There, scientists say, residents face a cancer risk 700 times greater than the EPA's goal of a 1-in-a-million, and seven times what the agency says is in the "upper limit" of what's acceptable for humans.

While those eye-popping numbers are a surprise for some, they seemed about right to 34-year-old Brad Trepagnier, who has been working at chemical plants since his early 20s.

But the situation is complicated, he said, because the chemical plants drive the economy for the towns most affected by the pollution.

"I've always been at risk, but I guess that's what I get for living close to all these plants," he said. "That's what we choose."

Across the river, Nolan Darensbourg has lived about a mile west of the Union Carbide plant his entire life.

He said he'd be "willing to move" if someone convinces him that ethylene oxide poses a real risk, but he's not yet convinced that his health is in peril.

"If it's proven that it's bringing up cancer and, you know, of course I'm moving," Darensbourg said. "Of course — there's no reason to stay here."

Others say they feel powerless against the industry, and have all but given up trying to fight it.

Nadine Washington can name several friends and neighbors who have been diagnosed with cancer in recent years, and she thinks the pollution had something to do with it.

But she has been in Killona her entire 59 years, in a home she inherited from her grandmother, and can't afford to leave, she said.

"We know they was releasing something that was going to kill us, and one day would have caused some kind of cancer," Washington said. "Ain't nothing we could've did about it. We didn't know how to go about doing anything about it."

Although some residents are quick to express concern and even outrage over the ethylene oxide emissions, few said they have made the effort to call their state legislators, congressional representatives or even local council members.

In the meantime, Sen. Edward Price, a Democrat from Gonzales, is one of the few state elected officials in Louisiana who have expressed willingness to take action after reading the EPA's report.

Price said that he had called on the Louisiana Department of Environmental Quality to work with the EPA on a future ambient air monitoring program in St. Charles and St. John parishes.

"Anytime you have anything like this there is a concern," Price said. "We have to make sure (the companies) are meeting emissions standards and aren't putting an overbearing burden on these parishes."

But officials with the DEQ say that as of now, they have no set plans to test for air quality. First, they're deferring to the EPA to set a national standard for emissions. In the meantime, said Greg Langley, a spokesman for DEQ, "residents don't need to be panicked" by the risk estimates so far.

"I think our message at this point is that even though this change has been made, it's a paper change," he said. "It's a change in a computation."

Officials with the EPA, who based the recent report on data from the chemical plants themselves, have said they may decide to do some monitoring. But even if they do, that process will take time, because first scientists have to figure out just how much of the chemical is permeating the air.

To achieve that, officials have to develop new monitoring techniques. Traditional air quality monitoring, they said, just isn't sensitive enough.

Based on the studies and evidence, the EPA determined that a very small amount of the chemical — just .003 micrograms per cubic meter — could cause a person to develop cancer if they have constant exposure over a lifetime, or 70 years.

"We just don't have anything to measure these emissions," Langley added. "It's essentially zero. If it's down as low as we want, we can't measure it."

Langley also said that official emissions standards have not been updated since ethylene oxide was reclassified as a carcinogen in 2016, so there is no way to "force" chemical companies to reduce their emissions.

In 2016, Dow's Union Carbide plant deliberately released an estimated 30,700 pounds of ethylene oxide into the air; an estimated unplanned 5,100 pounds of the produced gas also escaped into the air, according to an EPA database. The only U.S. industrial facility releasing more ethylene oxide into the air is in Port Neches, Texas.

A spokeswoman for Dow said their emissions have long been within legal limits, and Langley said the plant has been regulated in Louisiana for the last 20 years.

Most government officials who responded said they hadn't fully had a chance to review the EPA's findings, and so couldn't pass judgment on how they might affect their communities or constituents.

"The EPA report is alarming. That's what I have to say," said St. Charles Parish Councilwoman Marilyn Bellock. "I'm assuming we're going to get together as a council and talk about a plan of action."

To be sure, there are activists who are trying to organize residents in the most affected neighborhoods.

At a recent meeting held just blocks from Evonik Materials Corp. in Reserve, activist Robert Taylor called the EPA's recent report "devastating." The St. John Parish plant also produces ethylene oxide.

"For me it's hard to understand how this could take place," said Taylor, president of the Concerned Citizens of St. John. "Where is the government? Where are the regulatory people? Where are the agencies that are supposed to be protecting us from these people?"

He added: "It seems to me that they've completely abandoned us and that they're in collusion with the petrochemical plants. They're not regulating these people. How could this go on?"

Members of the group have long complained about health problems ranging from cancer to rashes and respiratory illnesses, and many were quick to blame the neighboring plants.

Their goal, they say, is to create a new group across the River Parishes that will be politically influential enough to field candidates in the 2019 legislative elections.

"The people are very disturbed," said Shondrell Perrilloux, a 40-year-old contractor. "Our government appears to be failing us. Their reactions are not as curious and as emboldened and concerned as the citizens' are."

The Hill: Greens challenge Trump's rollback of coal ash standards

https://thehill.com/policy/energy-environment/412524-greens-challenge-trumps-rollback-of-coal-ash-standards

BY TIMOTHY CAMA - 10/22/18 10:48 AM

Environmental groups are suing the Trump administration for rolling back parts of a major regulation governing how companies store coal ash.

The groups, led by Earthjustice, filed their lawsuit Monday against the Environmental Protection Agency (EPA) in the U.S. Court of Appeals for the District of Columbia Circuit.

The original 2015 rule from the Obama administration was the first national regulation regarding disposal of coal ash, a black sludge left over from burning coal that contains concentrated levels of heavy metals like arsenic, cadmium and chromium. Coal ash storage ponds are often adjacent to waterways, since coal-fired power plants need cooling water.

The amendments, rolled out in July, give more time for coal plant owners to clean up certain coal ash ponds that are leaking, and gives states new power to exempt companies from certain pollution-monitoring requirements.

"The risk that legacy impoundments and insufficiently lined coal ash ponds pose is too great to let another hurricane season go by without addressing the problem," Thomas Cmar, the lead Earthjustice attorney in the case, said in a statement.

"The dam breach at the Sutton Plant that spewed toxic coal ash into the Cape Fear River in the wake of flooding from Hurricane Florence should make it clear that there's no time to waste," he said, referring to an incident last month in which flood waters moved an unspecified amount of coal ash from a closed power plant into a North Carolina river. State tests later found that pollutant levels in the river had not exceeded state maximums.

"Throughout the country, in the absence of adequate regulation by EPA, coal ash has been irresponsibly disposed of," said Larissa Liebmann, staff attorney at the Waterkeeper Alliance, another party to the case. "This leaves communities and waterways vulnerable to long-term contamination, as well as spills like we saw in North Carolina with Hurricane Florence. EPA needs to stop catering to industry and start protecting the public."

The EPA did not respond to a request for comment.

But in rolling out the changes in July, acting EPA head Andrew Wheeler said they would provide necessary "flexibility" for states and companies.

"These amendments provide states and utilities much-needed flexibility in the management of coal ash, while ensuring human health and the environment are protected," he said. "Our actions mark a significant departure from the one-size-fits-all policies of the past and save tens of millions of dollars in regulatory costs."

The EPA estimated that the changes would save up to $31.4 million per year, from lower compliance costs for companies.

Some of the changes were mandated by Congress in a 2016 law.

In their statement, green groups highlighted an August ruling by the D.C. Circuit Court that found that some parts of the original 2015 rule were inadequate. Specifically, the judges said the EPA should not have exempted coal ash ponds at closed plants from certain standards, nor should it have let some unlined ponds remain.

The EPA said it is planning to propose further changes to the coal ash in the future.

--This report was updated at 11:24 a.m.

Common Dreams: Environmental Groups Challenge Trump Administration Coal Ash Rule Rollback in Court

https://www.commondreams.org/newswire/2018/10/22/environmental-groups-challenge-trump-administration-coal-ash-rule-rollback-court

Thom Cmar- October 22, 2018

In wake of recent court decision, EPA's watering down of coal ash regulations on weak footing

WASHINGTON - Today, Environmental groups filed a petition for review in the U.S. Court of Appeals for the D.C. Circuit challenging an EPA rule designed to gut coal ash disposal regulations that provide environmental safeguards for communities living near toxic coal ash waste dumps.

In March of 2018, the EPA proposed the rollbacks in response to an industry petition to the Trump Administration. The rule was finalized in July, and modifies the Obama-era Coal Ash Rule from 2015. Under the Trump Administration changes, power plant owners have more time to clean up leaking coal ash disposal sites that have been shown to have contaminated groundwater. The new rule also allows state-run coal ash permit programs to include loopholes such as allowing states to waive groundwater monitoring requirements under certain circumstances.

A recent court decision casts serious doubt on the legality of these rollbacks. In August, the D.C. Circuit ruled in favor of environmental groups' lawsuit challenging that the original Obama-era rule was unlawfully weak in several key respects. In particular, the court struck down provisions of the 2015 Coal Ash Rule that exempted impoundments at closed coal plants and allowed coal ash impoundments that are unlined or only underlain by inadequate clay liners to continue to operate. The EPA must now draft rules to address more than 100 "legacy" coal ash ponds at retired coal plant sites. The EPA is also now required to address the closure of over 600 unlined or clay-lined coal ash ponds in response to the court's decision.

"The risk that legacy impoundments and insufficiently lined coal ash ponds pose is too great to let another hurricane season go by without addressing the problem," said Thomas Cmar, deputy managing attorney for the coal program at Earthjustice. "The dam breach at the Sutton Plant that spewed toxic coal ash into the Cape Fear River in the wake of flooding from Hurricane Florence should make it clear that there's no time to waste."

"Throughout the country, in the absence of adequate regulation by EPA, coal ash has been irresponsibly disposed of," said Larissa Liebmann, staff attorney at Waterkeeper Alliance. "This leaves communities and waterways vulnerable to long-term contamination, as well as spills like we saw in North Carolina with Hurricane Florence. EPA needs to stop catering to industry and start protecting the public."

"The Trump EPA is a rogue agency, out of step with both its mission and the law," said Environmental Integrity Project attorney Abel Russ. "The courts are telling EPA that the coal ash rule is not strong enough, and meanwhile EPA is trying to weaken the rule. It's absurd. The American people deserve better."

Andrew Rehn, water resources engineer for the Prairie Rivers Network said, "Illinois needs the US EPA to step up it's protections on coal ash, not back away from them. Illinois's only National Scenic River is constantly being polluted by seepage from a coal ash pit at a closed power plant, and it's not the only waterway in Illinois with coal ash sitting on the riverbank."

"It's clear the Trump administration doesn't value protecting human health, especially if corporate special interests could be slightly inconvenienced," said Jennifer Peters, National Water Programs Director for Clean Water Action. "This outrageous scheme would let coal plants put communities, families, and water at risk with impunity. It's time for EPA to listen to the courts and the public and strengthen, not weaken coal ash safeguards."

"It's clear that former coal lobbyist and current acting EPA Administrator Andrew Wheeler has an open door policy when it comes to the coal industry," said Mary Anne Hitt, Senior Director of the Sierra Club's Beyond Coal Campaign. "Coal ash is a dangerous, widespread problem, but instead of safeguarding the public from its devastating effects, Wheeler is once again ignoring the issue in order to placate his former clients. Without strong federal coal ash regulations, polluters will continue to dump their toxic coal ash waste in unlined pits that will continue failing, endangering drinking water and public safety. The courts have already agreed the risks posed by coal ash can no longer be ignored, and that's why we're fighting this most-recent Wheeler roll back."

“The efforts of the current administration to roll back environmental safeguards are a direct threat to public health and safety,” Dr. Scott Williams, Executive Director of HEAL Utah said. “We can’t sit by idly and allow these rules to be eliminated. If we do, our most vulnerable populations – our elders and our children – will suffer needlessly from our lack of action.”

The petition was filed by Earthjustice, The Environmental Integrity Project, and Sierra Club, on behalf of Clean Water Action, Hoosier Environmental Council, Prairie Rivers Network, HEAL Utah, and Waterkeeper Alliance.

E&E News: Senate Dem says Wehrum has flouted ethics pledge

https://www.eenews.net/greenwire/2018/10/22/stories/1060104003

Sean Reilly- Published: Monday, October 22, 2018

EPA air chief Bill Wehrum has ignored President Trump's ethics pledge by meeting with former industry clients covered by his recusal statement, a Senate Democrat charged in letters released over the weekend.

Trump "campaigned on a promise to 'drain the swamp' of corruption in Washington," Sen. Sheldon Whitehouse (D-R.I.) said in a letter to acting EPA Administrator Andrew Wheeler.

"Allowing appointees to openly flout the terms" of that pledge "only serves to make a mockery of it," Whitehouse added in requesting answers to questions by the end of this week.

The pledge requires Trump's appointees to recuse themselves for two years from participation "in any particular matter involving specific parties that is directly and substantially related" to their former employer or specific clients.

But Wehrum — who was sworn in as head of the Office of Air and Radiation last November — signed the recusal statement only last month after Whitehouse had filed a legislative amendment that would have forced him to do so.

Among the clients Wehrum previously represented as an attorney in private practice are Duke Energy Corp., General Electric Co. and the Utility Air Regulatory Group. In the recusal statement, he acknowledged it covered "meetings or other communications" related to performance of his official duties.

But Wehrum returned last December to the offices of his old law firm to speak to a gathering that included representatives of Duke Energy, the Utility Air Regulatory Group and one other former client, Whitehouse wrote.

And in January, Wehrum twice met with General Electric representatives, while in February, he again participated in meetings with Duke Energy and the Utility Air Regulatory Group, the senator said, citing calendar entries released under the Freedom of Information Act. Under Trump's ethics pledge, the air chief's participation in all of those meetings would have been prohibited, he added.

Whitehouse's office also released a Sept. 29 letter from Kevin Minoli, then EPA's designated ethics official, which details a prolonged effort to prod Wehrum into furnishing the recusal statement. After Wehrum initially underwent ethics training last November, Minoli said he met with him in person, communicated by phone and email, and coordinated with Wehrum's staff about his ethics obligations, including the importance of signing a recusal statement.

"Mr. Wehrum initially chose to use other tools that he deemed effective in helping him to comply with the ethics requirements, such as use of a screening official," Minoli wrote.

For meetings involving his former firm, now known as Hunton Andrews Kurth LLP, or former clients, Wehrum's staff coordinates with EPA ethics officials, who provide "appropriate counsel and case-specific advice," Minoli wrote.

He added, however, that Wehrum has not received waivers or authorizations pursuant to Trump's ethics pledge, contained in an executive order issued early last year.

In his letter, Whitehouse, a member of the Senate Environment and Public Works Committee, asked Wheeler to explain how Wehrum would be allowed to meet with former clients without first getting a waiver or authorization. Whitehouse also asked whether it is "official EPA policy" to allow agency employees to choose their own compliance "tools" and whether Wehrum has faced any consequences.

Whitehouse's office released the Oct. 10 letter on Saturday, along with a similar letter to Trump, also dated Oct. 10.

The White House press office did not reply to an emailed request for comment this morning. Wehrum referred an inquiry to EPA's press office, where spokesman James Hewitt said the agency would reply to Whitehouse "through the proper channels."

E&E News: EPA's carbon rule sparks debate over cost-benefit analyses

https://www.eenews.net/greenwire/2018/10/22/stories/1060103977

Niina Heikkinen- Monday, October 22, 2018

Robin Lovett-Owen attended EPA's Chicago public hearing on the proposed replacement for the Obama-era Clean Power Plan to remind the Trump administration that the plan's projected increases in premature mortality and asthma attacks were more than numbers.

The student at the Lutheran School of Theology at Chicago recalled in the Oct. 1 hearing the two children she had met while working as a chaplain in a city hospital's pediatric unit — a teenage athlete and a nonverbal 8-year-old — who both died from asthma attacks.

"It's hard to imagine what those numbers mean when you read them from the comfort of your desk. It's all too easy to imagine what they mean when you've met and mourned children like Xander and Trey," Lovett-Owens said at the only hearing EPA has scheduled on its proposed Affordable Clean Energy rule.

"The deaths of black children in Chicagoland may seem like a far cry from the deaths of white coal miners and their families in Appalachia, but they point to the same truth: The true cost of polluting our air is the deaths of the most vulnerable people in our country, whether they be children of color in urban areas or workers in the hollers of the Smoky Mountains."

Lovett-Owen — a native of coal mining country in east Tennessee — was among many people to point to the 1,400 premature deaths the EPA's analysis says the rule would cause by 2030. How, they asked, could the Trump administration approve a rule that increased the risk of harm?

The answer lies in how the Trump administration has been weighing the costs of implementing the rule against its potential benefits.

As President Trump presses to reduce regulations across the government, EPA has been reworking how it tallies regulatory costs and benefits. Last week, the White House released its report on its rule-slashing efforts that showed EPA planning to proceed with a plan to "clarify interpretations" of cost-benefit analysis with a notice of proposed rulemaking next May (Greenwire, Oct. 17).

William Buzbee, a law professor at Georgetown University Law Center, said the administration is targeting a widely held assumption that regulations should prevent an increase in harm.

"It is rare that you see agency policy shifts that increase a risk," he said in a telephone interview.

Usually, federal agencies face legal hurdles to relaxing health protections. And states like California, known for strict environmental rules, tend to contradict any assertions that stricter standards aren't feasible.

Even businesses, Buzbee said, can undermine efforts to weaken standards by meeting pollution targets more cheaply and easily than expected.

"Why might you still see it? The reason why regulatory choices could lead to increased risks is that many statutes require consideration of a handful of factors, with health among them," he said.

"Health isn't the only criterion driving regulation," he said, but it "often is so central or an overarching goal that most agencies true to their congressionally assigned missions would on their own reject such a change."

Front and center in the Trump administration: the cost of regulating.

EPA released the proposed Affordable Clean Energy rule as the administration was in the midst of a sweeping effort to rein in regulatory spending.

Under Neomi Rao, the administrator of the White House Office of Information and Regulatory Affairs (OIRA), agencies have aggressively looked to cut regulatory costs in line with Trump's Executive Order 13771, which calls for eliminating costs of two regulations for every new rule enacted.

This fiscal year, agencies that succeeded at meeting that standard are being called on to push for even deeper reductions, cutting costs for three regulations for every rule enacted.

While not all federal agencies have kept that pace, the White House recently announced that in fiscal 2018, agencies cut about four significant regulations for every new significant rule put in place.

In a background press briefing last week, a senior administration official said the White House was "expressly focused" on reviewing significant rules that impose the greatest costs on the public.

Playing up costs

Critics of Trump's executive order say it undercuts the cost-benefit analysis process by pushing agencies to focus on cost but saying nothing about preserving rules that provide vital public health and environmental protections.

Sally Katzen, the former head of OIRA under the Clinton administration, notes that the order mentions costs 17 times but never mentions the word "benefits."

"That's not analytically sound," she said. "Not surprisingly, this has energized the left as well as conventional conservative economists to say this is wrong — cost-benefit analysis includes consideration of both sides of the equation, benefits as well as costs."

The administration's focus on cutting regulations also could have an influence on how OIRA reviews EPA's analysis of the power plant rule, according to another former chief of OIRA, which is responsible for reviewing agencies' draft and final regulations.

"At OIRA, the regulatory-policy guidance from cost-benefit analysis will always take a back seat to presidential priorities that relate to specific campaign commitments," John Graham, who led OIRA under President George W. Bush, said in an email.

One of OIRA's "key objectives" was to ensure that agencies honored presidential priorities and policy preferences.

"Thus, the premature deaths that might be induced by a deregulatory action are more of a concern in the judicial review of a regulatory action than they are in OIRA review of a White House-urged deregulatory action," Graham said.

Georgetown University's Buzbee also warned that regulatory revisions that would increase pollution or increase health threats compared with a previous rulemaking are "at high risk of flunking judicial arbitrary and capricious review."

Alan Krupnick, senior fellow at Resources for the Future and former senior economist on the President's Council of Economic Advisers under President Clinton, suggested that critics focusing on the increased risk of premature death under Trump's proposed power plant rule were taking a "strictly public health perspective," rather than looking at a cost-benefit assessment of whether the rule should go forward.

EPA in this case, he said, would look at the increased risk of death and put a dollar figure on it based on an estimate of "value of statistical life." Then that value, along with any other forgone benefits like reductions in emergency room visits or school absences, is compared with the cost savings of the rule to the power sector.

Those skeptical of the use of cost-benefit analysis to justify regulatory cuts say the administration's current approach has further eroded faith in that assessment tool.

"What we have seen in the economic analysis under this administration is that economic cost-benefit analysis is very easy to manipulate. That has been a clear takeaway," said Amit Narang, regulatory policy advocate at Public Citizen.

Narang suggested cost-benefit analysis should be used in an advisory way and agencies should use risk assessments that don't put a monetary value on life, because the methods of calculating that value are "barely transparent."

But Katzen stands by the analytical approach.

"Cost-benefit analysis is like democracy. It may have flaws, but it is better than anything else," she said. "It makes the decisionmaker consider all the consequences of a proposal in roughly comparable terms: apple and apples."

For Lovett-Owen, the woman preparing to be a Lutheran pastor, her hope was to make her testimony personal for the three EPA officials who oversaw the hearing.

Did she think her approach had an impact?

"When I said was from Appalachia, the man sitting in the middle gave me a thumbs-up and mouthed, 'Me, too,'" she said.

The Ecologist: Do we need to be concerned about asbestos again?

https://theecologist.org/2018/oct/22/do-we-need-be-concerned-about-asbestos-again

Emily Folk | 22 October 2018

The EPA in the US is planning to alter the current regulations on asbestos that may allow the harmful substance back into manufacturing.
What's really at stake is the larger role that the EPA will play in regulating chemicals, as the way that the agency handles asbestos may be an indication of how it will treat other potentially harmful substances.

The Environmental Protection Agency (EPA) in the United States announced a proposed new rule, called a Significant New Use Rule (SNUR), regarding asbestos in June.

The EPA claimed that the rule would increase restrictions, while critics - including some current and former EPA employees - worried it could open up loopholes. Others say it doesn't go far enough. The reality of what effect the law will have remains a little uncertain.

Asbestos is a type of naturally occurring mineral that was used widely in the United States and Europe until the 1970s, especially in the construction and automotive sectors.

Earlier rules

The material is resistant to fire and chemical corrosion and does not conduct electricity. It has been used in the production of cement, plastics, insulation, roofing shingles, floor tiles, paints and elsewhere.

Since the 1970s, however, the use of asbestos has declined significantly due to concerns about its health impacts. It is classified as a known human carcinogen and has been shown to cause mesothelioma and other forms of cancer.

The use of asbestos is banned in more than 60 countries, and the substance kills nearly 40,000 Americans a year, according to the Asbestos Disease Awareness Organization. It seems strange then that the EPA is proposing a rule that, if its critics are right, could bring more asbestos into the US. Here's the explanation.

There's room for such a rule because although the use of asbestos is restricted in the United States, it is not entirely banned.

Between 1970 and 1989, the US banned several specific uses of asbestos. In 1989, the EPA attempted to ban all new applications, meaning those developed before 1989 were exempt from the ban, except for those singled out and prohibited by earlier rules.

Boost asbestos

However, the US Circuit Court of Appeals overturned most of this ban in 1991. As a result, the only applications of asbestos the 1989 rules bans are new uses and five that the court did not overturn, which were already obsolete.

The new SNUR would require that certain non-banned uses of asbestos are subject to review by the EPA. Under the rule, companies wanting to use asbestos for these applications would have to notify the EPA, and the agency could decide to allow, regulate or prohibit the usage.

Specifically, the rule applies to what the EPA calls “currently unregulated former uses". These applications include those that had not been banned and were active at the time of the 1991 court ruling but are no longer in use.

Health concerns are the primary reasons these uses are no longer active. In the rule, the EPA lists 15 of these market uses. The EPA has noted that, without the new rule, companies could start using asbestos for these applications without notifying the EPA.

So, if the rule involves EPA reviews of asbestos uses, why do some believe it could boost asbestos use? The problems that critics point to include the language with which the agency wrote the SNUR and the way it plans to evaluate these applications. Some also believe that the proposal does not go far enough.

Stringent

According to internal EPA emails, some agency staff are worried that the language of the SNUR would allow some uses of asbestos to avoid review. Because the rule only lists 15 applications, currently unregulated uses that the EPA did not list could potentially avoid regulation.

Another concern is related to how the agency has indicated it will review the risks associated with asbestos. According to recently released EPA documents about how the agency plans to review potentially toxic chemicals, it will not consider the impacts of potential exposure due to a chemical's presence in the air, ground or water. It will instead look at risks associated with direct contact.

Critics say that this severely limits the scope of the review and would exclude the impacts of things like improper disposal.

The documents also narrow the definition of asbestos, meaning the EPA will not assess some asbestos-like fibers. It also will not review existing uses of asbestos or the disposal of asbestos and products containing it.

This limited scope suggests that any potential reviews of unregulated uses of asbestos likely won't be very stringent, according to critics.

Potentially harmful

The recent EPA actions, critics point out, represent a drastic change from the original intent of the Toxic Substances Control Act (TSCA), a law directing the EPA to review potentially harmful chemicals including asbestos.

When Congress strengthened the TSCA in 2016, some lawmakers saw it as a chance to try again to ban asbestos broadly. The recent EPA actions do not seem to support this notion.

The impact of these actions, especially regarding asbestos, is somewhat uncertain. There's reason to believe, though, they will change little for the average American.

The SNUR deals mostly with market uses of asbestos that are already obsolete, primarily due to their associated health concerns. These applications have been unregulated for decades, but businesses have still opted not to use them.

Using them is not only a health risk, it would also be a tremendous financial risk for the companies due to potential lawsuits. An estimated 100 companies have been forced into bankruptcy proceedings due to asbestos liabilities.

What's really at stake is the larger role that the EPA will play in regulating chemicals, as the way that the agency handles asbestos may be an indication of how it will treat other potentially harmful substances.

AgPro: EPA Told To Initiate Process To Allow Summertime E15 Sales

https://www.agprofessional.com/article/epa-told-initiate-process-allow-summertime-e15-sales

Rhonda Brooks- October 22, 2018 12:18 PM

Allowing the use of E15 year-round could save users between 3 cents and 10 cents per gallon in fuel costs for their vehicles. (Farm Journal)

U.S. consumers may soon have the opportunity to purchase gasoline with a higher ethanol blend year-round. Earlier this month, President Donald Trump announced he wants the sale of gasoline with 15% ethanol, typically referred to as E15, to go 12 months of the year.

"I want more [use] because I don't like $74," Trump said at a White House meeting, noting the price at the time for a barrel of crude oil. "That's what I want. I want low prices."

Trump has instructed Andrew Wheeler, Environmental Protection Agency (EPA) acting administrator, to initiate the process of lifting the summertime ban on the sale of E15. Sales are currently stopped between June and the middle of September. Opponents of E15 say the higher level of ethanol in it contributes to smog during hot summer days. They also contend that the ban cannot be changed without Congress being involved in the process.

Trade groups and USDA Secretary Sonny Perdue were quick to support the president's announcement.

"Year-round sale of E15 will increase demand for corn, which is obviously good for growers," Perdue said in a prepared statement. "This has been a years-long fight and is another victory for our farm and rural economies."

Allowing the use of E15 year-round could save users between 3 cents and 10 cents per gallon, because ethanol is cheaper than gasoline, notes the National Corn Growers Association (NCGA).

"With 9 out of 10 vehicles on the road today approved to use E15, consumers should have this lower-cost option year-round," says Lynn Chrisp, NCGA president.

E15 is approved for use in model year 2001 and newer passenger cars, light-trucks and medium-duty vehicles. 2001 and newer models account for 82% of vehicle miles traveled in the U.S.

In its October WASDE report, the USDA forecast that the use of corn for ethanol in 2018-19 will reach 5.65 billion bushels of the 14.827-billion-bushel harvest total projected for U.S. corn production this year.

If E15 is allowed to be sold year-round, David Hudak, general manager of POET Biorefining, said sales of the product will surge.

"Once [more] retailers come onboard, and then we get thousands of thousands of stations, then you'll see the product fly," he said on AgDay earlier this summer.

POET produces 1.8 billion gallons of ethanol in seven states, according to Hudak. With more than 200 biofuels facilities across the country, he has no doubts on supplying the demand.

Currently, approximately 1,300 gas stations in 28 states sell E15.

The oil industry is expected to challenge any decision by EPA to waive the summertime ban on E15, saying that Congress will have be involved to determine whether E15 can be sold year-round. Mike Sommers, American Petroleum Institute president, says the industry would fight such a decision in the courts using "all available legal remedies."

E&E News: Greens sue over EPA denial of cross-state ozone petitions

https://www.eenews.net/greenwire/stories/1060103985

Sean Reilly, - Monday, October 22, 2018

Eight environmental and public health advocacy groups are challenging EPA's rejection of bids from Maryland and Delaware for a federal crackdown on upwind power plant emissions from outside their borders.

"The Clean Air Act is clear that EPA must protect downwind states from dangerous air pollution that is encroaching on them from coal plants in neighboring states," Graham McCahan, a senior attorney with the Environmental Defense Fund, said in a news release late Friday announcing the lawsuit filed with the U.S. Court of Appeals for the District of Columbia Circuit. The lead plaintiff is the Chesapeake Bay Foundation. Among seven others are EDF, the Clean Air Council, the Adirondack Club and Physicians for Social Responsibility.

The two states petitioned EPA in 2016 to force coal-fired power producers in Pennsylvania and other states to curb releases of nitrogen oxides (NOx) that were allegedly making it harder for them to meet the 2008 ground-level ozone standard.

In a final decision published earlier this month, EPA rejected both states' "good neighbor" petitions, partly on the grounds that its 2016 Cross-State Air Pollution Rule Update was already addressing the problem.

The suit filed Friday does not spell out the grounds for the challenge. But in comments submitted to EPA last year, the Chesapeake Bay Foundation argued that tighter NOx curbs were warranted for some of the plants targeted in the petitions. The Environmental Defense Fund contended that both Delaware and Maryland had shown that out-of-state emissions were undercutting their efforts to comply with the 2008 ozone standard of 75 parts per billion.

Ozone, the main ingredient in smog, is formed by the reaction of NOx and volatile organic compounds in sunlight. EPA has deemed significant parts of Maryland and Delaware in nonattainment for both the 75-ppb threshold and the more stringent 70-ppb limit set in 2015.

The suit comes days after Maryland also went to the D.C. Circuit to challenge EPA's denial of its petition (E&E News PM, Oct. 17). The Delaware Department of Natural Resources and Environmental Control is weighing its legal options, a spokesman said in an email last week.

The remaining organizations joining in the new suit are the Chesapeake Climate Action Network, the Environmental Integrity Project and the Sierra Club. Many of the same groups had previously banded together in a suit filed last fall in U.S. District Court to compel EPA to make a decision on Maryland's petition after the agency failed to act by a statutory deadline (Greenwire, Oct. 5, 2017). A federal judge later set a timetable requiring EPA to take action by last month.

Slate: Trump's Biggest Attempts to Roll Back Environmental Regulations Remain at the Starting Gate

Our system is actually set up to prevent what Trump wants to do—which is erase regulation for no good reason.
https://slate.com/technology/2018/10/trump-methane-emissions-deregulation-is-failing.html
By DAVID J. HAYES- OCT 22, 20181:57 PM

Popular in Technology
The Creator of Godwin's Law Interviews David Simon About Nazis on Twitter and Much More
In Praise of Distracted Meditation
The Real Lie About Online Video Runs Deeper Than Facebook's False Metrics
Should You Buy a Mega Millions Ticket?
It is a popular fiction that despite its many foibles, the Trump administration has been successful in dismantling environmental regulations. Given the sheer number of attacks levied on energy and environmental regulations involving the coal, oil, gas, and automotive industries, casual observers might reasonably conclude that the administration is deploying a successful deregulatory strategy.

It is not. The administration has made multiple efforts to put Obama-era regulatory requirements on ice, but these efforts have largely failed. With its delay tactics in tatters, the administration is now taking a different approach—instead of just stopping old regulations, it's surfacing new replacement rules that would let industry walk away from environmental and public-health and safety obligations that have solid evidentiary support and broad appeal. But having squandered half of its four-year term, the White House faces an uphill climb in developing and finalizing many of its major environmental rollback initiatives, and getting them past now-skeptical courts, before the clock runs out.

The efforts to roll back the oil and gas industry's obligations to reduce methane emissions specifically illustrate this reality. Trump's Environmental Protection Agency and Interior Department tried, first, to quickly sideline methane regulations. The gambit failed. Progressive state attorneys general and environmental groups pounced, and won, exposing an impulsive administration that had failed to do its legal homework.

The Trump administration targeted two specific methane rules that the Obama administration had finalized before it left office: the EPA's New Source Performance Standard, or NSPS, and the Interior Department's methane-waste reduction rule. The Obama administration finalized the EPA methane rule in June 2016. It focuses primarily on detecting and repairing methane leaks for new oil and gas drilling activities. In May 2017, former EPA Administrator Scott Pruitt announced a 90-day delay for a key compliance deadline for the NSPS. Three weeks later, the EPA proposed a bare-bones "suspension" rule that sought to delay implementation of the existing rule for two years.

Many of the Trump administration's efforts to remove regulations that it complains are burdening the energy industry remain at the starting gate.
State attorneys general and environmental groups immediately petitioned for court review, arguing that the EPA had no authority to nullify key compliance requirements by administrative fiat. The District of Columbia Circuit Court agreed. It moved swiftly to strike down the EPA's compliance delay, ruling that "an agency issuing a legislative rule is itself bound by the rule until that rule is amended and revoked" through a formal, substantive "notice and comment" process. This ruling sent the EPA back to the drawing board for 14 months.

A similar pattern applies to the Interior Department's restrictions on methane emissions for oil and gas operations conducted on public lands. The department promulgated the methane "waste prevention rule" in November 2016 in response to evidence gathered by the Government Accountability Office and other watchdog groups that some oil and

gas drillers were wasting valuable, publicly owned assets by venting and flaring large quantities of unwanted methane in violation of the Mineral Leasing Act.

Like its efforts to sideline the EPA's methane restrictions, the Trump administration tried to nullify the Interior Department's methane-waste reduction rule. Initial efforts included a failed attempt to overturn the rule under the Congressional Review Act and an unsuccessful petition in the normally friendly U.S. District Court of Wyoming to enjoin operation of the rule. In June 2017, Interior Secretary Zinke announced that the department was delaying industry-compliance requirements under the rule. This effort also failed. A federal court in California agreed with California's and New Mexico's attorneys general, Xavier Becerra and Hector Balderas, that the Interior Department must complete a full rule-making process under the Administrative Procedure Act, or APA, before it can set aside the Obama rule. The court also noted that the Interior Department could not justify its change in direction by myopically focusing on the rule's compliance costs to industry. It also must consider "the benefits of the rule, such as decreased resource waste, air pollution, and enhanced public revenues."

Undeterred by its failures, Zinke's Interior Department moved forward with a "suspension" rule that purported to put off the waste-reduction rule for a full year. California's and New Mexico's attorneys general sued again, arguing that the suspension rule represented an unlawful attempt to circumvent the APA's substantive notice-and-comment rule-making process. In February, Judge Orrick in the Northern District of California agreed, noting, "The BLM's reasoning behind the Suspension Rule is untethered to evidence contradicting the reasons for implementing the Waste Prevention Rule."

inRead invented by Teads
After suffering one defeat after another in its repeated attempts to suspend or delay Obama-era methane regulations, the Trump administration's leadership at EPA and the Interior Department is finally taking on the much harder task of developing and justifying entirely new rules, instead of just rolling back the rules we have. Whether or not they will be successful in turning back the inevitable judicial challenges is murky at best.

The EPA recently proposed a replacement for the NSPS rule that would roll back inspections and repair requirements and drop requirements for third-party validation of leak-detection and repair activities. The new rule identifies modest cost savings to industry but offers no clear, fact-based rebuttal to the Obama administration's analysis of the benefits of the previous rule. Assuming that the EPA pushes forward to a similar final rule, it will confront a strong challenge to the foregone benefits associated with its weakened rule when it faces judicial scrutiny.

The Interior Department also is now moving forward with a replacement rule after the courts rejected the Trump administration's initial efforts to sideline its methane-waste reduction rule. More specifically, it promulgated a new final rule last month that purports to completely remove restrictions on the oil and gas industry's wasting of methane through excessive venting and flaring activities. Within hours of its release, attorneys general Becerra and Balderas challenged the final rule in federal court. In order to succeed in the rule's implementation, the Interior Department must overcome the substantial administrative record that addressed the necessity of regulations proposed in the old rule. (When overturning the department's attempted suspension of the methane-waste reduction rule, Judge Orrick emphasized the Supreme Court's teachings that new administrations cannot simply overturn policies that had been legally established by previous administrations and that the high court requires a "more detailed justification" for overturning existing regulations than what is required for the creation of wholly new regulations from scratch.)

Meanwhile, state attorneys general have put the EPA on the defensive on other methane-emissions-related developments. A coalition of state attorneys general sued the EPA in April for failing to comply with its obligation under the Clean Air Act to restrict methane emissions from existing oil and gas drilling operations. (The NSPS standards apply only to new oil and gas operations.) Eight state attorneys general also sued the EPA in May for failing to enforce

compliance deadlines set forth in a separate rule that applies to methane emissions from landfills. Those suits remain pending.

The same pattern seen in the Trump administration's efforts to roll back EPA and Interior Department requirements to reduce harmful methane emissions is playing out in other environmental and energy contexts. In recent weeks, federal courts struck down attempts by the administration to delay implementation of Obama-era rules protecting workers and first responders from exposure to dangerous chemicals during accidents in industrial chemical facilities as well as delays by the EPA in banning the use of a dangerous pesticide, chlorpyrifos, on food crops.

In all of these matters, Trump's initial deregulatory efforts failed. Some, like the ozone rule, are being dropped entirely. Others are wending their way forward, typically with a cloudy future in terms of their ultimate deregulatory success. Even very high-profile deregulatory matters like the Clean Power Plan and car-tailpipe emissions requirements are only now getting underway in earnest. While the Supreme Court stayed enforcement of the Obama administration's Clean Power Plan in 2016, EPA did not propose a replacement rule—the so-called Affordable Clean Energy Plan—until two months ago. It will be many months more before the replacement rule is finalized and subjected to judicial scrutiny. The same is true for the administration's proposed rules rolling back emissions reductions for cars and light trucks.

In short, many of the Trump administration's efforts to remove regulations that it complains are burdening the energy industry remain at the starting gate. In the coming months, courts will have an opportunity to weigh in and, for at least some of the new rules, send the administration back to the drawing board.

None of this is to say that the administration's onslaught against common-sense regulations and other protections that benefit the environment and public health and safety is not doing real harm. Agencies are taking actions outside the rule-making context, through guidance documents, lack of enforcement, and other administrative actions, to put industry interests above the public interest. But the clock is ticking on many of the administration's most serious rollback efforts, and increasingly, time is not on Trump's side.

UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF RHODE ISLAND

EMHART INDUSTRIES, INC., Plaintiff and Counterclaim Defendant, v. NEW ENGLAND CONTAINER COMPANY, INC; et al., Defendants and Counterclaim Plaintiffs.	C.A. No. 06-218 S
EMHART INDUSTRIES, INC., Plaintiff and Counterclaim Defendant, v. UNITED STATES DEPARTMENT OF THE AIR FORCE; et al., Defendants, Counterclaim Plaintiffs, and Third-Party Plaintiffs, v. BLACK & DECKER, INC.; et al., Third-Party Defendants.	C.A. No. 11-023 S

PHASE II FINDINGS OF FACT AND CONCLUSIONS OF LAW

I. Introduction

Dioxin and other toxic chemical pollution at the Centredale Manor Restoration Project Superfund Site ("Centredale Site" or

"Site") in North Providence, Rhode Island, has led to going on ten years of litigation over which parties are responsible and what is the appropriate remedy. The Court divided the litigation into three phases. (See Eighth Revised Case Management Order, ECF No. 295.)[1] At the close of trial in Phase I, the Court found Emhart jointly and severally liable for the release of dioxin at the Site. The Court has now concluded Phase II of the trial[2] and must provide findings of fact and conclusions of law addressing the following two issues: (1) whether the Environmental Protection Agency's ("EPA") remedy-selection process was arbitrary, capricious, or otherwise not in accordance with law; and (2) whether Emhart had sufficient cause to refuse to comply with EPA's June 10, 2014 Administrative Order. The necessary contributions, if any, of third-party defendants will be addressed in Phase III of the trial.

The Court provided a comprehensive background discussion and procedural history of this case in its Phase I opinion and need

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[1] The present case was also preceded by other insurance coverage litigation. The Centredale Site is truly a litigation gift that keeps on giving.

[2] The Court received evidence over the course of thirteen days, concluding on January 19, 2017. (See Trial Trs. vols. 1-13, ECF Nos. 448-57, 494-96.) This included thousands of pages of exhibits from both the United States (Bates numbered "US__") and Emhart (Bates numbered "Emhart__"). The parties supplied post-trial briefs thereafter. (See Emhart Post-Trial Brief, ECF No. 461; Gov't Post-Trial Brief, ECF No. 466; Emhart Post-Trial Reply Brief, ECF No. 497.) Lastly, the Court heard oral argument on April 4, 2017. (See Trial Tr. vol. 14, ECF No. 534.)

not repeat it here. (See Phase I Findings of Fact and Conclusions of Law ("Phase I Findings"), ECF No. 405.) However, since issuing the Phase I Findings, there have been three new and important developments in the case relating to the Phase II litigation. First, the parties have come to an agreement regarding the United States' past response costs in light of the Court's findings in Phase I. (See Stipulation Regarding United States' Past Response Costs, ECF No. 444.) As such, the Court need not determine the amount of past response costs.

Second, the Government moved to limit the scope of judicial review during Phase II. (Gov't Mot. to Limit Disc. and Scope of Review, ECF No. 415.) The Government argued that, because judicial review under CERCLA is strictly limited to the administrative record, Emhart should be prohibited from presenting any evidence or arguments not found in the administrative record. Emhart opposed the Government's motion, arguing that review under CERCLA is not so limited. (Emhart Obj., ECF No. 416.) The Court denied the Government's motion without prejudice, thereby allowing Emhart to conduct discovery and present the objected to evidence and arguments at trial. (Order Den. Mot. to Limit Disc. and Scope of Review 6, ECF No. 421.) However, the Court has reserved its judgment on the ultimate admissibility of the challenged evidence and arguments to this point. (Id. at 6.)

Third, and lastly, the Government moved to exclude certain portions of testimony provided by Emhart's expert, Mr. Jeffrey Loureiro. (Gov't Mot. to Exclude Certain Test. of Jeffrey Loureiro, ECF No. 447.) The Government argued that significant portions of Mr. Loureiro's opinions had not been disclosed in his expert report in violation of Rule 26(a)(2) of the Federal Rules of Civil Procedure. According to the Government, the admission of Mr. Loureiro's testimony would be "highly prejudicial . . . given the United States' inability to explore such opinions through discovery or to counter them effectively through rebuttal testimony." (Id. at 2.) In light of the Government's concerns, the Court decided to allow the testimony of Mr. Lourerio but provided the Government an opportunity to conduct additional discovery and to designate and utilize rebuttal experts to address the concerns highlighted in the Government's motion. The Court therefore denied that motion as moot. (Text Order of 10/07/2016.)

In this decision, the Court will set forth its findings of fact and conclusions of law with regards to the question of remedy, and address along the way the various issues reserved to this point. See Fed. R. Civ. P. 52(a)(1). The decision begins with an overview of the CERCLA process by which EPA may choose a response action, as well as the standards of judicial review for challenges to a response action. Next the Court provides specific findings of fact, starting with a history of EPA action at the Site, and then

moving into the topics which provide the bases for Emhart's challenge. After each topic the Court provides conclusions of law.

II. Remedy Selection

Congress enacted the Environmental Response, Compensation, and Liability Act ("CERCLA"), 42 U.S.C. § 9601 et seq., "in response to the serious environmental and health risks posed by industrial pollution." United States v. Bestfoods, 524 U.S. 51, 55 (1998). To address those risks, CERCLA "grants the President broad power to command government agencies and private parties to clean up hazardous waste sites." Id. (quoting Key Tronic Corp. v. United States, 511 U.S. 809, 814 (1994)). These cleanup efforts are called "response actions," and may require both "the cleanup or removal of released hazardous substances" as well as other "remedial action . . . to prevent or minimize the release of hazardous substances." 42 U.S.C. § 9601(23)-(25).

EPA acts on the President's behalf in choosing the appropriate response action. See Exec. Order No. 12580(b)(1), (g). In doing so, EPA must work within the framework provided by CERCLA and the National Contingency Plan ("NCP"). See 42 U.S.C. 9604(a)(1) (requiring EPA to act "consistent with the national contingency plan"); National Contingency Plan, 40 C.F.R. § 300, et seq. In addition, EPA has established various guidance documents to assist in the CERCLA process. While these guidance documents are non-binding on EPA, they do represent EPA's collective wisdom as to

best practices.[3] The steps required by CERCLA and the NCP, and implemented with the assistance of EPA guidance documents, are outlined below.

A. National Priorities List

The first step in the CERCLA process is placing a site on the National Priorities List ("NPL"). See 42 U.S.C. § 9605(a)(8)(b); 40 C.F.R. § 300.425. A site is appropriately included on the NPL if, for example, EPA determines that a hazardous substance poses "a significant threat to public health." Id. § 300.425(c)(3)(ii). A notice and comment period is required before a site is officially placed on the NPL. Id. § 300.425(d)(5). Once the notice and comment process is complete, and if EPA determines that NPL listing is still appropriate, EPA may begin the process of developing a response action for the site.

B. Remedial Investigation and Feasibility Study

EPA is required to conduct a remedial investigation ("RI") and feasibility study ("FS") before choosing a response action. The end goal of the RI/FS process is "to assess site conditions and evaluate alternatives to the extent necessary to select a remedy." Id. § 300.430(a)(2). The first step is the RI, which seeks to "collect data necessary to adequately characterize the

[3] (See, e.g., Ms. Taylor Test., Trial Tr. vol. 2, 5:3-9, ECF No. 449 (acknowledging that EPA guidance represents "the collective information and practices developed by EPA through its experience in implementing remedies").)

site for the purpose of developing and evaluating effective remedial alternatives." Id. § 300.430(d)(1). EPA is given significant leeway to develop a RI process specific to the site. But, at a minimum, EPA must conduct a field investigation (i.e., collect site-specific data) and create a baseline risk assessment. Id.

The data collected during the field investigation includes the physical characteristics of the site and the hazardous material present as well as the exposure pathways through which the hazardous material may affect human health and the environment. Id. § 300.430(d)(2)(i)-(vii). Part of this process involves estimating the reasonable maximum exposure that is likely to occur for both current and potential future land use at the site. (EPA, Interim Final Risk Assessment Guidance for Superfund ("IFRAGS"), vol. I, Emhart579-24.) EPA then uses this data in the baseline risk assessment to understand the extent to which hazardous materials pose a threat to human health and the environment, as well as what would be "acceptable exposure levels" for the site going forward. 40 C.F.R. § 300.430(d)(4).

At this point, EPA transitions from the RI process of collecting data to the FS process of finding a remedy.[4] "The

[4] While the RI and FS are labeled as separate steps in the CERCLA process, the RI does not end when the FS begins. Instead, "the RI and FS are interactive processes that are conducted concurrently," such that "field investigation activities will be

national goal of the remedy-selection process is to select remedies that are protective of human health and the environment, that maintain protection over time, and that minimize untreated waste." Id. § 300.430(a)(1)(i). As an aid to developing potential remedies that accomplish this overarching goal, EPA first establishes what are labeled Preliminary Remediation Goals ("PRGs") targeting "acceptable exposure levels that are protective of human health and the environment." Id. § 300.430(e)(2)(i). These PRGs must, among other things, comply with federal and state environmental laws[5] and limit the lifetime cancer risk from carcinogenic exposure. Id. § 300.430(e)(2)(i)(A)(1)-(2). EPA then develops a range of response alternatives that may achieve those PRGs.

In order to choose among the response options for the site, the various alternatives are initially screened using three criteria: (1) effectiveness; (2) implementability; and (3) cost. Id. § 300.430(e)(7)(i)-(iii). At this stage, alternatives that provide "significantly less effectiveness" or are "technically or

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ongoing during the development and screening of remedial action alternatives." EPA, The Remedial Investigation: Site Characterization and Treatability Studies, Emhart509-1; see also, 40 C.F.R. § 300.430(e)(1) ("Development of alternatives shall be fully integrated with the site characterization activities of the remedial investigation."); id. § 300.430(e)(1) ("Preliminary remediation goals should be modified, as necessary, as more information becomes available during the RI/FS.")

[5] These are referred to in CERCLA as Applicable and Relevant and Appropriate Requirements ("ARARs"). 40 C.F.R. § 300.505(d)(2)(iii).

administratively infeasible" may be eliminated. Id. § 300.430(e)(7)(i)-(ii). Additionally, "[c]osts that are grossly excessive compared to the overall effectiveness of alternatives may be considered as one of several factors used to eliminate alternatives." Id. § 300.430(e)(7)(iii).

Alternatives that survive the initial culling proceed to the "detailed analysis of alternatives." Id. § 300.430(e)(9). This requires "an assessment of individual alternatives against each of nine evaluation criteria and a comparative analysis that focuses upon the relative performance of each alternative against those criteria." Id. § 300.430(e)(9)(ii). The nine criteria used by EPA to compare alternatives are: (1) overall protection of human health and the environment; (2) compliance with federal and state environmental laws (i.e., "ARARs"[6]); (3) long-term effectiveness and permanence; (4) reduction of toxicity, mobility, or volume through treatment; (5) short-term effectiveness; (6) implementability; (7) cost; (8) state acceptance; and (9) community acceptance. See id. § 300.430(e)(9)(iii)(A)-(I). Once sufficient information has been gathered such that EPA can compare the alternatives based on the nine evaluation criteria, EPA may proceed with remedy selection.

[6] See id.

C. Remedy Selection

During remedy selection EPA places the nine evaluation criteria into three categories. The first category, labeled "threshold criteria," are the criteria "that each alternative must meet in order to be eligible for selection." Id. § 300.430(f)(1)(i)(A). The two threshold criteria are overall protection of human health and the environment and compliance with ARARs (criteria (1) and (2), above).[7]

Once EPA has screened out alternatives that do not meet the threshold criteria, EPA then compares the remaining alternatives based on the second category of criteria, labeled "primary balancing criteria." Id. § 300.430(f)(1)(i)(B). These include long-term effectiveness and permanence, reduction of toxicity, mobility, or volume through treatment, short-term effectiveness, implementability, and cost (criteria (3)-(7), above). Id. As the label suggests, these "primary balancing criteria" are balanced against one-another. In doing so, the NCP provides certain preferences. For instance, a remedy must be "cost-effective" in that "its costs are proportional to its overall effectiveness." Id. § 300.430(f)(1)(ii)(D). In addition, "balancing shall emphasize long-term effectiveness and reduction of toxicity,

[7] While compliance with ARARs is one of the "threshold criteria," there are limited circumstances where a chosen remedy need not meet those standards. See id. § 300.430(f)(1)(ii)(C).

mobility, or volume through treatment," thereby focusing on "permanent solutions . . . to the maximum extent practicable." Id. § 300.430(f)(1)(ii)(E). However, in the end, the NCP does not dictate exactly how the primary balancing criteria should be weighed. Instead, the NCP provides EPA with considerable discretion to select a remedy that "reflect[s] the scope and purpose of the actions being undertaken and how the action relates to long-term, comprehensive response at the site." Id. § 300.430(f)(a).

The last category EPA must consider is the "modifying criteria." Id. § 300.430(f)(1)(i)(C). The modifying criteria include state and community acceptance (criteria (8) and (9), above). Because the state and community provide formal input during the notice and comment period for the proposed plan, state and community acceptance is typically only considered, at this stage, "to the extent that information is available during the FS." EPA, A Guide to Preparing Superfund Proposed Plans, Records of Decision, and Other Remedy Selection Decision Documents, Emhart518-33. However, "after public comment is received on the Proposed Plan," the modifying criteria will be "fully considered." Id. This means that, "[i]n the final balancing of trade-offs between alternatives upon which the final remedy selection is based, modifying criteria are of equal importance to the balancing criteria." Id. This does not provide the state or community the power to veto a selected

remedy[8]; their input is simply considered along with the other balancing criteria.

After completing the "detailed analysis of alternatives" using the nine criteria, EPA chooses a remedy through a "two-step process." 40 C.F.R. § 300.430(f)(1)(ii). First, EPA "identifies a preferred alternative and presents it to the public in a proposed plan, for review and comment." Id. The proposed plan functions not only to "supplement the RI/FS," but also to "provide the public with a reasonable opportunity to comment on the preferred alternative for remedial action, as well as alternative plans under consideration, and to participate in the selection of remedial action at a site." Id. § 300.430(f)(2). To accomplish this, the proposed plan must, among other things, "[p]rovide a brief summary description of the remedial alternatives evaluated in the detailed analysis" as well as "[i]dentify and provide a discussion of the rationale that supports the preferred alternative." Id. The public is then given "a reasonable opportunity, not less than 30 calendar days, for submission of written and oral comments" as well as an "opportunity for a public meeting." Id. § 300.430(f)(3)(i)(C)-(D).

After receiving public input on the proposed remedy, the second step for remedy selection requires EPA to "review the public comments and consult with the state (or support agency) in order

[8] See, e.g., 42 U.S.C. § 9604(j); id. § 9621(e)(1); 40 C.F.R. § 300.400(d).

to determine if the [proposed plan] remains the most appropriate remedial action for the site or site problem." Id. § 300.430(f)(1)(ii). The NCP anticipates that public comments may provide "new information or points of view" that prompt EPA "to modify aspects of the preferred alternative or decide that another alternative provides a more appropriate balance." Id. § 300.430(f)(4). To the extent EPA makes any significant changes to the remedy, those changes must be documented.[9] EPA must then make the final remedy selection. Id. § 300.430(f)(4)(i).

Just as EPA is not required to remove all uncertainty at the RI/FS stage regarding the conditions at the site, EPA is also not required to provide complete details of the final remedy at the selection stage. The NCP envisions that EPA will fill in the details of the final remedy during the implementation (or "remedial design") phase and that the final remedy may require modifications. See generally id. § 300.435. The NCP also allows EPA to reserve decisions regarding how to handle certain portions of the remedy

[9] EPA must provide documentation in the record of decision where the final remedy "significantly differs from the original proposal." Id. § 300.430(f)(3)(ii). If the change is so drastic that it could not have been "reasonably anticipated by the public" based on the information in the proposed plan and administrative record, then EPA must allow for an additional notice and comment period on the revised plan. Id. § 300.430(f)(3)(ii)(B). If the changes to the plan could have been "reasonably anticipated," then EPA need only "[i]nclude a discussion in the record of decision of the significant changes and reasons for such changes." Id. § 300.430(f)(3)(ii)(A).

until remedial design. See, e.g., id. § 300.825(a)(1)-(2). This process is discussed below in the "Remedial Design and Remedial Action" section.

D. Documentation of the Remedy-Selection Process

After selecting the final remedy, EPA must "establish an administrative record that contains the documents that form the basis for the selection of a response action." 40 C.F.R. § 300.800. An important piece of the administrative record is the Record of Decision ("ROD"), which includes "all facts, analyses of facts, and site-specific policy determinations considered" by EPA in selecting the final remedy. Id. § 300.430(f)(5)(i). The ROD is essentially EPA's justification for its decision, explaining, for example, "[h]ow the selected remedy is protective of human health and the environment" and "provides overall effectiveness proportional to its costs." Id. § 300.430(f)(5)(ii)(A), (D). As part of this justification, the ROD also must include a "responsiveness summary," which is "a written summary of significant comments, criticisms, and new relevant information submitted during the public comment period and the lead agency response to each issue." Id. § 300.430(f)(3)(i)(F). Lastly, if EPA chooses to reserve certain decisions for a later date, it may, "[w]here appropriate, provide a commitment for further analysis." Id. § 300.430(f)(5)(iii)(D).

The administrative record is not a static document. Even after the final remedy is selected, EPA is responsible for updating the administrative record where necessary. For example, if the ROD did not address a portion of the response action or reserved certain decisions until the implementation phase, EPA must document those later decisions in the administrative record. Id. § 300.825(a)(1). Additionally, if EPA decides to modify the final remedy during remedial design, it must document those changes either through an "explanation of significant differences" or a ROD amendment, as appropriate. Id. § 300.825.[10]

E. Remedial Design and Remedial Action

The final step is "the development of the actual design of the selected remedy and implementation of the remedy through construction." Id. § 300.435(a). The NCP labels this as the "remedial design/remedial action (RD/RA) stage." Id. While the ROD establishes the final remedy, the NCP leaves it to the RD/RA stage for EPA to determine the remedy's final design. The "initial building block in developing" the final design is the information contained in the RI/FS, but EPA guidance also envisions that additional "data acquisition" and "sample analysis" will be necessary during RD/RA. EPA, Scoping the Remedial Design, Emhart516-2. EPA then recommends going through multiple design

[10] See infra note 11.

phases before coming to a "final design" and beginning construction. Id. at 0001. Once a final design is complete, EPA must - as a final notice to the public - "issue a fact sheet and provide, as appropriate, a public briefing prior to the initiation of remedial action." 40 C.F.R. § 300.435(c)(3). EPA may then implement the remedy, and as long as the "remedial action objectives and remediation goals in the ROD" are accomplished, the CERCLA response action process is largely complete. Id. § 300.435(f)(1); 42 U.S.C. § 9621(d) (stating that the response action must "attain a degree of cleanup . . . which assures protection of human health and the environment").

If strict adherence to the final design proves unworkable at any point, EPA guidance provides for significant "flexibility" to account for "any constraining factors of the particular site." EPA, Scoping the Remedial Design, Emhart516-1. The NCP also foresees that "[a]dditional work" may be "needed as a result of such unforeseen situations as newly discovered sources, types, or quantities of hazardous substances." 40 C.F.R. § 300.435(e)(1)(i). Because the "chief task" of RD/RA is "to achieve the goals of the Record of Decision . . . in a timely manner," EPA, Scoping the Remedial Design, Emhart516-1, as opposed to blind adherence to any particular design, EPA is permitted to change the remedial design

at any point. The NCP simply requires that sufficient notice and opportunity to comment is provided to the public.[11]

F. Unilateral Administrative Order

The parties responsible for the release of the hazard materials at the site are liable for the costs associated with the response action. See 42 U.S.C. § 9607(a). EPA can either complete the response action and seek reimbursement from the responsible parties or require the responsible parties to implement the response action themselves. Where EPA determines that "there may be an imminent and substantial endangerment to the public health or welfare or the environment," EPA is authorized to issue a unilateral administrative order ("UAO") requiring the responsible parties to implement the response action "as may be necessary to protect public health and welfare and the environment." Id. § 9606(a).

If a responsible party "willfully violates, or fails or refuses to comply with" the UAO, EPA may seek an order "in the

[11] If, after additional data collection or during construction, EPA determines that the final design will "differ[] significantly from the remedy selected in the ROD with respect to scope, performance, or cost," EPA must provide the public notice of this change. 40 C.F.R. § 300.435(c)(2). Where the differences "significantly change but do not fundamentally alter the remedy selected in the ROD," the lead agency will publish an "explanation of significant differences." Id. § 300.435(c)(2)(i). Alternatively, if EPA does "fundamentally alter" the final remedy in its final design, EPA must amend the ROD and provide for another period of notice and comment. Id. § 300.435(c)(2)(ii).

appropriate United States district court to enforce" the UAO. Id. § 9606(b)(1). Additionally, if the district court finds that the responsible party refused to comply with the UAO without "sufficient cause," the responsible party is subject to daily fines during the period of non-compliance as well as treble damages for any work EPA performed at the site. Id. § 9606(b)(1), 9607(c)(3). By regulation, the daily fine amount is $37,500 per day for every day of non-compliance between December 6, 2013 and November 2, 2015, and $54,789 per day thereafter. 40 C.F.R. §§ 19.2, 19.4.

III. Scope of Review

Having described the remedy-selection process as outlined by CERCLA and the NCP, the Court will now provide its findings of fact and conclusions of law regarding EPA's remedy-selection process at the Site. Before doing so, however, the Court must first determine what evidence and arguments it will consider, as well as the standard of review. As discussed previously, the Government moved, pre-trial, to limit the scope of discovery and judicial review. According to the Government, the Court should not consider evidence and arguments not contained in the administrative record when making its determination about the appropriateness of EPA's chosen remedy. The Court denied the Government's motion without prejudice, permitting Emhart to take discovery and present evidence and arguments at trial not contained in the administrative record. The Court "reserve[d] ruling on the admissibility of any

particular extra-record evidence until the time of trial." (Order Den. Mot. to Limit Disc. and Scope of Review 6.) The Government has reiterated its arguments at trial and in its post-trial briefs, and Emhart has again responded. (See Gov't Post-Trial Brief 8-13; Emhart Post-Trial Reply Brief 1-22.)

The two questions the Court must answer are: (1) whether the Court should consider evidence not contained in the administrative record; and (2) whether the Court should consider Emhart's arguments that were not made during the notice and comment period.

A. What Evidence Should the Court Consider?

CERCLA provides the following limitation to judicial review: "In any judicial action under this chapter, judicial review of any issues concerning the adequacy of any response action taken or ordered by the President shall be limited to the administrative record." 42 U.S.C. § 9613(j)(1) (emphasis added). CERCLA reiterates this limitation when describing the applicable standard of review: "In considering objections raised in any judicial action under this chapter, the court shall uphold the President's decision in selecting the response action unless the objecting party can demonstrate, on the administrative record, that the decision was arbitrary and capricious or otherwise not in accordance with law."

Id. § 9613 (j)(2) (emphasis added).[12] As these provisions make clear, "[u]nder CERCLA, judicial review normally is limited to the administrative record as it existed at the time of the challenged agency action." United States v. JG-24, Inc., 478 F.3d 28, 33-34 (1st Cir. 2007); see also Murphy v. Comm'r of Internal Revenue, 469 F.3d 27, 31 (1st Cir. 2006) ("[T]he Supreme Court has consistently stated that review of administrative decisions is ordinarily limited to consideration of the decision of the agency . . . and of the evidence on which it was based.") (internal quotations omitted). This ensures that the Court "take[s] into account 'neither more nor less information than did the agency when it made its decision.'" Linemaster Switch Corp. v. EPA, 938

[12] The administrative record is typically closed once the decision document has been signed, except in the following narrow circumstances:

> (a) The lead agency may add documents to the administrative record file after the decision document selecting the response action has been signed if: (1) The documents concern a portion of a response action decision that the decision document does not address or reserves to be decided at a later date; or (2) An explanation of significant differences required by § 300.435(c), or an amended decision document is issued, in which case, the explanation of significant differences or amended decision document and all documents that form the basis for the decision to modify the response action shall be added to the administrative record file.

40 C.F.R. § 300.825.

F.2d 1299, 1305 (D.C. Cir. 1991) (quoting Walter O. Boswell Mem'l Hosp. v. Heckler, 749 F.2d 788, 792 (D.C. Cir. 1984)).

However, while generally confining the scope of judicial review to the administrative record, CERCLA also provides that "[o]therwise applicable principles of administrative law shall govern whether any supplemental materials may be considered by the court." 42 U.S.C. § 9613(j)(1). Typically, "[c]ourts require a strong showing of bad faith or improper behavior before ordering the supplementation of the administrative record." Town of Norfolk v. U.S. Army Corps of Eng'rs, 968 F.2d 1438, 1458-59 (1st Cir. 1992); see also JG-24, Inc., 478 F.3d at 34 ("Normally, we do not allow supplementation of the administrative record unless the proponent points to specific evidence that the agency acted in bad faith."). This exception provides no assistance to Emhart, as there is no evidence that EPA crafted a remedy for the Site in bad faith or based on an improper motive, and EPA's "designation of the Administrative Record, like any established administrative procedure, is entitled to a presumption of administrative regularity." Nw. Bypass Grp. v. U.S. Army Corps of Eng'rs, No. CIV 06-CV-00258-JAW, 2007 WL 1498912, at *2 (D.N.H. May 14, 2007) (quoting Bar MK Ranches v. Yuetter, 994 F.2d 735, 740 (10th Cir. 1993)).

Emhart must therefore find some other legal avenue if it is to successfully inject evidence outside the administrative record

into this proceeding. Outside of a showing of bad faith, the First Circuit recognizes two other "exceptions to the rule against supplementation." Nw. Bypass Grp., 2007 WL 1498912, at *2.

First, "supplementation may be proper when . . . there is a record so inadequate that it prevents judicial review." Id. at *2-3 (citing Murphy, 469 F.3d at 31). This applies in very limited circumstances. For instance, the Supreme Court has recognized that, "where there are [no formal] administrative findings that were made at the same time as the decision, . . . it may be that the only way there can be effective judicial review is by examining the decisionmakers themselves." Citizens to Pres. Overton Park, Inc. v. Volpe, 401 U.S. 402, 420 (1971) (citing Shaughnessy v. Accardi, 349 U.S. 280 (1955)). This applies where the administrative agency's decision cannot be discerned or justified on the record. See Camp v. Pitts, 411 U.S. 138, 142-43 (1973) (reviewing whether "there was such failure to explain administrative action as to frustrate effective judicial review"). Therefore, supplemental materials are typically unnecessary when the administrative agency's judgment is based on a substantial record. See Nw. Bypass Grp., 2007 WL 1498912, at *3 (finding an administrative record "more than sufficient to allow for judicial review" because the administrative record was "hefty, 3,233 pages over seven volumes, with documents spanning from 1989 to 2006"). Where a substantial record is available, even if the administrative

agency's explanation is "curt," supplemental materials are unnecessary as long as the explanation "indicate[s] the determinative reason for the final action taken." Pitts, 411 U.S. at 143.

The second exception to the "rule against supplementation" applies where "additional testimony by experts" will "aid to understanding highly technical, environmental matters." Nw. Bypass Grp., 2007 WL 1498912, at *2 (quoting Valley Citizens for a Safe Env't v. Aldridge, 886 F.2d 458, 460 (1st Cir. 1989)). The Court may even allow "additional factual evidence as an aid to understanding." Valley Citizens for a Safe Env't, 886 F.2d at 460. The necessity of such evidence "is discretionary with the reviewing court." Id. However, it is important to note that the purpose of such evidence is "simply to help [the Court] understand matters in the agency record." Id. Therefore, the Court still "looks first and foremost at the record before the agency." Id.; see also Olsen v. United States, 414 F.3d 144, 155 (1st Cir. 2005) ("The focal point for judicial review should be the administrative record already in existence, not some new record made initially in the reviewing court.") (quoting Pitts, 411 U.S. at 142); United States v. Dravo Corp., No. 8:01CV500, 2003 WL 21434761, at *3 (D. Neb. June 20, 2003) (reviewing an EPA response action and refusing to "permit supplementation of the record absent a showing that it is

explanatory and not intended to invoke new material into the case").

In this case, given the extensive record compiled by EPA, as well as EPA's documentation of its decision-making process in the ROD, the Court does not find the record "so inadequate" as to require supplemental materials. However, the subject matter involved certainly falls under the umbrella of "highly technical, environmental matters" where the Court has discretion to consider "additional testimony by experts" and "additional factual evidence" as an aid to understanding the administrative record. Nw. Bypass Grp., 2007 WL 1498912, at *2 (quoting Valley Citizens for a Safe Env't, 886 F.2d at 460). The Court will therefore consider the expert testimony presented by both parties. With that said, in considering the expert testimony, the Court acknowledges that it must remain focused "first and foremost" on the administrative record, Valley Citizens for a Safe Env't, 886 F.2d at 460, as "the focal point for judicial review should be the administrative record already in existence, not some new record made initially in the reviewing court." Olsen, 414 F.3d at 155 (quoting Pitts, 411 U.S. at 142).

B. What Arguments Should the Court Consider?

Having established what evidence is admissible in this case, the Court must determine which of Emhart's arguments the Court will consider. According to EPA, the Court should not consider any

of Emhart's arguments that were not previously submitted during the official notice and comment period on the proposed remedy. Emhart, on the other hand, urges the Court to consider all of its arguments, including those made for the first time at trial.

There is limited precedent directly addressing this issue in the context of CERCLA. Generally speaking, arguments not made before an administrative agency are waived when a court reviews the administrative agency's decision:

> [w]e have recognized in more than a few decisions, and Congress has recognized in more than a few statutes, that orderly procedure and good administration require that objections to the proceedings of an administrative agency be made while it has opportunity for correction in order to raise issues reviewable by the courts.

United States v. L.A. Tucker Truck Lines, Inc., 344 U.S. 33, 36-37 (1952). This requirement is commonly referred to as "issue exhaustion." Sims v. Apfel, 530 U.S. 103, 107 (2000). As the First Circuit has explained, "this rule preserves judicial economy, agency autonomy, and accuracy of result by requiring full development of issues in the administrative setting to obtain judicial review." Pepperell Assocs. v. EPA, 246 F.3d 15, 27 (1st Cir. 2001) (citing Northern Wind, Inc. v. Daley, 200 F.3d 13, 18 (1st Cir. 1999)).

The doctrine of issue exhaustion was adhered to relatively recently by the First Circuit in Upper Blackstone Water Pollution Abatement Dist. v. EPA, 690 F.3d 9, 18 (1st Cir. 2012). That case

involved a sewage company ("petitioner") appealing a limitation imposed on it by EPA under the National Pollutant Discharge Elimination System. After notice and comment on the limitation, petitioner challenged EPA's decision in the First Circuit. Petitioner sought review of, among other things, "the limit placed on aluminum discharge, arguing that the EPA assembled and then relied upon an erroneous data set in deriving the limit." Id. at 33. The First Circuit refused to consider that argument, however, because it had not been raised during the notice and comment period.

The First Circuit explained that, by regulation, petitioner was required to "raise all reasonably ascertainable issues and submit all reasonably available arguments supporting their position by the close of the public comment period . . . ." Upper Blackstone, 690 F.3d at 30 (quoting 40 C.F.R. § 124.13). In light of this statutory requirement, the court found that petitioner had "waived the argument by failing to raise it during the public comment period of the permitting process. . . . By failing to give the EPA an opportunity to address the argument during the permitting process, [Upper Blackstone] has waived its claim." Id. (citing several cases, including L.A. Tucker Truck Lines, 344 U.S. 33, and Pepperell Assocs., 246 F.3d 15).

Emhart argues that Upper Blackstone is distinguishable from this case because, unlike the regulation at issue in Upper

Blackstone, CERCLA and its implementing regulations do not contain a clear issue exhaustion provision. The Court disagrees.

A determination of whether issue exhaustion applies to an administrative process "requires careful examination of the characteristics of the particular administrative procedure provided." Sims, 530 U.S. at 112-13 (O'Connor, J., concurring) (quoting McCarthy v. Madigan, 503 U.S. 140, 146 (1992)). And while the regulation reviewed by the First Circuit in Upper Blackstone may have been slightly more definitive than the language in CERCLA, the Court finds that CERCLA and its implementing regulations, when taken as a whole, clearly require interested parties to present arguments to EPA before bringing those issues before a federal court.

After identifying the "preferred" remedy, EPA is required to "present[] it to the public in a proposed plan, for review and comment." 40 C.F.R. § 300.430(f)(1)(ii). This ensures that the public has "a reasonable opportunity to comment on the preferred alternative for remedial action, as well as alternative plans under consideration, and to participate in the selection of remedial action at a site." Id. § 300.430(f)(2). At the completion of the notice and comment period, EPA is required to create a "responsiveness summary," which is "a written summary of significant comments, criticisms, and new relevant information submitted during the public comment period and the lead agency

response to each issue." Id. § 300.430(f)(3)(i)(F). The responsiveness summary is then placed in the administrative record. Id.

Importantly though, the responsiveness summary does not address comments made outside the public comment period. See id. § 300.825. As is mandated by CERCLA's implementing regulations, the responsiveness summary will not address comments made "after the close of the public comment period" unless the comments: (1) "contain significant information not contained elsewhere in the administrative record file"; (2) the information "could not have been submitted during the public comment period"; and (3) the information "substantially support[s] the need to significantly alter the response action." Id. § 300.825. Therefore, unless an issue raised after the public comment period falls under this exception, that issue will not be responded to in the responsiveness summary or included in the administrative record. And it is in this context that CERCLA explicitly limits judicial review to the information contained in the administrative record. See 42 U.S.C. § 9613(j)(1)-(2).

This statutory and regulatory scheme, when viewed as a whole, requires parties to make all of their known and available arguments regarding the merits of a remedy to EPA during the notice and comment period in the first instance. Only then, after EPA has had the opportunity to provide its response in the administrative

record, may a federal court review EPA's decision. See, e.g., JG-24, Inc., 478 F.3d at 33-34 ("Under CERCLA, judicial review normally is limited to the administrative record as it existed at the time of the challenged agency action."); Arco v. Travelers Ins. Co., 730 F. Supp. 59, 69 (W.D. Mich. 1989) ("[R]efusal to participate in this administrative process [under CERCLA] essentially allows the EPA a free-reign in dictating response methods since judicial review is limited to the administrative record."). To allow Emhart to make arguments it could have made directly to EPA for the first time on judicial review would frustrate this administrative scheme by depriving EPA of the opportunity to address Emhart's arguments in the first instance on administrative review. Upper Blackstone, 690 F.3d at 30.

However, while judicial review is generally limited to arguments presented during the notice and comment period, the Court also recognizes that there are some narrow exceptions to this rule. For instance, the Court of Appeals for the District of Columbia Circuit has recognized that EPA must justify "key assumptions" in its analysis, regardless of whether a party specifically objects during the notice and comment process:

> EPA has a preexisting duty to examine key assumptions as part of its affirmative burden of promulgating and explaining a non-arbitrary, non-capricious rule and therefore . . . must justify that assumption even if no one objects to it during the comment period.

Oklahoma Dep't of Envtl. Quality v. EPA, 740 F.3d 185, 192 (D.C. Cir. 2014) (quoting Appalachian Power Co. v. EPA, 135 F.3d 791, 818 (D.C. Cir. 1998)).[13]

While the First Circuit has not directly addressed this narrow exception, the Court agrees with the District of Columbia Circuit that issue waiver cannot absolve EPA of its responsibility to explain the key assumptions that underpin its remedy and that contain obvious mistakes. Therefore, while the Court will generally not consider Emhart's arguments that were not presented to EPA during the notice and comment period, the Court will consider several obvious issues relating to key assumptions that formed the basis of EPA's selected remedy.

C. Standard of Review

With the bounds of admissible evidence and argument established in the preceding sections, the Court turns to the applicable standard of review. The Court will uphold EPA's decision "unless the objecting party can demonstrate, on the administrative record, that the decision was arbitrary and capricious or otherwise

[13] Other Circuit Courts of Appeals have recognized similar exceptions for cases in which the allegedly waived issue should have been obvious to the reviewing agency. See, e.g., Sierra Club, Inc. v. Bostick, 787 F.3d 1043, 1048 (10th Cir. 2015) (holding that an issue not presented during the notice and comment period is not waived where that issue was "obvious"); Portland Gen. Elec. Co. v. Bonneville Power Admin., 501 F.3d 1009, 1024 (9th Cir. 2007) ("In general, we will not invoke the waiver rule in our review of a notice-and-comment proceeding if an agency has had an opportunity to consider the issue.").

not in accordance with law." 42 U.S.C. § 9613(j)(2). The "law" with which EPA must comply in selecting a remedial action is primarily found in CERCLA and the NCP. EPA's decisions made within that legal framework will qualify as "arbitrary and capricious" if EPA fails to "examine the relevant data and articulate a satisfactory explanation for its action." FCC v. Fox Television Stations, Inc., 556 U.S. 502, 513-14 (2009) (quoting Motor Vehicle Mfrs. Ass'n of United States, Inc. v. State Farm Mut. Auto. Ins. Co., 463 U.S. 29, 43 (1983)).

This is a "narrow" standard of review. Id. EPA's explanation need only be "plausible in light of the record as a whole" and "supported by substantial evidence in the record." Leahy v. Raytheon Co., 315 F.3d 11, 17 (1st Cir. 2002) (citations omitted). Furthermore, in reviewing the evidence, the Court is not permitted "to substitute its judgment for that of the agency." Fox Television Stations, 556 U.S. at 513-14 (quoting Bowman Transp., Inc. v. Arkansas-Best Freight Sys., Inc., 419 U.S. 281, 286 (1974)). Particularly when the Court reviews "a purely factual question within the area of competence of an administrative agency . . . and when resolution of that question depends on 'engineering and scientific' considerations," the Court must "recognize the relevant agency's technical expertise and experience, and defer to its analysis unless it is without substantial basis in fact." Browning-Ferris Indus. of S. Jersey, Inc. v. Muszynski, 899 F.2d

151, 160 (2d Cir. 1990) (quoting Federal Power Commission v. Florida Power & Light Co., 404 U.S. 453, 463 (1972)).

While certainly deferential, the arbitrary and capricious standard is not a shibboleth by which EPA may completely avoid judicial scrutiny. As the Supreme Court has explained, an action may qualify as arbitrary and capricious where the administrative agency: (1) "relied on factors which Congress has not intended it to consider," (2) "entirely failed to consider an important aspect of the problem," (3) "offered an explanation for its decision that runs counter to the evidence before the agency, or is so implausible that it could not be ascribed to a difference in view or the product of agency expertise," or (4) seeks to have its action upheld based on "post hoc rationalizations." Motor Vehicle Mfrs. Ass'n of U.S., Inc., 463 U.S. at 43. Courts also review whether an administrative agency has treated similar cases with "apparent irrational discrimination." Puerto Rico Sun Oil Co. v. EPA, 8 F.3d 73, 78 (1st Cir. 1993) (citing Green Country Mobilephone, Inc. v. FCC, 765 F.2d 235 (D.C. Cir. 1985)). Put simply, the Court must review EPA's analysis to ensure that it is "rational" and "makes sense." Penobscot Air Servs., Ltd. v. FAA, 164 F.3d 713, 720 (1999) (quotations, citations, and brackets omitted).

Emhart's arguments as to why EPA's actions qualify as arbitrary and capricious or otherwise not in accordance with law

address several aspects of EPA's processes as well as the merits of the selected remedy itself. Each of Emhart's arguments are addressed below.

IV. Case Specific Findings of Fact and Conclusions of Law

A. Background

1. Findings of Fact

In 1996 dioxin was discovered in fish collected from the Woonasquatucket River. (See Phase I Findings 12.)[14] An EPA investigation of the surrounding area - later labeled as the Centredale Manor Restoration Project Superfund Site - ensued. The Site covers a three-mile stretch of the Woonasquatucket River, which includes a nine-acre peninsula that has been identified as the "Source Area" of the Site's hazardous substances. (Id. at 10-11.) The Source Area contains two elderly housing facilities (Brook Village and Centredale Manor) and is bounded to the north by Smith Street, to the south by Allendale Pond, to the west by the Woonasquatucket River, and to the east by the "tailrace," a remnant of a narrow body of water used for water power by the mills that once occupied the peninsula. (Id.)

After passing by the Source Area, the Woonasquatucket River leads to the remainder of the Site. The river first runs into Allendale Pond, a .65-mile-long dammed pond that spans fifteen

[14] This and various other findings of fact are taken directly from the Court's Phase I Findings.

acres and has depths ranging from .5 to ten feet.[15] (ROD, US1444-12, 30.) The river next runs into Lyman Mill Pond, a .85-mile-long dammed pond spanning twenty-four acres with depths similar to Allendale Pond. (Id.) In between Allendale and Lyman Mill Ponds is the Oxbow Area, a forty-acre forested wetland habitat adjacent to the river channel. (U.S. Army Corps of Eng'rs, Oxbow Area Report, US1227-0005.) There are also various abutting residential and commercial properties throughout the Site. (Phase I Findings 10-11.)[16]

Ultimately, EPA determined that the entire Site, not just the Source Area, was polluted by a variety of contaminants, including dioxins (2, 3, 7, 8-TCDD, in particular), volatile organic compounds, polychlorinated biphenyls, semi-volatile organic compounds, polycyclic aromatic hydrocarbons, and various metals. (Phase I Findings 11-12.) This determination led EPA to list the Site on the National Priorities List of Superfund sites in 2000. (Id.)

The Site, in short, is complicated. It contains several types of toxic hazardous waste, and the Site's size and diversity require EPA to consider various types of flora, fauna, physical features, and human uses. As part of the cleanup effort EPA has required

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[15] Unless specified otherwise, the lengths, sizes, and depths discussed by the Court are approximated.

[16] The ROD contains a map of the Site. (ROD, US1444-0012.)

several removal and remedial actions. These involved the construction of three interim protective caps as well as one RCRA[17] cap over portions of the Source Area[18]; reconstruction of the Allendale Dam and restoration of Allendale Pond to prevent further downstream migration of contaminants; excavation and removal of one hundred cubic yards of soil from eleven areas along Allendale and Lyman Mill Ponds; and erection of fences along the residential properties adjacent to the Site in order to prevent access to the contamination. (ROD, US1444-15-16.) To varying degrees, Emhart has participated in each of these removal actions. (Phase I Findings n.110.)

Additionally, EPA has pursued a "comprehensive" remedial action at the Site that will address "all current and potential future risks caused by soil, sediment, groundwater and surface water contamination." (ROD, US1444-6.) The first step in this process (after listing the Site on the NPL) was the remedial

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[17] RCRA stands for the "Resource Conservation & Recovery Act." See 42 U.S.C. §§ 6901 et seq. "Congress enacted RCRA, a comprehensive environmental statute that governs the treatment, storage, and disposal of solid and hazardous waste, based . . . on its finding that waste disposal had become a national problem requiring federal involvement." AES Puerto Rico, LP v. Trujillo-Panisse, 857 F.3d 101, 103 (1st Cir. 2017) (quotations and citations omitted). RCRA governs the disposal of "hazardous waste" under subtitle C. Id. (citing 42 U.S.C. §§ 6921-6939g).

[18] The ROD contains a map of the work done on the Source Area. (ROD, US1444-13.)

investigation ("RI"). EPA's RI[19] utilized Site-specific data and modeling to characterize the nature and extent of contamination at the Site. (ROD, US 1444-16-17.) The culmination of this process was the RI report released in 2005. (ROD, US 1444-16-17; see also RI, US1098.) The RI included an assessment of the risks to human health (the Baseline Human Health Risk Assessment, or "BHHRA")[20] and ecology (the Baseline Ecological Risk Assessment, or "BERA")[21] posed by the Site.

Using the information collected during the RI, EPA developed remediation goals that, if achieved, would likely mitigate the risks to human health and the environment identified in EPA's risk assessment. (Feasibility Study, US1254-73-84.) EPA conducted an extensive feasibility study ("FS") in order to determine which remedial alternative would best achieve those targets.[22] The FS

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[19] The RI actually included a series of individual investigations conducted between 1999 and 2004. (See ROD, US 1444-16-17.)

[20] The BHHRA is comprised of several individual assessments, including: (1) 2005 BHHRA (US1101-1103); (2) 2011 Supplemental BHHRA & BERA (US1287); and (3) May 2012 Technical Memorandum on Impact of Dioxin Reassessment ("2012 Technical Memorandum") (US1392).

[21] The BERA is comprised of the 2004 BERA (US1040-1044) and the 2011 Supplemental BHHRA & BERA (US1287).

[22] In total, the FS consisted of 329 pages of analysis exclusive of references and appendices. Additionally, EPA issued an FS Addendum in September 2011 in order to incorporate information brought to EPA's attention after publication of the FS. (See FS Addendum, US1311.)

divides the Site into five "action areas," and provides potential remedial actions for each. (FS, US1254-10-14.) As is required by the NCP, the FS includes both an initial screening as well as a detailed analysis of the remedial alternatives for each action area. (FS, US1254-147-321.)

Based on the analysis in the FS, EPA drafted a Proposed Remedial Action Plan ("PRAP") in the fall of 2011. (See PRAP, US 1328.) This version of the remedy did not last long though. Soon after its publication, EPA released a nationwide change to its non-cancer toxicity value for dioxin. Since dioxin is present at the Site, EPA was forced to issue a "Technical Memorandum" updating the BHHRA, cleanup levels, and FS for the Site. (See 2012 Technical Mem., US1392.) These findings required several changes to the PRAP in the form of a PRAP Amendment. (See PRAP Amendment, US1393.) While much of the PRAP went unchanged, the PRAP Amendment did require, among other things, an expanded cleanup area at the Site. These changes were published in July, 2012.

Both the PRAP and PRAP Amendment were subject to notice and comment after their publication. The notice and comment period on the PRAP and PRAP Amendment went from November 14, 2011 to March 2, 2012, and July 19, 2012 to September 17, 2012, respectively. (ROD, US1444-24-25.) During that time EPA participated in public hearings and also accepted comments from a variety of sources,

including Emhart. (See Emhart Comments on PRAP, US1383; Emhart Comments on PRAP Amendment, US1418.)

On September 28, 2012, with the public comment period complete, EPA issued its Record of Decision ("ROD") explaining the remedial action plan. (See ROD, US1444.) The ROD provides EPA's justification for the chosen remedial action as well as a "Responsiveness Summary" that addresses significant public comments submitted to EPA on the PRAP and PRAP Amendment. In the end, the plan outlined in the ROD was substantially similar to the plan provided for in the PRAP and PRAP Amendment.

The remedy, as described in the ROD, contains the following basic characteristics. In the Source Area the ROD requires removal and off-Site treatment or disposal of waste material, installation of a RCRA C cap[23] over existing surfaces, and relocation of underground utilities into clean corridors. (ROD, US1444-7.) Sediment and floodplain soil in Allendale and Lyman Mill ponds will also be excavated. However, unlike in the Source Area, the majority of the excavated material in and around the ponds will be placed in a confined disposal facility ("CDF") near the Site, leaving only a small portion of the excavated material (estimated at approximately ten percent of the total) to be shipped off-Site[24]

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[23] See supra note 17.

[24] EPA requires off-Site disposal or treatment only for particularly toxic materials that exceed EPA's treatment

for disposal or treatment. (Id. at 7.) A thin-layer cover will be placed over the remaining contaminated areas in the Oxbow wetland area and, if necessary, over remaining contaminated sediment in the Woonasquatucket River. (Id.) Going forward, the ROD requires continuous institutional controls that limit certain activities at the Site, such as construction and use of groundwater. (Id.) Additionally, long-term maintenance and monitoring is required, including of the CDF, damns, sediment, water, and biota located at the Site. (Id.) In total, EPA estimates that the remedial action will cost approximately $104,600,000. (Id. at 213.)

Lastly, the ROD commits that EPA will collect additional information during the remedial design phase. For instance, EPA will have to determine the location of the CDF. (ROD, US1444-6.) EPA must also collect additional soil samples to determine the precise amount of excavation required. (ROD, US1444-304.) It is possible that the remedy will change based on this information.[25] However, EPA maintains that it has the capacity to adequately

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standards. Materials that do not exceed EPA's treatment standards may be disposed in a CDF.

[25] One example of this has already occurred since the ROD's publication. Several third party defendants collected additional information on the Source Area that led EPA to conclude that it "no longer expects that the removal of waste material and off-site treatment or disposal will be necessary in the Source Area." (Gov't Post-Trial Brief n.137 (citing Revised Draft Pre-Design Investigation Final Report, US1459).)

address such changes through, for example, an amendment to the remedial action plan or an "explanation of significant differences."[26]

EPA has compiled an extensive administrative record to document its remedy-selection process. The administrative record includes essentially all documents related to the development, creation, and implementation of the remedial action. The next step in EPA's process is to create a more in-depth design of the remedial action plan and implement it.[27] However, before the final design and implementation of the remedy occurred, Emhart challenged EPA's proposed remedy on several grounds.

2. Conclusions of Law

As a general matter, the Court finds that EPA followed the basic steps mandated by CERCLA and the NCP in developing its remedial action for the Site. These legal requirements were previously outlined by the Court. See supra Section II ("Remedy Selection"). However, Emhart argues that several of EPA's individual actions and decisions along the way were either arbitrary, capricious, or not in accordance with CERCLA or the NCP such that the remedy cannot withstand judicial scrutiny. The Court addresses each of Emhart's arguments below.

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[26] See supra note 9.

[27] The NCP labels this as the "remedial design/remedial action (RD/RA) stage." 40 C.F.R § 300.435(a).

B. Excavation and Treatment of Soil and Sediment

1. Findings of Fact

a. Excavation of Allendale and Lyman Mill Ponds

EPA's remedy calls for significant excavation of the sediment in Allendale and Lyman Mill Ponds. The goal of excavation is to achieve dioxin levels of approximately fifteen parts per trillion. (FS, US1254-75.) In order to determine how much excavation will be required to meet this target, EPA took sediment samples from both Allendale and Lyman Mill Ponds. Based on these samples, EPA estimates the average excavation depth in Allendale Pond required to achieve the target dioxin level is 2.2 feet. (ROD, US 1444-170.) For Lyman Mill Pond, the average excavation depth required to achieve EPA's target is 2.7 feet. (Id. at 170.) Both of these estimates assume .25 feet of over-excavation will occur. (Id.)

The data set used by EPA to come up with these estimates included 250 data records from Allendale Pond (ranging from 0.5 to twelve feet in depth) and 160 data records from Lyman Mill Pond (ranging from 0.5 feet to four feet in depth). (ROD, US1444-303; see also RI, US1098-29; FS, Tables G-3 and G-4, US1254-1458-1477.) The data most heavily relied upon by EPA were core samples taken in 2003 and 2005, which included ten sediment cores taken from Allendale Pond and sixteen sediment cores taken from Lyman Mill Pond. (FS, US1254-363-64.) Each core includes multiple soil samples all of which were collected with the specific objective of

detecting the vertical extent of contamination in the ponds. (Id. at 42, 679.) To that end, EPA conducted laboratory analysis using high resolution mass spectroscopy, a highly accurate method that can detect dioxin in parts per trillion. (Dr. Medine Test., Trial Tr. vol. 12, 13:16-14:18, ECF No. 495.)

Emhart's expert, Mr. Loureiro, testified that these samples were inadequate because only a small portion was taken at depths greater than one foot.[28] Furthermore, Mr. Loureiro pointed out that, in certain instances, dioxin levels were detected at levels above fifteen parts per trillion at depths greater than EPA's anticipated excavation depths.[29] As such, Mr. Loureiro opined that the available data was insufficient to accurately estimate the vertical extent of the contamination and, in turn, the amount of excavation that will be necessary to achieve EPA targets.

EPA agrees that additional sampling is needed. As EPA explained in the Feasibility Study, "[t]he proposed cleanup areas or remedial footprints are conceptual and more precise cleanup

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[28] Mr. Loureiro testified that he evaluated the data and found only forty samples from Allendale Pond and eighteen samples from Lyman Mill Pond at depths below one foot. (Mr. Loureiro Test., Trial Tr. vol. 6, 105:7-9, 113:23-117:1, ECF No. 453.)

[29] Because EPA's anticipated excavation depth is an average, Emhart is able to point to samples where dioxin is still above fifteen parts per trillion past the average excavation depth, and EPA is able to point to samples where dioxin is less than fifteen parts per trillion even before reaching the average excavation depth.

footprints will be developed during the remedial design. For example, additional coring will need to be performed at Allendale and Lyman Mill Ponds to confirm the vertical extent of the contamination." (FS, US1254-327-28.) For this reason, EPA committed itself in the ROD to "perform[ing] additional sampling and analysis closer to the time of remediation to confirm the sediment cleanup depth and volume." (ROD, US1444-304.)

Given the uncertainty as to the vertical extent of the contamination, the exact amount of excavation required will likely differ from the amount estimated in the ROD. Precision in this area is likely impossible, however. As Mr. Loureiro pointed out, "certainly in my experience with excavation of all types, even a robust data set aren't adequate to actually describe the conditions you run into in the field." (Mr. Loureiro Test., Trial Tr. vol. 6, 19:5-8.) This means that this component of the cost of the remedy is to some extent uncertain and could potentially be more expensive.

Emhart did raise this general issue during the notice and comment period on the PRAP. (Emhart PRAP Comments US1383-8 ("EPA fails to adequately define the volume of soil and sediment requiring excavation."); see also id. at 51.) EPA responded in the ROD's "Responsiveness Summary" by explaining that, in its opinion, sufficient data had been collected to provide excavation estimates, particularly in light of the 0.25-foot over-excavation

allowance built into the estimate. (ROD, US1444-303-04.) EPA also noted that additional sampling will be done during remedial design in order to refine those estimates. (Id.) Lastly, to the extent that the proposed level of excavation does not achieve target dioxin levels, EPA suggests that "a 6-inch soil cover on the sediment bottom" could be used where "additional excavation is not feasible." (Id.)

b. Excavation of the Oxbow Area and the Floodplain Soil of Allendale and Lyman Mill Ponds

In February 2012, after publication of the PRAP, EPA established a nation-wide non-cancer toxicity value for dioxin. This new information forced EPA to reevaluate its remedial design for the Site. EPA did so by issuing a Technical Memorandum on the Impact of Dioxin Reassessment that updated EPA's human health risk assessment and feasibility study. (2012 Technical Memorandum, US1392.) The analysis of each remedial action alternative did not change significantly. (See ROD, US1444-347 ("Impacts resulting from these changed conditions are presented in EPA's May 2012 Technical Memorandum and are generally consistent with evaluations presented in . . . the FS.").)

However, EPA did determine that certain areas, not previously identified in the PRAP, would require excavation. These areas were primarily located in the Oxbow Area and floodplain soil around Allendale and Lyman Mill Ponds. (See PRAP Amendment, US1393-002.)

EPA's expanded excavation plan was documented in the PRAP Amendment and later adopted in the ROD. (PRAP Amendment, US1393-7-9; ROD, US1444-176-192.) Emhart submitted comments on the PRAP Amendment in which it argued that EPA had not collected sufficient Site-specific data in order to adequately characterize contamination at the Site. (See Emhart Comments on PRAP Amendment, US1418-7-9.)[30]

With regards to the floodplain soils, EPA determined which areas would require excavation using two types of Site-specific data: soil samples and FEMA floodplain maps. The soil samples were collected during the remedial investigation on the eastern shore floodplains[31] of the Allendale and Lyman Mill Pond reaches. EPA collected 226 samples in total, 212 of which detected some level of dioxin. (ROD, US1444-345.) Of those 212 samples detecting dioxin, "there are approximately 100 sampling locations where floodplain residential-use soil samples have [dioxin] concentrations greater than cleanup levels." (Id.; see also 2012 Technical Mem., US1392-24.) Based on this data EPA expanded the

[30] A review of those comments reveals that Emhart provided no specifics for what level of sampling it believed was necessary going forward or where that sampling needed to occur. Emhart simply asserted that additional sampling is required.

[31] EPA defined "floodplain areas" as "the area of water and land inundated during the highest point of the base, or 100-year, flood using maps prepared by the Federal Insurance Administration of the Federal Emergency Management Agency . . . ." (ROD, US1444-306.)

area requiring excavation and estimated an average excavation depth of one foot. (See 2012 Technical Mem., US1392-62.)[32]

Having established that unsafe levels of dioxin had migrated into floodplain soils, EPA then used Federal Emergency Management Agency ("FEMA") floodplain maps to determine other areas that likely contain similar dioxin levels. FEMA floodplain maps cannot substitute for field samples in determining the precise nature or extent of dioxin contamination in a given area. (Mr. Loureiro Test., Trial Tr. vol. 5, 183:23-184:11, ECF No. 452.) However, as Dr. Medine explained, such maps are commonly used "to aid in designing sampling programs to characterize the nature and extent of floodplain contamination" because they can act as "a guide to where to look for contamination and serve as indicator of where contamination may have come to rest." (Dr. Medine Test., Trial Tr. vol. 12, 81:13-15, 21-22, ECF No. 495.)

As for the Oxbow Area, EPA relied primarily on two Site-specific data sets in order to estimate the amount of required excavation. The first was a data set collected by EPA during the remedial investigation. (FS, US1254-188.) The second was a data set collected by Emhart as part of its 2010 Oxbow Area

[32] EPA's limited subsurface data suggests that excavation will be limited to one foot in most areas, though some areas will also likely require deeper excavation. (2012 Technical Mem., US1392-24.) The ROD explains that, generally speaking, excavation below surface soils is unlikely because "dioxins do not migrate easily through the soil column." (ROD, US1444-346.)

Investigation. (ROD, US1444-318.) That investigation collected forty-four floodplain soil samples and twenty-eight sediment samples at depths ranging from zero to thirty-six inches. (ROD, US1444-36.) After EPA came out with its toxicity value for dioxin, EPA utilized both data sets in the FS Addendum. The FS Addendum determined that 5,600 cubic yards of material needs to be excavated in addition to the areas identified in the PRAP. (See PRAP Amendment, US 1393-7, 10.)

For both the Oxbow Area and the floodplain soils the ROD requires that EPA collect additional information during the remedial design phase to delineate the exact counters and depths of excavation. (See, e.g., ROD, US1444-176, 346.) This information will consist primarily of additional soil samples. (Id. at 176, 181-83.) Once excavation is complete, "confirmation sampling will be conducted to verify that the cleanup levels are achieved . . . ." (Id. at 177; see also id. at 183.)

c. Amount of Soil Requiring Incineration

Once contaminated soil and sediment are excavated, the ROD calls for those materials to be either stored in a CDF or shipped off-Site for treatment (i.e., incineration). (ROD, US1444-172-73.) Whether treatment is required depends on the toxicity of the material. Material that contains dioxin at or above ten parts per billion must be treated; material that contains dioxin below ten parts per billion can be stored in a CDF. (Id. at 172.)

At the Site, EPA estimates that ninety percent of the excavated soils can be disposed of in a CDF while ten percent will need to be shipped off-Site for treatment. (ROD, US1444-172.) As with EPA's excavation estimates, certainty in this area is likely impossible. (See, e.g., Mr. Loureiro Test., Trial Tr. vol. 6, 19:5-8.) Emhart's 2010 Oxbow Area Investigation exemplifies this uncertainty. EPA initially estimated that no Oxbow Area floodplain soil would require incineration. (ROD, US1444-318; FS Addendum, US1311-27.) However, after more extensive sampling was conducted in the 2010 Oxbow Area Investigation, EPA changed its opinion and now estimates that up to ten percent of the Oxbow Area floodplain soil may require treatment. (ROD, US1444-318; FS Addendum, US1311-27.) As this example demonstrates, additional sampling can reveal that more material requires treatment than initially estimated thereby increasing the cost of the overall remedy.

However, uncertainty does not necessarily mean that the ultimate remedy will involve additional treatment or increased cost compared to the estimates in the ROD. The Source Area provides an example where the amount of material requiring treatment decreased as a result of additional sampling. At the time the ROD was published, EPA "assumed that all of the excavated potential buried waste material [in the Source Area] would be taken to an off-site incinerator for treatment." (ROD, US1444-164; see also id. at 214.) However, the ROD also called for additional sampling

"to determine off-site treatment requirements." (Id. at 164.) After publication of the ROD, several third-party defendants collected additional information on the Source Area that led EPA to conclude that it no longer anticipates that the Source Area material will require off-Site treatment. (See Revised Draft Pre-Design Investigation Final Report, US1459.)

The lack of certainty in the ROD as to the amount of material requiring treatment was addressed by Emhart during the notice and comment period on the PRAP. As Emhart explained, "EPA's estimate that only 10% of the soil and sediment will exceed the alternative treatment standards for soil is based on limited data. . . . [I]t appears possible that a much higher percentage of soil and sediment will contain dioxin at concentrations above the standard, therefore requiring off-Site disposal." (Emhart PRAP Comments, US1383-71.)

EPA responded to Emhart's comment in the ROD's Responsiveness Summary. EPA explained that its estimates were based on approximately 400 sediment samples from Allendale and Lyman Mill Ponds and 250 floodplain soil samples (not including Source Area soil). (ROD, US1444-318.) Dr. Medine also described how those samples were used in combination with a contouring analysis in order to estimate the volume of material requiring treatment. (See Dr. Medine Test., Trial Tr. vol. 12, 28:23-29:13; FS, US1254-1478-79.) As discussed above, EPA has committed to refining these

estimates through further sampling and analysis during remedial design.

2. Conclusions of Law

Emhart argues that EPA failed to collect sufficient Site-specific data to conduct a feasibility study in accordance with the NCP. Specifically, Emhart argues that EPA did not collect enough Site-specific data to accurately estimate the amount of material that will have to be excavated or shipped off-Site for treatment. Emhart's comments on the PRAP and PRAP Amendment did not provide any specifics as to the number of samples it believes need to be collected from the Site in order to conduct a feasibility study under the NCP. However, Emhart did raise this issue as a general matter by discussing the need "to adequately define the volume of soil and sediment requiring excavation" or "off-Site disposal" (Emhart PRAP Comments, US1383-8, 71), and EPA had the opportunity to respond. (See, e.g. ROD, US1444-303-04, 318.) Under these circumstances the Court finds that Emhart adequately preserved this issue.

As to the merits of Emhart's arguments, both Emhart and EPA agree that additional sampling is needed in order to characterize the lateral and vertical extent of contamination at the Site and obtain a more accurate excavation and incineration volume estimate. The disagreement is about the timing of that additional data collection. Emhart argues that such data should have been

collected for use in the feasibility study. EPA contends that it collected sufficient data to conduct a feasibility study and that the necessary additional sampling can be done during remedial design.

While the NCP requires that EPA conduct a remedial investigation and feasibility study that includes the collection of site-specific data, it does not require a specific number of samples or data points. Instead, EPA must collect sufficient data "to adequately characterize the site for the purpose of developing and evaluating effective remedial alternatives." 40 C.F.R. § 300.430(d)(1); see also id. § 300.430(a)(2) (requiring EPA to "assess site conditions . . . to the extent necessary to select a remedy"). One of the factors that EPA must be able to "adequately characterize" is the cost of the remedy. Id. § 300.430(e)(7)(ii) (cost is an initial screening factor); see also id. § 300.430(e)(9)(iii)(G) (cost as one of nine factors to be balanced during remedy selection).

Beyond these general guidelines, EPA is given significant leeway to develop a remedial investigation and feasibility study process specific to a site. This leeway includes the option of reserving a certain amount of data collection for remedial design. The NCP specifically allows the ROD to reserve certain decisions for a later date and, "[w]hen appropriate, provide a commitment for further analysis . . . ." Id. § 300.430(f)(5)(iii)(D).

Therefore, while the information contained in the feasibility study provides the "initial building block in developing" the final design, EPA guidance also envisions that additional "data acquisition" and "sample analysis" may be necessary during the remedial design phase. EPA, Scoping the Remedial Design, Emhart516-1-2.

There is no doubt that the amount of soil that must be excavated or shipped off-Site for incineration will affect the cost of the remedy. The question before the Court is whether EPA collected sufficient data to "to adequately characterize" the remedial alternatives, including their estimated costs, such that those alternatives could be effectively compared during the feasibility study. The Court finds that EPA has met that standard.

As described in the findings of fact, EPA analyzed multiple data sets that include hundreds of soil and sediment samples from across the Site. In examining these samples EPA used high resolution mass spectroscopy to detect dioxin levels down to the parts per trillion. The results of this sample analysis were reviewed in conjunction with other types of information, such as FEMA floodplain maps and contouring analysis, to further refine EPA's estimates. It is true that in none of the Site areas discussed in this section - Allendale and Lyman Mill Ponds, the floodplain soils, or the Oxbow Area - has EPA collected sufficient data to fully implement the remedy. As EPA recognizes, additional

sampling and analysis will be required during remedial design. But this sort of commitment to further analysis is permitted by the NCP and EPA guidance. At this point in the remediation process EPA is only required to have collected sufficient data to "adequately characterize" the estimated excavation and treatment volumes such that remedial alternatives can be compared in a feasibility study, which EPA has done.

While the Court finds that the current state of EPA's excavation and treatment estimates does not violate the NCP, this finding is based, in part, on EPA's commitment to conduct additional sampling and analysis during remedial design. If information uncovered during remedial design reveals that the cost will differ significantly from the cost outlined in the ROD, EPA has the responsibility to update the administrative record as necessary. This could potentially require an "explanation of significant differences," a ROD amendment, or even an updated feasibility study. EPA has shown a willingness to take these sorts of actions in the past. (See, e.g., FS Addendum, US1311; PRAP Amendment, US1328.) And while CERCLA and the NCP leave it up to EPA to determine whether such actions are necessary in the first instance, the Court retains jurisdiction over this matter in the event that Emhart, in light of new evidence, seeks to challenge EPA's decision.

C. Location of the Confined Disposal Facility

1. Findings of Fact

The ROD calls for the majority of excavated soil to be placed in a CDF. (ROD, US1444-167.) EPA initially identified three locations at the Site that could potentially accommodate a CDF. (PRAP Amendment, US1328-21; see also FS, US1254-389.) However, as Emhart noted in its comments on the PRAP, EPA did not establish the final location of the CDF. (Emhart Comments on PRAP, US1383-65.) In addition, the Town of Johnston has made it clear that it does not want a CDF placed at or near the Site.[33]

Despite these concerns, the ROD states that a CDF will be part of the final remedy. The ROD explains that EPA "continues to believe the upland CDF disposal option is the best approach to address contaminated sediment/soil." (ROD, US144-172.) As for the exact location, the CDF could still be located on-Site. However, given the lack of community acceptance for this option, EPA has also "expanded the area where an upland CDF could be located to locations outside the Town of Johnston and beyond what is in very close proximity to the Site." (Id. at 172.) The ROD further commits

[33] The Town was initially supportive of the CDF being located in Johnston. (See, e.g., PRAP Hearing at Centredale Manor, US1333-30.) The Town has since sent a letter to EPA explaining that it "is no longer supportive of consideration of any upland disposal of contaminated material associated with the Centredale Manor Superfund Site." (Letter from Town of Johnston to EPA dated 12/29/2011, US1344-1.)

EPA "to identify additional locations where an upland CDF could be located" as part of remedial design. (Id. at 172.)

EPA has already begun this process. (See Technical Mem. for Assessment of CDF, US1475.) However, to date, EPA has not identified a location for the CDF. Whether EPA will be able to identify a location in close proximity to the Site that is acceptable to the local community is unclear. If a CDF is not utilized as part of the remedy the cost of the remedy will likely rise significantly. Given the local community's resistance to a CDF and the likely cost increase that would result if a CDF is not built, Emhart's expert, Mr. Loureiro, provided his opinion that "there should be a defined location for the CDF, whether it's purchased or under contract or part of the site itself" before the remedy is selected. (Mr. Loureiro Test., Trial Tr. vol. 10, 10:8-10.) Having a "defined location," according to Mr. Loureiro, is the only way to ensure that the proposed remedy is "feasible" and therefore a "viable alternative." (Id. at 10:11-12.) EPA disagrees and "believes a location can be identified [during remedial design] that addresses most or all of the concerns raised by the public." (ROD, US1444-172.)

2. Conclusions of Law

Emhart contends that the CDF discussed in the ROD "does not, and will never, exist." (Emhart Post-Trial Brief 175.) It argues that EPA's failure to identify a "defined location" for the CDF in

the ROD violates the NCP. (Id. at 175; see also Mr. Loureiro Test., Trial Tr. vol. 10, 10:8-13.) EPA disagrees and argues that the location of a CDF can be determined during remedial design. Emhart preserved this issue in its comments on the PRAP. (Emhart Comments on PRAP, US1383-65.)

The Court finds that EPA's decision to determine the location of the CDF during remedial design does not constitute a violation of the NCP. There are two problems with Emhart's argument. First, Emhart's claim that the CDF can "never" exist is premised on the idea that the Town of Johnston has the authority to prohibit EPA from constructing a CDF at the Site. But this is not the case. See, e.g., 42 U.S.C. § 9604(j); id. § 9621(e)(1); 40 C.F.R. § 300.400(d). And while the Town of Johnston has made clear that it does not wish to provide a space for a CDF, community acceptance is only one of nine factors that EPA must consider. See 40 C.F.R. § 300.430(e)(9)(iii)(A)-(I). Therefore, it is possible that EPA could determine, in its final analysis, that other factors outweigh a lack of community acceptance and override the Town of Johnston.

Second, there is no provision of the NCP requiring the ROD to provide a "defined location" for a CDF. Nor is there any requirement that the land on which a CDF will be located must be either purchased or under contract before publication of the ROD. Instead, EPA need only collect sufficient information to effectively compare remedial alternatives. EPA has expressed its

judgment that it will be able to find a suitable location for a CDF during remedial design and compared remedial alternatives accordingly. (ROD, US1444-172.) This does not violate the NCP.

Of course, as discussed in the preceding section, EPA is required to document all significant changes made during remedial design. Therefore, if EPA ultimately does not select a location for the CDF in accordance with the cost estimate and description in the ROD, EPA must document that fact in accordance with the NCP. This could potentially require additional analysis by EPA of remedial alternatives and possible challenges. At this point though, before EPA has completed its remedial design, the lack of a defined location for the CDF does not constitute a violation of the NCP.

D. Dewatering Sediment, Controlling Ground and Surface Water, and Constructing Haul Roads

1. Findings of Fact

The ROD calls for significant excavation of contaminated sediment at the Site. This will require some level of ground and surface water controls in order to access the contaminated sediment, the construction of haul roads to move the sediment after excavation, and the creation of a dewatering facility to remove the water from the sediment. At trial Emhart's expert, Mr. Loureiro, opined that these activities will require funding that is not provided for in EPA's cost estimate.

For instance, Mr. Loureiro testified that, while the remedy will require a dewatering facility, the ROD does not account for the cost of creating and removing that facility. (Id. at 147:4-12.) Mr. Loureiro also testified that controlling ground and surface water will require a complex system of bypass pumping, and sheet piling will be necessary to separate excavation areas from river flow. (See, e.g., Mr. Loureiro Test., Trial Tr. vol. 6, 7:11-8:8; Mr. Loureiro Test., Trial Tr. vol. 10, 23:2-19.) Lastly, Mr. Loureiro described how that the ROD does not allocate sufficient funds for the construction of haul roads that will be necessary to transport contaminated sediment and soil. (Id. at 145:9-147:4.)

Emhart never mentioned any of these costs in its extensive comments on the PRAP or PRAP Amendment. Additionally, while the Court found Mr. Loureiro's overall presentation persuasive, there is reason to believe that his cost estimates are somewhat exaggerated. EPA's expert, Dr. Medine, noted several instances where Mr. Loureiro may have overlooked less expensive alternatives.[34] Mr. Loureiro also did not address all of the cost allocations already provided for by EPA's estimate, including

[34] For example, Dr. Medine called into question whether Mr. Loureiro's proposed bypass pumping system was necessary in light of cheaper alternatives such as installing culverts. (See, e.g., Dr. Medine Test., Trial Tr. vol. 12, 71:24-72:10.) Dr. Medine also called into question whether the cost of Mr. Loureiro's proposed dewatering facility was necessary in light of a smaller (i.e., cheaper) alternative. (Id. at 51:24-52:2.)

approximately $10,800,000 for dewatering contaminated sediment[35], $3,400,000 for acquisition, installation and removal of sheet piling[36], and $1,800,000 for construction of haul roads.[37] Lastly, Mr. Loureiro's cost estimate for water management assumes that the river channel adjacent to the Source Area must be excavated despite the fact that the ROD calls for dredging, not excavation, of that area. (ROD, US1444-170; see also FS, US1254-228.)

2. Conclusions of Law

Emhart argues that EPA failed to take into account several costs associated with the remedy, including costs of dewatering sediment, controlling surface and ground water, and construction of haul roads. None of these arguments were provided to EPA during the notice and comment period on the PRAP or PRAP Amendment. (See Emhart Comments on PRAP, US1383; Emhart Comments on PRAP Amendment, US1418.) As such, Emhart's arguments related to these topics are waived. See Upper Blackstone, 690 F.3d at 30. In addition, Emhart

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[35] (See FS, US1254-1647:48-51 (describing approximately $8,613,000 in costs); see also id. at 1635 (providing for a 25% contingency).)

[36] (See FS Addendum, US1311-868:20-21 (estimating $804,600 to purchase the sheet piling and $1,933,742 to install and remove it); see also FS, US1254-1635 (providing for a 25% contingency).) Of note, EPA also allocated a "unit cost" for excavation, which could potentially provide funding for other water management requirements. (See FS Addendum, US1311-889.)

[37] (See ROD, US1444-198 (estimating cost for "mobilization and temporary roads"); see also FS Addendum, US1311-868 (explaining costs associated with haul roads).)

has failed to demonstrate that EPA miscalculated costs for the above-mentioned activities and that those miscalculations were "key assumptions" on which EPA based its selected remedy. See Oklahoma Dep't of Envtl. Quality, 740 F.3d at 192 (quoting Appalachian Power Co., 135 F.3d at 818).

EPA estimates that the remedy will cost approximately $104,600,000. (ROD, US1444-213.) This estimate includes funds for dewatering contaminated sediment, controlling ground and surface water, and constructing haul roads. While Mr. Loureiro believes EPA's estimates in these areas are low, it is unclear to what extent. But regardless of the exact number, any miscalculation by EPA in these areas likely constitutes a relatively small percentage of the total cost of the remedy, and any necessary recalculation is unlikely to fundamentally alter EPA's remedy selection. Under these circumstances, the Court finds that EPA did not fail to justify "key assumptions" such that an exception to issue exhaustion is warranted.

E. Baseline Ecological Risk Assessment

1. Findings of Fact

EPA conducted a remedial investigation of the Site that included an assessment of ecological risks. The results of that investigation can be found in EPA's Baseline Ecological Risk

Assessment, or "BERA."[38] The BERA characterized the risks to demersal fish, pelagic fish, piscivorous wildlife, and insectivorous wildlife, and the FS provided proposed cleanup goals based on those risks. (See FS, US1254-930-33.) However, EPA's remedial goals at the Site were primarily based on risks to human health, not risks to ecological receptors. (See, e.g., id. at 73 ("Biota PRGs are not presented in this FS because the sediment PRGs (based on fish consumption and direct contact/incidental ingestion) were used to determine proposed cleanup areas . . . ."); see also id. at 930-33; ROD, US144-120-22.) Furthermore, Emhart's objections to EPA's ecological risk calculations were not raised in its comments on the PRAP or PRAP Amendment. (See Emhart Comments on PRAP, US1383; Emhart Comments on PRAP Amendment, US1418.)

2. Conclusions of Law

At no point in Emhart's comments on the PRAP and PRAP Amendment did Emhart address potential flaws in EPA's ecological risk assessment, including EPA's findings on risks to demersal fish, pelagic fish, piscivorous wildlife, and insectivorous

[38] See supra note 21. The focus of the BERA is the "actual or potential impacts of site contaminants on plants and animals. . . . And the objectives of an ecological risk assessment . . . is to identify and characterize the current and potential threats to the environment" and "to identify cleanup levels that would protect those natural resources from risk." (Dr. Keenan, 138:24-139:8, ECF No. 451.)

wildlife. (See Emhart Comments on PRAP, US1383; Emhart Comments on PRAP Amendment, US1418.) Emhart's arguments related to these topics are therefore waived. Upper Blackstone, 690 F.3d at 30. In addition, Emhart has failed to demonstrate that EPA's determinations related to ecological risks constitute "key assumptions" on which EPA's selected remedy is based such that an exception to the doctrine of issue exhaustion is appropriate. See Oklahoma Dep't of Envtl. Quality, 740 F.3d at 192 (quoting Appalachian Power Co., 135 F.3d at 818).

A review of both the FS and the ROD reveals that EPA's remedial goals at the Site were primarily based on risks to human health, not risks to ecological receptors. (See, e.g., FS, US1254-73, 930-33; ROD, US144-120-22.) Even if the ecological risks were somehow mitigated, EPA has made clear that it would pursue essentially the same remedial design based on the risks to human health posed by the Site. (See, e.g., Gov't Post-Trial Brief 87 (explaining that "the results of the ecological risk assessment had little impact on the selection of the cleanup remedy" because "site cleanup was not driven by unacceptable ecological risk.").) As such, Emhart has failed to show how a change to EPA's ecological risk determinations would fundamentally change EPA's selected

remedy such that it constitutes a "key assumption" on which that remedy is based.[39]

F. Vernal Pool Habitats Located in the Oxbow Area

1. Findings of Fact

A vernal pool is a "seasonal pool of water that can provide [a] habitat for some plants and animals." (Dr. Keenan Test., Trial Tr. vol. 5, 92:9-10, ECF No. 452.) The 2004 BERA noted that "[s]everal likely vernal pools were observed in the forested floodplain area downstream from the Allendale damn." (2004 BERA, US1040-49.) The ROD also noted the potential presence of vernal pools in the Oxbow Area. (See ROD, US1444-181-82; 2011 Supplemental BHHRA & BERA, US1287-218.)

EPA's proposed remedy for the Oxbow Area includes excavation and the installation of a thin-layer cover. (ROD, US1444-7.) If vernal pools are found in the Oxbow Area, EPA recognizes that the current remedy "will face additional implementation issues." (FS, US1254-282.) The "implementation issues" consist primarily of taking "special care" not to disturb the vernal pool habitats during design and construction of the thin-layer cover. (ROD,

[39] The Court notes that, just as Emhart cannot establish that the remedy is invalid based on EPA's ecological risk calculations, EPA also cannot justify the proposed remedy based on those same ecological risk calculations. As EPA has argued, and the Court has agreed, EPA's selected remedy was the result of a process that focused on the risks to human health at the Site. EPA's selected remedy must therefore stand or fall based on those human health risk determinations.

US1444-156.) In light of these findings, EPA has committed to conducting "pre-design and design investigations [that] will include physical and ecological surveys to further . . . identify any potential vernal pools." (Id. at 181-82.)

Emhart did not raise any issues related to vernal pools in either its comments on the PRAP or the PRAP Amendment. Furthermore, at trial, Emhart's expert (Dr. Keenan) could not testify that EPA's remedy would cause damage to any potential vernal pools. (Dr. Keenan Test., Trial Tr. vol. 5, 92:12-16.) Instead, Dr. Keenan merely suggested that "it's something that needs to be investigated before it's implemented." (Id.)

2. Conclusions of Law

Emhart failed to address the potential presence of vernal pool habitats in the Oxbow Area in its comments on the PRAP and PRAP Amendment. (See Emhart Comments on PRAP, US1383; Emhart Comments on PRAP Amendment, US1418.) As such, Emhart's arguments on this topic are waived. See Upper Blackstone, 690 F.3d at 30. Moreover, Emhart has failed to demonstrate that EPA, by not confirming the presence of vernal pools, disregarded a "key assumption" on which the remedy is based. See Oklahoma Dep't of Envtl. Quality, 740 F.3d at 192 (quoting Appalachian Power Co., 135 F.3d at 818). The presence of vernal pools will not fundamentally change the remedy as a whole, but instead simply require that "special care" be taken not to disturb the vernal

pools in one of the five action areas at the Site. (ROD, US1444-156.) EPA has committed to further investigation of this issue during remedial design and can adjust the remedy as needed.

G. Classifying Site Soils and Sediments as Principal Threat Waste and F020 Listed Waste

1. Findings of Fact

EPA classified certain Site soils and sediments as Principal Threat Waste ("PTW")[40] and F020 listed waste.[41] Emhart challenged these classifications in their comments on the proposed remedy. (See Emhart Comments on Proposed Plan, US1383-83-90.) However, the ROD makes clear that the PTW and F020 classifications had no practical impact on remedy selection. (See, e.g., ROD, US1444-332 (explaining the PTW classification "had no practical effect on the selected remedy"); id. at 288 (explaining that the F020 classification "is not important for the purposes of the [conceptual site model]").) At trial Emhart's expert, Mr. Loureiro, essentially conceded this point. (See, e.g., Mr. Loureiro Test., Trial Tr. vol. 6, 161:12-15 ("And ultimately, at the end of the day it doesn't appear that [the PTW classification

[40] PTW is defined as material that is highly mobile and contains high concentrations of toxic compounds. 40 C.F.R. § 300.430(a)(1)(iii)(A).

[41] F020 listed waste is defined as "[w]astes . . . from the production or manufacturing use . . . of tri- or tetrachlorophenol, or of intermediates used to produce their pesticide derivatives." Id. § 261.31.

is] being used for any purpose in defining the scope of the work that needs to be done."); id. at 170:11-12 ("I don't think [the F020 classification has] any practical implication with regard to the costs of the remedy.").)

2. Conclusions of Law

Emhart argues that EPA's classification of certain soils and sediments as PTW and F020 listed waste was arbitrary and capricious. The Court finds that this question is moot. The ROD makes clear that these classifications had no practical impact on remedy selection. (See, e.g., ROD, US1444-288, 332.) Emhart agrees that the PTW classification "was not used for any purpose in defining the scope of work to be done." (Emhart Post-Trial Brief 228; see also Mr. Loureiro Test., Trial Tr. vol. 6, 161:12-15.) And Emhart's expert essentially conceded this same point with respect to the F020 classification. (See Mr. Loureiro Test., Trial Tr. vol. 6, 170:11-12.) The Court need not address the appropriateness of classifications that, if changed, would have no practical impact on the remedial action itself.

H. Classifying Source Area Ground Water as Drinking Water

1. Findings of Fact

For years the Rhode Island Department of Environmental Management ("RIDEM") has considered the Source Area groundwater to be so contaminated that it is unsuitable for potential use as drinking water. (FS, US1254-43.) The contamination is caused by

the Source Area, but also possibly by waste sites located close to the Woonasquatucket River upgradient from the Source Area. (ROD, US1444-58.) EPA's remedial investigation emphasized RIDEM's classification of the Source Area groundwater as non-potable and discussed several important uncertainties in its groundwater data, including the "vertical extent" of the contamination. (RI, US1098-88.) Based on this information, the FS determined that the groundwater was not a potential source of drinking water, which EPA labels as "Class III" groundwater. (FS, US1254-43.)

EPA later determined that its classification of the groundwater as Class III was not appropriate. As explained in the ROD, RIDEM's groundwater classification system had not "obtained EPA approval of a Comprehensive State Ground Water Protection Program." (ROD, US1444-57.)[42] EPA therefore could not base its classification decision on RIDEM's findings, and had to instead focus on the standards set by EPA guidance. (Id. at 343.) Citing these standards, the ROD explains that the Class III designation does not apply because the groundwater was not "so contaminated by naturally occurring conditions or the effects of broad-scale human activity (unrelated to a specific activity) that [it] cannot be

[42] This fact was brought to EPA's attention by the National Remedy Review Board, which "is a committee of EPA regional personnel that provide technical assistance and review large, complex, and costly Superfund sites." (Mr. Maccarone Test., Trial Tr. vol. 2, 169:25-170:9.)

cleaned up using treatment methods reasonably employed in public water supply systems." (Id. at 344.) This led EPA to label the Source Area groundwater as "Class II," which applies to "current and potential sources of drinking water." (Id.)

EPA made this determination despite the fact that EPA has recognized data gaps for Source Area groundwater. (See, e.g., RI, US1098-88.) For example, it is uncertain to what extent dense non-aqueous phase liquid ("DNAPL") is present in the Source Area and at what depths. (Compare Mr. Loureiro Test., Trial Tr. vol. 6, 172:9-173:6 with Dr. Medine Test., Trial Tr. vol. 12, 88:1-89:4.)[43] The vertical extent of that (and other) contamination is particularly important because the remedy - which partially relies on a RCRA C cap to prevent further contamination - will not be effective in stopping contamination sources located below the cap. (Mr. Loureiro Test., Trial Tr. vol. 6, 177:24-178:12; id. at 171:17-25; see also Mr. Loureiro Test., Trial Tr. vol. 5, 143:24-144:17.)

[43] EPA has collected some data that suggests DNAPL is not present in the Source Area groundwater. (See, e.g., RI, US1098-205-06; ROD, US1444-197.) However, EPA has also found some groundwater contamination in intermediate and deep samples in the southern end of the Source Area, the origin of which is not clear. (RI, US1098-54.) This issue was likely not more thoroughly investigated during the remedial investigation because, at that time, EPA did not consider the groundwater to be a potential source of drinking water.

In addition to possible DNAPL contamination, EPA recognizes that "there are numerous non-Superfund sources . . . that contribute or have the potential in the future to contribute to exceedances of drinking water standards away from the Source Area." (ROD, US1444-58.) Under these conditions, the ROD explains that "[f]uture groundwater uses are not expected to change significantly" and that water will continue to be supplied to the Source Area by outside sources. (Id.; id. at 62.) Nevertheless, EPA persists in classifying the groundwater as a potential source of drinking water.

The change in classification from Class III to Class II led EPA to adopt stricter cleanup goals for the Source Area groundwater. (FS Addendum, 1311-6, 79.) EPA also used the new classification as part of its justification for requiring a RCRA C cap over the Source Area. (ROD, US1444-339.) As explained in the ROD, the RCRA C cap will be used to prevent the migration of contamination from the soil to the groundwater. (ROD, US1444-7.) However, it is unclear whether this will be sufficient to prevent further Source Area groundwater contamination. (Id. at 342.) Therefore, the ROD calls for groundwater monitoring wells to be installed so that EPA can determine whether federal drinking water standards are achievable. (Id. at 7.)

2. Conclusions of Law

Emhart argues that EPA inappropriately classified Source Area groundwater as Class II as opposed to Class III. Emhart challenged EPA's classification of Source Area groundwater in its comments on the PRAP. (Emhart PRAP Comments, US1383-102-05; see also ROD, US1444-338.) Emhart has therefore preserved this issue for judicial review.

The NCP directs EPA "to return usable ground waters to their beneficial uses wherever practicable, within a timeframe that is reasonable given the particular circumstances of the site." 40 C.F.R. § 300.430(a)(1)(iii)(F). Where restoration is "not practicable" EPA need not restore the groundwater, but must instead take steps to "prevent further migration of the plume, prevent exposure to the contaminated ground water, and evaluate further risk reduction." Id. To that end, EPA has established guidelines that place groundwater into different "classes." (ROD, US1444-343.) Class II groundwater is considered a "current and potential sources of drinking water." (Id. at 344.) "Class III" groundwater, on the other hand, is "not considered potential sources of drinking water." (Id.) However, even where EPA guidelines would classify groundwater as Class II (i.e., a "potential" source of drinking water), the NCP still requires EPA to determine whether the application of drinking water standards is "technically impracticable." 40 C.F.R. § 300.430(f)(1)(ii)(C)(3); see also 42

U.S.C. § 9621(d)(4)(C). If restoration of the groundwater is impracticable, then a waiver is appropriate. Id.

In this case, EPA classified the groundwater as Class III in the vast majority of the Site. However, for the Source Area, EPA classified the groundwater as Class II. (ROD, US1444-342-43.) EPA explains that this finding is based on EPA guidelines, which permit the Class III label only where groundwater is "so contaminated by naturally occurring conditions or the effects of broad-scale human activity (unrelated to a specific activity) that [it] cannot be cleaned up using treatment methods reasonably employed in public water supply systems." (Id. at 344.)

As EPA has emphasized at several points in this litigation, EPA guidance is non-mandatory. (See, e.g., Gov't Post-Trial Brief 56-57.) Moreover, agency guidelines, while informative, cannot alter fundamental regulatory requirements. See United States v. S. Union Co., 643 F. Supp. 2d 201, 211-12 (D.R.I. 2009), aff'd, 630 F.3d 17 (1st Cir. 2010), rev'd and remanded on other grounds, 567 U.S. 343 (2012). Therefore, to justify the remedy, EPA cannot merely show that it followed EPA guidelines in classifying the groundwater as Class II. Instead, EPA must demonstrate that it complied with the NCP.

Unlike EPA guidelines, the NCP neither calls for nor permits a bright line rule that groundwater must be considered a potential source of drinking water anytime contamination is neither

"naturally occurring" nor the result of "broad-scale human activity." Instead, the NCP requires that EPA make a case-specific determination as to whether it is "practicable" to restore groundwater to its "beneficial use." 40 C.F.R. § 300.430(a)(1)(iii)(F). In addition, EPA is required to collect sufficient information to determine whether the remedy is likely to be "effective" in restoring the groundwater to any such use. Id. § 300.430(d)(1); see also id. § 300.430(e)(7)(i).

The evidence makes overwhelmingly clear that the Source Area groundwater is currently far too contaminated to provide a source of drinking water and that "[f]uture groundwater uses are not expected to change significantly." (ROD, US1444-58.) The ROD recognizes that there are likely several off-Site sources of contamination contributing to the contamination. (Id.) In addition, EPA has been on notice as far back as the remedial investigation that there are several important uncertainties in its data for the Source Area groundwater, including the "vertical extent" of the contamination. (RI, US1098-88.)

In light of these issues, the Court finds that EPA has not collected sufficient information or conducted sufficient analysis on which to base its finding that the Source Area groundwater is a potential source of drinking water or that the remedy is likely to effectuate that outcome. Specifically, there is currently insufficient analysis regarding (1) the vertical extent of the

contamination at the Site; and (2) the extent to which off-Site sources contribute to contamination in the Source Area groundwater. Going forward, EPA has broad discretion to determine how best to characterize the Site and make any necessary adjustments to the remedy. But, at a minimum, if EPA continues to classify the Source Area groundwater as a potential source of drinking water, EPA must present sufficient information and analysis to justify what are key findings under the NCP: that restoration of the groundwater is "practicable" and that the remedy will be "effective" in bringing about that restoration.

I. Requiring a RCRA C Cap in the Source Area

1. Findings of Fact

The Source Area, as its name suggests, contains a significant amount of contamination. Three interim protective caps as well as one RCRA cap have already been placed over several portions of the Source Area. (ROD, US1444-13.) Significant portions of what remains of the Source Area are covered by paved roads and parking lots that service two elderly housing facilities (Brook Village and Centredale Manor). (Id.)

EPA previously identified the existing caps and paved surfaces as a potential long-term solution. (See, e.g., EPA Action Mem. dated May 4, 1999, US605-14; 2004 Technical Mem. on Long-Term Remedy for Source Area Soils, US1048-25; 2005 BHHRA, US1101-57.) However, after EPA classified Source Area groundwater as potential

drinking water, EPA identified the RCRA C cap as the preferred remedy. (ROD, US1444-6.) Separate from remediation of the Source Area groundwater, EPA has presented evidence that the RCRA C cap is more durable and more likely to prevent direct human contact with contaminated soil. (Id. at 333.) However, it is unclear from the record whether, absent the change to groundwater classification, the ROD would have called for the installation of a RCRA C cap.

There are significant challenges to implementing a RCRA C cap on the Source Area. For example, installation of the cap will require removing paved surfaces and excavating substantial amounts of soil. This will inevitably cause inconvenience for the residents of Brook Village and Centredale Manor, and EPA will have to take special care to ensure that those residents are not exposed to the toxic soil that is being excavated in close proximity to their homes. (See, e.g., Mr. Loureiro Test., Trial Tr. vol. 6, 45:1-8 (discussing "life safety issues" for Site residents).) EPA considered many of these concerns in the FS. (See, e.g., FS, US1254-208, 313.)

2. Conclusions of Law

EPA is required to address whether a RCRA C cap is "relevant and appropriate" for the Source Area. See 42 U.S.C. § 9621(d). EPA determined that a RCRA C cap is "relevant and appropriate." (ROD, US1444-296.) Emhart concedes that the RCRA C cap is "relevant,"

but argues that it is not "appropriate." (Emhart Post-Trial Brief 190-91.) Emhart preserved this issue by bringing it to EPA's attention in its comments on the PRAP. (See generally Emhart Comments on PRAP, US1383-90-109.)

The Court already determined that EPA has not conducted sufficient analysis on which to base its finding that restoration of Source Area groundwater is practicable. On the current record, the Court also finds that EPA's decision to include a RCRA C cap as part of the remedy for the Source Area is inextricably intertwined with EPA's current remediation goals for Source Area groundwater. Therefore, EPA cannot justify the RCRA C cap absent further analysis with regards to the Source Area groundwater or a finding that the RCRA C cap is necessary regardless of EPA's groundwater remediation goals. This analysis will necessarily include a determination as to whether the benefits of the RCRA C cap outweigh the inconvenience and potential health and safety risks to Site residents. The Court reiterates that EPA has significant leeway in characterizing the Site going forward and making adjustments to the remedy as necessary. However, on the current record, the portion of the ROD requiring a RCRA C cap cannot survive judicial scrutiny.

J. Baseline Human Health Risk Assessment ("BHHRA")

1. Findings of Fact

a. Sampling of Residential Soils

EPA conducted a residential soil exposure risk assessment as part of the BHHRA. The goal was to evaluate the risks to human health caused by contact with the soils along Allendale and Lyman Mill Ponds. (See generally 2012 Technical Mem., US1392; PRAP Amendment, US1422.) Emhart submitted comments on the PRAP Amendment arguing that EPA had failed "to collect sufficient data in the area of the residential floodplain to derive appropriate exposure point concentrations for use in the [BHHRA]." (Emhart Comments on PRAP Amendment, US1418-14-15.)

During the remedial investigation conducted in 1999 EPA collected several samples from each of sixty-two residential lots located along the Woonasquatucket River. (RI, US1098-25.) EPA also collected a small number of additional samples in 2001. (See NTCRA, US1099-37-42.) In total, EPA evaluated 226 data records for dioxin in determining the potential human health risks posed by residential Site soils. (ROD, US1444-348-49.) Dioxin at concentrations "above the cleanup level for floodplain residential-use soil" was found "in approximately [forty-five percent] of the locations sampled along the eastern shore of Allendale and Lyman Mill Ponds." (Id. at 348-49.) While the ROD commits EPA to conducting additional sampling during remedial

design to define the exact contours of excavation, EPA determined that the current level of sampling was sufficient to assess the risk to human health posed by the Site. (Id. at 349.)

b. Estimates Used to Determine Risks from Recreational and Residential Pond and Soil Exposure

i. Relative Bioavailability of Dioxin

The BHHRA[44] considered the risks from dioxin exposure to recreational users and residents of the Site. This required that EPA estimate the relative bioavailabilty ("RBA") of dioxin in the soil and sediment. RBA is the availability of dioxin in the medium to which humans are likely being exposed (i.e., the soil and sediment) relative to the medium that was the source of the exposure. (Dr. Vorhees Test., Trial Tr. vol. 8, 65:14-25.) This number helps EPA estimate the rate at which humans are likely to absorb toxins at the Site. (Id.) EPA guidance suggests that RBA for dioxin "can be expected to be less than [one] hundred percent." (Id. at 66:21-24 (discussing EPA, Dioxin RBA Report, US1542-10).) Emhart therefore requested that EPA assume a RBA value of less than one hundred percent for the Site. (Emhart Comments on PRAP Amendment, US14418-12-14.)

EPA responded in the ROD that "there is not . . . a consensus protocol for determining a site-specific RBA for dioxin in soil, nor are such assessments a common practice." (ROD, US1444-357-58;

[44] See supra note 20.

see also Dr. Vorhees Test., Trial Tr. vol. 8, 66:25-67:5.) In the absence of a "consensus protocol" EPA declined to conduct any Site-specific RBA testing during the BHHRA. Instead, EPA used the "protective assumption[]" that that the Site soil and sediment had a RBA of one hundred percent. (Dr. Vorhees Test., Trial Tr. vol. 8, 68:7-11; see also ROD, US1444-357-58.) This is common practice for EPA at other CERCLA sites. (Id. at 67:10-22.) It is also consistent with EPA guidance. (See EPA, Dioxin RBA Report, US1542-10.)

ii. How Often People Use the Ponds

Having made a dioxin RBA assumption of one hundred percent, EPA then went on to consider the risks to persons swimming and wading in the ponds. In doing so EPA had to estimate how often people use the ponds. The BHHRA assumed that the Site is used for recreational activities from May through October, but specifically for wading and swimming only from June through August. (2005 BHHRA, US1101-81.) During these months the BHHRA used the Risk Assessment Guidance for Superfund to estimate the maximum exposure that would occur, which represents the upper percentile (95th-99.9th) of exposure for persons using the Site. (See EPA, Risk Assessment Guidance for Superfund ("RAGS"), vol. III, US1612-207.) The estimated maximum exposure frequency for wading was fifty-two days per year for young children (ages six and below) and thirty-nine days per year for adults and older children. (2005 BHHRA, US1101-

82.) Based on the "recommended exposure time for recreational swimming," EPA estimated that each exposure would last approximately one hour. (Id.)

Emhart submitted comments to EPA on the BHHRA. While Emhart conceded that the ponds were used recreationally, Emhart suggested that a lower frequency of use should be assumed because "[i]t is unlikely that most individuals will spend substantial amounts of time wading in the river or ponds that do not have beach areas." (Emhart Comments on BHHRA, US1151-47.) There is anecdotal evidence in the record suggesting that Allendale and Lyman Mill Ponds have not been frequently used for swimming and wading in the past. (See, e.g., 12/7/2011 Public Hearing, US1334-37-39; 8/15/2011 Email Correspondence, US1309.) This sentiment was echoed during trial by Emhart's expert. (Mr. Loureiro Test., Trial Tr. vol. 6, 196:14-197:1.) That visitors and residents would refrain from swimming and wading is understandable, as the ponds have been subject to historic industrial pollution (RI, US10998-57) and have portions that some consider unattractive. (See, e.g., Dr. Keenan Test., Trial Tr. vol. 4, 98:25-99:6; Mr. Loureiro Test., Trial Tr. vol. 6, 196:20-197:7.)

However, EPA presented evidence at trial demonstrating that the ponds are undoubtedly used for various recreational activities. Residents at the Site have yards that lead directly into the ponds, and various residents have placed docks and boats

in the ponds. (See Dr. Medine Test., Trial Tr. vol. 12, 58:25-59:6.) EPA's expert, Dr. Vorhees, also testified that she observed fisherman as well as evidence of other recreational activities at the Site. (Dr. Vorhees Test., Trial Tr. vol. 8, 11:21-12:19, 47:23-49:10.) Additionally, as a counter to Emhart's experts, Dr. Vorhees testified that she personally found various portions of the ponds to be "attractive." (Id. at 48:7-49:20.) This evidence suggests not only that the ponds are currently being used recreationally, but also that increased use could be expected after cleanup is complete.

iii. Incidental Ingestion Rates

In addition to estimating the frequency of pond use at the Site, EPA had to estimate the rate at which residents and recreational pond users incidentally ingest soils and sediments.[45] EPA estimated the upper range of incidental ingestion was a rate of approximately two hundred milligrams per day for young children and one hundred milligrams per day for older children and adults. (2012 Technical Mem., US1392-58.) These ingestion rates are based on EPA guidance. (See, e.g., IFRAGS, vol. I, US1620-13 (discussing "Standard Default Exposure Factors").) Dr. Vorhees testified at trial that the use of these ingestion rates is standard practice

[45] These ingestion rates were also used to estimate the risks from residential soil exposure.

at other CERCLA sites. (Dr. Vorhees Test., Trial Tr. vol. 8, 64:7-13.)

Emhart's expert, Dr. Keenan, testified at trial that these soil ingestion rates were unnecessarily high. Dr. Keenan explained that EPA's estimate is based primarily on research conducted by Dr. Edward Stanek and Dr. Edward Calabrese. (Dr. Keenan Test., Trial Tr. vol. 4, 103:16-21.) Those researchers have since cut their recommendations in half, suggesting a soil ingestion rate of approximately 100 milligrams per day for young children and fifty milligrams per day for older children and adults. (See Emhart Comments on BHHRA, US1151-61-64.) However, EPA has specifically considered this information and rejected the researchers' recommendations. (See EPA Response to Emhart Comments on the BHHRA, US1450-5.) As EPA's 2011 Exposure Factors Handbook explains, EPA has considered the available information (including the new research done by Dr. Stanek and Dr. Calabrese) and still recommends an upper percentile for soil ingestion of two hundred milligrams per day for young children. (See EPA, 2011 Exposure Factors Handbook, Emhart578-268-318.)

iv. Number of Days Per Year Residents are Exposed to Site Soils

As part of the residential soil risk assessment, EPA estimated the number of days Site residents are exposed to the soil surrounding their homes. EPA estimated that exposure occurs

approximately 350 days per year. (PRAP, US1393-5.) Emhart challenged this estimate during the notice and comment period on the PRAP, arguing that it was unrealistically high given seasonal weather changes in Rhode Island. (Emhart Comments on PRAP, US1418-11.) Emhart has also presented evidence that other CERCLA sites in New England have used the lower exposure frequency estimate of approximately 150 days per year. (ROD, US1444-356-357.)

There is no consensus way to determine the specific number of days EPA should use as the exposure frequency rate. (Dr. Vorhees Test., Trial Tr. vol. 8, 59:16-60:3.) EPA guidance provides for a national default exposure frequency rate of 350 days per year. However, EPA guidance also states that this default rate "may not be appropriate for all regions." (EPA, RAGS, vol. I, Emhart584-32.) For CERCLA sites in New England, EPA has previously recommended a lower exposure frequency of approximately 150 days per year. (Dr. Vorhees Test., Trial Tr. vol. 9, 4:21-6:15, 63:20-22.)

However, EPA released updated regional guidance in 2002 and since that time has typically used the national default rate of 350 days per year. (ROD, US1444-356-57 (citing EPA, Supplemental Soil Screening Guidance, US1637).) This is particularly true in Rhode Island where EPA's local counterpart (RIDEM) has also adopted a default exposure frequency rate of 350 days. (Dr. Vorhees Test., Trial Tr. vol. 8, 59:16-60:3.) For instance, the "Peterson Puritan"

site, located in Rhode Island, used an exposure frequency rate of 350 days. (Id. at 60:16-21.)

Furthermore, as noted in the ROD, there are reasons why EPA would assume that a 150-day estimate is too low for the Site. Unlike other cleanup locations, the Site contains a large residential population such that "access to this site is not restricted or otherwise limited." (EPA, IFRAGS, vol. I, Emhart579-116; see also id. ("[R]esidential land use is most often associated with the greatest exposure . . . ."); ROD, US1444-356.) As such, the residents (unlike recreational users) may have little choice but to venture outside and come into contact with the Site even in inclement weather. Additionally, because residents' houses are located on the Site, staying indoors does not prevent contact with contamination because Site soils and dust are easily tracked into the home. (Dr. Vorhees Test., Trial Tr. vol. 8, 60:5-15.)

c. Risks from Fish Consumption

EPA conducted a fish consumption risk assessment as part of the BHHRA. The goal was to determine the risks to human health posed by fish consumption at the Site and the cleanup levels of pond sediments necessary to mitigate those risks. To accomplish this goal, EPA had to estimate the frequency with which people consumed fish from the Site, the species of fish consumed, and the contamination levels present in the fish. EPA then determined the biota-to-sediment accumulation factor ("BSAF"), which is the ratio

between contamination levels in fish and contamination levels in the sediment. (Dr. Keenan Test., Trial Tr. vol. 4, 42:11-43:4.) By analyzing the frequency and toxicity of fish consumed in the ponds, in combination with the BSAF, EPA was able to make risk calculations and establish pond sediment remediation goals. Emhart challenged several aspects of EPA's fish consumption risk assessment in its comments on the BHHRA. (See Emhart Comments on BHHRA, US1151; see also EPA Response to Emhart Comments on BHHRA, US1450.)

i. Fish Sampling

Allendale Pond is sustained, in part, by a dam that prevents the majority of Allendale Pond waters from flowing downstream into Lyman Mill Pond. The dam has breached several times, including once in 1991 and twice in April and May of 2001. (See FS, US1254-43; Dr. Keenan Test., Trial Tr. vol. 4, 14:22-15:7.) The dam was restored after the 2001 breaches and by early 2002 Allendale Pond was restored to pre-breach water levels. (FS, US1254-43.)

Unfortunately, EPA's collection of fish samples in Allendale and Lyman Mill Ponds was conducted in July 2001, after the May 2001 breach but before Allendale pond had been restored. (ROD, US1444-71.) This led to two sampling issues. First, some of the fish collected from Lyman Mill Pond "may actually have been washed into Lyman Mill Pond from Allendale pond at the time of the

Allendale Dam breach." (MACTEC Mem., US1100-34.)[46] This means that the contamination levels of fish collected from Lyman Mill Pond "may not bear a meaningful relationship" to the sediment in Lyman Mill pond, such that EPA could not calculate an accurate BSAF. (CSTAG Recommendations, US1080-5.) The Contaminated Sediments Technical Advisory Group ("CSTAG")[47] highlighted this issue and "strongly recommend[ed] that new, co-located sediment and fish tissues samples be collected to develop a BSAF." (Id.)

EPA recognized this problem and employed MACTEC to find an adequate solution. MACTEC compared EPA's data collected after the Allendale dam breach to data collected before the breach and determined that, if certain steps were taken, "the available data appear to be sufficient for PRG development and additional sampling and analysis is not recommended at this time." (MACTEC 4/29/2005 Letter, US 1100.) The steps MACTEC recommended were for EPA to use fish tissue data and BSAFs from other parts of the Woonasquatucket River to determine PRGs. EPA complied with this recommendation. As EPA explained in the ROD, "[f]or [] Lyman Mill Pond, arithmetic mean BSAF for each fish species from five other exposure areas

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[46] MACTEC is an EPA contractor that was used during the risk assessment to analyze data collected at the Site.

[47] CSTAG is EPA's internal technical advisory group that monitors the progress and provides advice for certain large and complex Superfund sites involving contaminated sediment. (See Principles for Managing Contaminated Sediment Risks at Hazardous Waste Sites, US909.)

. . . for each contaminant was used in the derivation of species-specific fish-consumption-based sediment PRGs for Lyman Mill Pond." (ROD, US1444-71 n.6.) This allowed EPA to estimate a BSAF for Lyman Mill Pond without using the data points objected to by Emhart.

The second sampling issue caused by the Allendale dam breach involved the species of fish collected. EPA collected equal numbers of eel, white sucker, and largemouth bass from Lyman Mill Pond. (2005 BHHRA, US1101-60.) However, when EPA sampled Allendale Pond, EPA collected only eel and white sucker, but no largemouth bass. (Id.) Based on that data EPA's risk calculations assumed that largemouth bass are not typically present in Allendale Pond. (See id. at 60, 78-79.)

This assumption is likely erroneous. As MACTEC explained to EPA after reviewing the BHHRA, "[t]he absence of largemouth bass in Allendale Pond in July 2001 suggests that largemouth bass may [] have been washed out of Allendale Pond into Lyman Mill Pond." (MACTEC Mem., US1100-34.) EPA recognized that assuming the absence of largemouth bass in Allendale Pond "may have resulted in an over- or under-estimation" in its risk assumptions. (ROD, US1444-71.) However, unlike with its BSAF calculations, EPA did not adjust its species assumptions to take into account the likely effects of the Allendale dam breach.

ii. Species of Fish Consumed

In 1999 the Rhode Island Department of Health established a fish-consumption advisory at the Site in order to deter people from consuming contaminated fish. (Dr. Vorhees Test., Trial Tr. vol. 8, 26:18-24.) As discussed below, despite this fish-consumption advisory, people still fish and consume fish from the Site. In order to estimate the risk to human health from fish consumption at the Site, EPA came up with protective assumptions about the species of fish people currently consume from the Site as well as the species of fish people would consume under possible future conditions. (See, e.g., id. at 32:14-16.) This reasonable maximum exposure is essentially the highest exposure that could be reasonably expected to occur at the Site. (Id. at 37:16-19; see also id. at 40:18-19 (describing "reasonable maximum exposure" as "the high end of possible exposure").)

One common way of accomplishing this is through a survey of local anglers. However, an angler survey would be of limited value at the Site because of the fish-consumption advisory that has been in place since 1999. (Id. at 26:18-27:12, 33:7-14.) The effects were evident, for example, in EPA's 2001 survey of local anglers. In that survey various respondents admitted to catching and consuming fish from the Site. (2005 BHHRA, US1101-80.) However, "most respondents were aware of the current advisory against consumption of fish and other biota from the river" and "indicated

they no longer consume fish from the river." (Id.) Given the effect of the fish-consumption advisory, EPA reasonably concluded that a Site-specific angler survey would be of limited value in coming up with reasonable estimates of fish consumption, particularly in estimating the reasonable maximum exposure of likely future consumption.

This led EPA to use several other sources of information to estimate current and future fish-consumption rates at the Site. As discussed above, EPA sampled fish from Allendale and Lyman Mill Ponds to determine the types of fish typically caught at the Site. These included bass, white sucker, and eel. (Id. at 60.) EPA also used surveys of people living near the site, academic literature, and input from local stakeholders to confirm that people were consuming fish at the Site and were likely to do so in the future. (Id. at 80.) Based on this information, EPA determined that the group most likely to be the subject of maximum exposure was certain Asian populations living near the Site.

The academic literature suggested that various parts of New England (including Rhode Island) are home to Asian populations that fish both recreationally and as a form of subsistence. (See, e.g., id. at 576, 583-89.) These populations often either ignore or misunderstand fishing advisories and regularly consume their catches regardless of size. (See, e.g., id. at 583-4.) Additionally, they catch and consume a wide variety of species,

including species identified at the Site. (See, e.g., id. at 583; U.S. Army Corps of Eng'rs, Identification of Preferred Target Species, US847-3-4.) EPA, in consultation with a wide variety of local subject matters experts[48], considered these findings persuasive.

The information garnered from the academic literature and local experts also corresponded with some Site-specific data. For instance, two surveys conducted at or near the Site suggested that certain Asian populations fish in that area. The first is the 1998 Urban River Use Survey of the Woonasquatucket River, which observed a diverse set of people - including persons of Asian descent - fishing in the Woonasquatucket River. (Id. at 80.) The second was the Tool Kit for Urban Rivers, which focused on the demographics of nearby Providence and found the "Southeast Asian population (Hmong, Camobodian, Laotian, & Vietnamese) and other ethnic groups were . . . high consumers of fish, eel, and turtles from local waterways, including the Woonasquatucket River." (Id.; see also EPA, Tool Kit for Urban Rivers, US1029-3; Dr. Vorhees Test., Trial Tr. vol. 8, 31:6-21.)

[48] EPA consulted with private groups (such as universities and non-profits) as well as RIDEM, the Rhode Island Department of Health, and the Rhode Island Division of Fish and Wildlife. (See, e.g., BHHRA, US1102-577, 583; U.S. Army Corps of Eng'rs, Identification of Preferred Target Species, US847-3.)

EPA recognized that the lack of an angler survey made EPA's analysis of the data more difficult. This meant that "[t]here was no quantitative information available that could be used to quantify the potential combined fish diet in a more detailed manner." (EPA Response to Emhart Comments on BHHRA, US1450-3.) EPA was therefore forced to use its "professional judgment in identifying the composition of the hypothetical future combined fish diet." (Id.) Taking into account the fact that certain Asian populations consumed a wide variety of fish, EPA decided to make the conservative estimate that the species of fish found in Allendale and Lyman Mill Ponds were consumed in equal portions.

In its comments on the BHHRA Emhart challenged EPA's assumption that certain populations may consume white sucker and eel at the same rate as largemouth bass. (Emhart Comments on BHHRA, US1151-41.) In the absence of a Site-specific angler survey, Emhart suggested that EPA consult the Maine Angler Survey ("MAS"). The MAS is an assessment of fish consumption practices in Maine from 1992. EPA's Exposure Factors Handbook includes some MAS data when providing recommended consumption rates. (Dr. Keenan Test., Trial Tr. vol. 4, 49:17-50:21; EPA, 1997 Exposure Factors Handbook, Emhart598-458-461.) Specifically addressing the consumable species identified at the Site, the MAS recommends a consumption ratio of approximately 83:11:3 (largemouth bass - white sucker - eel).

EPA, however, determined that the MAS should not be relied upon at the Site. Instead, EPA opted to use its "professional judgment" to determine the reasonable maximum exposure of a "hypothetical future combined fish diet." (EPA Response to Emhart Comments on BHHRA, US1450-3.) As discussed above, EPA determined its consumption ratios using academic literature, input from subject matter experts, and surveys of people living near the Site.

iii. Parts of Fish Consumed

In the BHHRA, EPA had to consider which parts of the fish people might consume. For the purposes of determining the reasonable maximum exposure, EPA assumed that people consume the fillets of largemouth bass and the whole body of eels and white sucker. Based on this assumption, EPA used the contamination concentrations in the fillets of largemouth bass and the whole-body contamination concentrations for eel and white sucker in the BHHRA. (Dr. Vorhees Test., Trial Tr. vol. 8, 33:23-34:5.) This increased the estimated risk to human health because whole-body contamination concentrations are higher than the contamination concentrations of fillets due to the fact that contamination concentrates to a higher degree in fish organs than in the flesh. (Id. at 34:9-16, 41:3-6.)

Emhart objected to EPA's use of whole-body contamination concentrations for white sucker in its comments on the BHHRA. While recognizing that "it may be reasonable to assume that the entire

eel is consumed in certain instances," Emhart argued that EPA lacked sufficient Site-specific data on which to base its assumption "that individuals eat the entire sucker, which is an extremely bony fish." (Emhart Comments on BHHRA, US1151-42.) At trial, Emhart also cited EPA guidance which suggests that "[m]ost fishers in the United States consume fish fillets." (EPA, Assessing Chemical Contaminant Data for Use in Fish Advisories, vol. II, Emhart590-95.) Absent "specific data on fish preparation methods," this guidance "recommends using fillets as the standard sample type for analyzing chemical contaminants." (Id. at 312.)

EPA responded to this criticism by referring again to its research on certain Asian populations that consume fish at or near the Site. Academic literature suggests not only that those groups consume a wide variety of fish, but also that they consume the whole body of those fish. For example, one study found that certain Asian populations fishing in Rhode Island often do not remove the skin, fat or organs of the fish before cooking. (2005 BHHRA, US1102-584.) That study also found that "fish cooking methods" include "boiling" such that "the broth may be used for soup." (Id.) Another study provided a similar observation of Asian fishers on the Housatonic River in Connecticut, noting that "[i]n addition to fillets," those populations eat "eyes, skin, and organs." (Id. at 589.)

These conclusions were also supported by EPA guidance. The same EPA guidance cited by Emhart also notes that "[c]ertain populations, including some Asian-Americans . . . eat parts of the fish other than the fillet and may consume the whole fish. Recipes from many cultures employ whole fish for making soups or stews. As a result, more of the fish contaminants are consumed." (EPA, Assessing Chemical Contaminant Data for Use in Fish Advisories, vol. II, Emhart590-309.) Based on this information EPA "assumed that white sucker might be most likely to be consumed if it were a component of a fish stew or similar meal (as a whole body)." (EPA Response to Emhart Comments on BHHRA, US1450-3.)

iv. Consumption Amounts from the Site

In considering the risks to human health posed by fish consumption, EPA had to estimate the reasonable maximum consumption rate of fish at the Site. EPA did this, in part, by considering data from the MAS. (2005 BHHRA, US1101-79.) According to that data, the upper end (i.e., 90th percentile) of adult fishers who do not share the fish they catch can be expected to consume approximately "23 half-pound fish meals in a year or . . . about one fish meal every two to three weeks" from the Site. (Dr. Vorhees Test., Trial Tr. vol. 8, 38:5-7; see also MAS, US1541-7; 2005 BHHRA, US1101-79.) This equates to approximately fourteen grams of fish per day from the Site. (Id.)

In its comments on the BHHRA Emhart challenged these consumption rates for two reasons. (Emhart Comments on BHHRA, US1151-44.) First, Emhart argued that EPA was overestimating the amount of fish consumed by adults. (Id. at 45.) As Emhart noted, the MAS found that most anglers share their catch with other members of the household and therefore typically consume less than fourteen grams per day. (Id.) Under these circumstances, Dr. Keenan opined at trial that EPA should have assumed household sharing of fish in calculating reasonable maximum consumption rates. (Dr. Keenan Test., Trial Tr. vol. 4, 69:6-71:14.)

Dr. Vorhees disagreed and testified that EPA's assumptions were reasonable. As she explained, in calculating the reasonable maximum exposure EPA "obviously had a concern about the angler who does not share their catch and wanted to be sure to protect them." (Dr. Vorhees Test., Trial Tr. vol. 8, 40:13-15.) According to Dr. Vorhees, EPA's decision was therefore reasonable "because [EPA] wanted to protect consumers who, in fact, catch fish and don't necessarily share their catch." (Id. at 39:3-5.)

Emhart's second challenge to EPA's consumption rates was based on EPA's assumption that all fourteen grams consumed by the adult fisher came from the Site. (Emhart Comments on BHHRA, US1151-45.) EPA has explained that its "fish consumption rates include only fish caught from the water bodies at the Site, and do not include fish caught at other locations nor fresh or preserved fish

purchased for consumption." (2005 BHHRA, US1101-79.) This suggests that EPA is assuming that the adult fisher consumes fourteen grams per day from the Site and also consumes significant additional fish from other sources.

While this assumption may be reasonable, it does not conform to the MAS data on which EPA basis its analysis. (Id.) In the context of its fourteen-gram estimate, The MAS explains that

> the study was designed to collect data on consumption from all flowing bodies of water, and not just the . . . contaminated water. Thus, although individuals may fish in affected river reaches some of the time, it is highly unlikely that all fishing effort is focused on these waters, particularly because there are numerous alternative fisheries in close proximity to each river. . . . Consequently, whereas the estimates for rivers and streams include all consumed fish from rivers and streams during the season, it is likely that only a portion of the consumption can be attributed to a single water body.

(MAS, US1541-7.) As this makes clear, the MAS's fourteen-gram estimate assumes that those fourteen grams come from multiple sources.

2. Conclusions of Law

Emhart previously challenged the sufficiency of the BHHRA in comments submitted to EPA. However, several of Emhart's specific arguments presented at trial were not submitted during the official notice and comment period. Such arguments are typically waived under the doctrine of issue exhaustion. However, because the Court finds that the portions of the BHHRA challenged by Emhart were so

foundational to EPA's remedy-selection process, and because Emhart previously brought many of these issues to EPA's attention, a limited exception is warranted. EPA has emphasized that the BHHRA was the primary driver of EPA's cleanup standards. (See, e.g., Gov't Post-Trial Brief 87.) Under these circumstances, EPA must be able to justify the BHHRA's "key assumptions." See Oklahoma Dep't of Envtl. Quality, 740 F.3d at 192 (quoting Appalachian Power Co., 135 F.3d at 818). These "key assumptions" include EPA's conclusions regarding the human health risks posed by soil exposure, pond exposure, and fish consumption at the Site.

a. Sampling of Residential Soils

Emhart challenges the sufficiency of EPA's sampling process, describing it as "sparse." (Emhart Post-Trial Brief 79.) Emhart points to the fact that EPA collected fewer than four samples containing dioxin data for each property at the Site. (Id. at 79-80.) However, as the Court explained in previous sections, the NCP does not require a specific number of samples. Instead, EPA need only "collect data necessary to adequately characterize the site for the purpose of developing and evaluating effective remedial alternatives." 40 C.F.R. § 300.430(d)(1). In characterizing the risks from residential soil exposure EPA reviewed approximately 226 data points, which included several samples from each of sixty-two residential properties at the Site. Emhart has failed to

demonstrate how this level of sampling is somehow insufficient under the NCP.

b. Estimates Used to Determine Risks from Recreational and Residential Pond and Soil Exposure

Emhart challenges various estimates made by EPA in conducting the pond and soil risk assessments. Emhart argues that EPA used inappropriately high estimates in characterizing: (1) the relative bioavailability ("RBA") of dioxin; (2) the rates at which people incidentally ingest contaminants; (3) how often people use the ponds at the Site; and (4) how often residents are exposed to Site soils.

In each of these areas, EPA did not rely solely on Site-specific data, but instead developed "conservative" estimates (in the sense that EPA erred on the side of protecting people from exposure) based on available scientific literature, EPA guidance, and experience at other CERCLA sites. This practice does not inherently violate the NCP. While the NCP requires that EPA collect sufficient site-specific data to adequately compare remedial options, it plainly does not mandate that every piece of data used by EPA be the result of sampling at the site. And in the absence of clear direction from either the NCP or CERCLA, the development of broadly applicable estimates for use on either a regional or national basis falls squarely within the purview of EPA. See Fox Television Stations, 556 U.S. at 513-14; Florida Power & Light

Co., 404 U.S. at 463. The question is whether EPA's use of these estimates for the Site was somehow arbitrary and capricious. The Court finds that it was not.

EPA's estimates are not "without substantial basis in fact." Muszynski, 899 F.2d at 160 (quoting Florida Power & Light Co., 404 U.S. at 463). EPA has surveyed the available data and provided reasonably conservative estimates for use at CERCLA sites. To be sure, there is no universal consensus on how to estimate the RBA of dioxin, incidental ingestion rates, or pond and soil exposure frequency. But it is precisely in these areas that involve evolving scientific considerations that deference to EPA's "technical expertise and experience" is appropriate. Id. (quoting Florida Power & Light Co., 404 U.S. at 463). And while EPA's assumptions are certainly conservative, that is by design. The NCP mandates that EPA's "goal . . . is to select remedies that are protective of human health and the environment, that maintain protection over time, and that minimize untreated waste." 40 C.F.R. § 300.430(a)(1)(i). In so doing, EPA estimates the reasonable maximum exposure that is likely to occur for both current and potential future land use at the Site. (EPA, IFRAGS, vol. I, Emhart579-24.) The use of a conservative estimate under these circumstances - erring on the side of caution when it comes to the risk of cancer (and other maladies) for those living in and around

the Site - is neither arbitrary, capricious, nor a violation of the NCP.

c. Risks from Fish Consumption

Emhart challenges several aspects of EPA's fish consumption estimates in the BHHRA. With regards to EPA's estimates about the types and parts of fish consumed from the Site, Emhart's challenges focus primarily on EPA's decision to forego a Site-specific angler survey and to rely on data from outside the MAS. Neither the NCP nor EPA guidance mandates that EPA conduct an angler survey or use data from the MAS. Instead, as discussed above, EPA need only "collect data necessary to adequately characterize the site for the purpose of developing and evaluating effective remedial alternatives." 40 C.F.R. § 300.430(d)(1). After collecting that data, EPA must then draw conclusions that are not arbitrary or "without substantial basis in fact." Muszynski, 899 F.2d at 160 (quoting Florida Power & Light Co., 404 U.S. at 463).

In determining species preference at the Site EPA reasonably concluded that an angler survey would be of little use because of the fish-consumption advisory in place at the Site. EPA therefore looked to alternative sources of information, including local subject matter experts, academic literature on fish-consumption in Rhode Island and surrounding states, surveys, and fish samples collected from the Site. This level of data collection provided an adequate, non-arbitrary, basis on which to determine the likely

species preferences of the most vulnerable populations near the Site for the purposes of developing a reasonable maximum exposure estimate. EPA certainly had to make judgment calls along the way, but these decisions deserve deference considering EPA's "technical expertise and experience," id. (quoting Florida Power & Light Co., 404 U.S. at 463), as well as EPA's duty to make conservative estimates in order to protect human health. See 40 C.F.R. § 300.430(a)(1)(i).

With that said, regarding EPA's estimates for the amount of fish consumed from the Site, the Court finds two instances where EPA's analysis was arbitrary. First, EPA relied on knowingly non-representative sampling to assume that no largemouth bass are consumed from Allendale Pond. Despite being on notice that largemouth bass are likely present in Allendale Pond, EPA excluded them from its Allendale Pond risk calculation. Emhart has demonstrated that this misstep arbitrarily increased the risk calculation for Allendale Pond. See Motor Vehicle Mfrs., 463 U.S. at 50 (describing agency action as arbitrary where the agency "entirely failed to consider an important aspect of the problem").

Second, EPA assumed that certain populations consume fourteen grams of fish per day from the Site. While the fourteen-grams estimate is based on the MAS, the MAS provides the caveat that "only a portion of the consumption can be attributed to a single water body." (MAS, US1541-7.) While EPA may ultimately determine

that fourteen grams is the appropriate reasonable maximum consumption rate at the Site, on the current record (i.e., basing its estimate on the MAS data alone) EPA's decision is arbitrary. See Motor Vehicle Mfrs., 463 U.S. at 50 (describing agency action as arbitrary where the agency "offered an explanation for its decision that runs counter to the evidence before the agency.")

Both of these missteps must be remedied before moving forward. How best to address these issues and identify any consequent changes to the remedy is appropriately addressed by EPA in the first instance. That is EPA's prerogative, to be reviewed by the Court only as provided under CERCLA.

K. Notice and Reasonable Opportunity to Comment

1. Findings of Fact

EPA drafted a PRAP in the fall of 2011. (PRAP, US1328.) Based on subsequent analysis, EPA made several changes to the PRAP in the form of a PRAP Amendment, which was published the following summer. (PRAP Amendment, US 1393.) Both the PRAP and PRAP Amendment were subject to notice and comment after their publication. The notice and comment period on the PRAP and PRAP Amendment went from November 14, 2011 to March 2, 2012, and July 19, 2012 to September 17, 2012, respectively. (ROD, US1444-24-25.) EPA provided the public notice of the PRAP and PRAP Amendment through newspaper announcements. (Id.) EPA then participated in public hearings and considered comments from a variety of sources, including Emhart.

(Id.; see also Emhart Comments on PRAP, US1383; Emhart Comments on PRAP Amendment, US1418.)

2. Conclusions of Law

Emhart argues that EPA failed to provide the public notice and a reasonable opportunity to comment on the PRAP and PRAP Amendment. Before publication of the ROD, EPA is required to present a PRAP to the public "for review and comment." 40 C.F.R. § 300.430(f)(1)(ii). The PRAP must "briefly describe[] the remedial alternatives analyzed by the lead agency, propose[] a preferred remedial action alternative, and summarize[] the information relied upon to select the preferred alternative." Id. § 300.430. EPA must then provide the public "a reasonable opportunity, not less than 30 calendar days, for submission of written and oral comments" as well as an "opportunity for a public meeting." Id. § 300.430(f)(3)(i)(C)-(D). The purpose of this process is to provide "the public with a reasonable opportunity to comment on the preferred alternative for remedial action, as well as alternative plans under consideration, and to participate in the selection of remedial action at a site." Id. § 300.430(f)(2).

EPA complied with these NCP requirements. However, the Court has found several instances where EPA's analysis was flawed and the results of that analysis were included in the PRAP and PRAP Amendment. EPA has also committed to conducting further analysis during remedial design and reserved the right to modify the remedy.

EPA is therefore responsible for updating the administrative record as necessary to correct flaws in its analysis and document future analysis conducted during remedial design. Id. § 300.825(a)(1). To the extent EPA's corrections or the remedial design process lead to a significant change to the remedy[49], EPA must provide for an additional notice and comment period as required by the NCP.

L. Failure to Comply with the Unilateral Administrative Order

1. Findings of Fact

Emhart initiated this litigation in May 2006. (Phase I Findings 3-4.) EPA did not issue the ROD until over six years later, in September 2012. (See ROD, US1444.) EPA then issued its Unilateral Administrative Order ("UAO") on June 10, 2014. (See UAO, US1490.) The UAO ordered Emhart "to perform the Remedial Design, Remedial Action, and Operation and Maintenance for the selected remedy . . . as described in the Record of Decision." (Id. at 5.) That UAO currently remains in place.

Since EPA's issuance of the UAO the Court has completed Phase I and II of this litigation. The Phase I trial lasted over twenty days beginning on May 18, 2015 and resulted in Emhart being found liable under CERCLA. However, the Court also found that "the

[49] What constitutes a "significant change" includes, among other things, a significant increase in the estimated amount of time it will take to implement the remedy, costs, and potential disruption to local residents.

Government is not yet entitled to judgment in its favor on this claim because the issues of costs and whether the remedy selected by the EPA is consistent with CERCLA first need to be litigated in the second phase of this trial." (Phase I Findings 185-86.) Emhart has continued to refuse to comply with the UAO through Phase II of this litigation based on its objections to the selected remedy.

2. Conclusions of Law

CERCLA authorizes EPA to issue "such orders as may be necessary to protect public health and welfare and the environment." 42 U.S.C. § 9606(a). This includes situations where there "may be an imminent and substantial endangerment to the public health or welfare or the environment because of an actual or threatened release of a hazardous substance from a facility." Id. Any entity that "willfully violates" such an order "without sufficient cause" may be liable for fines and punitive damages. Id. §§ 9606(b)(1); 9607(c)(3). These penalties are not mandatory, but are instead authorized at the discretion of the Court. See id. §§ 9606(b)(1), 9607(c)(3) (explaining that violators "may" be liable for penalties); Gen. Elec. Co. v. Jackson, 610 F.3d 110, 119 (D.C. Cir. 2010) ("[T]he district court has authority to decide not to impose fines even if it concludes that a recipient 'without sufficient cause, willfully violate[d], or fail[ed] or refuse[d] to comply with' a UAO.") (citing 42 U.S.C. §§ 9606(b), 9607(c)(3)).

What constitutes "sufficient cause" under CERCLA has not been addressed by the First Circuit. However, multiple other Circuit Courts of Appeal have interpreted "sufficient cause" to mean a "good faith" or "objectively reasonable basis for believing that the EPA's order was either invalid or inapplicable to it." Solid State Circuits, Inc. v. EPA, 812 F.2d 383, 391 (8th Cir. 1987); see also, e.g., Gen. Elec. Co., 610 F.3d at 119; Emp'rs Ins. of Wausau v. Browner, 52 F.3d 656, 661 (7th Cir. 1995). A party may meet this standard by demonstrating "that the applicable provisions of CERCLA, EPA regulations and policy statements, and any formal or informal hearings or guidance the EPA may provide, give rise to an objectively reasonable belief in the invalidity or inapplicability of the clean-up order." Solid State Circuits, 812 F.2d at 392.

In this case, Emhart does not challenge EPA's finding that the Site qualifies as a potential "imminent and substantial" environmental threat sufficient to justify a UAO. Moreover, in the aftermath of the Phase I trial, Emhart cannot assert that the UAO is somehow inapplicable to it. Instead, Emhart argues that it has a "sufficient basis" to not comply with the UAO based on its good faith challenges to the validity of EPA's selected remedy.

The Court has determined that several aspects of EPA analysis qualify as arbitrary, including the classification of Site groundwater as a potential source of drinking water and portions

of the fish consumption risk assessment. Emhart made timely objections highlighting these issues in its comments on the PRAP. These sorts of missteps on the part of EPA do not automatically provide potentially responsible parties sufficient cause to disregard a UAO. However, given that Emhart has continuously objected to critical aspects of the remedial design that the Court has now found arbitrary, and Emhart's previous participation in the cleanup process, the Court finds that Emhart's challenge to the UAO up to this point has been pursued in objective good faith.

V. Conclusion

EPA has developed a remedial action using the process outlined in CERCLA and the NCP. That remedial action, if completed, will mitigate the risks to human health and the environment EPA has identified at the Site. However, in developing that remedial action, EPA made several decisions that the Court finds violated CERCLA because they were arbitrary, capricious, or otherwise not in accordance with law; these must be addressed before moving forward with the remedial action.

The Court finds that Emhart has met its burden in establishing that, on the record as currently constituted, the following decisions were arbitrary, capricious, or otherwise not in accordance with law: (1) labelling Source Area groundwater as a potential source of drinking water; (2) assuming that there are no largemouth bass in Allendale Pond; and (3) using fourteen grams as

the reasonable maximum consumption rate for anglers fishing at the Site. The UAO is stayed until these matters are resolved, and Emhart is not required to pay the fines and fees stemming from its non-compliance with the UAO that have accrued up to this point.

The Court can envision several ways EPA could approach these deficiencies. EPA could find that the issues identified by the Court require EPA to reopen the remedial investigation and feasibility study process and publish a PRAP Amendment, as it has done in the past. The Court takes no view as to the appropriate course of action at this time and leaves it to EPA to address these issues in the first instance. However, the Court retains jurisdiction in this matter in order to ensure that the issues are addressed in a manner consistent with the law and not arbitrary or capricious.

Lastly, the Court notes that its decision is based, in part, on EPA's commitment to further sampling and analysis during the remedial design phase. EPA concedes that information discovered during remedial design may require alterations to EPA's analysis and the chosen remedy. For instance, EPA's estimates may change with regards to the amount of money and time it will take to implement the remedy or the potential hazards and inconvenience to local residents caused by implementation. Again, the Court retains

jurisdiction over this process to ensure that EPA's actions are consistent with the law and not arbitrary or capricious.

IT IS SO ORDERED.

William E. Smith
Chief Judge
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The Search for Truth in Regulatory Science

featuring
Edward J. Calabrese, Professor of Environmental Health Sciences, School of Public Health, University of Massachusetts

facilitated by
Terence Kealey, Visiting Senior Fellow, Center for the Study of Science, Cato Institute

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One basis of modern medicine is that low doses of many chemicals are beneficial, while higher ones are toxic. And yet a great disparity has evolved with the regulation of many entities — including carcinogens, ionizing radiation, and toxics. Their regulation is based on the notion that that a single molecule or photon is as capable of inducing cancer as the billionth one. How has this disparity happened — with low, beneficial doses being treated and regulated the same way as higher ones? What is correct?

One highly compelling look at this subject has emerged from Dr. Edward Calabrese's painstaking and voluminous research. Using recently declassified Atomic Energy Commission documents, Calabrese details the establishment of the linear no-threshold model at the height of the Cold War, when thermonuclear bombs were routinely detonated in the atmosphere, resulting in radioactive fallout. His is a spellbinding story of what is often called "noble cause corruption" of science, and of

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The threshold vs LNT showdown: Dose rate findings exposed flaws in the LNT model part 2. How a mistake led BEIR I to adopt LNT

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ABSTRACT

This paper reveals that nearly 25 years after the National Academy of Sciences (NAS), Biological Effects of Ionizing Radiation (BEIR) I Committee (1972) used Russell's dose-rate data to support the adoption of the linear-no-threshold (LNT) dose response model for genetic and cancer risk assessment, Russell acknowledged a significant under-reporting of the mutation rate of the historical control group. This error, which was unknown to BEIR I, had profound implications, leading it to incorrectly adopt the LNT model, which was a decision that profoundly changed the course of risk assessment for radiation and chemicals to the present.

1. Introduction

Two years after the public was told by the U.S. National Academy of Sciences (Anonymous, 1956) that all radiation was dangerous and that the effects were cumulative and irreparable, William L. Russell, a member of that NAS Genetics Panel, published a paper in the journal *Science* documenting evidence of a significant discovery that threatened the underlying tenets supporting the linear no-threshold (LNT) dose response model. His sustained, respected, and painstaking work in observing and documenting the effects of radiation on mice indicated that the rate at which radiation dose was administered made a major difference in the measured mutation rate for the identical total dose. This research would show that lower dose rates reduced mutation rates by factors of 3 and 20 (Calabrese, 2016a) in certain germ cells of male and female mice, respectively.

Russell's (Russell et al., 1958) discovery of the dose-rate effect for ionizing radiation has had less of an impact than it should have had on risk assessment. That discovery revealed that fundamental beliefs/assumptions of the radiation genetics community from the 1940s through the 1950s concerning the induction by ionizing radiation of mutations in germ cells of mammals (i.e., especially in mouse spermatogonia and oocytes) were incorrect. That is, genetic damage was found to be highly dependent on dose-rate, whereas prior to this discovery it was assumed to depend on the total dose. These findings would challenge beliefs concerning the nature of the dose-response in the low dose zone. The present paper extends Part 1 (Calabrese, 2016a) by showing (1) how Russell's dose-rate findings affected genetic and cancer risk assessment principles and practices, (2) that estimates of genetic risk based on Russell's mouse specific locus test (SLT) were significantly affected by an undetected significant error in under reporting his control group mutation rate, (3) that the credibility of the LNT dose response recommendation of BEIR I (NAS, 1972) has accordingly been undermined by the undetected control group error, (4) that if this error had been identified and corrected at the time of BEIR I, the Russell data would have yielded either a threshold model or a hormetic model depending on the correction factor applied (Selby, 1998a, 1998b; Russell and Russell, 1996) and (5) that therefore the decision by BEIR I to recommend using the LNT dose response model was improperly based.

2. Russell finally challenges LNT-only after Muller's death

Russell created a significant niche whereby his research findings on dose-rate for ionizing radiation induced germ cell mutation in the mouse would 1) revise and refine beliefs that had been based on research reported for the fruit fly, 2) have more relevance for human risk assessment, 3) challenge beliefs and assumptions about the nature of the dose response in the low dose/dose-rate zone and 4) incorporate biological repair activities in the risk assessment process. While these research developments had the capacity to lead to confrontations with Hermann J. Muller by challenging the validity of the LNT dose-response model of which Muller was the chief proponent, Russell appears to have been reluctant to directly challenge Muller on the issue and implications of dose-rate in an intellectual sense. This was the case whether as a member of the BEAR Committee's Genetics Panel (see Calabrese, 2016a, Part 1), as an advisor to Karl Z. Morgan in development of the 1964 ICRP Report or in his publications. However, after the death of Muller in April of 1967, Russell became

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more eager to discuss his mega-mouse SLT research, its implications and impact on the mantra of the radiation geneticist much of which was based upon the research results of Muller and interpretations of the implications of those findings that Muller strongly promoted.[1]

In his 1970 presentation at the 14th International Congress of Radiation Research at Evian, France, Russell (1973) recalled that the original estimates of genetic risk (which were made by the BEAR I Genetics Panel (Anonymous, 1956) for radiation (and, as noted by Calabrese 2013), soon thereafter for chemical carcinogens were based on the two major assumptions that: (1) radiation-induced gene mutation frequencies in the fruit fly have extrapolative relevance to humans and (2) results from radiation experiments on fruit fly spermatozoa illustrate general principles of radiation genetics and thus can be applied to humans (i.e., the mantra of the radiation geneticist). What followed from these two overarching assumptions was a series of specific and fundamental risk-assessment tenets upon which genetic and, as noted by Calabrese (2013), cancer risk assessments were based. According to Russell (1973) they included that: 1) "Gene mutation rate is directly proportional to radiation dose; 2) Gene mutation rate is independent of radiation dose rate; 3) Gene mutation rate is independent of dose fractionation; 4) There is no repair of gene mutational damage; 5) There is no threshold below which no genetic damage occurs; and 6) There is no recovery from mutation with time after irradiation.".

After more than two decades of conducting genetics research on mice at Oak Ridge National Laboratory, Russell had evaluated the effects of ionizing radiation (up to that time) on well over a million animals (radiation-exposed and control groups combined) in the largest ever progressive/cumulative mammalian study in the history of science. From this vast experience, Russell concluded that, regarding the two major assumptions, "the first assumption is probably not valid, that the second is definitely incorrect, and that each one of the six 'general' principles does not apply to mouse spermatogonia and/or oocytes." During his presentation, Russell provided substantial scientific evidence supporting these conclusions. This should have been a major galvanizing event that led to substantial debate while offering the opportunity for a significant mid-course correction concerning the nature of the dose response in the low dose/dose-rate zone. However, it failed to do so. Even with the support of superior data and sounder reasoning, it seems that the correction of errant and entrenched dogma is much more difficult than it should be.

2.1. Russell's impact on risk assessment

Despite such a powerful and fundamental critique of the radiation geneticist's creed, these views of Russell never gained significant intellectual traction within the genotoxicology and risk assessment communities, in part due to the fact that the 1970 presentation was much like a one-and-done phenomenon. That is, Russell failed to adequately reinforce/expand upon this perspective and to elaborate on its broad health and societal implications at subsequent meetings and via other publications. For example, the article that was based on his 1970 presentation was delayed in publication for three years (Russell, 1973) and, when finally published, it was as a chapter in a monograph, thereby failing to get widespread citation and to have any significant impact. Nearly two decades later, Russell (1989) was still complaining that the publication of this manuscript was frustratingly delayed. However, the real issue was that Russell failed to provide the sufficient leadership to establish an influential following on this most critical issue, one on which he had extensive experience and copious data to support his argument.

It should, however, be realized that during this period there was a remarkable transformation in toxicology as researchers began to evaluate chemicals for their mutagenicity and carcinogenicity. As the spotlight shifted from ionizing radiation to chemical agents, an initial set of SLT studies was conducted to test five suspected mutagens (i.e., four methanesulfonates-methyl, ethyl, n-propyl and iso-propyl, and Myleran). Concerns were subsequently raised when the mutational responses of the spermatogonia to these agents were not statistically different from the controls (Russell, 1972) and yet these same agents were shown to be highly mutagenic in the new wave of relatively inexpensive short-term mutagenicity assays. Before long, the highly expensive and time-consuming mouse SLT, which did not address somatic cell mutation, began losing its relevance as it yielded significant ground to the Ames and other short-term tests. Protecting the mouse SLT from "losing its relevance" apparently became a more important consideration than providing leadership on the critical Threshold vs LNT dose-response issue. Indicative of such developments was the absence of any mention of Russell or the mouse SLT in the massive Handbook of Carcinogen Testing (Milman and Weisburger, 1985), which had included substantial coverage on short-term mutagenicity assays. A major hurdle that Russell faced in building an influential following was that vastly more scientists at numerous institutions applied the short-term tests and that, with the stiff competition for funding that existed, many of those scientists did not want to lose funding to the expensive SLT test. There was thus much incentive to challenge whether the SLT data should be viewed as conclusive when those data disagreed with the results of short-term tests. Russell (1984) provided a highly relevant analysis of all 11 "environmental" mutagens that had up to that time yielded no significant indication, by themselves, of inducing SLT mutations in mouse stem-cell spermatogonia. The sum total of the experimental data for those 11 mutagens (as judged from short-term tests) was 12 mutations in 285,329 offspring, which was a mutation frequency with a lower point-estimate than that of the SLT's historical control (43 mutations in 801,406 offspring). This finding conflicted in an unexpected manner with the commonly accepted argument that the SLT was too insensitive to detect effects induced by most chemical muta-

[1] Russell was acutely aware of the confrontational nature of Muller and his capacity to challenge others and generate professional hostility, having witnessed it firsthand as a member of the BEAR I, Genetics Panel (Crow, 1995). One such example was the prolonged strained relationship between Muller and James V. Neel, also of the BEAR Genetics Panels and one of its youngest members (i.e., 25 years younger than Muller). This conflict was started by Muller's response to a presentation by Neel at the WHO Study Group meeting on the Effects of Radiation on Human Heredity held in Denmark in August 1956. Based on his decade-long studies of atomic bomb survivors, Neel criticized a number of assumptions about radiation dose and mutation rates, thereby challenging some of the foundations of the LNT dose response model. While Neel felt that intellectual exchange was part of normal scientific debate/discussion, Neel claimed that "Dr. Muller apparently regarded it as an attack on his life's work." (Neel letter to Beadle, September 14, 1959). Muller tried to pressure both Neel and the conference leaders not to present the findings, leading other scientists to threaten to not participate should Muller have his way. The situation became so disruptive that Alexander Hollaender, the conference session Chair and Russell's supervisor, arranged for Muller and Neel to meet later in Oak Ridge, hoping that this now acrimonious situation could be resolved. The meeting took place on Sunday January 6, 1957 at Oak Ridge, Tennessee. Lindee (2013) reported that Hollaender acted as the referee. While the meeting led to nominal revisions of their respective papers for the conference proceedings, significant reconciliation was not achieved. The following comment on this meeting in a letter by Neel to Curt Stern (January 9, 1957) (Neel, 1957) is instructive:"I think I appreciated for the first time some of the facets that I had not previously understood as to why Muller was so upset. For years some zoologists have made critical remarks on man as an object of genetic study; this despite the fact that in my opinion many of them really did not have a feeling for human material or appreciate the possibilities. Now a human geneticist has turned around and pointed out some of the difficulties in working with *Drosophila*. This almost comes under the category of 'man bites dog'".
Neel would also inform Stern (Neel letter to Stern, December 11, 1956) (Neel, 1956b) how impossible it was to have a rational discussion with Muller on scientific points of dispute, with Muller often responding in highly emotional ways.
What emerged from these interactions with Muller was Neel's sense that Muller seemed to be intellectually threatened by human genetics and how it could affect Muller's leadership domain. This type of behavior and intimidation by Muller may well have contributed to the reluctance of Russell to directly challenge Muller on the LNT concept with his dose-rate findings (Neel to Beadle, September 14, 1959). In fact, on December 11, 1956, Neel wrote to Hollaender (Neel, 1956a) asking him to have Russell review the manuscript at the center of the dispute between Muller and himself. Thus, it is likely that Russell was well aware of the tensions between Muller and Neel.

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gens. One might have supposed that if the failure to find a significant increase was the insensitivity of the SLT, the total mutation frequency for all chemicals would have shown an increase—at least in the point-estimate. In addressing the apparent insensitivity of the mouse SLT Russell accordingly suggested "that committees involved in genetic risk estimation give more weight than they have in the past to the likelihood that negative findings in mouse stem-cell spermatogonia and arrested oocytes may be a result of repair." His suggestion was largely ignored. Also around this time, Russell et al. (1982) demonstrated that inspite of the extraordinarily high mutagenicity of ethylnitrosourea that he had discovered in mouse spermatogonia, those cells "are apparently capable of repairing at least a major part of the mutational damage when the repair process is not swamped by a high dose."

Also complicating the situation for application of the mouse SLT was the development over the next decade of a new vertebrate model in fish (i.e., Japanese medaka) using a SLT (Shima and Shimada, 1991) to compete with the mouse SLT. The new medaka model was less expensive and was claimed to be more efficient, could detect mosaic mutations by exposure to paternal germ cells (Shimada and Shima, 2001) and was applicable to chemical mutagens as well as ionizing radiation. With new tests came a new way of thinking and a new generation of genotoxicologists who either ignored or rejected Russell's criticisms of the radiation geneticist's mantra. Rather than retreating, the linearity doctrine was continuing and, in fact, was enthusiastically advancing onward. An example of this, already in the mid-1970s, was the proposal put forth by Committee 17 of the Environmental Mutagenesis Society when it advocated that a special "Delaney Amendment" be adopted exclusively for mutagens (Committee 17, 1975).

The process for evaluating chemical mutagens was apparently changing in such a way as to undermine Russell's newly acquired primacy in the field. In fact, Russell found his concept of dose rate, as applied to ionizing radiation (Russell and Kelly, 1982), under attack in the writings of high-level critics such as Abrahamson and Wolff (1976), Denniston (1982), and others, all of whom were colleagues of Crow and/or part of the Muller circle. By 1970, when had Russell finally used his data-based findings to launch a strong challenge to the geneticists' creed, he was within five years of reaching the mandatory retirement age of 65 at Oak Ridge National Laboratory, and the field was aggressively seeking new, quicker, and less expensive ways to address critical genotoxicity issues.

3. Bridging the gap- from BEAR to BEIR

In commenting on the 1956 report of the BEAR I Committee's Genetics Panel, the BEIR I Genetics Subcommittee (1972) (NAS/NRC BEIR, 1972) indicated on page 45 (column 1) of their report that the "principle of dose-rate independence has turned out to be wrong; it is now known that, for germ cell stages in the mouse that correspond to the human stage of the longest duration, radiation given at a very low rate or at widely spaced intervals produces fewer mutations than the same total dose given more rapidly."[2] In Note 4 (page 61, column 2) of its report, the BEIR I Genetics Subcommittee claimed that the mutation rate in spermatogonia for low dose rates of X-rays is about 1/3 as large as when the same total dose is given at a high dose rate. In mature oocytes, however, the mutation rate at low dose rates is approximately 1/20 the mutation rate for the same total dose given at a high dose rate. On page 97 of the BEIR I Genetics Subcommittee (1972) report, the findings of Russell et al. concerning dose rates for spermatogonia and oocytes were discussed further with respect to somatic cells. The Genetics Subcommittee speculated that the radiation-induced mutations in somatic cells may be similar to those produced in germ cells, thereby resulting in "deviations" from a linear dose response at low dose rates. If this were the case, then the findings of Russell might have important implications for cancer risk assessment.

The BEIR I Genetics Subcommittee also discussed other research developments that challenged the LNT dose response recommendation since the time of the BEAR I Report (Anonymous, 1956). Of particular note were the findings of Spalding et al. (1969) (see page 62 of NAS BEIR I, 1972) who exposed young adult males of an inbred mouse strain to 200 rads of whole-body radiation (delivered at 50 rads/min) per generation for 45 generations in order to assess potential adverse reproductive effects, especially those that may be difficult to detect within a few generations. Although these studies had certain limitations regarding sample size, statistical power and detection sensitivity, there were no significant radiation-induced effects observed on numerous traits, including on viability, fertility, growth, detectable abnormalities, and lifetime survival curves. This remarkable lack of radiation-induced effects across such a large range of traits, and at such a high dose, greatly impressed the BEIR I Genetics Subcommittee (NAS/NRC, 1972).

With respect to dose rate, the BEIR I Genetics Subcommittee (NAS/NRC BEIR, 1972) noted on page 88 of its report that the background radiation of 0.1 rem per year is a dose rate approximately one-hundred-million to one billion times lower than the high dose rates at which adverse effects have been reported in most population-based radiation studies. The subcommittee also reported that normal background radiation in these studies induced "ionizing events in cell nuclei" (i.e., mutations) in a single mammalian cell at a rate considerably less than one event per day even though the higher dose rates in these same studies induced this same kind of mutation in a single cell at an exponentially higher rate of about 2600 per s. This amounts to a mutation rate several million times higher than actually occurs at background exposure rates. According to the Genetics Subcommittee, this vast difference in exposure may have significant implications concerning the overall production of radiation-induced mutations and their repair. In fact, the Genetics Subcommittee argued that the risk of cancer at low doses and low dose rates will most likely be considerably smaller per unit dose than would occur at high doses and high dose rates, citing multiple studies that supported this conclusion

[2] The BEAR II Genetics Committee (1960) acknowledged the occurrence of the Russell et al. (1958) dose rate findings in mouse spermatogonia/oocytes, as well as in oogonia of *Drosophila*. The inclusion of this acknowledgement for mouse oocytes and *Drosophila* oogonia was a very late addition to the 1960 report, as Drs. Hollaender and Russell were very upset with both the draft final statement that nothing "spectacular" had been discovered since the 1956 BEAR I Genetics Panel report (Hollaender to Andrews letter, February 7, 1960) and that the findings of dose-rate had only mentioned mouse spermatogonia. Hollaender and Russell called George Beadle, Chair of this Committee, and the three agreed that the Genetics Section needed to be re-written. Hollaender and Russell, receiving Beadle's approval, quickly re-wrote the section and then informed the Executive Secretary, Howard L. Andrews, that he should insert the four pages of the retyped material/document (Russell to Andrews letter, February 5, 1960; Russell, 1960). The material sent to Andrews was substantially but not fully, incorporated into the final document. Thus, the BEAR 1960 (NAS, 1960) report acknowledged that the concept so strongly advocated by BEAR I (Anonymous, 1956), that mutation damage was

(footnote continued)
independent of dose-rate, was wrong, a judgment that would foreshadow the emergence of Russell's more extensive dose-rate findings and their potential risk assessment implications. Throughout 1959 there were multiple BEAR I Genetics Subcommittee drafts, with initial drafts not including the mouse oocyte and *Drosophila* oogonia information. However, subsequent drafts incorporated these findings. It is likely that Hollaender and Russell (especially), were upset to find that this key information of dose-rate had been removed in the "final" drafting by Bentley Glass (Glass Papers, BEAR II, APS). These Glass papers included a series of revised draft BEAR II Genetics Panel document and letter responses from multiple panelists such as Crow to Beadle (Oct. 31, 1959; Failla to Beadle-Oct. 26, 1959 and to Glass-Nov. 2, 1959 and Muller to Andrews-Feb. 8, 1960. Note that the letter of Muller chastised Andres with the statement that "This sentence ignores Russell's recent findings on the lower mutagenicity of chronic doses, confirmed on *Drosophila* by myself and associates." In addition, the 1960 BEAR II publication made no mention that the mouse oocyte findings reported by Russell at a June 22–26, 1959 international conference showed a reduction in mutational damage by nearly 18-fold as compared to the 3-fold decrease of the spermatogonia (Russell et al., 1960).

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(see page 88 NAS/NRC BEIR I, 1972).

As a result of these considerations, the BEIR I Genetics Subcommittee concluded that estimating mutation risks from radiation exposures to low doses and low dose rates while using an LNT dose response model derived from data collected at high doses and high dose rates "may well over estimate the risk of low LET radiation at low dose rates and may, therefore be regarded as upper limit of risk for low level low LET radiation" (see page 88 NAS/NRC BEIR I, 1972). According to the Genetics Subcommittee the actual risk from such exposures can therefore range from an estimated enhancement at a linear upper-limit of risk to a zero enhancement at a lower-limit of risk. The zero enhancement of risk at low doses and low dose rates is not inconsistent with data and would probably be the result of DNA repair efficiencies.

Despite the acknowledgment that the BEAR I Committee Genetics Panel had most likely overestimated risks substantially in view of subsequent research developments (such as the massive studies of the Russells and Spalding's multi-generation experiment) (Spalding et al., 1969), the BEIR I Genetics Subcommittee still decided to retain use of the LNT dose response model when determining policy for radiation protection. Given the need for relevant experimental data, the limited sensitivity of epidemiological studies to detect slight risks, and uncertainties when extrapolating from animal models to humans, this decision in the minds of the subcommittee members was appropriate and prudent. They emphasized that this recommendation would require clear and accurate explanations and qualifications of the assumptions and procedures used in such risk estimates. Interestingly, they also stated that such information was needed to prevent the acceptance of LNT as scientific dogma.[3]

Although the same LNT risk model was ultimately recommended, the monolithic tone of the BEAR I Genetics Panel (Anonymous, 1956) had clearly been transformed, and the concept of "dose response model uncertainty" was introduced and emphasized. The BEIR I Genetics Subcommittee acknowledged being guided by a Precautionary Principle which would be replacing a Mullerian generation of LNT-believing radiation geneticists from the BEAR I Committee Genetics Panel. It is interesting to speculate that the BEAR I Genetics Panel might possibly have arrived at this very same "err on the side of safety" position if it had been forced to provide the scientific basis underlying its original recommendations in 1956—16 years earlier—rather than obstinately refusing to do so (Calabrese, 2016b).

Although the dose-rate effect issue was placed on hold in a 1964 ICRP report, as noted in Part 1 (Calabrese, 2016a), it would gain standing in the regulatory communities over the next decade. For example, in the Reactor Safety Study (Nuclear Regulatory Commission (NRC), 1975) a dose rate effectiveness factor (DREF) was introduced with a maximum value of five to adjust for carcinogenic effects of low LET radiation. It was thought that at very low doses and low dose-rates the carcinogenic risk would be 5-fold less than reported at high dose-rates for the same total dose based on the LNT. A few years later, UNSCEAR (1977) recommended the use of a DREF of 2.5, with the suggestion that it could be larger under certain conditions. The DREF was subsequently subjected to a detailed review by the National Committee for Radiation Protection and Measurement (NCRPM), (1980) and has generated considerable interest internationally with application to the present time (Ruhm et al., 2015).

Thus, even though the presently recounted historical assessments did not result in the inclusion of the dose-rate concept in the risk assessment process (e.g., ICRP, 1964; NAS/NRC BEIR I, 1972), that concept did become adopted throughout the field of ionizing radiation (see Table 1 – Ruhm et al. (2015), for a detailed listing by organization) and was a broadly debated consideration for application to environmental mutagens as an LNT modifying factor (Committee 17, 1975). The findings of Russell would have impacted views on the LNT much more if the regression to control values at low dose rates that was demonstrated in oocytes had also occurred in spermatogonia. In fact, the findings that spermatogonia showed much less of a reduction than oocytes was a significant factor that influenced the continued use of the LNT recommendation by BEIR I, as discussed below.

4. Correcting the record

In 1995 and at the age of 85, William L. Russell found his work of many decades on the mouse SLT being challenged by Paul B. Selby, a radiation geneticist at Oak Ridge National Laboratory, who was Russell's only Ph.D. student and who was part of the mouse genetics program at Oak Ridge National Laboratory from 1966 to 1972 and 1975–1995. In late 1994 Selby uncovered a serious long-standing problem of the Russells not reporting spontaneous mutations arising as clusters of spontaneous mutations arising during the perigametic interval. Selby referred to the mice that produced these unreported clusters as first-cleavage gonadal mosaics (FCGMs) (Selby, 1998a). He realized that this problem, which dated back to Russell's first experiment reported in 1951 (which made no mention of the cluster) affected the mutation rate estimate of the historical control group of the Oak Ridge National Laboratory mouse SLT (Russell, 1951). Selby reported this long-standing problem to the Department of Energy, and an investigation resulted. Although the dispute between the Russells and Selby was significant and contentious, in the end the Russells (Russell and Russell, 1996) and Selby (1998a, 1998b) addressed the technical issues associated with the Selby claims. Both sides agreed that an adjustment upward was needed to correct the long-standing error. An increase of 1.7-fold for males and 2.2-fold for males and females combined was recommended by the Russells, while Selby presented extensive arguments based on available data from three laboratories and his extensive computer simulations that suggested an increase of 4.8-fold for males and 7.3-fold for males and females combined.

The spermatogonia mutation rate most closely preceding the BEIR I Committee report of November 1972 as based upon the cumulative historical control mutation rate of the Russells' research, was 7.5×10^{-6} mutations/locus (i.e., 28 mutations found in 531,500 offspring, with possible mutations at 7 loci) (Russell, 1965). When these Russell (1965) data are combined with those reported using the same animal model by Lyon et al. (1972a, 1972b) at the Harwell facility in the UK (i.e., 11 per 157,421 offspring) the mutation rate based on 688,921 offspring of control male mice becomes 8.09×10^{-6} mutations/locus. When these spontaneous mutation rate estimates are corrected using the 1.7 factor of the Russells the control mutation rate becomes 12.8×10^{-6} mutation/locus (all numbers expressed x 10^{-6} to facilitate comparisons) for Oak Ridge, while the combined Oak Ridge and Harwell historical control rate becomes 13.7×10^{-6} mutations/locus. These control values do not approach being statistically distinguishable from the mutation rates reported for the two lowest doses tested at the dose rate of 0.001 R/minute, namely 37.5 R (Lyon et al., 1972a) with a mutation rate of 11.6×10^{-6} and 86 R with a mutation rate of 14.3×10^{-6} (Russell, 1965). If Selby's recommendation of a 4.8-fold upward adjustment is applied, the control mutation rates for comparison become 36.1×10^{-6} mutations/locus for the Oak Ridge control and 38.8×10^{-6} mutations/locus for the combined Oak Ridge and Harwell findings. While the point estimates of these adjusted control frequencies are well above those of the experimental groups noted, there is too much uncertainty about the data on which the adjustments are based to be certain that the experimental frequencies are significantly lower. It should be noted that the Harwell data of Lyon et al. (1972a) were submitted to *Mutation Research* for publication in December 1971, and published in the June 1972, five months prior to the publication of

[3] The BEIR I (NAS/NRC, 1972) Genetics Subcommittee stated "... in view of its more conservative implications, the linear, nonthreshold hypothesis warrants use in determining public policy on radiation protection; however, explicit explanation and procedures involved in such risk estimates are called for to prevent their acceptance as scientific dogma." (p. 97)

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the BEIR I report.

While Selby and others would focus on understanding how this correction affected the doubling dose of human genetic risk (i.e., assuming a linear dose response) from radiation exposure, the mutation-rate adjustment for historical controls has significantly broader and fundamental implications for risk assessment. The Russells' correction appears to essentially convert a linear dose response to a threshold dose response, while the Selby correction reveals the possibility of a J-shaped dose response, suggestive of hormesis. In either case, the LNT estimate/interpretation would not have been supported if what was known about such clusters by the Russells in 1972 had been reported. (See Table 1 in either of the Selby, 1998a, 1998b papers for a listing of when the unreported clusters used in his analysis were actually found).

Although it may be possible to propose the use of model uncertainty when adjudicating the key issue of the Russell-Selby debate—which is the effect that those clusters of spontaneous mutations arising during the perigametic interval may have on any upward adjustment of the spontaneous mutation frequency of the mouse SLT—the weight of evidence over the past two decades has supported the Selby interpretation (Selby, 1998a, 1998b; Thompson et al., 1998; Woodruff et al., 1996; Woodruff and Thompson, 2005; Woodruff et al., 2015; Fu and Huai, 2003; Selby et al. unpublished manuscript; Sankaranarayanan and Chakraborty, 2000; Selby et al., 2004; NAS/NRC BEIR VII, 2006). As recently summarized by Woodruff et al. (2015) "Given the common nature of premeiotic clusters, all members of clusters must therefore be counted when determining mutation rates. This has been demonstrated theoretically by Fu and Huai (2003), who concluded mutation rate estimates should consider the number and size of clusters. That is, multiple offspring with the same new mutant allele from one parent cannot be counted as a single mutant as suggested by Russell and Russell (1996)...". This current synthesis by Woodruff and colleagues reinforces the original critique of Selby (1998a, 1998b) that the approach of the Russells of not reporting (until 1996) and, of course, accordingly not counting such clusters in the control mutation frequency significantly underestimated the control mutation frequency and thus led to a "considerable overestimate of risk" (Selby, 1998b). As pointed out by Selby and others, when the Russells' data were applied in genetic risk estimation by committees, this error yielded a profoundly lower doubling dose for radiation hereditary risk assessment, and, as stressed in the present paper, it also resulted in the adoption of the LNT dose response model in place of either a threshold model or hormesis.

The application of these dose-response adjustments, as employed for the first time in this paper, seriously challenge the decision made about 44 years ago by the BEIR I Genetics Subcommittee—a decision that incorrectly led to the adoption of the LNT model. On page 65 BEIR I (NAS/NRC, 1972) stated: **"The finding of a dose-rate effect for mutation induction in mouse spermatogonia and oocytes raised anew the question of whether there might be a threshold dose or dose rate below which all mutational damage would be repaired. Exploration of a range of dose rates provides no evidence of a threshold dose rate for mutation induction in mouse spermatogonia..... Therefore, we shall make the prudent assumptions that there is no threshold dose rate in the male and that the dose response at low dose rates is linear."**

After retrospectively applying the corrections of the Russells and Selby to the BEIR I report (1972), the data from both the oocytes and spermatogonia of mice in the massive studies on genetic risk provide no basis for rejecting either a threshold or a hormetic dose-response relationship. Further, it is reasonable to suggest that had these adjustments been available to the NAS/NRC BEIR Committee in 1972, the Committee could not have rationally concluded that "the dose response at low dose rates is linear"; it would have had to conclude instead that the data were consistent with a threshold and/or hormetic model.

The BEIR I Committee's error, caused by its not having full knowledge of the data it was using, led to serious errors by other groups that relied upon its evaluation. For example, the 1975 U.S. EPA radiation risk assessment policy was derived directly from the BEIR I (NAS/NRC, 1972) report. In a 1977 extension (US EPA, 1977) of the 1975 policy statement, the U.S. EPA explicitly addressed the issue of dose-rate: "EPA uses primarily the recommendations of the National Academy of Sciences Committee on the Biological Effects of Ionizing Radiation (BEIR) as expressed in the November 1972 report to arrive at dose to health conversion factors. Besides the concept of linearity expressed in the policy statement (i.e., EPA, 1975- EPA Policy Statement on Relationship between radiation dose and effect. 41 Federal Register, 28409), it is further assumed that health effects that have been observed at dose rates much greater than those represented in this report are indicative of radiation effects at lower dose rates. Any difference in biological recovery from precarcinogenic radiation damage due to low dose rates is neglected in the BEIR health estimates." Three years later, in 1980, the BEIR III Committee (NAS/NRC BEIR III, 1980) (whose membership included both of the Russells and Selby) used the Russells' same data to estimate mutation risks using the doubling dose that the 1972 BEIR I Committee had employed, following the same flawed course of action. It is unfortunate that the Russells' error has profoundly affected the theoretical and practical foundations on which cancer risk assessment is based and used by regulatory agencies. By its adoption of LNT for risk assessment based on error in the reporting of control group data, the BEIR I Committee established beliefs and affected the adoption of risk assessment practices—all based upon a flawed foundation.

It may seem strange that a mistake on such a critical question as estimation of mutation frequency was not detected and corrected early on. However, the mouse SLT was an example of what is called Big Biology, in which few groups possess the capacity to process massive numbers of animals (Bernd, 2013). In the U.S. the only group that could do this with mice was Russell's group at Oak Ridge National Laboratory using the mouse house facility (Russell, 2013). Thus, the Russells had few knowledgeable critics because very few in the national and international genetics research community were conducting similar studies. Russell's former Ph.D. student, Selby, who had spent a career at Oak Ridge and had unique insights and access to the Russell historical data, is a case in point in that he was closely associated with this program for decades before he realized that there was a serious problem with the historical control group.

Russell's purported error in estimating the mutation rate of his control animals was not widely publicized and was, more or less, retained within the Oak Ridge organizational structure as guarded information. With one minor exception, there was neither "leakage" to the press nor coverage/debate/discussion at professional meetings or their relevant journals prior to, during or following a detailed investigation involving an external panel of four geneticists. A resolution proposed by the external panel encouraged the principals to publish their findings/explanations in scientific articles (i.e., Russell and Russell, 1996, and Selby, 1998a, 1998b). This carefully controlled and detailed process further handicapped the scientific and regulatory agencies from understanding both the occurrence and the significance of the alleged error. For example, the Selby articles have been cited relatively few times despite their potential for academic scholarship and scientific significance. The process also greatly suppressed the possibility of DOE, Oak Ridge National Laboratory, and the involved parties from receiving substantial negative public attention. The result of such a process was that a story with profound risk assessment implications became of interest principally to only a limited number of geneticists who focused on spontaneous mutation and genetic mosaics and who may have feared that they themselves would become involved in a dispute they would have preferred to avoid. This secretive process delayed, until the present day, the uncovering of the BEIR I mistake of

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adopting LNT, a delay that affected risk assessment principles and practices for the past four decades.

5. Conclusions

Several factors have affected the application of, and the manner in which, the concept of the dose rate effect for ionizing radiation could have changed cancer risk assessment during the second half of the 20th century. These include: the strong opposition from an aging Muller; a BEIR I Genetics Subcommittee led by Muller's protégé James Crow that defaulted to the Precautionary Principle when the science supporting LNT became challenged; and an error in reporting mutations in the mouse SLT that resulted in the adoption of the LNT. The following six specific findings summarize the historic impact of the Russell mouse SLT model on assessing the risk of ionizing radiation and of chemical carcinogens:

- The discovery that a significant dose-rate effect on germ cell mutations for males and females in the mouse SLT challenged key assumptions of the LNT model including: 1) that all damage was cumulative and irreversible, and 2) that the dose response was linear at low dose. Russell also linked his dose-rate effects to the discovery/occurrence of DNA repair that was a concept contemporaneous with the mouse SLT findings.
- The Russells' research at Oak Ridge National Laboratory on the effects of ionizing radiation was an unprecedented massive effort involving well over two million mice over more than four decades.
- Russell determined that the underlying tenets of LNT as recommended by the BEAR I Genetics Panel did not apply to mouse spermatogonia and oocytes and that these germ cells had far more genetic relevance to somatic cells than mature spermatozoa (used for BEAR I dose response predictions for reproductive and somatic cells), which lacked DNA repair capabilities.
- While the dose-rate effects in oocytes suggested a threshold response, those in spermatogonia were less extreme, permitting retention of the LNT dose response model by BEIR I and enabling the subsequent creation of the dose-response effectiveness factor (DREF) to adjust for risk overestimation by the LNT dose response.
- An early error by Russell in reporting and estimating the mutation rate of the mouse control group (involving clusters of spontaneous mutations arising during the perigametic interval) affected all SLT-based risk assessments from the 1950s and was unknown until Selby reported it in the mid-1990s.
- If the error in control group mutation rate had been corrected prior to NAS/NRC BEIR I (1972), the course of cancer risk assessment might have been significantly affected, with consideration given to the threshold or hormetic dose-response models.

Since this paper has sought to provide historical perspective to factors that contributed to the maintaining and sustaining of the LNT concept in risk assessment practice, it seems appropriate that this paper should conclude with a "seasoned" perspective by Professor James Crow (Crow, 1987), an original member of BEAR I and Chair of BEIR I Genetics Subcommittee: "It is clear that Muller's alarmist views of the hazards of radiation have prevailed... In my opinion, Muller was too effective in cautioning against radiation risks, with the result that the public now has an irrational fear of low-level radiation relative to other risks. The fear, I suppose, has resulted more from the assumption of no threshold for carcinogenic effects than from the dread of genetic effects. In any event, the battle that Muller waged was certainly won: the present standards for radiation safety are more stringent than even he dared advocate." (p. 379).

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Philosophy, Ethics, and Humanities in Medicine

REVIEW Open Access

Muller's nobel prize research and peer review

Edward J Calabrese

Abstract

Background: This paper assesses possible reasons why Hermann J. Muller avoided peer-review of data that became the basis of his Nobel Prize award for producing gene mutations in male *Drosophila* by X-rays.

Methods: Extensive correspondence between Muller and close associates and other materials were obtained from preserved papers to compliment extensive publications by and about Muller in the open literature. These were evaluated for potential historical insights that clarify why he avoided peer-review of his Nobel Prize findings.

Results: This paper clarifies the basis of Muller's (Muller HJ, Sci 66 84-87, 1927c) belief that he produced X-ray induced "gene" mutations in Drosophila. It then shows his belief was contemporaneously challenged by his longtime friend/confidant and Drosophila geneticist, Edgar Altenburg. Altenburg insisted that Muller may have simply poked large holes in chromosomes with massive doses of X-rays, and needed to provide proof of gene "point" mutations. Given the daunting and uncertain task to experimentally address this criticism, especially within the context of trying to become first to produce gene mutations, it is proposed that Muller purposely avoided peer-review while rushing to publish his paper in *Science* to claim discovery primacy without showing any data. The present paper also explores ethical issues surrounding these actions, including those of the editor of *Science*, James McKeen Catell and Altenburg, and their subsequent impact on the scientific and regulatory communities.

Conclusion: This historical analysis suggests that Muller deliberately avoided peer-review on his most significant findings because he was extremely troubled by the insightful and serious criticism of Altenburg, which suggested he had not produced gene mutations as he claimed. Nonetheless, Muller manipulated this situation (i.e., publishing a discussion within *Science* with no data, publishing a poorly written non-peer reviewed conference proceedings with no methods and materials, and no references) due to both the widespread euphoria over his claim of gene mutation and confidence that Altenburg would not publically challenge him. This situation permitted Muller to achieve his goal to be the first to produce gene mutations while buying him time to later try to experimentally address Altenburg's criticisms, and a possible way to avoid discovery of his questionable actions.

Keywords: Gene mutation, X-ray, Nobel Prize, Hermann J. Muller, History of science, Peer review, Drosophila

Background

Hermann J. Muller [20] claimed that high doses of X-rays induced gene mutations in Drosophila in his paper entitled the "Artificial Transmutation of the Gene", introducing the concept of point mutation (page 87, left column). In a recent paper, I argued that Muller deliberately avoided peer review of his Nobel Prize data to win the race to become the first to report such uniquely important findings [12]. However, further examination of the underlying reasons for Muller's avoidance of the peer review process suggests it may not have been simply to win the race to publication. This paper offers a more nuanced and novel hypothesis that Muller feared his "landmark" paper may fail the peer review process since the critical gene mutation interpretation was not supported by data. This concern resulted in Muller devising a camouflaged process to avoid peer review while still retaining his worldwide acclaim thereby securing "primacy" for a discovery that would later yield a Nobel Prize.

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The new interpretation

Despite the rapid and massive acclaim Muller received following his presentation revealing X-ray induced gene mutation at the Fifth International Genetics Congress in September, 1927 [13], his striking gene mutation interpretation was not immune to criticism. In fact, Muller was acutely sensitive to insightful, yet private criticism, by his closest friend Edgar Altenburg [22] that he had "only" produced hundreds of transgenerational phenotypic changes with no proof of his gene mutation interpretation. While the production of massive numbers of such genetic changes in such a short period of time was overwhelming to many, for Altenburg it remained a potentially important observation in search of an explanation. He told Muller that he needed to show that the high dose mutation-inducing X-rays were not merely blasting holes in large sections of chromosomes, producing the heritable phenotypic changes via a chromosomal deletion rather than a gene mutation mediated process. For Altenburg this was the crucial question and Muller knew that he was correct.

For Muller this criticism was devastating as he had come so close to the goal of gene mutation discovery, only to be "denied" the satisfaction by his best friend, who offered a more objective perspective. While Muller needed to hear this criticism, he also was blinded by ambition to be first on perhaps the most critical question of the day in biology, that is, discovery of the mechanism of evolution.

In the years leading up to the key research of Muller on X-ray induced mutations he had made it clear that finding a means to induce mutation at the level of gene would be a profound advance as it may provide a mechanism that could drive evolution [8, 14]. Muller was dismissive of multiple claims of induced mutation during the early 1920s that were mediated at the level of chromosome rather than gene. Muller was firm that unless the induced changes occurred at the level of gene their significance would be very limited, not a qualitative advance. Altenburg was forcing Muller to be as self-critical as he was critical of numerous previously failed attempts by others to address the same issue.

Muller's [19] ground breaking report on July 22, 1927 in the journal *Science* that he had induced hundreds of gene mutations by X-rays was based upon the production of allomorphic (i.e. visual) transgenerational phenotypic changes and lethality. He made this gene mutation claim/interpretation in an article that discussed his findings, but failed to include any data. The scientific community would have to wait until the second week of September that year at the International Genetics Congress for Muller to present the actual findings [20].

A review of his Nobel Prize data notebook (Muller, Lilly Library, University of Indiana-Bloomington) reveals that findings were derived from three experiments with the second experiment being wrapped up with respect to data collection in the laboratory at the end of May, 1927. The dates of the third experiment were not recorded in the lab notebook although Muller [22] indicated that he ran out of time to include a control group, probably in order to prepare for and travel to the Genetics Congress. The data discussed in *Science* matches most closely to that reported in experiment # 1 that was completed in the laboratory in December, 1926.

While it is unknown when Altenburg precisely offered his criticism to Muller, the actions of Muller to introduce a profound advance on one of the most significant questions confronting biology in the journal *Science* without any proof was very brash under any circumstances. Permitting this to occur was also similarly flamboyant for the editor, James McKeen Cattell. A careful evaluation of the entire preserved correspondence record of Cattell, as an individual and *Science* editor did not reveal the arrangement between him and Muller. Likewise, this is the case following a careful review of the Muller correspondence files.

That Muller had the personality to manipulate the scientific community and the general public can be seen in this Nobel Prize Lecture (December 12, 1946) where he deliberately deceived the audience arguing that there was no possibility of a threshold dose response for radiation induced mutations when he had just observed data supporting such a conclusion from Caspari and Stern from the University of Rochester, one month prior to the Nobel Prize Lecture [4, 5]. Muller offered this deception to promote his long held ideology in support of a linear non-threshold dose response for mutation and cancer risks. Muller [24, 25] would also deliberately offer inaccurate and deceptive comments in the scientific literature to promote linear non threshold (LNT), expressly contradicting private correspondence between himself and Stern [6]. Such high stakes risk taking and bluffing behavior of Muller surfaced two decades prior to his Nobel Prize Lecture, when he published a description of these findings without data, knowing that he lacked proof for his key gene mutation conclusion. Muller had the chance to submit this most significant research for peer review but choose not to do so. As pointed out, the conference proceedings paper with the data lacked a methods section, provided no references cited and even failed to acknowledge the prior recent paper (January, 1927) by Gager and Blakeslee [15] which provided what appears to be the first evidence of radiation induced gene mutation. Until this point in his career, Muller published his papers within journals with a well-established peer review record as he was guided into this scientific acculturation by his Columbia University mentor, Thomas Hunt Morgan. Yet, for the paper of greatest significance he bizarrely published a discussion

of preliminary findings with no data and then avoided peer review with a poorly developed paper in the Congress proceedings [12].

It is likely that Muller was both devastated by the criticism of Altenburg and could not find any way to address it, at least not in the short term. Yet, he knew he was in a race to be first, even if Gager and Bakeslee may have some claim to the honor he could handle them with a series of Muller-like blistering challenges. It appears that Muller rolled the proverbial dice, ignored, at least for the time being, the criticisms of Altenburg, and went on to claim his "unequivocal" belief in having produced "gene" mutations, all the while knowing that he had not proven it. This was similar to his deceptive performance at the Nobel Prize Lecture, showing the same risk taking behavioral trait. Muller had confidence that his duplicity would not be revealed by those that knew of his scamming behaviors, such as Stern and Caspari and perhaps a few others at the University of Rochester for the Nobel Prize Lecture, and Altenburg for his Nobel Prize research.

Muller would find a means to address the criticism of Altenburg but it would take several years. He adopted the concept of reverse mutation and thought that it could provide indirect, but functional proof that his treatments would yield genes that would still be working and therefore not destroyed by the high doses of radiation via massive deletions. However, it is curious to note that Muller mentioned that he had not observed any reverse mutations in the *Science* paper. Thus, in his own experimental model and methods of mutation induction he had not observed the reverse mutation phenomenon. However, in the fall of 1927 he claimed to have observed two examples [18, 23] and then in early 1928 his colleague at Texas James Patterson found evidence of a third example [22]. These very limited observations offered Muller a glimmer of hope to perhaps address the criticism of Altenburg, but only time and experimental evidence would tell. Thus, even a year after the publication in *Science* Muller had no proof that he had induced gene mutation and was now going to try to support this interpretation via a collaboration with Patterson. At the December, 1927 [18] American Association for the Advancement of Science (AAAS) Conference in Nashville Muller provided the a glimpse into the emerging importance of the reverse mutation concept as he stated that the newly observed two reverse mutations should provide more information on gene structure than "gene" loses and be more significant in the study of evolution, the central theme of biology. In fact, the argument to support the claim of gene mutation would morph into that of reverse mutation [28].

This discussion provides context and a plausible explanation for why Muller deliberately avoided peer review. A case can be made that he accepted the validity of Altenburg's concerns. This would have suggested to him that there was the possibility/probability of this paper being rejected. Muller was caught in an ethical dilemma: he could address the criticisms of Altenburg or finesse his manuscripts and presentations to avoid possible devastating critical reviews while accepting the uncritical acclaim of the scientific and world communities who were overwhelmed and transformed with his capacity to produce transgenerational phenotypic changes; this same community then accepted his gene mutation interpretation based largely on an appeal to authority.

In a September 1928 publication (based on an April 15, 1928 presentation at the National Academy of Sciences) in the Proceedings of the National Academy of Sciences (PNAS) Muller acknowledged the criticisms of Altenburg [22]. He also indicated that Patterson had found an example of a reverse mutation. Muller then stated that he was now going to follow up on the suggestion of his wife, a mathematician, to address the Altenburg criticism via the study of reverse mutation. At this point Muller was seemingly aware of only three cases of what might be X-ray induced reverse mutation in the PNAS paper. It is also curious that Muller never acknowledged the two examples of reverse mutation that he referenced in the AAAS presentation nine months earlier.

Nearly three years after the Altenburg criticism Patterson and Muller [28] would provide support for the gene mutation interpretation using the reverse gene mutation. On page 574 they concluded that "the above (reverse mutations) demonstrates that mutations can be produced in both of two opposite directions at the same locus." They further claimed that these findings were "irreconcilable with the view that all mutational changes by X-rays consist of losses."

In retrospect the original "proof" of gene mutation using alleomorphic phenotypic changes-as both an observation and explanation-was not convincing to Muller following the Altenburg criticism. Thus, overtime Muller came to use the reverse mutation observations as the means to explain the induced transgenerational phenotypic changes. In some ways, this was a type of scientific bait and switch process that was nearly unrecognizable, yet critically important over the issue of gene mutation discovery. Nonetheless, the scientific community was mesmerized by the findings and publicity of Muller to such an extent that it quickly and uncritically came to believe that Muller had produced gene mutations based on an interpretation lacking scientific justification.

While Muller may have thought that the reverse mutation findings may have salvaged the gene mutation interpretation, even though three years too late, it too would be challenged very seriously by another thoughtful equal peer of Muller, the corn geneticist Lewis Stadler at the University of Missouri. In fact, Stadler [31] had been

only a few months behind Muller in the reporting of X-ray induced mutations. While Stadler at first accepted the gene mutation interpretation of Muller this would change some four years later.

In 1929 Barbara McClintock [16] developed a vastly improved cytogenetic method for assessing the characteristics and appearance of chromosomes of corn. At that time this technique was far more advanced than had been developed for Drosophila [8]. In 1931 [17], McClintock provided a cytogenetic evaluation of X-ray induced mutations in corn under the direction of Stadler. He had observed transgenerational phenotypic changes similar to those produced by Muller with Drosophila. However, McClintock's advanced cytogenetic methods revealed that the so called "gene" mutations that Stadler believed he had produced were most likely gross chromosomal deletions. Thus, McClintock concluded that the doses of X-rays used by Stadler were simply punching holes in the chromosomes and that he had not induced gene mutations as he had thought. After some deliberation, Stadler [32, 33] came to accept the new insights of McClintock and soon came to question whether this was also the case with Muller's gene mutations as first suggested by Altenburg. While Muller claimed that the X-ray induced reverse mutation explanation addressed the question, this position was not convincing to Stadler [34] and others (see [8] for a review) who offered a series of experimentally based alternative explanations (e.g. position effect, transpositional elemental effects) for Muller's reverse mutation phenotype findings without the need for gene mutation).

Overlooked in this debate was the dissertation of Clarence P. Oliver, Muller's student at the University of Texas. In his dissertation (i.e., Conclusion #10) [26] and in his journal publication (i.e., Conclusion #11) [27], Oliver stated "It is not possible to produce gene mutations without producing gene rearrangement, or vice versa". This statement undercuts Muller's conclusion that he produced "point mutations" at doses far higher than those used by Oliver [26]. Given this contradictory statement, it is curious that Muller approved this dissertation since this statement was very Stadler-like.

While most in the scientific community seemed to believe that Muller had induced gene mutation, Muller and Stadler would contest this point until Stadler died in 1954 with neither side conclusively winning this debate although the preponderance of the evidence favored Stadler [34]. In the end, Stadler adopted the perspective of Altenburg that Muller mistook an observation (i.e. transgenerational phenotypic changes) for an explanation (i.e., gene mutation). However, once nucleotide analysis methods were developed, he was shown to have achieved his transgenerational phenotypic changes via the "punching of holes" in chromosomes via modest to extraordinarily large deletions rather than via point mutations [8–11].

Discussion

The actions of Muller and their effects on other key figures are worth exploring for their historical and ethical considerations. In the case of Muller, we find the tactic of publishing on a transformative topic such as gene mutation without providing data as self-serving and not abiding by the generally accepted culture of scientific responsibility of successfully passing a responsible peer review. This failure was also coupled with his acceptance of worldwide acclaim and hoped for professional job offers (see letter to Carl Hartman, October 28, 1927 [21]). Thus, Muller was masterful in manipulating the scientific community without his manipulation being exposed.

In the case of Cattell, he was a close friend of TH Morgan, both being long time professors at Columbia University [1]. It is likely that Muller used their past Columbia association to approach Cattell. At that time Cattell personally owned the journal *Science* and may have sought to profit from the likely intense positive publicity. Thus, in some ways Muller and Cattell exploited each other for personal profit at the expense of society. Approximately two decades later Cattell would transfer ownership of *Science* to the AAAS [30].

In the case of Altenburg, he appears as a loyal friend and would remain so until both he and Muller died in the same year (1967), a few months apart. Their relationship was unique as seen when Muller attempted suicide in 1932. His suicide note was directed to Altenburg rather than his family [14]. Thus, for Altenburg loyalty to a close friend may have overwhelmed his responsibility to science and the broader community. It is both interesting and ironic that Altenburg [2] would eventually publicly side with the Stadler-McClinctock perspective that the high doses of radiation were mediating Muller's mutations most likely via McClintock's inducible transpositional element (i.e. the basis of her own Nobel Prize in 1981). Furthermore, Ratner et al. [29] confirmed this perspective later in studies closely replicating Muller's experiments.

Conclusion

The Muller story is a troubling one of excessive and unbridled ambition, self-serving manipulation, high risk behavior and an enabling authority (i.e. *Science* editor Cattell) and a very loyal/protective friend (i.e. Altenburg) along with a scientific community that failed to demand accountability and a Nobel Prize committee that inadequately evaluated Muller's findings. The implications of such actions are profound since it has been Muller's incorrect gene mutation interpretation and its legacy that created the LNT dose response model, leading to its recommendation by the US National Academy of Sciences in 1956 [3] and then subsequently adopted by all regulatory programs throughout the world. Thus, Muller's

many significant deceptions facilitated a long, continuing and influential reach with a profound impact on society which continues to the present without any action, acknowledgment or change by the scientific community and government regulatory agencies.

The recent detailed historical assessment [6–8] and the last two vignettes (this paper and [12]), document significant new scientific and ethical insights into the career of Hermann Muller and this era. The set of papers reveal Muller to be an inspiring and principled leader while, at other times, he appears as a person with distorted self-serving interests, highly ideological and partisan, and with little evidence of a moral compass, all the while being very self-righteous, within the context of a very intelligent, unrelenting, aggressive and at times disagreeable manner. This combination can be a dangerous personal and societal cocktail, making him capable of achieving much and often at the expense of many. Despite the documented limitations and flaws of Muller, the most significant criticisms and concern should be directed to the scientific and regulatory communities, such as the US Environmental Protection Agency, that have uncritically adopted and sustained Muller's findings as the foundation for cancer risk assessment and permitted this process to be corrupted by dominating ideological perspectives, failing in their congressionally mandated societal leadership.

Abbreviations
AAAS: American Association for the Advancement of Science; LNT: Linear non Threshold; PNAS: Proceedings of the National Academy of Sciences

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EJC conceived, researched, and wrote this contribution by himself. The author read and approved the final manuscript.

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See the attached new paper on additive to background and LNT....it is an important new effort.

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Review article

The additive to background assumption in cancer risk assessment: A reappraisal

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ARTICLE INFO

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ABSTRACT

The assumption that chemical and radiation induced cancers act in a manner that is additive to background was proposed in the mid-1970s. It was adopted by the U.S. Environmental Protection Agency (EPA) in 1986 and then subsequently by other regulatory agencies worldwide for cancer risk assessment. It ensured that cancer risks at low doses act in a linear fashion. The additive to background process assumes that the mechanism(s) resulting in induced (i.e., treatment related) and spontaneous (i.e., control group) cancers are identical. This assumption could not be properly evaluated due to inadequate mechanistic data when it was proposed in the 1970s. Using the findings of modern molecular toxicology, including oncogene activation/mutation, gene regulation, and molecular pathway analyses, the additive to background assumption was evaluated in the present paper. Based on published studies with 45 carcinogens over 13 diverse mammalian models and for a broad range of tumor types compelling evidence indicates that carcinogen-induced tumors are mediated in general via mechanisms that are not identical to those affecting the occurrence of the same type of spontaneous tumors in appropriate control groups. These findings, which challenge a fundamental assumption of the additive to background concept, have significant implications for cancer risk assessment policy, regulatory agency practices, as well as fundamental concepts of cancer biology.

1. Introduction

This paper assesses a critical, but overlooked area of cancer risk assessment (i.e., cancer dose-response assessment), the additive to background assumption, that essentially ensures low dose linearity in the estimates of carcinogen exposure risks. This assumption was proposed for application to cancer dose-response assessment by Crump et al. (1976). A decade later it was incorporated into governmental risk assessment policy and practices during 1986 (Anderson et al., 1983; Crump, 1984; U.S. EPA, 1986) and has continued to the present (U.S. EPA, 2005; EFSA, 2017). This assumption was proposed during the mid 1970s when it was not possible to assess its scientific validity with the oncogene revolution starting in the mid-1980s and the continued clarification of molecular mechanisms for spontaneous and induced tumors to the present. It is now possible to evaluate the scientific validity of the additive to background assumption. The present paper demonstrates that the additive to background assumption that spontaneous and induced tumors occur via identical mechanisms is not compatible with the vast body of modern molecular findings. Prior to assessing the additive to background hypothesis, a brief historical reconstruction of how linearity at low dose was adopted for cancer dose-response assessment by U.S. regulatory agencies during the 1970s is presented, providing the necessary scientific and regulatory contexts and introduction needed to assess the additive to background assumption.

2. Historical foundations of cancer risk assessment

2.1. The Thanksgiving Cranberry Scare of 1959

Within five years following the National Academy of Sciences (NAS) Biological Effects of Atomic Radiation (BEAR) I Genetics Panel report (NAS/NRC, 1956) recommending the use of linear dose response modeling in risk assessment, Nathan Mantel and Raymond Bryan (1961) would publish their landmark paper on cancer risk assessment. The modestly entitled paper, *Safety Testing of Carcinogenic Agents,* was based on the use of the tolerance distribution probit dose response model. The probit model was originally derived to assess non-carcinogenic responses (Zeise et al., 1987). However, Mantel and Bryan (1961) generalized its use, applying it to modeling responses of carcinogens. Their efforts followed by nearly two decades the earlier work of Bryan and Shimkin (1943) who applied the probit model to estimate cancer risks for several carcinogenic hydrocarbons based on chronic studies with male C3H mice, a study that suggested an hormetic dose response that was not addressed by the investigators.

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What stimulated the reemergence of interest in quantitative estimates of cancer risks was U.S. presidential politics. Mantel was employed as a biostatistician for the U.S. National Cancer Institute (NCI) during the time of the 1960 presidential election, pitting John F. Kennedy against Richard M. Nixon. During the run up to the election, there was the so-called Thanksgiving Cranberry Scare of 1959. The event proved to be both a major chemical scare for the American public and a chance for the two presidential candidates to demonstrate that they were not afraid of a small dose of the cancer causing (i.e. thyroid cancer) herbicide in their cranberry sauce or juice (i.e. Nixon had four servings of cranberry sauce while Kennedy had two drinks of cranberry juice-of course on the same day.) (http://coldwarstudies.com/2017/11/15/the-cranberry-scare-of-1959). The agent, 3-amino-1,2,4-triazole, which had been approved in 1957 for use on Cranberry bogs only after harvest, had been found in several sources of cranberries in the weeks leading up the Thanksgiving holiday (note that the farmers did not follow the instructions properly; they were only supposed to apply the herbicide after harvesting but applied it before). It became a political story when the Secretary of Health, Education & Welfare (HEW), Arthur Sherman Flemming went public on November 9, 1959 with the recommendation to the public not to buy cranberry products that year. His actions resulted in what might be called a consumer panic, which then threatened the livelihood of the cranberry industry. In an effort to prevent a similar public backlash in the future, Secretary Flemming asked the NCI for guidance on which cancer causing agents could be considered "safe" and what may be a safe or acceptable dose. To the rescue would come Mantel and the laboratory animal model cancer researcher Bryan, who were asked by the Director of the NCI to provide the needed guidance, including issues such as how to design appropriate animal bioassays and how to estimate risks and establish a means to distinguish between safe and unsafe. Little did the Secretary of HEW and the NCI Director realize that they had just opened a scientific version of Pandora's Box, with issues that still confront politicians, scientists and the general public.

In their publication, Mantel and Bryan (1961) would emphasize the generality of their dose response model approach for other agents and tumor endpoints. They introduced the concepts of no threshold and acceptable risk within a public health policy framework. In a manner to illustrate its practical utility they expressed the outcome of their model estimate in public health terms suggesting an acceptable risk with a value sufficiently low that few would have concerns over, that is, one cancer per 100 million people per lifetime. While this effort in 1961 by Mantel and Bryan was thought to have put a lid on concerns with chemical carcinogens, it was only the beginning, as Rachael Carson would publish her *Silent Spring* book a year later (Carson, 1962). The Carson publication, which was partially inspired by the efforts of radiation geneticist Hermann J. Muller, would galvanize the fledging environmental movement, lead to the creation of the National Environmental Protection Act (NEPA) (1969) and the EPA (1970) and help spark efforts to address the issue of cancer dose-response assessment about a decade later.

2.2. U.S. EPA, Cancer risk assessment, and low dose linearity

It would take about 12 years but the U.S. FDA would eventually restart its cancer risk assessment agenda by formally proposing the Mantel-Bryan (1961) model while still retaining the 1/100 million acceptable risk level in their July 19, 1973 (U.S. FDA, 1973) cancer risk assessment announcement in the *Federal Register*. As the regulatory stakes had changed since the Cranberry scare of 1959, this proposal was taken seriously, and became stalled in the U.S. regulatory apparatus. It finally emerged following what could only be seen as a rather elephantine-like gestational period in 1977 (U.S. FDA, 1977), having survived a presidential election and new political leadership. The Mantel-Bryan probit model approach had been largely retained, although with a number of alterations, including the adoption of a new acceptable risk value of one in a million.[1]

The practical significance of such actions was that it became the risk estimate below which no further governmental regulatory actions would be initiated. This recommendation was placed within the framework of a public health safety response to carcinogen residues in food products. Even though it had taken a long time to get an approved cancer risk assessment process through the regulatory system, the FDA-approved Mantel-Bryan model became the first cancer dose-response assessment model officially adopted by a U.S. federal regulatory agency. The next change would not take so long. About two years later, the U.S. FDA (1979) would alter its approach by dropping the tolerance distribution Mantel-Bryan model approach, replacing it with a linear dose response model. The rationale for such a decision was due to the more conservative risk estimates of the linear model along with its conceptual simplicity and ease of risk calculation (Anonymous, 1979). In the low dose zone, the one hit model as initially proposed by Timofeef-Ressovsky et al. (1935) yields very similar risk estimates as a simplified linear model. Getting a federal agency to change its cancer dose-response assessment model only two years after a long incubation period should raise the proverbial "why"? In fact, the FDA's actions were the direct offshoot of the recommendations of a multi-governmental agency panel with FDA technical representation (biostatistician David Gaylor) that published their linear dose response recommendation (Hoel et al., 1975). It was simply a matter of being more conservative, simplifying the process and timing.

While the U.S. FDA was pursuing its cancer risk assessment methods and issues, so to was the U.S. EPA. The posturing and approaches that emerged from this fledgling environmental regulatory agency seemed somewhat confusing to the outside reader and the regulated community. Much of the initial conceptualizing on the issue of regulation of cancer causing agents emerged from the Rachael Carson-inspired need to address the issue of risks from pesticides. Thus, during major pesticide hearings EPA staff attorneys presented an intellectual blueprint of what amounted to a set of Agency "cancer principles". The new "Principles" reflected the Agency view that carcinogen exposures should not be permitted.....that is, prevented from occurring in the first place. While the goal of this Principle was to ban carcinogenic agents from the market place, it was quickly seen as simply unrealistic, though it could remain a goal (Albert, 1994; Calabrese, 2009, 2013).

What emerged from this process was EPA adopting a set of non-regulatory guidelines that could be applied to a generic cancer risk assessment process (U.S. EPA, 1976). This system would have considerable practical importance, as it would employ quantitative risk assessment on chemicals and engineering-based processes. This conceptual framework would be the functional lead-in for a critical paper by the EPA's Carcinogen Assessment Group (CAG) (Albert et al., 1977), which reaffirmed the LNT concept and justified it based on epidemiological studies for smoking and ionizing radiation and the dose response pattern of induced genetic mutations based on the Ames assay with bacterial strains lacking DNA repair. This paper by

[1] During this regulatory "incubation" period within the FDA, Mantel et al. (1975) would update the original (Mantel and Bryan, 1961) application of the probit model with an "improved Mantel-Bryan procedure". The original Mantel and Bryan (1961) procedure incorporated Abbott's (1925) correction to adjust for spontaneous tumor background. This new procedure would account for background/spontaneous tumors via the introduction of a new estimated parameter "C", the expected (spontaneous) incidence in untreated animals, with the subsequent application of Abbott's correction (Abbott, 1925; Zeise et al., 1987). It is likely that the adoption of the independent of background approach using Abbott's formula by Mantel et al. (1975) lead to EPA accepting this approach several years later when it was incorporated into the single-hit model (Costle, 1979) and later into the multi-stage model (Anderson, 1983). Mantel et al. (1975) noted the possibility of an alternative to the independent of background model, by proposing a scheme similar to the additive to background concept. In this scheme, the spontaneous tumor rate "represents the response to the load of the test agents and its equivalent ALREADY in the environment. The total load for an individual or animal is then the sum of its administered dose and its environmental load. This was similar to that proposed earlier by Albert and Altshuler (1973) and later by Crump et al. (1976), except that Crump et al. (1976) tied the background and induced tumors via an identical mutation mechanism as discussed later in the text.

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the CAG (Albert et al., 1977), which offered no references to support LNT, provided the introduction for the key policy statement of the U.S. EPA Administrator, Russell E. Train (1977) to adopt the linear dose response for cancer risk assessment.

Two years later, on March 15, 1979, the new EPA administrator, Douglas Costle, reported in the *Federal Register* (U.S. EPA, 1979) that risk assessment for animal data is performed using the 'one-hit model' as given in the 1976 Interim EPA Guidance document (U.S. EPA, 1976). Costle would justify this decision on the basis that the one-hit model was endorsed by the four federal agencies of the Interagency Regulatory Liasion Group due to its very protective/conservative features, uncertainties in animal to human extrapolation, concerns over whether humans may be more susceptible than the animal model, the occurrence of human variability (i.e., interindividual variation) and "other unknown factors". However, use of the single-hit model was criticized in public comments of the proposed Water Quality Criteria for suspected carcinogens (U.S. EPA, 1979).[2] As a result, EPA changed from the single hit to the multi-stage model by November 1980 (Anderson, 1983).

In an historical assessment of this cancer risk assessment period, Albert (1994), chair of the CAG during the 1970s and early 1980s, indicated that the EPA accepted the LNT model that was being applied by the U.S. Atomic Energy Commission (AEC) to assess cancer risks from ionizing radiation data based on the Manhattan Project findings of Stern at the University of Rochester (Spencer and Stern, 1948; Uphoff and Stern, 1949; see Calabrese, 2015 for a review). Albert (1994) reaffirmed that the LNT model was a good fit for EPA since it had exquisite simplicity and therefore was readily understandable. This model also was attractive to the Agency since it had biological plausibility based on target theory as derived from a unique collaboration of leading physicists and radiation geneticists (Timofeef-Ressovsky et al., 1935). Of particular importance to the broad mission of EPA was that "any difference between chemical carcinogens and ionizing radiation could be waived aside as they both cause genetic damage…" The generalizing of the LNT for cancer risk assessment to include the domain of chemical carcinogens was a game changing decision that would significantly affect numerous societal and scientific issues.

This brief historical summary recapitulates how quantitative risk assessment evolved within the regulatory agencies from about 1960–1980. However, the initial quantitative modeling originated in the early 1950s and displayed divergent fundamental biological assumptions depending on the model. It is within the decade of the 1950s where the underlying biologically-driven quantitative models for both background/spontaneous and exogenously induced cancer incidence would provide the theoretical, mathematical and biological frameworks for the cadre of available approaches employed in regulatory agency based quantitative risk assessments that emerged in the mid to late 1970s. Despite the growing sophistication of quantitative modeling in the 1950s, these efforts received their intellectual and biological foundations from the seminal paper of Timofeef-Ressovsky et al. (1935) and follow-up papers (e.g. Zimmer, 1941) on the development of dose response modeling which was based on the capacity of X-rays to induce gene mutations in mature spermatozoa of *Drosophila* as originally reported by Muller (1927).

2.3. Cancer: Quantitative Risk Assessment and Biological Plausibility

The issue of quantitative risk assessment for carcinogens became a major consideration for regulatory agencies such as EPA in the mid-1970s. Such assessments were dominated with debates by biostatisticians concerning which biostatistical model to employ for risk assessment, with a significant priority being biological plausibility. The first efforts toward biological mechanism modeling were those of Iverson and Arley (1950) which were the direct offshoot of Timofeef-Ressovsky et al. (1935) and their subsequent papers. Their single stage model assumed that a key biological change or transformation occurs in a single target, typically the result of somatic gene mutation (i.e., somatic mutation theory – SMT). The model was built upon the use of a proportionality constant that represented the rate of cell transformation per unit of administered carcinogen, as well as the assumed carcinogen potency and the sensitivity/susceptibility of the target cells. The transformed cell increased cell number via an assumed monoclonal expansion that may become detected after a critical number of cell divisions.

While their model had serious biological limitations, it stimulated other efforts including those of Fisher and Halloman (1951) which transformed observations of age-dependent stomach cancer death incidence into a new biostatistical model for cancer risk assessment. The cancer incidence was estimated to occur with the 5th or 6th power of age. Fisher and Halloman (1951) assumed that this power law relationship could be mechanistically explained if there were 6 or 7 different cells that were altered/transformed/mutated (i.e., initiated) in a single tissue. These transformed cells would then become biologically integrated, yielding a developing tumor mass. This became known as the multi-cell theory (i.e., polyclonal model). This hypothesis developed a following in the biomedical community, being supported over several decades by prominent scientists as seen in the writings of Wright and Peto (1969), Jones and Grendon (1975) and others.

During the early 1950s, Muller (1951) and Nordling (1953) hypothesized that a single cell can transform into a tumor only if it experiences a number of mutational events (i.e., SMT application). From this theoretical basis would emerge the multistage model, with its quantitative foundations being developed soon thereafter by Stocks (1953) and Armitage and Doll (1954, 1957).

3. The additive to background assumption

3.1. Ensuring linearity at low dose

The origin of the additive to background cancer risk concept was first suggested by Platt (1955) in a letter-to-the-editor of the *Lancet*. He proposed that the principal effect of a carcinogenic agent is likely to alter the functioning of a cell in such a manner that its clonal expansive descendants proliferate at a greater rate than other nearby cells. He then suggested that if aging were seen as an extended process of numerous cell divisions then cancerous processes would be a function of such aging activities.

Based on this logic it was predicted that cancer would occur in the cellular descendants of the initially altered cells more quickly than in the neighboring "normal" tissue but only following a prolonged latent period. This initial suggestion of Platt (1955) was adopted,[3] formalized, and extended by Armitage and Doll (1957) into what became the additive to background hypothesis and the basis of the additive to

[2] Despite the apparent interagency convergence upon the LNT single-hit model, use of this model was criticized since it did not incorporate data from high doses. It derived the linear assessment via the use of a straight-line extrapolation employing the lowest statistically significant response. As a result of such dissatisfaction, the CAG modified its positon, switching from a single-hit to a multistage model because it employed data from each dosage, while yielding low dose linearity and having some vague measure of biological plausibility as cancer is a multistage disease process (Albert, 1994).

[3] The additive to background "concept" is an assumption that was first proposed by Robert Platt (1900–1978), a British physician who specialized in the area of hypertension and kidney function. He was president of the Royal College of Physicians (1957–1962), chairing the committee that published the first major assessment of the Society of Physicians on Smoking and Health, which assembled evidence for a causative relationship with lung cancer (Platt et al., 1962). This publication pre-dated the first report on smoking and lung cancer from the U.S. Surgeon General, which appeared in 1964. The statement of Platt (1955) on the concept of additive to background was a letter to the editor with no reference support. It was a brief commentary written in the form of reasonable but unsupported hypothesis. Perhaps because of his standing in the British medical community and/or the prestige of the journal Lancet, this unsubstantiated concept was adopted by two leading British researchers, Armitage and Doll, in their 1957 presentation of the multistage model of carcinogenesis. Prior to this time, background/spontaneous disease occurrences and/or susceptibilities were generally dealt with under an assumption of independence from background, using Abbott's (1925) correction, a widely adopted methodology in the biological and biomedical sciences, with over 8000 cumulative citations in the Web of Science through 2017.

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background multistage dose response model.[4] Armitage and Doll (1957) related the multistage model back to the incidence of cancer in the human population, which was linear when plotting the log of the disease incidence against the log of age. This developmental origin of the additive to background concept would become a cornerstone of low dose linearity as seen in cancer dose-response assessment models.

In the EPA era perhaps the first application of the additive to background concept was published by Albert and Altshuler (1973) in a presentation of the 12th Hanaford Biology Symposium on radionuclide carcinogenesis.[5] Of particular practical significance is that the lead author, Roy Albert, a professor at New York University, would become the director of the newly created unit within EPA called the Carcinogen Assessment Group (CAG) in 1976. Their paper modelled the effects of (1) radium-induced osteosarcoma in CF_1 female mice to a single iv dose of Radium 226 at age 70 days, (2) a three times weekly skin painting of dibenzanthracene (DBA) to mice across four treatments (1.0–9.0 μg) and (3) a smoking and lung mortality study of nearly 300,000 U.S. veterans. The methodology assumed the spontaneous and carcinogen-induced cancers were additive, induced by similar/identical mechanisms with the spontaneous cancers given an equivalent carcinogen dose rate. For example, using model based disease estimates, non-smokers were estimated to receive the equivalent of one cigarette/day while the control subjects in the osteosarcoma mouse study received the equivalent of 0.03 uc/kg radium 226. The effective dose rate is therefore the sum of the applied dose rate and the spontaneous equivalent dose rate. The procedure yields a more conservative risk estimate (i.e., higher tumor incidence) than an independent of background assumption, providing a higher cancer risk at a lower carcinogen dose rate. The Albert and Altshuler (1973) approach, which is based on Druckrey's dose-time response model, assumed that the additional incidence (i.e., treatment effect) had a time to tumor occurrence similar to the spontaneous tumor incidence. However, when the induced tumors were assumed to be independent of background, the induced tumors at low dose rates were predicted to occur after the survival cut off for the experiment, yielding lower overall tumor estimates. The linkage of time to tumor as being the same for spontaneous tumors and induced tumors in the additive to background framework but not in an independent of background approach is a unique but unexplored hypothesis. In the independent of background approach, the tumors appearance would be inversely related to dose and not related to the time of occurrence of spontaneous tumors. In the framework of Albert and Altschuler (1973) this could have considerable impact on carcinogen risk estimates. While the dose-time-response models of Blum et al. (1942), Blum (1959) and Jones and Grendon (1975) would have continuing appeal to Albert, they were not followed by Crump et al. (1976) and received criticism by Hoel, Schneiderman and others during the OSHA (1980) carcinogen hearings.

The additive to background concept would be assessed and refined by others in several influential publications during the mid-late 1970s, including by Crump et al. (1976), soon reinforced by Guess et al. (1977) and then integrated into the range of stochastic, mechanistic, and tolerance distribution models (see Szymczak and Szadkowska-Stanczyk, 2005 for a review). During the mid-1970s, mechanistic understandings of environmental induced tumor/cancer development were very limited as Crump et al. cited only two papers by Knudson (1973, 1974) to support their adoption of the additive to background assumption. A careful reading of these two papers provides no relevant direct support for the additive to background assumption, suggesting that the reviewers may never have assessed the Knudson papers cited by Crump et al. (1976). In a 1977 article in Science Cornfield (1977) noted that the "additivity [to background] assumption is a major one that lacks experimental support". This lack of experimental support for the additive to background assumption was noted by Munro and Krewski (1981) as well as the Food Safety Council (1980) which indicated this assumption "is far from compelling".[6] Despite these concerns and unresolved limitations this assumption became widely supported [e.g., Peto (1978), Zeise et al. (1987), Gaylor (1992, 1997), Crawford and Wilson (1996), Heitzman and Wilson (1997), Hoel (1980, 1997), Pollycove (1997), Crump (1997, 2017), Lovell (2000), Kodell (2001), Kopp-Schneider and Lutz (2001), Beninson (1988), and Wilson (1978, 2000, 2012a)].

The dominance of the independent of background/Abbott's correction in cancer risk assessment would end by the mid 1980s as seen in the EPA cancer risk assessment guidelines (U.S. EPA, 1986). The reasons for this change to an additive to background assumption were related to several factors. Hoel (1980) reported a profound difference in estimated cancer risk using the probit model when the two background assumptions (i.e., independent and additive) were compared. The additive to background assumption was far more conservative. Secondly, Hoel (1980) showed that even when the independent model assumption accounted for 99% of the background influence it was still completely dominated by the additive to background assumption. Thus, all one had to assume was that an additive to background model could account for just 1% of the cancer process to negate the influence of the independent of background assumptions. These two factors would soon lead to the EPA 1986 change (Table 1). These factors are due to the differential manner in which the background factors are mathematically accommodated without biological or mechanistic support.

Krewski et al. (1995) stated that the U.S. EPA (1986) cancer risk assessment guidelines, in effect, restated a major conclusion of the Crump et al. (1976) and Hoel (1980) papers. That is, according to Crump et al. (1976) "if the carcinogenesis by an external agent acts additively with an already ongoing process then under almost any model the response will be linear at low doses." Complementing this perspective Hoel (1980) noted that; "If even only a small portion of the background is additive then one is in the linear at low dose situation." The EPA (1986) report stated that; "If a carcinogenic agents acts by accelerating the same carcinogen process that leads to the background occurrence of concern, the added effect of the carcinogen at low dose is expected to be virtually linear." However, the EPA (1986) document only cited the Crump et al. (1976) paper in justifying the adoption of additive to background.

Wilson (2000) indicated that the multistage model of Armitage and Doll (1957), Crump et al. (1976), Guess et al. (1977) and others, which include the additive to background assumption, depends on the belief that cancers caused by the pollutant and background (i.e., spontaneous) are indistinguishable, and therefore, pollutant and the background act in a similar way. By "indistinguishable" Wilson (2012a) would later state that the spontaneous and induced cancers, "must be biologically indistinguishable and not merely what a pathologist cannot distinguish." Consistent with the view of Wilson (2012a) was the statement of Lovell (2000) that the additive to background assumption "only holds if the mechanisms are exactly the same, not just similar or variants of one

[4] The original model of Armitage and Doll went through a few iterations including incorporating the assumption that the rates of occurrence of the different changes were proportional to dose, thereby permitting the cumulative tumor incidence to be estimated via a simplifying equation. The facilitating procedure was then extended to incorporate a background incidence in the absence of dose (do). These modifications provided a vehicle to assess spontaneous tumor incidence at each stage. A different form of the multi-stage model by Crump et al. (1976) assumed that all carcinogenesis occurred via a common mutagen induced mechanism. The exogenous carcinogen treatment simply enhanced this ongoing process. As noted in the main body of the text, the Crump et al. model assumes that any dosage related treatment effect acts via identical mechanisms as that causing background disease incidence (Lovell and Thomas, 1996).

[5] The BEIR I Committee (NAS/NRC, 1972) qualitatively addressed the issue of background radiation (100 mrem/yr). They recommended keeping the additional radiation dose to the population from human sources (activities) below the normal background quantity. They claimed that this would assure that the additional consequences will neither differ in kind from those which people have experienced throughout human history nor exceed them in quantity. Thus, the natural background was employed as a type of comparison (NAS SDWC, 1977-page 879 for discussion).

[6] Recognition of these limitations likely contributed to the failure of the additive to background assumption being accepted by regulatory agencies during the late 1970s and early 1980s. Furthermore, the long history of Abbott's correction for background led to its initial acceptance by the FDA and EPA.

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Table 1
Supportive perspectives of additive to background assumption.

Crawford and Wilson (1996), page 305	"In 1976, Crump, Hoel, Langley, and Peto described how almost any dose-response relationship for carcinogens becomes linear at low doses when background cancers are taken into account. This has been used, by the U.S. Environmental Protection Agency, USEPA, as partial justification for a regulatory posture that assumes low-dose linearity.....The argument depends critically on the assumption that the pollutant and the background proceed by the same biological mechanism."
Crawford and Wilson (1996), pages 305 and 306	"Crump et al. (1976) and Guess, Crump, and Peto (1977) reminded us that when a small amount of pollutant is added to a large amount of the same pollutant, or to another pollutant operating in the same way as the first, a response linear with the incremental dose of pollutant can result. This was a simple and elegant idea that followed mathematically from the fact that the first derivative of a smooth curve is finite. This idea was applied to cancer risks and was used as a partial justification for the US. Environmental Protection Agency's (USEPA) use of low-dose linearity in regulating carcinogens."
Crawford and Wilson (1996), page 324	"A positive, favorable, effect (hormesis) has been suggested at low doses. A biological mechanism (repair) has been suggested to explain why the number is below the line. If there is such a favorable effect at low doses, there is then a nonmonotonic dose-response, and the argument becomes very complex. It would still be important to know whether radiogenic cancers are assumed to result from the same mechanism as naturally occurring cancers. If they do so result, there could be situations where hormesis could be biologically correct in the absence of background, but not true in real situation where the background had already brought the total dose to a region of positive slope of the dose-response curve."
Crump et al. (1976), page 2973, right column	"If the addition of the test carcinogen merely increases the rates of processes that were occurring anyway, then dose-response relationships will be linear at low dose levels.....we are chiefly discussing direct carcinogenic processes in which the compound or its metabolite acts at the cellular level to produce an irreversible and heritable (genetic or epigenetic) change."
Crump et al. (1976), page 2977, right column	"Cancers thought to be induced are generally indistinguishable from "spontaneous cancers". This obviously does not demonstrate that the cancers arise by a common mechanism, but it is consistent with a common pathway to "induced" and "spontaneous" carcinogenesis."
Crump et al. (1976), page 2977, right column	"The view of carcinogenesis as a fundamentally mutational phenomenon, as recently reviewed by Knudson (1973, 1974) supports the assumption that induced and spontaneous steps are mechanistically identical." This statement reflects very limited understanding of the process of carcinogenesis. While this perspective may have been widespread during the mid-1970s it has been superseded and replaced with modern molecular advances. The assumption of Crump et al. (1976) lacked adequate evidence and can no longer be used to support the additive to background assumption employed in low dose linearity.
Crump et al. (1976), page 2977, right column	"Small extra doses of a carcinogen will therefore elicit linear increases in risk for virtually any response model."
Crump et al. (1976), page 2978, left column	"Virtually all models of carcinogenesis that depict the exposure as affecting an already ongoing process will lead to linearity at low dose."
Crump et al. (1976), page 2978, left column	"....this assumption of dependence or common mechanism is not trivial. It can make orders of magnitude difference in the estimated risk associated with low dose exposure."
Crump (1997), BELLE Newsletter	"Based on the additivity to background argument, in order to justify a biologically–based model of underlying mechanisms that predicts a non-linear low-dose response, it is necessary to rule out completely the possibility that non-pollutant-related responses could ever occur via a common mechanism. This would appear to be a difficult task in many, perhaps most, situations. The argument also suggests that the shape of the dose response curve at low doseis inherently determined by the relationship between background responses and responses induced by the pollutant. This idea seems to have been largely ignored by researchers in designing and interpreting experiments with the goal of understanding low dose risk. Perhaps a fruitful line of research would be to develop data that could be used to better understand (and perhaps refute) the additivity to background argument for low-dose additivity."
Heitzmann and Wilson (1997), page 2	Crump et al. (1976) and Guess, Crump, and Peto (1977) pointed out that if a pollutant produces cancer via the same mechanism by which background cancers occur, then there results a linear incremental response to the incremental dose. Even if the biological response mechanism has a threshold, or is non linear, the existence of the background cancers shows that the threshold is already exceeded by a background pollutant. Then when a small amount of the same pollutant, or to another pollutant operation in the same way as the first, is added, an incremental response linear with the incremental dose of pollutant can result, almost independently of the particular biological mechanism relating dose and response. This simple and elegant argument follows mathematically from the fact that the first derivative of a smooth curve is always finite."
Heitzman and Wilson (1997), page 7	"....it is important not only measure the magnitude of the biological dose-response, but also to have some understanding of the underlying biological mechanism(s) in particular, whether the mechanisms are the same for pollutant- and background-induced effects. It is also clear that if the background and the pollutant effect operate by the same mechanism, the slope of the dose-response function at low doses can be much lower than the slope at high does if he background itself is small."
OSHA (1980), Carcinogen Hearings, page 5185, right column - Federal Register 45(15):5185 and 5186 (Peto/Rall)	"Mr. Richard Peto (Oxford Univ.) explained that the most models predict a linear dose-response relationship at low dose, because of the probable existence of "equivalent background", i.e., exposure to other background carcinogens to which the effects of an added carcinogenic agent will be linearly additive (Peto, S.1: Annex B; Peto 1978). He explained during his oral testimony: Now, if we admit the possibility of such a background, then it turns out that all the arguments as to whether we should have linear models, K-hit model, one-hit models, probit model, log normal models or whatever, becomes irrevelant to the mathematical estimation of the upper confidence limit on the risk associated with a particular low dose. If any of these models is formulated in the mathematical form that the risk is equal to some function—probit, log normal, whatever- of dose plus background dose, then the consequences will be the same. Where background dose now becomes a parameter to be estimated statistically and we always finish up with linear upper confidence limits; you know, that the risk just turns out to be proportional to the dose at low doses." (Peto, Tr. 2514)."
OSHA (1980), Carcinogen Hearings, page 5185, right column - Federal Register 45(15):5185 and 5186 (continued)	"Essentially the same point was made in detail in the original paper by Peto, Hoel and their colleagues in which the multi-stage model was developed (see comment by Hoel quoted above):

(*continued on next page*)

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Table 1 (*continued*)

	Virtually all models of carcinogenesis that depict the exposure as affecting an already ongoing process will lead to linearity at low dose. We have discussed the validity of this assumption above. This result then implies that, no matter what the biological mechanism we might imagine, if the carcinogen increases some part of the already ongoing process, then we should expect the response to be approximately linear at low dose. As pointed out above, this assumption of dependence or common mechanisms is not trivial. It can make orders of magnitude differences in the estimated risk associated with low dose exposure."
OSHA (1980), Carcinogen Hearings, page 5185, right column; page 5186, left column	"…if we conceive of the cell alteration process as a series of discrete single-cellular random events that can occur in sequence or in any given cell and that a dose-independent induction period follows, then we should expect dose-response over background to be linear. We have required neither that all steps be affected by the carcinogen (only some) nor that these steps be all mechanistically similar in quantity or quality. This general class incorporates most of the reasonable models that have been proposed. The keys to this result are the assumptions of the single-cell origin and the lack of any appreciable dose dependence in the induction period."
OSHA (1980), Carcinogen Hearings, page 5185, left column	"A further extension of the group of models allows the incorporation of threshold models into the class of "linear at low dose." We have indicated that, if we conceive of single cells as the biological unit at risk and that the initiation response is a threshold phenomenon, then by assuming that the threshold is randomly distributed in dose we find that the low dose response of the whole tissue over background will be approximately linear. If, rather implausibly, we do suppose that some sort of cellular thresholds exist, then clearly all cells do not have the same threshold since all cells do not all become cancers simultaneously. Here again, we have assumed that the carcinogen acts in conjunction with the "spontaneous" or background effects."
OSHA (1980), Carcinogen Hearings, page 5136, left column	Mr. Richard Peto (Oxford Univ.) developed this point at some length in his direct statement (S. 5-10 and Annex B) and presented a mathematical proof that under rather general conditions no threshold would be expected. The basic requirement for this proof is the existence of "carcinogenic background alternatives", i.e., the existence of other agents in the environment of the exposed animal which give rise to the same functional effect: The second assumption needed is that some carcinogenic background alternatives exist to the carcinogen being considered, i.e., *some* of the spontaneous cancers that arise do so by mechanisms which are functionally similar to those whereby the test carcinogen works. Again, I believe that for *most* carcinogens *some* carcinogenic background alternatives are likely to exist, although again I would expect a few counter-examples to occur. (The counter-examples would probably involve cases where the carcinogen produces some gross pathological effects, such as suppression of ovulation, which then affects cancer risk, rather than cases where it acts directly by causing a local DNA lesions.)"(Peto, S. 6). In introducing this section of his testimony, Mr. Peto explained that most arguments for the hypothesis of thresholds or extremely low risks associated with low doses are due to failure to recognize the possibility that "equivalent background" may exist. Accordingly, he referred to the concept of thresholds as "nonsense". (Peto, Tr. 2515)."
OSHA (1980), Carcinogen Hearings, page 5136, left column	"Dr. David Rall (Director, NIEHS) made essentially the same point, both in his opening statement (Rall, Tr. 354) and in response to a questions about protective mechanisms: …I think however, it becomes almost ridiculous when you recognize that 16% of the people in this room are going to die of cancer. I mean, this talk of a threshold is, to me, sort of an ethereal dream world. You know, 16% are going to die of cancer and about twice that will get cancer in their lifetime. I am sorry, but I think the threshold was exceeded a long time ago, and I just do not see any point in talking about it anymore, in terms of the population."(Rall, Tr. 397-398).
Wilson (2000), page 297, left column	"Crump et al. (1976) and Guess et al. (1977) pointed out that the argument for low-dose linearity is far more general than the Doll-Armitage theory and depends solely on the fact that cancers caused by the pollutant and background are indistinguishable, and therefore it is likely that the pollutant and the background act in a similar way." "These analyses were used by the U.S. Environmental Protection Agency (EPA) 25 years ago as a justification for assuming low-dose linearity as a general default."
Wilson (2012b), page 481-482	"…….the usual (conservative) model suggests low dose linearity. This comes from the realization that if a medical outcome of a pollutant or action is indistinguishable from one that occurs naturally, any addition to natural incidence is proportional to the dose at low doses (Crump et al., 1976; Guess et al., 1977; Crawford and Wilson, 1996). Indeed, this is also a consequence of the usual application of the multistage theory of cancer as described over 50 years ago (Armitage and Doll, 1954)." "….there are assumptions and approximations. In the justification I used the word "indistinguishable and not merely that a pathologist cannot distinguish."
Wilson (2012), page 2014, right column	"Low-dose linearity follows from a general argument enunciated by Guess et al (1977) and Crump et al (1976), which follows from Taylor's theorem (Hazewinkel, 2001). If a medical outcome caused by a pollutant is indistinguishable from an outcome that can occur naturally any small increase in dose is likely to lead to a corresponding small increase in effect (Crawford et al., 1996)."
Zeise et al. (1987), page 263, left column	"For very rare cancers, the effective background dose dB may be practically zero and the shape of the dose-response curve indistinguishable from that expected under the assumption of dose independence. On the other hand, if the cancer in question is common, with dB relatively large, the response at low doses of the external agent would be linear."
Zeise et al. (1987), page 263, left column	Commenting on a Krewski and Van Ryzin paper, they stated that "when the observed background doses were estimated and extrapolations down to an excess risk of 10^{-5} were performed and when the observed background tumor incidence was greater than about 1%, the predicted response at low dose was essentially linear in dose. However, "for background tumor incidence less than 1%, low dose linearity did not result when dose-response formula allowed for curvature, and the high dose data exhibited nonlinearity."
Zeise et al. (1987), page 262, right column	"An additive assumption: The spontaneous cancers are produced by the same mechanisms as the cancers produced by the xenobiotic…….this always gives a response linear in the external dose at sufficiently low doses if the first derivative of F is finite……The idea that the carcinogen may add to an effective background dose has been often used to support the claim that a linear dose-response formula is never overly conservative."

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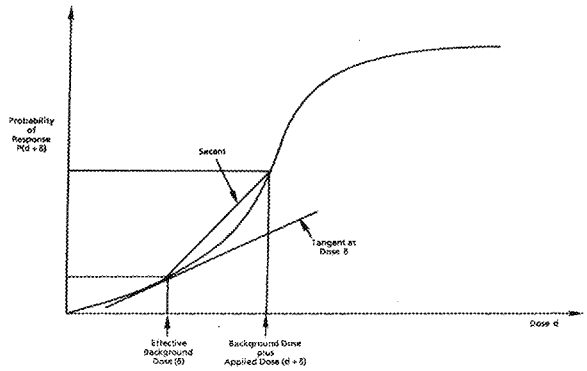

Fig. 1. The additive to background model (source: U.S. EPA, 1989).

another". As will be subsequently shown, this hypothesis became experimentally testable only well over a decade after Crump's et al. (1976) original formulation.

3.2. The additive to background rationale

Using the argument of Crump et al. (1976), Wilson and colleagues (Crawford and Wilson, 1996; Wilson, 1997) thought it reasonable to assume that most biological responses would be non-linear, but monotonically increasing. In a situation of zero background disease incidence, the dose-response relationship at low doses would reflect an obvious non-linearity as an infinitesimal dose would yield very slight (i.e., negligible) increase in response. Thus, this scheme assumes the existence of an "equivalent background dose" (do), that would create the background response. The general requirement for the additive to background based model demands that the toxicologically based dose response curve be smooth and monotonical while the background agent and toxic agents (i.e., pollutants) must act via the same mechanism(s) (Zeise et al., 1987; Krewski et al., 1995; Wilson, 1996, 1997). As noted by Krewski et al. (1995) and Rhomberg et al. (2011) the enhanced/extra risk over background is linear at low dose with the incremental/positive response over background being approximated by the tangent to the curve at zero dose (Fig. 1). As noted by EPA (1989) the spontaneous tumor rate increases as a result of an effective "background dose" the effects of which act additively to background via a dose related manner. The excess risk over background, which is linear at low doses, occurs due to the fact that the secant between doses of δ and (δ + d) converges to the tangent of the dose response curve when the dose d of the agent under evaluation becomes small. This situation creates a theoretical framework in which the unexposed population is, as noted by Rhomberg et al. (2011), already to some extent up the response curve at zero exposure to the agent under study/evaluation (Fig. 1). This would be the case even if the dose response mechanism for carcinogens acted via a threshold, linearizing the dose response for such agents (Zeise et al., 1987; Wilson (1997); Fisher (1984)). The general view was that humans display a relatively high cancer background incidence in the 20–25% range over a lifetime. New exposures to chemical/radiation-carcinogens were assumed to directly add to this background burden, resulting in an increased incidence of cancer and/or its accelerated appearance (i.e., shorter latency period) (Platt, 1955). This view was reinforced by Gaylor (1997) who noted that the most compelling argument supporting low dose linearity is the "situation where a chemical augments a background tumorigenic process… The presence of background tumors indicates that existing endogenous and/or exogenous factors already surpassed a threshold dose, if one exists".

When a chemical is designated as a human or probable human carcinogen and of regulatory concern, it may be evaluated further via the application of quantitative risk assessment. Of particular significance in this process is the derivation of the cancer slope factor,

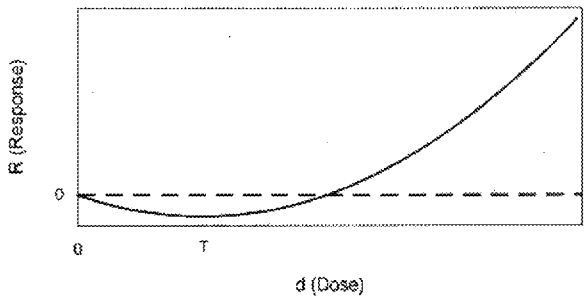

Fig. 2. Hormetic dose response: Additive to background low dose linearity response starts at the nadir (optimal treatment = T) of the hormetic (adapted from Crump, 1997).

which quantitatively defines the relationship between the dose and response. According to the EPA the cancer slope factor represents a plausible upper-bound estimate of the probability that an individual will develop cancer if exposed to a chemical for a lifetime of 70 years. The cancer dose-response assessment procedure determines a point of departure, which is typically the derivation of the lowest effective dose (LED) for a 10% treatment-related response corresponding to the lower 95% limit of the dose. This value, which is associated with the 10% risk adjusted for background (Wiltse and Dellarco, 2000), provides the practical significance of the additive to background with EPA risk assessment practices.

Krewski and van Ryzin (1981) showed that the additive to background assumption for multiple data sets revealed that when the background disease incidence > 1% the estimated risk at low dose was linear. When the background tumor incidence < 1%, low dose linearity did not occur. Thus, if the cancer under study is common, the predicted response at low dose would be linear. Since a large proportion of human cancers (i.e., breast, ovary, thyroid, colorectal, kidney-pelvic, leukemia, liver and bile duct, lung and bronchi, melanoma, non-Hodgkins lymphoma, oral cavity, pharynges, pancreas, prostate, stomach, and urinary bladder) (U.S. NCI 2011–2013 U.S. statistics) exceed the 1% background tumor incidence, the additive to background assumption would remain operational. An extensive follow up evaluation by Gaylor (1992) was supportive of the general perspectives offered by Krewski and van Ryzin (1981). For linearity at low dose to be dropped from the cancer risk assessment lexicon, the additive to background concept would have to be shown to be incorrect[7] or the hormesis model (Calabrese, 2008; Calabrese and Baldwin, 2003) would have to be adopted. In the case of hormesis, if additive to background were accepted, the response would start at the nadir of the J-shape dose-response, where the cancer risk is less than background creating, in effect,

[7] Hoel (1980) provided a biostatistical estimate of the impact of a mixture of independent and additive to background modeling for cancer risk assessments using the probit model. The exercise revealed that the additive to background assumption strongly dominated the risk estimated even when 99% of the sample was assumed to be independent. He indicated that even if additive to background was a minor factor it would still, in effect, drive the risk assessment to linearity at low dose. He stated that for the independent of background condition to have an important impact on the cancer risk it must be 100%, even 99% independent would not be influential. The assessment of Hoel was limited to assume a slope of 1 using the probit model. Whether the findings of Hoel (1980) would be generalizable to other slopes and/or models was not addressed. Yet, it was used by EPA (1986) as justification to continue its acceptance of the additive to background assumption in cancer risk assessment. According to Bogen (January 5, 2018-personal e-mail communication to Edward J. Calabrese) the argument of Hoel is independent of model and slope when the background response is non-zero. Despite such theoretical comments, Harris Fischer (1984) noted that "we will determine whether background acts additively or independently not by contemplation of dose-response relationships, but by whether we find such mechanisms at work in biological experiments. Presumably, this is something that we can eventually get a handle on by looking at the actual microbiological processes occurring within the DNA molecule." This comment by Fischer nearly 35 years ago anticipated the oncogene revolution and the intent of the present paper to evaluate the additive to background hypothesis with modern molecular biological mechanisms.

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Table 2
Critical perspectives on additive to background assumption.

Heitzman and Wilson (1997), page 6	"The usual assumption that background- and pollutant-induced biological effects proceed by the same biological mechanism may not be correct in the case of benzene. There is some evidence that benzene-induced leukemiasare always preceded by pancytopenia, which does not occur naturally in the population to any appreciable extent. If this is the case, background leukemias must be produced by an entirely different mechanism than benzene-induced leukemias, and the Crump et al. (1976) argument does not apply."
Davel G. Hoel (1997), BELLE Newsletter	"As one of the original authors (of the Crump et al., 1976) of the low-dose linearity paper, I am less enthusiastic than Crawford and Wilson are over its usefulness. The original paper was written some twenty years ago without benefit of much of today's biology. What is of concern is whether or not the original simple idea of background additivity is consistent with today's biology and whether the concept, if true, has any value for quantitative risk estimation."
Robert L. Sielken Jr. (1997)	He focused on the additive to background mechanism assumption: "First, the background dose and the "pollutant" are assumed to have exactly the same mechanism of action (that is, that the background dose and the pollutant dose are additive in the dose-response relationship). For example this means not just that the background dose and pollutant both cause liver tumors, but also that they both affect the same cell types, affect the same stage in a multiple stage process, and cause the same type of cellular activity (e.g. both cause the same adduct, both inhibit the same repair processes, or both impact cell proliferation or apoptosis in the same way).
James D. Wilson (1997)	This is a critique of the Heitzman and Wilson 1997: "…their conclusion rests on a mathematical analysis of a special case and a few examples, one an obvious and acknowledged exception. For science, the proof of their proposition is not persuasive. …. They and Crump et al. argue that the dose response is continuous and monotonically increasing. A relation that is discontinuous, a step function, for example, will obviously not produce a straight line when added to a background process. The result will still be a step function…..argues that the rate limiting step consists of induced proliferation in cells in the target organ, a process that is very tightly controlled, physiologically.

a dose response threshold (Fig. 2) (Crump, 1997).

3.3. The additive to background assumption and molecular mechanisms: How does this assumption hold up in the modern era

3.3.1. Early questions, concerns, and affirmations of additive to background

About two decades after the Crump et al. (1976) article on additive to background, Hoel (1997), a co-author of that paper, wrote **"what is of concern is whether or not the original simple idea of background additivity is consistent with today's biology and whether the concept, if true, has any value for quantitative risk estimation"**. According to Hoel (1997), troubling inconsistencies in the original additive to background concept as applied to risk assessment have been reported, such as that different carcinogens produce various cancerous subtypes. For example, cigarette smoking induces an acute myelogenous leukemia subtype M2, while benzene has a high relative risk for subtype M4. Moreover, other acute myelogenous leukemic subtypes are associated with ionizing radiation.

In the case of acute leukemia Taylor et al. (1992) found 15% had activated RAS oncogenes. While exposure to solvents has been associated with increased risk of RAS-activated acute leukemia it has not been associated with non-RAS acute leukemia. These complexities indicate that acute leukemia in humans displays multiple possible pathways and raises the likelihood that an agent may affect only a specific pathway. Based on the above findings, Hoel (1997) suggested that the existence of multiple pathways might affect the additive to background argument.

Hoel (1980) also challenged the additive to background concept for a cell proliferation tumor promotion mechanism, stating that since cells divide spontaneously, this assumption should apply. However, this was a position he found unpersuasive. This later criticism is important as it complements the principal focus of mechanisms inherently genetic/oncogene related. Table 2 provides a range of historical perspectives by leading experts that are supportive and critical of the additive to background assumption.

The additive to background features of the cancer dose-response assessment process were based on an assumption that was not verified nor experimentally explored prior to incorporating it into EPA and other regulatory agency risk assessment principles and practices. The molecular tools simply did not exist at that time to test the underlying hypothesis. It was also an assumption/belief that failed to become the object of an adequate timely follow-up review after its adoption by EPA.[8,9] It simply became the equivalent of a "codified" assumption, a "basic tenet", accepted without an adequate assessment and factual basis.

3.4. Testing the additive to background hypothesis

Contradicting the additive to background assumption, numerous papers have demonstrated that spontaneous tumors often are initiated and progress via different mechanisms (Table 3) than chemically/radiation-induced tumors of the same organ (e.g., hepatocellular carcinomas, lung tumors, forestomach tumors, Harderian gland tumors). This is the case despite similarities at the level of tumor location and histopathological evaluations (Table 4). The principal comparison of

[8] In a March 15, 1979 notice in the Federal Register, Douglas Costle, the EPA Administrator, indicated that the single-hit model "has been modified to account for spontaneous tumor incidence". An Appendix to this statement by Administrator Costle indicated it incorporated the concept of spontaneous control group tumor incidence via an independent of background assumption using Abbott's correction (Abbott, 1925), which was incorporated into the one-hit model. Abbott's correction was subsequently incorporated in the multi-stage model after its adoption by EPA in November 1980 as reported by Anderson (1983).

[9] In 1989 the EPA conducted a workshop on various aspects of carcinogen risk assessment. One aspect included the additive to background assumption. The additive to background assumption session activities were summarized in the workshop proceedings. While the conclusions reaffirmed past assumptions and practices, the workshop identified a series of key questions that guided and/or emerged from the workshop activities. The hypothetical questions posed were:

a. Is the additive-to-background position an assumption or are there data to suggest that it describes the underling biological truth?
b. How does the statistical argument that low-dose linearity is to be expected when mechanism is additive to background fare in view of knowledge of various mechanisms of carcinogenesis?
c. How can we distinguish cases of independent and additive background in practice?
d. What biological data can help in trying to make this distinction? What is known about the low-dose properties of dose-effect curves for elements of proposed mechanisms of carcinogenesis (e.g., mutation, cytotoxicity, receptor binding)?
e. Practically speaking, are we able to measure very small elevations in these processes over background so small that they imply trivial cancer consequences in order to detect a virtual (or practical) threshold?
f. For quantitative purposes, should a putative epigenetic carcinogen be treated as acting independently from or additively to low levels of other such agents in the human environment? Of genotoxic agents in the human environment?
g. In view of the above issues, under what circumstances might it be appropriate to assume that carcinogenesis has or does not have a dose threshold?
h. What criteria must be satisfied to treat a carcinogen as acting independently from background, and how should exposures to these substances be viewed vis-a-vis exposures to substances that may be additive to background?

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Table 3
Mechanism comparison of spontaneous vs induced tumors: Assessment of additive to background assumption.

Citation	Comparison Endpoint	Agent	Animal Model	Tumor	Evaluation/Comment
Antal et al. (2002)	Loss of heterogenecity (LOH)	Gamma irradiation	C57BL/6 female DBA2 male mice	Liver and lung tumors	LOH at D4Mitt77 (liver) and Acrb (lung) were identified in treated mice but not in spontaneous tumors.
Blackshear et al. (2015)	Gene upregulation	Vinylidene chloride (VDC)	F344/N male rats	Peritonael *meso*thelioma	Mesotheliomas from VDC-exposed rats ALONE displayed an over-representation of pro-inflammatory pathways and immune dysfunction including nuclear factor kappa chain-enhancer of activated B cells signaling pathway, IL-8, Il-signaling, interleukin responses, Fc receptor signaling and natural killer and dendritic cells signaling pathway, presentation of DNA damage and repair. (abstract) Mesotheliomas from VDC-exposed rats displayed upregulation of genes with tissue damage (Tlr2, Dpt, Mrcl, Pla2g2a) and damage-associated molecular pattern (DAMP) molecules (S100a*, S100a9, Mc1, and Lyvel). (page 176, right column) Mesotheliomas in all VDC-exposed rats were morphologically similar to spontaneous *meso*theliomas in F344N male rats. In many cases the expression of these genes were "fairly" similar between spontaneous and VDC *meso*thelioma, both displayed an overlap of genes associated with multiple molecular categories. (page 176, left column and page 1606, left column)
Chan et al. (1993)	Oncogene comparison	NDEA; NNK	B6C3F1 mouse	Liver tumor; lung tumor	Liver (NDEA): the mutation spectrum of activated H-ras genes was different than that reported for spontaneous mouse liver tumors. This was based on the proportion of mutations at the three locations of Codon 61 (CAA→AAA, CGA and CTA of the H-ras). There was also a dose dependent difference in the mutation spectra. This suggests that high doses do not predict responses at low doses well. Lung (NNK): As in the case with the liver, there was a significant difference in the specific location of activated K-ras for Codons 12 & 61 between the spontaneous and NNK treated mice. There also was a dose dependency issue for lung tumor oncogene responses as well.
Chinsky et al. (1985)	Location of tumor development	Methylcholanthrene CMA	RF/J mice; AKR mice	Thymic lymphomas	"…T-lymphocytes comprising tumors induced by MCA differ from the T-lymphocytes in spontaneous AKR tumors in the frequency with which they express c-*myc* and c-*myb* transcripts." (page 568, left column). Presented "evidence suggesting that thymic lymphomas induced in RF mice by skin painting with MCA differ significantly from spontaneous thymic lymphomas of AKR mice." (page 568, left column) "Spontaneous thymomas develop in thymic medulla while the MCA induced thymomas occur in the cortex. This happens in both models." (page 568, left column)
Devereux et al. (1991)	Oncogene comparison	NNK; NDMA	C3H mouse	Lung tumor	The activating mutation in all the pulmonary tumors from the NNK or NDMA-treated mice was a GC- > AT transition, located at the second base of codon 12 of the K-ras gene. However, K-ras activation was only reported in 3 of 7 spontaneous lung tumors: one GG to TA, (2nd base of codon 12), one GC to AT transition in the 2nd base of codon 12, and one AT to TA in the 3rd base of codon 61. (page 301, right column)
Donnelly et al. (1996)	Oncogene comparison	Aflatoxin B	AC3F1 (A/J x C3H/HeJ) mice	Lung tumor	All of the lung tumors from AFB1 treatment displayed K-ras codon 12/13 (exon 1) mutations at quanine residue while 65% of K-ras in the spontaneous tumors were located in codon 61 (exon 2) with mutations at the quanine and adenine residues. (page 1738, left column Nearly all lung tumors in AF1-treated mice had K-ras codon 12 mutations with most being at GTT followed by GAT and TGT. The controls showed a markedly different pattern with few mutations to GTT and TGT. (page 1738, left column)
Fox et al. (1990)	Oncogene comparison	Benzidene; Phenobarbital; chloroform; coprofibrate	C57B1/6 x C3H/He	Liver tumor	Liver tumors induced by these carcinogens had much lower frequency of H-ras activated than spontaneous liver tumors. These were also related to codon specificity.
Hayashi et al. (2001)	Oncogene comparison	1-amino-2,4-dibromoanthraquinone (ADBAQ)	B6C3F1	Forestomach tumor	Predominant H-ras codon 61 CAA- > CTA transversions were detected in ADBAQ-induced; these were not considered to be spontaneous because such mutations have not been detected in spontaneous forestomach tumors examined to date in B6C3F1 mice.
Hong et al. (2003)	Oncogene comparison	o-nitrotoluene; riddelliine	B6C3F1	Hemangiosarcomas (HS); skeletal muscle tumors; mesentery tumors	There was a lack of ras, p53 and B-catenin mutations in the spontaneous HS. It provides a foundation to compare with carcinogen-induced HS. A high proportion of K-ras codon 12 G to T transitions occurred in riddelliine-induced liver HS transversions. In controls, ras mutations were detected with o-nitrotoluene treatment but at a lower frequency. However, it showed high frequencies of B-catenin mutations. Both agents showed high incidence of p53 mutations. (page 233, top left column)

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Table 3 (*continued*)

Citation	Comparison Endpoint	Agent	Animal Model	Tumor	Evaluation/Comment
Hong et al. (2007)	Oncogene comparison	Ethylene oxide	B6C3F1	Lung tumors; Harderian gland tumors	The pattern of K-ras oncogene activation was significantly different between the spontaneous tumors of the controls and the tumors of the treated groups for Harderian gland neoplasms and lung tumors. There also appears to be a significant reprogramming within control groups for the lung and the Harderian gland tumors.
Hong et al. (2008)	Oncogene comparison	Comene	B6C3F1	Lung tumor	The comene treated mice displayed a markedly different mutation spectra than the controls across codons 12, 13, and 61. Major changes in the related frequency of codon mutations were seen in codon 12 (GAT and TT or codon 61 (CGA and CAT)
Hong et al. (2015)	Oncogene comparison	Cobalt metal	B6C3F1/N	Lung tumor alveolar/ bronchiolar carcinoma	The incidence of K-ras mutations in lung tumors from mice chemically exposed to cobalt was 67%. The majority of K-ras mutations were localized within codon 12 (43%) (30/69) followed by codon 61 (20%) and codon 13 (6%). The most common codon 12 mutations in lung tumors from all cobalt exposure dose groups was a G to T transversion.
Houle et al. (2006)	Oncogene comparison	Benzene; ethylene oxide	B6C3F1	Mammary gland carcinomas	The mutation spectra of H-ras codon 61 and P53 for codons revealed significant difference between the tumors of the controls and treated mice. Noteable suppression of control group oncogene activation was reported for both the H-ras and P53 codons.
Ozaka et al. (2010)	Chromosome aberrations	Gamma Ray	Sprague-Dawley	Mammary carcinomas	Characterized genomic copy number aberrations for radiation induced rat mammary carcinomas. Reported 14 carcinomas (2 Gy dose) and 26 aberrations, including trisomes of chromosomes 4 and 10 for three and one carcinomas, respectively, and an amplification of the chromosome region 1q12 in two carcinomas, and deletions of the chromosomal region 3q35q36, 5q32 and 7q11 in two, two and four carcinoma. These were not found in spontaneous mammary carcinomas. First report of array CGH (comparative genomic hybridization) analysis for radiation-induced mammary tumors showing distinct aberration patterns, different than spontaneous tumors. (abstract)
Imaoka et al. (2008)	Gene expression	Radiation Gamma-rays	Sprague-Dawley rats	Mammary Carcinomas	Gene expression analysis distinguished between spontaneous and radiogenic carcinomas, suggesting possible differences in their carcinogenic mechanisms. The data indicate that spontaneous and radiogenic mammary cancer developments involve distinct molecular and cellular mechanisms. 50 genes were identified that displayed different expression levels between spontaneous and radiogenic carcinomas. (abstract)
Kawano et al. (1996)	Oncogene comparison	NNK	A/J female mice	Lung tumors	K-ras gene mutations were found in 72.2% of all NNK-induced lung lesions; the principal mutations was a G to A transition at the 2nd base of codon 12. According to the authors the gene mutational change is "clearly different from the mutational pattern in untreated control mice." However, whether and how NNK induced mutations affect the process of carcinogenesis needs further clarification.
Liu et al. (2003)	Gene deletion comparison	ENU; Gamma Rays	HL-60 cells	Leukemia	The spectra of spontaneous mutations were notably different from that induced by ENU and radiation. Total exon deletion did not occur in a spontaneous mutation, but did so in gamma rays and ENU-induced mutations. The proportion of deletion mutations were very different between the treated and spontaneous mutation groups. Likewise, this was also the case for induced mutations at the hprt locus.
Lokhonov (1991)	Oncogene comparison	DMBA	CD-1 mice	Liver tumors	During transplacental carcinogenesis studies DMBA-induced lung tumors were associated with A to T transversions in codon 61, (position two) of H-ras. These mutations were not observed in the liver tumors of controls.
Manam et al. (1992)	Oncogene comparison	DMBA	CD-1 mice	Lung tumors	DMBA induced several types of K-ras mutations not found in spontaneous lung tumors in CD-1 mice. See codons 12, 13, and 61.
Manam et al. (1995)	Oncogene comparison	4-aminoazobenzene; N-OH-AAF; DEN; DMBA	CD-1 mouse	Liver tumors	Spontaneous liver tumors had a low frequency of ras mutations, all of which were in the Ha-ras codon 61, with the majority of mutations (~80%) C - > A mutations – only detectable after transfection. The pattern of ras mutations in AAB-, N-OH-AAF and DEN induced tumors were significantly different than spontaneous tumors. For example, 10 Ki-ras codon 12 (G-C) mutations were found in 37 single dose AAB-induced tumors. The number and distribution of mutation types were significantly different by dose, similar to other studies noted in this review. Multi-dose DMDA-induced liver tumors had A- > T mutations in the Ha-ras codon 61 and G- > C mutations in Ki-ras codon 13. These were similar to single dose responses. The DMBA treatment also had a high proportion of tumors with Ha-ras-codon 12 (G- > A) mutations. The Ha-ras codon 61 A- > T, Ha- and Ki-ras codon 13 G- > C and N-ras codon 12 and 13 G- > T mutations were believed to be induced by the initiating dose since they were not found in the spontaneous tumors.

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Table 3 (*continued*)

Citation	Comparison Endpoint	Agent	Animal Model	Tumor	Evaluation/Comment
Manjanatha et al. (2017)	Oncogene comparison	Ethylene oxide	Big Blue mouse	Lung tumors	The authors reported significant mutation spectra shift at the 200 ppm concentration of ethylene oxide at the 8 and 12 week time period.
Marxfield et al., 2006	Gene profiling	DMBA	Sprague Dawley	Mammary adenocarcinomas	The histologically similar spontaneous and DMBA-induced adenocarcinomas could be separated by gene profiling.
Mass et al. (1996)	Oncogene comparison	Benzo(b)fluoranthene	A/J mice	Lung tumors	Nearly 90% of chemically-induced tumors had a mutation in codon 12, the K-ras oncogene. These mutations had G- > T transversions in the first or second base on codon 12. In controls, the spontaneous tumors had a markedly different pattern GGT- > GAT (61%).
Melnikova et al. (2004)	Genomic alterations	DMBA	Murine, melanoma, cell line B16Fo	Melanoma	Inactivation of the pl9^{Arf} and P16^{INK4a} genes cooperating in the development of spontaneous mouse melanomas, whereas loss of P19^{Arf} or p53 tumor suppressors cooperate with ras and/or mAPK cascade to induce malignant transformation in carcinogen-induced melanomas.
Mittelstaedt et al. (1999)	Oncogene comparison	2-AAF	Hprt; lymphocytes in F334 rats	Lymphocytes studied	Different sequence specificity of Hprt lymphocyte mutation than controls. 2-AAF most common mutation was G:C- > T:A transversion (32% of all mutations); followed by 1-base pair (bp) deletion (19%); there were very few (5%) G:C- > A: transversions, but no 1 bp deletions.
Nesnow et al. (1995)	Oncogene comparison	Benzo(a)pyrene; benzo(b) fluoranthene; cyclopental(cd) pythene; dibenz(a,h)anthracene; 5-methylchrysene	A/J mouse	Lung tumor	A treatment-induced shift in tumor mutation frequency and in the mutation spectra. For example, in the spontaneous A/J mouse the codon 12 GAT mutation was reported to occur in 59% of tumors while codon 12 TGT initiation did not occur. In contrast, in the BAP treated mice the codon 12 GAT mutation occurred in only 19% of tumors and the codon 12 TGT mutation was observed in 50% of tumors.
Nishimura et al. (1999)	Oncogene comparison	Gamma-Rays	Scid mice	Thymic lymphomas	Neither activated K-ras or N-ras were found in the spontaneous lymphomas. However, K-ras mutations increased in a dose-dependent fashion in the radiation-induced lymphomas. Analysis of the spectrum of K-ras mutations revealed unique mutations in both codon 13 (GGC- > GAC) and codon 61 (CCA- > CTA) in addition to the more commonly observed substitutions of GAT- > GGT in codon 12 of K-ras.
NTP Tech Report 507 (2002)	Oncogene comparison	Vanadium pentoxide	B6C3F1 mouse	Lung tumor	Major mutation profile differences occurred with K-ras 12 codon; this is seen with GTT, TGT, and to a lesser extent with GAT mutations.
Reynolds et al. (1986)	Oncogene comparison	Liver carcinogens (not specifically identified)	B6C3F1 mouse	Liver tumors	"At present, any relationship between spontaneous tumors and the high frequency of chemically induced tumors in the liver of the B6C3fF Mouse is **unclear**." (p36- Column #2).
Reynolds et al. (1987)	Oncogene comparison	Furan and furfural	B6C3F1 mouse	Liver tumor	These two agents tested positive for tumor induction in the mouse liver 2-year carcinogenicity studies, but test NEGATIVE for induction of mutation in Salmonella assays. "The spectrum of activating mutations in the H-ras gene and the pattern of ras gene activation observed in the chemically-induced liver tumors differ significantly from those observed in the liver tumors of untreated animals. All of the activated ras genes detected in the spontaneous liver tumors were H-ras with activation by point mutations at Codon 61. However, only 60% and 40% of the ras oncogenes detected in the furan and furfural, respectively, were activated K-ras or H-ras genes with mutations and positions other than codon 61."
Reynolds et al. (1988)	Oncogene comparison		BC3F1 mouse	Liver tumors	"Activated Ki-ras genes were detailed in the chemically induced liver tumors but not in the spontaneous liver tumors." (refs. 9 & 10). "…all of the H-ras oncogenes detected in spontaneous liver tumors were activated by point mutations in codon 61 whereas H-ras oncogenes detected in the chemically induced tumors were activated by point mutations at a number of different codons." (see reference 10). In the F344/N rat strain of the NTP program with 20 benign spontaneous lung tumors there were no detectable oncogenes. However, 18 of 19 chemically induced lung tumors had detectable oncogenes in K-ras codon 12.
Ruasby et al. (1991)	Oncogene comparison	DEN; Phenobarbitone	C3H/He mice	Liver tumors	This study demonstrated a striking difference between tumors induced by DEN or PB and those that occur "spontaneously" in C3H/He mice. Codon 61 mutations were reported in 41% of DEN induced tumors (19/41), with either first base (CG- > AT, 12/19), a transversion, or the second base (AT- > GC, 7/19) a transition. Codon 61 mutations (all CG- > AT 6/21) were detected in 29% of spontaneous tumors. However, none were found in the PB induced tumors (0/15) and in normal tissue of untreated mice (0/30).
Sills et al. (1995)	Oncogene comparison	Ozone; methylene chloride; tetranitromethane, 1,3-butadiene	B6C3F1 mice	Lung tumors	The control group (concurrent) displayed a markedly different K-ras mutation pattern than ozone, MC, tetranitromethane and 1,3-butadiene for codons 12, 13, and 61. This was most evident in codon 12 for GTT and TGT mutations, codon 13 for CGC, and codon 61 for CTA mutation.

(*continued on next page*)

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Table 3 (*continued*)

Citation	Comparison Endpoint	Agent	Animal Model	Tumor	Evaluation/Comment
Sills et al. (1999a)	Oncogene comparison	Chloroprene	B6C3F1	Lung	While a significant mutation spectra difference occurred within the control and treated groups/ tumors for oncogene mutations, it was most evident for codon 61.
Sills et al. (2001)	Oncogene comparison	1,3-butadiene; isoprene; chloroprene	B6C3F1	Forestomach	The exposure to these agents induced changes in ras mutations which were a qualitatively and quantitatively different mutation spectrum than in spontaneously occurring neoplasms of the forestomach. The lack of H-ras codon 61 CTA mutations and the rare detection of K-ras codon 13 CGC mutations in the spontaneous forestomach neoplasm enhances the likelihood for a causal link with the chemical exposure.
Stanley et al. (1992)	Oncogene comparison	Vinyl carbamate	C57BL/6 J B6D2F1	Liver	These strains of mice are somewhat resistant to developing chemically-induced liver tumors and the frequency spectra of mutations for codon 61 was different in induced and spontaneous tumors, suggesting that different oncogene pathways were activated.
Ton et al. (2004)	Oncogene comparison	BMP 2,2-bis(bromomethyl1,3-propanediol) TNM	B6C3F1 mice	Lung tumors	The treated mice had lung tumors with K-ras mutations, most being GGT- > GTT mutations in codon 12, while none were observed in controls. The lung tumors typically show G- > A transitions (GGT- > GAT) at codon 12 (13/18), while none occurred with spontaneous lung tumors.
Unfried et al. (2002)	Oncogene comparison	Asbestos: crocidolite	(transgenic) F344 female rats, 8 weeks	Greater omentum mutations spectra lacl gene from DNA of omenta of lacl transgenic rats	The molecular assessment of the mutations showed marked differences (mutation types) between asbestos-induced mutations and spontaneous mutations. The authors concluded that a specific molecular mechanism induced by crocidolite differs from that induced by the generation of spontaneous mutations. G to A transitions occurred in the spontaneous mutations with a frequency of 57%. This occurred with only 20% frequency with crocidolite and 35% for BaP. Chromosome deletions were also 5 times more common with crocidolite than in spontaneous mutations.
Warshawsky et al. (1996)	Oncogene comparison	7H-dibenzo[c,g]carbazole	A/J	Lung tumor	K-ras mutations were very different for treated mice at codon 61 than spontaneous tumors. Multiple mutations at codon 12 for spontaneous tumors did not occur for treated mice.
Watson et al. (1995)	Oncogene comparison	Vinyl carbamate	B6C3F1	Hepatocellular tumor	Differences in mutation spectra for codon 61 are shown with CTA adenomas.
Wiseman et al. (1986)	Oncogene comparison	N-HO-AAF; VC (vinyl carbamate) HO-DHE (1′-hydroxy-2′,3′-dehydroestragole)	B6CeF1 male mice	Hepatoma	If the mutations included by these three agents occurred from spontaneous mutagen events, the distribution of mutations should have been similar regardless of the specific chemical treatment (see Discussion), which was not the case
You et al. (1989)	Oncogene comparison	BaP; MNU EC-ethylcarbamate	Strain A	Lung tumors	Spontaneous and chemically-induced lung tumors displayed a significant "different mutation spectra seen with codon 12 – TGT for BaP, codon 12 for GAT for MNU, and codon 61 for CTA for EC.
You et al. (1993)	Oncogene comparison	Benzotrichloride (BTC)	A/J mice	Lung tumors	The pattern of activating mutations in the K-ras gene seen of BTC-induced lung tumors differed markedly from the spontaneously occurring lung tumors. Activating mutations in spontaneous tumors were in both codon 12 (60%) and codon 61 (30%) with several types of codon substitute transitions. In contrast, 100% of the activating mutations detected in BTC induced lung tumors were in the 12th codon with exclusively GC- > AT transitions. Only 27% of the K-ras mutations seen in spontaneous tumors were GC- > AT transitions. These observations revealed that the activating mutations in the K-ras gene of BTC-induced lung tumors probably were induced by a genotoxic effect of the agent.

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spontaneous and induced tumor biology occurred as an outgrowth of the discovery of oncogenes at the level of molecular pathology (Tables 3 and 4). This research involved both the development of basic understanding of oncogenes in the process of carcinogenesis and using these findings to assess the predictive utility of the NTP cancer bioassay program (Maronpot et al., 1995).

Over the course of the now three decades, this area of research has expanded to include 45 agents (Table 5), multiple animal models (Table 6) and the assessment of multiple tumor types (Table 7). Furthermore, the mechanistic evaluation has progressed beyond initial oncogene activation to the occurrence of patterns of gene activation/molecular pathways (Blackshear et al., 2015), and the subsequent occurrence of additional mutations (Parsons et al., 2010) which further transform the altered tissue towards a more tumor-like progression, linked to metabolic reprogramming, creating progressively modified tumor phenotypes (Martinez-Outschoorn et al., 2013; Kerr et al., 2016). This mechanistic based knowledge indicated that tumors become progressively more diverse with individualistically appearing phenotypes, enhancing the difficulty for successful therapeutic applications (Salk et al., 2010). This general progress of knowledge of tumor development revealed that the induced tumors can be molecularly differentiated from similar appearing spontaneous tumors (Tables 3 and 4). This general biological/molecular framework does not support the assumption that one could expect additive to background for cancer assessment since spontaneous and induced tumors that develop within the same organ/tissue typically do not display the same mechanism(s).

The capacity to make molecular mechanism evaluations of the additive to background assumption emerged during the mid-1980s with the identification of proto-oncogenes and their related molecular pathways, as well as methodological developments such as polymerase chain reaction (PCR) in the late 1980s and other nucleotide assessment methods. None of the numerous subsequent experimental papers published on the issue of whether induced tumors employed the same mechanisms of background/spontaneous tumors using mutated/activated oncogenes related their findings back to the issue of cancer risk assessment, regulatory agency policy, or the additive to background papers of Amitage and Doll (1957), Crump et al. (1976), Guess et al. (1977), or to the guidance of the NAS SDWC (1977) or the U.S. EPA (1986, 1996, 2005) cancer risk assessment guidelines which reaffirmed the additive to background assumption.

3.5. Direct comparison: Spontaneous vs Induced Tumor Mechanisms

Data that supported the hypothesis that spontaneous and induced tumors were not mediated by the same mechanistic process emerged during the late 1980s to mid-1990s. A substantial number of the studies as summarized in Tables 8–18, and Figs. 3–10 compare spontaneous and induced tumors for oncogene mutation. When the carcinogen treatment induces different mutations and/or different mutation patterns than those of the spontaneous tumors it suggests that the carcinogen induced tumor occurred via different mechanism(s) than those involved in spontaneous tumors (Maronpot et al., 1995-page 132). An example of such a carcinogen induced altered mutation spectra is seen in Fig. 3, which compares spontaneous and NDEA-induced liver H-ras oncogene mutations in the B6C3F1 mouse with respect to codon 61 induced mutations at the three positions. The figure indicates that 64.6% of the spontaneous hepatocarcinomas display mutations in H-ras codon 61 whereas in the NDEA-induced tumors 46.7% have H-ras oncogene mutations (Chen et al., 1993). The codon 61 mutations of the treated mice were distributed in a manner that differed markedly from spontaneous tumors. None of the treatment induced tumor mutations were in position #1 (AAA) of codon 61 while position #2 (CGA) displayed nearly 93% of the mutated oncogenes. These data indicate that the induced tumors displayed significantly less mutated ras oncogenes at position #1 and a profoundly different overall mutation spectra pattern. These findings suggest that the tumor mechanisms are significantly different between the spontaneous and induced tumors. The findings of Nesnow et al. (1995) describe responses with the A/J mice strain lung tumor model for four polycyclic aromatic hydrocarbon carcinogens for codon 12 (Table 16). As was the case with the B6C3F1 mouse liver, these findings also illustrate marked differential patterns of oncogene involvement in tumor development between the chemically induced and spontaneous tumors. Tables 8–18 and Figs. 3–10 include a wide range of chemical agents with a predominant focus on hepatocellular carcinoma in the B6C3F1 mouse for K-ras mutations. However, the listing is also broadly inclusive of other animal models, tumor types, and oncogenes. While each mutation spectra is specific to the agent, model, and endpoint, the overall pattern of marked differences between induced and spontaneous tumor appears independent of the diverse experiment parameters (e.g., agent, model, tumor, etc.).

An assessment of numerous similar types of studies reveal marked carcinogen-specific oncogene mutation frequencies and spectra. Tables 8–18 and Figs. 3–10 show such cases of significantly differing mutation spectra for a wide range of chemical agents for induced versus spontaneous tumors. These findings indicate that the induced tumors are very unlikely to act via the identical mechanisms of the spontaneous tumors that relate to the specific oncogenes studied (Table 19). The tables reveal that the additive to background assumption was not credible for 45 agents, many of considerable environmental public health relevance (Table 5). These studies affected a broad range of animal models (Table 6) and tumor types (Table 7).

In a dose-time response extension of the above oncogene research, McKinzie and Parsons (2011) assessed oncogene-related mutation frequency over 32 weeks, with a temporal linkage of oncogene activation and colon cancer in the F344 rats treated with azoxymethane (AOM). The GAT and GTT mutations of codon 12 were not altered over the first 24 weeks in controls. However, by week 32, the control group displayed an increase in codon 12 mutations. The control developmental oncogene mutation pattern sharply differed from the AOM-treated rats, which displayed a significantly enhanced mutation rate of codon 12 after only one week of exposure. According to McKinzie and Parsons (2011), the findings reveal that the occurrence of cancer due to age in the control rats displays a different mechanism and progression pattern for the same type of tumor that results from the AOM treatment.

The concern expressed by Hoel (1997) about the compatibility of additivity to background assumption with advances in molecular biology may be illustrated further in the paper of Hisamoto et al. (2007), which compared oncogene mutations in cisplatin, NNK and BaP induced lung tumors of the A/J mouse. In the cisplatin-induced tumors there was an absence of K-ras codon 12 mutations, which is known to be the key mutations-induced by NNK or BaP for lung tumor development. The authors concluded that cisplatin induced the same type of lung tumor in the A/J mice as the NNK or BaP, but by a different mechanism. The same type of tumor in the same animal model may, therefore be produced by different mechanisms confirming the issue raised by Hoel (1997) that molecular biology advances may provide the means to assess the validity of the additive to background assumption.

3.6. Carcinogen-mediated background response reprogramming

Metabolic reprogramming by activated/mutated oncogenes has assumed an important role in tumorigenesis (Martinez-Outschoorn et al., 2013; Kerr et al., 2016). The process from oncogene activation to metabolic transformation often leads to glycolytic reprogramming of tumor cells. The rewiring is a progressive process, with the subsequent occurrence of newly activated and deactivated oncogenes, which mediate the creation of novel tumor phenotypes as well as the regression of tumorigenetic processes. Many of the studies in the present article that compare spontaneous and induced tumors reveal that carcinogen treated animals show a transformed mutation spectra. The carcinogen treatment not only induces a specific-mutation spectra signature, but often significant changes in a highly predictable/expected (i.e., background)

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Table 4
How decisions can be made concerning whether additive to background tumor risk can be assumed. Spontaneous vs chemically/radiation-induced tumor comparison.

Comparison Area #1	Comparison Area #2	Comparison Area #3	Comparison Area #4	Comparison Area #5
Origin/location of tumor in affected organ/tissue Example: Chinzky et al. (1985)	Global gene expression; profile of tumors Examples: Blackshear et al., (2014, 2015); Imaoka et al. (2008); Mansfeld et al. (2006);	Mutation profile of tumor based on codon comparison with oncogenes, often with Ha-Ras, K-Ras and N-Ras Examples: Codons 12, 13, 61; Manam et al., (1992, 1995); Rumsby et al. (1991); Fox et al. (1990); Yen et al., (1988, 1993); Sills et al., (1995, 2001); Donnelly et al. (1996); Mass et al. (1996); Hayashi et al. (2001); Tea et al. (2004); Hong et al. (2015); Watson et al. (1995); Stanley et al., 1992.	Mutation spectra comparison in target tissue for spontaneous and treatment induced mutations Examples: Mitelstaedt et al. (1999); Unfried et al. (2002); Hong et al. (2003); Reynolds et al., (1986, 1987, 1988); Nishimura et al. (1999); Ikaka et al (2010); Kawano et al. (1996); Chen et al. (1992)	Loss of heterozygenicity Example: Antal et al. (2012)

General Comparison Framework

- Identify Key Proto-oncogenes thought to be involved in the process of organ-specific spontaneous and chemically/radiation induced similar tumors (e.g., lung, liver).
- Compare proportion of tumors with specific oncogenes and oncogene subtypes. For example, RAS oncogenes and its subtype H-ras, K-ras, and N-ras.
- Assess/compare changes within specific sub oncogenes for changes within specific codons: For example, ras oncogene – codon 12, 13, and 61.

Five Comparison Areas: Anyone may be employed to assess additive to background within a specific study.

oncogenic activation spectra (Nesnow et al., 1995). Anna et al. (1994) refer to such carcinogen-induced changes as "selective inhibition and enhancements of subsets of spontaneously initiated hepatocellular neoplasms". Tables 16–18 provide examples of multiple carcinogen treatments that affect significant changes in oncogene activation of spontaneous tumors. These findings not only contradict the assumption of additivity to background, but also challenge the traditional concept of background after carcinogen treatment and whether the control group can serve as a legitimate reference group.

The carcinogen treatment, therefore, does not necessarily only add to the oncogene mutation spectra, but has the potential to fundamentally reprogram and transform it. The long-held assumption that the carcinogen treatment directly adds to identical spontaneous mutation frequency and spectra is not supported by a substantial number of studies assessed here. While spontaneous tumor oncogenic transformation/reprogramming occurs, specific spontaneous and induced tumor studies have only been assessed, to a limited extent, developmentally and/or for time course changes of this striking transformation (McKinzie and Parsons, 2011). Nevertheless, such reprogramming transformations may be generalized across biological model, organ, and inducing agent (Table 16).

While carcinogen exposures significantly alter the frequency of K- and H-ras oncogene mutations and tumor mutation spectra, there has been limited research with other oncogenes or other biological processes. Jackson et al. (2006) have produced a type of oncogene mutation meta-analysis for induced vs spontaneous tumors, for mouse liver H-ras and human liver TP53 mutations. This integrative summary of human studies re-enforces the above findings, illustrating fundamentally different general patterns of oncogene mutation responses between induced and spontaneous tumors. These findings strongly challenge the concept of additive to background. It is, however, also evident that the administration of the carcinogen treatment can alter the normal course of spontaneous oncogene activation, while imposing its own unique agent induced mutation spectra. These findings demonstrate that the carcinogen treatment in these instances does not only add to but may significantly modify the background response.

3.7. Dose-dependent oncogenic mutations

Significantly different mutation spectra may be induced by the same carcinogen in a dose dependent manner (Sills et al., 1999a; Hong et al., 2015; Cazoria et al., 1998; Zhuang et al., 2002; Manam et al., 1995; Nishimura et al., 1999; Warshawsky et al., 1996; Hecht et al., 1998; Chen et al., 1993). These findings indicate that different tumor-inducing mechanisms can operate at different doses. Both Bogen (2017) and Crump (2017) have acknowledged the occurrence of high and low dose slope divergence and its significant implications for modeling low dose effects. The presence of different mechanisms of oncogene activation across dose further challenges the process of decision making with respect to dose-response modeling which depends on the additive to background assumption (Blackshear et al., 2014, 2015; Imaoka et al., 2008). These findings show not only the evolving complexities of mechanistic understandings of induced vs spontaneous tumors, they also highlight the limitations of the initial additive to background assumption.

4. Discussion

The decision to adopt additive to background by the U.S. EPA for cancer risk assessment was influenced by the U.S. NAS SDWC (NAS, 1977) based on the Crump et al. (1976) paper, as well as the OSHA (1980) Carcinogen Hearings and Hoel (1980). Schneiderman, a member of that SDWC, summarized its recommendations in a follow-up paper, indicating that known carcinogens have never induced cancers in people that have never been seen before (i.e., novel cancers) (Schneiderman and Brown, 1978). With this logic, it was assumed that

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Table 5
Agents used to assess additive to background hypothesis for induced cancer.

	Agents
1	1,3-butadiene
2	2,2-bis(brom-methyl-1 to 3 propanediol
3	2AAF
4	4-amino azobenzene
5	5-methylchrysene
6	ADBA
7	Aflatoxin B
8	Asbestos crocidolite
9	Azoxymethane
10	Benzene
11	Benzo(a)pyrene
12	Benzo(b)fluoranthene
13	Benzidine
14	Benzotrichloride
15	Chloroform
16	Chloroprene
17	Ciprofibrate
18	Cobalt Metal
19	Cumene
20	Cyclopental(cd)pyrene
21	DEN
22	DMBA
23	Ethyl carbamate
24	Foran
25	Furfural
26	gamma-radiation
27	HO-DHE
28	Isoprene
29	Methylene chloride
30	MCA
31	MNU
32	N-HO-AAF
33	NDEA
34	NDMA
35	NNNK
36	O-nitrotoluene
37	Ozone
38	Phenobarbitone
39	Radionuclides
40	Riddelliine
41	TCDD
42	Tetranitromethane
43	Vanadium pentoxide
44	Vinyl carbamate
45	Vinylidene chloride

Table 6
Animal model utilized to assess additive to background hypothesis.

Animal Models
A/J mice
AC3F1 (A/J x C3H/He5)
B6C3F1 mice
C3H mice
C3H/Hp mice
C57BL/6 × C3H/He mice
C57BL/6 × DBA2 mice
CD-1 mice
RF/J/AKR mice
Scid mice
Sprague-Dawley rats
Strain A mice
F344 rats

carcinogens will act at least to some extent via an identical mechanism (s) in the same target cells as occurs with normal background conditions. This perspective lead Schneiderman and Brown (1978) (and the NAS SDWC) to conclude that exogenous carcinogens act in an additive to background manner within humans. Populations already exposed to endogenous and background environmental carcinogens were, therefore, expected to experience cancer risks in a continuous (i.e., linear-progressive-non-stepwise) manner with respect to dose, regardless of how low the dose. In their paper, Schneiderman and Brown (1978) acknowledged the assistance of several key individuals such as David Rall (the NIEHS Director), David Hoel (NIEHS biostatistician) and Sheldon Murphy (former President of the U.S. Society of Toxicology), all members of the NAS SDWC for the writing of his article, each of whom offered comments at the OSHA (1980) hearings except Murphy.

The above NAS SDWC recommendation and explanation of Schneiderman and Brown (1978) were subsequently challenged and largely contradicted by a substantial body of literature from the late 1980s to the present, showing that experimentally induced tumors display significantly different types of oncogene mutations and profoundly differing oncogene mutation spectra than spontaneous tumors (Figs. 3–10; Tables 8–18). The early and seemingly reasonable belief that when a chemical/ionizing radiation induces the same type of tumor in the same organ (e.g., lung, liver, etc) as does the control group, it does so via the same mechanism is not the case. In fact, these studies reveal no convincing evidence that induced and spontaneous tumors are even infrequently likely to be mediated by the same mechanisms. This conclusion is supported by observations that as the process of carcinogenesis progresses, more tumor specific changes occur, resulting in unique tumor specific phenotypes (see subsection entitled: Tumor Mutation Complexities) (Salk et al., 2010; Kerr et al., 2016). Further challenging the original additive to background decision is the growing view that most tumors are heterogeneous, suggesting that multiple clones of complementary mutant cells may influence the tumor progression (e.g., colon tumor) (Parsons, 2008; McKinzie and Parsons, 2011; Vogelstein et al., 2013).

The original additive to background assumption for cancer was based upon a mutation mechanism. It did not address the question of epigenetic carcinogens. Yet, the EPA procedure has made no distinction between genotoxic and epigenetic carcinogens with respect to the additive to background assumption. In 1994, Ray et al. (1994) reported that phenobarbital (PB)-induced liver tumors in a mouse model via epigenetic mechanisms. The molecular mechanisms that mediated the PB induced liver tumors were different than those of spontaneous tumors. This mechanistic divergence between epigenetic carcinogens and mechanisms and time of spontaneous tumors have been reported in other experimental studies (Hegi et al., 1993). Such findings suggest that the additive to background assumption may also not be applicable for chemically induced epigenetic tumors as well.

The limitations of the additive to background concept for environmental induced cancer was suggested by the results of a substantial series of ionizing radiation induced gene mutation studies. As has been the case with additive to background for chemically induced cancer, this same hypothesis for ionizing radiation induced mutations, as researched in considerable depth by Stadler, Muller, and others from the 1930s to 1950s, was not supported (Roman, 1988; Stadler, 1954; Stadler and Roman, 1948). Had the chemical toxicology and risk assessment communities of the 1970s and early 1980s better appreciated the history of the spontaneous and ionizing radiation induced mutation historical literature, the additive to background assumption may not have been incorporated into regulations by U.S. EPA (1986), reflecting the historical truism of the Spanish philosopher and novelist, George Santayana (1863–1952) that "who cannot remember the past is condemned to repeat it."

4.1. Reprogramming the disease background

The same tumor types/in the same organ of the same biological model for spontaneous and induced tumors typically show significant differences in oncogene activation and mutation spectra. Furthermore, the treatment with chemical carcinogens may significantly alter the occurrence of oncogene activation/mutation as would occur in

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Table 7
Tumor types utilized to assess additive to background hypothesis.

Tumor Types
Colon
Forestomach
Harderian gland
Liver
Lung
Lymphoma
Mammary
Mesothelioma
Thymic

spontaneous tumors, revealing a type of developmental cellular/tissue rewiring/reprogramming. The fundamental assumption of additive to background for carcinogens is, therefore, not compatible with the mutation spectra data. These developments are also incompatible with the concept of independence of background since the carcinogen treatment reprograms the normal spontaneous tumor response by changing the background of the animals allocated to the treatment groups. This observation challenges the traditional concept of a control group and how comparisons to a control group are to be made.

The carcinogen-induced reprogramming process may offer an insight to the findings of Gray et al. (2002) that many chronic bioassays display striking mixtures of organ-specific tumor and antitumor responses relative to the control. While the standard comparison to the control is valid in these cases, the issue of additive and independent of background will need to be re-evaluated within the context of the chronic bioassay related cancer dose-response assessment. The issue of carcinogen-induced reprogramming presents new challenges and opportunities that may affect model and dose selection, mechanism evaluation, carcinogen risk assessment modeling, and risk estimates currently used that affect numerous societal areas including health, medical, legal, and technology domains.

Table 8
Pattern of K-ras mutations in chloroprene and isoprene in lung neoplasms from female B6C3F1 mice (Source: Sills et al., 1999, Table 1, Page 659).

	Codon 12 (GGT)						Codon 61 (CAA)			
	gTt	gAt	Tgt	Cgt	CTt	ATt	cTa	caT	caC	cGa
Spontaneous	6.6	60.0	33.3	0	0	0	0.0	57.1	14.2	28.4
Chloroprene	20.0	50.0	0	10.0	10.0	10.0	88.0	0	0	12.0
Isoprene	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0

Note: Numbers in table represent percent of specific oncogene mutations within a specific codon. For example, the TGT mutation represents 33.3% of the total oncogene mutations for codon 12. The total mutations sums to 100% within each codon for spontaneous and agent induced tumors. Bolded, enlarged letters represent the location of mutation in codon for Table 8–Table 18.

Table 9
K-ras mutations in Harderian gland neoplasms and lung tumors for ethylene oxide-exposed B6C3F1 mice. (source: Hong et al., 2007).

Harderian Gland Tumors							
	Codon 12 (GGT)		Codon 13 (GGC)		Codon 61 (CAA)		
	gAt	Tgt	Cgc	Agc	cTa	cGa	caG
Spontaneous	0.0	0.0	0.0	0.0	100	0.0	0.0
Ethylene Oxide	3.3	26.6	50.0	3.3	3.3	10.0	3.3
Lung Tumors							
	Codon 12 (GGT)		Codon 13 (GGC)		Codon 61 (CAA)		
	gAt	Tgt	gTt	CgT	Agc	Cgc	cGa	caT	caC
Spontaneous	40.7	18.5	3.7	0.0	0.0	1.11	7.4	14.8	3.7
Ethylene Oxide	9	0	84	0	4	0	4	0	0

Note: Numbers in table represent percent of specific oncogene mutations within codons 12, 13, and 61. For example, the TGT mutation in codon 12 for Harderian gland tumors represents 26.6% of the total oncogene mutations for codon 12, 13, and 61 summed together. The total mutations sums to 100% across the three codons for spontaneous and agent induced tumors for Harderian gland and lung tumors.

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Table 10
Summary of K-ras mutations in alveolar/bronchiolar tumors in B6C3F1 mice from various NTP bioassays (source: Hong et al., 2015, Table 8, page 878).

	Codon 12 (GGT)					
	GAT	TGT	GTT	CGT		N
Spontaneous (Historical Control)	70	25	5	0.0		20
Cobalt metal dust[a,b]	16.6	3.3	76.6	3.3		30
1,3 butadiene[b]	Insignificant response	Insignificant response	Insignificant response	Insignificant response		Insignificant response
Ethylene oxide[a,b]	8.6	0.0	91.3	0.0		23
Cumene[b]	25.0	20.8	45.8	12.5		24
2,2-Bis(bromomethyl)-1,3-propanediol[c]	71.4	3.6	25.0	0.0		28
Tetranitromethane[a,b]	100.0	0.0	0.0	0.0		13
Isoprene[b]	0.0	0.0	0.0	0.0		0.0
Methylene Chloride[b]	-	-	-	-		-
	Codon 61 (CAA)					
	CAC	CTA	CGA	CAT	Other[d]	N
Spontaneous (Historical Control)	0	0	37.5	50.0	12.5	12
Cobalt metal dust[a,b]	0	0	35.7	28.5	35.7	14
1,3 butadiene[b]	0	66.6	33.3	0	0	9
Ethylene oxide[a,b]	Insignificant response	Insignificant response	Insignificant response	Insignificant response	Insignificant response	Insignificant response
Cumene[b]	Insignificant response	Insignificant response	Insignificant response	Insignificant response	Insignificant response	Insignificant response
2,2-Bis(bromomethyl)-1,3-propanediol[c]	Insignificant response	Insignificant response	Insignificant response	Insignificant response	Insignificant response	Insignificant response
Tetranitromethane[a,b]	0	88.0	12.0	0.0	0.0	25
Isoprene[b]		0.0	100.0	0.0	0.0	10
Methylene Chloride[b]	57.1	14.2	14.2	14.2	0.0	7

Note: Numbers in table represent percent of specific oncogene mutations within a specific codon. For example, the spontaneous TGT mutation represents 25% of the total oncogene mutations for codon 12. The total mutation percentages sum to 100% within each codon for spontaneous and agent induced tumors.
[a] If the same tumor had 2 point mutations, it was counted as 1.
[b] Exposure by inhalation for ~ 2 years.
[c] Exposure by feed for ~ 2 years.
[d] Corrected from original reference.

4.2. Pre- post-conditioning and "subtraction" from background

While the above assessment indicates that chemically and radiation-induced tumors typically act via mechanisms that differ from those of the same type of tumors in control groups (i.e., spontaneous tumors), other experimental evidence further weakens the additive to background assumption. These include the concepts of pre- and post-conditioning/adaptive response (Calabrese, 2016a, 2016b) and the lack of induced genetic changes at ionizing radiation doses far greater than background (Russell, 1969; Olipitz et al., 2012). In the case of preconditioning, a prior low dose of a vast array of toxic chemicals, radiation and other stressor conditions may reduce damage from a subsequent and more substantial toxic exposure. The decreased damage under optimized conditions is typically in the 30–60% range. The shape of the preconditioning/adaptive response follows an hormetic dose response (Calabrese, 2016a, 2016b). The preconditioning/adaptive response reflects a biological "subtraction" phenomenon, in which the subsequent exposure to mutagens and other toxic agents can be significantly less than additive, discrediting the additive to background assumption within such experimental settings. The preconditioning/adaptive response typically has two functional windows of protection over a several day period (i.e., short-term – 1 h. and then long term starting again at 12 h. and continuing for about 48–72 h). There is also a limited effective dose that typically involves a 25–200 fold range (Calabrese, 2016a, 2016b). While the preconditioning/adaptive response is highly generalizable, the specific experimental parameters may vary by dose, model, endpoint and agent. Furthermore, not only may damage be significantly mitigated by a prior low dose of numerous

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Table 11
K-ras mutations in lung neoplasms of B6C3F1 mice in a two-year inhalation study of cumene (source: Hong et al., 2008, Table 2, page 722.

	Cumene								
	Codon 12 (GGT)			Codon 13 (GGC)		Codon 61 (CAA)			
	GAT	TGA	GTT	CGT	CGC	CGA	CAT	CAC	CTA
Spontaneous (Historical Controls)	42.4	15.1	3.0	0	18.2	6.1	12.1	3.0	0
Cumene Treated Groups	13.3	11.1	24.4	6.6	8.8	28.2	0	4.4	2.2

Note: Numbers in table represent percent of specific oncogene mutations acrosscodons 12, 13, and 61. For example, the TGA mutation represents in codon 12 represents 15.1% of the total oncogene mutations for codons 12, 13, and 61 summed together. The total mutations sums to 100% across the three codons for spontaneous and agent induced tumors.

Table 12
H-ras mutation spectrum in benzene-induced B6C3F1 mouse mammary carcinomas (source: Houle et al., 2006, Fig. 4, page 759).

Benzene			
	Codon 61 - CAA		
	A to G	A to T	C to A
Spontaneous Control	71.0	14.3	14.3
Benzene	40.0	40.0	20.0

Note: Numbers in table represent percent of specific oncogene mutations within codon 61. For example, the A to G mutation in the spontaneous tumors represents 71% of the total oncogene mutations for codon 12. The total mutation percentages sum to 100% within codon 61 for spontaneous and agent induced tumors, respectively.

Table 13
p53 mutation base preference in benzene-induced B6C3F1 mouse mammary carcinomas (source: Houle et al., 2006, Fig. 5, page 759).

p53				
	Codons 5–8			
	G	A	T	C
Spontaneous Control	8	24	0	68
Benzene	44	28	14	14

Note: Numbers in table represent percent of specific oncogene mutations across codons 5–8 for the p53 gene. For example, the G mutation for the benzene treatment represents 44% of the total oncogene mutations for codons 5–8 summed together. The total mutations sums to 100% across codons 5–8 for spontaneous and agent induced tumors, respectively.

Table 14
H-ras mutation spectrum in ethylene oxide-induced B6C3F1 mouse mammary carcinomas (source: Houle et al., 2006, Fig. 4, page 759).

	Codon 61 (CAA)		
	A to G	A to T	C to A
Spontaneous Control	0.0	20.0	80.0
Ethylene Oxide	40.0	40.0	20.0

Note: Numbers in table represent percent of specific oncogene mutations within codon 61. For example, the A to G mutation in the ethylene oxide tumors represents 40% of the total oncogene mutations for codon 61. The total mutation percentages sum to 100% within codon 61 for spontaneous and agent induced tumors, respectively.

Table 15
p53 mutation base preference in ethylene oxide-induced B6C3F1 mouse mammary carcinomas (source: Houle et al., 2006, Fig. 5, page 759).

p53				
	Codons 5–8			
	G	A	T	C
Spontaneous Control	10.0	20.0	0.0	70.0
Ethylene Oxide	60.0	10.0	10.0	20.0

Note: Numbers in table represent percent of specific oncogene mutations across codons 5–8 for the p53 gene. For example, the G mutation for the ethylene oxide treatment represents 60% of the total oncogene mutations for codons 5–8 summed together. The total mutations sums to 100% across codons 5–8 for spontaneous and agent induced tumors, respectively.

toxic agents/carcinogen agents, a similar extensively documented phenomena (i.e., post-conditioning) can also diminish harmful effects when given after the toxic exposure within a limited time window. These significant scientific developments emerged one to two decades after the Crump et al. (1976) paper.

The conditioning treatment may act not only to reduce damage to a subsequent challenging dose but also to reduce damage from ongoing prior disease processes, creating a type of subtraction from background phenomenon (Calabrese, 2018). A post-conditioning type of subtraction from background was reported by Azzam et al. (1996). They found that a very low total dose of (0.1 cGy; 0.24 cGy/min) [i.e., equivalent to about one year of background (non-radon) gamma ray radiation delivered in less than one minute] upregulated repair mechanisms in C3H 1OTV2 cells, reducing neoplastic transformation by approximately 60–80%. The test dose of 0.1 cGy was selected to ensure that each cell that was hit received (on average) about one track. According to the Azzam et al. (1996) the exposure selected reflected a real-life increase of dose between zero (including background) and the dose deposited by one track (i.e., background does not yield one track for each cell on average). The single track dose in this cell system not only did not increase the frequency of neoplastic transformation but significantly reduced it over the range from 0.1 to 10 cGy. The findings of Azzam et al. (1996) were strongly supported by a detailed mechanistic

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Table 16
Differential pattern of oncogene involvement in PAH-induced lung cancer in the A/J mouse for spontaneous vs induced tumors for four carcinogens (source: Nesnow et al., 1995).

Codon 12 (GGT)								
Spontaneous Lung Tumor[1]				Induced Lung Tumor				
TGT	GTT	GAT	CGT		TGT	GTT	GAT	CGT
0.0	33	59	8	BaP[a]	57	24	19	0.0
0.0	33	59	8	B(b)F[b]	61	39	0.0	0.0
0.0	33	59	8	5MC[c]	50	22	0.0	28
0.0	33	59	8	CPP[d]	25	15	10	50

[1] Numerical values represent percent (%) of total mutations (100%) for the spontaneous tumors and agent-induced mutations of codon 12.
[a] BaP = benzo(a)pyrene.
[b] B(b)F = benzo(b)fluoranthene.
[c] 5MC = 5-methylchrysene.
[d] CPP = cyclopental[cd]pyrene.

Table 17
Pattern of H-ras gene mutations at codon 61 in liver tumors from male B6C3F1 mice treated with genotoxic chemicals (Source: Anderson et al., 1992).

		Codon 61 (CAA)		
Chemical	Tumors with Activated H-ras (%)	AAA	CGA	CTA
Spontaneous	61/91 (67%)	37 (62.6%)	16 (26.2%)	8 (13.1%)
Vinyl carbamate (VC)	29/37 (78%)	2 (5.4%)	1 (2.7%)	26 (89.6%)
dimethylbenz(a)anthracene (DMBA)	10/10 (100%)	0 (0.0%)	0 (0.0%)	10 (100%)
N-hydroxy-2-acetylaminofluorene (N-OH-AAF)	7/7 (100%	7 (100%)	0 (0.0%)	0 (0.0%)
benzidine (BZD)	13/22 (59%)	11 (84.6%)	1 (7.7%)	1 (7.7%)
N-nitrosodiethylamine (DEN)	14/33 (42%)	7 (50%)	3 (21.4%)	4 (28.6%)

Numerical values represent percent (%) of total mutations (100%) for the spontaneous tumors and agent-induced mutations of codon 61.

radiobiological model (Scholnberger et al., 2002), experimentally confirmed and significantly extended by others to lower doses (Redpath et al., 2001, 2003a, 2003b; Redpath and Elmore, 2007; Ko et al., 2004). These findings indicate that over a relatively broad dose range above background post-conditioning subtraction from background risk may occur.

The pre- post-conditioning/adaptive response phenomena are very general and have application to the additive to background assumption for both carcinogens and non-cancer endpoints.

4.3. Threshold responses and the additive to background concept

Within the context of additive to background, a continuous exposure to 0.0002 cGy/h., a dosage approximately 400 fold greater than background, for five weeks in a mouse model did not induce detectable changes in DNA nucleobase damage products. Likewise, neither was there evidence of DNA fragmentation in the micronucleus assay assuming double strand breaks. Further, at this dose rate a wide range of gene transcripts of DNA damage responses were not induced. Of significance is a dose-rate of about 30 fold higher than background exceeds permissible human exposures (FEMA, 2002). Olipitz et al. (2012) failed to show a response that is additive to background at a dosage some 400 fold greater than background even with very sensitive biomarkers of genetic damage. Similar findings were reported over a 400 day exposure period of 1 mGy/22 h day or 400 fold higher than background in a mouse model (Ono, 2013; Tatsumi and Tanooka, 2014). In addition, the massive data of Russell (1969) with the mouse specific locus test failed to induce mutations in oocytes at doses up to 27,000 fold greater than background. While these data of Russell (1969) were known to the NAS within the BEIR I Genetics Subcommittee (NAS/NRC, 1972) and the Safe Drinking Water Committee (1977) they had no discernable impact on their risk assessment recommendations.

4.4. The problem of background exposure and the dose-response relationship in the area of zero dose

In the case of ionizing radiation, it has long been assumed that one cannot ignore the occurrence of background exposure. Why? From before birth the individual has exceeded zero dose. By the time this

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Table 18
H-ras activation at codon 61 in hepatocellular adenomas and carcinomas following treatment with VC and/or TCDD (sexes combined) (source: Watson et al., 1995, page 1707).

Spontaneous Tumors				Induced Tumors		
Codon 61 - CAA				Codon 61 - CAA		
AAA	CGA	CTA		AAA	CGA	CTA
66.6	33.3	0.0	Vinyl Carbamate (VC)	18.5	18.5	63.0
			TCDD	69.5	17.5	12.5
			VC + TCDD	11.3	15.1	73.6

Numerical values represent percent (%) of total mutations (100%) for the spontaneous tumors and agent-induced mutations of codon 61.

person is 35–50 years of age background dose may be expected to be 70–100 mSy. According to the traditional radiation geneticist mantra, the body only recognizes the total dose received. Now if the person received an additional dose (i.e. see Beninson, 1988 - Fig. 5, Page 447) the body is assumed to simply add this to the background, proportionately enhancing risk. **"It is this incremental dose risk proportionality that is normally referred to as the linear non-threshold hypothesis"** (Beninson, 1988). The risk assessment "management system" for occupationally exposed people needs to deal with two type of exposures in such a manner that the summated risk is the same whether exposure A occurs first followed by exposure B or the other way around. For the risk management system to work the risk must be additive. If this were not the case, one could not add up the various dose increments that occur throughout the exposure period (e.g. a working week or year). It is the accepted convention of adding up the incremental doses that are received throughout the given period. This process creates the capacity to standardize and control the annual doses received by workers. This is the key management equity argument that legitimizes equal treatment across workers. This regulates the dose distribution (i.e. dose rate) within the work period (e.g. 1 year) (see Beninson, 1988 – Fig. 6, page 447). The risk associated with the total permitted dose is assumed within the given time period. According to Beninson (1988) "This is the basic condition for a workable dose control system in radiation protection".

This statement of Beninson (1988), who was chair of the International Commission of Radiological Protection (ICRP) at the time of the statement, is of relevance to the additive to background assessment. Beninson based his belief in linear cancer risks on the assumption that mutations also show a linear dose response down to a single ionization. He noted that these effects are contingent upon the transformation of a single cell and would therefore conform to a probabilistic/stochastic framework rather than a non-stochastic effect. He then indicated that the probability of inducing transformed cells will be equal to one minus an exponential function of dose, with the responses at low doses being "approximately linear." He later cited micro-dosimetric arguments indicating that the dose response should be linear even when there is even less than one energy track on average per cell.

With this as his theoretical background argument, he then focused on the response of humans exposed to ionizing radiation for several decades, having accumulated much dose and genetic damage, making a sizeable background, and therefore a sizeable proportional risk, that presumably might be detectable in an epidemiological sense. The new dose is then added to the accumulated background damage in a manner that is additive to background. This is then represented as such in the Beninson figure (see Beninson, 1988- Fig. 6). This argument is of course, framed, as he noted, to be applied to the practical realm of devising a worker protection plan.

The arguments of Beninson fail to address important empirical genetic damage findings that challenge and possibly contradict his premises. For example, the failure to detect ionizing radiation induced genetic damage in multiple sensitive experimental systems even at doses from several hundred to many thousands fold greater than background is highly problematic (Russell, 1969). Epidemiological evidence also does not support Beninson's application of the additive to background as seen with the series of essentially flat excess risk

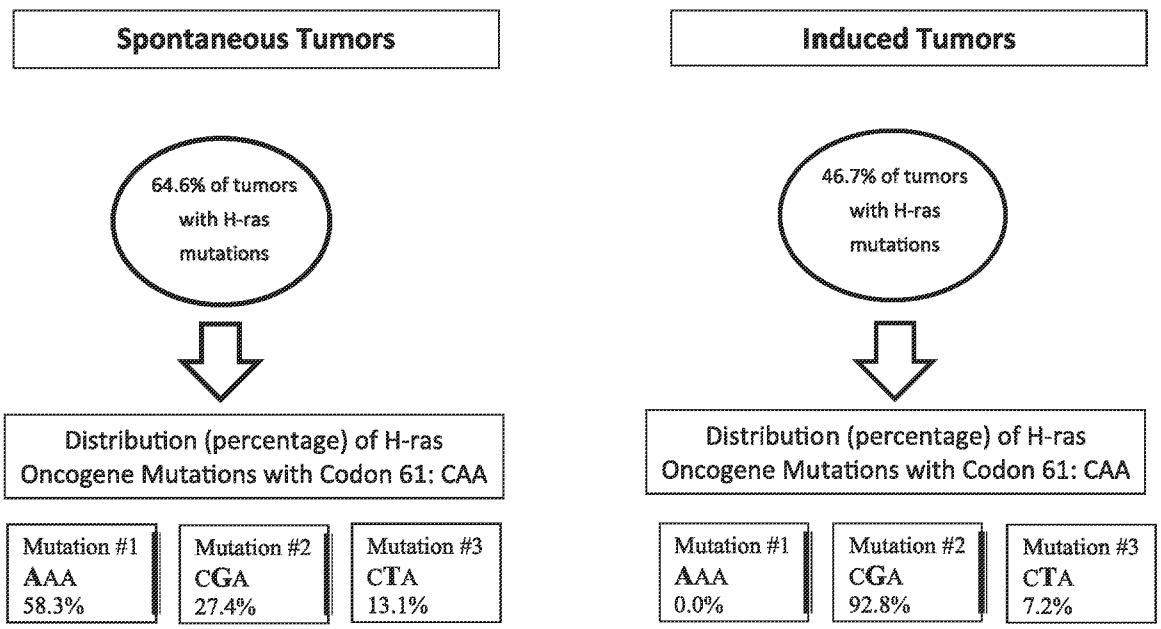

Fig. 3. Spontaneous vs NDEA induced liver tumors with H-ras oncogene mutations* in codon 61 of the B6C3F1 mouse (Source: Chen et al., 1993); marked boxes indicate oncogene mutations that differ between spontaneous and induced tumors. **Conclusion**: Induced tumors showed a significantly different mutation spectra as highlighted for mutations #1 and #2 than the spontaneous tumors. *Mutation percentages were summed across the three types of mutations in codon 61 for both spontaneous and induced tumors. Bolded, enlarged letters represent the location of mutation in codon in Figures 3–10.

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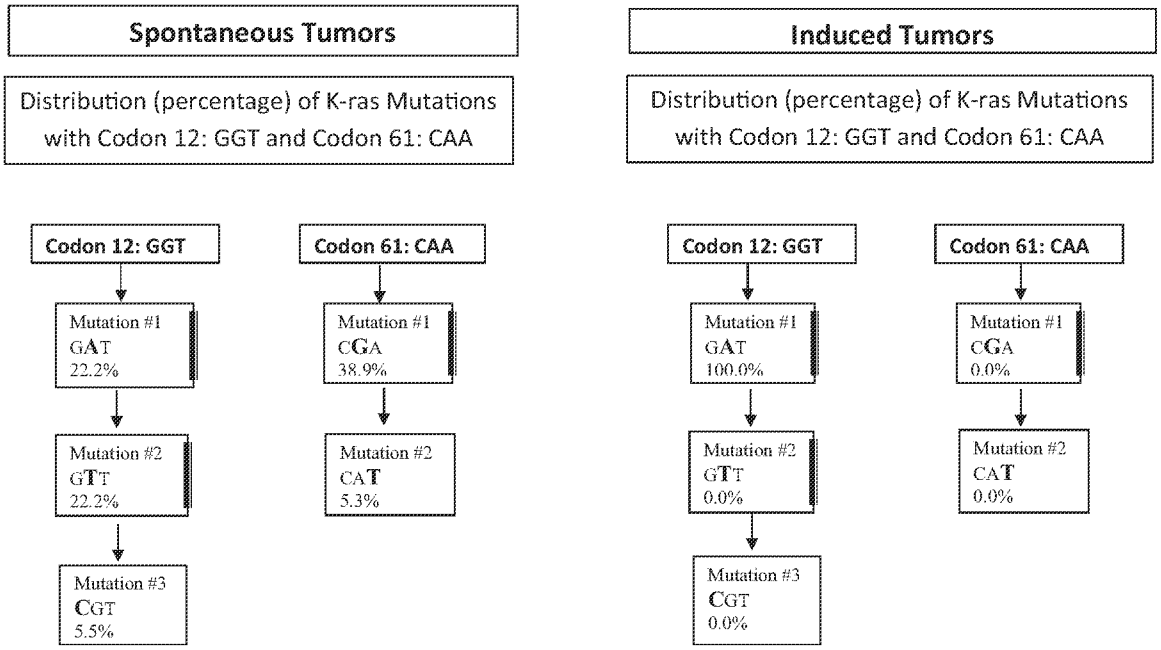

Fig. 4. Spontaneous vs NNK induced lung tumors with K-ras oncogene mutations* with codons 12 and 61 of the B6C3F1 mouse (Source: Chen et al., 1993);); marked boxes indicate oncogene mutations that differ between spontaneous and induced tumors. **Conclusion**: Induced tumors showed a significantly different mutation spectra as highlighted in codon 12 (i.e., mutations #1 and 2) and codon 61 (i.e., mutation #1) than the spontaneous tumors. *Mutation percentages were summed across the codons 12 and 61 for both spontaneous and induced tumors.

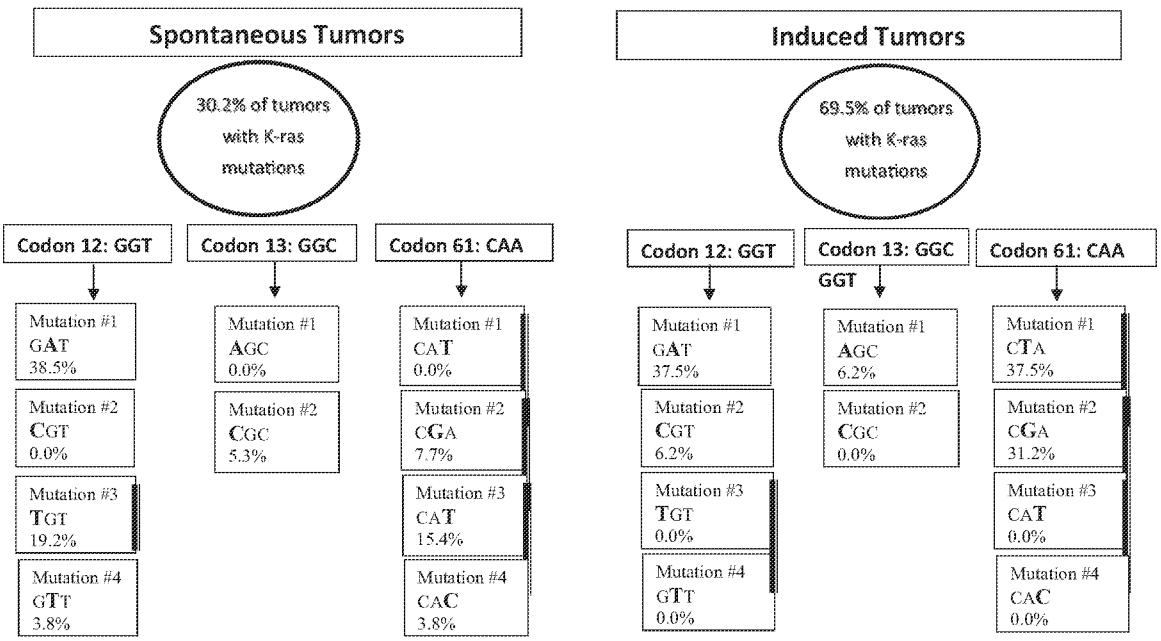

Fig. 5. Frequency/spectra of K-ras mutations* in lung tumors of B6C3F1 mouse study of ADBAQ (Source: Hayashi et al., 2001, Table 4, Page 426)' marked boxes indicate oncogene mutations that differ between spontaneous and induced tumors. **Conclusion**: Induced tumors showed a significantly different mutation spectra as highlighted in codon 12 (i.e., mutation #3) and codon 61 (i.e., mutations #1, #2, and #3) than the spontaneous tumors. *Mutation percentages were summed across the codons 12, 13, and 61 for both spontaneous and induced tumors.

responses across a broad range of radiation doses prior to reaching and exceeding a threshold (Ricci and Tharmalingam, 2018).

4.5. Tumor mutation complexity, hyperindividualistic tumor progression and challenges to additive to background

Of considerable relevance to the issue of additivity to background were a series of findings from large-scale efforts to systematically screen individual tumors for somatic mutation using human breast, colon, pancreas, and brain (i.e., glioblastoma) tumor samples (Sjoblom et al., 2006; Wood et al., 2007; Vogelstein et al., 2013). These tumor types were selected since they had a graded progression prior to reaching advanced, invasive stages. The guiding hypothesis was that there was a progressive mutational sequence that should be clinically related, reflecting an orderly, sequential pattern. The concept of cancer as a mutation-driven orderly disease as framed by Vogelstein et al. (2013) was born in the earlier multi-stage models of Armitage and Doll (1957) and Crump et al. (1976). The Vogelstein et al. studies are striking,

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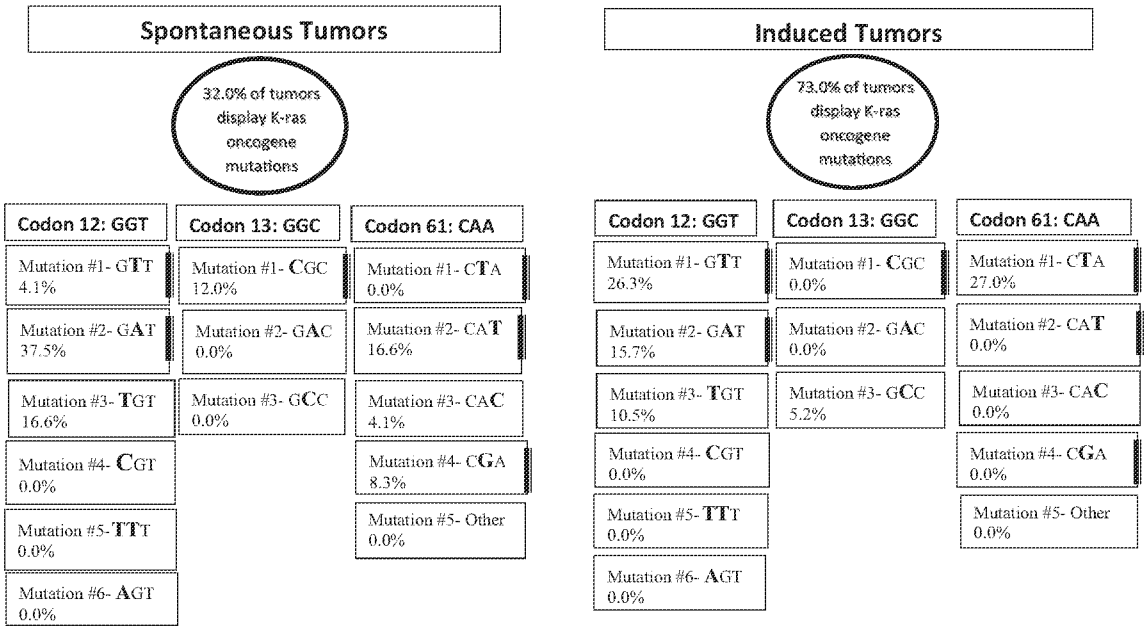

Fig. 6. Frequency and spectra of K-ras mutations* in controls and ozone exposed B6C3F1 mice (Source: Sills et al., 1995); marked boxes indicate oncogene mutations that differ between spontaneous and induced lung tumors. **Conclusion:** Induced tumors showed a significantly different mutation spectra as highlighted for Codon 12 (i.e., mutations #1 and #2), codon 13 (i.e., mutation #1) and codon 61 (mutation #1, #2, and #4) than the spontaneous tumors. *Mutation percentages were summed across the codons 12, 13, and 61 for both spontaneous and induced tumors.

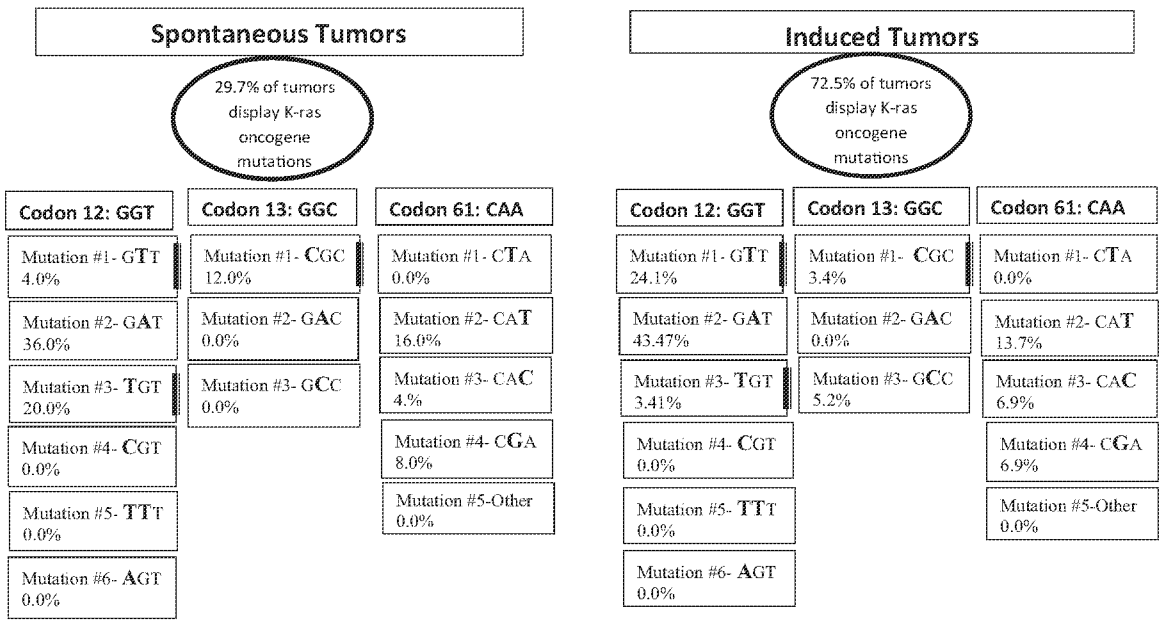

Fig. 7. Frequency and spectra of K-ras mutations in controls and vanadium pentoxide exposed B6C3F1 mice (Source: NTP Tech Rep 507, 2002; Ton et al., 2004, Table 2); marked boxes indicate oncogene mutations that differ between spontaneous and induced lung tumors. **Conclusion:** Induced tumors showed a significantly different mutation spectra as highlighted for Codon 12 (i.e., mutations #1, and #3), and codon 13 (i.e., mutation #1) than the spontaneous tumors. *Mutation percentages were summed across the codons 12, 13, and 61 for both spontaneous and induced tumors.

challenging this orderly progressive assumption. This perspective emerged from the substantial degree of complex intertumoral heterogeneity, and its progressively highly individualistic nature.

Driver mutations (i.e., genes that confer a selective growth advantage) appeared present for many cancer genes within the studied tumors. However, many infrequently altered non-driver passenger mutations (i.e., mutations with no direct or indirect effects of the selective growth advantage of the cell in which it occurred) when collectively combined make substantial contributions to tumor occurrence. In the case of breast cancer, the maximum number of mutated cancer genes (i.e., driver-like mutations) were six, along with about 50 possible passenger mutations. Nearly 30% of these tumors had a single driver mutation along with several passenger mutations, while about 5% had no identifiable oncogene plus tumor suppression gene mutations (Vogelstein et al., 2013).

While it is has long been established that cigarette smoking is a major risk of lung cancer, non-smokers still comprise about 10–15% of those displaying this disease (McCarthy et al., 2012). This relatively high occurrence of lung cancer in non-smokers offers the potential to evaluate the additive to background assumption within a population of

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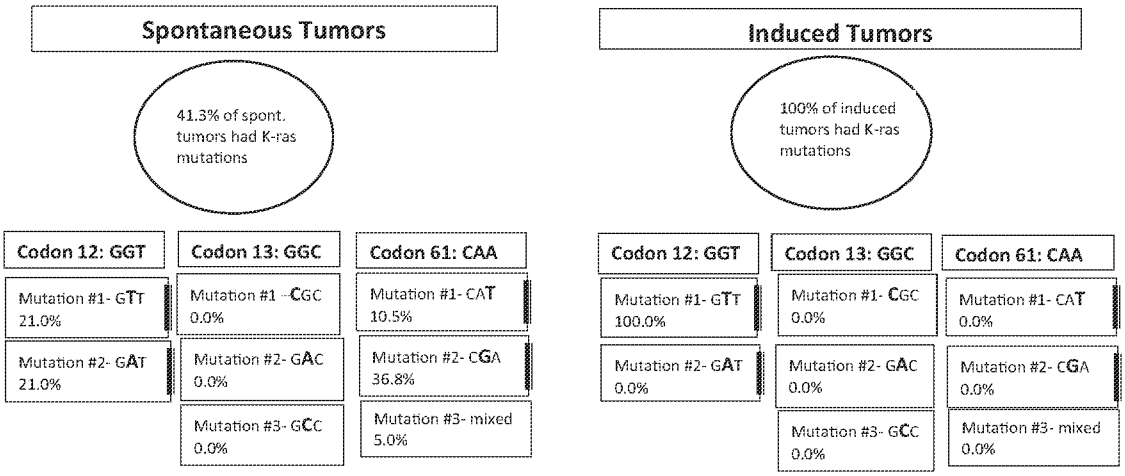

Fig. 8. Comparison of K-ras mutation spectra for spontaneous tumor and NNK-induced tumors in A/J lung tumors (Source: Matzinger et al., 1994; Ronai et al., 1993;Sills et al., 1999b); marked boxes indicate oncogene mutations that differ between spontaneous and induced tumors. **Conclusion**: Induced lung tumors showed a significantly different mutation spectra as highlighted for Codon 12 (i.e., mutations #1 and #2), and codon 61 (mutations #1 and #2) than the spontaneous tumors. *Mutation percentages were summed across the codons 12, 13, and 61 for both spontaneous and induced tumors.

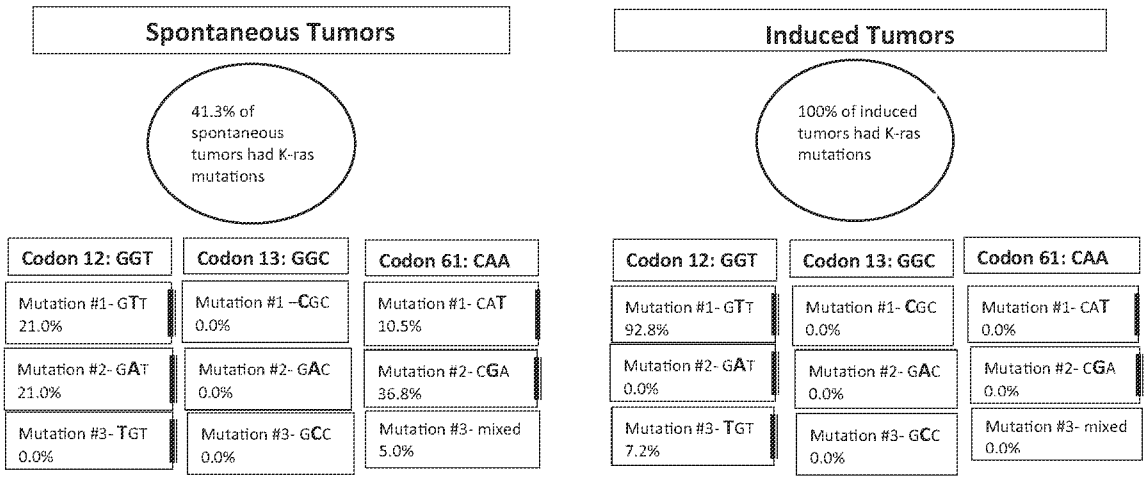

Fig. 9. Comparison of K-ras mutation spectra for spontaneous tumor and NNK-induced tumors in A/J lung tumors (Source: Matzinger et al., 1994; Ronai et al., 1993); marked boxes indicate oncogene mutations that differ between spontaneous and induced tumors. **Conclusion**: Induced lung tumors showed a significantly different mutation spectra as highlighted for Codon 12 (i.e., mutations #1 - #3), and codon 61 (mutations #1 and #2) than the spontaneous tumors. *Mutation percentages were summed across the codons 12, 13, and 61 for both spontaneous and induced tumors.

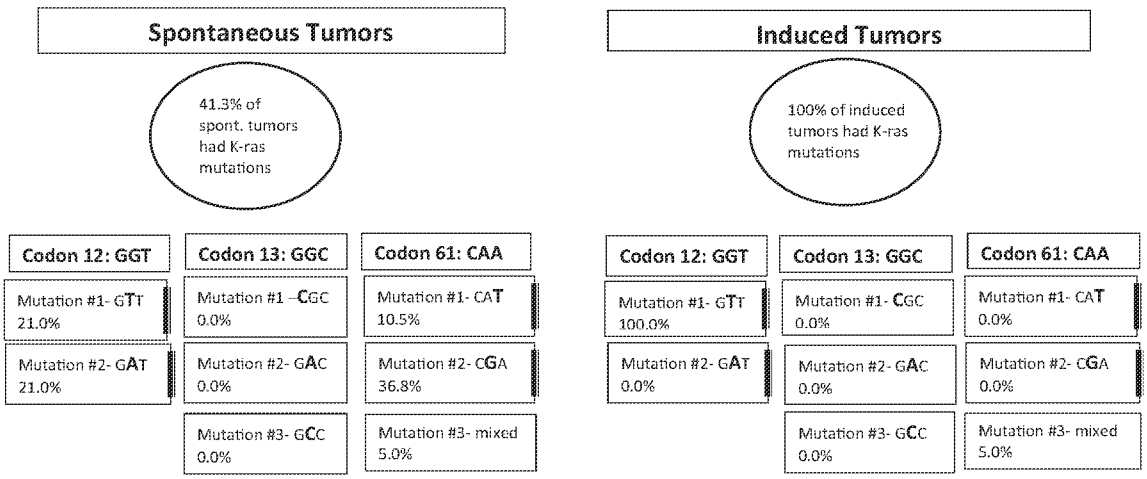

Fig. 10. Comparison of K-ras mutation spectra for spontaneous tumor and AMMN-induced tumors in A/J lung tumors (Source: Matzinger et al., 1994; Ronai et al., 1993; Sill et al., 1999b); marked boxes indicate oncogene mutations that differ between spontaneous and induced tumors. **Conclusion**: Induced lung tumors showed a significantly different mutation spectra as highlighted for Codon 12 (i.e., mutations #1 and #2), and codon 61 (mutations #1 and #2) than the spontaneous tumors. *Mutation percentages were summed across codons 12, 13, and 61 for both spontaneous and induced tumors.

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Table 19
Differentiating spontaneous vs induced mutations: Perspectives offered by leading researchers.

References	Quotes
Antal et al. (2002)	"In lung tumors…the frequencies of many alterations were different in radiation-induced and spontaneous tumors, suggesting that different oncogenic pathways were activated during spontaneous and radiation-induced lung carcinogenesis of mice." Page 122
Hayashi et al. (2001)	"Evaluation of ras mutations in ADBAQ-induced forestomach tumors showed a high frequency of chemical-specific point mutations. Predominant H-ras codon 61 CAA to CTA transversions were detected in ADBAQ-induced but not spontaneous tumors. The A- > T transversions were not considered to be spontaneous because such mutations have not been detected in spontaneous forestomach tumors examined to date from $B6C3F_1$ mice." Page 427, left column
Hoenerhoff et al. (2013)	"While spontaneous and GBE-treated HCC in this study were morphologically very similar, in terms of their gene expression and mutation spectra, these tumors are actually quite different. HCCs in mice exposed to GBE were characterized by dose-dependent *Ctnnb1* mutations, with an increased incidence of deletions and multiple mutations per tumor in some high-dose animals. These features are markedly different from spontaneous HCC in this strain, which does not typically harbor deletion mutations or multiple mutations, and has a relatively low concurrent (0%) and historical control (2%) incidence rate of *Ctnnb1* mutations (Hayashi et al., 2003)." Page 12, Discussion section "…while spontaneous and GBE-treated HCC in B6C3F1 mice are very similar at the morphologic level, we have shown that the molecular alterations in GBE-treated tumors are very different from those seen in spontaneous tumors. These include unique alterations in *Ctnnb1* gene and protein expression, structure, and function;…" Page 14
Hoenerhoff et al. (2013) (Continued)	"Additionally, marked differences in global gene expression profiling, including overrepresentation of cancer signaling pathways, xenobiotic metabolism, and oxidative stress, shows that while spontaneous and GBE-treated HCC are morphologically indistinguishable, they may be distinguished based upon their transcriptomic profiles. This is of considerable importance when distinguishing between a background tumor incidence and chemically induced neoplasms, particularly in strains with moderate background tumor rates." Page 14
Hong et al. (2003)	"Spontaneous hemangiosarcomas from control mice lacked both p53 and β-catenin protein expression and ras mutations. Our data indicated that p53 and β-catenin mutations in the *o*-nitrotoluene-induced hemangiosarcomas and K-ras mutations and p53 protein expression in riddelliine-induced hemangiosarcomas most likely occurred as a result of the genotoxic effects of these chemicals." Page 227, Abstract
Hong et al. (2003)	"…in some neoplasms the profiles of activation mutations in ras genes or inactivating mutations in the p53 gene are specific for particular chemicals and differ from those detected in spontaneous neoplasms." Page 228, left column
Hong et al. (2015)	"In summary, there was a significantly elevated incidence of Kras mutations, accompanied by a lower incidence of Egfr and Tp53 mutation in lung tumors from mice and rats chronically exposed to CMD compared to SL tumors. The mutations detected in the Kras, Egfr, and Tp53 genes in CMD-exposed mice and rats clearly imply those genetic events are related to chemical exposure, since those mutations were not detected in the concurrent spontaneous tumors in mice and SL tumors from previous NTP studies in rats." Page 880, left column
Houle et al. (2006)	"…the chemically induced tumors exhibited a distinct shift in the P53 and H-ras mutational spectra compared to spontaneous tumors suggesting that benzene and EtO exposure induced mammary specific alterations predisposing female mice to mammary tumor development. The difference in mutation profiles between spontaneous and chemically induced neoplasms suggest that different mechanisms are likely involved." Page 756, Discussion, right column
Houle et al. (2006)	"A comparison…the cumene-induced tumors displayed specific p53 mutations. P53 mutations were observed only in exon 5 (24/27, 89%) and exon 7 (3/27, 11%). The mutations observed in the p53 gene in cumene-exposed mice clearly imply this genetic event is related to chemical exposure, since these mutations were not detected in spontaneous tumors." Page 725, left column
Hong et al. (2007)	"The predominant [K-ras] mutations [in B6C3F1 mice] in EO-induced Harderian Gland (HG) neoplasms consisted of GGC to CGC transversions at codon 13 (15/18, 83%) and GGT to TGT transversions at codon 12 (8/18, 44%). Neither of these mutations was found in spontaneous HG neoplasms (0/27)." Page 82, right column
Iizuka et al. (2010)	"To clarify how ionizing radiation induces mammary carcinogenesis, we characterized genomic copy number aberrations for γ-ray-induced rat mammary carcinomas using micro-array-based comparative genomic hybridization. We examined 14 carcinomas induced by γ-radiation (2 Gy) and found 26 aberrations, including trisomies of chromosomes 4 and 10 for three and one carcinomas, respectively, an amplification of the chromosomal regions 3g35g36, Sg32 and 7911 in two, two and four carcinomas, respectively. These aberrations were not observed in seven spontaneous carcinomas". Page 206, abstract
Iizuka et al. (2010)	"…we found that the majority of radiation-induced carcinomas, but not spontaneous ones, had some form of DNA copy number aberration, illustrating the association between copy number aberrations and radiation-induced rat mammary carcinomas." Page 212, left column
Imaoka et al. (2008)	"Gene expression profiles of three spontaneous and four radiation-induced carcinomas, as well as those of normal mammary glands, were analyzed by microarrays…we identified 50 genes that had different expression levels between spontaneous and radiogenic carcinomas… Thus, gene expression analysis distinguished between spontaneous and radiogenic carcinomas suggesting possible difference in their carcinogenic mechanisms." Page 349, Abstract
Imaoka et al. (2008)	"We show for the first time that radiation-induced rat mammary cancer is distinguishable from spontaneous ones according to their gene expression." Page 357, left column
Imaoka et al. (2008)	"This data indicates that spontaneous and radiogenic mammary cancer development involves distinct molecular and cellular mechanisms." Page 359, left column
Kawano et al. (1996)	"In the present study, K-ras gene mutations were identified in 72.2% of all the NNK-induced lung lesions examined and the major mutation was G to A transition at the 2nd base of codon 12, which clearly differed from the mutational pattern in untreated control mice [tumors]." Page 48, Right column.

(*continued on next page*)

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Table 19 (*continued*)

References	Quotes
Liu et al. (2003)	"Total exon deletion did not exist in any spontaneous mutants but in γ-rays- and ENU-induced mutants. The proportions of deletion mutations were quite different between spontaneous mutants and induced mutants, and similar change of γ-rays- and ENU-induced mutants occurred at the hprt locus." Page 580, right column
Loktionov (1991)	"…A- > T transversions in the second position of codon 61 of Ha-ras were present only in liver tumors [CD− 1 mice] that developed in mice treated with DMBA…" Page 1189, Abstract
Lutz et al. (2002)	"The "additivity to background" concept which suggests low-dose linearity may also be questioned. The incremental DNA damage exerted by a genotoxic carcinogen may not always increase the probability of mutation. For instance, if exposure to a genotoxic agent induces DNA repair, and if the induced repair capacity also repairs background DNA damage, a non-monotonic (U-shaped) dose response might result. This type of dose response can no longer be dismissed (Calabrese and Baldwin, 2001a,b). It could also mean that a threshold-like dose response may in fact be U-shaped, and that the process of spontaneous carcinogenesis could even be slowed down at low dose of a "carcinogen"." Page 336, left column
Marxfeld et al. (2006)	"In this study, the histologically similar spontaneous and DMBA-induced adenocarcinomas (Figs. 1 and 2) could be separated by gene expression profiling." Page 159, right column
Maas et al. (1996)	"The distribution of these mutations is different from those in untreated animals from two previous studies." Page 1703 Note: These mutations are in lung tumors of A/J/ mice treated with benzo(b)fluoranthene
Nishimura et al., 1999	"To gain information on the possible role of Ras activation in development of thymic lymphomas in scid mice, we have examined both the frequency and the spectrum of Kras and Nras mutations in spontaneous and radiation-induced lymphomas. Neither activated Kras nor Nras genes were detected in spontaneous lymphomas, while Kras mutations increased in a dose-dependent manner in radiation-induced lymphomas." Page 142, Abstract
Schwartz et al. (1994)	"In the spontaneous mutants, most of the partial gene deletion mutations involved exons 4–6. In contrast, only exon 1 was deleted in the γ-induced partial deletion mutants so far, and in the α-induced spectra, deletion of exons 1–3 and 6–9 were found; no deletions of exons 4 or 5 were seen in either radiation-induced spectrum." Page 539, right column
Sills et al. (1995)	"…almost a third of the neoplasms induced by ozone exposure had an A- > T transversion in the second base of codon 61, a mutation not detected previously in 66 spontaneously arising lung neoplasms of B6C3F1 mice (19, 28) or in lung neoplasms from concurrent air controls." Page 1624, right column
Sills et al. (2001)	"…a high frequency of H-ras codon 61 CAA- > CTA transversion (10/41; 24%) was detected in chemically induced forestomach neoplasms, but none were present in the spontaneous forestomach neoplasms examined." Page 373, Abstract
Sills et al. (2001) (Continued)	"This is the first report in which the ras mutation frequency and spectra had been determined for spontaneous forestomach neoplasms of the B6C3F1 mouse… The exposure-related changes in ras mutations observed in this study were qualitative and quantitative, presenting both a different mutation spectrum from that of spontaneous occurring neoplasms of the forestomach, and an increase in the fraction of tumors containing ras mutations." Page 381 "The lack of H-ras codon 61 CTA mutations and the rare detection of K-ras codon 13 CGC mutations in the spontaneous forestomach neoplasms strengthens the hypothesis for a causal link between chemical exposure, ras mutations, and forestomach neoplasia following exposure to 1,3-butadiene and the structurally related analogs." Page 383
Tou et al. (2004)	"BMP-Induced Lung Tumors" "The predominant pattern of K-ras mutation detected in BMP-induced lung neoplasms consisted of G- > A transition (GGT- > GAT) at codon 12 (20/29)…it was significantly different from that observed in historical controls…" Page 17, right column
Tou et al. (2004)	"TNM-Induced Lung Tumors" "The predominant K-ras mutation detected in TNM-induced lung neoplasms consisted of G- > A transitions (GGT- > GAT) codon 12 (13/15) compared to none in spontaneous lung neoplasms from the inhalation control mice…" Page 18, left column

affected individuals that have been the object of substantial focused investigation with respect to this genomic landscape. Such investigations have revealed marked mutational differences in the tumors of smokers and non-smokers lungs with non-small cell lung cancer (Govindan et al., 2012). These include a different mutation spectrum, with C:G- > A:T predominating in smokers while in non-smokers C:G- > T:A predominated. There are also specific groups of mutations found in never smokers (i.e., EGFR mutations and ROS1 and ALK fusions) with KRAS, TP53, BRAF, JAK2, and JAK3 and mismatched repair gene mutations in smokers. These differences between smoker and non-smoker lung cancer subjects are therefore substantial and not linked to a few genes (Subramanian and Govindan, 2013). The findings indicate that induced and spontaneous lung cancers in humans are fundamentally different with respect to driver mutations.

These developments challenge both the biological foundation between spontaneous and induced tumors for driver mutations and the accompanying uncertainty of the additivity to background assumption in multiple ways, including the complexity of the process of carcinogenesis, its progressively hyperindividualistic tumor development, the presence of differing driver mutations between spontaneous and induced tumors, and the difference in the number and sequence of contributing mutations.

Given this complexity and the growing recognition of the different mutational signatures, there is no convincing biological justification for the use of the additive to background assumption as a default feature for cancer risk assessments. It is possible to speculate that a certain proportion of tumors may have some unknown mechanistic overlap, but how these occur and what an overlap means biologically, and how it may affect tumor progression for spontaneous and induced tumors is unknown. The closest linkage of these recent tumor complexity developments (Lin et al., 2007; Jones et al., 2008; Killela et al., 2013; Vogelstein et al., 2013) to the present additive to background

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assessment is that spontaneous and induced tumors have significant mutational differences which are likely to contribute to the individual tumor progression and hyperindividualisticity making the comparison between spontaneous and induced tumors highly tenuous and excessively speculative.

4.6. Additive to background for cancer vs non-cancer endpoints

The issue of additive to background was formulated to address low dose cancer risk assessment concerns. The validity of the additive to background assumption in this cancer risk assessment context is the focus of the present paper. However, there has been a debate over whether the additive to background concept would also apply to non-cancer endpoints. While this debate does not seem unlike the cancer risk assessment issue with discussion focusing on the occurrence of ongoing disease processes and heterogenicity within the human population, Rhomberg et al. (2011) argued that cancer risk from genotoxic agents is qualitatively different with the discrete and stochastic features of induced mutations, which is assumed to originate in a single cell, with subsequent monoclonal expansion. The cancer disease process is profoundly different from how most non-cancer effects seem to occur. However, even with such markedly different aspects to the disease processes for cancer and non-cancer effects, the additive to background assumption still would require that non-cancer endpoints occur via the same mechanisms for the inducing agents and the background disease processes. As seen in the present paper, advances in molecular biology have revealed that different mechanisms seem to be the rule rather than the exception. If this is the case for non-carcinogen endpoints, then current theoretical arguments made on behalf of this hypothesis (White et al., 2009) (see Crump, 2017 and Bogen, 2017 debate) would be challenged to provide the specific mechanistic data.

5. Conclusions

The question proposed in this paper is that posed by Hoel (1997), that is:

> "Whether or not the original simple idea of background additivity is consistent with today's biology and whether the concept, if true, has any value for quantitative risk estimation."

1. Multiple, complementary and converging lines of evidence indicate that the "original simple idea" of additive to background for the induction of tumors that became incorporated into regulatory agency (i.e., EPA) cancer risk assessment is **not** consistent with modern molecular biology and toxicology.
2. Studies from 45 carcinogens, over a dozen animal models and a broad range of organ- specific tumors contradict the additive to background assumption that induced and spontaneous tumors act via identical mechanisms.
3. Detailed genetic landscape studies of multiple human tumors, based on detailed assessments of driver and passenger mutations, support these animal bioassay studies by their illustration of the progressive hyperindividualistic tumor development.
4. Carcinogen treatment in the chronic bioassay commonly reprograms biological processes markedly altering oncogene activities, mutation frequency and the mutation spectra of normally developing spontaneous tumor development processes. These changes can profoundly alter spontaneous tumor incidence and phenotype and the control group reference for carcinogen treatment evaluation. These findings indicate that the traditional control group may not be a useful standard for comparison, since the biological/genetic reprogramming, due to carcinogen treatment, can significantly change the "background" tumor expectation observed in the treatment group. These findings challenge the validity and utility of the additive to background and the independent of background concepts.
5. While it is now known that the induced tumor mechanisms are very likely to be profoundly different than spontaneous tumor mechanisms, a principal risk assessment question is what proportion of the overall cancer mechanistic progress may overlap between these tumors and to what extent would any degree of overlapping affect the risk estimation. Since this area is extremely speculative and complex, it is not possible to provide specific qualitative and quantitative estimates. It would also not be possible to extrapolate information from one tumor type to another.
6. The present assessment reveals that the original additive to background assumption is not supported by modern molecular biology and toxicology. Numerous findings contradict the additive to background hypothesis of identical mechanisms for spontaneous/ induced tumors. There is also a lack of information concerning whether and to what extent there may be some degree of mechanistic overlap between the spontaneous/ induced tumor processes or even if this is an appropriate question. It is not known if similar partial pathway(s) occurring at different time(s) affect the cancer process, time to tumor, how this may be affected by dose and any biological implications if mediated by driver or passenger mutations in unique combinations and/or sequences.

Trying to formulate a modified additive to background policy on a hypothetic basis of what proportion of assumed processes might be identical for spontaneous/induced tumors adds an even more uncertain perspective which should caution against governmental risk assessment policy/procedures on such matters. Furthermore, observations that carcinogen treatment can reprogram spontaneous tumor endpoints may have significant risk assessment implications, contradicting the additive to background and independent of background assumptions. Neither the continued use of the additive to background assumption/procedure or a switch to an independent of background assessment process appears justified. In their absence this would return the evaluative process to a direct experimental comparison without underlying hypothetic assumptions.

Declaration of Interest

None.

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Edward J. Calabrese

Abstract

This historical analysis indicates that it is highly unlikely that the Nobel Prize winning research of Hermann J. Muller was peer-reviewed. The published paper of Muller lacked a research methods section, cited no references, and failed to acknowledge and discuss the work of Gager and Blakeslee (PNAS 13:75-79, 1927) that claimed to have induced gene mutation via ionizing radiation six months prior to Muller's non-data *Science* paper (Muller, Science 66(1699):84-87, 1927a). Despite being well acclimated into the scientific world of peer-review, Muller choose to avoid the peer-review process on his most significant publication. It appears that Muller's actions were strongly influenced by his desire to claim primacy for the discovery of gene mutation. The actions of Muller have important ethical lessons and implications today, when self-interest trumps one's obligations to society and the scientific culture that supports the quest for new knowledge and discovery.

Keywords: Nobel prize, Peer-review, Hermann J. Muller, Cancer risk assessment, Mutation

Background

Hermann J. Muller's reporting that X-rays induced gene mutations in the fruit fly has long been considered an interesting one [8]. Muller was in a tight race with at least three other research groups to be the first to induce gene mutations; the professional stakes were very high [4]. It was believed essential to be the first to report one's discovery to secure honors, awards and enhanced research support. Muller's groundbreaking paper was published in the July 22, 1927 issue of *Science* under the title, "The Artificial Transmutation of the Gene" [18]. Since the claimed findings were of fundamental importance, many researchers were extremely disappointed and puzzled that the paper contained no data, only a discussion of data that no one, apparently other than Muller, had seen. To only "discuss" one's data in this instance was seen as a poorly disguised effort to claim primacy for a novel and significant discovery that was premature. Why the editors at *Science* permitted Muller with their vehicle to manipulate the scientific community, ignoring the standard protocols/rules of scientific publication, has never been clarified by this journal, nor by Muller based on a review of publications and letter correspondence. So bizarre was this deviation from accepted protocol that Muller's former Ph.D. advisor at Columbia University, Thomas H. Morgan, suggested that perhaps Muller did not have the data to support his claims [8].

Muller's Nobel Prize data paper

The rampant speculation came to a head when Muller presented the data some three months after the *Science* publication at the 5th International Genetics Congress (September 11–18, 1927) in Berlin, finally silencing the critics. He showed that X-rays produced transgenerational phenotypic changes in the fruit fly, which he asserted were due to gene mutations. These changes were also induced with striking frequency. Muller became a highly recognized scientific leader within days as the media quickly grasped the potential significance of his discovery. However, he still had not published these research findings. These data, which would provide the basis for his eventual Nobel Prize in 1946, were subsequently published in 1928 in the Proceedings of the International Genetics Congress [20]. Despite its significance, this paper was not widely

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accessible, being found in few libraries worldwide. Thus, the "data-less/discussion-only" *Science* publication became the publication of note, even today, based on citation frequency. The data-based proceedings paper would be cited only about once/year in the basic Web of Science search until his Nobel Prize in 1946 and even less frequently thereafter.

Nearly a decade ago I obtained the Muller's 1928 Genetics Congress proceedings paper, being interested to learn more precisely how he verified the 1927 *Science* statements. However, this paper proved to be problematic as it failed to include a methods section, nor a normal discussion section that places his findings within the context of other relevant work, failing to cite any references. This omission was particularly glaring since Gager and Blakeslee [12] had reported in *Proceedings of the National Academy of Sciences* (PNAS) evidence for the first time of true gene mutations (via the use of ionizing radiation), some six months prior to Muller's *Science* paper. These same researchers also presented their mutation data at the Berlin conference with Muller and are also published in the same International Genetics Congress proceedings. The failure of Muller to acknowledge the January 1927 PNAS Gager and Blakeslee findings/claims is a serious issue, since both groups were claiming primacy for the induction of gene mutation.

Of further relevance was Muller's failure to address the possibility that his "gene" mutation findings could have been simply due to heritable chromosomal mutation, via the deleting of large gene segments. This issue was a crucial, yet initially hidden, concern of Muller. It was discussed privately by Muller and his colleague/friend Edgar Altenburg during the late summer/fall of 1927. He would eventually publicly discuss the matter at the annual American Association for the Advancement of Science (AAAS) meeting in December 1927 [19] and at a presentation to the National Academy of Sciences (NAS) in the spring of 1928 [21] as he tried to get control of an issue before it might undercut his striking success. However, this issue would not disappear, as Lewis J Stadler, from the University of Missouri, would challenge Muller's findings starting in 1931 until his death in 1954, claiming he did not induce point mutations but instead induced gapingly large chromosomal deletions [24–26].

In addition to the above issues, Muller's paper also seemed poorly constructed, requiring revision and clarification to enhance readability. The paper had other limitations of a more specific nature that could have been raised in a review process. While the purpose of the present paper is not to retrospectively review Muller's paper some 90 years later, but to present a few additional issues that may have warranted responses from contemporary peer-reviewers such as: failure to include a control group for experiment #3, and the use of only two doses, especially when using doses that were very high for experiments #2 and #3 with his new, more sensitive and then unvalidated fruit fly model. Furthermore, the initial experiment (#1) with a standard fruit model was performed with four doses, with the upper doses inducing substantial sterility while the lowest dose, which was still quite high, showed no evidence of transgenerational phenotypic (i.e. mutational) changes. These points were either only casually addressed, or ignored.

Upon reflection, it appeared that there was very little, if any, possibility that this paper had been peer reviewed due to its multiple critical information omissions, experimental design and evaluation questions and a lack of clarity in writing style. Despite its extraordinary gene mutational claims, the manuscript required considerable improvement prior to being acceptable for publication. These issues led to my obtaining a copy of Muller's research notebook, containing the data for his Nobel Prize research during the time period for the respective experiments. I also obtained numerous communications between Muller and colleagues to explore whether the manuscript was peer reviewed and if it was, what the evaluations were. The information revealed evidence within a letter from Muller to Edgar Altenburg on July 8, 1946 [22] that the manuscript that he read at the 5th International Genetics Congress in Berlin nearly 19 years earlier most likely did not undergo peer-review prior to publication. In that letter, Muller stated:

> **"This is to answer your question about when I first suggested the making of cytogenetic maps. In my paper given to the 1927 Congress in Berlin, it is stated on page 245 that the structural changes produced by x-rays should make it possible to obtain more direct evidence of the physical correctness of the linage maps. This paper, though printed first in 1928, was printed from a manuscript given (in) at the time of the Congress in 1927 where it was presented exactly as finally printed."**

Muller indicates that his presentation at the Congress in 1927 was published exactly as presented with no changes made. This statement reveals that the editor(s) of the Congress very likely published it without peer-review.

Discussion

A legitimate peer-review may have highlighted important methodological/study design concerns such as the requirement for a methods section, the need to discuss relevant research such as the claims of Gager and Blakeslee [12] for gene mutation findings, as well as addressing whether the high doses of radiation were simply poking large holes via deletions in the chromosomes rather than inducing point mutations. These and other possible

clarifications and the further reviewing of a revised manuscript would have delayed publication, possibly threatening Muller's claim to primacy. The outcome and time-duration of the review process are always difficult to predict. While the letter of July 8, 1946 between Altenburg and Muller as quoted above does not specifically address the issue of peer-review, it nonetheless leads one to reasonably conclude that there was little likelihood of peer review for his Nobel Prize findings.

The failure to receive appropriate peer review created the opportunity for Muller to quickly gain attention and fame, at the expense of others. It also seriously affected the direction of the field since he incorrectly linked the induction of transgenerational phenotypic changes induced by very high doses of X-rays with gene mutation and then to linear dose response modeling for risk assessment without convincing evidence. This critical belief came to dominate the field, misdirecting radiation genetics, environmental mutagenesis and cancer risk assessment until finally shown to be incorrect decades later [2, 3, 5–7]. Therefore, the failure to receive peer-review as a result of circumventing normal publication procedures at *Science* and publication of his critical data paper in a non-peer reviewed conference proceeding allowed Muller to side-step normal protocols by which the scientific community polices itself, to weed out inferior manuscripts and to enhance the quality of those deemed acceptable. These procedures not only aid society, they are intended to educate the researcher. Muller's actions were clearly manipulative and self-serving within a context of high stress and excitement. Further, his actions were unfair to the other competitors chasing the gene mutation prize and harmful to society that needs scientists to follow the procedure, even though the entire review process can be frustrating, time-consuming, and very imperfect.

The issue of peer review is relevant to Muller's Nobel Prize research activities. The question may be raised as to what was the nature of the peer review in the early decades of the twentieth century. While the history of peer in the early part of the twentieth century is not particularly clear due to inadequate record keeping, various biological/medical journals of the early twentieth century conducted their peer-review via a committee/editorial board system, which progressively expanded to include reviewers outside the Editorial Board. The *British Medical Journal* reports a modern sounding peer review process as early as 1893 [1]. A similar set of procedures was described for the journal *Surgery, Gynecology and Obstetrics* in 1905 [1].

An insight into how radiation geneticists viewed peer-review may be gained by a consideration of the actions/leadership of Stewart Gager and the activities of the *American Journal of Botany* (AJB). Gager, who was the co-author of the first paper to show gene mutation as noted above, was on the editorial board of the AJB starting in 1915, being its business manager for 21 years (until 1936). During this time, the journal developed a modern peer-review process with the goal of providing "a hopeful means of preventing publication of inferior papers and improving the quality of others". This process was in place by 1933. Of significance is that the AJB editorial board had representatives from a broad spectrum of affiliated professional societies, including the American Phytopathological Society, the Ecological Society of America, The American Society of Plant Physiologists, The Mycological Society of America and the Genetics Society of America. Referees were typically selected by an editorial board member with the closest subject matter expertise to the paper. Rejection of a paper had to be approved by at least one member of the board along with the editor-in-chief [23]. This historical reconstruction indicates that peer-review was well developed, sophisticated and built into the core framework of a broad spectrum of the biological sciences in the United States (U.S.) by the early 1930s.

The earliest historical linkage of peer-review to Muller may be seen with the *Journal of Experimental Zoology*. This journal was created in 1903 with the first issue published in 1904. Amongst the founding and longstanding editorial board members was Thomas H. Morgan, who would become Muller's Ph.D. advisor a decade later. In a retrospective recounting of the journal, the managing editor Ross G. Harrison [13] indicated that peer-review was incorporated from the onset. The paper specifically reported on the percentage of submitted manuscripts rejected over the course of the first 40 years of the journal. In the case of Muller, a search of the Web of Science indicates he published three genetics papers within the *Journal of Experimental Zoology* [15–17]. This information provides unequivocal evidence that Muller was part of a scientific culture of peer review, with an advisor who was a leader on this issue and that Muller personally experienced the peer review process in this journal. Other journals that Muller published in also had established peer-review processes such as *Genetics*, which T.H. Morgan also helped create in 1916 [11], with Muller publishing in this journal five times by 1928. There is also a documented incident of Muller resigning from the editorial board of *Advances in Genetics* over a dispute with the editor M. Dermerc. In this case, Muller was angry because a manuscript was published as a result of a favorable peer-review. Muller was frustrated that Demerec had not selected him as a reviewer. In their exchange of letters, Demerec ended up accusing Muller of attempting to impose his version of censorship [10]. There was, therefore a "culture" of peer-review, that existed and that Muller was part of it from his earliest days as a graduate student at Columbia. These examples are relevant to the Muller story, demonstrating peer-review activity

before and within the time period of Muller's significant work, linking it to the genetics community, to his research mentor T.H. Morgan, one of his rivals, Stewart Gager, and his own published research.

With respect to Muller, it was clear that he knew where his gene mutation interpretational weakness lay. In fact, the gene mutation criticism of Stadler [24, 25] was first brought up by Muller in December, 1927 at the AAAS meeting in Nashville but in a manner that he could control, direct and marginalize. When Stadler [24, 25] raised the issue it was based on new advances in cytogenetics as developed and applied by Barbara McClintock [14]. In fact, it was the Stadler-McClintock criticism that would stick and compel Muller's research attention for decades as he tried to defend his gene mutation interpretation.

Muller was focused on being the first to produce gene mutation. He knew it was critically important and so did others. The publication actions of Muller (i.e., *Science* journal discussion only and the non-peer-reviewed Congress proceedings) were directed toward being first, even if it meant skirting the protocol of the publication process. Muller also cited the date of publication of his key Proceedings paper incorrectly, making it 1927 rather than 1928. He did this in other subsequent publications, influencing many others to adopt the same publication date error, but all pointing to the notion that Muller was first. In the end, what this meant was that Muller won the race, got the prize and yet would have his key finding, that he produced gene mutations, recognized as incorrect even by his closest colleagues long after his death [9] (See [4] for a detailed review).

Would a professional peer-review had detected the error of Muller's gene mutation interpretation? While this is not known it is likely that reviewers would have demanded that Muller include a methods section, and a legitimate discussion component to the paper at the least. These would have involved delays. What would have happened in a review process will remain speculative. However, one can see in Muller's actions how they were not fair to this competitors as well as the scientific community and society who need scientific contributions vetted in an objective manner that ensures an appropriate evaluation of the submitted manuscript.

Conclusion

This paper reveals that the strategy of Hermann J. Muller to obtain primacy for his discovery of radiation-induced gene mutations led to his avoiding peer-review, publishing a paper that had significant limitations despite its Nobel Prize award quality. Muller's actions were troubling as he placed his prize-seeking goals ahead of his responsibility to the field and social obligation of playing by the same publication rules as other researchers. The peer-review process is important and even Muller's Nobel Prize winning research would have profited from this. Furthermore, we have now learned with modern nucleotide assessment technology, that his Nobel Prize winning findings of producing gene mutations by high doses of X-rays were not gene mutations, but gross gene deletions and other major chromosome rearrangements, a finding that confirmed the doubts of some leading geneticists of his era who had clearly suspected that he had incorrectly interpreted his findings.

Abbreviations

AAS: American Association for the Advancement of Science; AJB: American Journal of Botany; NAS: National Academy of Sciences; PNAS: Proceedings of the National Academy of Sciences; U.S.: United States

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Authors' contributions

Calabrese conceived, researched, and wrote this contribution by himself. The author read and approved the final manuscript.

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Not applicable

Competing interests

The author declares that he has no competing interests.

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From Muller to mechanism: How LNT became the default model for cancer risk assessment☆

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ABSTRACT

This paper summarizes the historical and scientific foundations of the Linear No-Threshold (LNT) cancer risk assessment model. The story of cancer risk assessment is an extraordinary one as it was based on an initial incorrect gene mutation interpretation of Muller, the application of this incorrect assumption in the derivation of the LNT single-hit model, and a series of actions by leading radiation geneticists during the 1946–1956 period, including a National Academy of Sciences (NAS) Biological Effects of Atomic Radiation (BEAR) I Genetics Panel (Anonymous, 1956), to sustain the LNT belief via a series of deliberate obfuscations, deceptions and misrepresentations that provided the basis of modern cancer risk assessment policy and practices. The reaffirming of the LNT model by a subsequent and highly influential NAS Biological Effects of Ionizing Radiation (BEIR) I Committee (NAS/NRC, 1972) using mouse data has now been found to be inappropriate based on the discovery of a significant documented error in the historical control group that led to incorrect estimations of risk in the low dose zone. Correction of this error by the original scientists and the application of the adjusted/corrected data back to the BEIR I (NAS/NRC, 1972) report indicates that the data would have supported a threshold rather than the LNT model. Thus, cancer risk assessment has a poorly appreciated, complex and seriously flawed history that has undermined policies and practices of regulatory agencies in the U.S. and worldwide to the present time.

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1. Introduction

While a role of the environment in affecting the occurrence of cancer has long been known (e.g., the occurrence of testicular cancer in chimney sweeps) (Pott, 1775), transitioning this recognition of concern into an experimental science proved to be difficult as seen in the series of failures to induce skin cancer in animal models during the early years of the 20th century. Finally, after many failed attempts, in 1918 Japanese researchers made the experimental breakthrough by the repeated administration of coal tars to the ears of rabbits to produce papillomas and carcinomas (Yamagiwa and Ichikawa, 1918). This seminal finding paved the way for experimental research to assess possible environmental causes of cancer.

In a similar manner, researchers early in the 20th century began to explore whether it was possible to induce mutations in plants and animals (Campos, 2015). While it took nearly three decades, Muller (1927a) reported that X-rays induced gene mutations in fruit flies, narrowly beating three independent teams of botanists who likewise reported inducing transgenerational phenotypic changes with X-rays/radium.[1] Muller's findings, like that of the Japanese cancer researchers, quickly transformed the field. For his discovery, Muller received the Nobel Prize in 1946. The current paper clarifies the historical foundations of the LNT single-hit dose-response model, its unique dependence upon the gene mutation interpretation of Muller in 1927, and how this interpretation became accepted by the scientific community and regulatory agencies. Most importantly, it will be shown that: (1) Muller's claim that the X-ray-induced transgenerational phenotypic changes were due to gene mutations was an interpretation lacking convincing evidence; (2) the induced transgenerational phenotypic changes

☆ This paper has been recommended for acceptance by B. Nowack.

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[1] In January 1927, in the *Proceedings of the National Academy of Sciences* (Communicated January 14, 1927), Gager and Blakeslee (1927) were the first to report cases of gene mutations. Thus, Muller's July 1927 publication was the second to report the gene mutation phenomenon. Muller gained acclaim because he produced many mutations quickly. However, Gager and Blakeslee repeatedly reminded the field of their primacy. In his effort to secure scientific honors, Muller (1927a, 1928a) failed to cite the earlier work of Gager and Blakeslee (1927).

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were due to chromosomal deletions and aberrations, not Muller's proposed gene "point mutations"; (3) these developments undermine the historical and scientific foundations of the LNT single-hit model since it was built upon Muller's gene mutation interpretation (see Calabrese, 2017a for a significantly expanded analysis of this issue); (4) Muller and other leading U.S. radiation geneticists would collude in a series of articles to promote acceptance of the LNT, making deliberate deceptions and misrepresentations of the scientific record; (5) the deceptive practices would infiltrate and culminate in the actions of the U.S. NAS BEAR I Genetics Panel that recommended adoption of the LNT model by regulatory and public health agencies in 1956 (Anonymous, 1956) (See Calabrese, 2015a, b, c); (6) the mouse data used to provide the experimental basis for the subsequent reaffirmation of the LNT for cancer risk assessment was similarly problematic, that is, the BEIR I NAS/NRC (1972) Committee used a flawed historical control group that significantly overestimated risk in the low dose zone, yielding a linear dose response (see Calabrese 2017b, c); (7) use of a corrected historical control value yields a threshold rather than the linear dose response and; (8) this new assessment indicates that the LNT has been flawed from the start, yet national and international regulations have continued to be based upon it (Calabrese, 2015a, 2017d).

2. Muller and mutation

Hermann J. Muller, a radiation geneticist at the University of Texas/Austin, truly burst upon the national and international scene following his presentation at the 5th International Genetics Congress in Berlin during September 1927. His highly anticipated presentation convincingly demonstrated to an eager and massive grouping of geneticists from around the world that X-rays could induce transgenerational phenotypic changes in *Drosophila* perhaps providing a mechanism for evolution. Muller claimed that these changes were the result of induced gene mutation, tiny genomic changes, with Muller coining the term "point mutation". Muller not only claimed to be the first to ever artificially induce gene mutation, he produced copious numbers of them. Muller's presentation drew especially great anticipation since his article in the journal *Science*, published about three months earlier, only discussed some of the new findings, inexplicably failing to show any data. Thus, Muller, with a flair for the dramatic, disproved the doubters and set himself on a path that 19 years later would result in another trip to Europe, Stockholm, to receive the Nobel Prize in Biology and Medicine.

Muller's stunning results soon inspired: (1) numerous laboratories to redirect their research to the assessment of ionizing radiation induced mutations (Campos, 2015); (2) the creation of the Genetics Society of America (GSA) (1931) a few years later, bringing zoologists and botanists who were researching genetics under one integrated professional society; (3) the concept of a Proportionality Rule that describes the linear dose response for the ionizing radiation induced mutation response (Muller, 1930a); (4) the interdisciplinary collaboration of leading physicists and radiation geneticists to create the first mechanism-based cancer risk assessment model (LNT single-hit model) using target theory (Timofeeff-Ressovsky et al., 1935) and (5) the discovery of chemically induced mutations by Charlotte Auerbach in the 1940s (Auerbach and Robson, 1946). The reach of Muller was long and influential, inspiring the focus of Carson (1962) in her seminal book *Silent Spring*, that is normally given credit for starting the environmental revolution of the late 1960s and 1970s and continuing to the present. Muller wrote a powerfully supportive review of *Silent Spring* in the New York Herald Tribune published on the Sunday prior to the book's publication four days later (Muller, 1962). Thus, the X-ray induced "gene" mutation findings of Muller and his leadership over the next 40 years would profoundly affect the environmental movement and the fields of genetic toxicology, cancer risk assessment and numerous medical, radiation and public health practices.

There is therefore little question that Muller had a major influence on the scientific community and the general public, originating from the belief that he had actually demonstrated that X-rays produce gene mutations in the fruit fly. While the above summary highlights some of the societal impact of Muller, there are important parallel concerns with Muller's scientific legacy. In brief, Muller (1927a) made the critical assumption that the numerous X-ray induced transgenerational/heritable phenotypic changes that he reported were the result of induced gene mutations. Muller knew that transgenerational/heritable phenotypic changes via X-ray-induced chromosomal aberrations was not a significant finding (Muller, 1928b). This had been reported previously and would not affect an understanding of basic biological themes such as evolution and its potential mechanism. This was why Muller (1927a) entitled his groundbreaking July 22, 1927 article in *Science* "The Artificial Transmutation of the Gene".

3. Point mutations vs gene deletions

Within three months of his presenting these findings at the Genetics Congress[2] in Berlin (September, 1927) (Muller, 1928a), Muller (1927b) would publically express concerns that some might think that all he had done was to shoot large holes (i.e., deletions) throughout the genome with the high doses of X-rays used, noting that such concerns/questions were initiated by his longtime friend, close colleague, collaborator and confidante, Edgar Altenburg, a professor of genetics at Rice University. Within this anticipatory defensive context, at the December 1927 AAAS meeting at Nashville, Tennessee and in an April 1928 presentation to the U.S. National Academy of Sciences (NAS) Muller (1928b) tried to discount the possibility that his reported transgenerational phenotypic changes were due principally to heritable chromosome changes, suggesting as proof observations of reverse mutations (e.g., X-ray-induced reversible changes in eye color – red to white). Patterson and Muller (1930) would subsequently publish a massive 82-page paper supporting his argument. This was proof enough for Muller that X-rays induced small mutations in genes rather than vast and large deletions as suggested by Altenburg. Muller used apparent reverse mutation findings to preempt potential challenges to his gene mutation interpretation. Muller argued further that the assumed point mutations closely mimicked the type of gene mutation changes underlying the mechanism of evolution as might be seen with spontaneous gene mutations, spending much of the next

[2] The proceedings of this Congress contains Muller's paper, which included the data used for the basis of the Nobel Prize in 1946. The Congress proceedings paper of Muller had substantial limitations, being somewhat sloppily written, having three experiments, each with important weaknesses. It also lacked a methods section and provided no references, including no acknowledgement of the report by Gager and Blakeslee (1927) that preceded his *Science* paper (Muller 1927a) for the reporting of ionizing radiation induced gene mutation by six months. The general substandard quality of the manuscript made me wonder whether the Nobel Prize paper of Muller from the Congress proceedings had ever been peer-reviewed. A July 8, 1946 letter from Muller to Altenburg (Muller 1946a) revealed that the manuscript that he read at the Congress was exactly the same as published in the subsequent proceedings. Thus, it is virtually certain that the Nobel Prize research of Muller was not peer-reviewed (Calabrese, 2018). However, Muller had been acculturated into the need for and process of peer-review by Thomas Hunt Morgan, his Ph.D. advisor at Columbia University. Morgan helped to create the *Journal of Experimental Zoology* in 1903, which had a modern peer-review process from the start. In fact, Muller would publish several articles in this journal by 1920 (Harrison, 1945). Thus, Muller was part of a culture of peer-review as a necessity and expectation. Yet, he avoided it for the seminal findings for which he would be honored with the Nobel Prize.

Table 1
Stadler's challenge to Muller. quotes from Stadler (1932, 1954).

Stadler (1932). **Proc 6th Intern Cong Genet 1:274–294**
"To state that an induced variation is a gene mutation is not to explain it but merely to label it."
Page 274-275
"We do not demonstrate that a chemical change has occurred; we simply infer, since no mechanical explanation can be found, that the variation must be due to this invisible mechanism."
Page 275
"We may define mutation as a transmissible change in the gene. But we identify mutation by experimental tests, and these tests are not such as to establish conclusively, in specific instances, that a change within the gene has occurred."
Page 275
"In effect, any Mendelizing variation which cannot be shown to be due to a change involving more than one gene is a mutation."
Page 275
"… the occurrence of reversion is not proof that the original mutation could not have been due even to a deficiency."
Page 292
Stadler (1954). **Science 120(3125):811–819**
"But there was no test to identify mutations due to a change within the gene; it was simply inferred that the mutants that could not be identified as the result of specific mechanical causes were, in fact, due to gene mutation in the ideal sense (11)."
Page 813

40 years in this quest for a mechanism for evolution.

While these findings would temporarily satisfy the questioning and doubtful Altenburg and others, supporting the X-ray-induced point mutation interpretation, this concern would not go away but actually grew principally due to the persistent questioning and new research insights of the plant radiation geneticist Stadler (1932, 1954), Muller's most staunch, yet objective, respected, competitor and critic (Calabrese, 2017e).

4. Stadler challenges gene mutation interpretation

4.1. Cytogenetic advances

At the time of his groundbreaking mutation publication, Muller's (1927a) research suffered from an acknowledged limited cytogenetic evaluative capacity which prevented fine structure chromosome resolution ("… Drosophila cytology is elusive in its finer details" – page 721, Muller, 1928b), and thereby a reduced capacity to detect chromosomal deletions. Markedly improved chromosome cytogenetic resolution capacity was developed by the Cornell plant cytogeneticist, Barbara McClintock, in the prophase stage of meiosis with maize (McClintock, 1929). Two years later she would apply this novel technique to Stadler's X-ray treated corn in the summer of 1931. It revealed that what was once believed to be X-ray induced "gene" mutagens were sizeable chromosomal deletions. While these findings would force Stadler to re-evaluate and challenge his previously published X-ray induced "gene" mutational findings in barley (Stadler, 1928), they would make him raise the question of whether Muller's gene mutation interpretation with fruit flies was also incorrect. While Stadler would cautiously share his new doubts with the research community in several 1931 publications (Stadler, 1931a,b) and in private correspondence with leaders in plant genetics research like Karl Sax (Stadler, 1931c), Stadler (1932) would finally challenge the Muller gene mutation interpretation in a very public manner during his Plenary Address at the Sixth International Genetics Congress at Cornell University in the presence of Muller (Table 1).

From this opening round of public debate, Muller and Stadler would challenge each other over whether Muller had induced true gene mutations in his highly publicized high dose X-ray experiments. This research-generated debate would continue until the death of Stadler in 1954 (Stadler, 1954), involving numerous radiation geneticists trying to resolve this fundamental question (Calabrese, 2017a ; Lefevre, 1950; Voss and Falk, 1973). Copies of Stadler's research grants and interim reports to the U.S. NRC that describe his progressive series of multi-year research plans, research methods and experimental developments reveal a focused, high quality and productive research activity with numerous publications that challenged Muller's gene mutation interpretation (State Historical Society of Missouri, Stadler Papers). An extensive review of Muller's gene mutation hypothesis along with supportive and non-supportive literature findings is provided in the dissertation of Lefevre (1949), Stadler's Ph.D. student. In this instance Stadler would show his flair for excitement and self-confidence by directing his student (with the assistance of *Drosophila* specialists and with some formal assistance of Muller) to challenge Muller's gene mutation interpretation with Muller's own biological model. In this extensive study, Lefevre (1949, 1950) found no support for Muller's gene mutation interpretation based on reverse mutations.

To the outside viewer it suggested two outstanding scientists locked in a scientific dispute, with Muller compelled to protect his reputation, future, and legacy. These longstanding competitive research activities of Stadler and Muller were much like a high-level chess match in which all moves (e.g., research publications, professional society presentations) contributed important information. By the late 1930s and/or early 1940s Stadler and others had methodically shown that Muller lacked the needed proof for his gene mutation assertions (Calabrese 2017a). The subsequent development of improved cytogenetic staining for *Drosophila* chromosomes by Painter (1934) would reveal that the use of the very high X-ray doses and dose rates similar to Muller's key findings, like that of Stadler's research with barley and corn, produced copious chromosome aberrations including a high proportion of deletions, along with few, if any, possible gene (i.e., "point") mutations.

Muller's use of the reverse mutation concept was also found unconvincing as multiple papers showed several mechanisms (e.g., position effect) by which reverse transgenerational phenotypic traits could occur without any change in the gene[3] (Bedford and Dewey, 2002; Lefevre, 1950). Thus, every move that Muller made was seemingly countered by the research of Stadler or spin-off ideas his research had inspired. Furthermore, Stadler's and related publications would yield insights that were incrementally more definite, insightful and over time, more convincing than Muller's, much like forcing Muller into a corner.

[3] See the discussion from Lefevre (1949) dissertation for a detailed assessment of reverse mutation and position effect as related to Muller's gene mutation interpretation.

4.2. McClintock's new X-Ray induced mutation mechanisms

Complementing the Stadler gene mutation criticism were new mechanistic findings of Barbara McClintock's study with her break-fusion-bridge-cycle model of X-ray induced genetic damage (Comfort, 1997, 2001) which then led to strikingly new and transformative transposable element induced mutational insights. Her novel mutable gene concept was particularly attractive to Muller's University of Indiana Colleague and future Nobel Laureate Salvadore Luria (McClintock, 1948; Muller, 1948) as well as Muller's closest colleague and friend, Edgar Altenburg. In the case of Altenburg, he would devote much effort to understand the scientific foundations of McClintock's findings and its role in spontaneous and exogenously induced mutations. The McClintock discovery had very broad biological and biomedical implications. However, it would also take Altenburg back to his 1927 suggestion that Muller had been blasting large holes in *Drosophila* chromosomes by high dose X-ray treatments. Extensive and detailed correspondence between Altenburg and McClintock in the early 1950s reveal the significance that Altenburg placed on her findings and how it stripped much significance from Muller's gene mutation model.

Altenburg would repeatedly encourage Muller to study and assimilate the findings of McClintock (Altenburg, 1952a,b,c, 1953a). Altenburg would provide Muller with a 25-page manuscript on McClintock's transpositional element concept and its relationship to X-ray-induced mutations (Altenburg, 1953a,b). However, Muller (1953) claimed he was too busy to read the manuscript while also being dismissive, claiming that no one could understand the "jumping gene" (i.e., transposable element) concept (Altenburg, 1953a; Muller, 1953), a common technique to distract attention from a perceived competitor while protecting one's legacy. However, Muller was not successful in drawing Altenburg back into his sphere of dominance, but rather, Altenburg (1957) would devote an entire chapter to McClintock's mutable gene (transposable element) concept in the second edition of his Genetics textbook. Altenburg, an excellent writer, made the challenging writings of McClintock readily understandable for geneticists and interested biologists. In this chapter, he claimed that a substantial proportion of high dose X-ray-induced mutations are due to chromosome deletions/rearrangements rather than Muller's "point mutations" and that such genetic damage was likely mediated by transposable elements (Table 2). The profound intellectual transformation of Altenburg to the McClintock model was a significant sign that the era of Muller was waning. During this same period Russell et al. (1958) would publish his highly influential dose rate challenge to Muller. With multiple scientific challenges facing him, Muller would transform his laboratory into one that would try to extend the findings of Russell into *Drosophila* rather than exploring the dramatic and more complex new ideas of McClintock. Within a month of the Russell et al. (1958) publication Muller was exploring dose rate. In the six years of redirected and intense research on this topic Muller's laboratory was plagued with a series of apparent false starts and a generally ambivalent finish. Thus, the final years of Muller's laboratory productivity were weak, perhaps a function of aging and health deterioration (Calabrese, 2017b).

Of further importance, as suggested above, was the discovery by McClintock (1950, 1951, 1953) that transposable chromosomal elements affected the occurrence of both spontaneous and exogenously induced mutations, including mutations induced by ionizing radiation and chemical mutagens such as mustard gas as used by Auerbach with *Drosophila*. Subsequent findings indicate that the early X-ray-induced transgenerational phenotypic findings of Muller (1927a) and Timofeeff-Ressovsky et al. (1935) were likely the result of X-ray activation of McClintock's transposition element process which induced massive chromosomal damage, such as small to massive deletions and other types of chromosomal aberrations (Ratner et al., 2001). These collective developments served to strongly reinforce the fundamental criticisms by Stadler of Muller's gene mutation interpretation, while supporting the McClintock transpositional element mediated mutation model.

5. LNT single-hit model, dose rate and the Manhattan Project

While Muller was in serious dispute with Stadler throughout the 1930s for his gene mutation interpretation, there was nonetheless a worldwide mesmerizing euphoria of Muller's mutation discovery (see Campos, 2015), one element of which resulted in a unique interdisciplinary collaboration between leading physicists and radiation geneticists as led by Delbruck and Timofeeff-Ressovsky, respectively. From the mid-1930s their research provided the LNT model with a hypothetical mechanistic basis via the use of target theory (Timofeeff-Ressovsky et al., 1935). This concept was then transformed into a biostatistical model (i.e., LNT Single-Hit model) which revealed that the shape of the dose response in the low dose zone was largely a function of the assumed number of target hits required to produce a gene mutation (Zimmer, 1941). The fewer the hits needed to produce gene mutations the closer the linear dose response for gene mutation was approached.

Since his X-ray induced gene mutation interpretation had experienced serious scientific challenges and setbacks through the 1930s, Muller needed another approach to redirect the mutation debate to restore support for his gene mutation interpretation and low dose linearity model and their integrative linkage. Muller's idea was an intriguing one that served, at least in part, both purposes, with a new application of a "dose x time = constant" experiment as seen in the Bunsen-Roscoe Law or with Haber's Law. Over the decade of the 1930s using his Proportionality Rule Muller had asserted that X-ray induced mutation damage was progressively cumulative and could not be repaired. As a result of these characteristics the damage should be predicted by the total dose, not by dose rate. If the total dose hypothesis were true, then the dose response for mutation should be linear at low dose, all the way down to a single ionization. Muller would test this idea in a

Table 2
Quote from Altenburg E. (1957). Genetics. Holt, Rhinehart and Winston, New York, NY.

Are all mutations due to chromosomal rearrangements?
… The possibility, therefore, arises that mutations might often be due to invisibly small deletions, rather than to an actual change in a gene–a change that we refer to as a "point" mutation. We cannot be sure, for example, that the yellow body-color mutant in Drosophila has a "yellow" gene in place of a "gray" (the normal allele of yellow). For all we know, the body color of the mutant might be yellow because the normal allele has been deleted. In fact, yellow mutants of independent origin differ somewhat in the intensity of their yellow pigmentation and, in the case of certain "extreme" yellow, it is very likely that the mutation is due to a very small deletion. In general, there is no way of telling from the outward appearance of a mutant what sort of genetic change caused the mutation. Inversions and duplications are also known to have mutant effects–inversion because of a "position" effect, and duplications either for the same reason or because of the genic unbalance they cause.
Now deletions, inversions, and duplications are all the results of chromosome breakage and rearrangement. Therefore, in the present state of our knowledge, all mutations might conceivable be due to such rearrangement and not to any actual alteration in the gene itself."
Page 303

dissertation by Ray-Chaudhuri at the University of Edinburgh using X-rays and mature spermatozoa of *Drosophila*. The findings of this dissertation matched up very well with Muller's predictions supporting the total dose/LNT hypothesis. These results provided support at a critical stage to Muller's gene mutation theory. In fact, during Muller's (1946b) Nobel Prize lecture, he cited the research of Ray-Chaudhuri (1939, 1944).

The problem with this newly adopted dose-rate vs total dose strategy to defend the gene mutation interpretation was that the study of Ray-Chaudhuri had a series of important design and execution limitations, requiring corrections, improvements and replication (Calabrese, 2011, 2017a). In fact, there were so many limitations (e.g., limited sample size, quality control issues, changing animal models during the experiment, lacked documentation of essential methods, major statistical errors, failure to collect critical information), it suggested that the normally critical Muller might have lowered his academic standards in order to provide support to his sagging gene mutation interpretation.

The Ray-Chaudhuri dissertation in some ways served as a pilot study for the far more substantial efforts lead by Curt Stern, University of Rochester, during the Manhattan Project starting in 1943. Stern would initially direct an acute study by Warren Spencer, a highly regarded *Drosophila* specialist who was on leave from his faculty position at the College of Wooster (Ohio, USA). While the Spencer part of the study went as planned, a significant problem for Muller, a paid consultant on this project, occurred when the data from the low dose chronic genetic toxicity study, led by Ernst Caspari, revealed a significant dose-rate effect and a threshold for mutagenicity, contradicting the Ray-Chaudhuri (1939, 1944) conclusions. These findings by themselves had the potential to land a severe blow to the LNT single-hit theory. These findings were just preceded by 15 years of research lead by Stadler that successfully weakened the plausibility of Muller's gene mutation interpretation and now along with new mechanistic insights of McClintock on X-ray-induced mutations. This situation became sufficiently threatening to the policy goals of key leaders of the radiation genetics community such as Muller and Stern who strongly advocated the adoption of the LNT single-hit model. What happened next to the field of radiation genetics could not have been predicted.

The above set of events, which collectively placed the LNT single-hit model at risk, set the stage for what is referred to as "LNTgate" (Calabrese, 2015c, 2016, 2017d), a series of obfuscations, deceptions, and misrepresentations of the scientific record all designed to ensure that the LNT single-hit theory would replace the threshold model for cancer risk assessment. This sequence of events has been reported in detail over the past seven years via a series of progressively informed historical discoveries (Calabrese 2011, 2013, 2015a,b,d, 2016, 2017b,c,e).

The LNTgate actions were mediated via the leadership of Curt Stern and Hermann J. Muller during the second half of 1946, continuing for more than a decade. These efforts lead to the actions of the NAS BEAR I Genetics Panel to sustain and integrate these successful manipulations into the scientific record and government regulatory policies. These ideologically directed activities would be guided by the academic "offspring" of Muller and Stern, such as Jim Crow, Bentley Glass, and other esteemed leaders of the radiation genetics community. The process became fully successful when the next generation uncritically accepted as scientific fact, the mistakes, deceptions, and misrepresentations handed down by the icons of the field. This is, in fact, the domain where key features of the fields of regulatory policy and cancer risk assessment are today.

6. Saving the hit model

The LNTgate process had an unexpected spontaneous origin. It began when Ernst Caspari informed Stern, his supervisor, that his dose-rate findings contradicted those of Ray-Chaudhuri (total dose). As noted above, the observation of a threshold response for mutation was not only not expected but, as it turned out, actually "not permitted", resulting in Stern refusing to accept the Caspari findings (Calabrese, 2011). Giving the appearance of objectivity, Stern blamed Caspari's threshold "discovery" on the use of a faulty control group that he insisted was aberrantly high. Stern did not provide any evidence to support this critical judgment. However, Stern was aware of earlier publications with control group responses for this model that supported the Caspari interpretation based on prior correspondence (Stern, 1938), but he either forgot this or refused to share it. Regardless, the Caspari year-long study had reached an impasse with the Stern judgement, a major crisis.

Showing some degree of independence, Caspari would not accept Stern's judgement that his control group displayed aberrantly high values. He dove into the literature and found a series of papers, which explicitly addressed the control group question, with all supporting his position (Calabrese, 2011). When Caspari assembled these findings, Stern withdrew the control group criticism. During this period, Caspari informed M. Demerec, head of the Genetics Department for the Carnegie Institute, of his mutation threshold dose-response findings and the problems it was creating. This prompted the influential Demerec to write Caspari asking "what can be done to save the hit model" (Caspari, 1947). This statement seemed to express what Stern and Caspari might well have been thinking. With the control group issue no longer a viable means to discredit the Caspari findings, the "save the hit model" strategy of Stern became publishing the manuscript, but framing the discussion to prevent the data from being accepted/used, while still showing competence of the research team, thereby securing the LNT/Ray-Chaudhuri framework. This seemed like the best possible outcome for Stern and Caspari.

The strategy adopted was to assert that the Caspari data could not be accepted or used until it could be determined why he obtained a threshold in the chronic study, while Warren Spencer obtained an apparent linear dose response a year earlier in an acute study with the same fruit fly model while working under Stern. This created a false standard, as the two studies had more than 25 methodological differences; there would be no possible practical means to determine why the studies differed (Calabrese, 2011). The only way that this highly nuanced perspective (i.e., the recommendation not to use the Caspari findings until it resolved the differences with the Spencer study) could have been published was if Stern was the journal (i.e., *Genetics*) editor and there was no peer-review, and this was most likely just what happened (Calabrese, 2011)! In fact, even though Stern proposed this unrealistic situation, no one, of course, ever explicitly accepted this challenge over the next 70 years, including himself, Caspari or Muller. It was a tactical move in the broader strategy to "save the hit model". So Caspari and Stern prepared this manuscript with this obfuscation and sent it to Muller for review on November 6, 1946 with Muller answering on November 12, 1946 (Calabrese, 2011). Muller indicated that he was upset that Caspari found a threshold since this could be a serious problem for LNT acceptance and Stern needed to replicate the study (not to explain why the Caspari study differed from the Spencer study as emphasized in the discussion as this was impossible to do). Thus, Muller was fully informed that the strongest study (i.e., chronic exposure to ionizing radiation) to date (i.e., Caspari experiment) showed a threshold for mutation one month prior to the Nobel Prize lecture of December 12, 1946 (Muller, 1946b). The linearity supporting acute exposure experiment of Spencer had a series of methodological limitations (e.g. inadequate temperature control, inexplicably combining different dose-rate groups with the same total dose, inadequate X-ray machine

calibration) that affected the reliability of the low dose study results (Calabrese, 2011). Yet Stern, Muller and others never identified such limitations, even in Muller's detailed review of this research (Muller, 1946c). These criticisms of the Spencer study (Spencer and Stern, 1948), were first reported more than six decades later (Calabrese, 2011).

In his crucial moment of making scientific history, Muller (1946d) deceived the world with his statement that there is no possibility for a threshold response ("no escape from the conclusion that there is no threshold") to ionizing radiation induced mutation and that risks needed to be assessed via the LNT single-hit model (Nobel Prize lecture, Dec 12, Muller, 1946b). Muller made this statement having seen the Caspari study and not offering any technical or other criticism (Muller, 1946e). Thus, a type of collusion began to take shape between Stern, Caspari, and Muller to do as Demerec urged. In a follow up letter to Stern (Muller, 1947) Muller supported publishing of the Caspari paper since there were enough caveats (i.e., obfuscations) and restrictions to make the paper non-threatening to the LNT acceptance.

In 1949 Stern manipulated or colluded with the leadership of *Science* to ensure LNT would be strongly promoted (Uphoff and Stern, 1949). This was similar to how Muller (1927a) was treated two decades earlier showing no data on his Nobel Prize experiments nor seven years later (1956) in the journal's dealings with the fraudulent NAS BEAR I Genetics Panel publication (Anonymous, 1956). Here is how it happened. While the Stern research team hoped that the follow-up replication studies would put an end to the Caspari study-created crisis, it simply created a new one. The first replication experiment (i.e., led by a new master's student Delta Uphoff) was unacceptable to Stern, this time because the control group was aberrantly low. The control group's values were so outside the norm that Stern had to check with Muller who strongly affirmed (in writing) that the Caspari control group values were appropriate while rejecting Uphoff's (see Calabrese, 2015a,b for the letter correspondence documentation). The troubled Stern would go so far as to blame her for having been biased [i.e., "may reflect a personal bias of the experimenter" (Uphoff and Stern, 1947)], with this leading to the low control group values (Calabrese, 2015b). This phrase was stated in the Discussion of the manuscript that was sent to the Atomic Energy Commission (AEC) (and which was immediately classified). This amazing statement should have raised a plethora of questions by the scientific community for Stern and Uphoff but it was hidden from view. For example, how did the alleged bias start? How long did it continue? How might it have affected other experiments, other team members and others, the data analysis and manuscript write up? A follow-up experiment by Uphoff also suffered the same fate with an aberrant control group value. This situation was turning into a professional disaster. So the question was not just what could be done to save the hit model but also the reputations of Stern, Caspari, and Uphoff and other members of the Manhattan Project at the University of Rochester. Stern would again show his creativity (or deviousness). Since essentially no one had read the classified material discounting the results and blaming Uphoff and her alleged biases leading to the uninterpretable findings, Stern used his contacts with the journal *Science* to publish a one page technical note of the experiments of Spencer, Caspari, and Uphoff. In this limited technical note, Stern showed no transparency, neglecting to inform the reader that he had found the low control studies of Uphoff unacceptable less than a year before and now he concluded these findings were fully acceptable. No criticisms of the Spencer study were mentioned despite its obvious significant limitations (Calabrese, 2011). Stern also reintroduced criticism of the Caspari study without evidence. In this mini-meta analysis, Stern restored the LNT model, literally "saving the hit model". In the final paragraph, Uphoff and Stern (1949) promised the *Science* readers to provide a comprehensive paper with methods, materials, missing data and other relevant information. Yet, they never did.

Muller and Stern actually promoted the discredited findings of Uphoff while marginalizing the Caspari paper. More specifically, at the time Stern asked Muller to help resolve the Caspari-Uphoff control group issue, Muller had been studying spontaneous mutations in the fruit fly in his ongoing disputes with Stadler concerning whether he induced gene mutation (Calabrese, 2017a). Thus, Muller was sitting on a treasure trove of control group spontaneous mutation data. As noted earlier, in multiple letters to Stern, Muller unequivocally sided with the Caspari findings while rejecting those of Uphoff (Calabrese, 2015a, b). With this as prologue we now fast forward a few years and find Muller (1950, 1954a) rejecting the Caspari study based on this control group being abnormally high, contradicting the literature, his own data/publications and his multiple letters to Stern, while never providing proof for his statements. The evidence reveals Muller dishonestly strove to discredit the Caspari study, and preserve LNT, while protecting himself from being accused of lying during his Nobel Prize Lecture. The 1950 paper of Muller was just preceded and perhaps inspired by an article by MIT's Robley P. Evans in *Science* (Evans, 1949) criticizing the LNT model, using the threshold findings of Caspari (Caspari and Stern, 1948). After Muller read the Evans article, he wrote to Stern criticizing the paper of Evans, blaming the criticism of LNT on the findings of Caspari (Muller, 1949). Muller urged Stern to contact Evans and discredit the Caspari work. No evidence has yet been found that Stern communicated with Evans on this matter.[4] However, shortly after that letter exchange with Stern, Muller published his false criticisms of Caspari's control group. Furthermore, on August 10, 1949 Altenburg (1949) wrote Muller about the Caspari threshold findings, acknowledged the reliability of the findings yet in search of a mechanistic explanation. Apparently, Muller had thought that Stern and his efforts had fully neutralized the threshold findings of Caspari, but this was not apparently the case.

7. LNT and the NAS BEAR Genetics panel

The next stage of the LNT story would take place with the NAS BEAR I Genetics Panel which first convened in early November, 1955 at Princeton University. As Muller had learned from many earlier frustrations, success within Advisory Committees is highly dependent upon who is selected. In the case of the BEAR I Genetics Panel, the answer was clear from the start, as the Panelist Tracy M. Sonneborn, a Muller colleague at the University of Indiana, read their radiation geneticist mantra into the recorded proceedings with no debate or dispute. All firmly believed that mutational damage was cumulative and irreversible with the dose response being linear down to a single ionization. Multiple notable radiation geneticists at that time were not advocates of the Muller perspective but they were either directed to other NAS BEAR I panels such as was the case of Ralph Singleton (agriculture panel) or not selected as was the case of McClintock. In retrospect, the deck was stacked along with an administrative leadership that would keep the panel focused on the big picture goals of the Rockefeller Foundation (RF) that both funded and directed the Panel while in

[4] The papers of Evans have been preserved at MIT. However, they have yet to be organized for scholarly use and it is unknown when they will be available. Of interest would be whether Stern ever sent Evans the letter Muller suggested. A check of the Stern files at APS revealed no record of a letter of Stern to Evans.

the administrative structure of the NAS.[5]

Despite the endorsement of the LNT single-hit model by leading research geneticists and physicists it was widely recognized that the fundamental data to support the LNT single-hit model was inappropriate. The model was dependent on point mutations, not large deletions, gene rearrangements, and other gross aberrations. In his final and masterful paper, published posthumously in *Science*, Stadler (1954) would illustrate how Muller's mutational data could not provide a credible biological basis for the LNT single-hit model. Despite the prominence of the journal *Science*, the stature of Stadler and the timeliness of the article, this criticism of the LNT single-hit model was never discussed by the NAS BEAR I Genetics Panel. In fact, not once in the transcribed pages of the Panel meetings were Stadler or McClintock's research on gene mutation ever mentioned.

At the second meeting of the Panel (in Chicago), Warren Weaver, Chair of the Genetics Panel and Director of Research for RF, tried to entice members of the Panel with RF funding if the Panel Report would support RF initiatives (e.g., LNT). Weaver indicated he would "try to get a very substantial amount of free support for genetics if at the end of this thing we have a case for it. I am not talking about a few thousand dollars, gentlemen, I am talking about a substantial amount of flexible and free support to geneticists" (Anonymous, 1956 - BEAR I Genetics Panel Transcript, February 5, 1956, page 35).[6] Weaver would further state that "There may be some very practical results – and here is the dangerous remark – don't misunderstand me, we are all just conspirators here together". The Weaver remarks obviously link the Panel deliverables to RF funding for geneticists, including those sitting in the room. Further discussions of the Panel during the February 5/6, 1956 meeting would reveal that to be successful in the eyes of Weaver, the Panel would need to present strong agreement/consensus for the estimation of genetic risks to the U.S. population assuming a linear dose response. However, an unanticipated problem came about 4–5 weeks later (March 1956) when the Panel members displayed multiple profound disagreements: they argued about whether it was possible to even estimate population risks, how to derive the estimations, how any derived estimates of damage related to true (real) risks, and what the risks actually were. With this confusion, the highly divergent results of the independent risk estimates that were carried out over 10 generations were seen as an unusable scientific "mess", such that Panel member, Jim Crow, would claim that no one would believe the policy recommendations of the Panelists since they could not agree amongst themselves. In a March 29, 1956 Letter to Warren Weaver, Crow (1956) stated that:

> "The limits presented on our estimates of genetic damage are so wide that the readers will, I believe, not have any confidence in them at all."

Lacking authority to do so, Crow, who was to organize the technical reports for Panel discussion, decided to arbitrarily drop the three lowest estimates of risk; by so doing he markedly reduced the variation, giving the false impression of more expert Panelist agreement than was the case. Even after dropping the three, there remained considerable uncertainty, being still too large to show to the scientific community and general public. One might have thought that the Panelists whose estimates were dropped would have strongly fought to have them retained. There is some evidence of significant disputes between Demerec and Muller on this matter based on a letter from Muller to Beadle in August 1956 (Muller, 1956) indicating that Muller did not want to be part of writing a scientific justification for their LNT recommendation. He indicated that he was already too frustrated with his debates with Demerec over the value of *Drosophila* versus bacteria in their risk estimations and did not want to air the so-called dirty laundry in public. He had thought that they had agreed to disagree. However, the available record does not reflect the details of this matter, as it likely occurred in the March 1956 meeting once Crow received the detailed write-ups for which there was no meeting transcript. Muller also noted his unresolved debates with the human geneticists of the Panel further confirming his unwillingness to seek a consensus report justifying their scientific recommendations. This lack of blatant open dispute/rebellion suggests that the group consensus was to present a united front that Weaver had earlier pointed out was necessary, perhaps using this funding carrot to achieve agreement. However, panelist James Neel, who refused to provide an estimate, strongly disputed the legitimacy of the proposed genetic damage estimation activity (Neel 1956 a, b). He argued that any consensus agreement was an illusion based on a self-fulfilling decision to reduce variability by forcing the use of similar models with similar process assumptions. Even with Crow stacking the deck, the risk estimates were still too variable, leading Weaver and Crow to encourage/coerce the Panel not to show their range of estimates to the outside world since it would destroy their credibility. The Panel would keep it private. There was no "minority" report nor leaking to the media. The "control" of the group was evident as those such as Demerec and Neel would not publically challenge the group view despite fundamental differences.

8. The NAS BEAR I Committee Genetics panel *science* publication story

The BEAR I Genetics Panel published a major article in *Science* (Anonymous, 1956) on their findings and recommendations. This paper had three significant misrepresentations of the Panel's research record. The first involved the Panel stating that the 12 geneticists of the Panel were invited to provide estimates of genetic risks for the entire U.S. population exposed to a certain dose of ionizing radiation, but only six accepted the challenge and provided the write up. Yet, nine of the 12 actually did, with Crow dropping three estimates as noted earlier.[7] In fact, I had obtained the nine detailed assessments. Second, the *Science* paper indicated that the minimum and maximum estimates of genetic damage range was ±10 or 100 fold. However, the actual average minimum-maximum damage range was about 750 fold. Third, the Genetics Panel *Science* paper neglected to report that three Panelists refused to participate, principally because they believed that such estimates could not be reliably done.

A written record exists that documents that the NAS BEAR I Committee Genetics Panel voted not to share their data with the scientific community and others (Calabrese, 2015a). After the Panel's publication in *Science* it was specifically challenged by

[5] Dr. Detlev Bronk was President of the Rockefeller Institute for Medical Research (later named Rockefeller University) and President of the National Academy of Sciences (NAS) during this time, confusing the roles of the Rockefeller Foundation and the NAS in this BEAR I Genetics Panel process.

[6] The concept of self-interest science (i.e., exaggerating fears of radiation to enhance research funding) of some members of the BEAR I Genetics Panel was documented via uncovered correspondence (Calabrese, 2014).

[7] It is interesting to note that the three estimates that Crow dropped (i.e., Demerec, Wright, and Kauffmann) were the areas with which Muller (1956) acknowledged serious issues in his letter to Beadle. Since Muller and Crow had a very close professional and personal relationship, it is tempting to speculate that Muller may have influenced Crow to drop the three estimates. This perspective is attractive since it is doubtful that Crow, one of the youngest members of the Panel, would have acted so precipitously without significant senior backup support. This would have been especially the case if he were doing Muller's bidding. Further documentation will be need to evaluate this hypothesis.

several leading U.S. academic researchers to share the scientific basis for the report and again the Panel formally voted not to do this as well (Calabrese, 2015a). Of significance is that the Panel had never even written such a scientific basis for their LNT recommendation. This should be seen as failed leadership by the NAS President Detlev Bronk and Chairman Weaver, a sign of scientific arrogance, or a type of defense posture. The Panel vote during August, 1956 not to provide a scientific basis for this major recommendation to adopt the LNT single-hit model for risk assessment was then passed on to NAS president Bronk, who accepted their decision. The NAS administration was therefore fully complicit in this process (Calabrese, 2015a).

The NAS BEAR I Committee Genetics Panel therefore falsified the research record, creating a significant cover up. Providing a detailed write up of their process would have revealed the deliberate misrepresentations of the research record. It would also have revealed a highly embarrassing fundamental lack of competence by such prestigious leading geneticists who simply could not properly address this risk estimation problem, as highlighted by Crow's amateurish and incorrect response (Calabrese, 2015a, b). It would also have taken considerable effort to complete such a report, something that should have been done during the activity of the Panel.

The goal of the NAS BEAR I Genetics Panel was to recommend adoption of the LNT in the U.S. and worldwide. Within about two years the LNT recommendation was adopted by national and international advisory committees, eventually becoming worldwide policy for cancer risk assessment. Thus, the most significant policy recommendation for cancer risk assessment lacked a written scientific basis. Most striking is that the Panel, including Muller, and the president of the NAS made this decision. It is ironic that the U.S. National Committee for Radiation Protection and Management (NCRPM) adopted LNT for cancer risk assessment in December 1958, based on the documentation-lacking NAS BEAR I Genetics Panel report days prior to the publication of Russell et al. (1958) demonstrating the existence of dose rate for ionizing radiation in the mouse model. Apparently, the status of the Genetics Panel and the NAS was so high that no documentation was needed for governments worldwide to adopt their transformative recommendations. As recently noted by Calabrese (2017a), seven of the members of the highly prestigious NAS BEAR I Committee Genetics Panel had no research experience with the effects of ionizing radiation on mutations. In fact, Crow, who had never published on the topic, made the decision on which estimates to retain. It is also ironic that Demerec and Neel, who were amongst the most appropriately experienced, did not contribute to the radiation risk estimates. Thus, the vision that the country was being guided by the most prestigious and experienced grouping of geneticists on the matter of radiation induced genetic damage was yet another myth to enhance acceptance of the LNT.

9. LNT, William Russell and the dose rate challenge

Within 2.5 years of the June, 1956 NAS BEAR I Genetics Panel *Science* publication, another *Science* publication would challenge one of the basic tenets of the BEAR I, Genetics Panel's recommendations. The paper was by William L. Russell of the Oakridge National Laboratory, also a member of the NAS BEAR I Genetics Panel. During June and July of 1958 Russell's group (Calabrese, 2017a, b) made a major discovery, that dose-rate, not total dose, was the key predictor of ionizing radiation induced mutation for mouse spermatogonia and oocytes. The Oak Ridge group kept this breakthrough discovery quiet, not presenting the findings at the International Genetics Congress in Burlington, VT in the middle of August. Russell did share the findings with a New York Times reporter during the Conference who wrote an article (Schmeck, 1958). The breakthrough paper was published on December 19, 1958 and with it was a timed release front page story by a Pulitzer Prize journalist (i.e., Nate Finney) for the Buffalo Evening News who specialized in atomic energy (note that the NY Times was then on strike) (Finney, 1958; Russell et al., 1958).

The Russell research revealed that damage from ionizing radiation was not cumulative, but reversible and had the potential to yield a threshold, suggesting the existence of DNA repair, a possibility that Altenburg shared with Muller soon after publication of the paper (Altenburg, 1958). In effect, Russell had discredited the mantra of the radiation geneticist community, creating a major problem. His strategy would be to promote the acceptance of his research while, at the same time, creating an impression of adhering to the radiation geneticist mantra. Russell did not want to be ostracized and marginalized from his field by his ideological radiation geneticist peers. Russell had seen the dominating and uncompromising personality of Muller in action many times while a member of the Genetics Panel (Crow, 1995) and with James Neel, whose paper Muller tried to prevent from being presented at an international genetics conference during the summer of 1956. In fact, Russell's supervisor, Alexander Hollaender, negotiated a follow up "reconciliation" meeting between Neel and Muller (January 1957) at Oakridge, essentially in the presence of Russell (Neel, 1956a, b; Neel, 1957a, b; Novitski, 1956) (Table 3). Thus, Russell knew only too well how hostile Muller could get if one deviated from the radiation genetics ideology. Russell would walk this dose-response tight rope until after the death of Muller in April 1967, after which Russell would unleash a profound set of criticisms of the radiation genetics mantra and the LNT concept (Russell, 1969, 1973).

Despite these findings, their massive expansion by Russell and their powerful challenge to the LNT single-hit recommendation of BEAR I, it would take some 14 years before a new powerful NAS Committee, now called the BEIR I Committee with the Genetics Subcommittee being chaired by Muller's protégé Jim Crow to reconsider the LNT recommendations of BEAR I. During this process the BEIR I Genetics Subcommittee re-examined the BEAR I report and made two clear initial determinations (Calabrese 2017a,b). The first was that the risk assessment recommendation of BEAR I (Anonymous, 1956) needed to be based on a mammalian model rather than on a fruit fly. The second factor was their acknowledgement that the BEAR I Genetics Panel (Anonymous, 1956) made a mistake in denying dose-rate. The recognition that dose-rate rather than the total dose best predicted mutation damage, meant that the radiation geneticist belief of cumulative and irreversible damage with each dose would be replaced. This finding also meant that linearity may be at risk of being replaced by the threshold dose response, reversing the 1956 position of the BEAR I Genetics Panel. However, despite these new challenges to the LNT model, the Genetics Subcommittee still had a strong disciple of Muller in charge with Crow[8] and would find some rationale to keep the linear dose response model as the default if possible.

Even though the findings of Russell revealed a true threshold for oocytes, the same could not be said for spermatogonia, where the dose-rate related damage, which was mediated by DNA repair, was only able to reduce total mutations induced acutely by 70% and not the 100% needed to achieve a threshold (Figure 1). The BEIR I Genetics Subcommittee therefore concluded that even though it was now known that an ionizing radiation threshold existed for mouse

[8] Toward the end of his career, Crow would acknowledge that Muller and he were amongst the strongest advocates of LNT and that they were too extreme in their views and actions (Crow, 1995).

Table 3
Quote from Neel (1959) letter to Beadle, September 14, 1959.

"There is no mind in science today for whose brilliance I have greater respect than that of Dr. Muller. In the first upsurge of concern concerning the effects of the increasing exposure of the human species to the radiation which followed World War II, it was Muller who had thought most about the problem, and Muller whose point of view dominated the picture. When Jack Schull and I pulled together our monograph on the findings in Japan, we felt obligated to try to fit these findings into the context of present knowledge. The outgrowth of that attempt, our Chapter 15, was a number of questions concerning Muller's argument. We couldn't prove that he was wrong, but we didn't feel he could prove that he was right. In other words, we felt that there were a number of unvalidated assumptions behind a good many of his points. One aspect of this evaluation of ours was a little critique of the significance of mutation rate studies. This critique I delivered at the WHO Study Group on the Effect of Radiation on Human Heredity which met in Denmark in the summer of 1956. I regarded it as part of the normal scientific interchange, but Dr. Muller apparently regarded it as an attack upon his life's work. There developed a rather strained relationship which persists until the present day, I am afraid, and keeps coming back to me in small ways which I consider beneath the dignity of a great man. Be that as it may, Alex Hollander was Chairman of that meeting in Denmark. Muller apparently insisted to Hollander that my statements were unacceptable and should be modified, to the point where Hollander arranged a meeting between Muller and myself at Oak Ridge, in an effort to reconcile the differences of opinion. At this point a number of the British participants in the WHO Study Group got wind of what was afoot, through no efforts of my own, and got their own backs up. It so happened that they agreed with my point of view and in effect transmitted the message that if any pressure were brought upon me, they would withdraw their own papers."

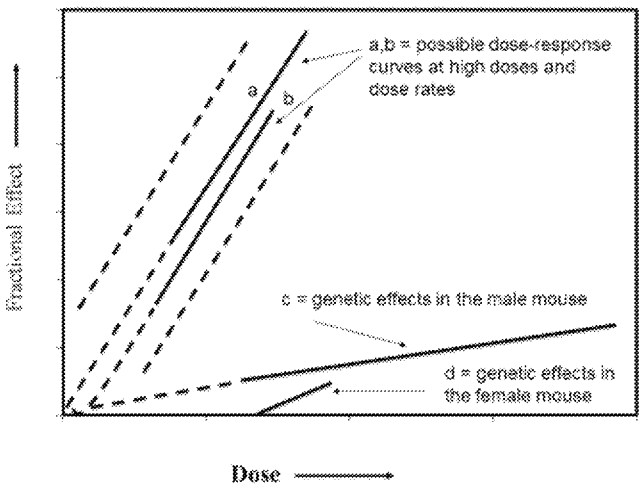

Figure 1. BEIR dose rate graph 1972. Hypothetical dose-response curves for leukemia and genetic effects (Source: NAS/NRC 1972 – page 98). Solid line = observed. Dashed extension of solid lines = unobserved. Line "a" and "b": possible dose-response curves at high doses and dose rates. Parallel dashed lines = rough limits of error for lines a and b. Lines c and d represent genetic damage in the male and female mice, respectively.

oocytes, the LNT would be based on responses of the mouse spermatogonia. While this logic was convincing to the Genetics Subcommittee one would have to wonder why this didn't require further evaluation. Could there be an evolutionary explanation for why oocytes might show a threshold while spermatogonia didn't? Do oocytes have a more efficient DNA repair system than spermatogonia? Are responses of reproductive cells directly applicable to somatic cells?

These above noted questions were not explored or debated by the BEIR I Genetics Subcommitee. The point here is that the Genetics Subcommittee failed to broadly consider the question and were directed by the Crow leadership to obtain the desired outcome. Thus, Crow and his Genetics Subcommittee retained the LNT based on the non-threshold mutation data of the mouse spermatogonia. These views were accepted by a non-inquisitive U.S. EPA in 1975 and reaffirmed in 1977 all with reference back to the Russell research (Calabrese, 2017c).

The findings of Russell were critical for modelling cancer risk assessment for ionizing radiation based on the Atomic Bomb Survivor data for cancer outcomes. However, these epidemiological findings have limited detectability at low doses (Taubes, 1995), and findings need to be extrapolated toward background exposure. In this key low dose extrapolation process the assumption of linearity was made by the BEIR I Genetics Subcommittee (NAS/NRC, 1972) with the findings of Russell serving as the biological dose-response "homing" device for the LNT model. In the late 1970s the U.S. EPA directly extended this linearity model based on ionizing radiation to chemical carcinogens (Albert et al., 1977). The EPA linear cancer risk assessment policy would be challenged in 2017 when Calabrese (2017b,c) reported that the Russell historical control had been found in error (Selby 1998a, b), and had been corrected for a massive error in 1996 by the Russells (Russell and Russell, 1996). Calabrese showed that if the corrected historical data had been used by the BEIR I (NAS/NRC, 1972) Genetics Subcommittee the male mouse would have shown a threshold while the female would show an hormetic response. These findings indicate that the basis for the LNT assumption was incorrectly formulated and that the adoption of LNT for risk assessment was incorrect.

10. Discussion

The present paper reveals that Muller did not discover what he claimed, that is, the "artificial transmutation of the gene" and this finding challenges the validity and application of the LNT single-hit model for cancer risk assessment (Calabrese, 2017a; Crow and Abrahamson, 1997). Muller was also incorrect on the issue of dose-rate (Russell et al., 1958) which had a significant impact on acceptance and promotion of the LNT single-hit theory (Calabrese, 2017b,c). Although complex, Muller's career was fundamentally centered on his quest to be the first to produce gene mutations, and then to defend this interpretation the rest of his life, against the findings of Stadler (1931a, b, 1932, 1954) and others and then over the remaining six years of his research career (1959–1964) on the issue of dose-rate (Calabrese, 2017a, b), while trying to avoid the alternative gene mutation model of McClintock (1950, 1951, 1953) and its advocacy by Altenburg (1957).

Current scientific understandings, therefore, reveal that Muller could not sustain the conclusion that his high dose X-ray induced artificial transmutations of the gene were "real" gene mutations. The strong preponderance of evidence in the 1930s suggested chromosome level heritable genetic changes based on advances in cytogenetic staining, findings that have been confirmed with nucleotide sequencing technologies (Calabrese, 2017a). Since Muller was incorrect with his gene mutation interpretations the LNT single-hit theory of Timofeeff-Ressovsky et al. (1935) lacked a scientific relationship with the data that was used as its foundation (as pointed out by Stadler, 1954). Despite being wrong on the fundamental biological issues, the Muller-led faction of the radiation genetics community was successful in achieving the adoption of LNT worldwide. This was largely due to its highly organized radiation geneticist network focus, profound exaggeration of risks, and collusions with the Rockefeller Foundation and the U.S. NAS (Calabrese, 2013, 2015a,b,d), and their massive LNT-promotion campaign immediately following BEAR I which affected

government, the scientific community, the media and the general public.

Since the deceptions (e.g., BEAR I) and significant errors (e.g., BEIR I) can be traced back to major scientific historical figures, Nobel Prize winners (i.e. Hermann Muller, George Beadle and Max Delbruck), prestigious U.S. NAS Committees (i.e. BEAR I and BEIR I) and at least one past NAS president (i.e. Detlev Bronk) (Calabrese, 2015a, b), it is important that the ideological history of cancer risk assessment in the U.S. be documented and become a part of the scientific and regulatory agency historical record to help ensure that vital public health policies and practices do not continue to be the offspring of a scientifically incorrect and dishonest past.

This historical assessment reveals a complicated dynamic amongst researchers, their colleagues, and rivals, all within a framework of politics, policies, social philosophies and personalities. Hermann Muller led the field, starting with redefining the concept of mutation and finding improved ways to assess it. Muller worked on these matters within a framework of wanting to be first, gaining recognition and its benefits and pushing this to extremes. One example of this obsession is seen when Muller claimed credit for an important discovery (i.e., first reported in *Drosophila* in which both genetic and cytological evidence of translocation were combined) that Curt Stern had made (Muller, 1929a, b; Muller and Painter, 1929; Stern 1926, Stern, 1929a, b). This resulted in getting the normally reserved Stern to confront Muller via correspondence. Muller was forced to publically apologize and correct the matter. However, symptomatic of this behavior and in this same general period, Muller would apparently manipulate an editor at *Science* to publish his discussion on X-ray induced mutation without providing any data, simply doing so as a means to ensure that he would be first - a tactic that was enormously rewarded.

Much of what Muller did over the next four decades was to preserve and defend the legacy of his breakthrough gene mutational findings/interpretation and the formulation of the Proportionality Rule (the LNT concept). In so doing, Muller would become the intellectual leader of the radiation genetics community, helping to ensure its importance and create new professional and funding opportunities. The principal challenge for Muller was the thoughtful reflections of Stadler and his capacity to create and test key hypotheses, the data from which would challenge Muller's interpretation of his "groundbreaking" findings. Stadler, who was unrelenting, objective and insightful, seemed to follow in the footsteps of Muller's Ph.D. advisor T.H. Morgan. These researchers, according to Muller (1946f), "abhorred what they termed "speculation", that they even distrusted the validity of the most essential lines of reasoning." Stadler and Morgan were leaders in that wave of skepticism whose participants "doubted the doubt 'til they doubted it out." (Muller, 1946f). In the end, Muller's interpretations were revealed via such follow up experimentation to be incorrect, that is, the very high doses he used produced heritable chromosomal, not gene, phenotype changes. More than 50 years later, with advances in nucleotide assessment methods, it would be shown that ionizing radiation could produce some gene mutations but at far lower doses (Asakawa et al., 2013; Colussi et al., 1998; Colussi and Lohman, 1997; De Serres, 1991; De Serres et al., 1967; Fossett et al., 1994; Furuno-Fukushi et al., 2003; Liu et al., 2003; Mognato et al., 2001; Nakamura et al., 2005; Nelson et al., 1994, 1995; Nohmi et al., 1999; Okudaira et al., 2010; Park et al., 1995; Russell and Hunsicker, 2012; Schwartz et al., 2000; Sudprasert et al., 2006; Thacker, 1986, 1992; Thacker et al., 1990; Toyokuni et al., 2009; Webber and De Serres, 1965; Yamada et al., 1996).

Muller loyalists, such as Charlotte Auerbach (1976) and others, would strain the limits of credibility by arguing that Muller was proven to be correct. These examples of revisionist history were based on an incorrect interpretation of his findings. Muller would excite the world with the claim he produced 40 gene mutations one weekend afternoon, more than the entire field had produced in a decade (Carlson, 1981). Yet, we now know that he was not producing gene mutations. In fact, Auerbach (1978) would eventually support Stadler noting that "Stadler tested many X-ray mutations of a particular gene in maize and found that all of them were deficiencies. Not long ago this conclusion was confirmed by experiments on a different gene in maze. Muller's evidence, gained from work with *Drosophila*, was less direct ..." (Auerbach, 1978). While Auerbach (1978) gave the proverbial nod to Stadler's perspective, this was done even more emphatically by two very close colleagues and friends of Muller. Crow and Abrahamson (1997) acknowledged that Stadler's deletion interpretations had been convincingly supported with modern analytical methods and that Muller was simply too stubborn, holding on too long to a discredited position. However, old deeply held and self-serving beliefs such as Muller's original error of interpretation, would mesmerize the scientific community making it impossible to change, as it became an accepted myth leading to the creation of the LNT single-hit model for cancer risk assessment, affecting vast changes in public health risk assessment policies and risk communication strategies, while being susceptible to political and ideological manipulation.

The Muller story reveals a conflicted character, the discoverer of an apparent major breakthrough, something that he greatly desired. At the same time, Muller was tortured with the possibility that he was wrong, spoke too soon, that his mutations were really only holes that the X-rays had poked in the chromosomes. He knew only too well that if his mutations were really only poked holes there really wasn't much new or great with his "breakthrough" discovery. Thus, we have a life that sought to "hold on", while trying to prove that he actually had produced "real" mutations.

Eventually the scientific story of Muller's chromosomal rather than gene mutations would progressively emerge, even if it would take up to five decades after he received his Nobel Prize. The influence of Muller continues to be dominantly reflected in current regulatory policy, which was based on poorly formulated science, in need of corrective transformation by major agencies, such as the U.S. EPA, which however have been unable or unwilling to do.

The story of Muller's discovery of gene mutation also speaks to the broader issue of science being self-correcting. Due to the courage and focus of Stadler, Muller's interpretations were challenged and tested in the laboratory. This inspired others, including perhaps a desperate Muller, to seek the truth.[9] These challenges would be tested in the domains of cytogenetics, position effects, transpositional elements, reverse mutations, and eventually with the use of the Southern Blot, PCR and other DNA technologies. We now know that Stadler was correct when he said that it was critical for the scientific community not to confuse the observation of transgenerational phenotypic changes at high doses with its unknown mechanism(s). In the end, Muller was trying in 1927 to discover the mechanism of evolution, and he "knew" that it must be gene mutation. However, he convinced the world (at least for a while), and maybe himself, that he had done so with his high dose *Drosophila* experimentation. However, the scientific community can thank Stadler and his collaborator McClintock for creating the necessary doubt that would eventually lead to science displaying a self-correction for Muller's claim. An important follow up question is whether regulatory agency "science", like that of experimental science, can be self-correcting. Now many years after Muller's

[9] In private letters with Altenburg (Altenburg, 1953c; Muller, 1953; 1954b,c), Muller would acknowledge problems with his reverse mutation explanation, the significant role of position effect and the influence of the mutable genes of McClintock.

incorrect interpretations were revealed, society still lives with a risk assessment model based on a mistaken set of Muller's interpretations. In 1995 Crow would reflect upon the impact of his generation of radiation geneticists in estimating ionizing radiation induced risks. With his then 20-20 hindsight Crow stated that Muller's leadership and action "oversold the dangers, and should accept some blame for what now seems, to me at least, to be an irrational emphasis by the general public and some regulatory agencies on low-level radiation …. ."

In the aftermath of the BEIR I (1972) recommendation and the adoption of the LNT perspective for regulatory agency policy and practice came a spate of biostatistical models offering estimates of cancer risk in the low dose zone following the linearized perspective. The broad range of linearized models were highly speculative attempts to estimate risks at very low doses often using some feature of enhanced biological plausibility, such as the number of theoretical stages in cancer development, the role of interindividual variation, the incorporation of carcinogen bioactivation and DNA repair and other approaches (Cornfield, 1977; Crump et al., 1976; Hoel et al., 1975; Krewski and Brown, 1980; Rai and Van Ryzin, 1981). This type of modeling started, for the most part, in 1961, with the Mantel and Bryan paper, based on the carcinogen contamination Cranberry scare during the Kennedy-Nixon election of 1960 followed by a hiatus until the mid-1970s after the creation of EPA and OSHA when legislative and regulatory activities intensified. These models were constrained by linear assumptions as provided by the BEAR I Genetics Panel, the BEIR I Committee and the official adoption of LNT from BEIR I in 1975 by EPA [see recommendation to support the LNT single-hit model by a subcommittee of the U.S. Department of Health & Welfare (Hoel et al., 1975)]. In between these two NAS committees there were many advisory groups of a national and international nature that followed BEAR I (Calabrese, 2013, 2015a). The linear assumption of these models in the mid-1970s and later were based on the predecessor NAS committees, with BEIR I having the latest and most direct impact since it was based on mice rather than fruit fly model of BEAR I. Given the above historical reconstruction, the risk assessment modeling activities would have been considerably different had EPA determined that the default should be a threshold or hormetic model. The rapid dominance of linear cancer risk assessment modeling in the late 1970s would not have occurred without the recommendations of the two NAS committees. These modeling activities were derived from biostatisticians who tried to derive more biologically motivated linearized models, not being aware of the plotting, scheming, deceptions, misrepresentations and mistakes of the two NAS committees. In the end, the real leaders were Muller, his radiation geneticist followers and their institutional partners. The subsequent linearized modeling was simply the following of the linearity script as written by the NAS BEAR I Genetics Panel.

These convergent entities reached a type of critical mass during the NAS BEAR I Committee Genetics Panel, facilitating no less than a scientific, social, psychological and politically-based risk assessment revolution within the U.S. and essentially all other countries adopting the LNT model for cancer risk assessment.

11. Conclusions

1. Muller incorrectly assumed he induced gene mutations in 1927 when he demonstrated that X-rays induced transgenerational phenotypic changes in *Drosophila* (Calabrese, 2017a).
2. The Muller findings had a major impact on the scientific community. His non-peer-reviewed data (Calabrese, 2018) and incorrect interpretations were widely accepted (Campos, 2015).
3. This incorrect gene mutation mechanistic interpretation lead to the development of the "Proportionality Rule" for dose response in 1930 by Muller and the LNT single-hit dose response model in 1935 by Timofeeff-Ressovsky et al. (Calabrese, 2017a).
4. Muller's gene mutation interpretations were strongly challenged in the genetics community, especially by Lewis J. Stadler and Barbara McClintock, who showed that Muller's gene mutation interpretation lacked scientific proof and could be explained by other mechanisms (Calabrese, 2017a).
5. Limited research directed by Muller supported a conclusion that X-ray induced mutations were best explained by total dose, not dose rate and the genetic damage was cumulative, irreversible and the dose response was linear (Ray-Chaudhuri, 1939,1944)
6. Muller's total dose findings were strongly challenged in Manhattan Project research with far stronger studies (Calabrese, 2011a). These findings were improperly marginalized by leaders of the U.S. radiation genetics communities including Stern and Muller who misrepresented the data via deceptions, false statements and obfuscations (Calabrese, 2011a, 2015b, 2016).
7. The inappropriate awarding of the Nobel Prize in 1946 to Muller for producing "gene" mutations gave an enormous credibility to the LNT risk assessment model, facilitating its acceptance within the scientific, medical, regulatory and political communities. It is likely that the award had long lasting societal impact that facilitated worldwide acceptance of LNT.
8. It was incorrectly assumed by the scientific/regulatory communities and prestigious advisory groups (e.g. U.S. NAS BEAR I Committee, Genetics Panel) (Anonymous, 1956) in the late 1950s that the responses of mature spermatozoa to ionizing radiation induced "gene" mutation which were linear at high doses and independent of dose rate and such doses could be generalized to all cell types, doses and dose rates (Calabrese, 2015b, 2016).
9. These assumptions were incorrect because it was later (i.e. early 1960s) determined that mature spermatozoa lacked DNA repair, thereby preventing its capacity to repair radiation and chemically induced mutation as could occur in somatic cells (Calabrese, 2017b, c).
10. The NAS BEAR I Genetics Panel deliberately misrepresented their own research findings and hid their contradictory findings to promote the acceptance of the LNT model for regulatory agency risk assessment (Calabrese, 2015b, 2016).
11. William L. Russell at the Oak Ridge National Laboratory starting in late 1958 demonstrated that ionizing radiation induced mutations in mouse spermatogonia and oocytes were dependent upon dose-rate, not total dose as had been assumed, due to their capacity to repair DNA damage (Calabrese, 2017b, c).
12. The BEIR I (NAS NRC, 1972) Genetics subcommittee acknowledged the "mistake" of the NAS BEAR I Genetics Panel on dose-rate but still retained the LNT recommendation because the significant reduction in mutation rate in the spermatogonia as shown by Russell et al. had not regressed to control values as in oocytes. Nonetheless, the BEIR I Genetics Subcommittee suggested that findings from spermatogonia had greater capacity for generalization to somatic cells, due to repair capacities, as compared to mature spermatozoa. Russell referred to failed DNA repair capacity as an "odd phenomenon, restricted to spermatozoa and

occasioned by the peculiar nature of the specialized spermatozoan cell." (Calabrese, 2017b,c)

13. Selby (1998a,b) in 1995 detected a significant error in the Russell mouse specific locus test historical control group. This error was subsequently acknowledged and corrected by Russell and Russell (1996) along with Selby (1998a,b). If this error had not been made or had been corrected prior to the creation of BEIR I the mouse spermatogonia data that was used to support continuance of the LNT model would have supported a threshold or hormetic model based on the Russell and Selby corrections, respectively (Calabrese 2017b,c).
14. Summary: The LNT for cancer risk assessment originated due to (1) a critical mistake by Muller that he had discovered X-ray induced "gene" mutation, (2) the adoption of the LNT single-hit model was based on this assumption, (3) a mistake in generalizing the use of the DNA-repair deficient mature spermatozoa for somatic cells by BEAR I (4) deceptions and misrepresentations of the scientific record by leaders of the radiation genetics community, including the NAS BEAR I Genetics Panel and (5) failure to detect the error in the Russell Mouse Specific Locus Test control group, which would have precluded support for LNT. EPA then extended the error by adopting LNT for cancer risk assessment, stating in 1975 and 1977 that it was based on the now recognized erroneous dose rate findings of Russell as cited in BEIR I (1972).
15. It is ironic that the misrepresentation of the scientific record by this NAS BEAR I Genetics Panel to promote their ideological agenda stands in sharp contrast to the memorialized quote on the Einstein statute on the very grounds of the U.S. NAS in Washington, DC. It states: "The right to search for truth implies also a duty; one must not conceal any part of what one has recognized to be true." As the historical record shows the NAS BEAR I Genetics Panel did not follow the guidance of Einstein.

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Conflict of interest

The authors declares no conflict of interest.

Declaration of interest

None.

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Message

From: McClintic, Howard [McClintH@ctc.com]
Sent: 4/27/2018 12:24:40 PM
To: Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]
Subject: RE: Your meeting request for EPA

Can't create time! What are the Labs doing?

I'll take you advice and keep you current. Of course, please promote our spot-on LNT Project internally and with whomever else externally, like the Chamber, among others.

Have a great weekend and many thanks,

Howard

Ex. 6

From: Beck, Nancy [mailto:Beck.Nancy@epa.gov]
Sent: Friday, April 27, 2018 8:16 AM
To: McClintic, Howard
Subject: RE: Your meeting request for EPA

I think it would be good for you to meet with Richard and Bruce. If there schedules are tight, there is not much I can do to create time.

Regards,
Nancy

Nancy B. Beck, Ph.D., DABT
Deputy Assistant Administrator, OCSPP
P: 202-564-1273
M: Ex. 6
beck.nancy@epa.gov

From: McClintic, Howard [mailto:McClintH@ctc.com]
Sent: Thursday, April 26, 2018 9:26 PM
To: Beck, Nancy <Beck.Nancy@epa.gov>
Subject: RE: Your meeting request for EPA

Thank you – YES that seems correct for Clint.

Can you organize another group any faster and is more exposure of more people to our LNT Project better?

I know your advice is valuable.

Many thanks,

Howard

Ex. 6

From: Beck, Nancy [mailto:Beck.Nancy@epa.gov]
Sent: Thursday, April 26, 2018 7:42 PM
To: McClintic, Howard
Subject: Re: Your meeting request for EPA

I think Clint Woods is who you are referring to. He is in the air office.

Regards
Nancy.

———————

Nancy B. Beck, Ph.D. DABT
Deputy Assistant Administrator, OCSPP
P: 202-564-1273
M: **Ex. 6**
Beck.Nancy@epa.gov

On Apr 26, 2018, at 3:44 PM, McClintic, Howard <McClintH@ctc.com> wrote:

Good Day Nancy,

Exciting times, right?

I had hoped to schedule a meeting with Assistant Administrator Bill Wehrum, whose reputation preceded

him – see my comments about him vis-à-vis our shared law firm friend. When I was told that I was being re-directed from Air & Radiation to ORD, I looked at their Staff Directory. In our conversation, you referenced three names: Richard Yamada; Brittany Bolen and Clint ____.

The week of June 4th seems distant! In most ways, I have no choice, so I guess I'll agree, expecting that it will be changed. Can you organize another group any faster and is more exposure of more people to our LNT Project better?

I value your advice.

Many thanks,

Howard

Ex. 6

From: Gentry, Nathan [mailto:Gentry.Nathan@epa.gov]
Sent: Thursday, April 26, 2018 3:07 PM
To: McClintic, Howard
Cc: **Ex. 6 R. Golden personal email**
Subject: RE: Your meeting request for EPA

Howard,

I've been asked to set up a meeting for you with the folks listed below. Schedules are very tight over the next month, and these

folks are going to be out of the office for most of the time. Could we look at something the week of **June 4**? I could set up some time the afternoon of June 5 or the morning of June 7.

EPA-ORD Participants
Richard Yamada, Deputy Assistant Administrator for Research and Development
Bruce Rodan, Associate Director for Science
Tina Bahadori, Director of the National Center for Environmental Assessment

Nathan Gentry
Scheduler for Jennifer Orme-Zavaleta, Richard Yamada, Chris Robbins and Bruce Rodan
Assistant Deputy Ethics Official
EPA Office of Research and Development
Phone: 202-564-9084
Fax: 202-565-2430

From: McClintic, Howard [mailto:McClintH@ctc.com]
Sent: Thursday, April 26, 2018 12:31 PM
To: Gentry, Nathan <Gentry.Nathan@epa.gov>
Cc: Ex. 6 R. Golden personal email
Subject: FW: Your meeting request for EPA

Good Day Nathan,

Dr. Bob Golden and I are pleased that we will be working together.

I'm not certain that this is the most current list of ORD personnel https://www.ecos.org/wp-content/uploads/2017/09/EPA-ORD-Contacts-for-ECOS-August-2017.pdf, but maybe it is the most

comprehensive? Who might you be thinking we should meet with at ORD? Also, with whom do you work?

I look forward to your response and am grateful for your guidance.

Most sincerely,

Howard

Ex. 6

From: McClintic, Howard
Sent: Thursday, April 26, 2018 8:56 AM
To: 'Lewis, Josh'; Bob Golden **Ex. 6 R. Golden personal email**
Cc: Gentry, Nathan; Atkinson, Emily
Subject: RE: Your meeting request for EPA

Good Morning Emily, Josh and Nathan,

Thank you for your email.

Curiously, I was just reading the Federal Register Notice about ***Strengthening Transparency in Regulatory Science*** and found the LNT referenced on the bottom of page 8. The Federal Register item was shared with Dr. Ed Calabrese by Administrator Pruitt, who is well aware of almost all aspects of this Project.

Dr. Bob Golden and I will coordinate with Nathan at ORD, no problem, Thank You. I had wanted to meet with Assistant Administrator Wehrum because of his certain knowledge of the LNT per his work at Hunton Andrews

Kurth LLP. Moreover, his colleague, Mark Bierbower, is a dear friend of mine and admirer of the Assistant Administrator – another time.

Anytime, starting next Tuesday, Bob and I are available for a Meeting.

Many thanks, most sincerely,

Howard

Ex. 6

Emily, thank you for your professionalism

From: Lewis, Josh [mailto:Lewis.Josh@epa.gov]
Sent: Thursday, April 26, 2018 8:18 AM
To: McClintic, Howard
Cc: Gentry, Nathan; Atkinson, Emily
Subject: Your meeting request for EPA

Hi Howard,

Following up on your meeting request below. In reviewing it, we think this meeting is more appropriate for our office of research and development. Nathan (cc'd) can be your point of contact there. Thank you.

Josh Lewis
Chief of Staff
Office of Air and Radiation
202 564 2095

From: McClintic, Howard [mailto:McClintH@ctc.com]
Sent: Tuesday, April 17, 2018 12:15 PM
To: Atkinson, Emily <Atkinson.Emily@epa.gov>
Cc: Ex. 6 R. Golden personal email
Subject: RE: Please Give Me a Brief Call at Your Convenience

Good Day Emily,

Thank you for your response and responsiveness.

Here is one way to answer your question, I think. In advance of our Meeting, I will share some background material, but I assume that the Assistant Administrator knows the topic, the LNT, very well. For example, he may wish to know "who" is on the Science Committee, which will be furnished.

The week of April 23rd is an example – please suggest dates and times therein or thereafter. We probably **need 45 minutes** because the subject is "rich" as well as broad-and-deep, spanning 60 years.

Many thanks for your assistance with scheduling this appointment Emily.

Most sincerely yours,

Howard

Ex. 6

WHO: Dr. Robert (Bob) Golden, Toxicologist, Co-Chair of the LNT Science Committee

Howard McClintic, Executive Director of the ***CTC Foundation***

WHAT: A Discussion about the Need to Objectively Evaluate the Linear No-Threshold (LNT) Risk Assessment Methodology

- The Science Committee's Mission, Purpose and Progress

- Next Steps: Completion; Peer-Review;

Publication;
Release

WHY: The Importance and Priority of This Project Should be Fully Appreciated and Realized

WHERE: Assistant Administrator Wehrum's Office

WHEN: TBD – Week of April 23rd? Time period 10:30 AM to 2:30 PM **Duration:** 45 Minutes

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From: Atkinson, Emily [mailto:Atkinson.Emily@epa.gov]
Sent: Tuesday, April 17, 2018 10:35 AM
To: McClintic, Howard
Subject: RE: Please Give Me a Brief Call at Your Convenience

Hi Howard,

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Once I have those details we will be able to look at scheduling options.

Thank you.

Emily

Emily Atkinson
Management Analyst/Office Manager

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Sent: Tuesday, April 17, 2018 10:28 AM
To: Atkinson, Emily <Atkinson.Emily@epa.gov>
Subject: Please Give Me a Brief Call at Your Convenience

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Good Day Ms. Atkinson,

I have a quick question for you.

Thank you in advance.

Most sincerely yours,

Howard

Howard G. McClintic

Executive Director

Ex. 6

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EPA/600/R-17/384F
September 2017

Update for Chapter 5 of the Exposure Factors Handbook

Soil and Dust Ingestion

National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC 20460

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5. SOIL AND DUST INGESTION

5.1. INTRODUCTION

This document is an update to Chapter 5 (Soil and Dust Ingestion) of the *Exposure Factors Handbook: 2011 Edition*. New information that has become available since 2011 has been added, and the recommended values have been revised, as needed, to reflect the additional information. The chapter includes a comprehensive review of the scientific literature through 2016. The new literature was identified via formal literature searches conducted by EPA library services as well as targeted internet searches conducted by the authors of this chapter. Appendix A provides a list of the key terms that were used in the literature searches. Revisions to this chapter have been made in accordance with the approved quality assurance plan for the *Exposure Factors Handbook*.

The ingestion of soil and dust is a potential route of exposure to environmental chemicals for both adults and children. Children, in particular, may ingest significant quantities of soil and dust due to their tendency to play on the floor indoors and on the ground outdoors and their tendency to mouth objects or their hands. For example, children may ingest soil and dust through deliberate hand-to-mouth movements, or unintentionally by eating food or mouthing objects that have dropped on the floor. Adults may also ingest soil or dust particles that adhere to food, cigarettes, or their hands. Other vulnerable populations may include pregnant women and populations engaging in wilderness and traditional rural lifestyles. Thus, understanding soil and dust ingestion patterns is an important part of estimating overall exposures to environmental chemicals.

Currently, knowledge of soil and dust ingestion patterns within the United States is limited. Only a few researchers have attempted to quantify soil and dust ingestion patterns in U.S. adults or children.

This chapter explains the concepts of soil and dust ingestion, soil pica, and geophagy; defines these terms for the purpose of this handbook's exposure factors; and presents available data from the literature on the amount of soil and dust ingested.

The Centers for Disease Control and Prevention's Agency for Toxic Substances and Disease Registry (ATSDR) held a workshop in June 2000 in which a panel of soil ingestion experts developed definitions for soil ingestion, soil pica, and geophagy to distinguish aspects of soil ingestion patterns that are important from a research perspective (ATSDR, 2001). This chapter uses the definitions developed by participants in that workshop:

Soil ingestion is the consumption of soil. This may result from various behaviors including, but not limited to, mouthing, contacting dirty hands, eating dropped food, or consuming soil directly.

Soil pica is the recurrent ingestion of unusually high amounts of soil (i.e., on the order of 1,000−5,000 mg/day or more).

Geophagy is the intentional ingestion of earths and is usually associated with cultural practices.

Some studies are of a behavior known as "pica," and the subset of "pica" that consists of ingesting soil. A general definition of the concept of pica is that of ingesting nonfood substances, or ingesting large quantities of certain particular foods. Definitions of pica often include references to recurring or repeated ingestion of these substances. Soil pica is specific to ingesting materials that are defined as soil, such as clays, yard soil, and flower-pot soil. Although soil pica has been observed among children and adults, information about the prevalence of pica behavior is limited. Gavrelis et al. (2011) reported that the prevalence of nonfood substance consumption varies by age, race, and income level. The behavior was most prevalent among children 1 to <3 years (Gavrelis et al., 2011). Geophagy, on the other hand, is an extremely rare behavior, especially among children, as is soil pica among adults. One distinction between geophagy and soil pica that may have public health implications is the fact that surface soils generally are not the main source of geophagy materials. Instead, geophagy is typically the consumption of clay from known, uncontaminated sources, whereas soil pica involves the consumption of surface soils, usually the top 2−3 inches (ATSDR, 2001).

Researchers in many different disciplines have hypothesized motivations for human soil pica or geophagy behavior, including alleviating hunger, nutritional deficiencies, or gastrointestinal distress (Young, 2010), a desire to remove toxins or self-medicate (Starks and Slabach, 2012), and other physiological or cultural influences (Danford, 1982). Bruhn and Pangborn (1971) and Harris and Harper (1997) suggest a religious context for certain geophagy or soil ingestion practices. Geophagy is characterized as an intentional behavior, whereas soil pica should not be limited to intentional soil ingestion, primarily because children can consume large amounts of soil from their typical behaviors

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and because differentiating intentional and unintentional behavior in young children is difficult (ATSDR, 2001). Some researchers have investigated populations that may be more likely than others to exhibit soil pica or geophagy behavior on a recurring basis. These populations might include pregnant women who exhibit soil pica behavior (Simpson et al., 2000), adults and children who practice geophagy (Vermeer and Frate, 1979), institutionalized children (Wong, 1988), and children with developmental delays (Danford, 1983), autism (Kinnell, 1985), or celiac disease (Korman, 1990). However, identifying specific soil pica and geophagy populations remains difficult due to limited research on this topic. ATSDR (2001) has estimated that 33% of children ingest more than 10 grams of soil 1 or 2 days a year. No information was located regarding the prevalence of geophagy behavior.

Because some soil and dust ingestion may be a result of hand-to-mouth behavior, soil properties that relate to adherence to the skin may be important. For example, soil particle size, organic matter content, moisture content, and other soil properties may affect the amount of soil that adheres to the skin and is available for ingestion. Soil particle sizes range from 50−2,000 μm for sand, 2−50 μm for silt, and are <2 μm for clay (USDA, 1999), while typical atmospheric dust particle sizes are in the range of 0.001−30 μm (U.S. OSHA, 1987). Studies on particle size have indicated that finer soil particles (generally <63 μm in diameter) tend to be adhered more efficiently to human hands, whereas adhered soil fractions are independent of organic matter content or soil origin (Choate et al., 2006; Yamamoto et al., 2006). For soils with higher moisture content, a greater number of large particles have been shown to adhere to the skin (Choate et al., 2006). Ikegami et al. (2014) found that approximately 90% of the particles of playground soil that adhered to children's hands were less than 100 μm in size. Beamer et al. (2012) and Bergstrom et al. (2011) found that concentrations of contaminants (e.g., metals) in soil may differ according to particle size. Cao et al. (2012) also described the importance of considering particle size when evaluating exposures to indoor settled dust.

In this handbook, soil, indoor settled dust, and outdoor settled dust are defined generally as the following:

Soil. Particles of unconsolidated mineral and/or organic matter from the earth's surface that are located outdoors, or are used indoors to support plant growth. It includes particles that have settled onto outdoor objects and surfaces (outdoor settled dust).

Indoor Settled Dust. Particles in building interiors that have settled onto objects, surfaces, floors, and carpeting. These particles may include soil particles that have been tracked or blown into the indoor environment from outdoors, as well as organic matter.

Outdoor Settled Dust. Particles that have settled onto outdoor objects and surfaces due to either wet or dry deposition. Note that it may not be possible to distinguish between soil and outdoor settled dust because outdoor settled dust generally is present on the uppermost surface layer of soil.

For the purposes of this handbook, soil ingestion includes both soil and outdoor settled dust, and dust ingestion includes indoor settled dust only.

Several methodologies related to soil and dust ingestion are represented in the literature. Two methodologies combine biomarker measurements with measurements of the biomarker substance's presence in environmental media. An additional methodology offers modeled estimates of soil/dust ingestion from activity pattern data from observational studies (e.g., videography) or from the responses to survey questionnaires about children's activities, behaviors, and locations.

The first of the biomarker methodologies is the *"tracer element"* methodology. This method uses measured quantities of specific elements present in feces, urine, food and medications, yard soil, house dust, and sometimes community soil and dust. This information is used in combination with certain assumptions about the elements' behavior in the gastrointestinal tract to produce estimates of soil and dust quantities ingested (Davis et al., 1990).

The second biomarker methodology is the *"biokinetic model comparison"* methodology. This method compares results from a biokinetic model of lead exposure and uptake that predicts blood lead levels, with biomarker measurements of lead in blood (Von Lindern et al., 2003). The model predictions are made using assumptions about ingested soil and dust quantities that are based, in part, on results from early versions of the first methodology. Therefore, the comparison with actual measured blood lead levels serves to confirm, to some extent, the assumptions about ingested soil and dust quantities used in the biokinetic model. Lead isotope ratios have also been used as a biomarker to

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study sources of lead exposures in children. This technique involves measurements of different lead isotopes in blood and/or urine, food, water, and house dust and compares the ratio of different lead isotopes to infer sources of lead exposure that may include dust or other environmental exposures (Manton et al., 2000). However, application of lead isotope ratios to derive estimates of dust ingestion by children has not been attempted. Therefore, it is not discussed any further in this chapter.

The third, *"activity pattern"* methodology, combines information from hand-to-mouth and object-to-mouth behaviors with microenvironment data (i.e., time spent at different locations) to derive estimates of soil and dust ingestion. Behavioral information often comes from data obtained using videography techniques or from responses to survey questions obtained from adults, caregivers, and/or children. Surveys often include questions about hand-to-mouth and object-to-mouth behaviors, soil and dust ingestion behaviors, frequency, and sometimes quantity (Barltrop, 1966). Moya and Phillips (2014) provide a review of these three methodologies used to estimate soil and dust ingestion.

A fourth methodology uses assumptions regarding ingested quantities of soil and dust that are based on a general knowledge of human behavior, and potentially supplemented or informed by data from other methodologies (Hawley, 1985; Kissel et al., 1998a; Wong et al., 2000). This methodology is not discussed in this chapter because it yields rudimentary estimates of soil ingestion.

Another approach used to estimate soil/dust ingestion consists of measurements of soil/dust loadings on surfaces (mass per surface area) and concentrations of contaminants on those surfaces. Estimates of soil/dust ingestion can be made by making assumptions about children's hand-to-mouth and object-to-mouth behavior, surfaces contacted, fraction of soil/dust transferred, exposure time, saliva extraction efficiency, and assumptions about the amount of soil/dust reloading on skin or surfaces. This approach results in a different metric of soil/dust ingestion in units of area contacted/day, which can then be used with corresponding contaminant concentrations of soil/dust per surface area. This approach is described in more detail by Wilson et al. (2016).

The recommendations for soil, dust, and soil + dust ingestion rates are provided in the next section, along with a summary of the confidence ratings for these recommendations. The recommended values are based on key studies identified by the U.S. Environmental Protection Agency (U.S. EPA) for this factor. As described in Chapter 1 of the *Exposure Factors Handbook: 2011 Edition* (U.S. EPA, 2011), key studies represent the most up-to-date and scientifically sound for deriving recommendations for exposure factors, whereas other studies are designated "relevant," meaning applicable or pertinent, but not necessarily the most important. For example, studies that provide supporting data or information related to the factor of interest (e.g., pica prevalence), or have study designs or approaches that make the data less applicable to the population of interest (e.g., studies not conducted in the United States) have been designated as relevant rather than key. Key studies were selected based on the general assessment factors described in Chapter 1 of the Handbook.

Following the recommendations, a description of the three methodologies used to estimate soil and dust ingestion is provided, followed by a summary of key and relevant studies. Because strengths and limitations of each one of the key and relevant studies relate to the strengths and limitations inherent of the methodologies themselves, they are discussed at the end of the key and relevant studies.

5.2. RECOMMENDATIONS

Table 5-1 provides the recommended soil and dust ingestion rates for use in human health risk assessments. The soil ingestion recommendations in Table 5-1 are intended to represent ingestion of a combination of soil and outdoor settled dust, without distinguishing between these sources. The source of the soil in these recommendations could be outdoor soil, indoor containerized soil used to support growth of indoor plants, or a combination of both outdoor soil and containerized indoor soil. The inhalation and subsequent swallowing of soil particles is accounted for in these recommended values; therefore, this pathway does not need to be considered separately. These recommendations are called "soil." The dust ingestion recommendations in Table 5-1 include soil tracked into the indoor setting, indoor settled dust, and air-suspended particulate matter that is inhaled and swallowed. "Dust" recommendations are provided in the event that assessors need recommendations for an indoor or inside a transportation vehicle scenario in which dust, but not outdoor soil, is the exposure medium of concern. The soil + dust recommendations would include soil, either from outdoor or containerized indoor sources, dust that is a combination of outdoor settled dust, indoor settled dust, and air-suspended particulate matter that is inhaled, subsequently trapped in mucous and moved from the respiratory

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system to the gastrointestinal tract, and a soil-origin material located on indoor floor surfaces that was tracked indoors by building occupants.

Many of the key studies predated the age groups recommended for children by U.S. EPA (2005) and were performed on groups of children of varying ages. As a result, central tendency and upper percentile recommendations could only be developed for some combined age categories, as shown in Table 5-1. Published estimates from the key studies have been rounded to one significant figure in Table 5-1.

An important factor to consider when using the recommended values described in the following sections is that they are limited to estimates of soil and dust quantities ingested. The scope of this chapter is limited to quantities of soil and dust taken into the gastrointestinal tract, and does not extend to issues regarding bioavailability of environmental contaminants present in that soil and dust. Information from other sources is needed to address bioavailability. In addition, as more information becomes available regarding gastrointestinal absorption of environmental contaminants, adjustments to the soil and dust ingestion exposure equations may need to be made to better represent the direction of movement of those contaminants within the gastrointestinal tract.

To place the recommended values into context, it may be useful to compare the soil ingestion rates to common measurements. For example, the central tendency recommendation of 40 mg/day or 0.040 g/day of either soil only or dust only for general population children 1 to <6 years old would be equivalent to approximately 1/8 of an aspirin tablet per day because the average aspirin tablet is approximately 325 mg. Likewise, the central tendency recommendation of 80 mg/day or 0.080 g/day, for soil and dust combined, would be equivalent to approximately 1/4 of an aspirin tablet. The 50 g/day ingestion rate recommended to represent geophagy behavior would be roughly equivalent to 150 aspirin tablets per day.

5.2.1 General Population Soil and Dust Ingestion Rates

The key studies described in Sections 5.3.2 and 5.3.3 were used to recommend values for soil and dust ingestion for adults and children in the general population. Table 5-1 shows the central tendency and upper percentile recommendations for daily ingestion of soil + dust, soil only, and dust only in mg/day. Section 5.5 and Table 5-34 provide additional details on the derivation of these recommended values. The recommended values for soil ingestion only and dust ingestion only are based on the assumption that 45% of the soil + dust ingestion can be attributed to soil and 55% can be attributed to dust. This assumption is based on the defaults used in EPA's Integrated Exposure and Uptake Biokinetic (IEUBK) model (U.S. EPA, 1994a). According to U.S. EPA (1994a), the assumption is based on the relative likelihood of contact with soil/dust in indoor and outdoor locations and "represents [EPA's] best judgement of a properly weighted ratio for this purpose." All recommended values have been rounded to one significant figure due to data limitations.

In general, the "central tendency" recommendations reflect an arithmetic mean (average) of estimated values within a study, across studies within a methodology, and across the three methodologies. However, in some of the tracer studies, (Stanek and Calabrese, 1995a), the central tendency value used was the average of the median values for the four best tracer elements or the average of the median of three tracers (see Section 5.3.3.2). For others (Calabrese et al., 1997a; 1997b) the central tendency value represents the average of the best tracer or the average based on aluminum and silicon. Upper percentile recommendations for daily ingestion are also provided in mg/day. Note that there is considerably more uncertainty related to the upper percentile soil and dust ingestion rate estimates than for the average estimates. Biases due to the errors (e.g., sampling errors, measurement errors, analytical errors) are more likely to affect the upper percentile estimates than the average estimates. Upper percentile recommendations for the general population are provided for soil, dust, and soil + dust ingestion. These values are based on the 95th percentile values from the key studies.

The recommended central tendency soil + dust ingestion estimate for general population infants 0 to <6 months old is 40 mg/day, and the central tendency estimate for 6 months to <1 year of age is 70 mg/day. If a central tendency estimate is needed for soil or dust only, the recommended values are both 20 mg/day for infants 0 to <6 months (i.e., 18 mg/day soil and 22 mg/day dust, both rounded to one significant figure is 20 mg/day). For infants 6 months to <1 year, the recommended soil only estimate is 30 mg/day and the dust only estimate is 40 mg/day.

For risk assessment involving children 1 to <2 and 2 to <6 years of age, the recommended central tendency soil + dust ingestion rates are 90 mg/day and 60 mg/day, respectively. For soil only, the recommended central tendency values are 40 mg/day for 1- to <2-year-olds and 30 mg/day for

2- to <6-year-olds, respectively. For dust only, the recommended central tendency values are 50 mg/day and 30 mg/day for the two age groups, respectively.

When assessing risks for children 1 to <6 years of age who are not expected to exhibit soil pica or geophagy behavior, the recommended central tendency soil + dust ingestion estimate is 80 mg/day. If an estimate for soil only or indoor dust only is needed, the recommendation is 40 mg/day.

For children 6 to <12 years old without pica, the recommended central tendency soil + dust ingestion rate is 60 mg/day. For either soil or dust alone, the estimate is 30 mg/day. For children 12 to <20 years and adults, the recommended central tendency values for use in risk assessment is 30 mg/day, rounded to one significant figure. This recommendation is based on data for adults from Davis and Mirick (2006) and data from Wilson et al. (2013) that indicated that central tendency soil + dust ingestion rates ages 12 to <20 years and adults are similar. For soil only, the recommended value is 10 mg/day, and for dust only, the recommended value is 20 mg/day.

The upper percentile recommendations for soil + dust ingestion among the general population, are based on the 95th percentile values obtained from key studies as shown in Table 5-1, rounded to one significant figure. The recommended values: are 100 mg/day for infants 0 to <6 months (50 mg/day soil and 60 mg/day dust), and 200 mg/day for children 6 months to <1 year, 1 to <2 years, 2 to <6 years, 1 to <6 years, and 6 to <12 years (90 mg/day soil and 100 mg/day dust). For ages 12 years through adults, the recommended upper percentile value is 100 mg/day (50 mg/day soil and 60 mg/day dust).

5.2.2. Soil Pica and Geophagy

Ingestion rates for "soil pica," and for individuals who exhibit "geophagy" are also provided in Table 5-1. The soil-pica and geophagy recommendations are likely to represent an acute high soil ingestion episode or behavior. The soil pica ingestion estimate in the literature for children up to age 6 years ranges from 1,000 to 6,000 mg/day, averaged over the study period (ATSDR, 2001; Barnes, 1990; Calabrese et al., 1989, 1991, 1997b; Stanek et al., 1998). Due to the short-term nature of these studies and the limited amount of data available for children exhibiting pica behavior, the lower end of this range of 1,000 mg/day is recommended for soil pica for children 1 to <6 years old. However, it is important to note that soil ingestion for these children exhibiting soil pica behavior has been reported as high as 20 to 25 g/day on any given day (Calabrese et al., 1997b). Note too that the recommended soil pica value may be more appropriate for acute exposures. Currently, no data are available for soil pica behavior for children less than 12 months or in children ages 6 to <21 years. Because pica behavior may occur among some children ages ~1 to 21 years old (Hyman et al., 1990), it is prudent to assume that, for some children, soil pica behavior may occur at any age up to 21 years. While pica may also occur among adults, no key studies were available for developing recommended intake rates for adults who exhibit pica.

The recommended geophagy soil estimate is 50,000 mg/day (50 grams) for both adults and children (Vermeer and Frate, 1979). It is important to note that this value may be more representative of acute exposures. Risk assessors should use this value for soil ingestion in for individuals or populations known to exhibit geophagy behaviors.

5.2.3. Wilderness or Traditional Rural Lifestyles

Information on soil ingestion among special populations, such as those engaging in wilderness lifestyles in Canada, are presented as relevant studies in Sections 5.3.4 (Doyle et al., 2012; Irvine et al., 2014). Data from these studies may be appropriate for high soil contact scenarios. For rural populations following traditional rural or wilderness lifestyles as described in these studies, adult soil ingestion rates may be somewhat higher than those of the general population. Based on these two studies the adult mean soil + dust ingestion rate is 50 mg/day and the upper percentile soil + dust ingestion rate is 200 mg/day. Based on personal communication with the authors of these two studies, the 95th percentile of the combined data sets was calculated to be 239 mg/day for aluminum and silicon (for all four tracers the value would be 243 mg/day) (personal communication between M. Stifelman, EPA, and J. Doyle, University of Ottawa, Canada). Rounding to one significant figure, the upper percentile value would also be 200 mg/day. Assuming that soil represents 45% and dust represents 55% of the soil + dust value, the mean and upper percentile soil only values would be 20 mg/day and 90 mg/day, respectively. The mean and upper percentile dust only values would be 30 mg/day and 100 mg/day, respectively.

5.2.4. Confidence Ratings

Section 5.4 gives a detailed explanation of the limitations of the various study methodologies, which are reflected in the confidence ratings for the recommendations shown in Table 5-2. Individual

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evaluations of the quality of studies are provided in the specific discussions for each of the individual studies. The confidence ratings are low due to the relatively limited data on which the recommendations are based and the limitations and uncertainties associated with tracer studies described in Section 5.4.1, and the assumptions needed to develop quantitative estimates using the biokinetic modeling (see Section 5.4.2) and activity pattern modeling approaches (see Section 5.4.3). Other uncertainties pertain to the representativeness of the populations studied. A more detailed discussion about the general assessment factors used to evaluate the confidence in the recommendations is provided in Chapter 1 of the Handbook. For the estimates of soil only and dust only, an additional uncertainty pertains to the assumption that the proportion of soil + dust represented by soil only (45%) and dust only (55%) is the same for all age groups.

Table 5-1. Recommended Values for Daily Soil, Dust, and Soil + Dust Ingestion (mg/day)[a]

	Soil + Dust		Soil[b]				Dust[c]	
Age Group	General Population Central Tendency[d]	General Population Upper Percentile[e]	General Population Central Tendency[f]	General Population Upper Percentile[f]	Soil Pica[g]	Geophagy[h]	General Population Central Tendency[f]	General Population Upper Percentile[f]
<6 months	40	100	20	50	—	—	20	60
6 months to <1 year	70 (60–80)	200	30	90	—	—	40	100
1 to <2 years	90	200	40	90	1,000	50,000	50	100
2 to <6 years	60	200	30	90	1,000	50,000	30	100
1 to <6 years	80 (60–100)	200	40	90	1,000	50,000	40	100
6 to <12 years	60 (60–60)[i]	200	30	90	1,000	50,000	30	100
12 years through adult	30 (4–50)[j]	100[j]	10	50	—	50,000	20	60

[a] Ranges are provided in parentheses, when applicable, and represent the range of means from the various studies. Ranges are not provided for age groups for which the recommendations are based on a single study.

[b] Includes soil and outdoor settled dust.

[c] Includes indoor settled dust only.

[d] Based on the average of the central tendency values from the various studies for each of the three methodologies (tracer, biokinetic modeling, activity pattern), averaged over the three methods. Recommendation for <6 months of age based on Wilson et al. (2013) (note that data for 0 to <7 months in Wilson et al. [2013] were used to represent the 0 to <6 months age group). Recommendations for children 6 months to <1 year based on the average of values from Hogan et al. (1998) and von Lindern et al. (2016). Recommendations for 1- to 2 year-olds and 2- to <6-year-olds based on von Lindern et al. (2016). Recommendations for children ages 1 to <6 years based on the average of values from Calabrese et al. (1989) as reanalyzed in Stanek and Calabrese 1995a (mean of the median values for the best 4 tracers for each child); Calabrese et al. (1997a) (average of the best tracer for each child); Calabrese et al. (1997b) (average of aluminum and silicon); Davis et al. (1990) as reanalyzed by Stanek and Calabrese, 1995a (mean of the median values for 3 tracers for each child); Hogan et al. (1998); Özkaynak et al. (2011); von Lindern et al. (2016); and Wilson et al. (2013). The recommendations for ages 12 years to adults are based on the average of data for teens (ages 12 to <20 years), adults, and seniors from Wilson et al. (2013) and on adults from Davis and Mirick (2006). All recommended values were rounded to one significant figure. See Table 5-34 for additional details.

[e] Based on the average of the 95th percentile values from the various studies for each of the three methodologies (tracer, biokinetic modeling, activity pattern), averaged over the three methods. Based on the 95th percentile values for the same studies as used for the central tendency estimates except for age 12 years through adults. Upper percentile recommendation for 12 years of age through adults based on the assumption that the ratio of the 95th percentile to the mean value for adults is the same as the average of the ratios of 95th percentiles to means for all other age groups (i.e., average ratio of the 95th percentile to mean recommendations = 3.2). See Table 5-34 for additional details.

[f] Estimates of soil and dust were derived from the soil + dust values assuming 45% soil and 55% dust, rounded to one significant figure.

[g] Professional judgement based on: ATSDR (2001); Barnes (1990); Calabrese et al. (1997b, 1991, 1989); Stanek et al. (1998).

[h] Vermeer and Frate (1979).

[i] Range based on two studies with estimates of 55 and 56 mg/day; both of these estimates round to 60 mg/day.

[j] Soil + dust ingestion rates may be higher for adults following a traditional rural or wilderness lifestyle. Based on Doyle et al. (2012) and Irvine et al. (2014) the central tendency adult soil + dust ingestion rates is 50 mg/day (20 mg/day soil and 30 mg/day dust) and the upper percentile rate is 200 mg/day (90 mg/day soil and 100 mg/day dust).

— = No data.

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Table 5-2. Confidence in Recommendations for Ingestion of Soil and Dust[a]

General Assessment Factors	Rationale	Rating
Soundness		Low
Adequacy of Approach	The methodologies have serious limitations. No single study captured all of the information needed (quantities ingested, frequency of high soil ingestion episodes, prevalence of high soil ingestion). Sample selection may have introduced some bias in the results (i.e., children near smelter or Superfund sites, volunteers in nursery schools). The total number of children in key studies were 241 (tracer studies; Calabrese et al., 1989; Davis et al., 1990; Calabrese et al., 1997a,b) and 2,599 (biokinetic modeling; Hogan et al., 1998; von Lindern et al., 2016). Modeled estimates were based on 1,000 simulated individuals (Özkaynak et al., 2011) or 200,000 trials (Wilson et al., 2013). Models may be sensitive to assumptions and the quality and availability of input variables. The response rates for in-person interviews and telephone surveys were often not stated in published articles. Only two key studies provided data for adults.	
Minimal (or defined) Bias	Numerous sources of measurement error exist in the tracer element studies and the biokinetic model comparison studies. Some input variables for the modeled estimates are uncertain. Some of the assumptions used in the modeling studies may underestimate soil ingestion rates. Knowledge of soil and dust contamination may have affected the results of some of the studies.	
Applicability and Utility		Low
Exposure Factor of Interest	The key tracer studies focused on the soil exposure factor, with little or no focus on the dust exposure factor. The biokinetic model comparison studies accounted for both soil and dust ingestion, but also addressed other factors (e.g., exposure via dietary intake, inhalation). The activity pattern studies focused on soil and dust ingestion.	
Representativeness	The study samples may not be representative of the United States in terms of race, ethnicity, socioeconomics, and geographical location; studies focused on specific areas. One key study was from Canada (Wilson et al., 2013), but some of the assumptions were derived from U.S. populations.	
Currency	Most of the tracer element studies were conducted in the 1980s and 1990s; activity pattern modeling studies are more recent; biokinetic modeling studies have more recent publication dates, but were generally based on older data.	
Data Collection Period	Tracer element studies' data collection periods may not represent long-term behaviors. Biokinetic model comparison and survey response studies represent longer term behaviors. Data used in modeled simulation estimates may not represent long-term behaviors.	

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Table 5-2. Confidence in Recommendations for Ingestion of Soil and Dust (Continued)

General Assessment Factors	Rationale	Rating
Clarity and Completeness		Medium
Accessibility	All key studies are available from the peer-reviewed literature.	
Reproducibility	For methodologies used by more than one research group, reproducible results were obtained in some instances.	
Quality Assurance	For some studies, information on quality assurance/quality control was limited or absent.	
Variability and Uncertainty		Low
Variability in Population	Tracer element and activity pattern methodology studies characterized variability among study sample members; the IEUBK model used in the biokinetic approach uses average soil ingestion rates. Day-to-day and seasonal variability was not very well characterized. Numerous factors that may influence variability have not been explored in detail.	
Minimal Uncertainty	Estimates are highly uncertain. Tracer element study designs appear to introduce biases in the results. Modeled estimates may be sensitive to input variables.	
Evaluation and Review		Medium
Peer Review	All key studies appeared in peer-reviewed journals.	
Number and Agreement of Studies	14 key studies, but some key studies are reanalyses of previously published data. Researchers using similar methodologies obtained generally similar results. While there is general agreement between researchers using different methodologies, estimates based on the activity pattern methodology generally yield somewhat lower estimates than both the tracer and biokinetic modeling approaches.	
Overall Rating		**Low**

[a] See Section 1.5.2 in Chapter 1 of the *Exposure Factors Handbook: 2011 Edition* for a detailed description of the evaluation criteria used in this table.

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5.3. KEY AND RELEVANT STUDIES

The key tracer element, biokinetic model comparison, and survey response studies are summarized in the following sections. Certain studies were considered "key" and were used as a basis for developing the recommendations, using judgment about the study's design features, applicability, and utility of the data to U.S. soil and dust ingestion rates, clarity and completeness, and characterization of uncertainty and variability in ingestion estimates. Because the studies often were performed for reasons unrelated to developing long-term soil and dust ingestion recommendations, their attributes that were characterized as "limitations" in this chapter might not be limitations when viewed in the context of the study's original purpose. However, when studies are used for developing a soil or dust ingestion recommendation, EPA has categorized some studies' design or implementation as preferable to others. In general, EPA chose studies designed either with a census or randomized sample approach over studies that used a convenience sample, or other nonrandomized approach, as well as studies that more clearly explained various factors in the study's implementation that affect interpretation of the results. However, in some cases, studies that used a nonrandomized design contain information that is useful for developing exposure factor recommendations (e.g., if they are the only studies of children in a particular age category), and thus may have been designated as "key" studies. Other studies were considered "relevant" but not "key" because they provide useful information for evaluating the reasonableness of the data in the key studies or provide supporting information, but in EPA's judgment they did not meet the same level of soundness, applicability and utility, clarity and completeness, and characterization of uncertainty and variability that the key studies did or they may not be representative of the U.S. general population. In addition, studies that did not contain information that can be used to develop a specific recommendation for mg/day soil and dust ingestion were classified as relevant rather than key. However, some studies classified as "relevant" may be used as the basis for recommendations for particular exposure settings (e.g., Doyle et al., 2012, Irvine et al., 2014 for populations engaging in rural or wilderness lifestyles).

Some studies are reanalyses of previously published data. For this reason, the sections that follow are organized into key and relevant studies of primary analysis (i.e., studies in which researchers have developed primary data pertaining to soil and dust ingestion) and key and relevant studies of secondary analysis (i.e., studies in which researchers have interpreted previously published results, or data that were originally collected for a different purpose).

The three methodologies described in this chapter to derive soil and dust ingestion rates have limitations. Because some of these limitations apply equally to all the studies within each methodology, they are discussed in more detail in Section 5.4 separately from the study summaries. Additional limitations specific to each study are described within each study summary. The discussion of limitations does not imply that the studies were conducted inappropriately, rather they are limitations inherent in these methodologies.

5.3.1. Methodologies Used in Key Studies

5.3.1.1. *Tracer Element Methodology*

The tracer element methodology attempts to quantify the amounts of soil ingested by analyzing samples of soil and dust from residences and/or children's play areas, and feces or urine. The soil, dust, fecal, and urine samples are analyzed for the presence and quantity of tracer elements—typically, aluminum, silicon, titanium, and other elements. A key underlying assumption is that these elements are not metabolized into other substances in the body or absorbed from the gastrointestinal tract in significant quantities, and thus their presence in feces and urine can be used to estimate the quantity of soil ingested by mouth. Although they are sometimes called mass balance studies, none of the studies attempt to quantify amounts excreted in perspiration, tears, glandular secretions, or shed skin, hair, or finger- and toenails, nor do they account for tracer element exposure via the dermal or inhalation routes, and thus they are not a complete "mass balance" methodology. Early studies using this methodology did not always account for the contribution of tracer elements from nonsoil substances (food, medications, and nonfood sources such as toothpaste) that might be swallowed. U.S. studies using this methodology in or after the mid to late 1980s account for, or attempt to account for, tracer element contributions from these nonsoil sources. Some study authors adjust their soil ingestion estimate results to account for the potential contribution of tracer elements found in household dust as well as soil.

Empirical estimates of soil ingestion rates in children have been made by back-calculating the

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mass of soil and/or dust a subject would need to ingest to achieve a tracer element mass measured in collected excreta (i.e., feces and urine). The following is a general expression for the trace element ("tracer") mass balance:

$$M_{ingested\ in\ soil} = M_{feces+urine} - M_{non-soil\ or\ dust}$$

(Eqn. 5-1)

where:

$M_{ingested\ in\ soil}$ = mass of tracer in soil or dust that is ingested (mg)

$M_{feces\ +\ urine}$ = mass of tracer measured in feces and urine (mg)

$M_{nonsoil\ or\ dust}$ = mass of tracer measured in nonsoil or dust (e.g., food, water, medicine, toothpaste) (mg)

Dividing the mass of tracer in soil or dust that is ingested by the measured tracer concentration in soil (mg/g) yields an estimate of the mass of soil ingested, S (g):

$$S = \frac{M_{ingested\ in\ soil}}{C_{soil\ or\ dust}}$$

where:

S = mass of soil or dust ingested (g)

$C_{soil\ or\ dust}$ = concentration of tracer in soil or dust (mg/g)

(Eqn. 5-2)

The U.S. tracer element researchers have all assumed a certain offset, or lag time between ingestion of food, medication, and soil, and the resulting fecal and urinary output. The lag times used are typically 24 or 28 hours (Davis and Mirick, 2006; Stanek et al., 2001a; Stanek and Calabrese, 1995b); thus, these researchers subtract the previous day's food and medication tracer element quantity ingested from the current day's fecal and urinary tracer element quantity that was excreted. When compositing food, medication, fecal, and urine samples across the entire study period, daily estimates can be obtained by dividing the total estimated soil ingestion by the number of days in which fecal and/or urine samples were collected. A variation of the algorithm that provides slightly higher estimates of soil ingestion is to divide the total estimated soil ingestion by the number of days on which feces were produced, which by definition would be equal to or less than the total number of days of the study period's fecal sample collection.

Substituting tracer element dust concentrations for tracer element soil concentrations yields a dust ingestion estimate. Because the actual nonfood, nonmedication quantity ingested is a combination of soil and dust, the unknown true soil and dust ingestion is likely to be somewhere between the estimates based on soil concentrations and those based on dust concentrations. Tracer element researchers have described ingestion estimates for soil that actually represent a combination of soil and dust, but were calculated based on tracer element concentrations in soil. Similarly, they have described ingestion estimates for dust that are actually for a combination of soil and dust, but were calculated based on tracer element concentrations in dust. Other variations on these general soil and dust ingestion algorithms have been published in attempts to account for time spent indoors, time spent away from the house, and other factors that might influence the relative proportion of soil versus dust.

Each individual's soil and dust ingestion can be represented as an unknown constant in a set of simultaneous equations of soil or dust ingestion represented by different tracer elements. To date, only two of the U.S. research teams (Lásztity et al., 1989; Barnes, 1990) have published estimates calculated for pairs of tracer elements using simultaneous equations.

The U.S. tracer element studies have been performed for only short-duration study periods, and only for 33 adults (Davis and Mirick, 2006) and 241 children (101 in Davis et al. [1990], 12 of whom were studied again in Davis and Mirick [2006]; 64 in Calabrese et al. [1989] and Barnes [1990]; 64 in Calabrese et al. [1997a]; and 12 in Calabrese et al. [1997b]). The studies provide information on quantities of soil and dust ingested for the studied groups for short time periods, but provide limited information on overall prevalence of soil ingestion by U.S. adults and children, and limited information on the frequency of higher soil ingestion episodes.

While there are advantages to using the tracer method (e.g., estimates are provided based on empirical data vs. modeling; direct measurements), there are also sources of uncertainty associated with this method. For example, error sources sometime cause individual soil or dust ingestion estimates for some tracers to be negative, and in some studies, this resulted in median or mean "mass balance" soil ingestion estimates that were also negative for some tracers. Authors of these studies have averaged both

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negative and positive numbers together in their estimation of soil ingestion rates. For soil and dust ingestion estimates based on each particular tracer, or averaged across tracers, the net impact of competing upward and downward sources of error is unclear. Other sources of error can influence the estimates in an upward direction (e.g., not accounting for all nonsoil/dust sources of the tracer elements). A more detailed discussion of the uncertainties and limitations associated with the tracer method is provided in Section 5.4.1.

5.3.1.2. *Biokinetic Model Comparison Methodology*

The Biokinetic Model Comparison methodology compares direct measurements of a biomarker, such as blood or urine levels of a toxicant, with predictions from a biokinetic model of oral, dermal, and inhalation exposure routes with air, food, water, soil, and dust toxicant sources. An example is to compare measured children's blood lead levels with predictions from the IEUBK model. Where environmental contamination of lead in soil, dust, and drinking water has been measured and those measurements can be used as model inputs for the children in a specific community, the model's assumed soil and dust ingestion values can be evaluated by comparing the model's predictions of blood lead levels with those children's measured blood lead levels. It should be noted, however, that such confirmation of the predicted blood lead levels would be confirmation of the net impact of all model inputs, and not just soil and dust ingestions. Under the assumption that (actual) blood lead levels of various groups of children studied were accurately measured, and those measured blood lead levels are consistent with biokinetic model predictions for those groups of children, then the model's default assumptions may correspond to the central tendency, or typical, children in an assessed group of children. Nevertheless, the model's default assumptions for biokinetics and intake rates can be useful for predicting outcomes for highly exposed children if the higher exposure occurs as increased concentrations in the relevant media, and if the default population variability is relevant for the group of children under consideration. Use of the IEUBK in this way assumes that blood lead can be used as a suitable biomarker for soil and dust ingestion. An advantage of this method is that it can be used to indirectly estimate long-term soil and dust intake. A detailed discussion on the limitations and uncertainties associated with this method is provided in Section 5.4.2.

5.3.1.3. *Activity Pattern Methodology*

The activity pattern methodology combines information on hand-to-mouth and object-to-mouth activities (microactivities) and time spent at various locations (microenvironments) with assumptions about transfer parameters (e.g., soil-to-skin adherence, saliva removal efficiency) and other exposure factors (e.g., frequency of hand washing) to derive estimates of soil and dust ingestion. This methodology has been used in U.S. EPA's stochastic human exposure and dose simulation (SHEDS) model. The SHEDS model is a probabilistic model that can simulate cumulative (multiple chemicals) or aggregate (single chemical) residential exposures for a population of interest over time via multiple routes of exposure for different types of chemicals and scenarios, including those involving soil ingestion (U.S. EPA, 2010).

The activity pattern methodology includes observational studies as well as surveys of adults, children's caretakers, or children themselves, via in-person or mailed questionnaires that ask about mouthing behavior and ingestion of various nonfood items and time spent in various microenvironments. There are three general approaches to gather data on children's mouthing behavior: real-time hand recording, in which trained observers manually record information (Davis et al., 1995); video-transcription, in which trained videographers tape a child's activities and subsequently extract the pertinent data manually or with computer software (Black et al., 2005); and questionnaire, or survey response, techniques (Stanek et al., 1998).

An advantage of this method is that it does not require collection of biologic samples. Also, soil and dust ingestion can be estimated separately. One of the limitations of this approach includes the availability and quality of the input variables. Özkaynak et al. (2011) found that the model is most sensitive to dust loadings on carpets and hard floor surfaces, soil-to-skin adherence factors, hand mouthing frequency, and hand washing frequency (Özkaynak et al., 2011). A detailed discussion of the limitations and uncertainties associated with this method is provided in Section 5.4.3.

5.3.2. Key Studies of Primary Analysis

The sections that follow provide summaries of key studies in which researchers have developed primary data pertaining to soil and dust ingestion.

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5.3.2.1. *Vermeer and Frate (1979)—Geophagia in Rural Mississippi: Environmental and Cultural Contexts and Nutritional Implications*

Vermeer and Frate (1979) performed a survey response study in Holmes County, MS in the 1970s (date unspecified). Questions about geophagy (defined as regular consumption of clay over a period of weeks) were asked of household members (N = 229 in 50 households; 56 were women, 33 were men, and 140 were children or adolescents) of a subset of a random sample of nutrition survey respondents. Caregiver responses to questions about 115 children under 13 years old indicate that geophagy was likely to be practiced by a minimum of 18 (16%) of these children; however, 16 of these 18 children were 1 to 4 years old, and only 2 of the 18 were older than 4 years. Of the 56 women, 32 (57%) reported eating clay. There was no reported geophagy among 33 men or 25 adolescent study subjects questioned.

In a separately administered survey, geophagy and pica data were obtained from 142 pregnant women over a period of 10 months. Geophagy was reported by 40 of these women (28%), and an additional 27 respondents (19%) reported other pica behavior, including the consumption of laundry starch, dry powdered milk, and baking soda.

The average daily amount of clay consumed was reported to be about 50 grams, for the adult and child respondents who acknowledged practicing geophagy. Quantities were usually described as either portions or multiples of the amount that could be held in a single, cupped hand. Clays for consumption were generally obtained from the B soil horizon, or subsoil rather than an uppermost layer, at a depth of 50 to 130 cm.

5.3.2.2. *Calabrese et al. (1989)—How Much Soil Do Young Children Ingest: An Epidemiologic Study/Barnes (1990)—Childhood Soil Ingestion: How Much Dirt Do Kids Eat?/Calabrese et al. (1991)—Evidence of Soil Pica Behavior and Quantification of Soil Ingested*

Calabrese et al. (1989) and Barnes (1990) studied soil ingestion among children using eight tracer elements—aluminum, barium, manganese, silicon, titanium, vanadium, yttrium, and zirconium. A nonrandom sample of 1-, 2-, and 3-year-olds (30 males and 34 females) from the greater Amherst, MA area was studied, presumably in 1987. The children were predominantly from two-parent households where the parents were highly educated.

The study was conducted over a period of 8 days spread over 2 weeks. During each week, duplicate samples of food, beverages, medicines, and vitamins were collected on Monday through Wednesday, while excreta, excluding wipes and toilet paper, were collected for four 24-hour cycles running from Monday/Tuesday through Thursday/Friday. Soil and dust samples were also collected from the children's homes and play areas. Study participants were supplied with toothpaste, baby cornstarch, diaper rash cream, and soap with low levels of most of the tracer elements. Quality control of the analysis yielded recoveries between 88.1% and 100.2% for all tracers except zirconium, which had a low recovery.

Table 5-3 shows the published mean soil ingestion estimates ranging from −294 mg/day based on manganese to 459 mg/day based on vanadium, median soil ingestion estimates ranging from −261 mg/day based on manganese to 96 mg/day based on vanadium, and 95th percentile estimates ranged from 106 mg/day based on yttrium to 1,903 mg/day based on vanadium. Maximum daily soil ingestion estimates ranged from 1,391 mg/day based on zirconium to 7,281 mg/day based on manganese. Dust ingestion estimates calculated using tracer concentrations in dust were often, but not always, higher than soil ingestions calculated using tracer concentrations in soil.

Data for the uppermost 23 subject-weeks (the highest soil ingestion estimates, averaged over the 4 days of excreta collection during each of the 2 weeks) were published in Calabrese et al. (1991). One child's soil pica behavior was estimated in Barnes (1990) using both the subtraction/division algorithm and the simultaneous equations method. On two particular days during the second week of the study period, the child's aluminum-based soil ingestion estimates were 19 g/day (18,700 mg/day) and 36 g/day (35,600 mg/day), silicon-based soil ingestion estimates were 20 g/day (20,000 mg/day) and 24 g/day (24,000 mg/day), and simultaneous-equation soil ingestion estimates were 20 g/day (20,100 mg/day) and 23 g/day (23,100 mg/day) (Barnes, 1990). By tracer, averaged across the entire week, this child's estimates ranged from approximately 10 to 14 g/day during the second week of observation, excluding zirconium, which presented limitations with the analytical protocol (Calabrese et al., 1991, see Table 5-4), and averaged 6 g/day across the entire study period. Additional information about this child's apparent ingestion of soil versus dust during the study period was published in Calabrese and Stanek (1992a).

5.3.2.3. *Davis et al. (1990)—Quantitative Estimates of Soil Ingestion in Normal Children between the Ages of 2 and 7 Years: Population-Based Estimates Using Aluminum, Silicon, and Titanium as Soil Tracer Elements*

Davis et al. (1990) used a tracer element technique to estimate soil ingestion among children. In this study, 104 children between the ages of 2 and 7 years were randomly selected from a three-city area in southeastern Washington State. Soil and dust ingestion was evaluated by analyzing soil and house dust, feces, urine, and duplicate food, dietary supplement, medication, and mouthwash samples for aluminum, silicon, and titanium. Data were collected for 101 of the 104 children during July, August, or September 1987. In each family, data were collected over a 7-day period, with 4 days of excreta sample collection. Dried soil samples were passed successively through a 20- and 60-mesh (850 and 250 µm, respectively; ASTM, 2017) stainless steel sieve. Participants were supplied with toothpaste with known tracer element content. In addition, information on dietary habits and demographics was collected to identify behavioral and demographic characteristics that influence soil ingestion rates among children. The amount of soil ingested on a daily basis was estimated using Equation 5-3:

$$S_{i,e} = \frac{(((DW_f + DW_p) \times E_f) + 2E_u) - (DW_{fd} \times E_{fd})}{E_{soil}}$$

(Eqn. 5-3)

where:

$S_{i,e}$ = soil ingested for child i based on tracer e (grams)
DW_f = feces dry weight (grams)
DW_p = feces dry weight on toilet paper (grams)
E_f = tracer concentration in feces (µg/g)
E_u = tracer amount in urine (µg)
DW_{fd} = food dry weight (grams)
E_{fd} = tracer concentration in food (µg/g)
E_{soil} = tracer concentration in soil (µg/g)

The tracer amount in urine (E_u) was multiplied by a factor of 2 to account for the fact that parents were asked to collect half of the total daily urine output. The soil ingestion rates were corrected by adding the amount of tracer in vitamins and medications to the amount of tracer in food and adjusting the food, fecal, and urine sample weights to account for missing samples. Food, fecal, and urine samples were composited over a 4-day period, and estimates for daily soil ingestion were obtained by dividing the 4-day composited tracer quantities by 4. Davis et al. (1990) reported that recoveries for most analyses were within the quality control limits, ±20% for laboratory samples and ±25% for the matrix spiked samples.

Soil ingestion rates were highly variable, especially those based on titanium. Mean daily soil ingestion estimates were 38.9 mg/day for aluminum, 82.4 mg/day for silicon, and 245.5 mg/day for titanium (see Table 5-5). Median values were 25.3 mg/day for aluminum, 59.4 mg/day for silicon, and 81.3 mg/day for titanium. The investigators also evaluated the extent to which differences in tracer concentrations in house dust and yard soil impacted estimated soil ingestion rates. The value used in the denominator of the soil ingestion estimate equation was recalculated to represent a weighted average of the tracer concentration in yard soil and house dust based on the proportion of time the child spent indoors and outdoors, using an assumption that the likelihood of ingesting soil outdoors was the same as that of ingesting dust indoors. The adjusted mean soil/dust ingestion rates were 64.5 mg/day for aluminum, 160.0 mg/day for silicon, and 268.4 mg/day for titanium. Adjusted median soil/dust ingestion rates were 51.8 mg/day for aluminum, 112.4 mg/day for silicon, and 116.6 mg/day for titanium. The authors also investigated whether nine behavioral and demographic factors could be used to predict soil ingestion. They found family income less than $15,000/year and swallowing toothpaste to be predictors with silicon-based estimates, residing in one of the three cities to be a significant predictor with aluminum-based estimates, and washing the face before eating significant for titanium-based estimates.

5.3.2.4. *Calabrese et al. (1997a)—Soil Ingestion Estimates for Children Residing on a Superfund Site*

Calabrese et al. (1997a) estimated soil ingestion rates for children residing on a Superfund site using a methodology in which eight tracer elements were analyzed. The methodology used in this study is similar to that employed in Calabrese et al. (1989), except that rather than using barium, manganese, and vanadium as three of the eight tracers, the researchers replaced them with cerium, lanthanum,

and neodymium. A total of 64 children ages 1 to 4 years (36 males, 28 females) were selected for this study of the Anaconda, MT area. The study was conducted for seven consecutive days during September or September and October, apparently in 1992, shortly after soil was removed and replaced in some residential yards in the area. Duplicate samples of meals, beverages, and over-the-counter medicines and vitamins were collected over the 7-day period, along with fecal samples. In addition, soil and dust samples were collected from the children's home and play areas. Soil samples were sieved through a 2-mm nylon mesh. A subsample was ground and sieved using a 200-mesh (75 µm; ASTM, 2017) screen. Dust samples were sieved to separate fine dust from larger pieces. Toothpaste containing nondetectable levels of the tracer elements, with the exception of silica, was provided to all of the children. Infants were provided with baby cornstarch, diaper rash cream, and soap, which were found to contain low levels of tracer elements.

Because of the high degree of intertracer variability, Calabrese et al. (1997a) also derived estimates based on the "best tracer methodology" (BTM). This BTM uses food:soil (F:S) tracer concentration ratios in order to correct for errors caused by misalignment of tracer input and outputs, ingestion of nonfood sources, and nonsoil sources (Stanek and Calabrese, 1995a). A low F:S ratio is desired because it minimizes transit time errors. The BTM did not use the results from cerium, lanthanum, and neodymium despite these tracers having low F:S ratios because the soil concentrations for these elements were found to be affected by particle size and more susceptible to source errors. Calabrese et al. (1997a) noted that estimates based on aluminum, silicon, and yttrium in this study may result in lower soil ingestion estimates than the true value because the apparent residual negative errors found for these three tracers for a large majority of subjects. It was noted that soil ingestion estimates for this population may be lower than estimates found by previous studies in the literature because of families' awareness of contamination from the Superfund site, which may have resulted in altered behavior.

Soil ingestion estimates were also examined based on various demographic characteristics. There were no statistically significant differences in soil ingestion based on age, sex, birth order, or house yard characteristics (Calabrese et al., 1997a). Although not statistically significant, soil ingestion rates were generally higher for females, children with lower birth number, children with parents employed as laborers, service professionals, homemakers, unemployed, and children with pets (Calabrese et al., 1997a).

Table 5-6 shows the estimated soil and dust ingestion by each tracer element and by the BTM. Based on the best tracer, the mean soil ingestion rate was 65.5 mg/day.

5.3.2.5. *Stanek et al. (1998)—Prevalence of Soil Mouthing/Ingestion among Healthy Children Aged One to Six/Calabrese et al. (1997b)—Soil Ingestion Rates in Children Identified by Parental Observation as Likely High Soil Ingesters*

Stanek et al. (1998) conducted a survey response study using in-person interviews of parents of children attending well visits at three western Massachusetts medical clinics in August, September, and October of 1992. Of 528 children ages 1 to 7 years with completed interviews, parents reported daily mouthing or ingestion of sand and stones in 6%, daily mouthing or ingestion of soil and dirt in 4%, and daily mouthing or ingestion of dust, lint. and dustballs in 1%. Parents reported more than weekly mouthing or ingestion of sand and stones in 16%, more than weekly mouthing or ingestion of soil and dirt in 10%, and more than weekly mouthing or ingestion of dust, lint and dustballs in 3%. Parents reported more than monthly mouthing or ingestion of sand and stones in 27%, more than monthly mouthing or ingestion of soil and dirt in 18%, and more than monthly mouthing or ingestion of dust, lint, and dustballs in 6%.

Calabrese and colleagues performed a follow-up tracer element study (Calabrese et al., 1997b) for a subset ($N = 12$) of the Stanek et al. (1998) children (ages 1 to 3) whose caregivers had reported daily sand/soil ingestion ($N = 17$). The time frame of the follow-up tracer study relative to the original survey response study was not stated; the study duration was 7 days. Of the 12 children in Calabrese et al. (1997b), one exhibited behavior that the authors believed was clearly soil pica; Table 5-7 shows estimated soil ingestion rates for this child during the study period. Estimates ranged from –10 mg/day to 7,253 mg/day depending on the tracer. The mean soil ingestion rate for the pica child, using aluminum and silicon as tracers, is approximately 1,000 mg/day (rounded to one significant figure).

Table 5-8 presents the estimated average daily soil and dust ingestion estimates for the 12 children studied. Estimates calculated based on soil tracer element concentrations only for the 12 subjects ranged from –15 to 1,783 mg/day based on aluminum, −46 to 931 mg/day based on silicon, and

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–47 to 3,581 mg/day based on titanium. Estimated average daily dust ingestion estimates ranged from −39 to 2,652 mg/day based on aluminum, −51 to 3,145 mg/day based on silicon, and −98 to 3,632 mg/day based on titanium. Quantities for soil and dust are presented separately and assume that the entire quantity of residual fecal tracers originates entirely from soil or dust. Calabrese et al. (1997b) questioned the validity of retrospective caregiver reports of soil pica on the basis of the tracer element results.

5.3.2.6. *Davis and Mirick (2006)—Soil Ingestion in Children and Adults in the Same Family*

Davis and Mirick (2006) calculated soil ingestion for children and adults in the same family using a tracer element approach. Data were collected one year after the Davis et al. (1990) study was conducted. Samples were collected and prepared for laboratory analysis and then stored for a 2-year period prior to tracer element quantification with laboratory analysis. Analytical recovery values for spiked samples were within the quality control limits of ±25%. The 20 families in this study were a nonrandom subset of the 104 families who participated in the soil ingestion study by Davis et al. (1990). Data collection issues resulted in sufficiently complete data for only 19 of the 20 families consisting of a child participant from the Davis et al. (1990) study ages 3 to 7, inclusive, and a female and male parent or guardian living in the same house. Duplicate samples of all food and medication items consumed, and all feces excreted, were collected for 11 consecutive days. Urine samples were collected twice daily for 9 of the 11 days; for the remaining 2 days, attempts were made to collect full 24-hour urine specimens. Soil and house dust samples were also collected. Soil and dust samples were passed successively through a 20- and 60-mesh (850 and 250 µm, respectively, ASTM, 2017) stainless steel sieves. Only 12 children had sufficiently complete data for use in the soil and dust ingestion estimates.

Tracer elements for this study included aluminum, silicon, and titanium. Toothpaste was supplied for use by study participants. In addition, parents completed a daily diary of activities for themselves and the participant child for 4 consecutive days during the study period.

Table 5-9 shows soil ingestion rates for all three family member participants. The mean and median estimates for children for all three tracers ranged from 36.7 to 206.9 mg/day and 26.4 to 46.7 mg/day, respectively, and fall within the range of those reported by Davis et al. (1990). Adult soil ingestion estimates ranged from 23.2 to 624.9 mg/day for mean values and from 0 to 259.5 mg/day for median values. This is based on 33 adults with complete food, excreta, and soil data. Adult soil ingestion estimates were more variable than those of children in the study regardless of the tracer. The authors believed that this higher variability may have indicated an important occupational contribution of soil ingestion in some, but not all, of the adults. As in previous studies, the soil ingestion estimates were the highest for titanium. Although toothpaste is a known source of titanium, the titanium content of the toothpaste used by study participants was not determined.

Only three of a number of behaviors examined for their relationship to soil ingestion were found to be associated with increased soil ingestion in this study:

- Reported eating of dirt (for children),
- Occupational contact with soil (for adults), and
- Hand washing before meals (for both children and adults).

Several typical childhood behaviors, however, including thumb-sucking, furniture licking, and carrying around a blanket or toy were not associated with increased soil ingestion for the participating children. Among both parents and children, neither nail-biting nor eating unwashed fruits or vegetables was correlated with increased soil ingestion. However, because duplicate food samples were used to "correct" for dietary intake of tracers, accounting for soil ingestion from eating unwashed fruits or vegetables was not possible. Although eating unwashed fruits or vegetables was not reflected in the soil ingestion estimates in this study, the authors noted that it is a behavior that could lead to soil ingestion. When investigating correlations within the same family, a child's soil ingestion was not found to be associated with either parent's soil ingestion, nor did the mother and father's soil ingestion appear to be correlated.

5.3.3. Key Studies of Secondary Analysis

The following sections provide summaries of key studies of secondary analysis (i.e., studies in which researchers have interpreted previously published results, or data that were originally collected for a different purpose).

5.3.3.1. *Calabrese and Stanek (1995)—Resolving Intertracer Inconsistencies in Soil Ingestion Estimation*

Calabrese and Stanek (1995) explored sources and magnitude of positive and negative errors in soil ingestion estimates for children on a subject-week and trace element basis. Calabrese and Stanek (1995) identified possible sources of positive errors as follows:

- Ingestion of high levels of tracers before the start of the study and low ingestion during the study period and
- Ingestion of element tracers from a nonfood or nonsoil source during the study period.

Possible sources of negative bias were identified as follows:

- Ingestion of tracers in food that are not captured in the fecal sample either due to slow lag time or not having a fecal sample available on the final study day and
- Sample measurement errors that result in diminished detection of fecal tracers, but not in soil tracer levels.

The authors developed an approach that attempted to reduce the magnitude of error in the individual trace element ingestion estimates. Results from a previous study conducted by Calabrese et al. (1989) were used to quantify these errors based on the following criteria: (1) a lag period of 28 hours was assumed for the passage of tracers ingested in food to the feces (this value was applied to all subject-day estimates), (2) a daily soil ingestion rate was estimated for each tracer for each 24-hour day a fecal sample was obtained, (3) the median tracer-based soil ingestion rate for each subject-day was determined, and (4) negative errors due to missing fecal samples at the end of the study period were also determined. Also, upper- and lower-bound estimates were determined based on criteria formed using an assumption of the magnitude of the relative standard deviation presented in another study conducted by Stanek and Calabrese (1995b). Daily soil ingestion rates for tracers that fell beyond the upper and lower ranges were excluded from subsequent calculations, and the median soil ingestion rates of the remaining tracer elements were considered the best estimate for that particular day. The magnitude of positive or negative error for a specific tracer per day was derived by determining the difference between the value for the tracer and the median value.

Table 5-10 presents the estimated magnitude of positive and negative error for six tracer elements in the children's study (conducted by Calabrese et al., 1989). The original nonnegative mean soil ingestion rates (see Table 5-3) ranged from a low of 21 mg/day based on zirconium to a high of 459 mg/day based on vanadium. The adjusted mean soil ingestion rate after correcting for negative and positive errors ranged from 97 mg/day based on yttrium to 208 mg/day based on titanium. Calabrese and Stanek (1995) concluded that correcting for errors at the individual level for each tracer element provides more reliable estimates of soil ingestion.

5.3.3.2. *Stanek and Calabrese (1995a)—Soil Ingestion Estimates for Use in Site Evaluations Based on the Best Tracer Method*

Stanek and Calabrese (1995a) recalculated soil ingestion rates for adults and children from two previous studies using data for eight tracers from Calabrese et al. (1989) and three tracers from Davis et al. (1990). Recalculations were performed using the BTM. This method selected the "best" tracer(s), by dividing the total amount of tracer in a particular child's duplicate food sample by tracer concentration in that child's soil sample to yield a (F:S) ratio. The F:S ratio was small when the tracer concentration in food was low compared to the tracer concentration in soil. Small F:S ratios were desirable because they lessened the impact of transit time error (the error that occurs when fecal output does not reflect food ingestion, due to fluctuation in gastrointestinal transit time) in the soil ingestion calculation.

For adults, Stanek and Calabrese (1995a) used data for eight tracers from the Calabrese et al. (1989) study to estimate soil ingestion by the BTM. The lowest F:S ratios were zirconium and aluminum and the element with the highest F:S ratio was manganese. For soil ingestion estimates based on the median of the lowest four F:S ratios, the tracers contributing most often to the soil ingestion estimates were aluminum, silicon, titanium, yttrium, vanadium, and zirconium. Using the median of the soil ingestion rates based on the best four tracer elements, the average adult soil ingestion rate was estimated to be 64 mg/day with a median of 87 mg/day. The 95th percentile soil ingestion

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estimate was 142 mg/day. These estimates are based on 18 subject weeks for the 6 adult volunteers described in Calabrese et al. (1989).

The BTM used a ranking scheme of F:S ratios to determine the best tracers for use in the ingestion rate calculation. To reduce the impact of biases that may occur as a result of sources of fecal tracers other than food or soil, the median of soil ingestion estimates based on the four lowest F:S ratios was used to represent soil ingestion.

Using the lowest four F:S ratios for each individual child, calculated on a per-week ("subject-week") basis, the median of the soil ingestion estimates from the Calabrese et al. (1989) study most often included aluminum, silicon, titanium, yttrium, and zirconium. Table 5-11 presents the soil ingestion estimates based on the median values for aluminum, silicon, and titanium for each child; the median of the best four tracers for each child, and the best tracer for each child. Based on the median of soil ingestion estimates from the best four tracers, the mean soil ingestion rate for children was 132 mg/day and the median was 33 mg/day. The 95th percentile value was 154 mg/day.

For the 101 children in the Davis et al. (1990) study, the mean soil ingestion rate was 69 mg/day and the median soil ingestion rate was 44 mg/day (see Table 5-11). The 95th percentile estimate was 246 mg/day. These data are based on the three tracers (i.e., aluminum, silicon, and titanium) from the Davis et al. (1990) study. When the results for the 128 subject-weeks in Calabrese et al. (1989) and 101 children in Davis et al. (1990) were combined, soil ingestion for children was estimated to be 104 mg/day (mean); 37 mg/day (median); and 217 mg/day (95th percentile), using the BTM.

5.3.3.3. *Hogan et al. (1998)—Integrated Exposure Uptake Biokinetic Model for Lead in Children: Empirical Comparisons with Epidemiologic Data*

Hogan et al. (1998) used the IEUBK model, to compare model predictions of blood lead levels with epidemiological data to serve as one component of the model validation. Environmental lead measurement data from 478 children (38 were 0.5 to <1 year; 440 were 1 to <7 years) across three epidemiological studies were used as input to the IEUBK model. Model results were compared to blood lead levels from the same children. These children were a subset of the entire population of children living in three historic lead smelting communities (Palmerton, PA; Madison County, IL; and southeastern Kansas/southwestern Missouri), whose environmental lead exposures (soil and dust lead levels) had been studied as part of public health evaluations in these communities. The study populations were, in general, random samples of children 6 months to 7 years of age. Children who had lived in their residence for less than 3 months or those reported by their parents to be away from home more than 10 hours per week (>20 hours/week for the Pennsylvania data set) were excluded due to lack of information regarding lead exposure at the secondary location. The nature of the soil and dust exposures for the residential study population were typical, with the sample size considered sufficiently large to ensure that a wide enough range of children's behavior would be spanned by the data. Comparisons were made for a number of exposure factors, including age, location, time spent away from home, time spent outside, and whether or not children took food outside to eat.

The IEUBK model is a biokinetic model for predicting children's blood lead levels that uses measurements of lead content in house dust, soil, drinking water, food, and air. Model users use default assumptions for the lead contents and intake rates for each exposure medium (including soil) when they do not have specific information for each child.

Hogan et al. (1998) compared children's measured blood lead levels with biokinetic model predictions (IEUBK version 0.99d) of blood lead levels, using the children's measured drinking water, soil, and dust lead contamination levels together with default IEUBK model inputs for soil and dust ingestion, relative proportions of soil and dust ingestion, lead bioavailability from soil and dust, and other model parameters. Thus, the default soil and dust ingestion rates, and other default assumptions in the model, were tested by comparing measured blood lead levels with the model's predictions for those children's blood lead levels. Most IEUBK model kinetic and intake parameters were drawn independently from published literature (White et al., 1998; U.S. EPA, 1994b). Elimination parameters in particular had relatively less literature to draw upon (few data in children) and were fixed through a calibration exercise using a data set with children's blood lead levels paired with measured environmental lead exposures in and around their homes, while holding the other model parameters constant.

Results for all community-wide children 6 months to 7 years of age were as follows: for Palmerton, PA ($N = 34$), the geometric mean measured blood lead levels (6.8 µg/dL) were slightly over-predicted by the model (7.5 µg/dL); for

southeastern Kansas/southwestern Missouri (N = 111), the blood lead levels (5.2 µg/dL) were slightly under-predicted (4.6 µg/dL), and for Madison County, IL (N = 333), the geometric mean measured blood lead levels matched the model predictions (5.9 µg/dL measured and predicted), with very slight differences in the 95% confidence interval. Geometric mean model predictions were within 1 µg/dL of the observed geometric mean blood lead levels in the three populations studied. Hogan et al. (1998) noted that results may vary depending on the ability to identify children's playing areas and the use of different environmental sampling methods. In addition, interactions between socioeconomic status, race, and sex can vary across communities and make generalizations of results difficult. For 31 children 6 to 12 months old in the Madison County, IL site only, Hogan et al. (1998) reported a predicted geometric mean blood lead level 1.4-fold higher than the geometric mean blood lead levels observed.

Default soil and dust ingestion rates used in this version of the IEUBK model were: 135 mg/day for 1-, 2-, and 3-year-olds; 100 mg/day for 4-year-olds; 90 mg/day for 5-year-olds; and 85 mg/day for 6-year-olds (U.S. EPA 1994b, 2007). These values represent mean soil and dust ingestion rates; distributional data are not used in the model. The time-averaged daily soil + dust ingestion rate for these 6 years of life was 113 mg/day. Hogan et al. (1998) did not provide information on the particle sizes of the soil analyzed for this study. Because particle size may be an important factor in estimating the concentrations of elements in soil, this adds uncertainties to the results. Regardless of this and other uncertainties, these results suggest that the combination of assumptions used as model input parameters, including soil and dust ingestion rates, are roughly accurate for 440 1- to <7-year-old children in the three locations studied.

5.3.3.4. *Özkaynak et al. (2011)—Modeled Estimates of Soil and Dust Ingestion Rates for Children*

Özkaynak et al. (2011) developed soil and dust ingestion rates for children 3 to <6 years of age using U.S. EPA's SHEDS model for multimedia pollutants (SHEDS-Multimedia). The authors had two main objectives for this research: (1) to demonstrate an application of the SHEDS model while identifying and quantifying the key factors contributing to the predicted variability and uncertainty in the soil and dust ingestion exposure estimates and (2) to compare the modeled results to existing tracer-element field measurements. The SHEDS model is a physically based probabilistic exposure model, which combines diary information on sequential time spent in different locations and activities drawn from EPA's Consolidated Human Activity Database (CHAD), with micro-activity data (e.g., hand-to-mouth frequency, hand-to-surface frequency), surface/object soil or dust loadings, and other exposure factors (e.g., soil-to-skin adherence, saliva removal efficiency). The SHEDS model generates simulated individuals, who are then followed through time, generally up to one year. The model computes changes to their exposure at the diary event level.

For this study, an indirect modeling approach was used in which soil and dust were assumed to first adhere to the hands and remain until washed off or ingested by mouthing. The object-to-mouth pathway for soil/dust ingestion was also addressed. For this application of the SHEDS model, however, other avenues of soil/dust ingestion were not considered. Outdoor matter was designated as "soil" and indoor matter as "dust." Estimates for the distributions of exposure factors such as activity, time outdoors, environmental concentrations, soil-skin and dust-skin transfer, hand washing frequency and efficiency, hand-mouthing frequency, area of object or hand mouthed, mouthing removal rates, and other variables were obtained from the literature. These input variables were used in this SHEDS model application to generate estimates of soil and dust ingestion rates for a simulated population of 1,000. Both sensitivity and uncertainty analyses were conducted. Based on the sensitivity analysis, the model results are the most sensitive to dust loadings on carpet and hard floor surfaces, soil-skin adherence factor, hand mouthing frequency, and mean number of hand washes per day. Based on 200 uncertainty simulations that were conducted, the modeling uncertainties were seen to be asymmetrically distributed around the 50th (median) or the central variability distribution.

Table 5-12 shows the predicted soil and dust ingestion rates. Mean total soil and dust ingestion was predicted to be 68 mg/day, with approximately 60% originating from soil ingestion, 30% from dust on hands, and 10% from dust on objects. It is important to note that this is different from the assumptions used in the IEUBK model of 55% dust and 45% soil. Hand-to-mouth soil ingestion was found to be the most important pathway, followed by hand-to-mouth dust ingestion, then object-to-mouth dust ingestion. The authors noted that these modeled estimates were found to be consistent with other soil/dust ingestion values in the literature, but

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slightly lower than the central tendency value of 100 mg/day recommended in EPA's *Child-Specific Exposure Factors Handbook* (U.S. EPA, 2008).

The SHEDS methodology can be applied to specific study populations of interest, a wide range of input parameters, and can generate a full range of distributions. One advantage of this methodology is that it produced both ingestion of soil and ingestion of dust. However, data for some of the input variables are lacking. Data needs include additional information on the activities and environments of children in younger age groups, including children with high hand-to-mouth, object-to-mouth, and pica behaviors, and information on skin adherence and dust loadings on indoor objects and floors, and information to evaluate temporal variability. Assumptions used, particularly for the transfer of dust from both bare floors and carpets to hands and soil loadings to hands while playing outdoors, may be considered low for these parameters compared to other experimental data available. The model also assumes that the same hand area is mouthed on each occasion, which may lead to lower dust intake rates. In addition, other age groups of interest were not included because of lack of data for some of the input variables.

5.3.3.5. *Wilson et al. (2013)—Revisiting Dust and Soil Ingestion Rates Based on Hand-to-Mouth Transfer*

Wilson et al. (2013) provided estimates of the ingestion rates of indoor dust and outdoor soil for Canadians using both deterministic and probabilistic methods. An indirect modeling approach was used. Based on data from multiple studies, Wilson et al. (2013) estimated dust and soil ingestion using measures of particle loading to indoor surfaces, the fraction transferred to the hands, hand surface areas, the fraction of hand surface area that may be mouthed or contact food, the frequency of hand-to-mouth contacts, the amount dissolved in saliva, and the exposure time. The following equations were used to estimate indoor dust and outdoor soil ingestion, respectively:

$$DIG = DSL \times FTSS \times SA_{hand} \times FSA_{fingers} \times FQ \times SE \times ET \quad \text{(Eqn. 5-4)}$$

and

$$SIR = SL_{hands} \times SA_{hand} \times FSA_{fingers} \times FQ \times SE \times ET \quad \text{(Eqn. 5-5)}$$

where:

DIG	=	dust ingestion rate (mg/day)
DSL	=	dust surface loading (mg/cm^2)
ET	=	exposure time (hours/day)
FQ	=	frequency of hand-to-mouth events (events/hour)
$FSA_{fingers}$	=	fractional surface area of hand mouthed
$FTSS$	=	fraction of dust transferred from surfaces to the skin
SA_{hand}	=	surface area of one hand (cm^2)
SE	=	saliva extraction factor (unitless)
SIR	=	soil ingestion rate (mg/day)
SL_{hands}	=	soil loading (mass of soil adhering to hands) (mg/cm^2)

The input parameters used in these equations are provided in Table 5-13. For FTSS, it was assumed that contact occurred with hard surfaces (e.g., nonporous floor surfaces such as tile or hardwood, countertops, tables, window sills) 50% of the time and with soft surfaces (e.g., carpets, sofas, beds) 50% of the time, except for infants for whom contact was assumed to occur with soft surfaces only (i.e., 100% of the time). In addition, it was assumed that the front surface of the four fingers and the thumb of one hand was the area mouthed.

Mean dust ingestion rates were similar within age groups when using either the deterministic or probabilistic approach based on 200,000 trials (see Table 5-14), ranging from 2.2 mg/day for teenagers (ages 12 to 19 years) to 41 mg/day for toddlers (ages 7 months to 4 years). Mean soil ingestion rates ranged from 1.2 mg/day for seniors (ages 60+ years) to 23 mg/day for children (ages 5 to 11 years) using the probabilistic approach (see Table 5-14). Mean soil ingestion rates using the deterministic approach were similar. Combined dust and soil ingestion rates ranged from 3.7 mg/day for teenagers (ages 12 to 19 years) to 61 mg/day for toddlers (ages 7 months to 4 years). The 95th percentile combined soil and dust ingestion rates ranged from 12.6 mg/day for teenagers to 204 mg/day for toddlers.

Ingestion rates were estimated for a wide range of age groups, and separate ingestion estimates were provided for both dust and soil. However, the study used input values from multiple studies to generate the dust and soil ingestion rates, and each individual study is expected to have its own shortcomings, which also apply to this analysis. Some of these limitations include uncertainties with regard to the methodologies for dust collection in hard and soft surfaces in the studies selected for the analysis,

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assumptions of a single hand contact with surfaces, assumptions about the parts of the hand mouthed, and not accounting for effects of soil and hand moisture on soil and dust loadings on the skin. Also, the model does not account for object-to-mouth contact. Many of these assumptions used by Wilson et al. (2013) in the model would tend to underestimate amount of soil and dust ingested.

5.3.3.6. *Von Lindern et al. (2016)—Estimating Children's Soil/Dust Ingestion Rates through Retrospective Analyses of Blood Lead Biomonitoring from the Bunker Hill Superfund Site in Idaho*

Von Lindern et al. (2016) conducted an analysis to estimate age-specific soil/dust ingestion rates using the IEUBK model for lead in children and soil and house dust lead bioavailability data from the Bunker Hill Mining and Metallurgical Complex Superfund Site in Idaho. A total of 271 soil/dust samples archived during 1986–2002, including 193 house dust samples, 73 yard soil samples, and 5 quality control samples, were retrieved from storage. The samples were matched with blood lead data and information on the child's age and sex, home location, and property remediation status. After sieving to 80-mesh (180 µm; ASTM, 2017), they were analyzed for total lead and in vitro bioaccessibility. In vivo relative and absolute bioavailability was calculated from in vitro bioaccessibility results. A total of 2,176 records of blood/soil/dust/lead concentrations were available.

Structural equations modeling (SEM), which is a statistical multivariate methodology, was used to determine soil and dust partitions, age-specific soil and dust intake rates, and lead uptake from sources other than soil and dust (i.e., air, diet, and water). Von Lindern et al. (2016) calculated the total lead uptake by dividing the measured blood lead levels (µg/L) by the age-specific biokinetic slope factors in the IEUBK model. Total lead uptake is the sum of the uptakes from the four components: air, water, diet, and soil/dust. Soil and dust intake rates were calculated by assigning partition coefficients (i.e., fractional contributions to total soil/dust ingestion by each source) using Equation 5-6.

$$IR_{sd} = 1{,}000 \times \{UP_{sd}/[(C_d \times PT_d \times ABS_d) + (C_{ys} \times PT_{ys} \times ABS_{ys}) + (C_{cs} \times PT_{cs} \times ABS_{cs}) + (C_{ns} \times PT_{ns} \times ABS_{ns})]\}$$

(Eqn. 5-6)

where:

IR_{sd} = soil/dust intake rate (mg/day)
UP_{sd} = uptake from soil/dust (µg/day)
C = concentration from the various sources (i.e., d = dust; ys = yard soil; cs = community soil; ns = neighborhood soil) (mg/kg)
PT = partition coefficient for the various sources (unitless)
ABS = absolute bioavailability for the various sources (unitless)

Soil and dust intake rates were calculated for four source partition (PT) scenarios: (1) the IEUBK model default of 55% dust and 45% soil (55/45); (2) the original Bunker Hill Superfund Site model of 40% dust, 30% yard soil, and 30% geometric mean community soil (40/30/30GM); (3) the same partition as in scenario 2, but using the arithmetic mean (40/30/30AM); and (4) the SEM using 50% dust, 25% yard soil, 10% neighborhood soil, and 15% community soil (50/25/10/15). Mean soil and dust absolute bioavailability (ABS) was estimated to be 33% (SD ± 4%) and 28% (SD ± 6%), respectively. These values are similar to the 30% value recommended in the IEUBK model as a default.

Central tendency age-specific soil and dust intake rates for the four partition scenarios were estimated using Equation 5-5, and are presented in Table 5-15. All partition scenarios produced similar central tendency intake rates (von Lindern et al., 2016). For children ages 0.5 to 9 years, the mean soil and dust ingestion rates ranged from 47 mg/day to 100 mg/day. Among the age groups evaluated, children ages 1 to <2 years had the highest mean soil and dust ingestion rates (i.e., 89–100 mg/day), and children older than 2 years, had estimated soil and dust ingestion rates averaging approximately 60 mg/day. Age-specific distributions of soil and dust intake rates for the four partition scenarios are shown in Table 5-16. The 95th percentile soil and dust intake rates ranged from 120 mg/day to 493 mg/day. The 55/45 partition scenario consistently produced higher 95th percentile soil and dust intake rates compared to the other scenarios. These age specific soil/dust intake rates were input to the IEUBK model to compare predicted and observed blood lead levels (von Lindern et al., 2016). Linear regression analyses indicated that the 50/25/10/15 and the 40/30/30 average intake rates were the partition scenarios that best fit the blood lead levels predicted by the IEUBK model to the observed blood lead levels. The data from these two

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partition scenarios were used in developing the recommended soil and dust ingestion values shown in Tables 5-1 and 5-34.

This study provides soil and dust intake rates for various age groups including very young children. Blood lead data were collected for a number of consecutive years. The strengths of this study are that the soil and dust ingestion estimates are based on a large number of samples, and that the estimates represent long-term exposures. One of the limitations of this study is that partition coefficients that were derived may not be representative of all age groups, all neighborhoods in the study, or other populations. Partition of soil and dust materials contributing to an individual child's lead uptake can be expected to vary strongly among residences and among children. The authors also acknowledged that education and intervention programs may have resulted in children's temporary reduction in soil/dust ingestion rates at this site (von Lindern et al., 2016). Increased knowledge and concern about lead exposure may lead to residents taking steps to reduce their child's lead exposure through more frequent hand washing, more household vacuuming, and changing the areas where children play. This is evidenced by a decline in the estimated average ingestion rates from 1988 to 2002 when the analysis was performed for the different partition scenarios by year. Other uncertainties include not accounting for the variability in the children's blood lead levels with time, a limited number of soil and dust measurements, the representativeness of the soil and dust measurements of the children's play areas, and measurement error. In addition, the equations derived by von Lindern et al. (2016) for the analysis would be highly sensitive to low lead media concentrations and less sensitive to higher lead concentrations.

5.3.4. Relevant Studies of Primary Analysis

The following studies are classified as relevant rather than key. They either do not provide a quantitative estimate of soil ingestion, or they are estimates generated for a population that may not be representative of the U.S. general population. Studies that provide data for special populations such as those engaging in wilderness lifestyles in Canada (Doyle et al., 2012; Irvine et al., 2014) are presented in this section. Data from these studies may be appropriate for high soil contact scenarios. The general population tracer element studies described in this section are not designated as key because the methodology to account for nonsoil tracer exposures (e.g., food, medicine) was not as well developed as the methodology in the U.S. tracer element studies described in Sections 5.3.2 and 5.3.3. However, the method of Clausing et al. (1987) and data of van Wijnen et al. (1990) were used in developing biokinetic model default soil and dust ingestion rates (U.S. EPA, 1994a) used in the Hogan et al. (1998) study and von Lindern et al. (2016), which were designated as key. In most cases, the survey response studies were of a nonrandomized design, provided insufficient information to determine important details regarding study design, or provided no data to allow quantitative estimates of soil and/or dust ingestion rates.

5.3.4.1. *Dickins and Ford (1942)—Geophagy (Dirt Eating) among Mississippi Negro School Children*

Dickins and Ford (1942) conducted a survey response study of rural Black school children (4th grade and above) in Oktibbeha County, MS in September 1941. A total of 52 of 207 children (18 of 69 boys and 34 of 38 girls) studied gave positive responses to questions administered in a test-taking format regarding having eaten dirt in the previous 10 to 16 days. The authors stated that the study sample likely was more representative of the higher socioeconomic levels in the community because older children from lower socioeconomic levels sometimes left school in order to work and because children in the lower grades, who were more socioeconomically representative of the overall community, were excluded from the study. Clay was identified as the predominant type of soil eaten.

5.3.4.2. *Ferguson and Keaton (1950)—Studies of the Diets of Pregnant Women in Mississippi: II Diet Patterns*

Ferguson and Keaton (1950) conducted a survey response study of a group of 361 pregnant women receiving health care at the Mississippi State Board of Health, who were interviewed regarding their diet, including the consumption of clay or starch. All of the women were from the lowest economic and educational level in the area, and 92% were Black. Of the Black women, 27% reported eating clay and 41% eating starch. In the group of White women, 7 and 10% reporting clay- and starch-eating, respectively. The amount of starch eaten ranged from 2–3 small lumps to 3 boxes (24 ounces) per day. The amount of clay eaten ranged from one tablespoon to one cup per day.

5.3.4.3. *Cooper (1957)—Pica: A Survey of the Historical Literature as Well as Reports from the Fields of Veterinary Medicine and Anthropology, the Present Study of Pica in Young Children, and a Discussion of Its Pediatric and Psychological Implications*

Cooper (1957) conducted a nonrandomized survey response study in the 1950s of children age 7 months or older referred to a Baltimore, MD mental hygiene clinic. For 86 out of 784 children studied, parents or caretakers gave positive responses to the question, "Does your child have a habit, or did he ever have a habit, of eating dirt, plaster, ashes, etc.?" and identified dirt, or dirt combined with other substances, as the substance ingested. Cooper (1957) described a pattern of pica behavior, including ingesting substances other than soil, being most common between ages 2 and 4 or 5 years, with one of the 86 children ingesting clay at age 10 years and 9 months.

5.3.4.4. *Barltrop (1966)—The Prevalence of Pica*

Barltrop (1966) conducted a randomized survey response study of children born in Boston, MA between 1958 and 1962, inclusive, whose parents resided in Boston and who were neither illegitimate nor adopted. A stratified random subsample of 500 of these children was contacted for in-person caregiver interviews, in which a total of 186 families (37%) participated. A separate stratified subsample of 1,000 children was selected for a mailed survey in which 277 (28%) of the families participated. Interview-obtained data regarding caregiver reports of pica (in this study is defined as placing nonfood items in the mouth and swallowing them) behavior in all children ages 1 to 6 years in the 186 families (N = 439) indicated 19 had ingested dirt (defined as yard dirt, house dust, plant-pot soil, pebbles, ashes, cigarette ash, glass fragments, lint, and hair combings) in the preceding 14 days. These data do not appear to have been corrected for unequal selection probability in the stratified random sample, nor were they corrected for nonresponse bias. Interviews were conducted in the March/April time frame, presumably in 1964. Mail-survey obtained data regarding caregiver reports of pica in the preceding 14 days indicated that 39 of 277 children had ingested dirt, presumably using the same definition as above. Barltrop (1966) mentions several possible limitations of the study, including nonparticipation bias and respondents' memory, or recall, effects.

5.3.4.5. *Bruhn and Pangborn (1971)—Reported Incidence of Pica among Migrant Families*

Bruhn and Pangborn (1971) conducted a survey among 91 low income families of migrant agricultural workers in California in May through August 1969. Families in two labor camps (Madison camp, 10 miles west of Woodland, and Davis camp, 10 miles east of Davis) were of Mexican descent, and families in one camp (Harney Lane camp 17 miles north of Stockton) were "Anglo." Participation was 34 of 50 families at the Madison camp, 31 of 50 families at the Davis camp, and 26 of 26 families at the Harney Lane camp. Respondents for the studied families (primarily wives) gave positive responses to open-ended questions such as "Do you know of anyone who eats dirt or laundry starch?" Bruhn and Pangborn (1971) apparently asked a modified version of this question pertaining to the respondents' own or relatives' families. They reported 18% (12 of 65) of Mexican families' respondents as giving positive responses for consumption of "dirt" among children within the Mexican respondents' own or relatives' families. They reported 42% (11 of 26) of "Anglo" families' respondents as giving positive responses for consumption of "dirt" among children within the Anglo respondents' own or relatives' families.

5.3.4.6. *Robischon (1971)—Pica Practice and Other Hand-Mouth Behavior and Children's Developmental Level*

A survey response sample of 19- to 24-month-old children examined at an urban well-child clinic in the late 1960s or 1970 in an unspecified location indicated that 48 of the 130 children whose caregivers were interviewed, exhibited pica behavior (defined as "ate nonedibles more than once a week"). The specific substances eaten were reported for 30 of the 48 children. All except 2 of the 30 children habitually ate more than one nonedible substance. The soil and dust-like substances reported as eaten by these 30 children were: ashes (17), "earth" (5), dust (3), fuzz from rugs (2), clay (1), and pebbles/stones (1). Caregivers for some of the study subjects (between 0 and 52 of the 130 subjects, exact number not specified) reported that the children "ate nonedibles less than once a week."

5.3.4.7. *Bronstein and Dollar (1974)—Pica in Pregnancy*

The frequency and effects of pica behavior was investigated by Bronstein and Dollar (1974) in 410 pregnant, low-income women from both urban

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(N = 201) and rural (N = 209) areas in Georgia. The women selected were part of the Nutrition Demonstration Project, a study investigating the effect of nutrition on the outcome of the pregnancy, conducted at the Eugene Talmadge Memorial Hospital and University Hospital in Augusta, GA. During their initial prenatal visit, each patient was interviewed by a nutrition counselor who questioned her food frequency, social and dietary history, and the presence of pica. Patients were categorized by age, parity, and place of residence (rural or urban).

Of the 410 women interviewed, 65 (16%) stated that they practiced pica. A variety of substances were ingested, with laundry starch being the most common. There was no significant difference in the practice of pica between rural and urban women, although older rural women (20–35 years) showed a greater tendency to practice pica than younger rural or urban women (<20 years). The number of previous pregnancies did not influence the practice of pica. The authors noted that the frequency of pica among rural patients had declined from a previous study conducted 8 years earlier, and attributed the reduction to a program of intensified nutrition education and counseling provided in the area. No specific information on the amount of pica substances ingested was provided by this study, and the data are more than 30 years old.

5.3.4.8. *Hook (1978)—Dietary Cravings and Aversions during Pregnancy*

Hook (1978) conducted interviews of 250 women who had each delivered a live infant at two New York hospitals; the interviews took place in 1975. The mothers were first asked about any differences in consumption of seven beverages during their pregnancy, and the reasons for any changes. They were then asked, without mentioning specific items, about any cravings or aversions for other foods or nonfood items that may have developed at any time during their pregnancy.

Nonfood items reportedly ingested during pregnancy were ice, reported by three women, and chalk from a river clay bank, reported by one woman. In addition, one woman reported an aversion to nonfood items (specific nonfood item not reported). No quantity data were provided by this study.

5.3.4.9. *Binder et al. (1986)—Estimating Soil Ingestion: The Use of Tracer Elements in Estimating the Amount of Soil Ingested by Young Children*

Binder et al. (1986) used a tracer technique modified from a method previously used to measure soil ingestion among grazing animals to study the ingestion of soil among children 1 to 3 years of age who wore diapers. The children were studied during the summer of 1984 as part of a larger study of residents living near a lead smelter in East Helena, MT. Fecal samples from diapers were collected over a 3-day period from 65 children (42 males and 23 females), and composited samples of soil were obtained from the children's yards. Both excreta and soil samples were analyzed for aluminum, silicon, and titanium. These elements were found in soil but were thought to be poorly absorbed in the gut and to have been present in the diet only in limited quantities. Excreta measurements were obtained for 59 of the children. Soil ingestion by each child was estimated on the basis of each of the three tracer elements using a standard assumed fecal dry weight of 15 g/day, and the following Equation 5-7:

$$T_{i,e} = \frac{f_{i,e} \times F_i}{S_{i,e}} \qquad \text{(Eqn. 5-7)}$$

where:

$T_{i,e}$ = estimated soil ingestion for child i based on element e (g/day)
$f_{i,e}$ = concentration of element e in fecal sample of child i (mg/g)
F_i = fecal dry weight (g/day)
$S_{i,e}$ = concentration of element e in child i's yard soil (mg/g)

The analysis assumed that (1) the tracer elements were neither lost nor introduced during sample processing, (2) the soil ingested by children originates primarily from their own yards, and (3) that absorption of the tracer elements by the children occurred in only small amounts. The study did not distinguish between ingestion of soil and house dust, nor did it account for the presence of the tracer elements in ingested foods or medicines.

The arithmetic mean quantity of soil ingested by the children in the Binder et al. (1986) study was estimated to be 181 mg/day (range 25 to 1,324) based on the aluminum tracer, 184 mg/day (range 31

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to 799) based on the silicon tracer, and 1,834 mg/day (range 4 to 17,076) based on the titanium tracer (see Table 5-17). The overall mean soil ingestion estimate, based on the minimum of the three individual tracer estimates for each child, was 108 mg/day (range 4 to 708). The median values were 121 mg/day, 136 mg/day, and 618 mg/day for aluminum, silicon, and titanium, respectively. The 95th percentile values for aluminum, silicon, and titanium were 584 mg/day, 578 mg/day, and 9,590 mg/day, respectively. The 95th percentile value based on the minimum of the three individual tracer estimates for each child was 386 mg/day.

The authors were not able to explain the difference between the results for titanium and for the other two elements, but they speculated that unrecognized sources of titanium in the diet or in the laboratory processing of stool samples may have accounted for the increased levels. The frequency distribution graph of soil ingestion estimates based on titanium in the Binder et al. (1986) paper (not provided here) shows that a group of 21 children had particularly high titanium values (i.e., >1,000 mg/day). The remainder of the children showed titanium ingestion estimates at lower levels, with a distribution more comparable to that of the other elements.

5.3.4.10. *Clausing et al. (1987)—A Method for Estimating Soil Ingestion by Children*

Clausing et al. (1987) conducted a soil ingestion study with Dutch children using a tracer element methodology. The study measured aluminum, titanium, and acid-insoluble residue (AIR) contents of fecal samples from children aged 2 to 4 years attending a nursery school, and for samples of playground dirt at that school. Over a 5-day period, 27 daily fecal samples were obtained for 18 children. Soil samples from the direct surroundings of the nursery school were sieved through a 250 µm screen. Using the average soil concentrations present at the school, and assuming a standard fecal dry weight of 10 g/day, soil ingestion was estimated for each tracer. Six hospitalized, bedridden children served as a control group, representing children who had very limited access to soil; eight daily fecal samples were collected from the hospitalized children.

Recoveries from analytical analyses ranged from 54–89% for titanium and aluminum. Without correcting for the tracer element contribution from background sources, represented by the hospitalized children's soil ingestion estimates, the aluminum-based soil ingestion estimates for the school children in this study ranged from 23 to 979 mg/day, the AIR-based estimates ranged from 48 to 362 mg/day, and the titanium-based estimates ranged from 64 to 11,620 mg/day. As in the Binder et al. (1986) study, a fraction of the children (6/18) showed titanium values above 1,000 mg/day, with most of the remaining children showing substantially lower values. Calculating an arithmetic mean quantity of soil ingested based on each fecal sample yielded 232 mg/day for aluminum; 129 mg/day for AIR, and 1,431 mg/day for titanium (see Table 5-18). Based on the limiting tracer method (LTM) and averaging across each fecal sample, the arithmetic mean soil ingestion was estimated to be 105 mg/day with a population standard deviation of 67 mg/day (range 23 to 362 mg/day); geometric mean soil ingestion was estimated to be 90 mg/day. Use of the LTM assumed that "the maximum amount of soil ingested corresponded with the lowest estimate from the three tracers" (Clausing et al., 1987).

The hospitalized children's arithmetic mean aluminum-based soil ingestion estimate was 56 mg/day; titanium-based estimates included estimates for three of the six children that exceeded 1,000 mg/day, with the remaining three children in the range of 28 to 58 mg/day (see Table 5-19). AIR measurements were not reported for the hospitalized children. Using the LTM method, the mean soil ingestion rate was estimated to be 49 mg/day with a population standard deviation of 22 mg/day (range 26 to 84 mg/day). The geometric mean soil ingestion rate was 45 mg/day. The hospitalized children's data suggested a major nonsoil source of titanium for some children and a background nonsoil source of aluminum. However, conditions specific to hospitalization (e.g., medications) were not considered.

Clausing et al. (1987) estimated that the average soil ingestion of the nursery school children was 56 mg/day, after subtracting the mean LTM soil ingestion for the hospitalized children (49 mg/day) from the nursery school children's mean LTM soil ingestion (105 mg/day), to account for background tracer intake from dietary and other nonsoil sources.

5.3.4.11. *Van Wïjnen et al. (1990)—Estimated Soil Ingestion by Children*

In a tracer element study by Van Wïjnen et al. (1990), soil ingestion among Dutch children ranging in age from 1 to 5 years was evaluated using a tracer element methodology. Van Wïjnen et al. (1990) measured three tracers (titanium, aluminum, and AIR) in soil and feces. The authors estimated soil ingestion based on the LTM, which assumed that soil

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ingestion could not be higher than the lowest value of the three tracers. LTM values represented soil ingestion estimates that were not corrected for dietary intake. Recoveries for aluminum and titanium from soil materials mixed with fecal samples were 94 and 97%, respectively.

An average daily feces dry weight of 15 grams was assumed. A total of 292 children attending daycare centers were studied during the first of two sampling periods and 187 children were studied in the second sampling period; 162 of these children were studied during both periods (i.e., at the beginning and near the end of the summer of 1986). A total of 78 children were studied at campgrounds. Soil samples were sieved through a 2-mm mesh. The authors reported geometric mean LTM values because soil ingestion rates were found to be skewed and the log-transformed data were approximately normally distributed. Geometric mean LTM values were estimated to be 111 mg/day for children in daycare centers and 174 mg/day for children vacationing at campgrounds (see Table 5-20). For the 162 daycare center children studied during both sampling periods the arithmetic mean LTM was 162 mg/day, and the median was 114 mg/day. For the 78 children at the campgrounds, the overall arithmetic mean LTM was 213 mg/day and the median was 160 mg/day.

Fifteen hospitalized children were studied and used as a control group. These children's LTM soil ingestion estimates were 74 (geometric mean), 93 (mean), and 110 (median) mg/day. The authors assumed the hospitalized children's soil ingestion estimates represented dietary intake of tracer elements, and used rounded 95% confidence limits on the arithmetic mean, 70 to 120 mg/day (midpoint 95 mg/day), to correct the daycare and campground children's LTM estimates for dietary intake of tracers. Although the authors suggested that corrections should be made to the soil ingestion values obtained from the daycare and campground subjects by subtracting values obtained from the hospitalized children (i.e., background), they do not appear to have made these adjustments to the arithmetic means. These corrections would result in soil ingestion rates of 67 mg/day (162 mg/day minus 95 mg/day) for daycare children and 118 mg/day (213 mg/day minus 95 mg/day) for campers. Van Wijnen et al. (1990) showed corrected geometric mean soil ingestion to range from 0 to 90 mg/day, with a 90th percentile value of up to 190 mg/day for the various age categories within the daycare group and 30 to 200 mg/day, with a 90th percentile value of up to 300 mg/day for the various age categories within the camping group.

AIR was the limiting tracer in about 80% of the samples. Among children attending daycare centers, soil ingestion was also found to be higher when the weather was good (i.e., <2 days/week precipitation) than when the weather was bad (i.e., >4 days/week precipitation) (see Table 5-21).

5.3.4.12. *Calabrese et al. (1990)—Preliminary Adult Soil Ingestion Estimates: Results of a Pilot Study*

Calabrese et al. (1990) studied six adults to evaluate the extent to which they ingest soil. This adult study was originally part of the children soil ingestion study (Calabrese et al., 1989) and was used to validate part of the analytical methodology used in the children's study. The participants were six healthy adults, three males and three females, 25−41 years old. Each volunteer ingested one empty gelatin capsule at breakfast and one at dinner Monday, Tuesday, and Wednesday during the first week of the study. During the second week, they ingested 50 mg of sterilized soil within a gelatin capsule at breakfast and at dinner (a total of 100 mg of sterilized soil per day) for 3 days. For the third week, the participants ingested 250 mg of sterilized soil in a gelatin capsule at breakfast and at dinner (a total of 500 mg of soil per day) during the 3 days. Duplicate meal samples (food and beverage) were collected from the six adults. The sample included all foods ingested from breakfast Monday, through the evening meal Wednesday during each of the 3 weeks. In addition, all medications and vitamins ingested by the adults were collected. Total excretory output was collected from Monday noon through Friday midnight over three consecutive weeks.

Data obtained from the first week, when empty gelatin capsules were ingested, were used to estimate soil intake by adults. On the basis of recovery values, aluminum, silicon, yttrium, and zirconium were considered the most valid tracers. The mean values for these four tracers were: aluminum, 110 mg/day; silicon, 30 mg/day; yttrium, 63 mg/day; and zirconium, 134 mg/day. A limitation of this study is the small sample size. Thus, this study was classified as relevant.

5.3.4.13. *Cooksey (1995)—Pica and Olfactory Craving of Pregnancy: How Deep Are the Secrets?*

Postpartum interviews were conducted between 1992 and 1994 of 300 women at a mid-western hospital, to document their experiences of pica behavior. The majority of women were Black and low-income, and ranged in age from 13 to 42 years.

In addition to questions regarding nutrition, each woman was asked if during her pregnancy she experienced a craving to eat ice or other things that are not food.

Of the 300 women, 194 (65%) described ingesting one or more pica substances during their pregnancy, and the majority (78%) ate ice/freezer frost alone or in addition to other pica substances. Reported quantities of items ingested on a daily basis were three to four 8-pound bags of ice, two to three boxes of cornstarch, two cans of baking powder, one cereal bowl of dirt, five quarts of freezer frost, and one large can of powdered cleanser.

5.3.4.14. *Smulian et al. (1995)—Pica in a Rural Obstetric Population*

In 1992, Smulian et al. (1995) conducted a survey response study of pica in a convenience sample of 125 pregnant women in Muscogee County, GA, who ranged in age from 12 to 37 years. Of these, 73 were Black, 47 were White, 4 were Hispanic, and 1 was Asian. Interviews were conducted at the time of the first prenatal visit, using nondirective questionnaires to obtain information regarding substances ingested as well as patterns of pica behavior and influences on pica behavior. Only women ingesting nonfood items were considered to have pica. Ingestion of ice was included as a pica behavior only if the ice was reported to be ingested multiple times per day, if the ice was purchased solely for ingestion, or if the ice was obtained from an unusual source such as freezer frost.

The overall prevalence of pica behavior in this study was 14.4% (18 of 125 women), and was highest among Black women (17.8%). There was no significant difference between groups with respect to age, race, weight, or gestational age at the time of enrollment in the study. The most common form of pica was ice eating (pagophagia), reported by 44.4% of the patients. Nine of the women reported information on the frequency and amount of the substances they were ingesting. Of these women, 66.7% reported daily consumption and 33.3% reported pica behavior three times per week. Soap, paint chips, or burnt matches were reportedly ingested 3 days per week. One patient ate ice 60 times per week. Women who ate dirt or clay reported ingesting 0.5−1 pound per week. The largest amount of ice consumed was five pounds per day.

5.3.4.15. *Grigsby et al. (1999)—Chalk Eating in Middle Georgia: A Culture-Bound Syndrome of Pica?*

Grigsby et al. (1999) investigated the ingestion of kaolin, also known as white dirt, chalk, or white clay, in the central Georgia Piedmont area as a culture-bound syndrome. A total of 21 individuals who consumed kaolin at the time or had a history of consuming kaolin were interviewed, using a seven-item, one-page interview protocol. All of those interviewed were Black, ranging in age from 28 to 88 years (mean age of 46.5 years), and all were female except for one.

Reasons for eating kaolin included liking the taste, being pregnant, craving it, and to gain weight. Eight respondents indicated that they obtained the kaolin from others, five reported getting it directly from the earth, four purchased it from a store, and two obtained it from a kaolin pit mine. The majority of the respondents reported that they liked the taste and feel of the kaolin as they ate it. Only three individuals reported knowing either males or White persons who consumed kaolin. Most individuals were not forthcoming in discussing their ingestion of kaolin and recognized that their behavior was unusual.

The study suggests that kaolin-eating is primarily practiced by Black women who were introduced to the behavior by family members or friends, during childhood or pregnancy. The authors concluded that kaolin ingestion is a culturally transmitted form of pica, not associated with any other psychopathology. Although information on kaolin eating habits and attitudes were provided by this study, no quantitative information on consumption was included, and the sample population was small and nonrandom.

5.3.4.16. *Ward and Kutner (1999)—Reported Pica Behavior in a Sample of Incident Dialysis Patients*

Structured interviews were conducted with a sample of 226 dialysis patients in the metropolitan Atlanta, GA area from September 1996 to September 1997. Interviewers were trained in nutrition data collection methods, and patients also received a 3-day diet diary that they were asked to complete and return by mail. If a subject reported a strong past or current food or nonfood craving, a separate form was used to collect information to determine whether this was a pica behavior.

Pica behavior was reported by 37 of the dialysis patients studied (16%), and most of these patients (31 of 37) reported that they were currently practicing some form of pica behavior. The patients'

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race and sex were significantly associated with pica behavior, with Black patients and women making up 86% and 84% of those reporting pica, respectively. Those reporting pica behavior were also younger than the remainder of the sample, and two patients described a persistent craving for ice. Other pica items reportedly consumed included starch, dirt, flour, or aspirin.

5.3.4.17. *Simpson et al. (2000)—Pica During Pregnancy in Low-Income Women Born in Mexico*

Simpson et al. (2000) interviewed 225 Mexican-born women, aged 18−42 years (mean age of 25 years), using a questionnaire administered in Spanish. Subjects were recruited by approaching women in medical facilities that served low-income populations in the cities of Ensenada, Mexico (N = 75), and Santa Ana, Bakersfield and East Los Angeles, CA (N = 150). Criteria for participation were that the women had to be Mexican-born, speak Spanish as their primary language, and be pregnant or have been pregnant within the past year. Only the data for the women in the United States are included in this handbook.

Pica behavior was reported in 31% of the women interviewed in the United States. Table 5-22 shows the items ingested and the number of women reporting the pica behavior. Of the items ingested, only ice was said to be routinely eaten outside of pregnancy, and was only reported by U.S. women, probably because none of the low-income women interviewed in Mexico owned a refrigerator. Removing the 12 women who reported eating only ice from the survey lowers the percentage of U.S. women who reported pica behavior to 23%. Women said they engaged in pica behavior because of the taste, smell, or texture of the items, for medicinal purposes, or because of advice from someone, and one woman reported eating clay for religious reasons. Magnesium carbonate, a pica item not found to be previously reported in the literature, was reportedly consumed by 17% of women. The amount of magnesium carbonate ingested ranged from a quarter of a block to five blocks per day; the blocks were approximately the size of a 35-mm film box. No specific quantity information on the amounts of pica substances ingested was provided in the study.

5.3.4.18. *Obialo et al. (2001)—Clay Pica Has No Hematologic or Metabolic Correlate to Chronic Hemodialysis Patients*

A total of 138 dialysis patients at the Morehouse School of Medicine, Atlanta, GA, were interviewed about their unusual cravings or food habits. The patients were Black and ranged in age from 37 to 78 years. Obialo et al. (2001) suggested that the stress caused by end-stage renal disease may provide a stimulus for pica, especially for those with cultural predispositions.

Thirty of the patients (22%) reported some form of pica behavior, while 13 patients (9.4%) reported clay pica. The patients with clay pica reported daily consumption of 225−450 g of clay.

5.3.4.19. *Klitzman et al. (2002)—Lead Poisoning among Pregnant Women in New York City: Risk Factors and Screening Practices*

Klitzman et al. (2002) interviewed 33 pregnant women whose blood lead levels were >20 µg/dL as reported to the New York City Department of Health between 1996 and 1999. The median age of the women was 24 years (range of 15 to 43 years), and the majority were foreign born. The women were interviewed regarding their work, reproductive, and lead exposure history. A home visit was also conducted and included a visual inspection and a colorimetric swab test; consumable items suspected to contain lead were sent to a laboratory for analysis.

Thirteen women (39%) reported pica behavior during their current pregnancies. Of these, 10 reported eating soil, dirt, or clay; 2 reported pulverizing and eating pottery; and 1 reported eating soap. One of the women reported eating approximately one quart of dirt daily from her backyard for the past three months. No other quantity data were reported.

5.3.4.20. *Doyle et al. (2012)—A Soil Ingestion Pilot Study of a Population Following a Traditional Lifestyle Typical of Rural or Wilderness Areas*

Doyle et al. (2012) conducted a pilot study to estimate soil ingestion among a Canadian Aboriginal community living in a wilderness area. The study was conducted over a 3-week period during 2011 in the Nemiah Valley of British Columbia. The study was conducted on traditional lands in cooperation with the Xeni Gwet'in First Nation. Seven adults were recruited, and four of these adults were members of the Xeni Gwet'in community. During the study, the subjects participated in traditional

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daily activities (e.g., fishing, clearing, scouting, hiking, cutting firewood, etc.). Soil ingestion was estimated using a mass balance tracer methodology. The tracers used for this study were aluminum, barium, cerium, lanthanum, manganese, silicon, thorium, titanium, uranium, vanadium, yttrium, and zirconium. Daily soil ingestion was calculated for each subject based on tracer concentrations in fecal and soil samples, assuming a 24-hour transit time. Soil ingestion rates were corrected for tracers ingested via the diet and in medications. Participants recorded their food and medicine intake in daily logs. Soil, food, water, and fecal samples were analyzed at a commercial laboratory.

Soil ingestion rates calculated for the four most reliable tracers (aluminum, cerium, lanthanum, silicon) are shown in Table 5-23. The most reliable tracers were considered those that are poorly absorbed in the gastrointestinal tract and those with the lowest food:soil ratios. For all four tracers combined, the mean, median, and 90th percentile soil ingestion rates were 75, 50, and 211 mg/day, respectively. This study provides quantitative soil ingestion estimates for an adult Canadian population following a traditional wilderness lifestyle. However, as a pilot study, the sample population was very small, and is not expected to be representative of the U.S. population.

5.3.4.21. *Irvine et al. (2014)—Soil Ingestion Rate Determination in a Rural Population in Alberta, Canada Practicing a Wilderness Lifestyle*

Irvine et al. (2014) estimated soil ingestion rates among a group of First Nations people inhabiting a wilderness area of Alberta, Canada using the mass balance tracer method. The study was conducted over a 3-week period in August 2012. Nine adults within the Cold Lake First Nations Reserve who practiced traditional activities (e.g., hunting, fishing, and gathering) participated in the study. The tracers used for this study were aluminum, barium, cerium, lanthanum, manganese, silicon, thorium, titanium, uranium, vanadium, yttrium, and zirconium. Soil were sieved into multiple particle size fractions to separate soil particles of <63 µm. Daily soil ingestion was calculated for each subject based on tracer concentrations in fecal and soil samples. A 24-hour transit time was assumed, and soil ingestion rates were corrected for tracers ingested via the diet. Four tracers were considered to be the most reliable (aluminum, cerium, lanthanum, and silicon) because of their low food:soil ratios, but aluminum and silicon had lower coefficients of variance. Table 5-24 provides the estimated soil ingestion rates based on aluminum and silicon, and on all four of the most reliable tracers (aluminum, cerium, lanthanum, and silicon combined). Mean soil ingestion rates for these tracers ranged from 32 to 68 mg/day for this adult population; 90th percentile values ranged from 152 to 231 mg/day. This study provides quantitative soil ingestion estimates for an adult Canadian population following a traditional wilderness lifestyle. However, as a pilot study, the sample population was very small, and is not expected to be representative of the U.S. population.

5.3.4.22. *Lumish et al. (2014)—Gestational Iron Deficiency is Associated with Pica Behaviors in Adolescents*

Lumish et al. (2014) examined pica behavior and iron status among 158 pregnant adolescents (<18 years of age) receiving prenatal care at a health clinic in Rochester, NY in 2006−2009. The women were mostly African-American (two-thirds) and about 25% were Hispanic. At each visit, the women were asked whether they craved any nonfood items, and were asked to provide detailed information on the items that they craved or ate. A total of 18 different items were reported to have been ingested, including: ice; raw starches (flour and cornstarch); powder (dust, vacuum powder from vacuum cleaner bags, and baby powder); soap (soap, bar soap, laundry soap, and powdered cleansers); plastic/foam (stuffing from pillows/sofas and sponges); paper (writing paper, toilet paper, and tissues); baking soda/powder; and other (dirt and chalk). Overall, 46% of the teens reported ingesting one or more of these items. Ice was the nonfood item most often consumed (37% of all the pregnant adolescents), while only 1.3% of the teens reported ingestion of dirt/chalk. Lumish et al. (2014) also observed that a significantly larger proportion of African-American teens reported pica behavior than Caucasian teens. This study provides prevalence data for a population of pregnant adolescents. These data may not be entirely representative of the U.S. population as a whole or of pregnant women in general. Also, the study provides prevalence data only. No information on the quantity of substance ingested is provided.

5.3.4.23. *Jang et al. (2014)—General Factors of the Korean Exposure Factors Handbook*

Jang et al. (2014) reported on a soil ingestion study that was conducted in Seoul, South Korea. Feces samples were collected from 63 children, ages 0 to 7 years and analyzed for the following tracer elements: aluminum, barium, manganese, silicon,

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titanium, vanadium, yttrium, and zirconium. Soil samples collected from the areas where the children spent most of their time were analyzed for the same tracer elements. Five children who spent no time outdoors were used as controls to account for exposures to tracer elements from sources other than soil (e.g., food, medicine). Using the LTM, aluminum was used to estimate soil ingestion rates. Jang et al. (2014) reported that, after adjusting for the environmental background levels based on the control group, the estimated arithmetic mean soil ingestion rate was 118 mg/day, and the geometric mean was 29 mg/day. The 90th percentile value was 286 mg/day and the 95th percentile value was 898 mg/day. This study provides quantitative data for a study population in South Korea. While it does not appear that tracer elements in food or medicines were accounted for, a control population was used to adjust for exposures from sources other than soil. A limitation of this study is that the study population may not be representative of children in the United States.

5.3.4.24. *Chien et al. (2015)—Soil Ingestion Rates for Children under 3 Years Old in Taiwan*

Chien et al. (2015) conducted a soil ingestion tracer study of 66 children (33 boys and 33 girls) under 3 years of age recruited from health centers from northern, central, southern, and eastern Taiwan from May 2011 to November 2012. Duplicate 24-hour food and liquid samples were collected for each child on Day one. All feces were collected beginning on Day 2 through Day 4 of the study. Diapers were collected for those children who wore diapers and feces were removed in the laboratory. Urine samples were not collected. Outdoor soil samples from the top 0–5 cm of soil were collected from typical play areas. Household dust samples were collected from the living room, bedroom, and kitchen floors using a handheld vacuum cleaner. Both soil and household dust samples were sieved using a 100-mesh (150 µm; ASTM, 2017) screen. Samples were analyzed for titanium and silicon. Soil ingestion rates were calculated using Equation 5-7 adapted from Davis and Mirick (2006).

$$S_{i,e} = \left(\frac{A_f - A_{food}}{E_{soil}}\right) \qquad \text{(Eqn. 5-8)}$$

where:

$S_{i,e}$ = amount of soil ingested (mg/day)

A_f = daily concentration of tracer element in the feces (µg/day)

A_{food} = daily concentration of tracer element in the foods (µg/day)

E_{soil} = concentration of tracer elements in soils (g/kg)

The following equation was used to adjust the daily fecal tracer concentration to account for missing fecal samples.

$$A_f = \left(C_{f,2} \times \frac{DW_{f,2}}{DW_{f,2} + DW_{f,3} + DW_{f,4}} + C_{f,3} \times \frac{DW_{f,3}}{DW_{f,2} + DW_{f,3} + DW_{f,4}} + C_{f,4} \times \frac{DW_{f,4}}{DW_{f,2} + DW_{f,3} + DW_{f,4}}\right) \times DW_{f,ave}$$

(Eqn. 5-9)

where:

$C_{f,2}$ = concentration of tracer element in the feces on Day 2 (µg/day)

$C_{f,3}$ = concentration of tracer element in the feces on Day 3 (µg/day)

$C_{f,4}$ = concentration of tracer element in the feces on Day 4 (µg/day)

$DW_{f,2}$ = dry weight of the feces on Day 2 (g)

$DW_{f,3}$ = dry weight of the feces on Day 3 (g)

$DW_{f,4}$ = dry weight of the feces on Day 4 (g)

$DW_{f,ave}$ = average dry weight of the feces on Day 2 through Day 4 (g)

Activity pattern data were also collected by videotaping each child for 2 hours and by administering a questionnaire to parents or caregivers. Most of the children spent more than four times a week outdoors. About half of the children spent under one hour a day outdoors. Children washed their hands and took a bath or shower an average of 3.98 and 1.24 times a day, respectively.

Recoveries for the analyses were 100 ± 20%. The soil intake rates ranged from 0–82.6 mg/day for silicon and 36.4−1,850 mg/day for titanium, with an average of 9.6 mg/day (SD = 19.2 mg/day) and 957.1 mg/day (SD = 477 mg/day) for silicon and titanium, respectively (Chien et al., 2015). These estimates excluded children with soil intake rates that were considered outliers. Negative soil intake rate values were replaced by 0 mg/day in the

estimation of the average soil intake rates. Soil intake rates based on silicon were positively correlated with the total hand-to-mouth frequency for both indoors and outdoors. Soil intake rates for children with hand-to-mouth contact ≥15 contacts/hr was estimated to be 15.9 mg/day compared to 5.46 mg/day for children with <15 contacts/hour. Soil intake rates were also statistically significantly greater for children with higher hand washing frequency (≥4 events/day, mean = 17.4 mg/day) compared to children with less hand washing frequency (<4 events/day, mean = 5.6 mg/day). While not suggested as an explanation for this observation by Chien et al. (2015), this may due to re-loading of soil on hands and subsequent ingestion. Chien et al. (2015) found no significant difference between children's age and gender and the estimated soil intake rates. The authors stated that titanium may not be a suitable tracer for estimating soil intake rates in the study because other sources of titanium were not considered, including: peeling paint chips, toothpaste, and candy. They also noted that soil intake rates estimated in this study are lower than those in the United States. This may be due to several factors. The authors stated that dust loadings in the Taiwanese homes are lower than those in U.S. homes. As in most other Asian countries, the Taiwanese remove their shoes before entering the house, therefore, reducing the amount of household dust. The authors also found that the cleaning frequency in the study homes was higher than those found in studies performed in the United States. This study found correlations between soil intake rates and hand-to-mouth frequency behavior. The results, however, may not be representative of children's behavior in the United States. Another limitation is that the sources of tracer elements, other than food, were not accounted for in estimating of soil intake rates.

5.3.4.25. *Wang et al. (2015)—Quantification of Soil/Dust (SD) on the Hands of Children from Hubei Province, China Using Hand Wipes*

Wang et al. (2015) estimated soil and dust ingestion for three age ranges of children: kindergarten (ages 3 to <7 years), primary school (7 to <13 years), and middle school (13 to <17 years) in Hubei Province in Central China. Hand wipe samples and background area soil samples were both analyzed for three tracer elements (cerium, vanadium, and yttrium). Age-specific estimates of soil/dust adhering to the hands (in mg) were calculated as the amount of tracer element on the hand (in ng) divided by the amount of tracer element in the soil (in mg/kg). Based on the three tracer elements, the amount of soil/dust on the hands was estimated to range from 0.59–0.64 ng for kindergarten-aged children, 1.42–1.64 ng for primary school children, and 1.04–1.52 ng for middle school children. Wang et al. (2015) used these hand soil/dust estimates, along with assumptions about the number of hand-to-mouth contacts that occur over a 12-hour period, the proportion of the hand area that contacts the mouth, and the efficiency at which soil/dust is transferred from the hand to the mouth to estimate soil/dust ingestion rates. Hand-to-mouth contact was assumed to occur 15, 7, and 2 times per hour for kindergarten, primary, and middle school children, respectively. The proportion of the hand that contacts the mouth was assumed to be 0.1 (10%), and the transfer efficiency was assumed to be 0.159. The mean rates of hand soil/dust ingestion were estimated to be 1.79, 2.12, and 0.49 mg/day for kindergarten, primary, and middle school children, respectively. This study provides a novel method for estimating the amount of hand soil/dust ingested. However, these soil/dust ingestion estimates do not account for soil/dust ingested from other pathways (e.g., object-to-mouth contact, food-to-soil contact), and the population evaluated in this study may not be representative of the U.S. population. Additional limitations relate to the uncertainties associated with the input parameters for estimating soil/dust intake (e.g., transfer efficiency, proportion of the hand contacting the mouth).

5.3.4.26. *Lin et al. (2015)—Pica during Pregnancy among Mexican-Born Women: A Formative Study*

Lin et al. (2015) formed nine focus groups involving 76 Mexican-born pregnant or ≤2-year postpartum women. Three of the focus groups were in central California (N = 23), and six were held in Mexico (N = 53). The aim of the focus group discussions was to obtain information on the frequency and types of pica behavior among the women. Pica was defined as "eating items that were not food." Among the women in the California focus group, 10 (43%) indicated that they had engaged in pica, and 6 (60%) of these women reported pica behavior during pregnancy. Among the Mexican focus group, 18 (34%) reported engaging in pica, and 16 (80%) of these women reported engaging in pica during pregnancy. Commonly eaten items were earth, bean stones, and adobe. Among the California group, 5 (22%) had eaten earth. Among the Mexican

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group, 6 (11%) had eaten earth. Some women discussed their pica behavior in the context of micronutrient deficiencies, or perceived health consequences of unfulfilled cravings. This study provides information on the pica behavior for Mexican-born women living in the United States and Mexico. However, this population may not be representative of the general population of the United States. Also, the study provides prevalence data only. Data on the quantity of pica substances ingested are not provided.

5.3.4.27. *Ma et al. (2016)—Estimation of the Daily Soil/Dust (SD) Ingestion Rate of Children from Gansu Province, China via Hand-to-Mouth Contact Using Tracer Elements*

Ma et al. (2016) estimated soil and dust ingestion among 60 children between the ages of 3 and 12 years in the Gansu Province of China. An activity pattern modeling approach was used to estimate the soil and dust ingestion from hand-to-mouth contact using the following equation:

$$IR_{handSD} = TA_{handSD} \times TE \times SAC \times EF \quad \text{(Eqn. 5-10)}$$

where:

IR_{handSD} = daily ingestion of soil and dust from hands (mg/day)
TA_{handSD} = theoretical amount of hand soil and dust (mg)
TE = transfer efficiency at each contact
SAC = proportion of the hand surface area contacting the mouth at each event
EF = frequency of contact in a day (day^{-1})

The amount of soil on the hands was estimated by collecting two hand wipes from each child (one in the morning and one in the afternoon), and analyzing them for three tracer elements (cerium, yttrium, and vanadium). Background levels of these tracers were also measured in soil samples from the Gansu Province. Using these data, the amount of soil and dust on the children's hands (TA_{SD}) was estimated as follows:

$$TA_{SD} = C_{tracer\text{-}hands} / C_{tracer\text{-}soil} \quad \text{(Eqn. 5-11)}$$

where:

$C_{tracer\text{-}hands}$ = the amount of tracer on the hands (ng)
$C_{tracer\text{-}soil}$ = the amount of tracer in background soil (mg/kg)

Transfer efficiency (TE) was assumed to be 0.159, based on a study by Kissel et al. (1998b), the proportion of the hand surface contacting the mouth per event was assumed to be 0.1, and the frequency of contact (EF) was assumed to be 15 and 7 contacts/hour, for kindergarten and primary school children, respectively, over a 12-hour exposure period. The estimated mean soil and dust ingestion rates from hand-to-mouth contact ranged from 6 to 10 mg/day and 95th percentile rates ranged from 12 to 18 mg/day.

The estimates provided by Ma et al. (2016) are based on various assumptions related to hand-to-mouth exposure (i.e., surface areas of the hand contacted, transfer efficiency, the number of contacts per day), which introduce a certain degree of uncertainty. The amount of soil on the hands was based on measurements of three tracers (i.e., cerium, yttrium, and vanadium) from hand wipe samples, which the authors believed to "more accurately estimate the amounts of [soil and dust] on the hands" than simply weighing the hand wipes before and after wiping the children's hands because other substances such as grease and other organic ingredients are eliminated. The estimates account for soil and dust ingestion from hand-to-mouth contact only, and the population of children studied may not be representative of children in the United States.

5.3.5. Relevant Studies of Secondary Analysis and Other Relevant Information

The following studies are classified as relevant rather than key. This section includes studies of secondary analysis using the three methodologies discussed in Section 5.3.1, and data on the prevalence of the soil ingestion behavior. The secondary analysis literature on soil and dust ingestion rates also gives important insights into methodological strengths and limitations of the studies. These methodological issues include attempts to determine the origins of apparent positive and negative bias in the methodologies, including: food input/fecal output misalignment; missed fecal samples; assumptions about the weight of children's feces; particle sizes of, and relative contributions of soils and dusts to total soil and dust ingestion; and attempts to identify a "best" tracer

element or combination of tracer elements. The secondary analysis literature also provides insights into the data needs for assessing soil and dust ingestion using activity pattern approaches. Potential error from using short-term-study estimates for long-term soil and dust ingestion behavior estimates is also discussed.

5.3.5.1. *Wong (1988)—The Role of Environmental and Host Behavioral Factors in Determining Exposure to Infection with* **Ascaris lumbricoides** *and* **Trichuris trichiura***/Calabrese and Stanek (1993)—Soil Pica: Not a Rare Event*

Calabrese and Stanek (1993) reviewed a tracer element study conducted by Wong (1988) to estimate the amount of soil ingested by two groups of children. Wong (1988) studied a total of 52 children in two government institutions in Jamaica. The younger group included 24 children with an average age of 3.1 years (range of 0.3 to 7.5 years). The older group included 28 children with an average age of 7.2 years (range of 1.8 to 14 years). One fecal sample was collected each month from each subject over the 4-month study period. The amount of silicon in dry feces was measured to estimate soil ingestion.

An unspecified number of daily fecal samples were collected from a hospital control group of 30 children with an average age of 4.8 years (range of 0.3 to 12 years). Dry feces were observed to contain 1.45% silicon, or 14.5 mg Si per gram of dry feces. This quantity was used to correct measured fecal silicon from dietary sources. Fecal silicon quantities greater than 1.45% in the 52 studied children were interpreted as originating from soil ingestion.

For the 28 children in the older group, soil ingestion was estimated to be 58 mg/day, based on the mean minus one outlier, and 1,520 mg/day, based on the mean of all the children. The outlier was a child with an estimated average soil ingestion rate of 41,000 mg/day (41 g/day) over the 4 months.

Estimates of soil ingestion were higher in the younger group of 24 children. The mean soil ingestion of all the children was 470 ± 370 mg/day. Due to some sample losses, of the 24 children studied, only 15 had samples for each of the 4 months of the study. Over the entire 4-month study period, 9 of 84 samples (or 10.5%) yielded soil ingestion estimates in excess of 1 g/day.

Of the 52 children studied, 6 had 1-day estimates of more than 1,000 mg/day. Table 5-25 shows the estimated soil ingestion for these six children. The article describes 5 of 24 (or 20.8%) in the younger group of children as having a >1,000 mg/day estimate on at least one of the four study days; in the older group one child is described in this manner. A high degree of daily variability in soil ingestion was observed among these six children; three showed soil pica behavior on 2, 3, and 4 days, respectively, with the most consistent (4 out of 4 days) soil pica child having the highest estimated soil ingestion, 3.8 to 60.7 g/day.

5.3.5.2. *Calabrese and Stanek (1992b)—What Proportion of Household Dust is Derived from Outdoor Soil?*

Calabrese and Stanek (1992b) estimated the amount of outdoor soil in indoor dust using statistical modeling. The model used soil and dust data from the 60 households that participated in the Calabrese et al. (1989) study, by preparing scatter plots of each tracer's concentration in soil versus dust. Correlation analysis of the scatter plots was performed. The scatter plots showed little evidence of a consistent relationship between outdoor soil and indoor dust concentrations. The model estimated the proportion of outdoor soil in indoor dust using the simplifying assumption that the following variables were constants in all houses: the amount of dust produced every day from both indoor and outdoor sources, the proportion of indoor dust due to outdoor soil, and the concentration of the tracer element in dust produced from indoor sources. Using these assumptions, the model predicted that 31.3% by weight of indoor dust came from outdoor soil. This model was then used to adjust the soil ingestion estimates from Calabrese et al. (1989).

5.3.5.3. *Stanek and Calabrese (1995b)—Daily Estimates of Soil Ingestion in Children*

Stanek and Calabrese (1995b) presented a methodology that links the physical passage of food and fecal samples to construct daily soil ingestion estimates from daily food and fecal trace-element concentrations. Soil ingestion data for children obtained from the Amherst study (Calabrese et al., 1989) were reanalyzed by Stanek and Calabrese (1995b). A lag period of 28 hours between food intake and fecal output was assumed for all respondents. Day 1 for the food sample corresponded to the 24-hour period from midnight on Sunday to midnight on Monday of a study week; Day 1 of the fecal sample corresponded to the 24-hour period from noon on Monday to noon on Tuesday. Based on these definitions, the food soil

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equivalent was subtracted from the fecal soil equivalent to obtain an estimate of soil ingestion for a trace element. A daily overall ingestion estimate was constructed for each child as the median of trace element values remaining after tracers falling outside of a defined range around the overall median were excluded.

Table 5-26 presents adjusted estimates, modified according to the input/output misalignment correction, of mean daily soil ingestion per child (mg/day) for the 64 study participants. The approach adopted in this paper led to changes in ingestion estimates from those presented in Calabrese et al. (1989).

Estimates of children's soil ingestion projected over a period of 365 days were derived by fitting log-normal distributions to the overall daily soil ingestion estimates using estimates modified according to the input/output misalignment correction (see Table 5-27). The estimated median value of the 64 respondents' daily soil ingestion averaged over a year was 75 mg/day, while the 95th percentile was 1,751 mg/day. In developing the 365-day soil ingestion estimates, data that were obtained over a short period of time (as is the case with all available soil ingestion studies) were extrapolated over a year. The 2-week study period may not reflect variability in tracer element ingestion over a year. This study was classified as relevant because, while Stanek and Calabrese (1995b) attempted to address the variability through modeling of the long-term ingestion, new uncertainties were introduced through the parametric modeling of the limited subject day data.

5.3.5.4. *Calabrese et al. (1996)—Methodology to Estimate the Amount and Particle Size of Soil Ingested by Children: Implications for Exposure Assessment at Waste Sites*

Calabrese et al. (1996) examined the hypothesis that one cause of the variation between tracers seen in soil ingestion studies could be related to differences in soil tracer concentrations by particle size. This study, published before the Calabrese et al. (1997a) primary analysis study results, used laboratory analytical results for the Anaconda, MT soil's tracer concentration after it had been sieved to a particle size of <250 µm in diameter (it was sieved to <2 mm soil particle size in Calabrese et al. [1997a]). The smaller particle size was examined based on the assumption that children principally ingest soil of small particle size adhering to fingertips and under fingernails. For five of the tracers used in the original study (aluminum, silicon, titanium, yttrium, and zirconium), soil concentration was not changed by particle size. However, the soil concentrations of three tracers (lanthanum, cerium, and neodymium) were increased 2- to 4-fold at the smaller soil particle size. Soil ingestion estimates for these three tracers were decreased by approximately 60% at the 95th percentile compared to the Calabrese et al. (1997a) results.

5.3.5.5. *Stanek et al. (1999)—Soil Ingestion Estimates for Children in Anaconda Using Trace Element Concentrations in Different Particle Size Fractions*

Stanek et al. (1999) extended the findings from Calabrese et al. (1996) by quantifying trace element concentrations in soil based on sieving to particle sizes of 100−250 µm and to particle sizes of 53 to <100 µm. The earlier study (Calabrese et al., 1996) used particle sizes of 0−2 µm and 1−250 µm. This study used the data from soil concentrations from the Anaconda, MT site reported by Calabrese et al. (1997a). Results of the study indicated that soil concentrations of aluminum, silicon, and titanium did not increase at the two finer particle size ranges measured. However, soil concentrations of cerium, lanthanum, and neodymium increased by a factor of 2.5 to 4.0 in the 100−250 µm particle size range when compared with the 0−2 µm particle size range. There was not a significant increase in concentration in the 53−100 µm particle size range.

5.3.5.6. *Stanek and Calabrese (2000)—Daily Soil Ingestion Estimates for Children at a Superfund Site*

Stanek and Calabrese (2000) reanalyzed the soil ingestion data from the Anaconda study (Calabrese et al., 1997a) to provide estimates of variability between days and subjects, and daily soil ingestion rates over a longer period. Stanek and Calabrese (2000) attempted to address several sources of uncertainties in this reanalysis including identification of outliers and the estimation of soil ingestion rates based on particle size of <250 µm. Tracer-specific soil ingestion rates were estimated for 8 tracer elements for up to 7 subject days for each of the 64 children. The mean and median values were estimated for all tracers for each day, and median and mean estimates were generated over all subject days. Table 5-28 summarizes these soil ingestion estimates.

Assuming a log-normal distribution for the soil ingestion estimates in the Anaconda study, average long-term soil ingestion rates were predicted for children for each of the eight trace elements. Using

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"best linear unbiased predictors," the authors predicted 95th percentile soil ingestion values over time periods of 7 days, 30 days, 90 days, and 365 days. The 95th percentile soil ingestion values were predicted to be 133 mg/day over 7 days, 112 mg/day over 30 days, 108 mg/day over 90 days, and 106 mg/day over 365 days. Based on this analysis, estimates of the distribution of longer term average soil ingestion are expected to be narrower, with the 95th percentile estimates being as much as 25% lower (Stanek and Calabrese, 2000). This study was classified as relevant because, while Stanek and Calabrese (2000) attempted to characterize the variability between days for a given subject and between subjects, the derivation of usual (long term) intake based on limited short term subject day data introduces new uncertainties.

5.3.5.7. *Stanek et al. (2001a)—Biasing Factors for Simple Soil Ingestion Estimates in Mass Balance Studies of Soil Ingestion*

To identify and evaluate biasing factors for soil ingestion estimates, the authors developed a simulation model based on data from previous soil ingestion studies. The soil ingestion data used in this model were taken from Calabrese et al. (1989) (the Amherst study), Davis et al. (1990) (southeastern Washington State), Calabrese et al. (1997a) (the Anaconda study), and Calabrese et al. (1997b) (soil pica in Massachusetts), and relied only on the aluminum and silicon trace element estimates provided in these studies.

Of the biasing factors explored, the impact of study duration was the most striking, with a positive bias of more than 100% for 95th percentile estimates in a 4-day tracer element study. A smaller bias was observed for the impact of absorption of trace elements from food. Although the trace elements selected for use in these studies are believed to have low absorption, whatever amount is not accounted for will result in an underestimation of the soil ingestion distribution. In these simulations, the absorption of trace elements from food of up to 30% was shown to negatively bias the estimated soil ingestion distribution by less than 20 mg/day. No biasing effect was found for misidentifying play areas for soil sampling (i.e., ingested soil from a yard other than the subject's yard).

5.3.5.8. *Stanek et al. (2001b)—Soil Ingestion Distributions for Monte Carlo Risk Assessment in Children*

Stanek et al. (2001b) developed "best linear unbiased predictors" to reduce the biasing effect of short-term soil ingestion estimates. This study estimated the long-term average soil ingestion distribution using daily soil ingestion estimates from children who participated in the Anaconda, MT study. Trace element specific estimates on a subject day were simulated assuming a normal distribution. In this long-term (annual) distribution, the soil ingestion estimates were: mean 31, median 24, 75th percentile 42, 90th percentile 75, and 95th percentile 91 mg/day. This study was classified as relevant because of the uncertainties introduced by the assumptions used to derive long-term average soil ingestion distribution. For example, the methodology assumes that the variance of the trace element estimates on a subject-day is identical for all subjects and days and that the variance between days in soil ingestion for a subject is the same for all subjects.

5.3.5.9. *Von Lindern et al. (2003)—Assessing Remedial Effectiveness through the Blood Lead:Soil/Dust Lead Relationship at the Bunker Hill Superfund Site in the Silver Valley of Idaho*

Similar to Hogan et al. (1998), Von Lindern et al. (2003) used the IEUBK model to predict blood lead levels in a nonrandom sample of several hundred children ages 0–9 years in an area of northern Idaho from 1989–1998 during community-wide soil remediation. Von Lindern et al. (2003) used the IEUBK default soil and dust ingestion rates together with observed house dust/soil lead levels (and imputed values based on community soil and dust lead levels, when observations were missing). The authors compared the predicted blood lead levels with observed blood lead levels and found that the default IEUBK soil and dust ingestion rates and lead bioavailability value over-predicted blood lead levels, with the over-prediction decreasing as the community soil remediation progressed. The authors stated that the over-prediction may have been caused either by a default soil and dust ingestion that was too high, a default bioavailability value for lead that was too high, or some combination of the two. They also noted under-predictions for some children, for whom follow up interviews revealed exposures to lead sources not accounted for in the model simulations (e.g., recreational exposures from outside the site). In addition, some of these children were socioeconomically disadvantaged, highly mobile, and cared for at multiple locations.

Von Lindern et al. (2003) developed a statistical model that apportioned the contributions of community soils, yard soils of the residence, and

house dust to lead intake; the models' results suggested that community soils contributed more (50%) than neighborhood soils (28%) or yard soils (22%) to soil found in house dust of the studied children.

5.3.5.10. *Layton and Beamer (2009)—Migration of Contaminated Soil and Airborne Particulates to Indoor Dust*

Layton and Beamer (2009) developed a modeling and measurement framework to assess the transport of contaminated soils and airborne particulates indoors at a residence from outdoor sources. The model accounted for the resuspension and deposition processes, and the removal of particles by cleaning and exhalation. The model consisted of a two-compartment model (i.e., air and floor) that simulated the dust fall, accumulation, and loss of particles on floor surfaces using the concentration of an inorganic tracer in those two media. Monitoring data from the National Human Exposure Assessment Survey (NHEXAS) in six Midwestern states and a city in The Netherlands were used to characterize the transport parameters of the model. The analysis assumed a lognormal distribution of soil track-in indoors with a geometric mean of 0.1 g/day (GSD = 3) based on data from the published literature. Results of the model indicated that 60% of the arsenic indoors originated from ambient air, while 40% was a result of soil tracked-in.

5.3.5.11. *Gavrelis et al. (2011)—An Analysis of the Proportion of the U.S. Population That Ingests Soil or Other Non-Food Substances*

Gavrelis et al. (2011) evaluated the prevalence of the U.S. population that ingests nonfood substances such as soil, clay, starch, paint, or plaster. Data were compiled from the National Health and Nutrition Examination Survey (NHANES) collected from 1971 to 1975 (NHANES I) and 1976 to 1980 (NHANES II), which represent a complex, stratified, multistage, probability-cluster design, and include nationwide probability samples of approximately 21,000 and 25,000 study participants, respectively. NHANES I surveyed people aged 1 to 74 years and NHANES II surveyed those 6 months to 74 years. The study population included women of childbearing age, people with low income status, the elderly, and preschool children, who represented an oversampling of specific groups in the population that were believed to have high risks for malnutrition.

The survey questions were demographic, socioeconomic, dietary, and health-related queries, and included specific questions regarding soil and nonfood substance ingestion. Survey questions for children under 12 years asked whether they consumed nonfood substances including dirt or clay, starch, paint or plaster, and other materials (NHANES I) or about consumption of clay, starch, paint or plaster, dirt, and other materials (NHANES II). For participants over 12 years of age, the survey questions asked only about consumption of dirt or clay, starch, and other materials (NHANES I) or about nonfood substances including clay, starch, and other materials (NHANES II).

Age groupings used in this analysis vary slightly from the age group categories established by EPA and described in *Guidance on Selecting Age Groups for Monitoring and Assessing Childhood Exposures to Environmental Contaminants* (U.S. EPA, 2005). Other demographic parameters included sex (including pregnant and nonpregnant females), race (White, Black, and other), geography (urban and rural, with "urban" defined as populations >2,500), income level (ranging from $0−$9,999 up to >$20,000, or not stated), and highest grade head of household (population under 18 years) or respondent (population >18 years) attended. For statistical analysis, frequency estimates were generated for the proportion of the total U.S. population that reported consumption of dirt, clay, starch, paint or plaster, or other materials "considered unusual" using the appropriate NCHS sampling weights and responses to the relevant questions in NHANES I and II. NHANES I and II were evaluated separately because the data sets did not provide components of the weight variable separately (i.e., probability of selection, nonresponse adjustment weight, and poststratification weight).

Although the overall prevalence estimates were higher in NHANES I compared with NHANES II, similar patterns were generally observed across substance types and demographic groups studied. For NHANES I, the estimated prevalence of all nonfood substance consumption in the United States for all ages combined was 2.5% (95% confidence interval [CI]: 2.2−2.9%), whereas for NHANES II, the estimated prevalence of all nonfood substance consumption in the United States for all ages combined was 1.1% (95% CI: 1.0−1.2%). Table 5-29 provides the prevalence estimates by type of substance consumed for all ages combined. By type of substance, the estimated prevalence was greatest for dirt and clay consumption and lowest for starch. Figures 5-1, 5-2, and 5-3, respectively, show the prevalence of nonfood substance consumption by

age, race, and income. The most notable differences were seen across age, race (Black vs. White), and income groups. For both NHANES I and II, prevalence for the ingestion of all nonfood substances decreased with increasing age, was higher among Blacks (5.7%; 95% CI: 4.4−7.0%) as compared to Whites (2.1%; 95% CI: 1.8−2.5%), and was inversely related to income level, with prevalence of nonfood consumption decreasing as household income increased. The estimated prevalence of all nonfood substances for the 1- to <3-year age category was at least twice that of the next oldest category (3 to <6 years). Prevalence estimates were 22.7% (95% CI: 20.1–25.3%) for the 1- to <3-year age group based on NHANES I and 12% based on NHANES II. In contrast, prevalence estimates for the >21-year age group was 0.7% (95% CI: 0.5–1.0%) and 0.4% (95% CI: 0.3–0.5%) for NHANES I and NHANES II, respectively. Other differences related to geography (i.e., urban and rural), highest grade level of the household head, and sex were less remarkable. For NHANES I, for example, the estimated prevalence of nonfood substance consumption was only slightly higher among females (2.9%; CI: 2.3−3.5%) compared to males (2.1%; CI: 1.8−2.5%) of all ages. For pregnant females, prevalence estimates (2.5%; 95% CI: 0.0−5.6%) for those 12 years and over were more than twice those for nonpregnant females (1.0%; 95% CI: 0.7−1.4%).

5.3.5.12. *Stanek et al. (2012a)—Meta-Analysis of Mass-Balance Studies of Soil Ingestion in Children*

Stanek et al. (2012a) conducted a meta-analysis of four major mass-balance soil ingestion studies in U.S. children between 1 and 7 years of age. The four studies included the Amherst study (Calabrese et al., 1989), the Washington State study (Davis et al., 1990), the Washington Family study (Davis and Mirick, 2006), and the Anaconda study (Calabrese et al., 1997a).

Data for only two trace elements were included (Al and Si). Excluded from the study were 10% of subjects identified as outliers. In addition, study subjects with high soil intake (soil pica) were excluded. Additional sources of variability and bias between the four studies included in the meta-analysis are discussed in Stanek et al. (2012b). Mean, median, and 95th percentile soil ingestion estimates based on 216 children were 25.5, 32.6, and 79.4 mg/day, respectively. Soil ingestion rates for males and females were similar. As shown in Table 5-30, soil ingestion appeared to increase with age with the youngest age group (1 to <2 years) having the lowest soil ingestion rate.

This study provides age-specific soil ingestion estimates based on an analysis of four U.S. mass-balance soil ingestion studies for children. However, this study was classified as relevant because high-end values that were considered to be biased were excluded. In addition, data for a child with pica were also excluded. The study subjects were all from a limited study area (northern United States only) and data were collected only in the summer and early fall.

5.3.5.13. *Wilson et al. (2015)—Estimation of Sediment Ingestion Rates Based on Hand-to-Mouth Contact and Incidental Surface Water Ingestion*

Wilson et al. (2015) used a similar mechanistic approach to that of Wilson et al. (2013) to estimate sediment ingestion rates, except that greater adherence of sediment to the hands was assumed than for soil and dust. These sediment ingestion rates were intended for use in recreational exposure scenarios involving contact with sediments in aquatic areas. Sediment ingestion was assumed to occur as a result of direct contact with the sediment and subsequent hand-to-mouth contact, as well as incidental ingestion of surface water containing suspended sediments. Both deterministic and probabilistic methods were used to estimate sediment ingestion based on the following equations:

$$SDIR_{HM} = SL_{hands} \times SA_{hand} \times FSA_{fingers} \times FQ \times SE \quad \text{(Eqn. 5-12)}$$

and

$$SDIR_{WC} = SS \times SWIR \quad \text{(Eqn. 5-13)}$$

where:

FQ = frequency of hand-to-mouth events (1/hour)
$FSA_{fingers}$ = fractional surface area of the hands (unitless)
SA_{hand} = surface area of the hand (cm^2)
$SDIR_{HM}$ = sediment ingestion rate from hand-to-mouth contact (mg/hr)
$SDIR_{WC}$ = sediment ingestion from incidental water consumption (mg/hr)
SE = saliva extraction factor (unitless)

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SL_{hands} = sediment adherence on hands (mg/cm^2)
SS = suspended sediment concentration (mg/L)
$SWIR$ = surface water ingestion rate (L/hr)

Table 5-31 presents the age-dependent input values used in these calculations. Both deterministic and probabilistic methods were used to estimate sediment ingestion rates. The results based on the probabilistic assessment are presented in Table 5-32. The estimated sediment ingestion rates based on hand-to-mouth contact using the probabilistic approach were 72 mg/hour for toddlers 7-months to 4 years old, 57 mg/hour for children 5 to 11 years old, 18 mg/hour for teens 12 to 19 years old, and 20 mg/hour for adults and seniors ages 20 to 60+ years. The sediment ingestion rate based on incidental surface water ingestion was 7.7 mg/hour for all age groups. Slightly lower values were observed using the deterministic approach.

This study provides sediment ingestion rates in units of mg/hour which can be used to estimate exposure to contaminants in sediment. Its limitations are based on the uncertainties associated with the input parameters used to model dust ingestion. Also, as indicated by the author, the age groups and receptor characteristics were intended to represent the Canadian population, which may or may not be representative of U.S. populations.

5.3.5.14. *Wilson et al. (2016)—Estimation of Dust Ingestion Rates in Units of Surface Area per Day Using a Mechanistic Hand-to-Mouth Model*

Wilson et al. (2016) estimated dust ingestion rates for various age groups of Canadian children on the basis of surface area (m^2/day). These dust ingestion rates are intended to be used with measured contaminant loadings in surface dust ($\mu g/m^2$) to estimate exposure from ingestion of surface dust. Wilson et al. (2016) used a similar approach and input parameters as used in Wilson et al. (2013). Dust ingestion was calculated using the following equation:

$$DIG = FTSS \times SA_{hand} \times FSA_{fingers} \times FQ \times SE \times ET$$

(Eqn. 5-14)

where:

DIG = dust ingestion rate (m^2/d)
ET = exposure time (h/d)
FQ = frequency of hand-to-mouth events (events/hr)
$FSA_{fingers}$ = fractional surface area of hand mouthed
$FTSS$ = fraction of dust transferred from indoor surfaces to hands
SA_{hand} = surface area of one hand (cm^2)
SE = saliva extraction factor (unitless)

The age-dependent parameters used were the same as those used in Wilson et al. (2013), as shown in Table 5-13. Age-specific exposure time (ET) values were estimated as 24 hours/day minus the time spent sleeping and the time spent outdoors. It was assumed that contact occurred with hard surfaces (e.g., carpets, countertops) 50% of the time and with soft surfaces (e.g., carpets, sofas) 50% of the time, except for infants for whom contact was assumed to occur with soft surfaces only (i.e., 100% of the time). Both deterministic and probabilistic methods were used to estimate dust ingestion rates. Results based on the probabilistic approach are presented in Table 5-33. Dust ingestion rates for children <11 years old were estimated to range from 0.025 m^2/day for infants 0–6 months to 0.061 m^2/day for toddlers 7 months to 4 years.

This study provides dust ingestion rates in units of m^2/day which can be used with surface loadings of dust (and corresponding contaminant concentrations in the dust) in $\mu g/m^2$ to estimate exposure. Its limitations are based on the uncertainties associated with the input parameters used to model dust ingestion. The use of these dust ingestion rates assumes that the surface loading measurements are representative of the surfaces contacted.

5.3.5.15. *Fawcett et al. (2016)—A Meta-Analysis of the Worldwide Prevalence of Pica during Pregnancy and the Postpartum Period*

Fawcett et al. (2016) conducted a meta-analysis using information found in 70 studies published through February 2014 to develop worldwide pica prevalence estimates among pregnant and postpartum populations. Fawcett et al. (2016) also characterized variations in prevalence based on moderating variables (e.g., educational level, geographic region, and ethnicity). Of the studies evaluated, 33.8% were from North America, 5.6% were from South America, 33.8% were from Africa, 18.3% were from the Middle East, 4.2% were from

Asia, and 4.2% were from Europe. Fawcett et al. (2016) defined pica as "the purposeful consumption of nonfood or nonnutritive substances," including "earth (geophagia), starch (amylophagia), ice (pagophagia), and a vast number of additional substances (e.g., baking soda)." Postpartum was defined as up to 12 months after delivery. The overall prevalence of pica was estimated to be 27.8%, but Fawcett et al. (2016) suggested that this is a poor indicator of pica prevalence among the general population of pregnant and postpartum because of the heterogeneity across studies. African women had the highest prevalence of pica at 44.8%, followed by North/South American at 23.0%, and Eurasia at 17.5%. Based on nine U.S. studies that included data on ethnicity, African-American women in the United States were also more likely to experience pica than non-African-American women. Based on a subset of 29 studies that reported the educational level of the women, education had a negative association with pica prevalence (i.e., higher educational level is associated with lower prevalence of pica). A subset of 31 studies reported on pica behavior among women with anemia. These women were one-and-a-half times more likely to report pica than nonanemic women.

This study estimated pica based on a wide range of studies, and provides information on the factors that may affect pica prevalence among pregnant and postpartum women. However, as stated by the author, the overall pica prevalence of 27.8% is not a good indicator of prevalence among the general population due to the wide range of values observed in the various studies evaluated. Also, pica was defined as ingestion of nonfood substances and did not represent soil/dust ingestion alone, but Fawcett et al. (2016) indicated that the moderating variables for geophagia did not differ from those of overall pica.

5.4. LIMITATIONS OF STUDY METHODOLOGIES

The three types of information needed to provide recommendations to exposure assessors on soil and dust ingestion rates among U.S. children include quantities of soil and dust ingested, frequency of high soil and dust ingestion episodes, and prevalence of high soil and dust ingesters. The methodologies provide different types of information: The tracer element, biokinetic model comparison, and activity pattern methodologies provide information on quantities of soil and dust ingested; the tracer element methodology provides limited evidence of the frequency of high soil ingestion episodes; and the survey response methodology can shed light on prevalence of mouthing behavior and frequency of high soil ingestion episodes. The biokinetic model comparison methodology has the advantage of reflecting longer term exposures. However, the methodologies used to estimate soil and dust ingestion rates and prevalence of soil and dust ingestion behaviors have certain limitations when used for the purpose of developing recommended soil and dust ingestion rates. These limitations may not have excluded specific studies from use in the development of recommended ingestion rates, but have been noted throughout this handbook. This section describes some of the known limitations, presents an evaluation of the current state of the science for U.S. children's soil and dust ingestion rates, and describes how the limitations affect the confidence ratings given to the recommendations.

5.4.1. Tracer Element Methodology

This section describes some previously identified limitations of the tracer element methodology as it has been implemented by U.S. researchers, as well as additional potential limitations that have not been explored. Some of these same limitations would also apply to the Dutch and Jamaican studies that used a control group of hospitalized children to account for dietary and pharmaceutical tracer intakes.

Binder et al. (1986) described some of the major and obvious limitations of the early U.S. tracer element methodology as follows:

> [T]he algorithm assumes that children ingest predominantly soil from their own yards and that concentrations of elements in composite soil samples from front and back yards are representative of overall concentrations in the yards...children probably eat a combination of soil and dust; the algorithm used does not distinguish between soil and dust ingestion....fecal sample weights...were much lower than expected...the assumption that aluminum, silicon and titanium are not absorbed is not entirely true...dietary intake of aluminum, silicon and titanium is not negligible when compared with the potential intake of these elements from soil...Before accepting these estimates as true values of soil ingestion in toddlers, we need a better understanding of the metabolisms of aluminum, silicon and titanium in children, and the validity of the

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assumptions we made in our calculations should be explored further.

The subsequent U.S. tracer element studies (Calabrese et al., 1997a, 1989; Barnes, 1990; Davis et al., 1990; Davis and Mirick, 2006) made some progress in addressing some of the Binder et al. (1986) study's stated limitations.

Regarding the issue of nonyard (community-wide) soil as a source of ingested soil, one study (Calabrese et al., 1989; Barnes, 1990) addressed this issue to some extent, by including samples of children's daycare center soil in the analysis. Calabrese et al. (1997a) attempted to address the issue by excluding children in daycare from the study sample frame. Homogeneity of community soils' tracer element content would play a role in whether this issue is an important biasing factor for the tracer element studies' estimates. Davis et al. (1990) evaluated community soils' aluminum, silicon, and titanium content and found little variation among 101 yards throughout the three-city area. Stanek et al. (2001a) concluded that there was "minimal impact" on estimates of soil ingestion due to mis-specifying a child's play area.

Regarding the issue of soil and dust both contributing to measured tracer element quantities in excreta samples, the key U.S. tracer element studies all attempted to address the issue by including samples of household dust in the analysis, and in some cases estimates are presented in the published articles that adjust soil ingestion estimates on the basis of the measured tracer elements found in the household dust. The relationship between soil ingestion rates and indoor settled dust ingestion rates has been evaluated in some of the secondary studies (Calabrese and Stanek, 1992b). An issue similar to the community-wide soil exposures in the previous paragraph could also exist with community-wide indoor dust exposures (such as dust found in schools and community buildings occupied by study subjects during or prior to the study period). A portion of the community-wide indoor dust exposures (due to occupying daycare facilities) was addressed in the Calabrese et al. (1989) and Barnes (1990) studies, but not in the other three key tracer element studies. In addition, if the key studies' vacuum cleaner collection method for household and daycare indoor settled dust samples influenced tracer element composition of indoor settled dust samples, the dust sample collection method would be another area of uncertainty with the key studies' indoor dust-related estimates. The survey response studies suggest that some young children may prefer ingesting dust to ingesting soil. The existing literature on soil versus dust sources of children's lead exposure may provide useful information that has not yet been compiled for use in soil and dust ingestion recommendations.

Regarding the issue of fecal sample weights and the related issue of missing fecal and urine samples, the key tracer element studies have varying strengths and limitations. The Calabrese et al. (1989) article stated that wipes and toilet paper were not collected by the researchers, and thus fecal quantities, and soil and dust ingestion may have been underestimated. Calabrese et al. (1989) stated that cotton cloth diapers were supplied for use during the study; commodes apparently were used to collect both feces and urine for those children who were not using diapers. Barnes (1990) described cellulose and polyester disposable diapers with significant variability in silicon and titanium content and suggested that children's urine was not included in the analysis. Thus, it is unclear to what extent complete fecal and urine output was obtained for each study subject. The Calabrese et al. (1997a) study did not describe missing fecal samples and did not state whether urinary tracer element quantities were used in the soil and dust ingestion estimates, but stated that wipes and toilet paper were not collected. Missing fecal samples may have resulted in negative bias in the estimates from both of these studies. Davis et al. (1990) and Davis and Mirick (2006) were limited to children who no longer wore diapers. The authors made adjustments to the soil and dust ingestion estimates based on assumptions regarding the quantities of feces and urine in missed samples. These adjustments may have affected those studies' estimates, but the direction of the bias is uncertain. Adjustments for missing fecal and urine samples could introduce errors sufficient to cause negative estimates if missed samples were heavier than the collected samples used in the soil and dust ingestion estimate calculations.

Regarding the issue of dietary intake, the key U.S. tracer element studies have all addressed dietary (and nondietary, nonsoil) intake by subtracting calculated estimates of these sources of tracer elements from excreta tracer element quantities, or by providing study subjects with personal hygiene products that were low in tracer element content. Applying the food and nondietary, nonsoil corrections required subtracting the tracer element contributions from these nonsoil sources from the measured fecal/urine tracer element quantities. To perform this correction required assumptions to be made regarding the gastrointestinal transit time, or the time lag between inputs (food, nondietary nonsoil, and soil) and outputs (fecal and urine). The gastrointestinal transit

time assumption introduced a new potential source of bias that some authors (Stanek and Calabrese, 1995b) called input/output misalignment or transit time error. Stanek and Calabrese (1995a) attempted to correct for this transit time error by using the BTM and focusing estimates on those tracers that had low food:soil tracer concentration ratios. The lag time may also be a function of age. Davis et al. (1990) and Davis and Mirick (2006) assumed a 24-hour lag time in contrast to the 28-hour lag times used in Calabrese et al. (1989), Barnes (1990), and Calabrese et al. (1997a). ICRP (2002) suggested a lag time of 37 hours for 1-year-old children and 5- to 15-year-old children. Stanek and Calabrese (1995b) describe a method designed to reduce bias from this error source. Lag times that are shorten or longer than what is assumed may result in misalignment of the intake of tracers and their output. The direction of these biases is difficult to predict.

Regarding gastrointestinal absorption, the authors of three of the studies appeared to agree that the presence of silicon in urine represented evidence that silicon was being absorbed from the gastrointestinal tract (Davis et al., 1990; Calabrese et al., 1989; Barnes, 1990; Davis and Mirick, 2006). There was some evidence of aluminum absorption in Calabrese et al. (1989) and Barnes (1990); Davis and Mirick (2006) stated that aluminum and titanium did not appear to have been absorbed, based on low urinary levels. Davis et al. (1990) stated that silicon appears to have been absorbed to a greater degree than aluminum and titanium, based on urine concentrations. Absorption of these tracer elements would likely bias the estimated soil and dust ingestion rates low, because intake of the tracer elements would not be accounted for in the excretia if they had been absorbed. More research to better understand the uptake of these tracer elements may be warranted.

Aside from the gastrointestinal absorption, lag time, and missed fecal sample issues, Davis and Mirick (2006) offered another possible explanation for the negative soil and dust ingestion rates estimated for some study participants. Negative values result when the tracer amount in food and medicine is greater than that in urine/fecal matter. Given that some analytical error may occur, any overestimation of tracer amounts in the food samples would be greater than an overestimation in urine/feces because the food samples were many times heavier than the urine and fecal samples. Overestimating the amount of tracers ingested via food and medicines would tend to negatively bias soil and dust ingestion estimates while underestimating the amount of tracers ingested via foods and medicines would tend to positively bias soil and dust ingestion estimates. Interactions among elements in the gastrointestinal tract, in particular fluoride from ingested toothpaste, and how these may affect the absorption of tracer elements are not available based on the studies reviewed for this chapter.

Another limitation on the accuracy of tracer element-based estimates of soil and dust ingestion relates to inaccuracies inherent in environmental sampling and laboratory analytical techniques. The "percent recovery" of different tracer elements varies (according to validation of the study methodology performed with adults who swallowed gelatin capsules with known quantities of sterilized soil, as part of the Calabrese et al. [1989, 1997a] studies). Digestion/extraction efficiencies also vary by media type (e.g., food, soil, medicine). Estimates based on a particular tracer element with a lower or higher recovery than the expected 100% in any of the study samples would be influenced in either a positive or negative direction, depending on the recoveries in the various samples and their degree of deviation from 100% (Calabrese et al., 1989). Soil/dust size fractions, and digestion/extraction methods of sample analysis may be additional limitations.

Davis et al. (1990) offered an assessment of the impact of swallowed toothpaste on the tracer-based estimates by adjusting estimates for those children whose caregivers reported that they had swallowed toothpaste. Davis et al. (1990) had supplied study children with toothpaste that had been preanalyzed for its tracer element content, but it is not known to what extent the children actually used or swallowed the supplied toothpaste. Similarly, Calabrese et al. (1989, 1997a) supplied children in the Amherst, MA and Anaconda, MT studies with toothpaste containing low levels of most tracers, but it is unclear to what extent those children used or swallowed the supplied toothpaste. Not accounting for the consumption of toothpaste or other materials that contain the tracer elements would tend to bias soil and dust ingestion rates in the positive direction, but over-predicting the amount of tracers consumed in these materials would have the opposite effect.

Other research suggests additional possible limitations that have not yet been explored. First, lymph tissue structures in the gastrointestinal tract might serve as reservoirs for titanium dioxide food additives and soil particles, which could bias estimates either upward or downward depending on the tracers' entrapment within, or release from, these reservoirs during the study period (ICRP, 2002; Shepherd et al., 1987; Powell et al., 1996). Second,

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gastrointestinal uptake of silicon may have occurred, which could bias those estimates downward. There is an increasing body of evidence that silicon is an essential nutrient and that it plays a role in the initiation of the mineralization process and bone formation (Chen et al., 2016; Nielsen, 2014; Price et al., 2012; Jugdaohsingh, 2007; Carlisle, 1980). However, absorption of silicon in the gastrointestinal tract is not well understood (Jugdaohsingh et al., 2002). Van Dyck et al. (2000) suggests a possible negative bias in the silicon-based soil ingestion estimates, depending on the quantities of silicon absorbed by growing children. Third, regarding the potential for swallowed toothpaste to bias soil ingestion estimates upward, commercially available toothpaste may contain quantities of titanium and perhaps silicon and aluminum in the range that could be expected to affect the soil and dust ingestion estimates. Fourth, for those children who drank bottled or tap water during the study period, and did not include those drinking water samples in their duplicate food samples, slight upward bias may exist in some of the estimates for those children because drinking water may contain small, but relevant, quantities of silicon and potentially other tracer elements. Fifth, the tracer element studies conducted to date have not explored the impact of soil properties' influence on toxicant uptake or excretion within the gastrointestinal tract. Nutrition researchers investigating influence of clay geophagy behavior on human nutrition have begun using in vitro models of human digestion (Dominy et al., 2003; Hooda et al., 2004). A recent review (Wilson, 2003) covers a wide range of geophagy research in humans and various hypotheses proposed to explain soil ingestion behaviors, with emphasis on the soil properties of geophagy materials.

5.4.2. Biokinetic Model Comparison Methodology

It is possible that the IEUBK biokinetic model comparison methodology contained sources of both positive and negative bias, like the tracer element studies, and that the net impact of the competing biases is not known. There may be several significant sources of bias with the biokinetic model comparison methodology. One source of potential bias was the possibility that the biokinetic model cannot account for sources of lead exposure that are important for certain children due to incomplete exposure characterizations. For these children, the model might under-predict blood lead levels compared to actual measured lead levels. However, this result may actually mean that the default assumed lead intake rates via either soil and dust ingestion, or another lead source that is accounted for by the model, are too high. Another source of potential bias includes not accounting for the variability in the children's blood lead levels with time. There are also uncertainties with regard to the representativeness of the media concentrations in the children's play areas. For example, there is potential bias when predicting blood lead levels in children who have not spent a significant amount of time in the areas characterized as the main sources of environmental lead exposure. Modeling this population could result in either upward or downward biases in predicted blood lead levels. Comparing upward-biased predictions with actual measured blood lead levels and finding a relatively good match could lead to inferences that the model's default soil and dust ingestion rates are accurate, when in fact the children's soil and dust ingestion rates, or some other lead source, were actually higher than the default assumption. Von Lindern et al. (2016) attempted to address the issue of representativeness by assuming different partition models (i.e., percentage of time spent at various soil-contact locations).

Additionally, there is uncertainty with the assumption within the model itself regarding the biokinetics of absorbed lead, which could result in either positively or negatively biased predictions and the same kinds of incorrect inferences as the second source of potential bias. Another source of bias is the education and intervention programs implemented at contaminated sites which can potentially result in children's temporary reduction in soil/dust ingestion rates (see Section 5.4.4) (von Lindern et al., 2016).

In addition, there was no extensive sensitivity analysis. The calibration step used to fix model parameters limits the degree that most parameters can reasonably be varied. Second, the IEUBK model was not designed to predict blood lead levels greater than 25−30 µg/dL; there are few data to develop such predictions and less to validate them. If there are site-specific data that indicate soil ingestion rates (or other ingestion/intake rates) are higher than the defaults on average (not for specific children), the site-specific data should be considered. EPA considers the default IEUBK value of 30% bioavailability reasonable for most data sets/sites. Bioavailability has been assayed for soils similar to those in the calibration step and the empirical comparison data sets; 30% was used in the calibration step, and is therefore recommended for similar sites. The default provides a reasonable substitute when there are no specific data. Speciation of lead compounds for a particular exposure scenario

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could support adjusting bioavailability if they are known to differ strongly from 30%. The use of the 30% bioavailability was further supported by von Lindern et al. (2016). In general, EPA supports using bioavailability rates determined for the particular soils of interest if available.

5.4.3. Activity Pattern Methodology

The limitations associated with the activity pattern methodology relate to the availability and quality of the underlying data used to model soil ingestion rates. Some examples where data are limited or lacking include: information on the activities and environments of very young children (e.g., hand-to-mouth, object-to-mouth, and pica behaviors), information on skin adherence and dust loadings on indoor objects and floors (bare and carpeted), as well as the transfer of dust from both bare floors and carpets to hands, soil loadings to hands while playing outdoors, and information to evaluate temporal variability. Soil properties that may also be important include: soil particle size, organic content, moisture content, and other properties that affect soil adherence to the skin.

Real-time hand recording, where observations are made by trained professionals (rather than parents), may offer the advantage of consistency in interpreting visible behaviors and may be less subjective than observations made by someone who maintains a care-giving relationship to the child. On the other hand, young children's behavior may be influenced by the presence of unfamiliar people (Davis et al., 1995). Groot et al. (1998) indicated that parent observers perceived that deviating from their usual care-giving behavior by observing and recording mouthing behavior appeared to have influenced the children's behavior. With video-transcription methodology, an assumption is made that the presence of the videographer or camera does not influence the child's behavior. This assumption may result in minimal biases introduced when filming newborns or when the camera and videographer are not visible to the child. However, if the children being studied are older than newborns and can see the camera or videographer, biases may be introduced. Ferguson et al. (2006) described apprehension caused by videotaping and described situations where a child's awareness of the videotaping crew caused "play-acting" to occur, or parents indicated that the child was behaving differently during the taping session. Another possible source of measurement error may be introduced when children's movements or positions cause their mouthing not to be captured by the camera. Data transcription errors can bias results in either the negative or positive direction (i.e., underestimating mouthing behavior may bias results low while overestimating mouthing behavior may bias estimates high). Also, measurement error can occur if situations arise in which caregivers are absent during videotaping and researchers must stop videotaping and intervene to prevent risky behaviors (Zartarian et al., 1995). Finally, the videography studies have relatively small sample sizes, and the number of children studied and their method of selection may not be entirely representative of the population of the US.

Survey response studies rely on responses to questions about a child's mouthing behavior posed to parents or caregivers. Measurement errors from these studies could occur for a number of different reasons, including language/dialect differences between interviewers and respondents, question wording problems and lack of definitions for terms used in questions, differences in respondents' interpretation of questions, and recall/memory effects.

Other data collection methodologies (in-person interview, mailed questionnaire, or questions administered in "test" format in a school setting) may have had specific limitations. In-person interviews could result in either positive or negative response bias due to distractions posed by young children, especially when interview respondents simultaneously care for young children and answer questions. Other limitations include positive or negative response bias due to respondents' perceptions of a "correct" answer, question wording difficulties, lack of understanding of definitions of terms used, language and dialect differences between investigators and respondents, respondents' desires to avoid negative emotions associated with giving a particular type of answer, and respondent memory problems ("recall" effects) concerning past events. Positive biases would tend to occur when respondents overestimate behaviors that could result in soil and dust ingestion; negative biases would tend to occur when respondents underestimate behaviors that could result in soil and dust ingestion. Mailed questionnaires have many of the same limitations as in-person interviews, but may allow respondents to respond when they are not distracted by childcare duties. An in-school test format is more problematic than either interviews or mailed surveys because respondent bias related to teacher expectations could influence responses.

One approach to evaluating the degree of bias in survey response studies may be to make use of a surrogate biomarker indicator providing suggestive

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evidence of ingestion of significant quantities of soil (although quantitative estimates would not be possible). The biomarker technique measures the presence of serum antibodies to *Toxocara* species, a parasitic roundworm from cat and dog feces. Two U.S. studies have found associations between reported soil ingestion and positive serum antibody tests for *Toxocara* infection (Marmor et al., 1987; Glickman et al., 1981); a third (Nelson et al., 1996) has not, but the authors stated that reliability of survey responses regarding soil ingestion may have been an issue. Further refinement of survey response methodologies, together with recent NHANES data on U.S. prevalence of positive serum antibody status regarding infection with *Toxocara* species, may be useful.

5.4.4. Environmental and Household Interventions

Some of the studies discussed in this chapter were conducted near hazardous waste sites where soil contamination was present (e.g., smelting and mining waste containing lead). Environmental and household educational interventions have occurred at many of these sites. Several studies have been published in the literature to evaluate the effectiveness of educational and environmental interventions for reducing blood lead levels in children. It may be reasonable to assume that environmental interventions (e.g., soil removal) would not have had an effect on soil and dust ingestion rates because blood lead levels may come down as a result of a reduction of lead concentrations and not a change in behavior. However, awareness of contamination and educational interventions may affect behaviors (e.g., increase in frequency of hand washing and household cleaning); thus, reducing soil ingestion rates. Researchers have studied the effectiveness of educational interventions alone in reducing blood lead levels in children (Brown et al., 2006; Jordan et al., 2003; Wasserman, 2002; Rhoads et al., 1999). These studies found a decline in blood lead levels in the intervention groups compared to the control groups. However, Rhoads et al. (1999) and Jordan et al. (2003) concluded that educational intervention was only partially effective in reducing or maintaining lower blood lead levels. Yeoh et al. (2014) conducted a meta-analysis of five studies of educational interventions (Lanphear et al., 1996; Lanphear et al., 1999; Brown et al., 2006; Jordan et al, 2003; and Wasserman, 2002). The meta-analysis of the log-transformed data from these five studies showed that there was no statistical significant reduction in blood lead levels (Yeoh et al, 2014). However, another factor that may affect the results observed in these intervention studies is that bone turnover in children occurs at a higher rate than that of an adult, resulting in longer half-life of lead in blood (8 to 11 months for acute exposure and 20 to 38 months for chronic exposures), thus, providing a continuous source of lead in blood (Yeoh et al., 2014). With the exception of Jordan et al. (2003), who conducted quarterly booster sessions with participants for 2 years after the first year of educational intervention, the duration of these intervention studies was 12 months or less.

5.4.5. Key Studies: Representativeness of the U.S. Population

Limitations regarding the key studies performed in the United States for estimating soil and dust ingestion rates in the entire population of U.S. children ages 0 to <21 years fall into the broad categories of geographic range and demographics (age, sex, race/ethnicity, socioeconomic status).

Regarding geographic range, the two most obvious issues relate to soil types and climate. Soil properties might influence the soil ingestion estimates that are based on excreted tracer elements. The Davis et al. (1990), Calabrese et al. (1989), Barnes (1990), Calabrese et al. (1997a), and Davis and Mirick (2006) tracer element studies were conducted in locations with soils that had sand content ranging from 21−80%, silt content ranging from 16−71%, and clay content ranging from 3−20% by weight, based on data from USDA (2008). The location of children in the Calabrese et al. (1997b) study was not specified, but due to the original survey response study's occurrence in western Massachusetts, the soil types in the vicinity of the Calabrese et al. (1997b) study are likely to be similar to those in the Calabrese et al. (1989) and Barnes (1990) study.

The Hogan et al. (1998) study included locations in the central part of the United States (an area along the Kansas/Missouri border, and an area in western Illinois) and one in the eastern United States (Palmerton, PA). The Davis et al. (1990) study was conducted in Washington State, Von Lindern et al. (2016) was conducted in Idaho, and Wilson et al. (2013) was conducted in Canada. The only key study conducted in the southern part of the United States was Vermeer and Frate (1979).

Children might be outside and have access to soil in a very wide range of weather conditions (Wong et al., 2000). In the parts of the United States that experience moderate temperatures year-round, soil ingestion rates may be fairly evenly distributed

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throughout the year. During conditions of deep snow cover, extreme cold, or extreme heat, children could be expected to have minimal contact with outside soil. All children, regardless of location, could ingest soils located indoors in plant containers, soil derived particulates transported into dwellings as ambient airborne particulates, or outdoor soil tracked inside buildings by human or animal building occupants. Davis et al. (1990) did not find a clear or consistent association between the number of hours spent indoors per day and soil ingestion, but reported a consistent association between spending a greater number of hours outdoors and high (defined as the uppermost tertile) soil ingestion levels across all three tracers used.

The key tracer element studies all took place in northern latitudes. The temperature and precipitation patterns that occurred during these four studies' data collection periods were difficult to discern due to no mention of specific data collection dates in the published articles. The Calabrese et al. (1989) and Barnes (1990) study apparently took place in mid to late September 1987 in and near Amherst, MA; Calabrese et al. (1997a) apparently took place in late September and early October 1992, in Anaconda, MT; Davis et al. (1990) took place in July, August, and September 1987, in Richland, Kennewick, and Pasco, WA; and Davis and Mirick (2006) took place in the same Washington state location in late July, August, and very early September 1988 (raw data). Inferring exact data collection dates, a wide range of temperatures may have occurred during the four studies' data collection periods (daily lows from 22−60°F and 25−48°F, and daily highs from 53−81°F and 55−88°F in Calabrese et al. [1989] and Calabrese et al. [1997a], respectively, and daily lows from 51−72°F and 51−67°F, and daily highs from 69−103°F and 80−102°F in Davis et al. [1990] and Davis and Mirick [2006], respectively) (NCDC, 2006). Significant amounts of precipitation occurred during Calabrese et al. (1989) (more than 0.1 inches per 24-hour period) on several days; somewhat less precipitation was observed during Calabrese et al. (1997a); precipitation in Kennewick and Richland during the data collection periods of Davis et al. (1990) was almost nonexistent; there was no recorded precipitation in Kennewick or Richland during the data collection period for Davis and Mirick (2006) (NCDC, 2006).

One key biokinetic model comparison study (Hogan et al., 1998) targeted three locations in more southerly latitudes (Pennsylvania, southern Illinois, and southern Kansas/Missouri) than the tracer element studies. The other key biokinetic model comparison study was conducted in Idaho. The biokinetic model comparison methodology had an advantage over the tracer element studies in that the study represented long-term environmental exposures over periods up to several years that would include a range of seasons and climate conditions.

A brief review of the representativeness of the key studies' samples with respect to sex and age suggested that males and females were represented roughly equally in those studies for which study subjects' sex was stated. Children up to age 12 years were studied in the key studies with an emphasis on younger children.

A brief review of the representativeness of the key studies' samples with respect to socioeconomic status and racial/ethnic identity suggested that there were some discrepancies between the study subjects and the current U.S. population of children age 0 to <21 years. The single survey response study (Vermeer and Frate, 1979) was specifically targeted toward a predominantly rural Black population in a particular county in Mississippi. The tracer element studies are of predominantly White populations, apparently with limited representation from other racial and ethnic groups. The Amherst, MA study (Calabrese et al., 1989; Barnes, 1990) did not publish the study participants' socioeconomic status or racial and ethnic identities. The socioeconomic level of children studied by Davis et al. (1990) was reported to be primarily of middle to high income. Self-reported race and ethnicity of relatives of the children studied (in most cases, they were the parents of the children studied) in Davis et al. (1990) were White (86.5%), Asian (6.7%), Hispanic (4.8%), Native American (1.0%), and Other (1.0%), and the 91 married or living-as-married respondents identified their spouses as White (86.8%), Hispanic (7.7%), Asian (4.4%), and Other (1.1%). Davis and Mirick (2006) did not state the race and ethnicity of the follow-up study participants, who were a subset of the original study participants from Davis et al. (1990). For the Calabrese et al. (1997a) study in Anaconda, MT, population demographics were not presented in the published article. The study sample appeared to have been drawn from a door-to-door census of Anaconda residents that identified 642 toilet-trained children who were less than 72 months of age. Of the 414 children participating in a companion study (out of the 642 eligible children identified), 271 had complete study data for that companion study, and of these 271, 97.4% were identified as White and the remaining 2.6% were identified as Native American, Black, Asian, and Hispanic (Hwang et al., 1997). The 64 children in the Calabrese et al. (1997a) study apparently were a

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stratified random sample (based on such factors as behavior during a previous study, the existence of a disability, or attendance in daycare) drawn from the 642 children identified in the door-to-door census. Presumably these children identified as similar races and ethnicities to the Hwang et al. (1997) study children. The Calabrese et al. (1997b) study indicated that 11 of the 12 children studied were White.

In summary, the geographic range of the key study populations was somewhat limited. Of those performed in North America, U.S. locations include Massachusetts, Kansas, Montana, Missouri, Illinois, Washington, Pennsylvania, and Idaho. Canada is also represented. The two most obvious issues regarding geographic range relate to soil types and climate. Soil types were not always described, so the representativeness of the key studies related to soil types and properties is unclear. The key tracer element studies all took place in northern latitudes. The only key study conducted in the southern part of the United States was Vermeer and Frate (1979).

In terms of sex and age, males and females were represented roughly equally in those studies for which study subjects' sex was stated, while the majority of children studied were under the age of eight. The tracer element studies are of predominantly White populations, with a single survey response study (Vermeer and Frate, 1979) targeted toward a rural Black population. Other racial and ethnic identities were not well reported among the key studies, nor was socioeconomic status.

5.5. DERIVATION OF RECOMMENDED SOIL AND DUST INGESTION VALUES

Table 5-34 summarizes the soil and dust ingestion estimates from the key studies for general population children, by age range, based on the tracer, biokinetic modeling, and activity patterns approaches. These three methods were given equal weight in deriving the recommendation because of the inherent limitations in all of the methods (see Section 5.4). Also, there is no supportive evidence to suggest that one method provides more reliable estimates than the other.

The mean and upper percentile recommendations were derived by averaging the values for each age group for each of the three study methodologies and then taking the average of the three study types, as follows:

$$IR_{soil+dust} = (IR_t + IR_b + IR_a) / 3 \qquad \text{(Eqn 5-15)}$$

Where:

$IR_{soil+dust}$ = age-specific mean (or 95th percentile) soil + dust ingestion rate (mg/day)
IR_t = average of the age-specific mean (or 95th percentile) soil + dust ingestion rates from the various tracer studies (mg/day);
IR_b = average of the age-specific mean (or 95th percentile) soil + dust ingestion rates from the various biokinetic modeling comparison studies (mg/day);
IR_a = average of the age-specific mean (or 95th percentile) soil + dust ingestion rates from the various activity pattern modeling studies.

For example, the mean soil + dust ingestion rate for children 1 to <6 years was estimated as follows (see Table 5-34 for additional details):

$$IR_{soil+dust} = (IR_t\,[99\text{ mg/day}] + IR_b\,[90\text{ mg/day}] + IR_a\,[65\text{ mg/day}]) / 3$$

$$IR_{soil+dust} = 84\text{ mg/day}$$

Where:

IR_t = average of 132, 69, 66, and 129 mg/day (means from 4 tracers studies that represent children 1 to <6 years of age) = 99 mg/day;
IR_b = average of 113 and 67 mg/day (means from 2 biokinetic modeling studies that represent children 1 to <6 years of age) = 90 mg/day;
IR_a = average of 68 and 61 mg/day (means from 2 activity pattern studies that represent children 1 to <6 years) = 65 mg/day.

Using the number of study participants in the various studies to weight the means and upper percentile estimates would not change the recommended values, when rounded to one significant figure. Also, although there might be alternatives to averaging upper percentile values to get an upper percentile value, it does not appear that other approaches would significantly change the upper percentile values for these data because the upper percentile values from all study types are similar when rounded to one significant figure.

As stated earlier in this chapter, the key studies were used as the basis for developing the soil and dust ingestion recommendations shown in Table 5-1. The following sections describe in more detail how the recommended soil and dust ingestion, soil pica, and geophagy values were derived. Appendix B

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provides a comparison of data from the tracer studies conducted in Amherst, MA, Washington State, Anaconda, MT, and western MA. Appendix C provides a detailed summary of the key studies used in developing the recommended soil and dust ingestion rates. Appendix D provides a detailed summary of the studies on the prevalence of pica.

5.5.1. Central Tendency Soil and Dust Ingestion Recommendations

In general, "central tendency" recommendations in this chapter reflect an arithmetic mean (average) of measured values within a study, across studies within a methodology, and across the three methodologies. For some studies, arithmetic means were not available from the study, and some other central estimate was used. For example, for some of the tracer studies, the means represent the average of the median values for multiple tracers for each child. As noted, when describing how different estimates were averaged, there is uncertainty as to what is a central estimate consumption rate. Also, for some studies, the age groups evaluated did not match the age stratifications in EPA's *Guidance on Selecting Age Groups for Monitoring and Assessing Childhood Exposures to Environmental Contaminants* (U.S. EPA, 2005). However, for the purpose of providing recommended values for use in risk assessment, the ages were assumed to represent the age categories that they most closely matched.

For infants of 0 to <6 months in age, the recommended central tendency soil + dust ingestion estimate for use in risk assessments is 40 mg/day (36 mg/day rounded to one significant figure). This value is based on a single key activity pattern modeling study that provided data for infants 0 to <7 months (Wilson et al., 2013). Based on the assumption that 45% of the soil + dust ingestion can be attributed to soil and 55% can be attributed to dust, as in EPA's IEUBK model for lead in older children (U.S. EPA, 1994a), the central tendency soil ingestion rate for infants 0 to <6 months of age is 18 mg/day and the dust ingestion rate is 22 mg/day. Rounded to one significant figure, both soil and dust mean ingestion estimates for infants 0 to <6 months of age are 20 mg/day. Because the Wilson et al. (2013) study did not include exposures from object-to-mouth contact, the recommended values provided here may underestimate soil + dust ingestion among this age group.

For infants, 6 months to <1 year, data from two key biokinetic modeling studies were used to estimate a central tendency soil + dust ingestion rate of 70 mg/day; no key tracer studies or activity pattern modeling studies were available for this age group. This value is based on the average of the central estimate generated by von Lindern et al. (2016) and an EPA-adjusted central estimate from Hogan et al. (1998). Using the IEUBK model, Hogan et al. (1998) reported a predicted geometric mean blood lead level that was 1.4-fold higher than the geometric mean blood lead levels observed for 31 children 6- to 12-months old from a site in Illinois. The default soil and dust intakes for the 6- to 12-month old infants in the model (U.S. EPA, 1994b) are 38 mg soil/day and 47 mg house dust/day, for a total soil + dust intake of 85 mg/day for this life stage (U.S. EPA, 1994a). Assuming all other model input parameters are roughly accurate, EPA adjusted the default ingestion rate of 85 mg/day by a factor of 1.4 to estimate a soil + dust ingestion rate of 61 mg/day. Also using the biokinetic modeling approach, von Lindern et al. (2016) reported a mean value of 81 mg/day for children 6 months to <1 year of age, based on the average of the two best-fit model runs, as described in Section 5.3.3.7. The average of the estimates from these two biokinetic modeling studies is 71 mg/day (rounded to 70 mg/day). Based on the assumption that 45% of the soil + dust ingestion can be attributed to soil and 55% can be attributed to dust, as in EPA's IEUBK model for lead (U.S. EPA, 1994a), the central tendency soil ingestion rate for infants 6 months to <1 year of age is 32 mg/day and the dust ingestion rate is 38 mg/day. Rounded to one significant figure, the mean soil-only estimate is 30 mg/day and the mean dust-only estimate is 40 mg/day.

For children 1 to <2 years and children 2 to <6 years, the recommended central tendency soil + dust ingestion estimate for use in risk assessments are 90 mg/day (92 mg/day rounded to one significant figure) and 60 mg/day (62 mg/day rounded to one significant figure), respectively. These values are based on a single key biokinetic modeling study that provided data for these age groups (von Lindern et al., 2016). The averages of the two best-fit model runs were used for these age groups (see Section 5.3.3.7). Based on the assumption that 45% of the soil + dust ingestion can be attributed to soil and 55% can be attributed to dust, as in EPA's IEUBK model (U.S. EPA, 1994a) for lead, the central tendency soil ingestion rate for children 1 to <2 years of age is 41 mg/day and the dust ingestion rate is 49 mg/day. Rounded to one significant figure, the soil ingestion rate is 40 mg/day and the dust ingestion rate is 50 mg/day for children 1 to <2 years old. For children 2 to <6 years, the soil-only estimate is 27 mg/day and the dust-only value is 33 mg/day. Rounded to one significant

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figure, both soil and dust mean ingestion estimates for infants 2 to <6 years of age are 30 mg/day.

For children ages 1 to <6 years the recommended central tendency ingestion rate for use in risk assessment is 80 mg/day. This value is based on estimates provided by studies conducted using tracer, biokinetic modeling, and activity pattern modeling approaches. Four key tracer studies (Calabrese et al. [1989] as reanalyzed by Stanek and Calabrese [1995a]; Davis et al. [1990] as reanalyzed by Stanek and Calabrese [1995a]; Calabrese et al. [1997a,b]) estimated central tendency soil and dust ingestion rates for children ranging in age from 1 to <8 years (N = 241). The estimates ranged from 66 to 132 mg/day, with an average of 99 mg/day. For the Calabrese et al. (1989) study, as reanalyzed by Stanek and Calabrese et al. (1995a), the average of the median of the best four tracers for each child was used in developing the recommendations for children 1 to <6 years of age. Stanek and Calabrese et al. (1995a) suggest that this is the most reliable estimate for this data set. For the Davis et al. (1990) study, as reanalyzed by Stanek and Calabrese (1995a), data for only three tracers were available, and the average of the median of these three tracers was used. For the Calabrese et al. (1997a) study, the average based of the best tracer for each child was used. This estimate was assumed to be more reliable than the average of the best four tracers because the central tendency estimates for some of the four individual tracers were negative. For Calabrese et al. (1997b), data were available for aluminum, silicon, and titanium. However, only the data for aluminum and silicon were used, given the high degree of variability in the titanium estimates. The results of two biokinetic modeling studies were used (Hogan et al., 1998; von Lindern et al., 2016) in developing the recommendations for children 1 to <6 years of age. In the Hogan et al. (1998) study, blood levels for 471 children were similar to those predicted by the IEUBK model. The IEUBK default soil + dust ingestion values used in the Hogan et al. (1998) biokinetic modeling study were 135 mg/day for 1-, 2-, and 3-year-olds; 100 mg/day for 4-year-olds; 90 mg/day for 5-year-olds; and 85 mg/day for 6-year-olds (U.S. EPA 1994b, 2007). The time-averaged daily soil + dust ingestion rate for these 6 years of life was 113 mg/day. Also, using the biokinetic modeling approach, von Lindern et al. (2016) estimated a mean soil ingestion rate of 68 mg/day for children 1 to <6 years of age (N = 1,075), based on the average of the two best-fit model runs, as described in Section 5.3.3.6. The average based on these two biokinetic model studies is 91 mg/day. The two activity pattern modeling studies provide somewhat lower soil + dust ingestion estimates for this life stage. Özkaynak et al. (2011) provided an estimate of 68 mg/day for 3- to <6-year-old children, and Wilson et al. (2013) provided an estimate of 61 mg/day for children aged 7 months to <5 years. The mean of these two estimates is 65 mg/day. Averaging the soil + dust ingestion estimates from the three approaches yields a soil + dust ingestion estimate of 84 mg/day which was rounded to 80 mg/day. Based on the assumption that 45% of the soil + dust ingestion can be attributed to soil and 55% can be attributed to dust, the central tendency estimates for soil and dust ingestion alone are both 40 mg/day, rounded to one significant figure (i.e., 36 mg/day soil and 44 mg/day dust) for children 1 to <6 years old. Although children 1 to <6 years old have been grouped together for the purposes of deriving this recommendation, it is important to note that children ages 1 to <2 years have been reported to have higher blood lead levels compared to other children (Hogan et al., 1998). This age group also presents higher mouthing behavior frequency (i.e., hand-to-mouth and object-to-mouth) than other age groups based on the available data (See Chapter 4 of this handbook). Default soil ingestion rates used in the IEUBK model are higher for this age group than for older aged children, and von Lindern et al. (2016) showed that soil ingestion rate for this age group was the highest among all of the age groups evaluated up to <10 years of age, with an estimated value of 93 mg/day. The recommended soil ingestion rate for 1- to <2-year-olds for use in risk assessment is 90 mg/day, based on the von Lindern et al. (2016) study, as discussed above.

For children 6 to <12 years old, the recommended central tendency soil + dust ingestion rate is based on data from one biokinetic modeling study (von Lindern et al., 2016) and one activity pattern modeling study (Wilson et al., 2013). Based on biokinetic modeling, soil + dust ingestion was estimated to be 56 mg/day for 6- to <10-year-olds, based on the average of two best-fit model runs conducted by von Lindern et al. (2016) (see Section 5.3.3.7). The estimate for 5- to <12-year-olds, predicted by Wilson et al. (2013) using the activity pattern modeling was also 55 mg/day. The average of these two estimates is 56 mg/day. Rounded to one significant figure, the central tendency soil + dust ingestion rate for this life stage is 60 mg/day. Again, assuming that 45% of the soil + dust ingestion can be attributed to soil and 55% can be attributed to dust, the central tendency estimates for soil and dust ingestion alone are both 30 mg/day, rounded to one significant figure, for 6- to <12-year-old children.

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For older children (i.e., ages 12 to <19 years) and general population adults (i.e., ages ≥19 years), the recommended central tendency soil + dust ingestion rate is 30 mg/day. This is based on data from one key tracer study for adults (Davis and Mirick, 2006) and one activity pattern modeling study (Wilson et al., 2013). Davis and Mirick (2006) reported a mean soil + dust ingestion rate of 52 mg/day for 33 adults, based on average estimates using aluminum and silicon as tracers. Wilson et al. (2013) used an activity pattern modeling approach to estimate soil + dust ingestion among various age groups, including teens (ages 12 to <20 years), adults (ages 20 to 59 years), and senior (ages 60+ years). The estimated soil + dust ingestion rates were 3.7 mg/day for teens, 4.2 mg/day for adults, and 3.8 mg/day for seniors. Given the similarity in these estimates, the three ages group were combined into one category (i.e., 12 years through adults), with a central tendency soil + dust ingestion estimate of 4 mg/day. Averaging the estimates from the Davis and Mirick (2006) tracer study and the Wilson et al. (2013) activity pattern modeling study results in a central tendency soil + dust ingestion estimate of 28 mg/day. Rounded to one significant figure, this value is 30 mg/day. Assuming that 45% of the soil + dust ingestion can be attributed to soil and 55% can be attributed to dust, the central tendency estimate for soil is 13 mg/day and the estimate for dust is 17 mg/day. Rounded to one significant figure, the central tendency estimates are 10 mg/day and soil and 20 mg/day dust.

For rural populations following traditional rural or wilderness lifestyles as described by Doyle et al. (2012) and Irvine et al. (2014) adult soil ingestion rates may be somewhat higher than those of the general population (see Sections 5.3.4.20 and 5.3.4.21). Doyle et al. (2012) conducted a tracer study in Canada in which the estimated mean soil + dust was 42 mg/day based on the average values for aluminum and silicon tracers. Irvine et al. (2014) also conducted a tracer study in Canada and estimated a mean soil + dust ingestion rate of 52 mg/day, based on the aluminum and silicon tracers. The average of these two values is 47 mg/day. Rounded to one significant figure, the soil + dust ingestion estimate is 50 mg/day (20 mg/day soil and 30 mg/day dust).

5.5.2. Upper Percentile, Soil Pica, and Geophagy Recommendations

In general, there is considerably more uncertainty related to the upper percentile soil and dust ingestion rate estimates than for the average estimates. Biases due to the errors (e.g., sampling errors, measurement errors, analytical errors, etc.) are more likely to affect the upper percentile estimates than the average estimates. The use of data obtained from short-term studies to represent long-term usual behavior also introduces biases that may have a more considerable effect on the upper percentile estimates.

The upper percentile soil + dust ingestion rate for infants 0 to <6 months of age was estimated to be 100 mg/day. This value is based on a single key activity pattern modeling study that provided a soil + dust ingestion rate of 140 mg/day for infants 0 to <7 months (Wilson et al., 2013). Rounded to one significant figure, this value is 100 mg/day. Based on the assumption that 45% of the soil + dust ingestion can be attributed to soil and 55% can be attributed to dust, as in EPA's IEUBK model for lead in older children (U.S. EPA, 1994a), the upper percentile soil ingestion rate for infants 0 to <6 months of age is 45 mg/day and the dust ingestion rate is 55 mg/day. Rounded to one significant figure, the soil ingestion rate is assumed to be 50 mg/day for soil and 60 mg/day for dust for infants 0 to <6 months of age. An uncertainty associated with this estimate is that the Wilson et al. (2013) study did not include exposures from object-to-mouth contact. Thus, the recommended values provided here may underestimate soil + dust ingestion among this age group.

The recommended upper percentile soil + dust ingestion rate for infants 6 months to <1 year is 200 mg/day. This value is based on data from one biokinetic modeling study. Von Lindern et al. (2016) provided a 95th percentile value of 208 mg/day, based on the average of two best-fit model runs (see Section 5.3.3.6), which has been rounded to 200 mg/day. It is assumed that 90 mg/day represents soil ingestion and 110 mg/day represents dust ingestion, based on the assumption that 55% of the 200 mg/day is soil and 45% is dust. Rounded to one significant figure the recommended upper percentile ingestion rates for infants 6 months to 1 year of age are 90 mg/day soil and 100 mg/day dust.

The von Lindern et al. (2016) biokinetic modeling study was also used as the basis for the upper percentile soil + dust ingestion rate for children 1 to <2 years of age and 2 to <6 years of age. For 1- to <2-year-olds, the upper percentile soil + dust ingestion rate was 240 mg/day, based on the average of two best-fit model runs (see Section 5.3.3.6). Rounded to one significant figure, the soil + dust ingestion recommendation for use in risk assessment is 200 mg/day (90 mg/day soil; 100 mg/day dust). For children ages 2 to <6 years, von

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Lindern et al. (2016) reported an upper percentile soil + dust ingestion rate of 162 mg/day, based on the average of two best-fit model runs. Rounded to one significant figure, the soil + dust ingestion recommendation for use in risk assessment is also 200 mg/day (90 mg/day soil; 100 mg/day dust).

For children 1 to <6 years old, upper percentile soil + dust ingestion rates were derived from three tracer studies that used the BTM approach, one biokinetic study, and two activity pattern studies. The 95th percentile soil + dust ingestion estimates from the three tracer studies ranged from 154 to 282 mg/day, with a mean of 227 mg/day ($N = 229$) (Calabrese et al. [1989] as reanalyzed by Stanek and Calabrese [1995a]; Davis et al. [1990]; Calabrese et al. [1997a]). Von Lindern et al. (2016) estimated a 95th percentile rate of 178 mg/day. Özkaynak et al. (2011) and Wilson et al. (2013) reported similar estimates of the 95th percentile value (i.e., 224 mg/day and 204 mg/day for age groups 3 to <6 years and 7 months to <5 years, respectively). The average of these two values is 214 mg/day. Averaging the estimates from the three approaches gives an estimated 95th percentile soil + dust ingestion rate of 206 mg/day for children ages 1 to <6 years. Rounding to one significant figure, the recommended upper percentile estimate of soil + dust ingestion is 200 mg/day (90 mg/day soil; 100 mg/day dust).

Similar upper percentile estimates were provided for older children by von Lindern et al. (2016) and Wilson et al. (2013), and the recommended soil + dust ingestion rate for risk assessment purposes is also 200 mg/day. This upper percentile recommendation is the average of the von Lindern et al. (2016) estimate of 155 mg/day for children ages 6 to <10 years and the Wilson et al. (2013) estimate of 185 mg/day for children ages 5 to <12 years. The average of these two values is 170 mg/day. Rounded to one significant figure the soil + dust ingestion recommendation for use in risk assessment is 200 mg/day (90 mg/day soil; 100 mg/day dust).

Data that could be used to develop an upper percentile soil + dust ingestion rate for ages 12 years through adult were limited. For example, an upper percentile rate was not provided in the only adult tracer study. The only data available were from a single activity pattern study that provided an upper percentile rate (i.e., 14 mg/day) for teens, adults, and seniors that is inconsistent with the recommended central tendency rate of 30 mg/day. Therefore, an upper percentile recommendation for 12 years of age through adults was developed by assuming that the ratio of the 95th percentile value to the mean value for adults is the same as the average of the ratios of 95th percentiles to means for all other age groups (i.e., average ratio of the 95th percentile recommendations to the mean recommendation for all ages groups <12 years = 3.2). It should be noted that this assumes that the variance is the same for children and adults. Because estimates for adults are lower than those of children, they are likelier to have a lower variance. Applying this ratio to the central tendency estimate of 30 mg/day for the 12 years through adult age group gives an estimated upper percentile value of 100 mg/day (i.e., 30 mg/day × 3.2 = 94 mg/day, rounded to one significant figure). If upper percentile soil- or dust-only values are needed, the recommended rates are 50 mg/day soil and 60 mg/day dust. These values are rounded to one significant figure from 45 mg/day and 55 mg/day, assuming that 45% of the soil + dust estimate is soil and 55% is dust.

For rural populations following traditional rural or wilderness lifestyles the data from tracer studies conducted in rural Canadian locations and reported in Doyle et al. (2012) and Irvine et al. (2014) (see Section 5.3.4.20 and 5.3.4.21) may be used to estimate an upper percentile soil + dust ingestion rate for this population. Doyle et al. (2012) reported a 90th percentile soil + dust ingestion rate of 124 mg/day, based on the average of the results using aluminum and silicon as tracers. Irvine et al. (2014) reported a 90th percentile value of 220 mg/day based on the average of these same two tracers. Averaging the results from these two studies gives an upper percentile estimate of 172 mg/day. Rounded to one significant figure, the upper percentile soil + dust ingestion rate for these populations would be 200 mg/day (90 mg/day soil and 100 mg/day dust).

For the upper percentile soil pica and geophagy recommendations shown in Table 5-1, two primary lines of evidence suggest that at least some U.S. children exhibit soil pica behavior at least once during childhood. First, the survey response studies of reported soil ingestion behavior that were conducted in numerous U.S. locations and of different populations consistently yield a certain proportion of respondents who acknowledge soil ingestion by children. The surveys typically did not ask explicit and detailed questions about the soil ingestion incidents reported by the caregivers who acknowledged soil ingestion in children. Responses conceivably could fall into three categories: (1) responses in which caregivers interpret visible dirt on children's hands and subsequent hand-to-mouth behavior as soil ingestion; (2) responses in which caregivers interpret intentional ingestion of clay, "dirt," or soil as soil ingestion; and (3) responses in which caregivers

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regard observations of hand-to-mouth behavior of visible quantities of soil as soil ingestion. Knowledge of soils' bulk density allows one to infer that these latter observed hand-to-mouth soil ingestion incidents are likely to represent a quantity of soil that meets the quantity part of the definition of soil pica used in this chapter, or 1,000 mg. Occasionally, what is not known from survey response studies is whether the latter type of survey responses include responses regarding repeated soil ingestion that meets the definition of soil pica used in this chapter. The second category probably does represent ingestion that would satisfy the definition of soil pica as well as geophagy. The first category may represent relatively small amounts that appear to be ingested by many children based on the biokinetic modeling studies and the tracer element studies. Second, the U.S. tracer studies report a wide range of soil ingestion values. Due to averaging procedures used, for 4-, 7-, or 8-day periods, the rounded range of these estimates of soil ingestion behavior that apparently met the definition of soil pica used in this chapter is from 1,000 to 6,000 mg/day averaged over the study period. Due to the short-term nature of these studies and the limited amount of data available for children exhibiting pica behavior, the lower end of this range of 1,000 mg/day is recommended for use in risk assessments involving soil pica for children 1 to <6 years old. However, it is important to note that soil ingestion for these children exhibiting soil pica behavior has been reported as high as 20 to 25 g/day on a given day (Calabrese et al., 1997b).

Although no tracer element studies or biokinetic model comparison studies have been performed for children 15- to <21-year-olds in which soil pica behavior has been investigated, EPA is aware of one study documenting pica behavior in a group that includes children in this age range (Hyman et al., 1990). The study was not specific regarding whether soil pica (vs. other pica substances) was observed, nor did it identify the specific ages of the children observed to practice pica. In the absence of data that can be used to develop specific soil pica ingestion recommendations for children aged 15 years and 16 to <21 years, EPA recommends that risk assessors who need to assess risks via soil and dust ingestion to children ages 15 to <21 years use the same soil ingestion rate as that recommended for younger children, in the 1 to <6, 6 to <11, and 11 to <16-year age categories.

Researchers who have studied human geophagy behavior around the world typically have studied populations in specific locations, and often include investigations of soil properties as part of the research (Wilson, 2003; Aufreiter et al., 1997). Most studies of geophagy behavior in the United States were survey response studies of residents in specific locations who acknowledged eating clays. Typically, study subjects were from a relatively small area such as a county, or a group of counties within the same state. Although geophagy behavior may have been studied in only a single county in a given state, documentation of geophagy behavior by some residents in one or more counties of a given state may suggest that the same behavior also occurs elsewhere within that state.

A qualitative description of amounts of soil ingested by geophagy practitioners was provided by Vermeer and Frate (1979) with an estimated mean amount, 50 g/day that apparently was averaged over 32 adults and 18 children. The 18 children whose caregivers acknowledged geophagy (or more specifically, eating of clay) were (N = 16) ages 1 to 4 and (N = 2) ages 5 to 12 years. The definition of geophagy used included consumption of clay "on a regular basis over a period of weeks." EPA is recommending this 50 g/day value for geophagy. This mean quantity is roughly consistent with a median quantity reported by Geissler et al. (1998) in a survey response study of geophagy in primary school children in Nyanza Province, Kenya (28 g/day, range 8 to 108 g/day; interquartile range 13 to 42 g/day).

Several studies are available that investigated pica behavior among pregnant women. Many of these studies focus on the prevalence of the behavior, and very few provide data on amounts ingested (Fawcett et al., 2016; Lin et al., 2015; Lumish et al., 2014; Klitzman et al., 2002; Simpson et al., 2000; Smulian et al., 1995; Cooksey, 1995; Bronstein and Dollar 1974; Ferguson and Keaton, 1950). Studies of pica among pregnant women in various U.S. locations (Rainville, 1998; Corbett et al., 2003; Smulian et al., 1995) suggest that clay geophagy among pregnant women may include those less than 21 years old (Smulian et al., 1995; Corbett et al., 2003). Smulian provides a quantitative estimate of clay consumption of approximately 200−500 g/week, for the very small number of geophagy practitioners (N = 4) in that study's sample (N = 125). If consumed on a daily basis, this quantity (approximately 30 to 70 g/day) is roughly consistent with the Vermeer and Frate (1979) estimated mean of 50 g/day.

Johns and Duquette (1991) describe use of clays in baking bread made from acorn flour, in a ratio of 1 part clay to 10 or 20 parts acorn flour, by volume, in a Native American population in California and in Sardinia (~12 grams clay suspended in water added

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to 100 grams acorn). Either preparation method would add several grams of clay to the final prepared food; daily ingestion of the food would amount to several grams of clay.

5.6. REFERENCES FOR CHAPTER 5

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Table 5-3. Soil, Dust, and Soil + Dust Ingestion Estimates for Amherst, MA Study Children (Ages 1 to <4 Years)

		Ingestion (mg/day)				
Tracer Element	*N*	Mean	Median	SD	95th Percentile	Maximum
Aluminum						
soil	64	153	29	852	223	6,837
dust	64	317	31	1,272	506	8,462
soil/dust combined	64	154	30	629	478	4,929
Barium						
soil	64	32	–37	1,002	283	6,773
dust	64	31	–18	860	337	5,480
soil/dust combined	64	29	–19	868	331	5,626
Manganese						
soil	64	–294	–261	1,266	788	7,281
dust	64	–1,289	–340	9,087	2,916	20,575
soil/dust combined	64	–496	–340	1,974	3,174	4,189
Silicon						
soil	64	154	40	693	276	5,549
dust	64	964	49	6,848	692	54,870
soil/dust combined	64	483	49	3,105	653	24,900
Vanadium						
soil	62	459	96	1,037	1,903	5,676
dust	64	453	127	1,005	1,918	6,782
soil//dust combined	62	456	123	1,013	1,783	6,736
Yttrium						
soil	62	85	9	890	106	6,736
dust	64	62	15	687	169	5,096
soil/dust combined	62	65	11	717	159	5,269
Zirconium						
soil	62	21	16	209	110	1,391
dust	64	27	12	133	160	789
soil/dust combined	62	23	11	138	159	838
Titanium						
soil	64	218	55	1,150	1,432	6,707
dust	64	163	28	659	1,266	3,354
soil/dust combined	64	170	30	691	1,059	3,597

N = Number of subjects.
SD = Standard deviation.

Source: Calabrese et al. (1989).

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Table 5-4. Amherst, MA Soil Pica Child's Daily Ingestion Estimates by Tracer and by Week (mg/day)

Tracer Element	Estimated Soil Ingestion (mg/day)	
	Week 1	Week 2
Aluminum	74	13,600
Barium	458	12,088
Manganese	2,221	12,341
Silicon	142	10,955
Titanium	1,543	11,870
Vanadium	1,269	10,071
Yttrium	147	13,325
Zirconium	86	2,695

Source: Calabrese et al. (1991).

Table 5-5. Estimated Soil Ingestion for Sample of Washington State Children (2–7 years; *N* = 101)[a]

Element	Mean (mg/day)	Median (mg/day)	Standard Error of the Mean (mg/day)	Range (mg/day)[b]
Aluminum	38.9	25.3	14.4	279.0 to 904.5
Silicon	82.4	59.4	12.2	–404.0 to 534.6
Titanium	245.5	81.3	119.7	–5,820.8 to 6,182.2
Minimum	38.9	25.3	12.2	–5,820.8
Maximum	245.5	81.3	119.7	6,182.2

[a] Excludes three children who did not provide any samples.
[b] Negative values occurred as a result of correction for nonsoil sources of the tracer elements. For aluminum, lower end of range published as 279.0 mg/day in article appears to be a typographical error that omitted the negative sign.

Source: Adapted from Davis et al. (1990).

Table 5-6. Soil Ingestion Estimates for 64 Anaconda Children (Ages 1–4 Years)

	Estimated Soil Ingestion (mg/day)							
	Percentile							
Tracer	1st	50th	75th	90th	95th	Max	Mean	SD
Aluminum	–202.8	–3.3	17.7	66.6	94.3	461.1	2.7	95.8
Cerium	–219.8	44.9	164.6	424.7	455.8	862.2	116.9	186.1
Lanthanum	–10,673	84.5	247.9	460.8	639.0	1,089.7	8.6	1,377.2
Neodymium	–387.2	220.1	410.5	812.6	875.2	993.5	269.6	304.8
Silicon	–128.8	–18.2	1.4	36.9	68.9	262.3	–16.5	57.3
Titanium	–15,736	11.9	398.2	1,237.9	1,377.8	4,066.6	–544.4	2,509.0
Yttrium	–441.3	32.1	85.0	200.6	242.6	299.3	42.3	113.7
Zirconium	–298.3	–30.8	17.7	94.6	122.8	376.1	–19.6	92.5
BTM; median of best 4 tracers	NA	–2.4	26.8	73.1	159.8	380.2	6.8	74.5
BTM; best tracer-soil	NA	20.1	68.9	223.6	282.4	609.9	65.5	120.3
BTM; median of best 4 tracers-dust	NA	–5.5	62.8	209.2	353.0	683.9	16.5	160.9
BTM; best tracer-dust	NA	26.8	198.1	558.6	613.6	1,499.4	127.2	299.1

BTM = Best tracer methodology.
NA = Not available.
SD = Standard deviation.
Note: Negative values are a result of limitations in the methodology.

Source: Calabrese et al. (1997a).

Table 5-7. Soil Ingestion Estimates for Massachusetts Child Displaying Soil-Pica Behavior (mg/day)

Study Day	Aluminum-Based Estimate	Silicon-Based Estimate	Titanium-Based Estimate
1	53	9	153
2	7,253	2,704	5,437
3	2,755	1,827	2,007
4	725	534	801
5	5	–10	21
6	1,452	1,373	794
7	238	76	84

Note: Negative values are a result of limitations in the methodology.

Source: Calabrese et al. (1997b).

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Table 5-8. Average Daily Soil and Dust Ingestion Estimate for Children Ages 1–3 Years (mg/day)

Type of Estimate	Soil Ingestion			Dust Ingestion		
	Aluminum	Silicon	Titanium	Aluminum	Silicon	Titanium
Mean	168	89	448	260	297	415
Median	7	0	32	13	2	66
SD	510	270	1,056	759	907	1,032
Range	–15 to 1,783	–46 to 931	–47 to 3,581	–39 to 2,652	–351 to 3,145	–98 to 3,632

SD = Standard deviation.
Note: *N* = 12. Negative values are a result of limitations in the methodology.

Source: Calabrese et al. (1997b).

Table 5-9. Mean and Median Soil Ingestion (mg/day) by Family Members

Participant	Tracer Element	Estimated Soil Ingestion[a] (mg/day)			
		Mean	Median	SD	Maximum
Children (ages 3–7 years)[b]	Aluminum	36.7	33.3	35.4	107.9
	Silicon	38.1	26.4	31.4	95.0
	Titanium	206.9	46.7	277.5	808.3
Mother[c]	Aluminum	92.1	0	218.3	813.6
	Silicon	23.2	5.2	37.0	138.1
	Titanium	359.0	259.5	421.5	1,394.3
Father[d]	Aluminum	68.4	23.2	129.9	537.4
	Silicon	26.1	0.2	49.0	196.8
	Titanium	624.9	198.7	835.0	2,899.1

[a] For some study participants, estimated soil ingestion resulted in a negative value. These estimates have been set to 0 mg/day for tabulation and analysis.
[b] Results based on 12 children with complete food, excreta, and soil data.
[c] Results based on 16 mothers with complete food, excreta, and soil data.
[d] Results based on 17 fathers with complete food, excreta, and soil data.
SD = Standard deviation.

Source: Davis and Mirick (2006).

Table 5-10. Positive/Negative Error (Bias) in Soil Ingestion Estimates in Calabrese et al. (1989) Study: Effect on Mean Soil Ingestion Estimate (mg/day)[a]

	Negative Error						
Tracer	Lack of Fecal Sample on Final Study Day	Other Cause[b]	Total Negative Error	Total Positive Error	Net Error	Original Mean	Adjusted Mean
Aluminum	14	11	25	43	+18	153	136
Silicon	15	6	21	41	+20	154	133
Titanium	82	187	269	282	+13	218	208
Vanadium	66	55	121	432	+311	459	148
Yttrium	8	26	34	22	–12	85	97
Zirconium	6	91	97	5	–92	21	113

[a] How to read table: for example, aluminum as a soil tracer displayed both negative and positive error. The cumulative total negative error is estimated to bias the mean estimate by 25 mg/day downward. However, aluminum has positive error biasing the original mean upward by 43 mg/day. The net bias in the original mean was 18 mg/day positive bias. Thus, the original 153 mg/day mean for aluminum should be corrected downward to 136 mg/day.

[b] Values indicate impact on mean of 128 subject-weeks (64 children ages 1–4 years) in milligrams of soil ingested per day.

Source: Calabrese and Stanek (1995).

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Table 5-11. Comparison of Soil Ingestion Estimates (mg/day) from Two Sites

	Min	Percentile									Max	Mean	SD
		1st	5th	10th	25th	50th	75th	90th	95th	99th			
Amherst, MA; 64 children 1 to <4 years[a]													
Median of Aluminum, Silicon, Titanium	–169	–79	–19	–11	6	30	72	188	253	435	11,874	147	1,048
Median of best 4 tracers	–97	–32	–13	–6	9	33	72	110	154	226	11,415	132	1,006
Best tracer	–12	–10	–3	1	10	34	58	100	217	2,782	11,874	176	1,083
Washington State; 101 children 2–7 years[b]													
Median of Aluminum, Silicon, Titanium	–404	–242	–94	–52	15	44	116	210	246	535	905	69	146
Best tracer	–131	–59	–22	3	26	68	177	531	1,320	2,846	6,182	274	750

[a] Based on data from Calabrese et al. (1989).
[b] Based on data from Davis et al. (1990).
Max = Maximum.
Min = Minimum.
SD = Standard deviation.

Source: Stanek and Calabrese (1995a).

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Table 5-12. Predicted Soil and Dust Ingestion Rates for Children Age 3 to <6 Years (mg/day)

Scenario	*N*	Mean	Percentile 5	25	50	75	95	100
Dust ingestion/hand-to-mouth	1,000	19.8	0.6	3.4	8.4	21.3	73.7	649.3
Dust ingestion/object-to-mouth	1,000	6.9	0.1	0.7	2.4	7.4	27.2	252.7
Total dust ingestion[a]	1,000	27	ND	ND	13	ND	109	360
Soil ingestion/hand-to-mouth	1,000	41.0	0.2	5.3	15.3	44.9	175.6	1,367.4
Total ingestion	1,000	67.6	4.9	16.8	37.8	83.2	224.0	1,369.7

[a] Email from Haluk Özkaynak (NERL, U.S. EPA) to Jacqueline Moya (NCEA, U.S. EPA) dated 3/8/11.
N = Number of model runs.
ND = data not provided.

Source: Özkaynak et al. (2011).

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Table 5-13. Age-Dependent Probability Density Functions Used to Estimate Dust and Soil Ingestion Rates via the Activity Pattern Modeling Approach

Parameters	Age Groups					
	Infants 0–6 Months	Toddlers 7 Months–4 Years	Children 5–11 Years	Teens 12–19 Years	Adults 20–59 Years	Seniors 60+ Years
DSL_{hard} (mg/cm^2)	NA	AM 0.052 ± 0.065, LN	AM 0.052 ± 0.065, LN	AM 0.052 ± 0.065, LN	AM 0.052 ± 0.065, LN	AM 0.052 ± 0.065, LN
DSL_{soft} (mg/cm^2)	AM 0.139 ± 0.305, LN	AM 0.139 ± 0.305, LN	AM 0.139 ± 0.305, LN	AM 0.139 ± 0.305, LN	AM 0.139 ± 0.305, LN	AM 0.139 ± 0.305, LN
ET (hr/d)	24 hr/d-ST	24 hr/d-ST-TO	24 hr/d-ST-TO	24 hr/d-ST-TO[a]	24 hr/d-ST-TO[b]	24 hr/d-ST-TO[c]
ST (hr/d)	12; 13; 15, TRI	10.5 ± 2.78, LN	9.9 ± 2.6, LN	9.1 ± 2.4, LN	8.4 ± 2.2, LN	8.5 ± 2.2, LN
TO (hr/d)	NA	0; 1.2; 3.0, TRI	0; 2.2; 4.0, TRI	1.4 ± 1.2, LN	1.4 ± 1.3, LN	1.3 ± 1.4, LN
FQ (events/hr)	28 ± 22, LN	16 ± 9.9, LN	9.1 ± 6.8, LN	1.0 ± 0.50, LN	1.0 ± 0.50, LN	1.0 ± 0.50, LN
$FSA_{fingers}$ (unitless)	0.05; 0.08; 0.10, TRI	0.04; 0.07; 0.10, TRI	0.04; 0.07; 0.10, TRI	0.04; 0.05; 0.06, TRI	0.04; 0.05; 0.06, TRI	0.04; 0.05; 0.06, TRI
$FTSS_{hard}$ (unitless)	NA	0.7 ± 0.1, LN	0.7 ± 0.1, LN	0.4 ± 0.1, LN	0.4 ± 0.1, LN	0.4 ± 0.1, LN
$FTSS_{soft}$ (unitless)	0.14 ± 0.02, LN	0.14 ± 0.02, LN	0.14 ± 0.02, LN	0.08 ± 0.02, LN	0.08 ± 0.02, LN	0.08 ± 0.02, LN
SA_{hand} (cm^2)	160 ± 15, LN	215 ± 25, LN	295 ± 40, LN	400 ± 50, LN	445 ± 55, LN	450 ± 55, LN
SE (unitless)	0; 0.5; 1.0, TRI	0; 0.5; 1.0, TRI	0; 0.5; 1.0, TRI	0; 0.5; 1.0, TRI	0; 0.5; 1.0, TRI	0; 0.5; 1.0, TRI
SL_{hands} (mg/cm^2)	GM 0.1 ± 1.8, LN	GM 0.1 ± 1.8, LN	GM 0.1 ± 1.8, LN	GM 0.1 ± 1.8, LN	GM 0.1 ± 1.8, LN	GM 0.1 ± 1.8, LN

[a] 93.3% of teens were assumed to spend time outdoors and 6.7% were assumed to spend no time outdoors.
[b] 89.5% of adults were assumed to spend time outdoors and 10.5% were assumed to spend no time outdoors.
[c] 71.8% of seniors were assumed to spend time outdoors and 28.2% were assumed to spend no time outdoors.
AM = Arithmetic mean.
DSL = Dust surface loading.
ET = Exposure time.
FQ = Frequency of hand to mouth events.
FSA = Fraction of surface area of hands.
FTSS = Fraction of dust transferred from surfaces to skin.
GM = Geometric mean.
LN = Lognormal distribution.
NA = Not applicable.
SA = Surface area of the hand.
SE = Saliva extraction fraction.
SL = Soil loading.
ST = Sleep time.
TO = Time outdoors.
TRI = Triangular distribution.

Source: Wilson et al. (2013).

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Table 5-14. Soil and Dust Ingestion Rates, Estimated Using a Probabilistic Activity Pattern Modeling Approach[a]

Age Group	mg/day, Mean ± Standard Deviation (95th Percentile)		
	Soil	Dust[b]	Soil + Dust
Infant (0 to 6 months)	NA	36 ± 130 (140)	36 (140)
Toddler (7 months to 4 years)	20 ± 26 (64)	41 ± 71 (140)	61 (204)
Child (5 to 11 years)	23 ± 32 (75)	32 ± 59 (110)	55 (185)
Teenager (12 to 19 years)	1.5 ± 2.6 (5.3)	2.2 ± 3.6 (7.3)	3.7 (12.6)
Adult (20 to 59 years)	1.6 ± 2.9 (5.9)	2.6 ± 4.2 (8.6)	4.2 (14.5)
Senior (60+ years)	1.2 ± 2.7 (4.8)	2.6 ± 4.2 (8.7)	3.8 (13.5)

[a] Based on 200,000 trials.
[b] Dust ingestion rate assuming 50% hard and 50% soft surfaces; except infants who were assumed to spend all their indoor and outdoor awake time in contact with soft surfaces only.

Source: Wilson et al. (2013).

Table 5-15. Age-Specific Central Tendency Soil/Dust Ingestion Rates for Four Scenarios That Best Predict Observed Blood Lead Levels (mg/day)[a]

Age (Years)	55/45[b,c]	40/30/30[d]	40/30/30[e]	50/25/10/15[f]	Average All Models
0.5 to <1	92	82	76	86	84
1 to <2	100	89	90	94	93
2 to <3	72	64	66	67	67
3 to <4	65	58	62	63	62
4 to <5	69	62	63	67	65
5 to <6	54	49	50	52	51
6 to <7	54	49	54	55	53
7 to <8	51	47	50	51	50
8 to <9	57	53	61	63	59
9 to <10	58	54	57	59	57

[a] A total of 2,176 records of blood/soil/dust/lead concentrations were available for the analysis.
[b] Geometric mean ingestion rate. The IEUBK model default of 55% dust and 45% soil (55/45)
[c] Dust/yard soil.
[d] Dust/yard/community soil. The original Bunker Hill Superfund Site model of 40% dust, 30% yard soil, and 30% community soil (40/30/30GM); geometric mean.
[e] Dust/yard/community soil. The original Bunker Hill Superfund Site model of 40% dust, 30% yard soil, and 30% community soil (40/30/30AM); Arithmetic mean.
[f] Dust/yard/neighborhood/community soil. The SEM using 50% dust, 25% yard soil, 10% neighborhood soil, and 15% community soil (50/25/10/15); arithmetic mean.

Source: von Lindern et al. (2016).

Table 5-16. Age-Specific Distributions of Soil and Dust Intake Rates for the Four Partition Scenarios (mg/day)[a]

Partition	Age (years)	*N*	Percentile						
			5	10	25	50	75	90	95
55/45[b]	0.5 to <1	60	21	34	49	98	163	265	370
	1 to <2	190	17	24	56	106	209	331	493
	2 to <3	226	14	22	38	80	139	236	313
	3 to <4	225	13	18	32	67	135	219	305
	4 to <5	208	11	15	39	75	142	249	307
	5 to <6	226	10	16	29	63	107	171	224
	6 to <7	229	9	14	27	56	109	184	284
	7 to <8	239	9	13	27	53	107	169	233
	8 to <9	270	4	15	29	68	132	234	305
	9 to <10	255	8	18	32	63	111	201	303
40/30/30GM[c]	0.5 to <1	60	22	34	46	89	138	210	298
	1 to <2	190	18	30	56	91	159	262	323
	2 to <3	226	14	22	37	66	113	190	229
	3 to <4	225	13	18	35	60	111	160	206
	4 to <5	208	12	19	37	66	118	178	240
	5 to <6	226	11	15	26	55	94	140	166
	6 to <7	229	9	15	26	56	93	149	217
	7 to <8	239	9	14	27	51	88	132	185
	8 to <9	270	3	19	30	61	110	185	231
	9 to <10	255	9	20	32	61	98	169	212
40/30/30AM[c,d]	0.5 to <1	60	16	24	36	58	88	173	195
	1 to <2	190	16	23	40	67	110	196	229
	2 to <3	226	11	17	27	50	80	145	171
	3 to <4	225	9	13	26	46	79	123	160
	4 to <5	208	10	14	30	51	80	120	197
	5 to <6	226	9	11	20	38	73	103	128
	6 to <7	229	7	11	20	40	68	112	151
	7 to <8	239	7	12	19	38	66	98	129
	8 to <9	270	2	14	25	42	85	131	170
	9 to <10	255	7	17	25	43	79	119	159

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Table 5-16. Age-Specific Distributions of Soil and Dust Intake Rates for the Four Partition Scenarios (mg/day) (Continued)

Partition	Age (years)	*N*	Percentile 5	10	25	50	75	90	95
50/25/10/15[d,e]	0.5 to <1	54	17	27	38	72	94	165	221
	1 to <2	174	16	22	42	69	123	188	250
	2 to <3	202	10	19	28	53	82	140	178
	3 to <4	209	10	14	26	47	76	130	156
	4 to <5	192	11	15	32	53	86	122	182
	5 to <6	208	10	12	23	41	74	102	126
	6 to <7	218	7	11	21	41	68	116	171
	7 to <8	228	7	12	21	41	68	105	120
	8 to <9	258	2	14	25	44	80	134	170
	9 to <10	245	8	17	25	43	80	116	171

[a] A total of 2,176 records of blood/soil/dust/lead concentrations were available for analysis.
[b] dust/yard soil.
[c] dust/yard soil/community soil.
[d] Partition scenarios that best fit the blood lead levels predicted by the IEUBK model to the observed blood lead levels.
[e] dust/yard soil/neighborhood soil/community soil.
GM = Geometric mean.
AM = Arithmetic mean.
N = Number of observations.

Source: von Lindern et al. (2016).

Table 5-17. Estimated Daily Soil Ingestion for East Helena, MT Children Ages 1–3 years (*N* = 59)

Estimation Method	Mean (mg/day)	Median (mg/day)	Standard Deviation (mg/day)	Range (mg/day)	95th Percentile (mg/day)	Geometric Mean (mg/day)
Aluminum	181	121	203	25–1,324	584	128
Silicon	184	136	175	31–799	578	130
Titanium	1,834	618	3,091	4–17,076	9,590	401
Minimum	108	88	121	4–708	386	65

Source: Binder et al. (1986).

Table 5-18. Estimated Soil Ingestion for Sample of Dutch Nursery School Children, Ages 2–4 Years

Child	Sample Number	Soil Ingestion as Calculated from Titanium (mg/day)	Soil Ingestion as Calculated from Aluminum (mg/day)	Soil Ingestion as Calculated from AIR (mg/day)	Limiting Tracer (mg/day)
1	L3	103	300	107	103
	L14	154	211	172	154
	L25	130	23	—	23
2	L5	131	—	71	71
	L13	184	103	82	82
	L27	142	81	84	81
3	L2	124	42	84	42
	L17	670	566	174	174
4	L4	246	62	145	62
	L11	2,990	65	139	65
5	L8	293	—	108	108
	L21	313	—	152	152
6	L12	1,110	693	362	362
	L16	176	—	145	145
7	L18	11,620	—	120	120
	L22	11,320	77	—	77
8	L1	3,060	82	96	82
9	L6	624	979	111	111
10	L7	600	200	124	124
11	L9	133	—	95	95
12	L10	354	195	106	106
13	L15	2,400	—	48	48
14	L19	124	71	93	71
15	L20	269	212	274	212
16	L23	1,130	51	84	51
17	L24	64	566	—	64
18	L26	184	56	—	56
Arithmetic Mean		1,431	232	129	105

— = No data.
AIR = Acid insoluble residue.

Source: Adapted from Clausing et al. (1987).

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Table 5-19. Estimated Soil Ingestion for Sample of Dutch Hospitalized, Bedridden Children, Ages 2–4 Years

Child	Sample	Soil Ingestion as Calculated from Titanium (mg/day)	Soil Ingestion as Calculated from Aluminum (mg/day)	Limiting Tracer (mg/day)
1	G5	3,290	57	57
	G6	4,790	71	71
2	G1	28	26	26
3	G2	6,570	94	84
	G8	2,480	57	57
4	G3	28	77	28
5	G4	1,100	30	30
6	G7	58	38	38
Arithmetic Mean		2,293	56	49

Source: Adapted from Clausing et al. (1987).

Table 5-20. Van Wijnen et al. (1990) Limiting Tracer Method (LTM) Soil Ingestion Estimates for Sample of Dutch Children

		Daycare Center			Campground		
Age (years)	Sex	*N*	GM LTM (mg/day)	GSD LTM (mg/day)	*N*	GM LTM (mg/day)	GSD LTM (mg/day)
Birth to <1	Girls	3	81	1.09	NA	NA	NA
	Boys	1	75		NA	NA	NA
1 to <2	Girls	20	124	1.87	3	207	1.99
	Boys	17	114	1.47	5	312	2.58
2 to <3	Girls	34	118	1.74	4	367	2.44
	Boys	17	96	1.53	8	232	2.15
3 to <4	Girls	26	111	1.57	6	164	1.27
	Boys	29	110	1.32	8	148	1.42
4 to <5	Girls	1	180		19	164	1.48
	Boys	4	99	1.62	18	136	1.30
All girls		86	117	1.70	36	179	1.67
All boys		72	104	1.46	42	169	1.79
Total		162[a]	111	1.60	78[b]	174	1.73

[a] Age and/or sex not registered for eight children; one untransformed value = 0.
[b] Age not registered for seven children; geometric mean LTM value = 140.
GM = Geometric mean.
GSD = Geometric standard deviation.
LTM = Limiting tracer method.
N = Number of subjects.
NA = Not available.

Source: Adapted from Van Wijnen et al. (1990).

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Table 5-21. Estimated Geometric Mean Limiting Tracer Method (LTM) Soil Ingestion Values of Children Attending Daycare Centers According to Age, Weather Category, and Sampling Period

		First Sampling Period		Second Sampling Period	
Weather Category	Age (years)	*N*	Estimated Geometric Mean LTM Value (mg/day)	*N*	Estimated Geometric Mean LTM Value (mg/day)
Bad (>4 days/week precipitation)	<1	3	94	3	67
	1 to <2	18	103	33	80
	2 to <3	33	109	48	91
	4 to <5	5	124	6	109
Reasonable (2−3 days/week precipitation)	<1			1	61
	1 to <2			10	96
	2 to <3			13	99
	3 to <4			19	94
	4 to <5			1	61
Good (<2 days/week precipitation)	<1	4	102		
	1 to <2	42	229		
	2 to <3	65	166		
	3 to <4	67	138		
	4 to <5	10	132		

LTM = Limiting tracer method.
N = Number of subjects.

Source: Van Wijnen et al. (1990).

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Table 5-22. Items Ingested by Low-Income Mexican-Born Women (Ages 18–42 Years) Who Practiced Pica during Pregnancy While in the United States (*N* = 46)

Item Ingested	Number (%) Ingesting Items
Dirt	11 (24)
Bean stones[a]	17 (37)
Magnesium carbonate	8 (17)
Ashes	5 (11)
Clay	4 (9)
Ice	18 (39)
Other[b]	17 (37)

[a] Little clods of dirt found among unwashed beans.
[b] Including eggshells, starch, paper, lipstick, pieces of clay pot, and adobe.
N = Number of individuals reporting pica behavior.

Source: Simpson et al. (2000).

Table 5-23. Soil Ingestion Rates for the Four Most Reliable Tracers (Aluminum, Silicon, Lanthanum, and Cerium), Aluminum and Silicon Combined, and All Four Tracers Combined (mg/day)

Tracer	*N*	Mean	SD	50th Percentile	75th Percentile	90th Percentile
Aluminum	43	36.9	51.9	31	61	110
Cerium	43	72.2	179.5	51	142	217
Lanthanum	43	132.6	158.6	104	211	343
Silicon	30	49.4	73.7	40	124	145
Aluminum and silicon	73	42.0	61.6	32	89	124
All 4 tracers	159	74.7	119.5	50	130	211

N = Number of fecal samples from the seven adult subjects.
SD = Standard deviation.

Source: Doyle et al. (2012).

Table 5-24. Soil Ingestion Rates for Aluminum, Silicon, and the Four Most Reliable Tracers (Aluminum, Silicon, Lanthanum, and Cerium) Combined (mg/day)

Tracer	*N*	Mean	SD	50th Percentile	90th Percentile
Aluminum	87	36	117	7	161
Silicon	87	68	152	37	231
Aluminum and silicon	87	52	119	37	220
All 4 tracers	87	32	88	18	152

N = Number of fecal samples from the nine adult subjects.
SD = Standard deviation.

Source: Irvine et al. (2014).

Table 5-25. Estimated Soil Ingestion for Six Jamaican Children Displaying Soil Pica[a]

Child	Month	Estimated Soil Ingestion (mg/day)
11	1	55
	2	1,447
	3	22
	4	40
12	1	0
	2	0
	3	7,924
	4	192
14	1	1,016
	2	464
	3	2,690
	4	898
18	1	30
	2	10,343
	3	4,222
	4	1,404
22	1	0
	2	—
	3	5,341
	4	0
27	1	48,314
	2	60,692
	3	51,422
	4	3,782

[a] Based on study of children 0.3 to 14 years.
— = No data.

Source: Calabrese and Stanek (1993).

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Table 5-26. Distribution of Average (Mean) Daily Soil Ingestion Estimates per Child for 64 Children, Ages 1 to <4 Years[a] (mg/day)

Type of Estimate	Overall	Aluminum	Barium	Manganese	Silicon	Titanium	Vanadium	Yttrium	Zirconium
Number of samples	64	64	33	19	63	56	52	61	62
Mean	179	122	655	1,053	139	271	112	165	23
25th Percentile	10	10	28	35	5	8	8	0	0
50th Percentile	45	19	65	121	32	31	47	15	15
75th Percentile	88	73	260	319	94	93	177	47	41
90th Percentile	186	131	470	478	206	154	340	105	87
95th Percentile	208	254	518	17,374	224	279	398	144	117
Maximum	7,703	4,692	17,991	17,374	4,975	12,055	845	8,976	208

[a] For each child, estimates of soil ingestion were formed on days 4–8, and the mean of these estimates was then evaluated for each child. The values in the column "overall" correspond to percentiles of the distribution of these means over the 64 children. When specific trace elements were not excluded via the relative standard deviation criteria, estimates of soil ingestion based on the specific trace element were formed for 108 days for each subject. The mean soil ingestion estimate was again evaluated. The distribution of these means for specific trace elements is shown.

Source: Stanek and Calabrese (1995b).

Table 5-27. Estimated Distribution of Individual Mean Daily Soil Ingestion Based on Data for 64 Subjects (Ages 1 to <4 Years) Projected over 365 Days[a]

Range	1–2,268 mg/day[b]
50th Percentile (median)	75 mg/day
90th Percentile	1,190 mg/day
95th Percentile	1,751 mg/day

[a] Based on fitting a log-normal distribution to model daily soil ingestion values.
[b] Subject with pica excluded.

Source: Stanek and Calabrese (1995b).

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Table 5-28. Distribution of Daily Soil Ingestion (mg/day) Over 7 Days, 64 Children (Ages 1–4 years) from Anaconda, MT[a]

Over days	Over elements within a day	Mean	SD	Percentile					Max
				25th	50th	75th	90th	95th	
Median	Median[b]	13	49	14	8	30	82	107	136
Mean	Median[b]	31	56	–3	17	53	111	141	219
Median	Mean[c]	14	59	–14	4	26	120	128	151
Mean	Mean[c]	36	72	–7	16	72	151	160	283

[a] Based on 7 of 8 tracer elements (excluding titanium), and 28-hour lag time.
[b] Estimates correspond to the median from the 7 trace elements for each subject day.
[c] Estimates correspond to the mean.

Source: Stanek and Calabrese (2000).

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Table 5-29. Prevalence of Nonfood Consumption by Substance from NHANES I and NHANES II

	NHANES I (age 1−74 years) *N* (sample size) = 20,724 (unweighted); 193,716,939 (weighted)			NHANES II (age 6 months−74 years) *N* (sample size) = 25,271 (unweighted); 203,432,944 (weighted)		
Substance	*N* Unweighted (Weighted)	Prevalence[a]	95% Confidence Interval	*N* Unweighted (Weighted)	Prevalence[a]	95% Confidence Interval
Any nonfood substance	732 (4,900,370)	2.5%	2.2−2.9%	480 (2,237,993)	1.1%	1.0−1.2%
Clay				46 (223,361)	0.1%	0.1−0.2%
Starch	131 (582,101)	0.3%	0.2−0.4%	61 (450,915)	0.2%	0.1−0.3%
Paint and plaster	39 (195,764)	0.5%[b]	0.3−0.7%	55 (213,588)	0.6%[c]	0.4−0.8%
Dirt				216 (772,714)	2.1%[d]	1.7−2.5%
Dirt and clay	385 (2,466,210)	1.3%	1.1−1.5%			
Other	190 (1,488,327)	0.8%	0.6−0.9%	218 (1,008,476)	0.5%	0.4−0.6%

[a] Prevalence = Frequency (*n*) (weighted) ÷ Sample Size (*N*) (weighted).
[b] NHANES I sample size (<12 years): 4,968 (unweighted); 40,463,951 (weighted).
[c] NHANES II sample size (<12 years): 6,834 (unweighted); 37,697,059 (weighted).
[d] For those aged <12 years only; question not prompted for those ≥12 years.
Unweighted = raw counts.
Weighted = adjusted to account for the unequal selection probabilities caused by the cluster design, item nonresponse, planned oversampling of certain subgroups, and adjustments to ensure data are representative of the civilian noninstitutionalized census population in the coterminous United States.

Source: Gavrelis et al. (2011).

Table 5-30. Results of Meta-Analysis on Soil Ingestion (mg/day)

Population Group	Number of Studies	Number of Subjects	Number of Subject-Weeks	Mean[a]	SE	95% UCL	50th Percentile	95th Percentile	Adjusted Mean[b]
Subjects	4	216	266	25.5	15.5	73.1	32.6	79.4	31.3
Male	4	114	137	27.7	15.5	74.7	27.1	88.8	31.2
Female	4	102	129	22.2	18.4	78.7	33.5	66.7	31.3
Age 1 to <2 years	2	39	58	3.8	11.8	154	9.2	18.1	6.8
Age 2 to <3 years	3	55	76	20.6	24.0	115	21.9	94.5	28.3
Age 3 to <4 years	4	47	57	32.2	22.3	104	57.0	71.3	46.3
Age 4 to <5 years	3	75	75	40.9	17.0	257	36.2	104	41.3
Excluding Anaconda data[c]	3	156	206	43.4	4.5	52.3	40.8	90.0	43.7
All subjects except pica child[d]	4	240	303	36.5	17.1	88.7	39.4	114	43.6

[a] Calculation of the mean includes negative values for some subjects.
[b] Estimates of soil ingestion less than zero are set equal to zero.
[c] Excludes data from the Anaconda study (Calabrese et al., 1997a) because the children lived near a Superfund site and it could be assumed that soil ingestion at that site could be different from at other sites because additional effort may be taken to limit soil ingestion at this site.
[d] Excludes subject exhibiting pica behavior.

Source: Stanek et al. (2012a).

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Table 5-31. Age-Dependent Probability Density Functions Used to Estimate Sediment Ingestion Rates Using an Activity Pattern Modeling Approach[a]

Parameters	Age Groups				
	Toddlers 7 months–4 years	Children 5–11 years	Teens 12–19 years.	Adults	Seniors
FQ (events/hr)	16 ± 9.9, LN	9.1 ± 6.8, LN	3.0 ± 1.5, LN	3.0 ± 1.5, LN	3.0 ± 1.5, LN
$FSA_{fingers}$ (unitless)	0.04; 0.07; 0.10, TRI	0.04; 0.07; 0.10, TRI	0.04; 0.05; 0.06, TRI	0.04; 0.05; 0.06, TRI	0.04; 0.05; 0.06, TRI
SA_{hand} (cm^2)	215 ± 25, LN	295 ± 40, LN	400 ± 50, LN	445 ± 55, LN	450 ± 55, LN
SE (unitless)	0; 0.5; 1.0, TRI	0; 0.5; 1.0, TRI	0; 0.5; 1.0, TRI	0; 0.5; 1.0, TRI	0; 0.5; 1.0, TRI
	All Ages				
SL_{hands} (mg/cm^2)	GM 0.88 (95% CI = 0.35 to 2.2), LN				
SS (mg/L)	39; 847; 5,146, TRI				
SWIR (L/hr)	AM 0.0037 (95th percentile 0.0112), LN				

[a] Based on 200,000 trials.
AM = Arithmetic mean.
CI = Confidence interval.
FQ = Frequency of events.
FSA = Fraction of surface area of hands.
GM = Geometric mean.
LN = Lognormal distribution.
SA = Surface area.
SE = Saliva extraction.
SL_{hands} = Sediment adherence factor.
SS = Suspended sediment concentration.
SWIR = Surface water ingestion rate.
TRI = Triangular distribution.

Source: Wilson et al. (2015).

Table 5-32. Estimated Sediment Ingestion Rates (mg/hour) Using an Activity Pattern Modeling Approach

Age Group	Deterministic Estimate Arithmetic Mean	Probabilistic Estimate[a] Arithmetic Mean ± SD (95th percentile)
Sediment Ingestion Due to Hand-to-Mouth Contact		
Toddlers (7 months–4 years)	59	72 ± 89 (300)
Children (5–11 years)	46	57 ± 78 (250)
Teens (12–19 years)	15	18 ± 20 (70)
Adults (20–59 years)	16	20 ± 23 (80)
Seniors (60+ years)	17	20 ± 23 (80)
Sediment Ingestion Due to Surface Water Intake		
All age groups	3.1	7.7 ± 89 (44)

[a] Based on 200,000 trials.
SD = Standard deviation.

Source: Wilson et al. (2015).

Table 5-33. Dust Ingestion Rates at Residential Settings[a] Based on an Activity Pattern Modeling Approach

Age Group	Mean ± SD (95th percentile) Dust Ingestion Rates (m^2/d)		
	100% Hard Surfaces	100% Soft Surfaces	50% Hard/50% Soft Surfaces
Infant (0–6 months)	—[b]	0.025 ± 0.024 (0.088)	0.025 ± 0.024 (0.088)[b]
Toddler (7 months–4 years)	0.10 ± 0.092 (0.34)	0.020 ± 0.018 (0.067)	0.061 ± 0.055 (0.20)
Child (5–11 years)	0.078 ± 0.081 (0.29)	0.016 ± 0.016 (0.059)	0.047 ± 0.048 (0.18)
Teen (12–19 years)	0.0053 ± 0.0042 (0.016)	0.0011 ± 0.00084(0.0032)	0.0032 ± 0.0025 (0.0094)
Adult (20–59 years)	0.0062 ± 0.0050 (0.019)	0.0012 ± 0.00098 (0.0038)	0.0037 ± 0.0029 (0.0093)
Senior (60+ years)	0.0063 ± 0.0051 (0.019)	0.0013 ± 0.0010 (0.0039)	0.0038 ± 0.0030 (0.012)

[a] Based on probabilistic approach (200,000 trials).
[b] Infants were assumed to contact soft surfaces only.

Source: Wilson et al. (2016).

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Table 5-34. Summary of Estimates of Soil and Dust Ingestion by General Population Children and Adults from Key Studies Using the Tracer Study, Biokinetic Modeling, and Activity Pattern Methodologies (mg/day)[a]

Population	Sample Size	Age	Mean	p50	p95	Reference
		Tracer Studies				
Amherst, MA	64	1 to <4 years	132[b]	33[b]	154[b]	Calabrese et al. (1989) reanalyzed by Stanek and Calabrese (1995a)
Southeastern, WA	101	2 to 7 years	69[c]	44[c]	246[c]	Davis et al. (1990) reanalyzed by Stanek and Calabrese (1995a)
Anaconda, MT	64	1 to 4 years	66[d]	20[d]	282[d]	Calabrese et al. (1997a)
Western MA	12	1 to 3 years	129[e]	4	NR	Calabrese et al. (1997b)
Southeastern, WA	33	Adults	52	7	NR	Davis and Mirick (2006)
Tracer studies		**1 to <8 years**	**99[f]**		**227[g]**	
Age-specific averages		**Adults**	**52**		**NR**	
		Biokinetic Model Comparison Studies				
Lead smelting site: Illinois	31	0.5 to <1 year	61[h]	NR	NR	Hogan et al. (1998)
Lead smelting sites: Pennsylvania, Illinois, Kansas/Missouri	440	1 to <7 years	113	NR	NR	Hogan et al. (1998)
Bunker Hill site, Idaho	60	0.5 to <1 year	81[i]	65	208[i]	von Lindern et al. (2016)
Bunker Hill site, Idaho	190	1 to <2 years	92[i]	68	240[i]	von Lindern et al. (2016)
Bunker Hill site, Idaho	885	2 to <6 years	61[i]	47	162[i]	von Lindern et al. (2016)
Bunker Hill site, Idaho	1,075	1 to <6 years	67[i,j]	52	178[i,j]	von Lindern et al. (2016)
Bunker Hill site, Idaho	993	6 to <10 years	56[i]	42	155[i]	von Lindern et al. (2016)
Biokinetic model comparison studies		**0.5 to <1 year**	**71**		**208**	
Age-specific averages		**1 to <2 years**	**92**		**240**	
		2 to <6 years	**62**		**163**	
		1 to <6 years	**91[k]**		**178**	
		6 to <10 years	**57**		**155**	

Table 5-34. Summary of Estimates of Soil and Dust Ingestion by General Population Children and Adults from Key Studies Using the Tracer Study, Biokinetic Modeling, and Activity Pattern Methodologies (mg/day)[a] (Continued)

Activity Pattern Modeling Studies						
Simulated population	1,000[l]	3 to <6 years	68	38	224	Özkaynak et al. (2011)
Canada	—[m]	0 to <7 months	36[n]	NR	140	Wilson et al. (2013)
Canada	—[m]	7 months to <5 years	61[n]	NR	204	Wilson et al. (2013)
Canada	—[m]	5 to <12 years	55[n]	NR	185	Wilson et al. (2013)
Canada	—[m]	12 years through adults	4[n]	NR	14	Wilson et al. (2013)
Activity pattern modeling studies		**0 to <7 months**	**36**		**140**	
Age-specific averages		**7 months to <5 years**	**65[o]**		**214[p]**	
		5 to <12 years	**55**		**185**	
		12 years through adults	**4**		**13**	
All study types		**0 to <7 months**	**36**		**140**	**Wilson et al. (2013)**
Age-specific averages		**0.5 to <1 year**	**71**		**208**	**Hogan et al. (1998) (mean only); von Lindern et al. (2016)**
		1 to <2 years	**92**		**240**	**von Lindern et al. (2016)**
		2 to <6 years	**61**		**162**	**von Lindern et al. (2016)**
		1 to <6 years	**84**		**206**	**Calabrese et al. (1989) reanalyzed by Stanek and Calabrese (1995a); Davis et al. (1990) reanalyzed by Stanek and Calabrese (1995a) (mean only); Calabrese et al. (1997a); and Calabrese et al. (1997b); Hogan et al. (1998); Ozkaynak et al. (2011); von Lindern et al. (2016); Wilson et al. (2013)**
		6 to <12 years	**56**		**170**	**Wilson et al. (2013); von Lindern et al. (2016)**
		12 years through adults	**28**		**—**	**Davis and Mirick (2006); Wilson et al. (2013)**
Recommended values (rounded to one significant figure)		**0 to <6 months**	**40**		**100**	
		6 months to <1 year	**70**		**200**	
		1 to <2 years	**90**		**200**	
		2 to <6 years	**60**		**200**	
		1 to <6 years	**80**		**200**	
		6 to <12 years	**60**		**200**	
		12 years through adults[q]	**30**		**100[r]**	

Table 5-34. Summary of Estimates of Soil and Dust Ingestion by General Population Children and Adults from Key Studies Using the Tracer Study, Biokinetic Modeling, and Activity Pattern Methodologies (mg/day)[a] (Continued)

a	See Appendix B for additional details.
b	Estimates adjusted by Stanek and Calabrese (1995a) based on data from Calabrese et al. (1989) using BTM (average of the median of the four best tracers for each child).
c	Estimates from Davis et al. (1990) were adjusted by Stanek and Calabrese (1995a) using the BTM (average of the median of three tracers for each child).
d	Estimates based on BTM (average of the best tracer for each child).
e	Estimates based on aluminum and silicon.
f	Average of the means.
g	Average of the 95th percentiles.
h	Adjusted from model default of 85 mg/day under the assumption that the geometric mean model predicted blood lead level was higher than the geometric mean blood lead by a factor of 1.4 due only to individual soil + dust ingestion rates.
i	Average of two best-fit models from Table 5-15.
j	Average of ages 1 to <2, 2 to <3, 3 to <4, 4 to <5, 5 to <6 years.
k	Average of 113 and 68 mg/day.
l	Simulations.
m	Wilson et al. (2013) data based on 200,000 trials.
n	Does not include object-to-mouth exposure to soil and dust.
o	Average of 68 and 61 mg/day.
p	Average of 224 and 204 mg/day.
q	Soil + dust ingestion rates may be higher for adults following a traditional rural or wilderness lifestyle (see Sections 5.3.4.20 and 5.3.4.21). Based on Doyle et al. (2012) and Irvine et al. (2014) the central tendency adult soil + dust ingestion rates is 50 mg/day (20 mg/day soil and 30 mg/day dust) and the upper percentile rate is 200 mg/day (90 mg/day soil and 100 mg/day dust).
r	Upper percentile value for adults estimated by multiplying the average of the ratios of 95th percentiles to means for all other age groups times the adult central tendency estimate (i.e., 30 mg/day × 3.2 = 100 mg/day).
NR	= Not reported.
P	= Percentile.

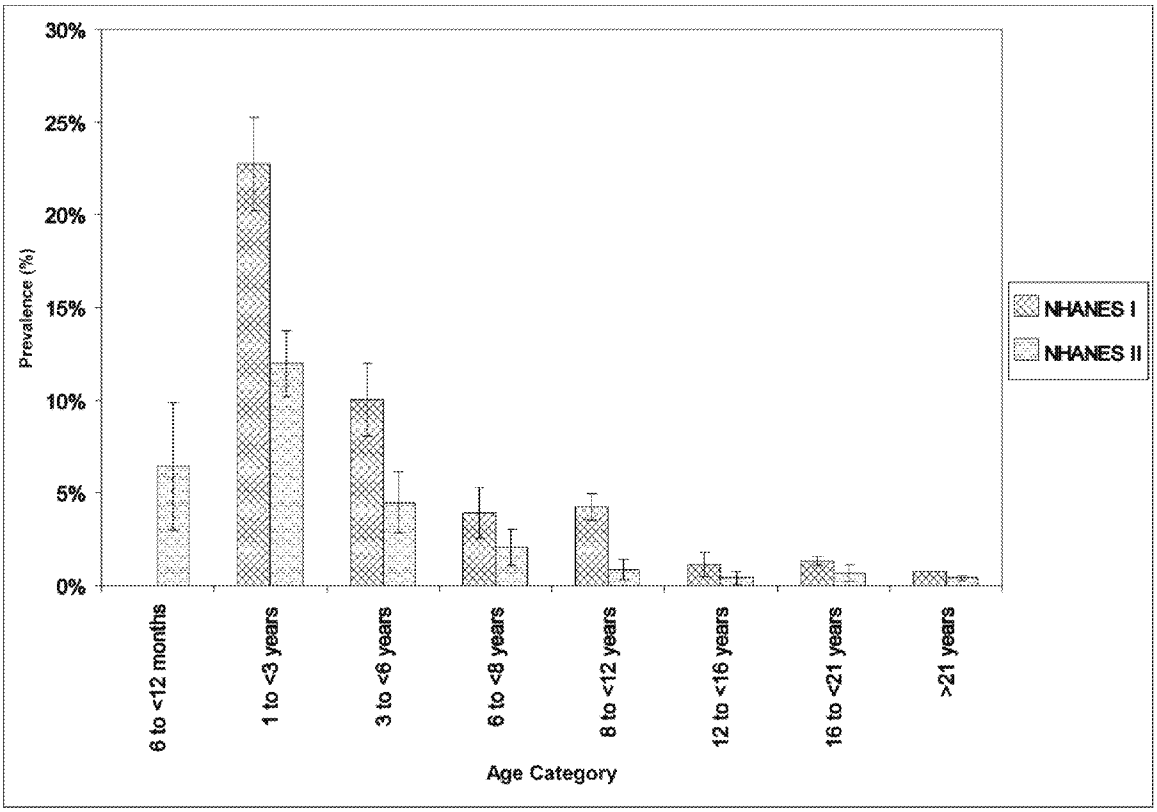

Figure 5-1. Prevalence of Nonfood Substance Consumption by Age, NHANES I and NHANES II.

Source: Gavrelis et al. (2011).

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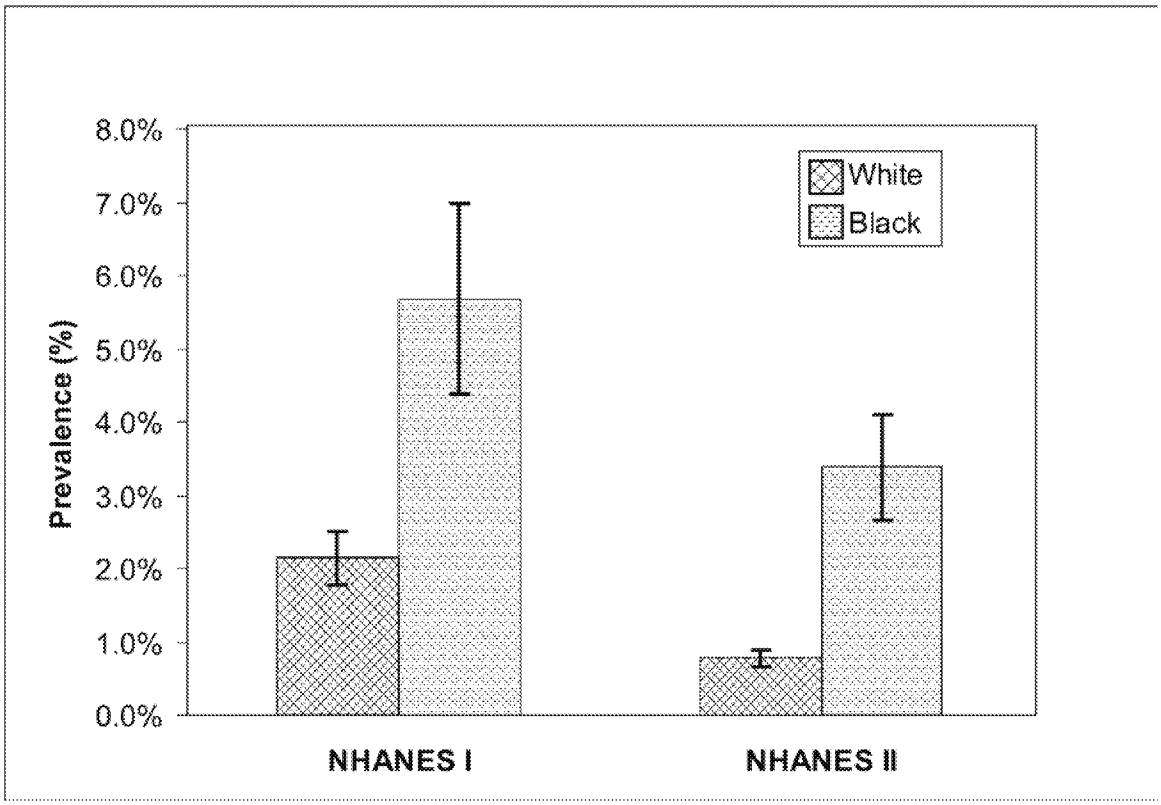

Figure 5-2. Prevalence of Nonfood Substance Consumption by Race, NHANES I and NHANES II.

Source: Gavrelis et al. (2011).

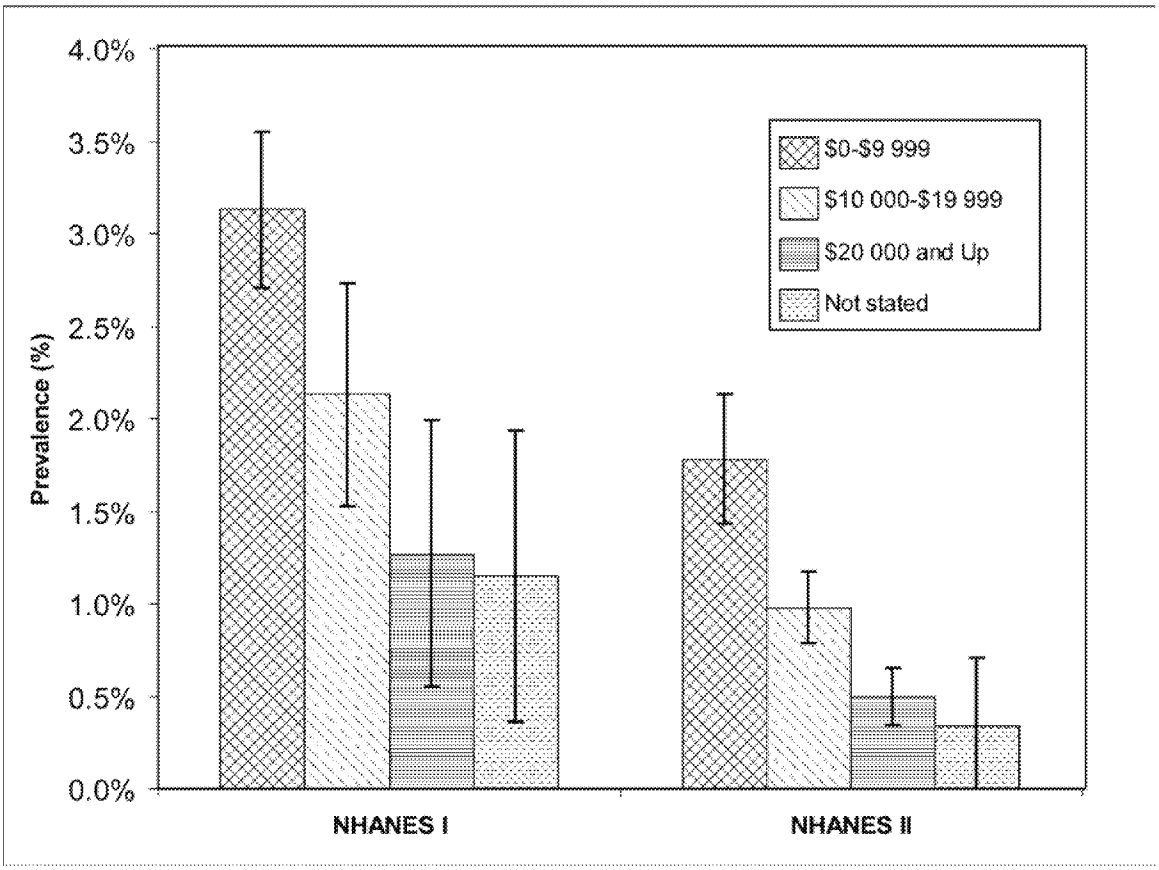

Figure 5-3. Prevalence of Nonfood Substance Consumption by Income, NHANES I (1971–1975) and NHANES II (1976–1980).

Source: Gavrelis et al. (2011).

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APPENDIX A

Table A-1. Terms Used in Literature Searches
Soil ingestion
Dust ingestion
Soil/dust ingestion prevalence
Pica
Soil pica
Geophagy
Indoor settled dust
Outdoor settled dust
Tracer element methodology
Biokinetic soil/dust model/methodology
Activity pattern soil/dust model/methodology
Chalk/dirt/starch/kaolin/magnesium carbonate/pottery/plaster/paint chip eating/ingestion
Ingestion of nonfood substances
Vermeer DE
Frate DA
Davis S
Mirick D
Calabrese EJ
Stanek EJ
Hogan K

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APPENDIX B

Table B-1. Distributions of Soil Ingestion Rates (mg/day) Based on Different Methods of Analyzing Data from the Tracer Studies

Summary Statistic	Amherst, MA (*N* = 64; children 1 to <4 years) Calabrese et al. (1989)				Washington State (*N* = 101; children 2−7 years) Davis et al. (1990)		Anaconda, MT (*N* = 64; children 1−4 years) Calabrese et al. (1997a)			Western MA (*N* = 12; children 1−3 years) Calabrese et al. (1997b)
	Average of Median Al, Si, Ti[a]	Average of Median of Best 4 Tracers[a]	Average of Best Tracer[a]	Daily Average[b]	Average of Median Al, Si, Ti[c]	Average of Best Tracer[c]	Average of Median of Best 4 Tracers[d]	Average of Best Tracer[d]	Daily Average[e]	Average of Mean values for Al and Si[f]
Min	<0	<0	<0	—	<0	<0	<0	0	<0	—
Max	11,874	11,415	11,874	7,703	905	6,182	380	610	219	—
Mean	147	132	176	179	69	274	7	66	31	129
SD	1,048	1,006	1,083	—	146	750	75	120	56	510 (Al); 270 (Si)
Percentile										
5th	<0	<0	<0	—	<0	<0	<0	<0	<0	—
10th	<0	<0	1	10	<0	3	<0	<0	<0	—
25th	6	9	10	45	15	26	<0	2	<0	—
50th	30	33	34	88	44	68	<0	20	17	7 (Al); 0 (Si)
75th	72	72	58	—	116	177	27	69	53	—
90th	188	110	100	186	210	530	73	224	111	—
95th	253	154	217	208	246	1,320	160	282	141	—

[a] Based on a reanalysis of the Calabrese et al. (1989) data using the BTM. Median of Al, Si, and Ti; median of best 4 of 8 tracers (i.e., 4 lowest F/S ratios); or best tracer (lowest F/S ratio) (see Table 6, Stanek and Calabrese, 1995a). The average of the best 4 tracers for each child was used in developing the recommended soil and dust ingestion rates.

[b] Based on average (mean) daily soil ingestion estimates (mg/day) per child (Table 5, Stanek and Calabrese, 1995b).

[c] Best Tracer Method; median of Al, Si, and Ti or best tracer (Table 9, Stanek and Calabrese, 1995a). The average of the 3 tracers for each child was used in developing the recommended soil and dust ingestion rates.

[d] Best Tracer Method; median of best 4 of 5 tracers (i.e., lowest F/S ratios) or best tracer for a given subject-week (Table 12, Calabrese et al., 1997a). The average of the best tracer for each child was used in developing the recommended soil and dust ingestion rates.

[e] Based on the mean of daily soil ingestion estimates based on the median values for 7 trace elements (Table 2, Stanek and Calabrese, 2000).

[f] Average of mean soil ingestion values (Table 3, Calabrese et al., 1997b).

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APPENDIX C

Table C-1. Key Soil Ingestion Studies Used in Developing Soil + Dust Ingestion Recommendation for Use in Risk Assessment

Tracer Studies					
Reference	Location	Population	Tracers	Study Design	Results
Calabrese et al. (1989); Barnes (1990)	Amherst, MA	64 children (1 to <4 years)	aluminum, barium, manganese, silicon, titanium, vanadium, yttrium, zirconium	Duplicate samples of food, beverages, medicines, vitamins, excreta collected over 2-week period; soil/dust samples from children's homes/play areas; participants supplied with toothpaste, baby cornstarch, diaper rash cream, and soap with low levels of most tracer elements; fecal/urine samples collected.	Mean soil + dust ingestion ranged from –496 mg/day based on manganese to 483 mg/day based on silicon. The 95th percentiles range from 159 for both yttrium and zirconium to 3,174 for manganese. Stanek and Calabrese (1995a) reanalyzed the data using the best tracer method (BTM) and the lowest four food:soil ratios for each child, calculated on a per-week ("subject-week") basis. Based on the median of soil ingestion estimates from the best four tracers, the mean soil ingestion rate for children was 132 mg/day and the median was 33 mg/day. The 95th percentile value was 154 mg/day.
Davis et al. (1990)	3-city area in southeastern Washington	101 children (2 to 7 years)	aluminum, silicon, titanium	Collected soil/house dust, duplicate food, dietary supplements/medications, and mouthwash samples over 7 days; urine/feces collected over 4 days; toothpaste with known tracer element content was supplied; information on dietary habits and demographics collected.	Mean soil ingestion rates were 39 mg/day for aluminum, 82 mg/day for silicon, and 245 mg/day for titanium; median values were 25 mg/day for aluminum, 59 mg/day for silicon, and 81 mg/day for titanium. Adjusted mean soil + dust ingestion: 65 mg/day for aluminum, 160 mg/day for silicon, 268 mg/day for titanium; median values were: 52 mg/day for aluminum, 112 mg/day for silicon, 117 mg/day for titanium. Stanek and Calabrese (1995a) reanalyzed the data using the BTM and the lowest four food:soil ratios for each child, calculated on a per-week ("subject-week") basis. The soil + dust ingestion values were 69 mg/day (mean), 44 mg/day (median), and 246 mg/day (95th percentile).
Calabrese et al. (1997a)	Anaconda, MT	64 children (1 to 4 years) at a Superfund site	aluminum, cerium, lanthanum, neodymium, silicon, titanium, yttrium, zirconium	Duplicate samples of meals/beverages and over-the-counter medicines/vitamins collected; feces collected over 7 days; soil/and dust collected from the children's homes/play areas; toothpaste containing nondetectable tracer levels (except silica) provided; infants provided with baby cornstarch, diaper rash cream, and soap with low levels of tracers.	Mean ranged from −544 mg/day based on titanium to 270 mg/day based on neodymium; 95th percentile estimates ranged from 69 mg/day based on silicon to 1,378 mg/day based on titanium. Using the BTM (average of best tracer for each child), the mean value for soil was 66 mg/day; the median was 20 mg/day; and the 95th percentile value was 282 mg/day.

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Table C-1. Key Soil Ingestion Studies Used in Developing Soil + Dust Ingestion Recommendation for Use in Risk Assessment (Continued)

Calabrese et al. (1997b)	Western Massachusetts	12 children 1 to 3 years old observed to have frequent soil ingestion in previous study.	aluminum, silicon, titanium	Mass balance tracer study with duplicate food sampling; both soil and dust samples collected.	Estimates calculated based on soil tracer element concentrations only for the 12 subjects ranged from –15 to +1,783 mg/day based on aluminum, −46 to +931 mg/day based on silicon, and –47 to +3,581 mg/day based on titanium. Mean soil estimates: 168 mg/day based on aluminum, 89 mg/day based on silicon, 448 mg/day based on titanium. Mean dust ingestion estimates: 260 mg/day based on aluminum, 297 mg/day based on silicon, 415 mg/day based on titanium. Based on the average of aluminum and silicon, the mean estimate is 129 mg/day. One child exhibited pica behavior.
Davis and Mirick (2006)	3-city area in southeastern Washington	Subset of Davis et al. (1990): 33 adults	aluminum, silicon, titanium	Duplicate samples of food/medications; feces collected for 11 consecutive days; urine samples collected; soil/house dust samples collected.	Mean for the three tracers ranged from 23 to 625 mg/day; calculated by setting negative estimates to zero. Based on the average of aluminum and silicon, the mean and median values are 52 mg/day and 7 mg/day respectively.
Biokinetic Modeling Comparison Studies					
Reference	Location	Population Studied	Study Design		Results
Hogan et al. (1998)	Historic lead smelting communities: Palmerton, PA; southeastern Kansas and southwestern Missouri; Madison Co., IL.	478 children ages 0.5 to <7 years Pennsylvania, Illinois, and Kansas/Missouri with blood lead measurements and related soil and dust lead levels; 31 of these children from the Illinois site were 0.5 to <1 year old	Compared IEUBK-predicted blood lead levels with observed blood lead levels using observed house dust/soil lead levels, and default soil and dust intake rates, and other model parameters.		The default IEUBK model mean soil + dust soil intake rates averaged over ages 1 to <7 years was approximately 135 mg/day. The geometric mean blood lead levels at one site were slightly over-predicted by the model; blood lead levels were slightly under-predicted at a second site, and the blood lead levels predicted by the model were roughly accurate at the third site. The default IEUBK model mean soil + dust soil intake rates averaged over ages 0.5 to <1 year was approximately 85 mg/day. For children 6 to 12 months old in the Illinois site only, Hogan et al. (1998) reported a predicted geometric mean blood lead level 1.4-fold higher than the geometric mean blood lead levels observed.

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Table C-1. Key Soil Ingestion Studies Used in Developing Soil + Dust Ingestion Recommendation for Use in Risk Assessment (Continued)

Von Lindern et al. (2016)	Northern Idaho; site of community-wide soil remediation	3,203 children (ages 0 to <10 years) with blood lead measurements and related lead levels in yard, neighborhood and community soil, and house dust.	Compared IEUBK-predicted blood lead levels with observed blood lead levels; the soil and dust ingestion values used in the model were developed using a statistical model that apportioned the contributions of community soils, yard soils of the residence, and house dust to lead intake; soil + dust ingestion was estimated based on four partition scenarios that used different combinations of the proportions of dust + soil attributed to different sources (e.g., dust, yard soil, neighborhood soil).	All four partition scenarios produced similar central tendency intake rates. For children ages 0.5 to <10 years, the mean soil and dust ingestion rates ranged from 47 mg/day to 100 mg/day. Among the age groups evaluated, children ages 1 to <2 years had the highest mean soil and dust ingestion rates (i.e., 89–100 mg/day). The 95th percentile soil and dust intake rates ranged from 120 mg/day to 493 mg/day for ages 0.5 to <10 years of age. Based on the average of all four scenarios, the mean soil + dust ingestion rates ranged from 50 mg/day for 7- to <8-year-olds to 93 mg/day for 1- to <2-year-olds.
			Activity Pattern Modeling Studies	
Reference	Location	Population Studied	Study Design	Results
Özkaynak et al. (2011)	United States	Simulated population of children ages 3 to <6 years.	Used EPA's SHEDS-Multimedia model to estimate soil and dust ingestion rates using distributions of exposure factor values for hand-to-mouth activities; assumed soil and dust adhered to hands and remained until washed off or ingested by mouthing; object-to-mouth pathway for soil/dust ingestion was also addressed; outdoor matter was designated as "soil" and indoor matter as "dust."	Mean total soil and dust ingestion: 68 mg/day; approximately 60% originating from soil ingestion, 30% from dust on hands, and 10% from dust on objects; 95th percentile: 224 mg/day. The predicted soil and dust ingestion values fit a log-normal distribution.
Wilson et al. (2013)	Canada	Simulated populations of infants aged 0 to <7 months; toddlers 7 months to <5 years; children 5 years to <12 years; teens 12 years to <20 years; adults 20 years to <60 years; seniors 60+ years.	Modeling approaches (deterministic and probabilistic) used to estimate soil + dust via hand-to-mouth contact; object-to-mouth exposures were not considered. The models used measures of particle loading to indoor surfaces, the fraction transferred to the hands, hand surface areas, the fraction of hand surface area that may be mouthed or contact food, the frequency of hand-to-mouth contacts, the amount dissolved in saliva, and the exposure time. Model parameters used were representative of the Canadian population. Contact was assumed to occur with hard surfaces 50% of the time and with soft surfaces 50% of the time, except for infants for whom contact was assumed to occur with soft surfaces only.	Mean soil + dust ingestion rates ranged from 4 mg/day for teens, adults, and seniors to 61 mg/day for toddlers (7 months to <5 years of age). The 95th percentile soil + dust ingestion rates ranged from 14 mg/day for teens, adults, and seniors to 204 mg/day for toddlers (7 months to <5 years of age). Infants (0 to <7 months in age) were assumed to consume dust only at mean rate of 36 mg/day (95th percentile: 140 mg/day).

Source: Adapted from Moya and Phillips (2014).

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APPENDIX D

Table D-1. Studies on the Prevalence of Ingesting Soil, Dust, or Other Nonfood Substances

Reference	Location	Population	Results
Dickins and Ford (1942)	Oktibbeha County, MS	207 rural Black school children (≥4th grade)	52 of the children ate dirt in the previous 10 to 16 days; clay was predominant type of soil eaten.
Ferguson and Keaton (1950)	Mississippi	361 pregnant women; primarily Black, low economic and educational level	27% of the Black women reported eating clay and 41% reported eating starch. 7% of the White women reported eating clay and 10% reported eating starch.
Cooper (1957)	Baltimore, MD	784 children (≥7 months) referred to a mental hygiene clinic	Parents/caretakers of 86 children responded positively to "Does your child have a habit, or did he ever have a habit, of eating dirt, plaster, ashes, etc.?"
Baltrop (1966)	Boston, MA	439 children (1−6 years): interview	19 children ingested dirt (defined as yard dirt, house dust, plant-pot soil, pebbles, ashes, cigarette ash, glass fragments, lint, and hair combings) in the preceding 14 days.
		277 children (1−6 years): mail survey	39 children ingested dirt in the 14 days prior to the survey.
Bruhn and Pangborn (1971)	California	91 Mexican and "Anglo" low-income families of migrant agricultural workers	12 of 65 Mexican and 11 of 26 "Anglo" respondents indicated consumption of "dirt" among their family members.
Robischon (1971)	Unspecified Location	130 children (19−24 months) from urban well-child clinic	48 "ate nonedibles more than once a week"; substances eaten for 30 of the children were: ashes (17), "earth" (5), dust (3), fuzz from rugs (2), clay (1), and pebbles/stones (1).
Bronstein and Dollar (1974)	Georgia	410 pregnant, low-income women: urban $N = 201$; rural $N = 209$	65 (16%) of the women practiced pica; a variety of substances were ingested, with laundry starch being the most common; there was no significant difference in the practice of pica between rural and urban women.
Hook (1978)	New York	250 who had delivered live infants	Nonfood items reportedly ingested during pregnancy were ice, reported by three women, and chalk from a river clay bank, reported by one woman.
Vermeer and Frate (1979)	Holmes County, MS	50 households (229 people; 140 children or adolescents)	Geophagy (regular consumption of clay over a period of weeks) in 16% of children under 13 years of age; average daily amount of clay consumed estimated at 50 g for both adults and children.
Cooksey (1995)	Midwest	350 postpartum women; majority Black, low income 13 to 42 years old	194 (65%) ingested one or more pica substances during their pregnancy, and the majority (78%) ate ice/freezer frost alone or in addition to other pica substances.
Smulian et al. (1995)	Muscogee County, GA	125 pregnant women ages 12 to 37 years; 73 Black, 47 White, 4 Hispanic, and 1 Asian.	14.4% (18 of 125 women) practiced pica; pica prevalence was highest among Black women (17.8%). The most common form of pica was ice eating (pagophagia), reported by 44.4% of the patients.
Stanek et al. (1998)	Western Massachusetts	528 children (ages 1−7 years) at well medical clinics	Daily mouthing or ingestion: 6% for sand and stones; 4% for soil and dirt; 1% for dust, lint, and dustballs. More than weekly mouthing or ingestion: 16% for sand and stones; 10% for soil and dirt; 3% for dust, lint, and dustballs. More than monthly mouthing or ingestion: 27% for sand and stones; 18% for soil and dirt; 6% for dust, lint, and dustballs.

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Table D-1. Studies on the Prevalence of Ingesting Soil, Dust, or Other Nonfood Substances (Continued)

Reference	Location	Population	Results
Grigsby et al. (1999)	Middle Georgia	21 Black individuals (20 of whom were women) who had reported eating kaolin	Reasons for eating kaolin included liking the taste, being pregnant, craving it, and to gain weight.
Ward and Kutner (1999)	Metropolitan Atlanta, GA	226 dialysis patients	37 (16%) reported pica behavior; Black patients and women made up 86% and 84% of those reporting pica, respectively. Pica items reportedly consumed included ice, starch, dirt, flour, or aspirin.
Simpson et al. (2000)	Santa Ana, Bakersfield, and East Los Angeles, CA	150 Mexican-born, low-income, pregnant or postpartum women	46 (31%) of the women interviewed in the United States reported pica behavior; pica substances included dirt, bean stones, magnesium carbonate, ashes, clay, ice, and other substances; ice was the most consumed substance.
Obialo et al. (2001)	Atlanta, GA	138 Black dialysis patients; 37–78 years old	30 (22%) reported some form of pica behavior; 13 (9.4%) reported clay pica.
Klitzman et al. (2002)	New York City	33 pregnant women whose blood lead levels were >20 µg/dL; majority were foreign born; 15—43 years of age	13 women (39%) reported pica behavior during their current pregnancies; 10 reported eating soil, dirt or clay, 2 reported pulverizing and eating pottery, and 1 reported eating soap.
Gavrelis et al. (2011)	United States nationwide	~21,000 individuals (ages 1–74 years)	Prevalence of consuming nonfood substances was 22.7% for the 1- to <3-year age group based on NHANES I (1971–75) and 12% based on NHANES II (1976–80).
		~25,000 individuals, (ages 0.5−74 years)	Prevalence estimates for the >21-year age group was 0.7% and 0.4% for NHANES I (1971–75) and NHANES II (1976–80).
Lumish et al. (2014)	Rochester, NY	158 pregnant adolescents (<18 years of age); mostly African-American and ~25% Hispanic	46% reported ingesting one or more items, including raw starches (flour and cornstarch); powder (dust, vacuum powder from vacuum cleaner bags, and baby powder); soap (soap, bar soap, laundry soap, and powdered cleansers); plastic/foam (stuffing from pillows/sofas and sponges); paper (writing paper, toilet paper, and tissues); baking soda/powder; and other (dirt and chalk). Ice was the nonfood item most often consumed (37% of all the pregnant adolescents), while only 1.3% of the teens reported ingestion of dirt/chalk.
Lin et al. (2015)	Central California and Mexico	76 Mexican-born pregnant or ≤2-year postpartum women	In the California, 10 (43%) had engaged in pica; 6 during pregnancy. In the Mexican group, 18 (34%) had engaged in pica; 16 during pregnancy. Commonly eaten items were earth, bean stones, and adobe. In the California group, 5 (22%) had eaten earth; in the Mexican group, 6 (11%) had eaten earth.

Source: Adapted from Moya and Phillips (2014).

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Message

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Subject: EPA Administrator Pruitt Proposes Rule To Strengthen Science Used In EPA Regulations

U.S. ENVIRONMENTAL PROTECTION AGENCY

NEWS RELEASE

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EPA Administrator Pruitt Proposes Rule To Strengthen Science Used In EPA Regulations

WASHINGTON (April 24, 2018) - Today, U.S. Environmental Protection Agency (EPA) Administrator Scott Pruitt signed a proposed rule to strengthen the science used in regulations issued by EPA. The rule will ensure that the regulatory science underlying Agency actions is fully transparent, and that underlying scientific information is publicly available in a manner sufficient for independent validation.

"The era of secret science at EPA is coming to an end," **said EPA Administrator Scott Pruitt**. "The ability to test, authenticate, and reproduce scientific findings is vital for the integrity of rulemaking process. Americans deserve to assess the legitimacy of the science underpinning EPA decisions that may impact their lives."

This proposed rule is in line with the scientific community's moves toward increased data sharing to address the "replication crisis"—a growing recognition that a significant proportion of published research may not be reproducible. The proposal is consistent with data access requirements for major scientific journals like *Science*, *Nature*, and *Proceedings of the National Academy of Sciences* as well as recommendations from the Bipartisan Policy Center's Science for Policy Project and the Administrative Conference of the United States' Science in the Administrative Process Project.

The proposed rule builds upon President Trump's executive orders on regulatory reform and energy independence:

- **Executive Order 13777**, issued in March 2017, provides that regulatory reform efforts shall attempt to identify "those regulations that rely in whole or in part on data, information, or methods that are not publicly available or that are insufficiently transparent to meet the standard of reproducibility."
- **Executive Order 13783**, also issued in March 2017, provides that "It is the policy of the United States that necessary and appropriate environmental regulations comply with the law, are of greater benefit than cost, when permissible, achieve environmental improvements for the American people, and are developed through transparent processes that employ the best available peer-reviewed science and economics."

Chairman Lamar Smith (R-TX): "Administrator Pruitt's announcement ensures that data will be secret no more. For too long, the EPA has issued rules and regulations based on data that has been withheld from the American people. It's likely that in the past, the data did not justify all regulations. Today, Administrator Pruitt rightfully is changing business as usual and putting a stop to hidden agendas."

Senator Mike Rounds (R-SD): "Sound, reliable science is vital to helping us make important policy decisions that impact the health of American families and their livelihoods. Inserting new levels of transparency in the EPA rulemaking process will help make the agency more accountable to the American people and help everyone understand the impact of EPA's decisions. Today's directive is a significant step toward making sure these decisions are not made behind closed doors with information accessible only to those writing the regulations, but rather in the full view of those who will be affected."

Dr. Edward J. Calabrese, Professor, Environmental Health Sciences, University of Massachusetts: "The proposal represents a major scientific step forward by recognizing the widespread occurrence of non-linear dose responses in toxicology and epidemiology for chemicals and radiation and the need to incorporate such data in the risk assessment process."

Dr. Louis Anthony (Tony) Cox, President, Cox Associates; Member, National Academy of Engineering; and Editor-in-Chief of the Journal *Risk Analysis*: "I believe that transparency and independent reproducibility of analyses and conclusions are bedrock principles of sound science. Some commentators have expressed concerns that making the data behind policy conclusions and recommendations accessible and transparent might threaten the privacy of individuals. But this concern can be fully met by applying current privacy-protection techniques for data analysis. These techniques have been developed and used successfully for years at the Census Bureau and elsewhere. Thus, we can have the scientific benefits of accessible data while protecting individual privacy."

Dr. Jason Scott Johnston, Director, Olin Law and Economics Program, University of Virginia School of Law: "EPA's proposed rule, Strengthening Transparency in Regulatory

Science, is badly needed "Best practice among peer-edited scientific journals is to require that data and statistical routines used in published papers be posted online and/or made publicly available. To apply the same standards to research that EPA says justify regulations affecting billions of dollars in economic activity and millions of human lives is essential for those regulations to truly be scientifically based."

Bruno Pigott, Commissioner of the Indiana Department of Environmental Management (IDEM): "IDEM supports transparency in rulemaking. Good, sound science leads to better regulations."

Dr. George Wolff, Principal Scientist, Air Improvement Resource, Inc., and former Chairman of EPA's Clean Air Scientific Advisory Committee (1992 - 1996): "In the development of regulations based on environmental studies, numerous subjective assumptions and choices must be made regarding the selection of data and models that have a profound impact on the strength of any statistical associations and even whether the associations are positive or negative. The appropriateness of the assumptions and choices are not adequately evaluated in the standard peer review process. That is why it is essential that the data and models be placed in the public domain for a more rigorous evaluation by qualified experts. The proposed regulation, Strengthening Transparency in Regulatory Science, will provide an opportunity for such evaluations."

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ORGAN TOXICITY AND MECHANISMS

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High concordance of drug-induced human hepatotoxicity with in vitro cytotoxicity measured in a novel cell-based model using high content screening

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Abstract To develop and validate a practical, in vitro, cell-based model to assess human hepatotoxicity potential of drugs, we used the new technology of high content screening (HCS) and a novel combination of critical model features, including (1) use of live, human hepatocytes with drug metabolism capability, (2) preincubation of cells for 3 days with drugs at a range of concentrations up to at least 30 times the efficacious concentration or 100 μM, (3) measurement of multiple parameters that were (4) morphological and biochemical, (5) indicative of prelethal cytotoxic effects, (6) representative of different mechanisms of toxicity, (7) at the single cell level and (8) amenable to rapid throughput. HCS is based on automated epifluorescence microscopy and image analysis of cells in a microtiter plate format. The assay was applied to HepG2 human hepatocytes cultured in 96-well plates and loaded with four fluorescent dyes for: calcium (Fluo-4 AM), mitochondrial membrane potential (TMRM), DNA content (Hoechst 33342) to determine nuclear area and cell number and plasma membrane permeability (TOTO-3). Assay results were compared with those from 7 conventional, in vitro cytotoxicity assays that were applied to 611 compounds and shown to have low sensitivity (<25%), although high specificity (~90%) for detection of toxic drugs. For 243 drugs with varying degrees of toxicity, the HCS, sublethal, cytotoxicity assay had a sensitivity of 93% and specificity of 98%. Drugs testing positive that did not cause hepatotoxicity produced other serious, human organ toxicities. For 201 positive assay results, 86% drugs affected cell number, 70% affected nuclear area and mitochondrial membrane potential and 45% affected membrane permeability and 41% intracellular calcium concentration. Cell number was the first parameter affected for 56% of these drugs, nuclear area for 34% and mitochondrial membrane potential for 29% and membrane permeability for 7% and intracellular calcium for 10%. Hormesis occurred for 48% of all drugs with positive response, for 26% of mitochondrial and 34% nuclear area changes and 12% of cell number changes. Pattern of change was dependent on the class of drug and mechanism of toxicity. The ratio of concentrations for in vitro cytotoxicity to maximal efficaciousness in humans was not different across groups (12 ± 22). Human toxicity potential was detected with 80% sensitivity and 90% specificity at a concentration of 30× the maximal efficacious concentration or 100 μM when efficaciousness was not considered. We conclude that human hepatotoxicity is highly concordant with in vitro cytotoxicity in this novel model and as detected by HCS.

Keywords HCS · Hepatotoxicity · Human · Sublethal · Multi-parameter

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Introduction

Drug-induced liver injury is a major cause of attrition in preclinical and clinical drug development, and when a drug reaches the market. Hepatotoxicity is the most frequent reason cited for labelling drugs with a black box warning, and for withdrawal of an approved drug (Fung et al. 2001). Hepatotoxicity accounts for one-third to one-half of cases of acute liver failure (Andrade et al. 2004; Kaplowitz 2001; Lewis 2002; Russo et al. 2004), and for 15% of associated liver transplantations. Hepatic reactions occur in less than 1 per 10,000 persons exposed, in individuals using therapeutic doses after a

variable latency period, and are usually considered to be idiosyncratic (Kaplowitz 2005). They typically occur on a background of a higher rate of mild, asymptomatic and transient liver injuries, such as indicated by a threefold increase in serum alanine aminotransferase (ALT) greater than the upper limit of normal.

Human hepatotoxicity has not been predictable because of its low concordance with either standard in vitro cytotoxicity screening assay results (O'Brien et al. 2003; Xu et al. 2004) or regulatory animal study findings (Olson et al. 1998, 2000). Whereas conventional cytotoxicity assays frequently have more than 80% specificity, they have less than 25% sensitivity for detection of human hepatotoxicity potential (O'Brien et al. 2003; Xu et al. 2004). Along with hypersensitivity and cutaneous reactions, hepatotoxicity has the poorest correlation with regulatory animal toxicity tests (Olson et al. 1998, 2000). In only approximately half of the new pharmaceuticals that produced hepatotoxicity in clinical drug development was there any concordance with animal toxicity studies (Olson et al. 1998, 2000). This contrasted remarkably with the high correlation between animal and human toxicities affecting the cardiovascular, hematologic and gastrointestinal systems.

Despite the poor predictivity, there is reasonable understanding of the general pathophysiological mechanism of most drug-induced hepatotoxicities (Boelsterli 2003a, b; Jaeschke et al. 2002; Lee 2003; Xu et al. 2004). Also, there are in vitro methods available for detection of the various pathogenetic mechanisms. The major mechanistic classifications of hepatotoxicity include inhibition of mitochondrial function, disruption of intracellular calcium homeostasis, activation of apoptosis, oxidative stress, inhibition of specific enzymes or transporters and formations of reactive metabolites that cause direct toxicity or immunogenicity.

Conventional cytotoxicity assays have had poor sensitivity for several reasons (Xu et al. 2004). Firstly, they have measured lethal events in late stages of toxicity, despite that serious toxicities may not be lethal by themselves or that detection of this advanced endpoint may be not be possible with drugs of limited solubility. Secondly, in vitro cytotoxicity frequently takes several days to express itself (Slaughter et al 2002; Lewis et al. 2003; Xu et al. 2004; Schoonen et al. 2005a, b) and most cytotoxicity assays do not include preincubation of cells with drugs for multiple days. Most assays evaluate only one endpoint, whereas there are multiple mechanisms of toxicity that need to be tested for by different methods, including use of morphological and biochemical or functional parameters. Measurements need to be made directly at the individual cell level to minimize artefact and ensure they reflect cell effects. Cells need to be human and with capacity to metabolise drugs. Finally, tests need to be conducted at concentrations of drugs that are relevant to concentrations having efficacious effects in vivo.

High content screening (HCS) may be an important predictive tool for application of the above mechanistic understanding to drug discovery for the assessment of compound potential for human hepatotoxicity (O'Brien et al. 2003; Xu et al. 2004; Abraham et al. 2004; Giuliano et al. 2003), and for optimisation and prioritisation of a compound's safety. HCS is a recent advance in the automation of quantitative epifluorescence microscopy and image analysis, and in the application of microfluorescent, multiprobe technology (Abraham et al. 2004; Giuliano et al. 2003; Haskins et al. 2001; Plymale et al. 1999). It enables kinetic monitoring in vitro of live cells in real time for multiple cellular biomarkers of processes that are critically involved in the pathogenesis of toxicity. These include inner mitochondrial membrane potential, intracellular free Ca^{2+} membrane permeability, as well as nuclear number and size (Giuliano et al. 2003; Haskins et al. 2001; Plymale et al. 1999).

This study tested the hypothesis that clinical occurrence of human hepatotoxicity concorded with in vitro cytotoxicity assessed in a cell-based model with a novel combination of critical features and using HCS.

Materials and methods

Materials

Chemicals and supplies

HepG2 cells that had undergone 84 passages were acquired from the American type cell culture (ATCC;#HB-8065) and stored in liquid nitrogen as a local cell bank for distribution as needed. Dulbecco's modified eagle's medium (DMEM;#21969–035), fetal bovine serum (FBS;#10108–165), L-glutamine (#25030–024) and penicillin–streptomycin mixture (#15140–122) were acquired from Gibco, Invitrogen. Poly-D-lysine hydrobromide (PDL, MW = 30,000–70,000,#P7280) and non-essential amino acids mixture (#M7145) were acquired from Sigma. All dyes were from molecular probes. Black-walled, 96-well plates with lids were obtained from packard (Packard ViewPlate#6005182). All other drugs and chemicals were acquired from Sigma-Aldrich unless stated otherwise, and were of the highest purity possible.

Cell culture

Human hepatocellular carcinoma cells (HepG2) were subcultured less than ten times after being acquired from the local cell bank. Their doubling time was 29 ± 3 h. They were grown according to ATCC instructions (http://www.lgcpromochem.com/atcc/) in flat-bottomed culture flasks (T25), with bottom surface areas of 25 cm^2. The flasks were manually coated with PDL and the cells grown in DMEM supplemented with 10% heat-inactivated FBS, 1% penicillin–streptomycin, 2 mM glutamine and 1% non-essential amino acids mixture. Cells were grown in a standard, cell culture incubator at 37°C and 5% CO_2 with a water reservoir for humidity

control. Cell counts were determined by hemocytometry for addition of cells to 96-well plates.

Drugs and controls

Drugs that have been marketed for use in man were classified into four categories according to the severity of human hepatotoxicity they produce. Severe human hepatotoxicity was ascribed to drugs producing more than 1% frequency of increased serum ALT plus two of either (1) jaundice, (2) more than three reports of liver failure, or (3) a black box warning. Moderate human hepatotoxicity was ascribed to drugs producing 0.1–1% frequency of increased serum ALT plus either jaundice or a label of occurrence of adverse effect. Non-toxic or minimally toxic drugs were defined as those with less than 0.1% frequency of increased ALT, and associated with no clinical symptoms. The fourth category consisted of drugs that were not known to be hepatotoxic but were known to have other organ toxicities. Additionally, a wide selection of toxic chemicals and chemicals known to be non-toxic were tested.

Twelve drugs were selected to represent drugs causing idiosyncratic hepatotoxicity: acetaminophen, diclofenac, felbamate, hydralazine, leflunomide, methyldopa, minocycline, nitrofurantoin, rifampicin, sulindac, terbinafine and valproate (Kaplowitz 2005). Eighteen drugs were selected to represent drugs causing toxicity by virtue of their metabolism to reactive metabolites: acetaminophen, chloramphenicol, danazol, diclofenac, flutamide, ibuprofen, imipramine, indomethacin, isoniazid, hydralazine, nitrofurantoin, piroxicam, procainamide, sulphamethoxazole, tacrine, tamoxifen, terbinafine and valproate (Kalgutkar et al. 2005).

HCS analyser specifications and settings

Plates were analysed by epifluorescence microscopy using an automated, microplate-reading analyser (Kinetic-Scan® HCS Reader, KSR; Cellomics, Pittsburgh, PA). The system is equipped with an incubator to maintain constant temperature, CO_2 and humidity during analysis. The instrument enables fully automated monitoring of fluorescence intensity at four wavelengths as well as image analysis (Compartmental Analysis Bioapplication) and data viewing (Cellomics vHCS™: View)

The imaging system of the KSR (Carl Zeiss) was controlled entirely via a personal computer (Dell Precision 650 Workstation), with dual (Xenon) 2.0 GHz processors, with 4 Gb of random access memory (RAM) and 4 Gb of virtual memory allocation. Of the more than 600 cellular ratios calculated per field, only 10 were selected for this study. The KSR computer RAM and virtual memory were upgraded to 4,000 MB each to improve performance. Image and database files were spooled to and stored on a remotely located server, which required approximately 2 GB storage space per 96-well plate. To avoid possible conflicts with the Cellomics software, no other software was loaded onto the computer excepting for computer virus scanning.

Unless stated otherwise, fluorescence was monitored kinetically for the four dyes for 3 h with a single cell count made at the end of the assay. The 20× objective was used to collect images for all four fluorescence channels with an appropriate filter set (XF93). Dyes were excited and their fluorescence monitored at excitation and emission wavelengths of, respectively: (1) 365 ± 25 and 515 ± 10 nm for Hoechst 33342 on channel 1, (2) 549 ± 4 and 600 ± 12.5 nm for TMRM on channel 2, (3) 475 ± 20 and 515 ± 10 nm for fluo-4 on channel 3 and (4) 655 ± 15 and 730 ± 25 nm for TOTO-3 on channel 4. The channels for TMRM and fluo-4 were set to auto-exposure. With this feature, the exposure time is automatically determined based on the cellular fluorescence of negative control wells, and then set constant for the whole plate. The exposure for Hoechst was fixed to 100 ms for convenience. Exposure for TOTO-3 was set to a fixed exposure of 1 s. In the standard assay, each well is monitored at four fields-of-view per well each hour for 3 h.

The KSR's incubator was set to 37°C and maintained at 5% carbon dioxide. Microplate lids were left on the assay plate during kinetic monitoring to prevent evaporation. A study of evaporation occurring when lids were not placed on plates indicated losses of $21 \pm 4\%$ of well volume.

Cell counts were made after monitoring fluorescences for 3 h. Ten fields per well were imaged and analysed using the 10× objective. Fluorescence from an average of 41 cells using the 20× objective was measured for each of the four microscopic fields-of-view per well. Control wells had more cells, whereas wells with toxic doses of drugs had many fewer cells measured per field, depending on the cytotoxicity of the drug.

Methods

Conventional cytotoxicity assay methods

Seven independent, conventional cytotoxicity methods were evaluated for their sensitivity and specificity for detection of human hepatotoxicity. For all, positive test results were considered to be those that produce at least a 50% effect in the assay at a concentration of 30 μM. New DNA synthesis was assayed by liquid scintillation detection of pulse incorporation of ^{3}H-thymidine (Meselson and Stahl 1958). New protein synthesis was assayed by liquid scintillation of pulse-incorporation of ^{14}C-methionine (Colombo et al. 1965). Glutathione depletion was assayed by fluorometric detection of monobromobimane-conjugated, buthionine sulfoximine-inhibitable cytoplasmic thiols (Barhoumi et al. 1995). Superoxide secretion was assayed by spectrophotometric detection of cytochrome c reduction (Lorico et al. 1986). Caspase-3 activity was assayed by spectrophotometric detection of DEVD-pNA substrate

cleavage (Gurtu et al. 1997). Membrane integrity was assayed by fluorometric detection of ethidium homodimer-DNA conjugation in response to membrane damage (Levesque et al. 1995). Cell viability was assayed in HepG2 cells for 48 h by fluorometric detection of reduction of resazurin (Alamar Blue) to resorufin in response to mitochondrial activity (Nociari et al. 1998).

HCS, sublethal, cytotoxicity assay method For preliminary studies, the HCS assay was conducted immediately after addition of drugs to cells. However, in most cases, the HCS assay was conducted after a period of preincubation of the cells with drug. Usually, this was for 3 days, although the effects of incubation for 7 days were also determined. For studies in which there was no preincubation of drugs and cells, drug effects were tested only to 100 μM. However, for preincubation of drugs and cells, the drug effects were tested to at least 100 μM and to a minimum concentration of 30 times the maximum total concentration (C_{max}) of drugs reported circulating at the therapeutic dose. The effects of protein binding were not considered for determining the maximum concentration for testing. Protein binding of drugs would be minimal in the assay, because of the low content of plasma protein in the culture medium.

Drugs were initially dissolved as concentrated stock solutions in water or DMSO. When DMSO was used to dissolve drugs, it was added to the same final concentration of 0.5% (volume/volume) for all wells used for determining the drug response.

In a preliminary experiment with 16 toxic drugs the optimal time for treatment of cells prior to assay was determined. Cells were preincubated with the drug for 0, 3 or 7 days. Initial seeding densities of the cells were adjusted so that there would be sufficient cells for analysis but not overgrowth of cells: 5,000, 3,000 and 1,000 cells per well, respectively. For all other experiments, cells were treated for a period of 3 days before the assay. For determining drug effects without preincubation, the assay was started at drug addition and cells were analysed every 36 min for ten determinations; six determinations were used for preincubated cells. In this preliminary experiment, cells were analysed for only one microscopic field-of-view. The first time point is not included in the figures since the dilution effect of adding the drug to the cells obscured any drug effects; thus the first time point illustrated is 36 min after drug addition.

Preparation of plates for HCS, sublethal, cytotoxicity assay Depending on the duration of drug exposure, 1,000–5,000 cells in 100 ul media were added to each well of a 96-well plate ("cell plate") and incubated for 16–24 h, which ensured attachment of the cells to the bottom of the plate before drug treatment. Prior to drug addition, 50 ul of media were removed from each well. Drugs were added in 100-ul volumes to each well from a separate 96-well plate ("drug plate"). This was prepared with 1.5-fold the final drug concentration needed in the cell plate. Each row had a different drug whose concentration halved from serial dilution, from the highest concentration in column 12 to the lowest in column 2. Wells in column 1 had no drug added.

Incorporation of fluorescent probes for HCS, sublethal, cytotoxicity assay Cells were simultaneously loaded with 0.8 μM Hoechst 33342, 20 nM TMRM, 1 μM fluo-4 AM and 1 μM TOTO-3. Cells were loaded in media containing 10% FBS for 1 h at 37°C.

Data capture The following data were collected. Nuclear size was defined as the area of Hoechst 33342 fluorescence and measured as the mean object size for channel 1 in the nuclear region. Cellular mitochondrial membrane potential was defined as the TMRM fluorescence intensity in punctate cytosolic regions around the nucleus and was measured as the mean ring spot average intensity for channel 2. To assess intracellular free calcium concentration, Fluo-4 fluorescence intensity was measured in a large intracellular circular region centered at the nucleus as the mean circular average intensity for channel 3. To assess plasma membrane permeability, TOTO-3 fluorescence intensity in the nuclear region was measured as the mean circular average intensity for channel 4. Selected object count was used for cell counting.

Quality control For positive controls, three chemicals with known effects were added in triplicate to each plate to confirm quality of testing for the plate and to determine the maximum responses for TMRM, Fluo-4 and TOTO-3 dyes. The three chemicals used were: the mitochondrial uncoupler FCCP (100 μM), the calcium ionophore ionomycin (10 μM) and the membrane-perturbing detergent, Triton X-100 (0.05%). Column 1 of each row contained drug solvent but no drug, and was used as a negative control. Fluorescence values for up to 3 of the 11 wells with the same drug were considered outliers and excluded if there were more than two cv's (of the repeated measure of controls–see section on assessment of precision) different from those of both adjacent wells. Tests in which more than three of the wells had outlying values, or wherever there were equivocal results, were repeated.

Data reduction and statistical analysis The dose-response relationship for each parameter was quantified where possible using the IC_{50}, the concentration causing 50% inhibition. For most compounds, the standard dose response curve of drug dose was used to model the response. Data curves were generated using least-squares fitting routines with IC_{50} values determined using variable-slope, sigmoidal, curve-fitting routines, using a log scale for the drug dose on the horizontal axis and a

linear scale for the response on the vertical axis. This was the four-parameter logistic curve versus logarithm. However for 43% of compounds with a positive test response, there was hormesis (low dose enhancement). A modified version of the Brain and Cousens (1989) curve was used to model this response. The hormesis dose response equation used is presented as Eq. (1) below.

$$y = \frac{(C + A \times \text{Dose})}{\left(1 + \left(1 + \frac{2 \times A}{C} \times \exp(\text{IC}_{50})\right)\left(\frac{\text{Dose}}{\text{IC}_{50}}\right)^{B}\right)} + \text{Bottom}$$

Both curves were fitted using non-linear regression (which uses least squares) and the compound IC_{50}'s estimated where possible. The precision of the IC_{50}'s was also found where possible, and can be presented as the percentage coefficient of variation. The regressions were carried out using the statistical packages Genstat and Graphpad Prism.

Assessment of predictivity Sensitivity is the proportion of toxic drugs testing positive, TP/(TP + FN), where TP is the number of toxic compounds testing positive and FN is the number of toxic drugs testing negative. Specificity is the proportion of non-toxic drugs testing negative, TN/(TN + FP), where TN is the number of non-toxic drugs testing negative and FP is the number of non-toxic drugs testing positive.

Assessment of assay imprecision Imprecision was studied in order to distinguish between a drug effect and random variation or artefact. Imprecision could occur at four levels: the cell, the field of view, the well and the plate. Accordingly, the imprecision in parameter measurements was determined: (a) from cell to cell within field-of-view; (b) from field-of-view to field-of-view within well; (c) from well to well within plate and (d) from plate to plate. In order to compare the different sources of imprecision, each type was estimated based on a similar number of measures and this estimate was repeated a similar number of times. For this comparison of imprecision sources, each parameter measure was made seven times, except when limited by the number of measures made. This limitation occurred across field-of-view because only four fields-of-view were used per well. The imprecision was defined as the SD divided by the mean. Each imprecision estimate was made seven times and was expressed as the mean ± SD.

KSR parameter variance between control wells on the same plate and between control wells on different plates was evaluated after three days. Negative control wells were used for assessing nuclear area, cell count and TMRM. Positive control wells were used for assessing calcium after addition of 10 μM ionomycin and for assessing membrane permeability after addition of 0.25% Tween 20. Plates used were OP17, OP18, OP19, OP20, OP21, OP22. To determine variance between plates, the CV of the well means for each of the six plates was calculated. Outliers were defined as values more than three standard deviations away from the mean of the others, and were excluded, resulting in some plates not being used and a smaller n. For every control well, mean, SD and CV were calculated for each plate. The average CV was then used to determine variance between wells.

Definition of positive and negative test responses The result of the HCS sublethal cytotoxicity assay of a compound is reported in terms of the (1) degree of positivity, (2) the ratio (TI) of the lowest cytotoxic concentration to the total, maximal drug concentration (C_{max}) in serum that is associated with efficacy (where this information is available) and (3) the concentrations at which clear effects are first observed with each parameter.

Discrimination of a positive test result in the HCS assay was based on several criteria. Firstly, drug-induced effects had to be greater than the variance in the measure of the parameter across wells within plate. When a change was two and four times, respectively, the cv for determination of that parameter in controls, the test result was designated positive or strongly positive. For those compounds where biphasic changes (hormesis, see below) were identified in cell number, nuclear area and mitochondrial potential, the change had to be from the baseline level of the signal and not from the maximal point of increase in the signal. Drugs producing a strongly positive test were considered to be markedly cytotoxicity. Secondly, at least two parameters had to show this effect. Thirdly, there needed to be clear demonstration of a concentration-response relationship for the parameter. This included occurrence of the effect in at least one successively higher concentration, and absence of the effect in at least one lower concentration. Alternatively, if the effect occurred at the end of the concentration range, then its occurrence was supported by an effect in at least one other parameter within one concentration point on the dose-response curve. Biphasic effects were identified by a clear increase by two cv followed by a clear decrease. Finally, the magnitude of the effect had to be considered biologically relevant.

When the parameter change was between 1 and 2 cvs, test results were considered to be equivocal and weakly positive. A negative test result was designated to be a result where the above were absent, there was not a dose-response relationship, and all differences from baseline and control values were less than one CV, unless these could be unequivocally attributed to an experimental artefact affecting the well in question.

Results

Conventional cytotoxicity assays and their predictivity

Table 1 compares the predictivity of various conventional cytotoxicity assays (see Materials and methods)

applied to 611 drugs: 42 drugs causing severe hepatotoxicity, 283 drugs causing moderate hepatoxicity and 286 non-hepatotoxic drugs. Table 1 demonstrates that these assays have only half the sensitivity that regulatory animal tests have. Although the in vitro assays were still relatively insensitive at detecting hepatotoxicity, they were highly specific, so that when drugs tested positive in these assays there was high probability that the drug produced human toxicity.

Comparison on the performance of the conventional cytotoxicity assays with the new multiparametric assay (Table 2) on identical compounds indicated similar results as when the conventional assays were applied to the larger data set of 611 drugs.

Although none of the conventional assays for cytotoxicity had adequate concordance with in vivo human toxicity, there were notable differences between assays. The assay with greatest sensitivity, 19%, for detection of human toxicity potential was glutathione depletion, being twice as sensitive as the next most effective assays, namely DNA synthesis, as assessed by tritiated thymidine incorporation, and cell viability, as assessed by mitochondrial redox cycling activity. The caspase-3 induction test for apoptosis, protein synthesis, superoxide induction test for oxidative stress and the membrane integrity test were simply ineffective.

Table 1 Predictivity of drug-induced human hepatotoxicity by conventional, in vitro cytotoxicity assays and by regulatory animal testing in vivo

Sl. no.	Predictive test	Sensitivity	Specificity
1	DNA synthesis	10	92
2	Protein synthesis	4	97
3	Glutathione depletion	19	85
4	Superoxide induction	1	97
5	Caspase-3 induction	5	95
6	Membrane integrity	2	99
7	Cell viability	10	92
8	Combination of above tests 1, 3, 7	25	83
9	Regulatory animal toxicity studies	52	–

Sensitivity (percentage of hepatotoxic drugs detected) and specificity (percentage of drugs testing positive that are hepatoxic) are tabulated for seven conventional, cytotoxicity in vitro assays, for the optimal combination of these assays, and for regulatory, in vivo, animal toxicity studies. The in vitro tests were applied to 611 drugs, including: (a) 42 drugs causing severe human hepatotoxicity, defined as producing more than 1% frequency of increased serum ALT plus at least two of either jaundice, more than three reports of liver failure or a black box warning; (b) 283 drugs producing moderate human hepatotoxicity, defined as producing 0.1–1% frequency of increased serum ALT, plus jaundice or a label of occurrence of adverse effect and (c) 286 drugs producing negligible human hepatotoxicity, defined as less than 0.1% frequency of increased ALT and absence of clinical symptoms. Sensitivity for regulatory animal toxicity studies is based on retrospective examination of outcomes in animal studies for drugs found in clinical studies to produce human hepatotoxicity (Olson et al. 2000)

Cellular effects in the HCS, sublethal, cytotoxicity assay

When cells were seeded prior to incubation with drugs, 3,000 cells were added per well. This corresponds to about 500 cells in ten fields at the 10× objective used for the cell count. This number did not change significantly on the following day when incubation began, indicating that the stresses of plating had a cytostatic effect. In the four Channel Assay using a 20× objective this seeding density corresponds to 12 cells per field. This data can be used to assess whether a drug is merely halting cells in their cell cycle, or actually killing them (i.e. cytostatic versus cytotoxic). Cell counts at Day 0 (i.e. 24 h after plating) were slightly more than the theoretical amount that should be counted when they are first plated.

Typical cytotoxic changes are illustrated in Fig. 1. See HCS analyser specifications and settings for details on settings and procedures for image analysis. Furazolidone-induced decreases in cell number, nuclear area and mitochondrial membrane potential are readily visible, as are the increases in cell calcium and plasma membrane permeability.

Effects of duration on preincubation of cells with drugs on cytotoxicity

HCS assays for drug-induced cytotoxicity conducted on 23 toxic drugs and 6 non-toxic drugs at concentrations up to 100 μM were far more effective when cells were preincubated with drugs prior to testing. Assays in which cells had not been preincubated with the drug, produced positive test results for only 17% of the toxic drugs (Fig. 2, top). However, cytotoxicity was seen for 70% of these toxic drugs after preincubation at up to 100 μM for 3 days (Fig. 2, bottom). Toxic effects were induced after 3 days preincubation by amodiaquine, cerivastatin, diclofenac, fenfluramine, furazolidone, kanamycin, pamidronate, primaquine, pyrmethamine, rosiglitazone, tacrine and zidovudine. Cytotoxicity was not seen, even after 3 days of incubation with up to 100 μM, for acetaminophen, cisapride, erythromycin, isoniazide, lamivudine, sulphanilamide and vidaribine. However, in subsequent studies (Table 2) in which these six drugs were tested to higher concentrations of up to 30-fold the C_{max}, cytotoxicity was also detected for acetaminophen, erythromycin and lamivudine. There were no effects detected for any of the non-toxic drugs tested: flufenamate, flumazenil, glimepiride and zomepirac. Effects were not found for foscarnet nor primidone, although in later studies in which these compounds were tested to 30-fold C_{max}, their toxicity was revealed.

Preincubation of cells with toxic drugs for 3 days produced a fourfold greater frequency of change than when cells were not preincubated with drugs. Toxicity of the following drugs was not detectable without preincubation with cells for 3 days: amodiaquine, cerivastatin, diclofenac, fenfluramine, foscarnet, furazolidone,

Table 2 Cytotoxic effects of drugs causing human toxicity

Sl. no	Drug	Toxicity	Old tests	HCS test	First signal	Cell number	Mitochondrial potential	Ca	Mem perm	Nuclear area	C_{max} (μM)	TI	PPB (%)	Mechanism
Severely hepatotoxic drugs														
1	[i,r]Acetaminophen	X,FH,HI,Ht	–	Str +	#	500↑4,000↓	4,000	–	–	7,800	130	4	8	OS
2	Aminosalycilate	0.005,W,X J	–	+	#	24↑750↓	100	–	–	–	49	0.5	68	IM
3	Amitryptilline	<10,H,C,LD	–	+	#	13	50	25	50	50	0.3	43	95	
4	Amiodarone	0.25,BBW,H	+	Str +	#	6↑25↓	100	13	25	25	0.81	7	99	OP
5	Amodiaquine	Wd,FH,HI	+	Str +	C	25	50	13	25	25	0.42	31	95	IM
6	Cerivastatin	Wd,I,H	ND	Str +	#,M	0.1↑1.6↓	0.1↑0.8↓	0.2	0.4	1.6	0.03	3	99	M,Ca,Ap
7	Cyclosporin A	>3,C,Ht	+	Str +	M	25	2↑	13	13	13	0.2	10	93	OS
8	Danazol	50,W	–	Str +	#,N	13	50↑100↓	25	50	13	0.16	81		
9	Dantrolene	0.012,BBW,H,X,LD	–	Str +	#	25	100	50	50	50	7.9	3	85	IM
10	[i,r]Diclofenac	0.04,W,H,FH,J	–	Str +	#	16↑126↓	250	–	126	126↑	4.2	4	99	OP,Ap
11	Didanosine	65,BBW,H,FH,X	ND	+	#	2↑	–	–	–	–	12	0.2	5	M
12	Disulfiram	H,FH,X	+	Str +	#	13	25	25	25	25	5.4	2	50	OS
13	Efavirenz	8,Ht	ND	Str +	#	50	100	100	100	100	13	4	99.5	M
14	Etoposide	20,H,HI,	ND	Str +	#,N	0.5	505	16	4	0.5↑	17	0.03	96	Syn
15	[i,r]Felbamate	BBW,X,FH,H	–	Str +	M	315	160↑	–	–	315	42	4	24	IM
16	Fialuridine	Wd,X,Ht	ND	Str +	#	2	–	–	500	125↑	1	2		M
17	Flutamide	<1,w,H,C,J,X,FH	–	Str +	#,C,M,P	50	50	50	50	–	6	8	90	M
18	Indomethacin	W,FH,X,I	–	+	N	190	47	–	–	2↑	6	0.3	90	IM, OS
19	Imipramine	<22,H,LD,C	–	Str +	#	0.8↑50↓	100	50	50	50	0.6	1	100	IM, M
20	[r]Isoniazide	20,BBW,X,FH,H	–	+	M	–	50↓	–	–	–	40	1	0	IM, OS
21	Itraconazole	X	ND	+	M	2	0.2	–	2	3	0.4	0.5	99.8	IM
22	Ketoconazole	40,BW,X,H,C	+	Str +	N	100	25↑100↓	25	50	13↑50↓	7	2	99	M
23	Ketorolac	BW,FH,H,C	–	Str +	M,N,CP	430↑	220↑	220	220	220	7	0.25	99	IM
24	Labetalol	W,I,H,FH,X,C,HI	–	Str +	N	13↑ 100↓	50↑	50	100	6	0.4	15	50	
25	Lamivudine	4, BBW,X,	–	+	#	125	–	–	–	–	17	7	36	M
26	Mercaptopurine	40, W,	+	+	N	–	–	–	–	0.2	1	0.2	19	Syn
27	Methapyrilene	Ht	–	+	M	–	150	–	–	–	115	1.4		M,OP
28	Methotrexate	BW,Ht,I,Ci,F	ND	Str +	#,N	0.02	–	–	–	0.02	0.02	1		Syn
29	[i,r]Methyldopa	W,I,H,FH	ND	Str +	All	330	330	330	330	330	11	30	15	
30	[i]Minocycline	W,I,H,B	–	+	#	13	25	–	–	50↑	8	2	76	IM, M
31	Niacin	<5,W,FH,C,J	ND	+	#,N	3800↑	7500	–	–	3800↑	126	30		
32	Nimesulide	X,Ht	ND	+	#,N	340	680	1370	1370	340	15	23	99	M
33	[i]Nitrofurantoin	W,X,H	–	Str +	N	12	50	50	50	0.7↑	6	0.1	62	IM, OS, M
34	Novobiocin	BBW,I	–	Str +	#,P	100	400	400	100	400	1	100		IM
35	Phenylbutazone	Wd	–	+	#,M,C	6,600	6,600↑13,000↓	6,600	–	–	438	15	99	
36	[r]Piroxicam	2,W,H,X	–	+	#,N	5	160	–	–	5↑	5	1	99	
37	Propythiouracil	<30,W,H,FH,J	–	+	#	25	–	–	–	–	42	0.6	85	IM
38	Pyrazinamide	W,I,	ND	+	#	2400↑	4800↑	–	–	–	325	7	50	
49	Stavudine	BBW,H,FH	ND	Str +	#	250	–	500	1,000	–	4	63	5	M
40	[i]Sulindac	<1,W,H,X	–	Str +	M	285	140	–	–	570↑	19	8	94	IM, OS
41	[i,r]Valproate	44,BBW,B,X	–	Str +	#	1,000	4,000↑	–	4,000	8,000	540	2	93	OS, M
42	Zileuton	W,Ht	ND	+	#	100	–	–	–	–	17	6	94	OS
Sensitivity			20%	100%										
Moderately hepatotoxic drugs														
43	Aspirin	I,H,Ht	–	Str +	M,C,N	6,250	1,300	1,300	6,250	1,300	1,650	0.8	30	OS, M
44	Azathioprine	<1,B,Ht	–	+	#	0.8	–	–	25	–	0.34	2	30	OS
45	Bupropion	<1,W,C,H,LD	–	Hi TI –	#	300	1,200	600	600	600	0.5	600	84	

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Table 2 (Contd.)

Sl. no	Drug	Toxicity	Old tests	HCS test	First signal	Cell number	Mitochondrial potential	Ca	Mem perm	Nuclear area	C_{max} (μM)	TI	PPB (%)	Mechanism
46	Captopril	W,I,H,B	–	+	N	–	–	–	–	55↑110↓	4	14	30	
47	Ceftazidime	I,C	+	Str +	#	12	24	48	48	48	220	0.05	21	
48	Chloramphenicol	C,J,HI	–	Str +	#	260	–	–	–	–	57	5	53	OS
49	Chlorpromazine	0.75,	ND	Str +	#	3	25	13	13	6	0.5	6	98.5	IM, M
50	Ciprofibrate	I,H,HI	–	+	#	100	–	–	–	–	5	20		M
51	Clofibrate	I,Hm,C	–	+	M	14,000	7,100↑	–	–	–	470	15		M
52	Colchicine	HI,	ND	+	#,N	0.1	–	–	–	0.1	0.016	6	40	
53	Cyclophosphamide	J	–	+	#,N	2,300	–	–	–	2,300↑	143	16	13	
54	Diethylcarbamazine	X,H,HI	–	Weak +	#	100	–	–	–	–	1.3	77	0	
55	Doxorubicin	< 1,H,C	+	Str +	#,C,P	0.1	1↑ 2↓	0.1	0.1	0.4	0.2	0.5	76	OS, M
56	Enalapril	W,I,H,B,C	ND	+	N,M	100	1.6↑	–	–	1.6	0.4	4	55	M
57	[r]Erythromycin	W,I,H,c,J,LD	–	+	#	1.6	50↑	–	–	–	11	0.15	84	OS
58.	Ethylestrenol	Ht	–	+	#	6	–	100	–	100	3	2		C
69	[r]Estradiol	W,C,J	+	Hi TI –	#,N	25	50	–	–	25↑	0.0006	42,000	98	C
60	Famotidine	< 1,C,J	–	+	M	–	3	–	–	25	0.3	10	17	
61	Fenofibrate	0.063,W,H,C	–	Str +	#	250	–	1,000	1,000	1,000	25	10	99	M
62	Furazolidone	C,HI	–	Str +	#	6	25	25	50	100	4	1.5		M
63	[r]Furosemide	C,J	–	Str +	M	1,100	570	2,300	2,300	2,300	16	35	99	M
64	Fusidic Acid	B,Ht,C	–	+	M	–	516	–	–	–	17	30	95	
65	Glyburide	I,H,C	–	+	M	50	2↑	–	100	50↑	0.2	10	99.8	
66	[i,r]Hydralazine	0.005,HHI,C,	ND	Str +	#,N	67	135↑	270	–	75	5	13.5	87	IM
67	Ibuprofen	< 1,H,J	–	+	#	50	–	–	–	–	250	0.2	99	IM, M
68	Ifosfamide	0.03,B,C	–	+	#	1,500	–	–	–	–	203	8	0	
69	Isotretinoin	0.15,W,H	ND	+	#,M	100	100				8	13		
70	Ketoprofen	H,C,LD	–	Str +	M	47	3	–	–	–	15	0.2	98.7	
71	[i,r]Leflunomide	0.05,W,B	+	+	#	25	–	–	–	–	340	0.07	99.3	IM
72	Lovastatin	0.019,W,H,C	ND	+	N	1.6↑25↓	3↑100↓	13	6	0.4	0.01	40	95	M, Ap
73	Mesalamine	I,H,B,FH,C,HI	–	+	#	6	10	–	–	25↑	12	0.5	50	IM
74	Methacycline	H,C	–	+	#	13	50	–	–	–	5	3	85	
75	Naproxen	< 1,FH,HI,C	ND	Hi TI –	M,N	100	50↑	–	–	50	0.2	250	99	IM
76	Nizatidine	I,H,HI,C	–	+	N	–	–	–	–	26	4	7	35	
77	Norfloxacin	1.6, H	–	Str +	N	28	7	–	–	0.5↑	8	0.8	18	IM
78	Paclitaxel	0.19,B,HI	ND	Str +	#	0.1	–	50	50	25↑	4.3	0.02	94	
79	Paroxetine	I,H,FH,HI	+	Str +	#	0.8↑25↓	13	6.3	13	13	0.2	4	95	M
80	Pravastatin	0.045,W,H,B,HI	ND	Hi TI–	#	100	–	–	–	–	0.1	1,000	50	Ap
81	[r]Procainamide	I,H,C	ND	Str +	N	630	630↑	630	–	320	12	27	18	IM
82	Pyrimethamine	C,J,HI	ND	Str +	#	3	–	10	10	10↑100↓	3.3	1		Syn
83	Quinacrine	H	+	Str +	C	2	3	0.4	3	3	1	0.4	90	
84	Quinidine	H,HI,Ht	ND	Str +	C	8	30	0.5	1	15	9	0.06	90	M
85	Quinine	H	+	Str +	#,P,C,N	36	70	36	36	36	19	2	93	
86	[i]Rifampicin	W,I,H	+	+	N	50	–	100	100	13↑	9	1	81	
87	Rosuvastatin	< 0.5,	+	+	N	0.4↑	0.4↑1.6↓3↑	50	–	0.2↑0.4↓1.6↑6↓	0.01	20		M, Ca, Ap
88	Simvastatin	0.05,W,H	ND	+	#,M,N	0.2↑50↓	0.2↑0.9↓3↑50↓	6	6	0.2↓0.9↑3↓	0.02	10	94	M,IM
99	[r]Sulfamethizole	W,H	–	+	M	–	1000	–	–	–	222	5	90	
90	[r]Sulfamethoxazole	W,H,B,C,J,HI	–	+	#	1,100↑	–	–	–	–	217	5	62	IM, M
91	[r]Sulfaphenazole		ND	+	N	100	100	–	–	50	78	0.6		
92	[r]Tacrine	0.29,W,H,B,Ht	–	Str +	N	25	50↑100↓	50	25	13↑50↓	0.1	130	55	OS, M
93	[r]Tamoxifen	W,I,H,B,C,HI	+	Str +	N	100↑	25	–	25	0.4↑25↓	0.4	1	98	M

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Table 2 (Contd.)

Sl. no	Drug	Toxicity	Old tests	HCS test	First signal	Cell number	Mitochondrial potential	Ca	Mem perm	Nuclear area	C_{max} (μM)	TI	PPB (%)	Mechanism
94	[i,r]Terbinafine	C,J,Ht,LD	ND	Str +	#	3	6	6	6	13	4	0.8	99	
95	Terfenadine	I,H,C,J		Hi TI –	C	6	6	3	6	13	0.01	300		CA
96	Tetracycline	I,Ht,C	–	+	M,C,P	–	560	560	560	–	9	62	65	IM
97	Tolazamide	C	–	+	N	50	–	–	–	2	90	0.02	94	
98	Tolbutamide	X,C,J	–	+	#	30	–	–	–	–	590	0.05	96	
99	Trifluoperazine	H,C	+	Hi TI –	C	13	25	6	13	13	0.003	2,000	99	
100	Warfarin	I,H,C,J	–	+	#,M,N	100	100	–	–	100	7	14	99	IM
101	[i]Zarfirlukast	W,Ht	ND	+	M,N	100	13	–	100	13↑	1	13	99	
102	Zidovudine	BW,H,HI	–	Str +	#	63	–	500	–	–	4	16	25	M
Sensitivity			24%	88%										
Non-toxic drugs														
103	Acetylcysteine	HI	–	–	–	–	–	–	–	–	1,900	> 1	80	
104	Aminobenzoate		–	–	–	–	–	–	–	–	50	> 30		
105	Bambuterol		–	–	–	–	–	–	–	–	0.015	> 6,700	45	
106	Betaine		–	–	–	–	–	–	–	–	940	> 30		
107	Biotin		–	–	–	–	–	–	–	–	< 1	> 100		
108	Bisacodyl		–	–	M	–	100	–	–		0.15	667		
109	Buspirone	1	ND	–	#,N	100	–	–	–	100	0.01	1,000	95	
110	Carbidopa		+	–	#	520	–	–	–	–	2	260	36	
111	Citicoline		–	–	–	–	–	–	–	–	700	> 30		
112	Cromolyn	I (poss Alerg)	–	–	–	–	–	–	–	–	0.016	> 6,600	68	
113	Cyanocobalamin		–	–	#,M	0.4	0.4	–	–	0.8	0.001	400		
114	Dexamethasone		–	–	M,N	100	50	–	–	50↑	0.23	217	77	
115	Dimethylsulfoxide		ND	–	#	35,000	70,000	–	70,000	70,000	< 1,000	> 30		
116	Diphenhydramine		–	–	#	1,300	2,500	2,500	2,500	2,500	0.3	4,200	98.8	
117	Flumazenil		+	–	P	–	–	–	50	–	0.1	500	45	
118	Eserine		ND	–	M	–	6↑	–	–	–	0.22	> 450		M
119	Fexofenadine		ND	–		–	–	–	–	–	0.57	> 175		
120	Folate		ND	–	–	–	–	–	–	–	3.4	> 30		
121	Folinic acid		ND	–	–	–	–	–	–	–	3	> 30		
122	Glimepiride	H (1 case),C	–	–	#,P	100	–	–	100	–	0.73	> 137x2	99	
123	Isoproterenol		ND	–	#	6↑100↓	–	–	–	–	0.006	1,000	68	
124	Isosorbide dinitrate		–	–	–	–	–	–	–	–	0.0008	130,000	28	
125	Ketotifen		–	–	#,P,C	50	100↑	50	50	100	0.0001	500,000		
126	Moxisylyte		ND	–	M	–	100↑	–	–	–	1.65	> 61		
127	Myo-inositol		ND	–		–	–	–	–	–	22	> 30		
128	Oxyphenonium		ND	–	M,N	–	100	–	–	100	0.3	300		
129	Pargyline		ND	–	#	100	–	–	–	–	0.3	333		
130	Picotamide		ND	+	M	13	2	100	100	6↑	50	0.04		
131	Pinacidil		ND	–	#,M,N	100	100	–	–	100↑	0.17	588	40	
132	Pioglitazone		–	–	–	–	–	–	–	–	2.6	> 38	99	M
133	Praziquantel		–	–	–	–	–	–	–	–	1.8	> 56	80	
134	Propranolol	HI	–	–	C,P,N	50	–	25	25	25	0.1	250	87	
135	Pyridoxine		ND	–	–	–	–	–	–	–	1.1	> 91		
136	Rosiglitazone		ND	–	#	50	100	–	–	80	0.67	> 75x10	100	M
137	Thiamine		ND	–	–	–	–	–	–	–	6.8	> 30		

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Table 2 (Contd.)

Sl. no	Drug	Toxicity	Old tests	HCS test	First signal	Cell number	Mitochondrial potential	Ca	Mem perm	Nuclear area	C_{max} (μM)	TI	PPB (%)	Mechanism
Specificity			88%	97%										
Drugs toxic to other organs														
138	Astemizole	CA	+	Hi TI –	All	6	6	6	6	6	0.01	600	97	IM
139	Bezafibrate	I	–	+	#	50	–	–	–	–	17	3	95	M
140	Bupivacaine	Myo	ND	+	#,C	6	–	6	–	–	0.7	9	96	OS
141	Caffeine		–	+	#	1,250	–	–	2,500	2,500↑	42	30	36	
142	Capreomycin	I,R,O	ND	Str +	#	150↑	300	–	–	–	40	4		
143	Chloroquine	I,HI	+	Str +	#,C	6	26↑50↓	6	13	13	0.48	13	61	
144	Chlorpheniramine		ND	+	N	50	50↑100↓	100	–	0.4↑6↓	0.2	2	72	
145	Ciglitizone		ND	+	M	–	3	–	–	6↑	5	0.6		M
146	Cisapride	I,H, CA	–	Hi TI -	–	–	–	–	–	–	0.12	>810	98	CA
147	Clioquinol		–	+	#,N	64	2,000	500	2,000	64	69	1		
148	Dipyrone		–	+	#,P	1,000	–	–	1,000	–	34.5	29	60	IM, Ag
149	Fenfluramine		–	+	#	6	25	50	50	50	0.1	60	30	Card
150	Flufenamate		–	Str +	M,N	–	100	–	–	100↑	46	2	90	
151	Flucytosine	I,HI,LD	ND	Str +	#,P	6	–	–	6	–	780	0.008		RM, Syn
152	Fluvastatin	0.011,W,Myo	ND	Str +	C	1.6	3↑6↓13↑50↓	0.8	1.6	13	0.45	2	99	M, Ap
153	Foscarnet	5,R	–	+	C,N	4,400	8,700↑17000↓	2,200	8,700	2,200	580	4	20	R
154	Gentamycin	R,O	ND	+	N	185	–	185	185	95↑	13	7	28	R
155	Halothane		ND	+	N	13	3	–	–	2↑	10	1		RM, OS
156	Indoprofen		ND	+	#	2,100	–	–	–	–	72	30	99	
157	Isoxicam	H,D,GI	ND	False–	–	–	–	–	–	–	20		97.7	IM
158	Kanamycin	HI,R,O	–	+	#,M	11	11	–	–	25↑	47	0.2	0	Trans
159	Lidocaine	CNS	–	+	M	1,300	300	2,500	2,500	2,500	36	8	70	IM
160	Memantine	N	–	Str +	#	25	50	100	100	–	0.2	125		
161	Metformin	Pan	ND	+	M	1,700	220	–	–	440	116	2		
162	Metoclopramide	I,Ht	–	+	N	–	50	–	–	13	0.4	32	65	
163	Menadione		+	Str +	#	6	13	13	13	25	5	1		OS
164	Mevastatin		ND	+	N	3	0.2↑0.4↓3↑	0.4	13	0.1↑0.2↓				Ap
165	Mibefradil	Wd	ND	Str +	#	0.4↑6↓	6	6	50	6	1.1	0.4		Ca
166	Nialamide	CNS	ND	Str +	N	–	–	–	–	100	14	7		
167	Nicotine		ND	+	M	27	0.4	–	–	100	0.09	5	5	
168	Nocodazole		ND	+	#	0.1	–	25	25	0.4	0.5	0.2		
169	Nomifensine		ND	+	#	100	–	–	–	–	1.2	83		
170	Pamidronate	R	–	Str +	#	3↑100↓	100	100	100	–	11	0.3	30	Trans
171	Phenacetin		ND	+	#	25	100	–	–	100↑	12	2	33	R
172	Phenformin		ND	+	#,M	13	13	–	–	25↑	0.63	21	20	LA
173	Pimozide	I	+	Str +	#,C,P	6	13	6	6	13	0.11	55	99	
174	Primaquine		–	+	#,	3	50	25	25	50	0.11	27		
175	Primidone		–	+	#	350	690	–	–	–	23	15	20	
176	Propythiouracil		–	Str+	M	630	313	–	–	1,300	42	7		
177	Sodium chloride, mM		ND	Str +	N	13	100	50	50	3↑	145	NA	0.1	
178	Streptomycin	R,O	–	+	M	1,600	780	–	–	–	52	15	50	
179	Streptozocin		ND	?	N	–	–	–	–	100↑				
180	Sulfabenzamide		–	?	#	25	100	–	–					
181	Sulfanilamide		–	?	M,P	–	100	–	100	–	–		70	IM
182	Telenzipine		–	+	M	–	1	–	–	3↑	0.02	50		
183	Temozolomide	BM	–	+	#	50	–	–	–	–	6	8		Syn

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Table 2 (Contd.)

Sl. no	Drug	Toxicity	Old tests	HCS test	First signal	Cell number	Mitochondrial potential	Ca	Mem perm	Nuclear area	C_{max} (μM)	TI	PPB (%)	Mechanism
184	Theophylline	I,H	–	+	N	–	–	–	–	25	85	0.3	56	
185	Topiramate	X,CNS	ND	+	#,M	300	300↑				20	15	13	
186	Vancomycin		ND	+	#,M	285	285↑				19	15		
187	Vidarabine	I,B	–	+	N	100	50	–	–	6	17	0.35	25	M
188	Vincamine	HI,CA	ND	Hi TI –	M	–	100↑	–	–	–	0.44	230		Hem
189	Zalcitabine		ND	+	#	63	–	–	500	–	0.37	170	4	M
190	Zomepirac		–	False–	-	–	–	–	–	–	5.6		92	R, IM
191	Zonisamide		ND	+	N	–	–	–	–	6	24	0.25	50	
Sensitivity			14%	92%										
Positive controls: chemicals causing toxicity														
192	Acetamidofluorene		ND	+	#,M	100	100	–	–	–	NA	NA		
193	Aflatoxin B1		ND	Str +	#	0.1	0.2↑	3.2	3.2	0.2↑	NA	NA		OS
194	Alloxan		ND	+	M		100				NA	NA		OS
195	Ally alcohol		ND	+	N	–	3	–	–	0.4↑	NA	NA		
196	Benzopyrene		ND	Str +	#	0.1	1.6↑	1.6	0.8	1.6	NA	NA		
197	Betaine HCl (acidic)		ND	Str +	#	7,100	28,000	–	–	–	NA	NA		
198	Bromobenzene		ND	+	#	100	–	–	–	–	NA	NA		OS
199	p-Bromophenol		ND	+	#	630	2,500	1,300	1,300	1,300	NA	NA		
200	Buthionine		ND	+	M	50	0.2	–	50	–	NA	NA		OS
201	Butylhydroxytoluene		ND	+	#	50	–	–	–	–	NA	NA		OS
202	Carbon tetrachloride		ND	+	N	–	50↑	–	–	0.8	NA	NA		OS
203	Chloroform		ND	+	#	100	–	–	–	–	NA	NA		OS
204	m-Cresol		ND	+	M	–	0.8	–	–	3	NA	NA		
205	p-Cresol		ND	+	M,N	50	0.2	–	–	0.2↑	NA	NA		
206	Cytochalasin B		ND	Str +	N	2	0.8	3	50	0.1	NA	NA		
207	Cytochalasin D		ND	Str +	#	0.4	100	100	50	6	NA	NA		
208	Diaminotriazole		ND	Str +	N	3,100	–	–	–	1,560	NA	NA		
209	Dichloroethylene		ND	Str +	M,N	–	25↑	–	–	25	NA	NA		
210	rDiethylmaleate		ND	+	N	50	25	–	–	13	NA	NA		OS
211	rDimethylformamide		ND	+	#,N	3	13	6	25	3	NA	NA		
212	Diquat		ND	Str +	#	13	50	50	100	100	NA	NA		OS
213	Ethionine		ND	+	N	–	–	–	–	3	NA	NA		M
214	rEugenol		ND	+	M	–	13↑	–	–	–	NA	NA		OS
215	FCCP		ND	Str +	N	25	50	50	50	13↑	NA	NA		M,OS
216	Formaldehyde		ND	+	M, N	–	25↑	–	–	25	NA	NA		
217	Galactosamine		ND	Str +	#	780	12,500	13,000	1,600	13,000	NA	NA		Syn
218	Glucosamine		ND	Str +	N	750	–	–	–	5↑	NA	NA		
219	Hydrochloric acid		ND	Str +	#	10↑	–	–	–	–	NA	NA		
220	Ionomycin		ND	Str +	M	0.16	0.04	0.08	0.08	0.16	NA	NA		Ca
221	Isopropylphenol		ND	+	#,M	100	100	–	–	–	NA	NA		
222	Malonic acid		ND	+	#	2,500	–	–	–	–	NA	NA		M
223	Methoxyphenol		ND	+	N	50	–	–	–	13	NA	NA		
224	Monensin		ND	Str +	#	0.1	–	0.8	1.6	0.8	NA	NA		M
225	Napthylisothiocyanate		ND	+	#,M,C,P	100	100	100	100		NA	NA		
226	Nitrosodimethylamine		ND	+	M,N	100	6	–	–	6↑	NA	NA		
227	Nitroproprionic acid		ND	+	#	600	–	–	2,500	–	NA	NA		
228	Pentachlorophenol		ND	+	M,N	25	6	–	100	6	NA	NA		OS
229	Pyrazinamide		ND	Str +	#,M	19,000	19,000	–	–	–	NA	NA		

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Table 2 (Contd.)

Sl. no	Drug	Toxicity	Old tests	HCS test	First signal	Cell number	Mitochondrial potential	Ca	Mem perm	Nuclear area	C_{max} (μM)	TI	PPB (%)	Mechanism
230	Rotenone		ND	Str +	#,P	0.1	0.2	6	0.1	0.4	50	0.002		M,OS
231	Ryanodine		ND	False–	-	–	–	–	–	–	NA	NA		Ca
232	Sodium hydroxide		ND	+	#	5,000	–	–	–	–	NA	NA		
233	Taurocholic		ND	+	N	–	–	–	–	25	NA	NA		M
234	Taurodeoycholic		ND	+	N	1,250	2,500	–	–	310	NA	NA		M
235	Taurolithocholic		ND	+	N	310	–	–	–	156	NA	NA		M
236	Thioacetamide		ND	+	M	100	6	–	–	–	NA	NA		
237	Trichloroethylene		ND	+	M	100	6	–	–	–	NA	NA		
Sensitivity			ND	98%										
Negative controls: chemicals non-toxic to humans (at concentration up to 100 μM)														
238	3-Acetamidophenol		ND	–	–	–	–	–	–	–	NA	NA		
239	Ascorbate		–	–	–	–	–	–	–	–	NA	NA		
240	Culture media		ND	–	–	–	–	–	–	–	NA	NA		
241	Ethanol		ND	–	–	–	–	–	–	–	NA	NA		
242	Lactose		ND	–	–	–	–	–	–	–	NA	NA		
243	Sorbitol		–	–	–	–	–	–	–	–	NA	NA		
Overall sensitivity (drugs)			21%	93%										
Overall specificity (drugs)			90%	98%										

About 243 drugs and compounds are tabulated (column 1) in different categories of human toxicity and compared with respect to how they tested in conventional cytotoxicity assays (column 3), the toxicity they produce (column 2), and how they tested in the HCS, sublethal, cytotoxicity assay (columns 4–10). Drugs and compounds are categorised according to their human hepatotoxicity as: (1) severely and (2) moderately hepatotoxic drugs, (3) non-toxic drugs, (4) chemicals toxic to other organs, (5) toxic chemicals, and (6) non-toxic chemicals. Results for the HCS cytototoxicity assay are tabulated for test outcome and degree of positivity in column 4, parameter affected at the lowest concentration in column 5, and in columns 6–10 for the concentrations causing effects on cell number, mitochondrial membrane potential, intracellular ionised calcium concentration, membrane permeability and nuclear area, respectively. The maximal plasma total concentration of drugs associated with efficacy (C_{max}) is indicated in column 11. The ratio of lowest cytotoxic concentration to C_{max} is indicated in column 12 (TI). The percentage of drug that is bound to plasma proteins is also indicated (column 13), and where known the cellular mechanism of cytotoxicity (column 14). The sensitivities of the conventional assays and the HCS assay are indicated in the row at the bottom of the list of drugs in each category

ND not determined, *NA* not applicable, *?* not tested to 30× C_{max} and therefore diagnosis uncertain. Mechanisms of toxicity are designated as follows: *Ap* apoptosis; *Ca* calcium dyshomeostasis, *Card* cardiotoxicity, *Hem* hematologic, *IM* immune-mediated, *LA* lactic acidosis, *M* mitochondrial, *OP* oxidative phosphorylation, *OS* oxidative stress, *Syn* DNA-synthesis, *Trans* membrane transporter inhibition, *Superscript i* idiosyncratic hepatotoxicity, *Superscript r* reactive metabolite, ↑ signal increased. Toxicities (column 2 and 14): the number in this column refers to the % incidence of patients with increased liver function tests, *Ag* agranulocytosis, *B* hyperbilirubinemia, *H* hepatitis, *BBW* black box warning, *BM* myelotoxic, *BW* boxed warning, *CA* cardiac arrhythmia; *C* cholestasis, *Ci* cirrhosis, *D* dermatotoxic, *F* fibrosis, *FH* fulminating hepatitis, *GI* gastrointestinal toxicity, *HI* Hepatocellular injury (with necrosis), *Hm* hepatomegaly, *Ht* hepatotoxicity, *I* incidence of elevated liver function tests (but % not given), *J* jaundice, *LD* liver dysfunction, *Myo* myotoxic, *N* neurotoxic, *O* ototoxic, *Pan* pancreatic, *R* renotoxic, *St* steatosis, *W* regulatory warning, *Wd* withdrawn, *X* deaths

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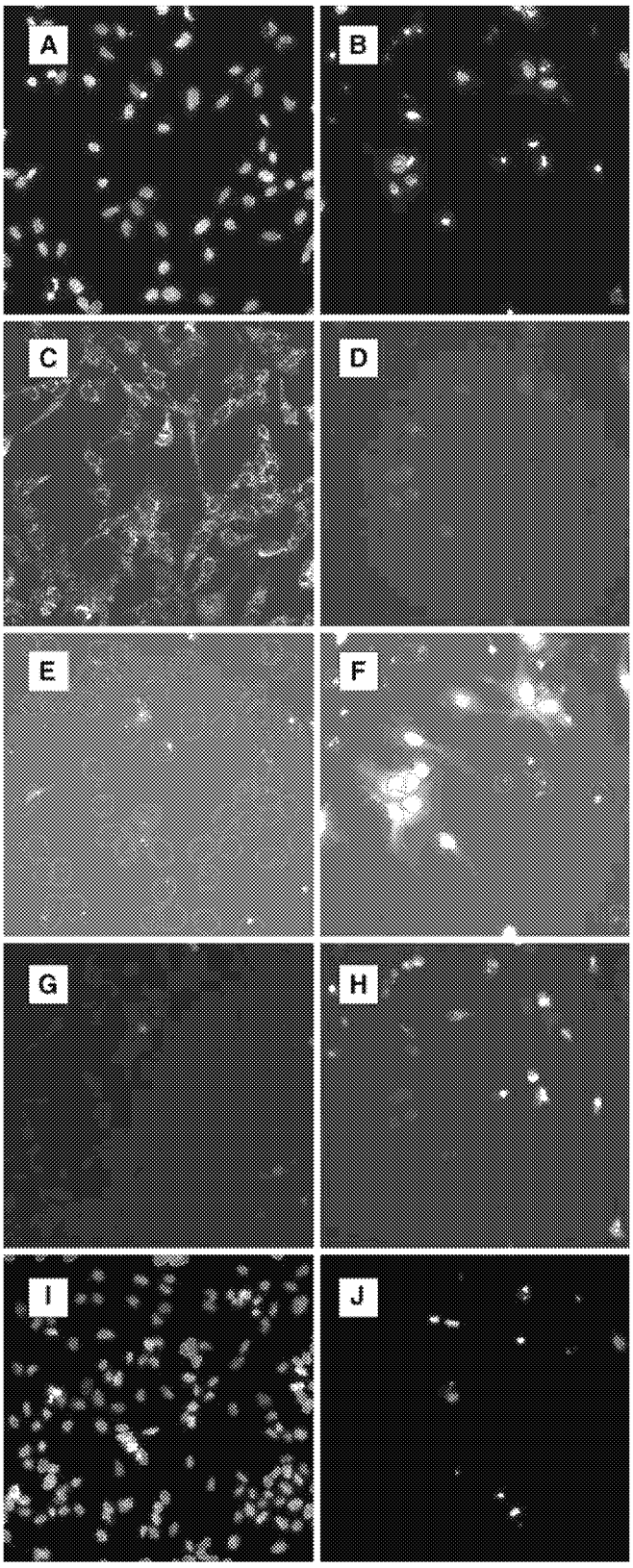

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Fig. 1 Photomicrographs of furazolidione-induced changes in prelethal cytotoxicity parameters. Effects of preincubation of HepG2 cells with 100 μM furazolidone, compared to controls, respectively, on nuclear area (**a**, **b**), mitochondrial membrane potential (**c**, **d**), calcium (**e**, **f**), membrane permeability (**g**, **h**) and cell number (**i**, **j**). Furazolidone produced a marked reduction in cell number, nuclear area, and mitochondrial membrane potential, and marked increases in intracellular calcium and membrane permeability. See HCS analyser specifications and settings for details of instrument settings and procedures. Circular outlines indicate the areas within cells in which fluorescence intensity is measured. Cells are viewed with the 20× objective for fluorescence intensity quantitation and the 10× objective for cell counting

pamidronate, primaquine, pyrimethamine, rosiglitazone, sulphanilamide and tacrine.

Preincubation of cells for 7 days resulted in preparations from which little data could be obtained. Cell counts obtained were highly variable and substantially reduced. This was attributed to the low seeding density, combined with lengthy preincubation time, resulting in the clonal expansion of small numbers of cells into irregular clumped colonies for which individual cells could not be distinguished.

Based on the low frequency of detection of toxicity without drug preincubation and on the clumping of cells grown for 7 days, a three-day exposure protocol was selected for standardisation of the assay protocol for the collection of data reported in Tables 2 and 3 and Fig. 2 (bottom), 3 and 4. In Table 2, cytotoxic effects of drugs in conventional and the new multiparametric assay are tabulated.

Time-course of cytotoxic change

Above a threshold drug concentration, cytotoxic effects progressively increased with time of incubation during the 3.4 h period that the cells were monitored for. These effects occurred more rapidly, and followed a dose-response pattern. The threshold concentration producing effect, the magnitude of the effect, the sequence in which parameters were affected and whether they were increased or decreased, varied across drugs.

With the exception of TMRM, cell fluorescence intensities were constant over time for the negative controls and for cells exposed to drugs not producing acute toxicity, or exposed at non-toxic concentrations of toxic drugs. Fluorescence of TMRM decreased gradually over time, possibly due to its extrusion by the p-glycoprotein transporter, and/or to photobleaching. Toxic effects were considered to occur only when the rate of change of fluorescence was unmistakeably greater than for the negative controls.

In Fig. 3, the time course of change in fluorescence parameters is indicated at the critical concentration where each drug produced toxic effects. Effects are demonstrated for a representative single cell and for a population of cells for each drug at the concentration producing toxicity. The patterns of change are quite similar for single cells and populations of cells, although the former is more precise in determining the sequence of events. For example, for amiodarone and dantrolene, individual cell data confirmed that calcium was the last

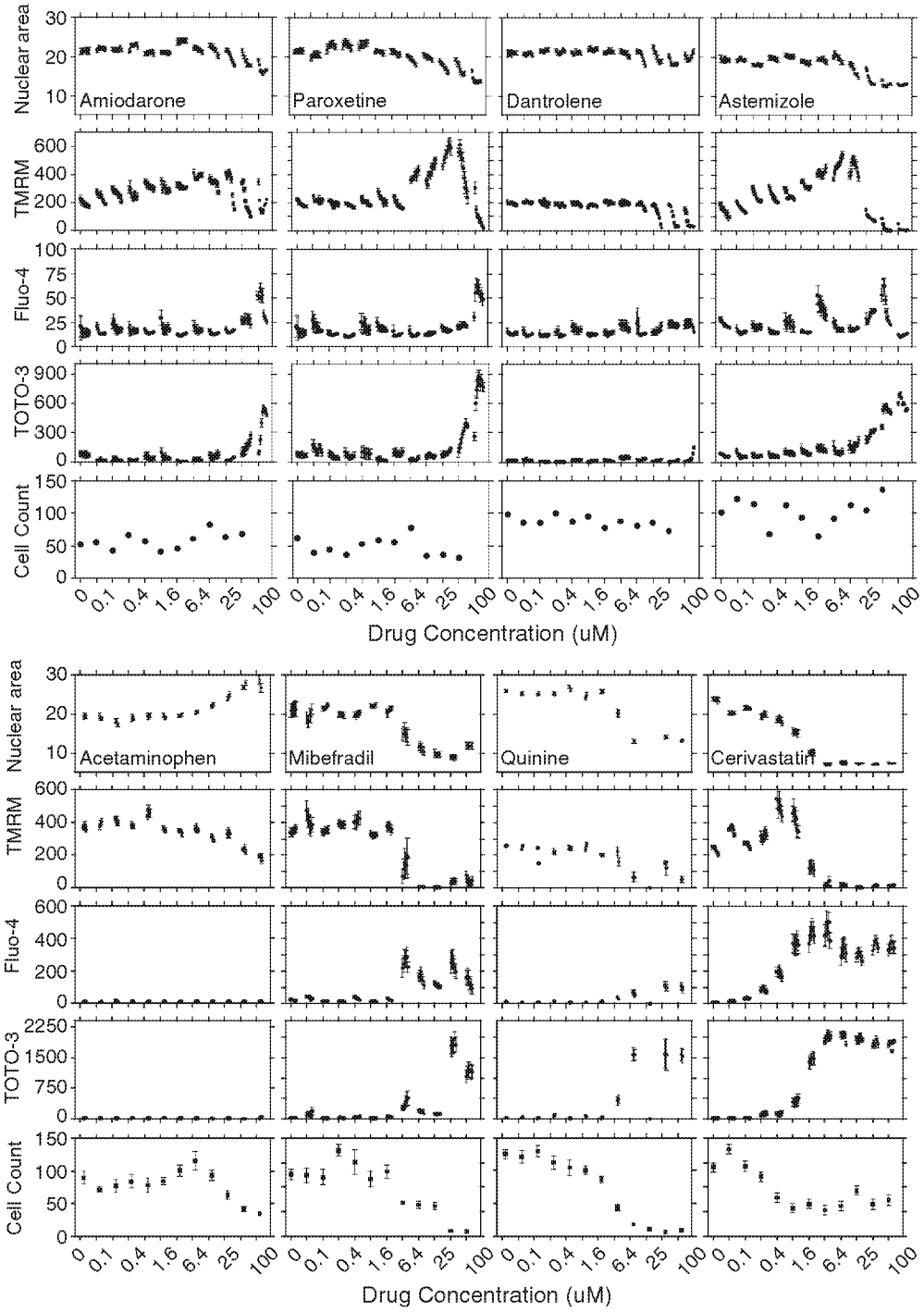

Fig. 2 Cytotoxic effects of specific drugs before and after 3-day preincubation of cells with drugs. Combined concentration-response and time-course of changes are shown in cell proliferation, mitochondrial function, intracellular calcium homeostasis, cell membrane permeability and nuclear area for drugs producing immediate toxicity without preincubation (top half; amiodarone, paroxetine, dantrolene, astemizole) and for drugs producing toxicity after 3-day preincubation of drugs and cells (bottom half; acetaminophen, mibefradil, quinine, cerivastatin). At each concentration indicated, nine measurements on approximately 40 cells in a single field-of-view were made with 36 min intervals for nuclear area (Hoechst 33342; top), mitochondrial membrane potential (TMRM; second from top), intracellular ionised Ca (Fluo-4; third from top), plasma membrane permeability (TOTO-3; second from bottom) and cell number (bottom). The wells without drug addition served as negative controls. The effects of FCCP for TMRM, Tween-20 for TOTO-3 and nuclear area and ionomycin for Fluo-4 served as positive controls. Toxic effects occur at the concentration producing a downward time-curve for TMRM (greater than that occurring for the negative control) or nuclear area or an upward time-curve for Fluo-4 or TOTO-3. Data is expressed as mean ± SEM

parameter to change, and that mitochondrial membrane potential change substantially preceded membrane permeability change.

As TMRM is known to be a substrate for the multidrug resistance (MDR) p-glycoprotein that extrudes cations, the drug concentration causing fluorescence increase was compared to the concentration at which the drug inhibits MDR. Maximal TMRM fluorescence increases were 100, 200, 0 and 150% by, respectively, amiodarone, paroxetine, dantrolene and astemizole. Although all four of these drugs inhibit MDR, there was not a correlation between the degree of TMRM increase and potency of inhibition, 34, 16, 22 and 89%, respectively.

Antiproliferative effect could only be measured in assays in which cells were exposed to drug for multiple days. For the four drugs that produced effects with and without preincubation for multiple days (acetaminophen, mibefradil, quinine and cerivastatin), the most conspicuous difference in patterns of parameter change

Table 3 Sources of imprecision in HCS, sublethal, cytotoxicity assay

Imprecision due to well and plate variation

KSR assay parameter	Variation between wells within plate		Variation between plates	
	(% CV)	*N*	(% CV)	*N*
Mitochondrial membrane potential	9.36 ± 1.33	7	14.60	5
Nuclear area	5.16 ± 1.80	7	6.99	6
Calcium	7.25 ± 4.15	3	18.35	4
Membrane permeability	9.30 ± 7.02	3	16.64	6
Cell count	16.45 ± 4.30	7	16.10	5

Imprecision due to field-of-view and cell variations (data for representative plate OP18)

KSR assay parameter	Variation between fields		Variation between cells		Variation between wells	
	(% CV) ± SD	*N*	(% CV) ± SD	*N*	(% CV)	*N*
Mitochondrial membrane potential	8.50 ± 4.04	4	30.23 ± 10.44	7	9.83	7
Nuclear area	2.84 ± 1.24	4	17.13 ± 8.14	7	6.03	7
Calcium	10.28 ± 0.02	4	41.57 ± 20.17	7	5.67	3
Membrane permeability	9.02 ± 3.96	4	35.74 ± 9.47	7	10.65	3
Cell count	29.81 ± 9.25	10	N/A	N/A	21.47	7

The cell-to-cell, field-to-field, well-to-well and plate-to-plate imprecisions are compared for the parameters measured in the HCS assay. Imprecision due to well and plate variation. For well-to-well variance, a mean, SD, and CV were calculated for every control well for each assay parameter. An average CV was then used to show the well-to-well variance for that parameter. Plate-to-plate variance was then estimated using the well-to-well data for each plate. The well-to-well mean for each assay parameter for all six plates was averaged and a CV calculated. This CV value then shows the plate-to-plate variance for each parameter. Imprecision due to field-of-view and cell variations (data for representative plate OP18): To estimate the field-to-field variance, the average value of each parameter for each individual field was calculated for all seven control wells on the plate. This gave a CV for each well, which when averaged gave the field-to-field variance for that plate. For nuclear area, TMRM, calcium and membrane permeability for four fields were used (as this was the maximum number of fields used for the assay), whilst ten fields were used for the cell count analysis. To estimate the cell-to-cell variance the KSR parameter value for individual cells was recorded. Seven groups of seven cells were randomly chosen (using a random number generator) within the same field of view and within the same well. The mean, SD and CV were then calculated for each group of seven cells and an average CV value recorded

was the effect of cell number (Fig. 3). This was the least affected of the measured parameters with acute toxicity. With chronic toxicity, it was the parameter affected at the lowest toxic concentration of dantrolene and astemizole and at intermediate toxic concentrations for amiodarone.

Sequence and direction of drug-induced change (Fig. 3)

The sequence of change in parameters was similar with the four different drugs that caused toxicity without the need for preincubation (Fig. 2, top). Nuclear area usually changed early and marginally, then mitochondrial potential, with or without permeability change, then permeability change if it had not already occurred and finally Ca effects occurred last. Three of the four drugs, excepting dantrolene, had dual and opposing effects on TMRM fluorescence, showing an initial increase followed by a decrease. This dual biphasic effect was also noted for nuclear area for amiodarone and paroxetine (see Hormesis).

For amiodarone, the sequence of change in the dose-response curves (Fig. 2) was TMRM fluorescence increase at 0.2–0.8 μM, nuclear shrinkage at 6 μM, mitochondrial inhibition at 13 μM, plasma membrane permeabilisation at 50 μM, then Ca rise at 100 μM. Potential was decreased by one-third at 13 μM, and by two-thirds at 25 μM and higher. However, at lower concentrations than this, potential was increased progressively up to 100% at 12 μM. Nuclear area decreased by 10% at 6 μM, 15% at 13 μM and by 30% at 100 μM. Membrane permeability was increased to about half of that of the positive controls at 100 μM. Cell counts were not adversely affected.

Paroxetine was tested on the same plate as amiodarone. The sequence of changes were: nuclear shrinkage at 3–6 μM, TMRM fluorescence increase at 6 μM, mitochondrial inhibition at 25–50 μM, permeabilisation at 25–50 μM, Ca rise at 100 μM and cell count decrease at 50–100 μM. Nuclear area decreased 10% at 3 μM, 15% at 6 μM and 30% at 25 μM. TMRM signal increased by 20% at 6 μM and by 80% at 25 μM. At 50 μM, it rose over time by 200% to its maximum value and then fell to half of its baseline value; at 100 μM it fell to zero. Membrane permeability started to increase at 25 μM, reached half-maximal values at 50 μM and maximal values at 100 μM. Calcium rose a slight amount and only at 100 μM. Cell counts apparently decreased mildly at 50 and 100 μM.

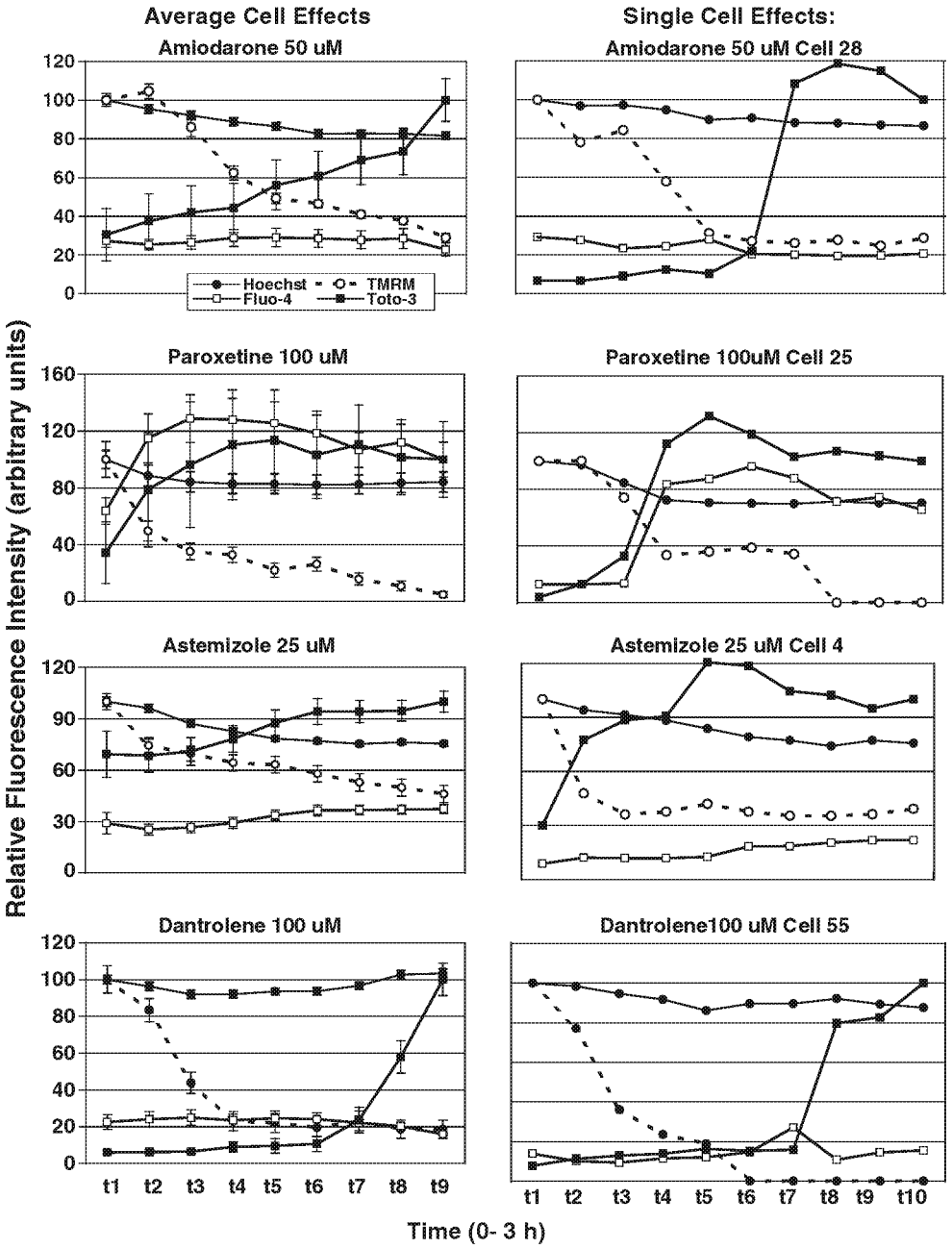

Fig. 3 Kinetics of averaged compared to individual cellular change for drugs producing acute toxicity. See Fig. 2 for details of assay. The patterns of change in fluorescences of the different dyes for a population of cells are similar to that of individual cells. However, the sequence of change can be more precisely determined at the single cell level

Dantrolene effects were in the sequence: nuclear area decrease at 6–13 μM, potential decrease at 13 μM and membrane effects were much less and occurred only at 100 μM. There was no clear change in Ca. Cell counts were not adversely affected.

Astemizole effects were in the sequence: TMRM fluorescence increase at 0.2 μM, nuclear shrinkage at 6 μM, permeability increase at 13–25 μM and Ca rise at 25 μM. The TMRM signal increased by 50% at 0.2 μM, 100% at 1.6 μM and by 150% at 6 μM. However at 13 μM it rose over time to this level then fell to baseline. At 25 μM it had decreased 90% from the maximum. Nuclei were apparently, transiently enlarged by 10% at 6 μM and shrunk by one-third at 13 and 25 μM. Membrane permeabilisation began at 13 μM and at 25 μM was about one-quarter that of the positive controls. Cell counts were not adversely affected.

Hormesis

As occurred without preincubation, some drugs produced dual effects, with increases followed by decreases on TMRM fluorescence in 26% drugs. This was especially conspicuous for those drugs that were strong inhibitors of the MDR pump (such as cyclosporine A and quinacrine), where TMRM progressively increased by approximately 100 and 150%, respectively (from 1.6

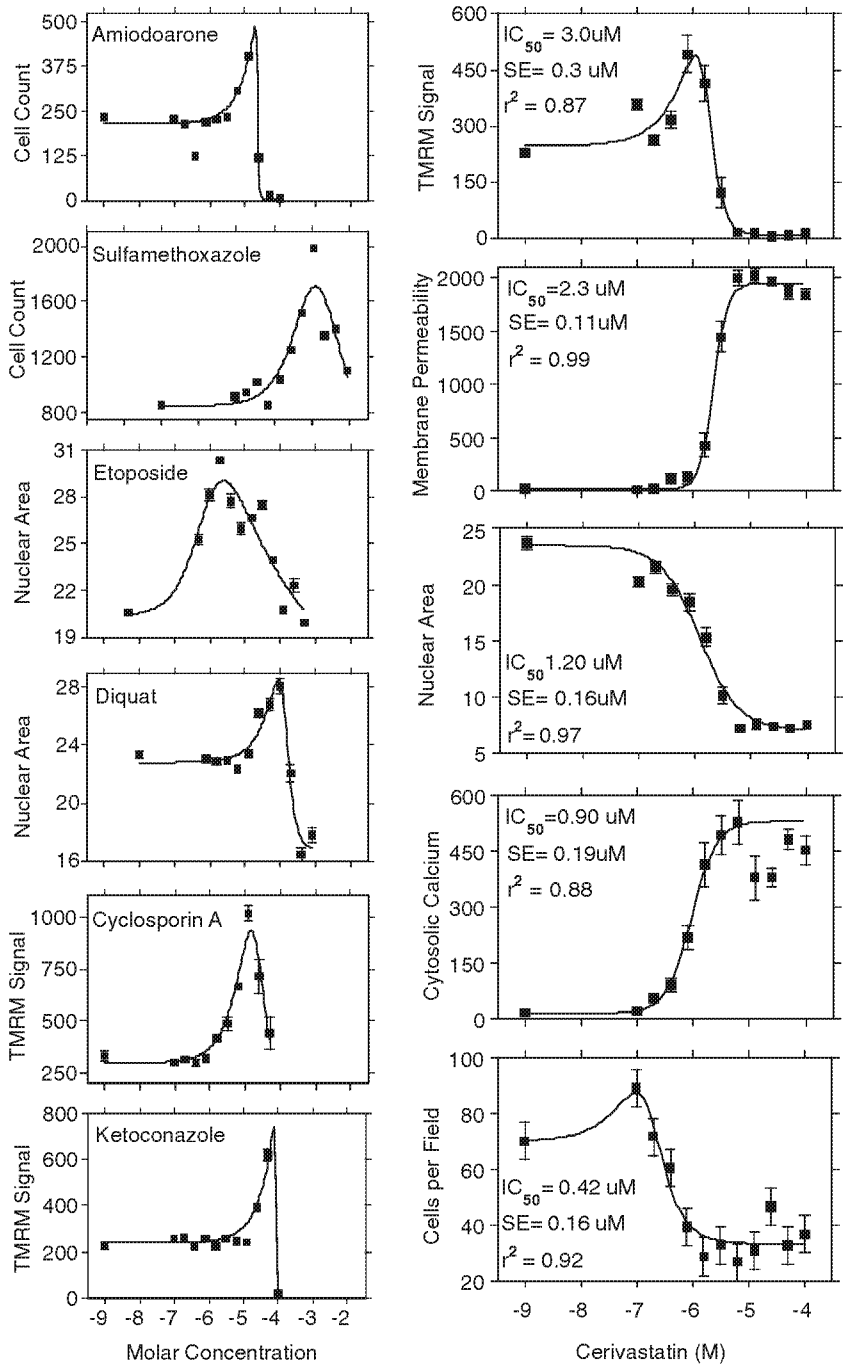

Fig. 4 Hormesis in dose-response for drug-induced cytochemical effects. IC_{50} for cerivastatin-induced hepatotoxicity. Biphasic dose-response curves occurred clearly for cell count, nuclear area and mitochondrial potential. Representative examples are shown. Curve-fitting to the dose-response data for cerivastatin cytotoxicity is shown for each of the five parameters assessed, with IC_{50} indicated as mean and error estimate

to 13 μM, and 0.1 to 0.2 μM). Dual effects were also noted for cell number for 12% compounds showing positive responses, and especially for nuclear area, where 34% of compounds showing positive response.

Figure 4 (left) demonstrates dual, biphasic responses for cell number, nuclear area and mitochondrial membrane potential for several drugs. This phenomenon was seen with 43% drugs and could not be attributed to artefacts of the position of wells within plates.

Hormesis could not be detected for calcium nor membrane permeability, likely because measures of these parameters in healthy cells could only be measured in one direction.

Sources of imprecision

Cell-to-cell, field-to-field, well-to-well and plate-to-plate variances after 3 days of drug treatment are shown in Table 3. The greatest imprecision was in measurements between cells within field-of-view. Next most variable was measurements between field-of-views, then between

plates. Measurements between wells within plate were usually the most precise, varying between wells by approximately 5% for nuclear area, 10% for mitochondrial membrane potential, calcium and membrane permeability and 15% for cell count. Nuclear area was by far the most precise measurement for all types of variance. Mitochondrial membrane potential was next most precise across cells, fields-of-view and across plates. Most variable of parameters was calcium, especially between cells.

The high variance could be attributed in part to not collecting data greater than a threshold value that would ensure only viable cells were assessed (thresholding). Because of the rapid rise and fall in Ca compared to the speed of analyses, timing of data acquisition once ionophore was added was neither precise nor consistent, resulting in counts including dead or dying cells that had lost fluorescent dye. This could be overcome by ensuring that positive controls are in wells spread across the whole of the plate at regular intervals. Because of the time it takes for the KSR to scan through each well, positive controls are measured approximately 40 min after addition of ionomycin. Thresholding could also be used for exclusion of dying cells from the cell count. Dead cells are assumed to have lifted off the well surface and therefore not counted. Cell count variance is high both between wells and between plates, due to clumping of proliferating cells resulting in an uneven distribution.

IC_{50} Determination

Estimates of IC_{50} values could be made for those drugs for which a near complete range of toxic effects could be determined in the concentration range studied, up to 30-fold C_{max} or 100 μM (Table 4). IC_{50} values could be obtained for 39 of 82 toxicants (48%). The IC_{50} was highly correlated with the concentration at which effects were first seen. For cell number the correlation coefficient was 0.75 ($P < 0.0001$) and the IC50 was 1.27 ± 0.19 fold this concentration.

Based on an IC_{50} analysis, the parameter that was most sensitive in detection of cytotoxicity was cell count for 56% of toxicants. The next most sensitive parameters were nuclear area and calcium, detecting 33 and 26% of toxicants, respectively. The least sensitive parameter was mitochondrial membrane potential. The frequency with which a parameter was the most sensitive or second most sensitive was 73 % each for cell count and nuclear area, 48% each for calcium and membrane permeability and 28% for mitochondrial membrane potential.

The ranking of the sensitivities of the different parameters for detection of cytotoxicity was somewhat different when assessed based on determination of IC_{50} values than when based on the concentration at which a clear effect was detected. Both approaches indicated that cell number and nuclear area were the most sensitive parameters. However, mitochondrial membrane potential was ranked as the least sensitive based on IC_{50} values, whereas membrane permeability was ranked as the least sensitive based on the parameter to first change. This difference likely reflects the fact that the point of first clear effect measures low dose enhancement which apparently delays the point of 50% loss of signal compared to baseline.

In Fig. 4 (right), concentration-response curves with IC_{50} values are shown for each of the five parameters measured for cerivastatin. Half-maximal inhibition occurred first for cell number at 0.4 μM, then intracellular Ca and nuclear area at about 1 μM, then membrane permeability and mitochondrial potential at 2–3 μM. Hormesis is seen to occur for both TMRM and cell number resulting in these parameters being affected at doses of 1 and 0.1 μM, respectively.

Predictivity of the HCS, sublethal, cytotoxicity assay

Comparison of effectiveness of different cytotoxicity parameters

Table 2 demonstrates that cell number was by far the parameter most frequently found to be affected by 3 days exposure to toxic drugs and the most sensitive indicator of cytotoxicity. About 86% of the 201 drugs that produced a positive test response affected this parameter, although it was not directly determined whether this was from antiproliferative or cell lytic effects. In contrast, only 70% of drugs testing positive affected nuclear area and mitochondrial membrane potential, and only 45% affected membrane permeability and intracellular calcium concentration. Cell number was the first parameter affected in 56% of drugs testing positive, with mitochondrial membrane potential and nuclear area being affected first for 30%, and cell permeability and calcium affected first for only 10 and 7%, respectively.

Cell permeability was the least sensitive parameters for detection of cytotoxicity, being the first parameter affected in only 7% of drugs (Table 1). As for all the parameters, this frequency was independent of the severity of hepatotoxicity and was the same for toxicities affecting other organs.

The measurement of nuclear size was the most precise of all parameters. It decreased at toxic concentrations by 50% for compounds such as astemizole, chloroquine, chlorpromazine, danazol, disulfiram, furazolidone, ketoconazol, mibefradil, paroxetine, primaquine, quinacrine, quinidine, quinine, tacrine, tamoxifen and valproate. It decreased by about 25% for amodiaquine, cyclosporine A, dantrolene, fenfluramine, imipramine, labetalol, methyldopa and novobiocin. However, it increased by 20% for niacin, norfloxacin and vidarabine, and by 50% for acetaminophen.

Sensitivity and specificity

The sensitivity for detection of severely hepatotoxic, moderately hepatotoxic and other organ toxic drugs

Table 4 IC_{50}s for concentration-response curves for drug induced effects on cell proliferation, mitochondrial function, intracellular calcium homeostasis, cell membrane permeability and nuclear area

Drug	Nuclear area	Mitochondrial potential	Calcium	Membrane permeability	Cell count
Amiodarone	**9.8 ± 0.33**	19.2 ± 0.42	17.2 ± 0.04	*15.8 ± 0.29*	25.6 ± 5.2
Amodiaquine	20.0 ± 0.25	35.8	**13.5**	*17.6 ± 0.34*	24.8
Astemizole	**2.6 ± 0.49**	5.6	*4.1*	*4.1 ± 0.14*	10.1 ± 4.9
Cerivastatin	*1.2 ± 0.16*	3.0 ± 0.30	*0.9 ± 0.19*	2.3 ± 0.11	**0.4 ± 0.11**
Chlorpromazine	*12.6*	20.7	24.4 ± 3.6	20.8 ± 0.4	**6.4 ± 1.0**
Chloroquine	**11.1 ± 0.22**	27.2 ± .02	22.4 ± 0.11	22.9 ± 0.21	*16.0 ± 6.3*
Cyclosporin A	**10.1 ± 0.3**	> 100	35.1	78.8 ± 0.6	*14.8 ± 3.8*
Danazol	*58.8 ± 0.1*	73.6	**43.1 ± 1.7**	81.4 ± 0.1	**39.2 ± 19.3**
Dantrolene	*52.1 ± 0.4*	105.0 ± 0.1	67.1	> 100	**14.5 ± 31.6**
Diquat	85.8 ± 6.6	**43.2 ± 5.4**	*79.2 ± 8.1*	*75.8 ± 8.2*	**39.6 ± 11.2**
Doxorubicin	*0.7 ± 0.34*	3.1	**0.2 ± 0.23**	**0.4 ± 0.20**	**0.2 ± 0.03**
Etoposide	> 500	*534.6*	1927 ± 553	772.7 ± 173.3	**0.14 ± 0.05**
Fenfluramine	*77.1 ± 10.0*	> 100	261.8 ± 25.6	195.8 ± 22.1	**62.9 ± 8.7**
Fluvastatin	**11.9 ± 0.55**	92.6 ± 0.20	**13.4 ± 0.75**	*51.6 ± 0.39*	106.7
Furazolidone	90.9 ± 0.14	86.8 ± 0.74	76.2	*58.2 ± 0.12*	**28.7 ± 13.5**
Furosemide	*2286*	4285	2890 ± 181	*2360 ± 8*	**1208 ± 177**
Imipramine	> 100	84.9	**35.4 ± 0.4**	*76.6 ± 0.3*	153.8
Ketoconazole	85.9 ± 13.1	80.2	*71.6 ± 0.2*	**62.2 ± 0.2**	89.7 ± 61.2
Ketorolac	**215.7 ± 77.3**	> 450	> 450	693.3 ± 163.8	**247.7 ± 47.2**
Ketotifene	91.2 ± 15.8	> 100	*70.0 ± 0.5*	86.5 ± 0.7	**27.6 ± 6.1**
Labetalol	*95.2 ± 18.6*	> 100	**81.9 ± 1.2**	106.0 ± 8.0	**80.2 ± 9.0**
Lovastatin	**14.9 ± 0.78**	*65.5*	**17.9 ± 1.11**	86.3 ± 0.49	104.2 ± 54.1
MethylDOPA	*257.7 ± 42.8*	**187.3 ± 23.0**	> 670	> 670	*257.4 ± 30.8*
Mevastatin	**25.2 ± 0.7**	330.4	**24.7 ± 1.0**	*97.7 ± 2.7*	> 100
Mibefradil	5.8 ± 0.17	5.6	16.9 ± 0.60	29.5	*8.8 ± 2.4*
Novobiocin	397.3 ± 89.2	*202.0 ± 49.9*	458.9 ± 18.7	*227.3 ± 52.6*	**183.2 ± 19.9**
Pamidronate	**52.9**	70.1	110.0 ± 0.1	*63.3*	*63.2 ± 15.7*
Paroxetine	*7.1*	**4.7 ± 0.93**	14.3 ± 0.05	*7.7*	17.6 ± 4.4
Picotamide	> 100	**12.5 ± 5.4**	93.3 ± 51.6	104.5 ± 67.2	*19.6 ± 3.8*
Primaquine	**28.1**	**27.8**	*58.9 ± 0.08*	*55.2 ± 0.14*	**31.4 ± 11.6**
Propranolol	**13.9 ± 0.41**	> 100	*57.7*	61.6	*49.0 ± 10.4*
Pyrimethamine	212.3 ± 128.2	> 100	124.2 ± 10.8	*77.8 ± 3.8*	**3.8 ± 0.6**
Quinacrine	*2.3 ± 0.18*	*2.6 ± 0.51*	**1.4 ± 0.31**	*2.7 ± 0.32*	**1.4 ± 0.17**
Quinidine	*72.8*	118.8 ± 26.2	118.3 ± 8.2	102.5 ± 1.5	**14.7 ± 2.4**
Quinine	*38.3 ± 2.8*	65.0 ± 26.8	> 600	*40.5 ± 1.3*	**22.3 ± 2.4**
Simvastatin	**16.1 ± 0.42**	51.7	**18.2 ± 0.56**	*40.2*	51.4 ± 25.0
Sodium chloride	> 100 mM	*71.6 mM*	106.6 mM	95.1 mM	**20.0 mM**
Tacrine	*54.3*	99.8	*58.3 ± 0.06*	87.5	**37.2 ± 18.1**
Valproic acid	*6546 ± 1309*	> 16200	> 16200	15930 ± 915	**1520**
Most sensitive assay (%)	**33**	**15**	**26**	**5**	**56**
2nd most sensitive (%)	**38**	**13**	**20**	**44**	**18**
1st or 2nd most sensitive (%)	**72**	**28**	**46**	**49**	**74**

Values are reported as mean or, when there was sufficient data, mean ± SE. The parameter with the lowest and second lowest IC_{50} for each drug is indicated in bold lettering or by italics, respectively

was, respectively, 20, 24 and 14% for the conventional cytotoxicity assays, and 100, 88 and 92% for the multiparameter, HCS cell health assay. Specificity for detection of organ toxicity was 98% for the 35 non-toxic drugs and 100% for the 6 different negative controls. Overall sensitivity and specificity were 90 and 98%, respectively, for the multiparametric assay.

Safety margin estimation

The ratio of the cytotoxic concentration in vitro to the in vivo concentration associated with efficacy was assumed to provide an indication of the safety margin or therapeutic index for the drug. This ratio was independent of whether toxicity was direct or metabolite-mediated. The ratio was 12 ± 22 for hepatotoxic compounds testing positive and 20 ± 34 for compounds toxic to other organs.

Variation of assay sensitivity with drug concentration

Assay sensitivity was found to be dependent on the absolute drug concentration tested and the safety margin (Fig. 5): 30% at 1 μM or a TI of 10 μM; 60% at 30 μM or a TI of 10 μM and 80% at 100 μM or a TI of 30 μM. Plots of the false positive rate versus the false negative rate, that is, receiver operating characteristic curves, indicate the optimal sensitivity that the assay can provide without compromising specificity (Fig. 5). Sensitivity falls off rapidly for specificity greater than about 93%.

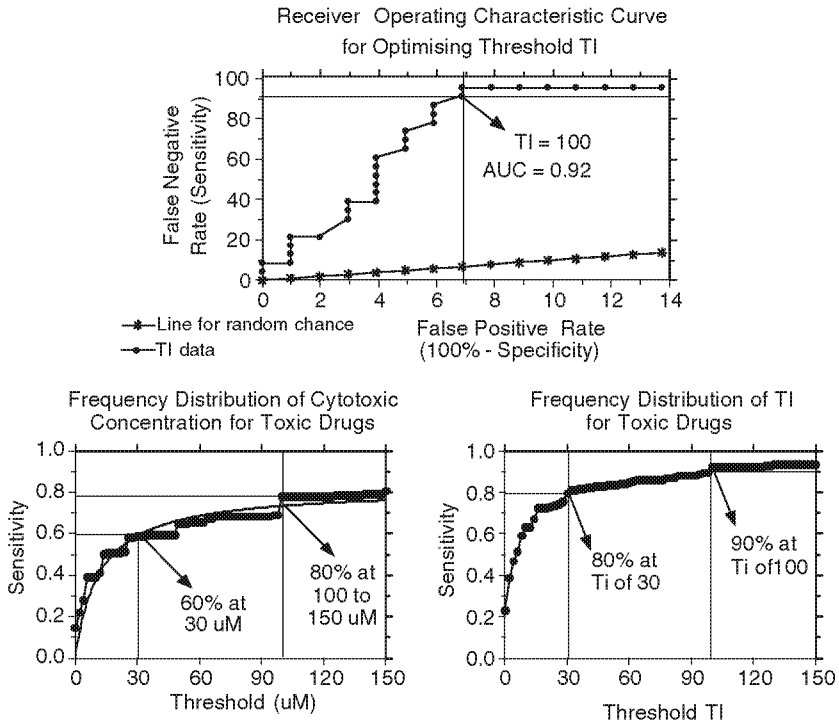

Fig. 5 Balancing assay sensitivity with specificity in defining diagnostic cut-off cytotoxic drug concentrations relative to efficacious concentration (TI). Receiver-operator characteristic curve for TI from 102 hepatotoxicants and the 23 non-toxic drugs for which a TI was determined are plotted. Curve was only slightly different when all toxicants were included. A cutoff TI of 100 gave 93% specificity and 90% specificity. Area under the curve (AUC) was 0.92, corresponding to a toxic drug have 92% probability of having a higher TI than a non-toxic drug. Frequency distribution curves show the proportion of toxic drugs that are identified at increasing cut-off cytotoxic concentrations or TI's (cytotoxic concentration/efficacious concentration [C_{max}]) and indicate that a cut-off based on TI has superior diagnostic value than based on concentration alone

Discussion

Conventional cytotoxicity assays

This study demonstrates that conventional cytotoxicity assays have poor concordance with human toxicity. This poor predictivity of cell-based assays for in vivo toxicity likely reflects at least in part on the lateness of the point in the sequence of pathogenic events that they assess (Table 2). Assays that target late events in the process of cell injury, when the cell is near death, are more likely to miss toxicities that require chronic exposure or exert adverse but non-lethal effects. Many of the conventional cytotoxicity assays (e.g. LDH release, cell rupture, membrane blebbing) are for late-stage toxicity and cellular events associated with a lethal apoptotic or necrotic effect (Jaeschke et al. 2002; Olson et al. 2000). Such assays have low sensitivity (Table 1) for detection of adverse cellular effects and furthermore provide little mechanistic understanding of the toxicologic effects in humans. Conversely, assays that provide early assessment of specific toxicologic mechanisms in cells (Table 2), prior to the onset of the late stages of non-specific degeneration and apoptotic or necrotic death, should theoretically have greater predictive power and extrapolatability across models and species. While these retrospective analyses support the value of conventional in vitro cytotoxicity screens in identification of the "overtly" toxic compounds, it points to the need of further refinement in the method to predict subtle or sub-lethal adverse events that account for the majority of side effect profiles of human pharmaceuticals.

Although none of the conventional cytotoxicity assays had adequate concordance with in vivo human toxicity, it is important to note which of the endpoints used by these tests were most predictive. Glutathione assay for oxidative stress was by far the most sensitive (Table 1), supporting the proposal of the important role of oxidative stress in drug toxicity (Xu et al. 2004). The two indicators that were next most sensitive were the Alamar Blue assay for mitochondrial reductive activity and the DNA assay for cell proliferation, both having sensitivities an order of magnitude greater than the tests for membrane integrity, protein synthesis, superoxide and caspase-3. These findings support the inclusion in the HCS assay of parameters reflective of cell proliferation, mitochondrial function and oxidative stress. The latter though is currently not yet incorporated into HCS screening, although studies are under way to do this (Phillips et al. 2005).

An additional important finding in this study of conventional cytotoxicity assays is that multiple and different early measurements of toxicity mechanisms are

critical for detection of human toxicity potential. Predictivity was additive for several of the conventional assays when combined, e.g. mitochondrial activity, glutathione and cell proliferation. The data from the HCS cytotoxicity assay also indicate the need for measurement of multiple, independent and early mechanisms of cytotoxicity. As well, similar conclusion that multiple different toxicity endpoints need to be measured has recently been made by others (Schoonen et al. 2005a, b).

Retrospective analysis of the predictivity of testing strategies using a set of marketed drugs that have already been screened for those that were thought to be toxic, can only result in substantial underestimation. However, such approach provides a common ground for comparisons across testing strategies. Thus, whereas predictivity for human hepatotoxicity (Table 1 and 2) of regulatory animal toxicity testing, conventional cytotoxicity tests and HCS sublethal cytotoxicity tests must necessarily be underestimated using this approach, this underestimation should be proportionately the same for all testing strategies assessed.

Predictivity of the HCS, sublethal cytotoxicity assay

Hormesis

The concentration-response curves for many drugs with toxic effects were characterised by early positive responses in cell proliferation, nuclear area and mitochondrial potential, prior to negative responses in these parameters. Hormetic effects were seen only after 3 days of preincubation with drug, with the exception of mitochondrial membrane potential, for which biphasic effects occurred acutely as well as after preincubation (see Fig. 2). The occurrence of these positive responses prior to degenerative effects is suggestive of them being compensatory, adaptational and protective (Olson et al. 2001; Calabrese and Baldwin 2002). The basis for the hormetic effect on nuclear area is unknown, but may relate to a specific inhibition of the cell cycle in which nuclear area is increased followed by a degenerative effect in which the nucleus shrinks. The hormetic effects for cell proliferation and mitochondrial potential resulted in IC_{50} values being higher (see Fig. 4, right) because they were defined as the concentration at which basal activities were decreased by 50% rather than the concentration at which maximal activities were decreased by 50%.

Hormesis was found for approximately half the drugs, for nuclear area in 34% of the 136 drugs affecting it, for mitochondrial membrane potential in 26% of the 141 drugs affecting this parameter, but for cell number in only 12% of the 173 drugs affecting it.

Requirement for exposure to drugs for multiple days

The current study clearly demonstrates that preincubation of cells with drugs for multiple days is typically needed for expression of their toxicity. Predictivity was increased by an order of magnitude by preincubating cells with drugs for 3 days. This finding reasonably explains why sensitivity of the HCS cytotoxicity assay is an order of magnitude greater than that of conventional cytotoxicity assays. Support of this can be found in recent work where cells were preincubated with drugs for 3 days then subjected to conventional cytotoxicity assays performed independently (Schoonen et al. 2005a, b). This finding of the effect of preincubation of cells and drugs for multiple days is also consistent with our previous observations (Slaughter et al. 2002; O'Brien et al. 2003; Xu et al. 2004) and recent studies by Schoonen et al. (2005a, b). Whereas the preincubation time was not titrated, the finding that cell proliferation was the most highly sensitive (although least specific) indicator of toxicity, indicates that preincubation should extend over multiple doubling times.

Identification of idiosyncratic hepatotoxicity and hepatotoxicity due to reactive metabolites

The HCS sublethal cytotoxicity assay correctly identified drugs that produce toxicity via metabolites and drugs that produced idiosyncratic hepatotoxicity. Surprisingly, after 3 days of pre-incubation with drug, the assay was able to detect toxicity thought to be mediated by reactive metabolites of the parent drug equally as well as for drugs that produced their toxicity directly. Most of these toxicities are thought to be caused by formation of reactive metabolites that are frequently linked with oxidative stress (Kalgutkar et al. 2005). Also surprising was that the assay was able to detect most of the 12 drugs that have been associated with idiosyncratic hepatotoxicity. It is noteworthy, however, that there were relatively few drugs that were clear in these categories. Cell count was affected by all of these compounds and mitochondrial membrane potential and nuclear area was affected by 11 of them. Calcium and membrane permeability was affected by only half of them.

Most sensitive parameters are cell proliferation, mitochondria and nuclear area; membrane permeability and calcium are least sensitive

Cell proliferation inhibition in most cases preceded the changes in other parameters. The high sensitivity of this parameter may reflect its dependency on the normal functioning of a wide range of physiological processes. On the other hand, this sensitivity comes at the expense of specificity for the toxicologic mechanism. It is noteworthy that cell proliferation was assessed by measurement of cell number, which could be affected not only by effects on cell proliferation but also by cell death. Thus a decrease in cell number could be attributed to a cytostatic effect or both. However, in most cases, cell proliferation was affected prior to any discernible effect on the sub-lethal cytotoxicity parameters, indicating that

the effects on cell number were sub-lethal rather than lethal. Results from this study support the idea that cytostatic effects are a consistent and early effect of sub-lethal cytotoxicity. However, cytostatic effects may occur in the absence of cytotoxic effects, as is shown by effects of specific inhibitors of cell proliferation that have no other adverse effects, such as azathioprine, colchicines, cyclophosphamide and methotrexate. Thus, additional measures of adverse effects on cells are needed to interpret the cytostatic effect. These drugs did, however, affect the nuclear area at the same time as cell number.

Nuclear area was found to have comparable sensitivity as cell count for detection of toxicants. The least sensitive parameter, based on occurrence of a significant change, indicated that cell membrane permeability was least sensitive. However, ranking of parameters' sensitivities for cytotoxicity detection based on IC_{50} values indicated that mitochondrial membrane potential was the least sensitive. The basis for these contradictory findings likely relates to the hormesis effect occurring in the dose-response relationship for mitochondrial membrane potential but not detected for membrane permeability. Based on the FCCP control well results from a random analysis of ten plates, the background signal contributes to 24.4% (SD = 8.0%) of the total TMRM signal. Therefore, the useful dynamic range of the assay to measure mitochondrial uncoupling may limit sensitivity.

The finding that membrane permeability and intracellular calcium were the least sensitive parameters is expected on the basis that membrane integrity and maintenance of an intact barrier to the extracellular milieu are essential for cell function and are maintained with highest priority by the cell. Rise in intracellular calcium is an important final event in the live of a cell. It is noteworthy that a more sensitive dye with greater accuracy and precision for measurement of intracellular calcium at basal levels, rather than at cytotoxic levels, may be a more effective biomarker for cytotoxicity. For example, studies with indo-1 have shown that there may be subtle elevations in resting intracellular calcium and impairments in calcium regulatory capacity with genetic and toxicologic cellular pathologies (O'Brien et al. 1989, 1990).

Whereas intracellular calcium was the second least sensitive parameter for detection of toxic effect, it was an early indicator for several classes of drugs, such as the statins (cerivastatin, fluvastatin, mevastatin), local anaesthetics, (bupivacaine, lidocaine), antimalarials (amodiaquine, chloroquin, quinacrine, quinidine, quinine), neuroleptics (amitryptilline, trifluoperazine, paroxetine), anthracyclines (doxorubicin), amiodarone, tacrine and captopril. The basis for this early involvement of calcium dyshomeostasis is uncertain, but may relate to the mechanism of cytotoxicity.

Cell membrane permeability change was an early effect in only 7% of drugs causing a positive response, e.g. doxorubicin, pimozide, dipyrone, flucytosine, foscarnet, novobiocin, rotenone and sulphanilamide. Such disruption of the barrier to the extracellular medium inevitably produced immediate and rapid increases in mitochondrial permeability and in intracellular calcium. In contrast, mitochondrial changes did not produce immediate and rapid changes in cell membrane permeability.

Cytotoxicity test results reported herein are supported by the literature. Comparison of the HCS results with those for 7 conventional, independent, cytotoxicity assays from recent studies by Schoonen et al. (2005a, b) is possible because of overlap for 30 of the drugs and compounds studied (acetaminophen, amiodarone, aspirin, benzopyrene, bromobenzene, carbon tetrachloride, chlorpromazine, colchicine, cyclophosphamide, dantrolene, dexamethasone, diclofenac, diethylmalemide, doxorubicin, erythromycin, estradiol, flutamide, gentamycin, imipramine, indomethacin, isoniazide, ketoconazole, labetalol, nitrofurantoin, quinidine, rotenone, sulphaphenazole, tacrine, tamoxifen, tetracycline). There was high correlation of results between studies for all parameters, especially Alamar Blue, glutathione and membrane permeability ($r \sim 0.8$), although lesser with DNA, ATP and NADPH ($r \sim 0.6$) and least with reactive oxygen species ($r = 0.46$). This correlation supports the findings of the current study, especially for use of a mitochondrial function biomarker and glutathione.

Assessment of human toxicity potential: TI, PPB, AUC

The significance of the cytotoxic signals should be interpreted in terms of the ratio of cytotoxic concentration to the concentration causing efficacy. The latter was estimated by comparing with the maximal total concentration of the drug in human serum that is associated with administration of the drug at an efficacious dose. However, the degree of plasma protein binding was not considered in this study. As there is only one tenth as much plasma protein in the in vitro system compared to in vivo, significant protein binding would be expected to result in an overestimate of the circulating free drug compared to in vitro, with consequent proportionate underestimate of the safety margin and overestimate of the toxicity potential. For example, rosiglitazone's safety margin was underestimated without consideration of the high plasma protein binding. Thus, this ratio should be considered an estimate of the minimal safety margin. Finally, in this context, the best estimates of safety margin for most drugs should be based on drug exposure to free concentration per unit time (i.e. area under the concentration time curve, AUC). However, these values were not available for the in vitro studies.

The risk associated with a low safety margin needs to be considered with respect to the indication and to the dose being used. Lower safety margins will be accepted for drugs intended for treatment of life-threatening diseases for which there are no equivalent alternatives. Lower safety margins may also be accepted for drugs in

which the ingestion is limited by the bulk required for toxicity or by side-effects such as vomiting.

It may also be relevant to interpret the significance of the signal based on the degree of change and the number of parameters affected, and the mechanism and the steepness of the concentration-response curve.

Application of prelethal cytotoxicity testing

Whereas drug-induced cytotoxicity may indicate potential for in vivo human hepatotoxicity, it is not predictive of such. Cytotoxic effects in vivo may be limited or even aggravated, compared to those occurring in vitro. Cytotoxicity models are limited by their incomplete modelling of the cell type's structure and function as it occurs in vivo, by their incomplete modelling of other cell types and of cell functions and interactions within a tissue, organ, systems and whole body, e.g. (a) drug properties, concentrations, protein binding and transport may differ in vivo; (b) pharmacokinetic characteristics of absorption, distribution, metabolism and excretion can have a major influence on which target organ is affected and the severity of toxicity; (c) toxicities occurring at the tissue or organ level such as cholestasis, cataractogenesis and myelotoxicity cannot be effectively predicted from cellular systems; (d) toxicities may occur secondarily to direct cytotoxicity and due to the interaction of organs and systems, and other processes such as inflammation, immune-mediated hypersensitivity, plasma volume expansion and endocrine effects.

Prelethal cytotoxicity assay as first tier of laboratory testing for organ toxicity potential Although cytotoxicity assays cannot predict human hepatotoxicity and cannot be unequivocally used for "go - no go" decision-making in the progression of drug discovery, they nevertheless have value. Knowledge of the cytotoxicity of drugs early in drug discovery can create opportunities for ranking and prioritizing, or developing alternatives with lower toxicity potential and can also flag the need for further scholarship and experimental safety assessments if the compound is to progress to preclinical development. Such assessments would need to include conduct of more mechanism-specific, or possibly tissue-specific, in vitro assays, as well as in vivo animal studies. These in vitro models may also provide valuable mechanistic understanding of the toxicity.

Cytotoxicity testing throughput Throughput is an important consideration in assessment of the practicality of an assay to be implemented for screening compounds in drug discovery. Throughput of the assay in which cells are exposed for 3 days to 12 concentrations of each drug then measured at three different time points over 3 h is 14 drugs per standard workweek for a single operator. However, the assay can be abbreviated so that only one time point is measured and only one or a few concentrations are measured (such as the minimally acceptable multiple of the concentration at efficacy that would have no effect) then throughput can be increased up to many hundreds of assays per week. Further throughput enhancement would likely require adaptation of the assay to a 384 well-plate format.

Optimal drug concentration and safety margin for predicting human toxicity potential The current study indicates the concentration of drug needed for assessment of human toxicity potential. At a concentration of 30 μM, 60% of drugs with human toxicity potential were identified, whereas 100 μM identified about 80% (Fig. 5). These concentrations are considerably lower, that is the assay is more sensitive, than previous reported assays (Bugelski et al. 2000; Schoonen et al. 2005a, b). Assessment of toxicity potential was more accurate when the concentration of drug tested was based on multiples of the total efficacious concentration (C_{max} for marketed drugs), with 80% of cytotoxicities being detected at a concentration of 30 times the efficacious concentration, C_{max}. In the current study, toxicities occasionally went undetected by the HCS cytotoxicity test when the C_{max} had not been determined and the drug was not tested up to 30-fold the efficacious concentration.

False test results in the HCS prelethal cytotoxicity assay Examination of the false positives and negatives may provide understanding of what toxicities the HCS cytotoxicity assay is not relevant for. As described above, idiosyncratic heptoxicants and heptotoxicities attributed to reactive metabolites were detected. However, there were several specific examples of toxicities that were not detected in the current study. For some of these drug toxicities, this could be attributed to the specific known effect of the drug on molecular targets that are not found in heptocytes. For example, the assay applied to HepG2 cells did not detect cholestatic effects of estradiol, calcium-channel effects of ryanodine, potassium channel effects of astemizole and terfenadine, renal toxicity of zomepirac, dermatotoxicity of isoxicam and hematologic toxicity of vincamine. Additionally, the human toxicity potential was not detected at relevant concentrations in the HCS cytotoxicity assay for bupropion, diethylcarbamazine, naproxen, pravastatin and trifluoperazine. Furthermore, whereas picotamide is essentially nontoxic, it was found to be highly toxic in the cyotoxicity assay. The number of such false test results was too small to draw definitive conclusions, and additional work would be needed to confirm these proposals.

Limitations and need for further studies

Although the HCS sub-lethal cytotoxicity assay had greater than 90% concordance with human toxicities, it failed to detect up to 10% of these. The basis for the

failure of the HCS cytotoxicity test for the other drugs is unknown, but may be partly attributable to either incomplete metabolic competence of the cell-based model used or else to toxicities being caused by, or mediated by, extra-hepatocytic effects that require involvement of some other molecular transporter or process, cell, tissue, organ or system that cannot be represented by a hepatocyte cell line model. However, that the assay was positive for most drugs that produce their toxicities by a reactive metabolite argues against metabolic competence being significantly limiting. The extrahepatic toxicities may involve (a) cytokine, growth factor or hormone production, (b) immune-mediated toxicity and (c) extrahepatocytic, tissue-specific transporters which are found in hepatobiliary or renal tubular cells.

Metabolic competence Developing further metabolic competence, either by using extracellular incubation of drugs with S9 microsomal fractions, or by using cells that are more competent metabolically, may enhance predictivity. Primary human hepatocytes would theoretically provide the best model of metabolic competence for cytotoxicity assays. However, current culture methods in 96-well plates do not stabilise the phenotype of primary cells and prevent their rapid dedifferentiation over the several days of incubation needed for most drug toxicities to be expressed. Furthermore, primary cells are also less suitable for cytotoxicity assessment because of their low rates of cellular proliferation, which is the most sensitive measure of hepatotoxicity potential.

Given the need for a proliferating cell model for predictive cytotoxicity studies, the effectiveness of the choice of HepG2 cells as a cell line to use is supported by other studies. Schoonen et al. (2005a, b) found them slightly more predictive than HeLa, ECC-1 and CHO-k1 cells. Others have made similar findings (Bugelski et al. 2000). However, a cell line with metabolic competence and morphology more representative of the in vivo state would likely further enhance the predictivity of cytotoxicity assays.

Data processing We have identified two areas where improvement in analysis would result in increased efficiency with regard to performing larger scale cytotoxicity screens on a routine basis. First, the number of cellular measurements generated by the image analysis algorithms used far exceeded those relevant for this assay. This required handling and archiving of more data than was required. We are currently pursuing the use of streamlined assay algorithms that generate only the required assay output. In addition, we are investigating ways to further automate data analysis of large volumes of kinetic data, especially the calculation of IC_{50} values using user-definable mathematical models.

Assay refinement The Cellomics KSR was used successfully to apply automated HCS assays to investigate a large set of experimental variables including cell plating density, cytotoxic indicators, drugs, drug concentrations and incubation times. However, further improvement may be obtainable by refinement and optimisation of cell culture factors such as cell substrate, media and drug replacement during preincubation, and assessment of drug transport and stability. Imprecision will likely be decreased and diagnostic sensitivity improved by refinement of culture conditions to facilitate cell attachment and more uniform spreading across the well surface, and by reducing evaporation and uneven heating and oxygenation of wells from the edges to the center of the plates.

Further studies are needed to define optimal strategies for cytofluorescent monitoring of relevant cytotoxicity biomarkers. The current study indicates significant room for improvement in dye and parameter choice, especially with respect to membrane permeability and also intracellular calcium concentration, at least as measured with a non-ratiometric dye with relatively low calcium-binding affinity compared to basal, intracellular concentration of calcium. The effectiveness and mechanistic information of the HCS cytotoxicity assay may be enhanced by replacement of these dyes with others that could measure glutathione or reactive oxygen species, lysosomal mass, safety-signal transduction, additional morphological cell features, cell cycle information indicated by nuclear staining or the mass, reductive activity or DNA content of mitochondria. The specific need to incorporate an oxidative stress biomarker is also indicated by the results herein on conventional cytotoxicity assays, our preliminary studies currently underway (Phillips et al. 2005), and by the importance of oxidative stress in drug-induced toxicity (Xu et al. 2004).

Further studies are also needed to distinguish artefact from toxic response from adaptive responses (eg mitochondrial potential dyes); to better model in vivo metabolic competency; to sensitise to cytotoxicity and further enhance assay sensitivity; and to quantitate predictivity and correlation with human hepatotoxicity and its mechanistic basis. Finally, more non-toxic drugs need to be tested to more accurately assess the frequency of false positives and the appropriate safety margin to interpret cytotoxicity.

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Message

From: POLITICO Pro Energy [politicoemail@politicopro.com]
Sent: 10/3/2018 9:54:47 AM
To: Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]
Subject: Morning Energy, presented by Growth Energy: Mountain Valley Pipeline stumbles again — PJM's new proposal for FERC — NOPEC's moment?

By Annie Snider | 10/03/2018 05:52 AM EDT

With help from Ben Lefebvre, Alex Guillen and Darius Dixon.

PROGRAMMING NOTE: Morning Energy will not publish on Monday Oct. 8. Our next Morning Energy newsletter will publish on Tues. Oct. 9. Please continue to follow Pro Energy issues here.

MORE LEGAL TROUBLE FOR MOUNTAIN VALLEY PIPELINE: The 4th Circuit U.S. Court of Appeals dealt a major blow to the West Virginia pipeline project Tuesday when it vacated its Army Corps of Engineers permit, finding that the project didn't meet the requirements for a nationwide permit to harm streams and wetlands. The court vacated that verification and said it would explain its reasoning more fully in a forthcoming opinion, Pro's Ben Lefebvre reports.

Sierra Club Executive Director Michael Brune called on FERC to halt construction of the pipeline in light of the court's decision. "In their haste to make a quick buck, MVP rushed essential processes because they knew there was no way their dirty project would ever satisfy commonsense protections for water and health," he said in a statement.

"Disappointed" but not defeated: The project's developer, Mountain Valley Pipeline LLC said in a statement that it is assessing whether it can continue construction in areas that don't impact streams and wetlands along the 160 miles of the route covered by the vacated permit, and that it plans to apply for a new Army Corps permit following changes to permit when West Virginia's state certification process. The company said it expects to secure a new permit in early 2019 and hopes to have the pipeline fully in service by the fourth quarter of 2019.

PJM OFFERS NEW PITCH TO SATISFY FERC: Tuesday was the deadline to submit the initial round of comments to FERC's contentious order requiring that the nation's largest power market, PJM Interconnection, rewrite its capacity market rules to account for state-level policies like nuclear or renewable subsidies. Nobody expected PJM's response to be simple, but the proposal adds even more layers to the already complex capacity market, which effectively pays power plant owners to be available when the grid needs them most. In addition to setting up a price floor for generators to bid in, PJM suggests creating a "resource carve-out" mechanism, which would let states support a subsidized plant but remove them from the capacity auctions, as well as an "extended resource carve-out" for FERC to consider if they don't think that goes far enough.

PJM gave everyone a lot to chew on so you'll have to make the most of the time you have between now and Nov. 6 to tell FERC what you think.

A little help on the way? A couple of ME tipsters tell us that the White House is primed to announce its next pick for the FERC leadership, Bernie McNamee, as soon as this week.

WELCOME TO WEDNESDAY! I'm your guest host, Annie Snider, and am eternally grateful for all the bagel suggestions. The ME hivemind has spoken and Bullfrog is clearly the place to service our NYC bagel

needs (the whitefish salad, and jalapeno cream cheese were specifically endorsed). We'll see about that! But ME would be remiss if we didn't mention some honorable mentions: So's Your Mom, and Buffalo and Bergen.

Today's trivia question: Name the scientist who coined the phrase "global warming." Ben takes the reins to carry us until Kelsey's much-awaited return, so send your guesses, along with your tips, energy gossip and comments, to blefebvre@politico.com, and remember to follow us on Twitter @Morning_Energy and @POLITICOPro.

PRESIDENT DONALD TRUMP SAYS A RED WAVE IS COMING ON ELECTION DAY. Is he right, or will the tide turn blue? Compete against the nation's top political minds in the POLITICO Playbook Election Challenge, by correctly picking the winning candidates in some of the most competitive House, Senate and gubernatorial races in the country. Win awesome prizes and eternal bragging rights. Sign up today! Visit politico.com/playbookelectionchallenge to play.

NOPEC GAINING? Gas prices are rising, and so is animosity toward OPEC. The oil cartel, which President Donald Trump bashed at the United Nations last week, may find itself in the crosshairs again today at a Senate Judiciary subcommittee hearing on antitrust laws. The hearing will not focus explicitly on the Judiciary Chairman Chuck Grassley's NOPEC bill, S. 3214 (115) (115), which would allow the attorney general to bring antitrust lawsuits against OPEC members in U.S. courts. Sen. Mike Lee, who chairs the antitrust subcommittee holding today's hearing, is a co-sponsor of the bill, as is subcommittee ranking member Amy Klobuchar. Today's hearing may offer members of the Trump administration a chance to weigh in on the bill, and committee aides tell ME they expect it will "probably" come up.

Versions of NOPEC have floated around for years without becoming law, but supporters may now have an ally in the president, who has complained on Twitter about OPEC for years. Just last week, Trump accused OPEC of "giving us high oil prices," in his speech to the U.N. general assembly. "OPEC is a pet peeve for him," Joe McMonigle, a senior energy policy analyst at Hedgeye Potomac Research told Reuters. "Everybody thinks he could easily support NOPEC." The average U.S. gasoline price reached $2.90 a gallon this week, up about 40 cents from a year ago, according to price tracking website Gasbuddy.com.

Assistant Attorney General Makan Delrahim, of the Justice Department's Antitrust Division, will be among today's witnesses; in 2008, while working in private practice, he endorsed an earlier version of the NOPEC bill. Grassley and House Judiciary Committee Chairmen Bob Goodlatte wrote to Delrahim in August asking for his input on this year's bill, and the committee said Tuesday it never received a reply.

And what if it becomes law? The U.S. could challenge Saudi Arabia and other cartel members under anti-trust laws if the NOPEC bill were ever enacted, but it's not necessarily clear to ME what could be won. Some analysts fear any lawsuit would simply provoke backlash from the cartel. "I have seen this type of proposal in the past, especially when prices rise," said Andy Lipow, president of Lipow Oil Associates, told ME. "The fear might be that if the U.S.A. litigated or seized assets, OPEC would simply take more oil off the market, sending prices higher. In the worst case, they stop selling to the U.S.A."

COMPLEX OZONE LAWSUIT GOES TO COURT: The D.C. Circuit Court of Appeals hears oral arguments today over a 2016 rule incorporating the 2015 ozone standard into the Cross-State Air Pollution Rule, which seeks to curb pollution from upwind states. Many of the issues are highly technical, and have to do with how pollution is measured, patterns are modeled, or emissions allowances were allocated. But the rule is under attack from two sides. Environmental and public health groups say the rule wasn't stringent enough, while industry and state challengers say it went too far. The case will be heard by Judges Sri Srinivasan, Patricia Millett and Robert Wilkins, all Barack Obama appointees.

One big reason the Trump administration is defending this Obama rule: EPA has relied on this ozone update to justify recent decisions not to require upwind states to cut even more emissions. In July, EPA said it

believes upwind states have done enough because of this regulation, and thus do not need to find further ways to curb pollution. And last month EPA rejected petitions from Maryland and Delaware asking it to require upwind states to do more about their pollution, saying those smokestacks are already covered under the 2016 update.

If you go: 9:30 a.m. at the E. Barrett Prettyman Courthouse; the hearing will also be audiostreamed on the court's website.

** **A message from America's ethanol producers and farm supporters at Growth Energy:** E15 ethanol blends are cleaner, higher in octane, and approved for use in nine out of 10 cars on the road. They also can save consumers up to 10 cents per gallon. Learn more at https://e15now.com **

MURKOWSKI, BISHOP HUDDLING ON LWCF: Senate Energy Chairman Lisa Murkowski (R-Alaska) has held some initial conversations with House Natural Resources Chairman Rob Bishop (R-Utah) about a possible path forward on reauthorizing the Land and Water Conservation Fund, she told reporters Tuesday. Both Murkowski and Bishop have raised concerns about the mandatory funding included in the measure to permanently reauthorize the LWCF, S. 569 (115), which was advanced by Senate Energy earlier Tuesday. "I want this measure to pass," Murkowski told reporters. "Working with our colleagues in the other body is going to be important as we advance what I think we all agree is a national priority — and it should be a national priority. We'll figure it out." More from Anthony Adragna here.

IT'S STILL GOOD, IT'S STILL GOOD: Alaska Gasline Development Corp. — the company behind the state's proposed Alaska LNG export terminal and pipeline — wants everyone to know that there's still room for its project after Shell announced yesterday it would build its own LNG terminal in British Columbia. Shell's LNG Canada, with a planned capacity of up to 14 million metric tons a year to be built by the middle of next decade, would target buyers in Asia, the same market the Alaska Gasline project is shooting for. In response, Alaska Development said it "reaffirmed" its agreement it signed last year with Chinese chemical company Sinopec, the Bank of China and China Investment Corp. to pursue an LNG project. "The supplemental agreement reaffirms the parties' intent to negotiate and conclude definitive agreements by December 31, 2018," Alaska Development said in a prepared statement.

APPEALS COURT OPENS DOOR TO PFAS LITIGATION: Residents whose drinking water has been contaminated by the military's use of firefighting foam can sue for government-funded medical monitoring, the U.S. Court of Appeals for the 3rd Circuit ruled Tuesday, poking a hole in the government's immunity defense. The joined cases were brought by two families in eastern Pennsylvania who say their drinking water wells were contaminated with the chemicals at levels far above EPA's health advisory level. A federal district court had dismissed the cases as barred under the Superfund law, but on appeal the 3rd Circuit held that the families could proceed with a portion of their case and seek medical monitoring.

SEE YA IN SEATTLE: Acting EPA Administrator Andrew Wheeler is in Seattle today , and will tour construction of the first major water infrastructure project to receive funding under the WIFIA loan program. The EPA in April approved up to $134.5 million in loans for the Georgetown Wet Weather Treatment Station in Washington state's King County. The project is designed to collect and treat up to 70 million gallons of wastewater and stormwater each day that would otherwise spill into the Duwamish River, and ultimately Puget Sound, during heavy storms. On Thursday, Wheeler will hold a press conference at EPA's Region 10 office.

SECRET SCIENCE ON THE HILL: A Senate Environment and Public Works subpanel takes up the topic of scientific transparency today, as EPA considers how to finalize the controversial rule it proposed this spring that would block the agency from using many studies looking at the effects of pollution on human health.

One of the less discussed aspects of the EPA proposal may also get some airtime — the move to change the default assumption that there is no safe level of exposure for carcinogens, which has been the focus of a major battle between the chemicals industry and public health advocates for years. Witness Edward Calabrese, a

toxicologist at the University of Massachusetts, Amherst, hailed the change as "correct and long overdue" in comments on the rule, while advocates have argued it runs counter to increasing scientific evidence that there is no "safe threshold" of exposure to toxic chemicals.

Implications for radiation? The Associated Press reported Tuesday that those changes could affect how the federal government regulates radiation exposure for everything from workers at nuclear facilities to people living next to Superfund sites to medical workers doing X-rays and CT scans. Calabrese "has said weakening limits on radiation exposure would save billions of dollars and have a positive impact on human health," the AP reports.

If you go: The hearing is at 2:15 p.m. in Dirksen 406.

ON ONE HAND: Ardent Trump supporter Rep. Steve King (R-Iowa) tweeted a picture of his agenda — including E15 — written on his hand for what he said was a 75-minute private meeting with President Donald Trump.

GET 'EM OUTTA HERE: The Army Corps of Engineers, one of the country's main dam builders, took a major step to incentivize the removal of obsolete dams Tuesday, issuing guidance to its district offices on how to count the environmental benefits of removing dams when issuing mitigation credits. Developers spend billions of dollars each year on mitigation required by the Army Corps to offset damage to streams and wetlands. Those credits can be earned by restoring or creating wetlands and streams, but that has rarely included removing dams, which the Corps said in a statement are often more than a century old and create not just environmental harm but also public safety hazards.

LAME-DUCK FUNDING FIGHT LOOMS: When the House returns after the Nov. 6 elections, Speaker Paul Ryan and his deputies will have just four weeks to pass funding bills that keep the federal government open through the holidays — and the way they played the last funding fight this fall stands to make their job that much harder, Budget & Approps' Sarah Ferris reports this morning.

Leaders gave up their best leverage for enticing votes from both sides of the aisle by already approving an entire year of military funding and the vast majority of the government's non-defense spending. Without that to use as a bargaining chip, House GOP negotiators will be expected to deliver far more Republican policy victories to gain the votes from their own party. And if Democrats take the House, partisan battle lines are likely to harden on everything from President Donald Trump's demand for a border wall to environmental policies at the Interior Department. Read the full story here.

MORE CRES ENDORSEMENTS: The GOP clean energy group Citizens for Responsible Energy Solutions will make its third round of congressional endorsements this morning. Republicans winning the group's backing are Reps. Don Bacon (Neb.), Mike Coffman (Colo.), John J. Faso (N.Y.), Chuck Fleischmann (Tenn.), Garret Graves (La.), John Katko (N.Y.), Adam Kinzinger (Ill.), Leonard Lance (N.J.), Markwayne Mullin (Okla.), Francis Rooney (Fla.), Mimi Walters (Calif.), Kevin Yoder (Kan.), Ted Yoho (Fla.) and Lee Zeldin (Fla.), as well as Dusty Johnson, a candidate for South Dakota's House seat.

CAP ON TONGASS: Ahead of today's public hearing on whether to exempt the nation's largest national forest from federal rules limiting construction in national forests, the Center for American Progress has a report arguing that timber production in the Tongass national forest is a waste of taxpayer dollars and harms other industries, including fisheries and tourism.

QUICK HITS

— "Trump's Import Tariffs Will Make U.S. Wind Power More Expensive." Bloomberg

— "PJM: FirstEnergy can shut 4 GW of fossil plants without harming reliability." UtilityDive

— "Elon Musk's Ultimatum to Tesla: Fight the SEC or I quit." New York Times

— "In about-face, Gov. Bruce Rauner calls for Sterigenics shutdown after weeks of downplaying cancer risks." Chicago Tribune

HAPPENING TODAY

9:30 a.m. — Natural Gas Supply Association winter outlook briefing. Bloomberg Room at the National Press Club Press, 529 14th Street N.W.

1:30 p.m. — U.S. Forest Service public hearing on exempting the Tongass National Forest from the federal Roadless. Holiday Inn Capitol, 550 C Street S.W.

2:15 p.m. — Senate Environment and Public Works subcommittee on Superfund, Waste Management, and Regulatory Oversight hearing titled "EPA Oversight of the Environmental Protection Agency's Implementation of Sound and Transparent Science in Regulation." 406 Dirksen.

CORRECTION: The Oct. 2 edition of Morning Energy misidentified the organization that helps energy companies address methane leaks. It is the Oil and Gas Climate Initiative.

That's all for ME!

**** A message from America's ethanol producers and farm supporters at Growth Energy:** Outdated EPA regulations block many retailers from offering E15 fuel blends year-round, but President Trump has promised a fix. That means consumers will be able to take advantage of ethanol blends that are cleaner, higher in octane, and approved for use in nine out of 10 cars on the road. E15 can also save consumers up to 10 cents per gallon. America's biofuel leaders at Growth Energy are calling on the EPA to act quickly on the president's plan to open year-round competition at the fuel pump. Learn more at https://e15now.com **

To view online:
https://subscriber.politicopro.com/newsletters/morning-energy/2018/10/mountain-valley-pipeline-stumbles-again-360685

Stories from POLITICO Pro

Court delivers new setback for Mountain Valley pipeline Back

By Ben Lefebvre | 10/02/2018 07:28 PM EDT

A U.S. appeals court vacated an Army Corps of Engineers permit for the Mountain Valley Pipeline in West Virginia today, a potentially major setback for the developer of the proposed natural gas conduit.

The order from the 4th Circuit U.S. Court of Appeals in West Virginia said the Corps improperly verified the pipeline complied with a nationwide permit. The court vacated that verification and said it would explain its reasoning more fully in a forthcoming opinion.

The ruling may hinder Mountain Valley's ability to continue construction of the pipeline in what has already been a litigious, on-again-off-again project. Mountain Valley Pipeline LLC said in late September that court-

ordered work stoppages along the route caused it to bump the project's total cost estimate to $4.6 billion. A company spokesperson did not immediately reply to questions.

"Because the court has vacated the [permit] in its entirety, we anticipate that FERC is likely to issue a broad stop work order, halting construction on all incomplete water crossings (unless there are strong environmental reasons not to do so)," ClearView Energy partners wrote in a note to clients.

The Sierra Club and other environmental groups that opposed the pipeline cheered the court's decision.

"In their haste to make a quick buck, MVP rushed essential processes because they knew there was no way their dirty project would ever satisfy commonsense protections for water and health," said Sierra Club Executive Director Michael Brune in a statement. "Now, FERC must require MVP to immediately stop construction on the pipeline."

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Sources: DOE's McNamee to get FERC nod Back

By Eric Wolff and Darius Dixon | 08/08/2018 04:07 PM EDT

The White House plans to nominate Energy Department official Bernard McNamee to fill the FERC leadership seat being vacated by departing Commissioner Rob Powelson, three sources familiar with discussions tell POLITICO.

McNamee helped roll out Energy Secretary Rick Perry's proposal last year to save struggling coal and nuclear power plants — an issue that sources have said served as a key litmus test for Trump administration officials evaluating a replacement for Powelson, who is set to resign Friday.

FERC in January unanimously voted down that plan, which sought to create special payments for power plants capable of holding 90 days of fuel on-site. But the administration has been considering additional options such as invoking rarely used emergency powers to force power plants to run, which would potentially give McNamee a chance to provide the pivotal vote on the subsequent rates and rules as a commissioner.

It is unclear when President Donald Trump would formally nominate McNamee, and the vetting process still seems to be underway. It would likely take the Senate several months to confirm him, a process that would start with hearings at the Energy and Natural Resources Committee.

Neither the White House nor DOE immediately responded to requests for comment Wednesday.

McNamee, who runs the DOE's Office of Policy, has been in and out of the agency under Trump. He was deputy general counsel for energy policy last year when he worked on Perry's ill-fated proposal to FERC. In February, he left DOE for a senior post with the Texas Public Policy Foundation, a conservative think tank with ties to Perry, before returning to DOE in May.

Before joining the Trump administration, McNamee previously worked at McGuireWoods, as chief of staff to Texas Attorney General Ken Paxton and as an aide to Sen. Ted Cruz (R-Texas).

To view online click here.

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Murkowski says she's spoken with Bishop on LWCF Back

By Anthony Adragna | 10/02/2018 03:28 PM EDT

Senate Energy Chairman Lisa Murkowski (R-Alaska) said today she's held some initial conversations with House Natural Resources Chairman Rob Bishop (R-Utah) about a possible path forward to reauthorizing the Land and Water Conservation Fund.

"I want this measure to pass," she told reporters. "Working with our colleagues in the other body is going to be important as we advance what I think we all agree is a national priority — and it should be a national priority. We'll figure it out."

The Energy and Natural Resources Committee earlier today advanced legislation, S. 569 (115), permanently reauthorizing LWCF and providing mandatory funding despite Murkowski's serious concerns with that approach. Bishop has warned that mandatory spending would likely doom the Senate bill in the House, and he moved his own bill, H.R. 502 (115), without it in September.

"My concern is we won't be successful with this measure if we can't address that part," Murkowski said, adding that an offset would be required under Congressional Budget Office rules.

"Some may say that's just moving money from one pot to another," she said. "Yes, that is true, but when you talk about how items score around here, that is a reality."

To view online click here.

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Lame-duck funding woes await House GOP leaders Back

By Sarah Ferris | 10/03/2018 05:00 AM EDT

Republican leaders dodged an October shutdown. But the way they played that first round of funding could make it hard to finish the job come December — especially if the midterms don't sway in their favor.

When the House returns after the Nov. 6 elections, Speaker Paul Ryan and his deputies will have just four weeks to pass funding bills to keep the government fully functioning through the holidays.

Complicating that task is the fact that they have given up their best leverage to entice votes from both sides of the aisle by clearing the deck for an entire year on military funding and the vast majority of the government's non-defense spending.

Add to that a midterm outcome that flips the House to a Democratic majority and it could be nearly impossible to muscle through the seven outstanding bills for fiscal 2019, at best prompting another stint of stopgaps and at worst stoking a pre-Christmas shutdown with Republicans in charge.

"That's the danger in not getting more bills done," said Rep. Tom Cole (R-Okla.), a senior appropriator, before House lawmakers departed. "Maybe we can get it done, but the election will impact what happens."

With five weeks until polls close, budget observers are eyeing a handful of factors that will determine the fate of funding for those agencies:

Democrats take the House:

If the GOP is forced to surrender its eight-year House majority, partisan battle lines are likely to harden ahead of the Dec. 7 deadline for funding the many departments left hanging while others received full-year spending levels last month. That could heighten already well-trodden disputes on everything from President Donald Trump's demand for a border wall to environmental policies at the Interior Department and abortion restrictions for foreign aid.

A House that's even more polarized would make it far more difficult for leaders of both parties to strike a final funding deal, marking an abrupt end to Congress' most productive appropriations cycle in two decades and ensuring more spending uncertainty for the departments of Homeland Security, Commerce, Transportation, Housing and Urban Development, Justice, State, Agriculture and Interior, as well as foreign operations, the IRS, science programs and the EPA.

Last-minute GOP demands:

Unlike this summer, Republican leaders can't dangle funding for the Pentagon or the Department of Veterans Affairs to get their fellow conservatives on board with bipartisan funding bills, since they already spent that bargaining chip for fiscal 2019. So House GOP negotiators will now be expected to deliver more Republican policy victories, or risk falling short on votes from their own party, according to multiple lawmakers and aides.

"A lot of us who are defense and fiscal hawks are really getting put in some tough situations right now. It's frustrating, and I don't like it," Rep. Mark Walker (R-N.C.), chairman of the roughly 170-member Republican Study Committee, told POLITICO last week. He added that he'll have "some issues" to raise in any lame-duck spending package.

Roughly a half-dozen House Freedom Caucus members also suggested last week that they plan to stir up an immigration fight when Congress takes on funding for the Department of Homeland Security.

"We should be doing what we said," Rep. Jim Jordan (R-Ohio) said in a last-minute push for border wall funding, one day before the House passed the main fiscal 2019 spending package, H.R. 5895 (115). "That's the single biggest issue voters voted us into power to accomplish, and we haven't yet done that."

Appetite for shutdown:

Because Trump has already signed spending bundles that make up about 75 percent of all federal funding for the current fiscal year, hard-line Republicans could be more inclined to force a partial government shutdown, since it wouldn't hurt the VA or the military.

Whether the president would be willing to shut down the government over demands for more money to fund a wall along the U.S.-Mexico border is still an open question, though. Trump said this week that he has "a big decision to make after the election as to whether or not we go for it."

Dragging out the deadline:

Given early conservative backlash against the remaining spending bills, Republican leaders will almost certainly require help from Democrats to pass bipartisan measures on the floor.

GOP leaders could have trouble enticing that support, however, if Democrats are newly empowered by election results and insist on punting all funding decisions until they have ushered in a new majority in January.

"Their temptation might be to hold off until the new majority shows up. I advise them to not do that," Cole said. "We did that, waiting for the president. It doesn't get you anything more and just creates a lot of work and anger."

Another omnibus:

When the House adjourned last month, spending leaders were still negotiating an unfinished "minibus," H.R. 6147 (115), that includes funding for the departments of Agriculture, Interior, Transportation, and Housing and Urban Development, plus the IRS and EPA.

Conference negotiators could easily abandon work on that spending package now and simply wrap it up into a catchall omnibus with the remaining three fiscal 2019 bills that fund the departments of Homeland Security, Justice, State and Commerce.

"I don't think there is as much incentive," one House GOP aide said on the possibility of continuing talks on that four-bill package through November.

GOP appropriators like Sen. Richard Shelby (R-Ala.) have pushed to keep the "minibus" negotiations open through the fall, insisting that lawmakers are "very close" to a deal. But even Shelby acknowledged there is little chance of anything more than "some talk" getting done while House lawmakers are back home campaigning.

Lawmakers and aides have said that package has been repeatedly held up by policy issues within the Financial Services bill, while the other three measures — Transportation-HUD, Agriculture and Interior-Environment — are largely negotiated.

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Goodbye to the bioassay

Jay I. Goodman

It is time to say goodbye to the standard two-year rodent bioassay. While a few, primarily genotoxic, compounds which are clearly associated with human cancer test positive in the bioassay, there is no science-based, sound foundation for presuming it provides either a valid broad (across different chemicals) capability for discerning potential human carcinogens or a valid starting point for making human risk assessment decisions. The two basic assumptions underlying the bioassay are: (1) rodent carcinogens are human carcinogens; and (2) results obtained at high doses are indicative of results that will occur at lower, environmentally relevant, doses. Both of these assumptions are not correct. Furthermore, a reevaluation of National Toxicology Program bioassay data has revealed that if the dose group size were increased from 50 to 200 rodents per group the number of bioassays deemed to be positive would increase from approximately 50% to very close to 100%. Thus, under the extreme conditions of the bioassay (*e.g.*, high doses, lifetime exposure and, at times, a non-physiological route of administration) virtually all chemicals tested could be made into rodent carcinogens. In recent years there have been a number of proposals to move away from the standard bioassay. In particular, a recently formulated decision tree (Cohen, 2017), which places an emphasis on dose–response relationships and invites the use of MOA information, provides a sound basis for moving on from the bioassay and towards a rational approach to both identify chemicals which appear to have the potential to cause cancer in humans and take dose–response relationships into consideration in order to place the extent, if any, of the risk they might pose into proper perspective.

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Introduction

The first indications that chemicals could cause cancer were based on observations in humans. John Hill, in 1761, found that people who used tobacco snuff exhibited an unusual high rate of nasal cancer,[1] and in 1775 Percivall Pott described scrotal cancer as an occupational cancer in chimney sweeps, due to contamination of the skin by chimney soot.[2] However, 140 years passed until, in 1915, experimental carcinogenesis in animals began with the demonstration that application of coal tar to the ears of rabbits caused malignant skin tumors.[3] This observation lead to the use of animals as proxies for evaluating the potential of chemicals to cause human cancer. Justification for the use of animal testing to identify human carcinogens was based, in part, on observations that a number of compounds (typically exhibiting a potential to cause genotoxicity) which sound epidemiology studies indicate are clearly associated with human cancer have been shown to cause cancer in rodents.[4] However, beyond this rather small group of chemicals, there is no convincing body of evidence to justify a statement that the opposite is true. The rodent bioassay has not been validated.

Long-term, approaching life-time, studies in rodents were viewed as a way to identify chemicals that had the potential to be carcinogenic to humans. A historical review of the rodent bioassay indicates that while several long-term bioassays were conducted in the 1930s, guidelines did not appear until the 1950s and it was not until the late 1960s that cancer bioassays as we know them today really began.[5] At that time the National Cancer Institute (NCI) was charged with evaluating the potential of a rather large number of chemicals for carcinogenic activity.[5] The NCI developed guidelines which were published in 1976.[6] Chemicals were considered to be either carcinogens or noncarcinogens.[7] Chronic rodent bioassay testing became more formalized with the development of the National Toxicology Program in 1984, the Food and Drug Administration's guidelines (referred to as the "Red Book") in 1982 and revised in 1993, and the Environmental Protection Agency in 1987 and the Organization for Economic Cooperation and Development.[5]

Virtually all of the chemicals tested early-on in the bioassay were DNA-reactive electrophiles, or capable of being metabolized to a DNA-reactive (electrophilic) metabolite, able to form covalent adducts with DNA and referred to as genotoxic

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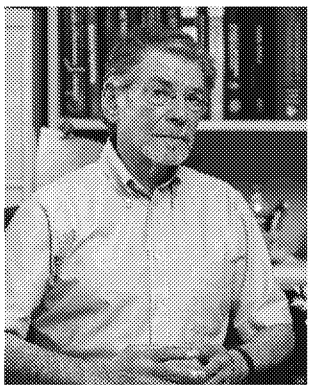
chemicals.[8] A DNA adduct is not a mutation; however, it can be a substrate for mutagenesis depending upon the degree to which it is capable of causing miss-pairing of DNA-bases, the capacity for DNA repair and rate of cell proliferation. The belief that mutagenesis is the basis for carcinogenesis was enhanced when Bruce Ames and colleagues developed a bacteria-based assay for mutagens, showed that a number of carcinogens tested positive in this assay and proclaimed that "carcinogens are mutagens".[9] However, by the mid 1990s it became quite clear that the notion that all carcinogens are mutagens is overly simplistic since numerous non-mutagens were testing positive in the bioassay, *e.g.*, saccharin, 1,1′-(2,2,2-trichloroethane-1,1-diyl)bis(4-chlorobenzene) (DDT), 2,3,7,8-tetrachlorodibenzodioxin (TCDD) and D-limonene.[10] The first, highly attractive, proposed mechanism underlying carcinogenesis which explains how non-genotoxic chemicals, *e.g.*, non-mutagens, can cause cancer was put forth by Cohen and Ellwein in 1991.[11] These investigators hypothesized that chemicals which increased cell proliferation (either by acting as mitogens or causing necrosis leading to compensatory hyperplasia) could cause cancer in a secondary fashion due to errors in DNA replication (DNA polymerase is not 100% accurate) leading to accumulation of critical mutations. The practical significance here is that if the dose of the chemical in question is kept below that which leads to increased cell proliferation cancer is not expected to arise, *e.g.*, the chemical exhibits a threshold.[11]

Additionally, altered epigenetic parameters leading to aberrant gene expression that can facilitate the transformation of a normal cell into a frank malignancy is another mechanism underlying carcinogenesis which is not, necessarily, based on mutagenesis.[12–15]



Jay I. Goodman

Dr Jay I. Goodman, a past President of the Society of Toxicology, is a professor of Pharmacology and Toxicology at Michigan State University and a member of the University's Center for Integrative Toxicology. He is a Diplomate of the American Board of Toxicology and a Fellow of the Academy of Toxicological Sciences. Dr Goodman's research interests are focused on discerning mechanisms underlying, and biomarkers for, non-genotoxic chemical-induced carcinogenesis, and testing the hypothesis that susceptibility to carcinogenesis is related inversely to the capacity to maintain the normal epigenetic status. Dr Goodman is an Associate Editor of Toxicology Sciences, *and of* Regulatory Toxicology and Pharmacology. *Dr Goodman served as a member of the Advisory Committee to the Director of the Centers for Disease Control and Prevention, the Board of Scientific Counselors of the National Toxicology Program, the Board of Directors of the American Board of Toxicology, the Board of Directors of the Academy of Toxicological Sciences, Chair of the Board of Trustees of the International Life Sciences Institute's (ILSI) Health and Environmental Sciences Institute (HESI), and the Board of Scientific Counselors, NIH, National Institute of Environmental Health Sciences. Dr Goodman's honors and awards include: Distinguished Alumnus Award, Long Island University, College of Pharmacy, 1998; Distinguished Alumnus Award, Doctoral Program in Pharmacology, The University of Michigan, 2000; Recipient of the John Barnes Prize Lecture, awarded by the British Toxicology Society, 2005; Recipient of the George H. Scott Memorial Award, awarded by the Toxicology Forum, 2007; Recipient of the Society of Toxicology's Merit Award, 2014; Recipient of the International Society of Regulatory Toxicology and Pharmacology's International; Achievement Award, 2014. Dr Goodman holds a Ph.D. in Pharmacology from The University of Michigan, and was a postdoctoral fellow in the McArdle Laboratory for Cancer Research, University of Wisconsin.*

Assumptions underlying the use of the bioassay are not valid

The use of the rodent bioassay as a predictor of the ability of the chemical in question to cause cancer in humans is based on two assumptions, which should be questioned: (1) the animal model result is relevant to humans (interspecies extrapolation); and (2) the tumor response at doses used in animal models are relevant to human exposure levels (dose extrapolation).[16] The validity of these assumptions for the two-year bioassay had been based on the tumor response for potent DNA reactive carcinogens. However, for non-genotoxic chemicals, one or both of these assumptions have been demonstrated to be incorrect.[17] The use of a linear no threshold (LNT) assumption for carcinogens appears to have its basis in radiation genetics and recent, in-depth reviews provide convincing evidence that this conjecture is not valid.[18,19]

The National Toxicology Program (NTP) Board of Scientific Counselors reviewed the NTP in 1992.[20] There were three working groups and the report of the Carcinogenesis Working Group formed the basis of a subsequent publication.[21] An aspect of the use of bioassay results which drew particular attention was the implicit assumptions that underlie a linear extrapolation from doses in the vicinity of the highest dose used (the maximum tolerated dose (MTD)): (1) pharmacokinetics are not dose-dependent; (2) the dose–response relationship is linear; (3) DNA repair is not dependent on dose; (4) response is not dependent on age; and (5) the test doses need not bear a relationship to human exposure (*e.g.*, there is no need to employ doses which are in the vicinity of anticipated human exposure). These assumptions were evaluated in a critical manner. The Board concluded that "the implicit assumptions underlying extrapolation from the MTD ... do not appear

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to be valid".[20,21] Thus, it is wrong to place mathematics in front of biology, the biology should drive the mathematics.

Too many rodent carcinogens

It is instructive to reflect on the observation that a high percentage of chemicals tested in the bioassay are positive only at the MTD. Approximately 50% of chemicals that were bioassayed were deemed to be rodent carcinogens.[22,23] Additionally, all too frequently the NTP carcinogens would not have been considered to be carcinogens if the MTD was not included in the study. For example: (1) an evaluation of the carcinogenic effects detected when the diet was the route of administration, the route used in a majority of NTP bioassays,[23] revealed that two-thirds of the positive bioassays were positive only when the MTD was employed;[24] and (2) an evaluation of 127 positive bioassays (using various routes of administration) indicated that in 43% of these tests statistically significant increases in tumor incidences occurred only at the MTD.[23] Furthermore, in those bioassays where a positive effect was observed at a dose below the MTD, this "lower" dose (typically one-half to one-fourth of the MTD) is so close to the MTD relative to the multiple orders of magnitude associated with extrapolation down to a dose that is purported to represent, for example, a one in a million added cancer risk that, for practical purposes, the extrapolation can be considered as based on one data point. There is no valid biological basis for doing this. In this context, it is of interest to consider that: (1) in freshman chemistry laboratory we are taught to not extrapolate beyond a standard curve; and (2) drawing a straight line from a single data point would not be deemed acceptable in a basic geometry class.

Dose influences mechanism and over a wide range of doses one can expect that mechanism will change with changing doses. Thus, a carcinogenic effect observed at a high dose is not necessarily expected to occur at lower doses,[25,26] especially when dealing with nongenotoxic compounds.[27] It is likely that critical, limiting steps in a mechanistic pathway might become overwhelmed with increasing dose leading to the emergence of new modalities of toxic tissue injury at these higher doses.[28,29] Indeed, a variety of examples of dose-dependent transitions in mechanisms of toxicity have been reported.[28,29] This includes a demonstration of the influence of DNA repair on non-linear dose-responses for mutation.[30] Thus, one should not presume there is no threshold for mutagenicity.[18,19,30]

A key, very legitimate concern regarding the validity of the bioassay as a predictor of human carcinogenicity is that positive results are basically a high dose artifact, *e.g.*, secondary to toxicity which would not occur at lower doses, particularly doses in the vicinity of what humans might be exposed to.[31,32] Nongenotoxic chemicals that cause cancer in rodents at high doses typically increase cell proliferation in their target organ (s). A biologically-based description of carcinogenesis was used to demonstrate that increased cell proliferation can account for the carcinogenicity of nongenotoxic compounds and that an increased rate of cell proliferation also plays a role in the carcinogenic dose–response for genotoxic chemicals.[11,33] Thus, doses below that which are capable of causing increased cell proliferation are expected to not be carcinogenic, *e.g.*, there is a threshold. The important role that increased cell proliferation can play in carcinogenesis is not incompatible with the view that a chemical's ability to affect epigenetic parameters is crucial, too. An analysis of over 150 NTP bioassays was performed with the aim of estimating the probability that a statistically significant ($p < 0.01$) dose–response trend would be obtained at one or more sites in either sex of rats or mice if 200 rather than 50 rodents were used per dose group.[34] In this series of chemicals 97/156 (62%) were considered to be rodent carcinogens by the NTP. With an increase of the number of animals per dose group from 50 to 200 it was estimated that 92% would be deemed to be rodent carcinogens. Many of the chemicals are not genotoxic. The analysis suggests that almost all of the chemicals evaluated would be viewed as carcinogens when tested at the maximum tolerated dose (MTD) with a larger sample size.[34] Thus, the rodent bioassay could be just a screen for cytotoxins at the MTD rather than a screen specifically for carcinogenicity.[34]

What about evidence of chemoprevention?

An aspect of the rodent bioassay which typically is not afforded the attention it deserves is the fact that it often results in tumor decreases as well as increases. An analysis of 31 NTP bioassays indicated a similar frequency of tumor increases and decreases.[35] However, an evaluation of 218 NTP bioassays revealed that the vast majority (<90%) of the chemicals tested showed at least one statistically significant ($p < 0.05$) decrease in site-specific tumor incidence.[36] The anticarcinogenic responses in rodent bioassays were shown to not be explained by random effects and the authors of this study point out that: (1) the bioassay is actually rather insensitive for detecting anticarcinogenicity because so few tumor types have background rates high enough to be able to detect a statistically significant decrease; and (2) the high doses used might so perturb the animal's physiology that the responses, both positive and negative, have little meaning for lower doses.[37] There have been some suggestions that anticarcinogenic effects observed are due to decreased body weight and/or decreased survival of treated rodents.[35,36] However, there are a number of chemicals that are anticarcinogenic with no indication of weight or survival depression and many chemicals cause weight or survival decreases with no apparent anticarcinogenic effects.[38] Clearly, there has been insufficient discussion regarding how to factor anticarcinogenic effects into the overall evaluation of bioassay results.

Rodents are not small humans

Concordance between outcomes in rodents and humans is a fundamental tenet of the rodent bioassay. However, there are

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numerous examples which demonstrate that this assumption is not correct. Defining the mode(s) of action by which chemicals induce tumors in laboratory animals has become a key to judgments about the relevance of such tumor data for human risk assessment. Mode of action analytical frameworks depend on both qualitative and quantitative evaluations of relevant data and information: (1) presenting key events in the animal mode of action, (2) developing a "concordance" table for side-by-side comparison of key events as defined in animal studies with comparable information from human systems, and (3) using data and information from mode of action analyses, as well as information on relative sensitivity and exposure, to make weight-of-evidence judgments about the relevance of animal tumors for human cancer assessments.[39] The International Program on Chemical Safety (IPSC) has developed a framework for analyzing the relevance of a cancer mode of action (MOA) for humans.[40] A variety of nongenotoxic compounds which cause mouse liver tumors (*e.g.*, phenobarbital), a classical activator of the constitutive androstane receptor (CAR);[41,42] sulfoxaflor;[43] and compounds which activate the nuclear receptor peroxisome proliferator-activated receptor-alpha (PPAR-alpha)[44] have been shown to act through a MOA which is not relevant to humans and, therefore, these compounds are not considered to be potential human carcinogens.

Goodbye bioassay

In light of the discussion presented above it is not surprising to see that, in recent years, a variety of publications have made compelling statements to the effect that the time has come to cease performing/requiring the standard 2-year rodent bioassay because it does not provide reliable data for assessing the potential of chemicals to cause human cancer. The mouse arm of the bioassay is viewed as being not scientifically justifiable for the safety assessment of pesticides,[45] while some go further and take the position that inclusion of the mouse bioassay is no longer necessary when dealing with nongenotoxic compounds.[46] A strong case has been made regarding the bioassay as being no longer necessary for the evaluation of chemicals to potentially cause human liver tumors.[47] Additionally, a commentary concerning cancer risk assessment at the US Food and Drug Administration's Center for Drug Evaluation and Research raises the question as to whether the era of the 2-year bioassay is drawing to a close.[48]

The serious concerns regarding the ability to identify the significance, if any, that the standard 2-year bioassay has for human safety assessment have led to some carefully thought out proposals for alternatives. An evaluation of pharmaceuticals indicated that the value added by conducting a rat two-year carcinogenicity study is dubious, at best, for compounds that lack: (1) histopathologic risk factors for rat neoplasia in chronic (*e.g.*, 6 month) toxicology studies, (2) evidence of hormonal perturbation, and (3) positive genetic toxicology results. Based on these criteria, a decision paradigm was proposed which has the potential to eliminate over 40% of rat two-year testing on new pharmaceuticals without compromise to patient safety.[49] This approach is supported by a thorough study which evaluated carcinogenicity data of medical products for human use that have been authorized by the European centralized procedure (CP) between 1995 and 2009.[50] The majority of tumor findings in rodent carcinogenicity studies were considered not to be relevant for humans. Appropriately, the authors questioned the value of the rodent bioassay and called for a revision of the carcinogenicity testing paradigm.[50] Additionally, a recent study focused on the rat arm of the bioassay, regarding pharmaceuticals, concluded that juxtaposing a consideration of the pharmacological properties of the compound in question with histopathology findings following treatment for 6-months could enhance the ability to define conditions under which two-year rat carcinogenicity studies would not add value.[51]

Consideration of alternatives to the standard rodent bioassay extends beyond a focus on pharmaceuticals. A retrospective study involving an evaluation of sub-chronic toxicity studies of 163 nongenotoxic chemicals in comparison with tumor responses in 2-year rat carcinogenicity studies supported that notion that chemicals showing no histological risk factors for neoplasia in a sub-chronic study do not require further testing in a bioassay.[52] A tiered strategy for cancer hazard identification has been proposed.[53] The authors indicate they are well aware of the fact that, at this time, we do not have a good handle on short-term approaches for detecting nongenotoxic compounds that might be carcinogenic. In addition to genotoxic potential, they rely on histopathology, evidence of hormone perturbation and, as yet to be developed, toxicogenomics approaches to discern nongenotoxic carcinogens.[53] My emphasis on the "as yet to be determined toxicogenomics approaches" is not intended to connote criticism. While surely being a worthy pursuit, the task of developing a human-relevant toxicogenomics approach to discern nongenotoxic chemicals which are potential human carcinogens will be, to put it mildly, very difficult. Additionally, the authors[53] focus on hazard identification and we know that the necessary transition to safety assessment requires an understanding dose–response relationships. The dose makes the poison. The tiered strategy is well thought out and the authors deserve a lot of credit for putting it forward.

In this author's view, the decision tree for evaluating the potential of chemicals to cause a cancer response in humans proposed by Cohen (2017) is the current best, comprehensive proposal for moving away from the standard two-year rodent bioassay.[17] It builds upon and advances earlier proposals.[49–53] The decision tree starts with an evaluation of a chemical's potential to be DNA-reactive (*i.e.*, a genotoxic compound) If the answer is "yes" it might be a human carcinogen, and there is a need to take human exposure and dose–response relationships into consideration. If the answer is "no", then one asks if the compound causes significant immune suppression and/or significant estrogenic activity (I would phrase it in a broader sense, hormonal perturbation) and/or significant increased

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cell proliferation. If the answer to any of these is "yes" then the compound is presumed to be a possible human carcinogen, depending on human exposure and dose–response relatonships. If the answers to all these are "no", the compound is presumed to be a noncarcinogen with regard to humans. This decision tree,[17] which places an emphasis on dose–response relationships and invites the use of MOA information, provides a sound basis for moving on from the bioassay and towards a rational approach to both identify chemicals which have the potential to cause cancer in humans and take dose–response relationships into consideration in order to place the extent, if any, of the risk they might pose into proper perspective. Thus, we have a sound basis for saying "goodbye to the bioassay".

While there is an emphasis on genotoxic potential of compounds of interest, it needs to be understood that not all genotoxic compounds are expected to be carcinogens and not all carcinogens are expected to be genotoxic compounds capable of providing substrates for mutagenesis. Mutagenesis does not equal carcinogenesis, carcinogenesis involves more than mutagenesis, and altered epigenetic parameters play a key role in the transformation of normal cells into frank malignancies.[12–15] However, a thorough evaluation (which includes giving due consideration to dose–response relationships and the existence of thresholds) of the genotoxic potential of compounds in question, with an understanding that high quality *in vivo* data trumps *in vitro* data, is warranted. Irrespective of the cancer issue, we do not want to expose humans to significant levels of genotoxic compounds.

Conclusions

It is time to say goodbye to the standard two-year rodent bioassay. The two basic assumptions underlying the bioassay are: (1) rodent carcinogens are human carcinogens; and (2) results obtained at high doses are indicative of results that will occur at lower, environmentally relevant, doses are not correct. In this context, it is ironic to note that the first author of a key paper which describes an algorithm for low-dose extrapolation of carcinogenicity data is an individual named "Guess".[54] While a few, primarily genotoxic, compounds which are clearly associated with human cancer test positive in the bioassay, there is no science-based, sound foundation for presuming it provides either a valid broad (across different chemicals) capability for discerning potential human carcinogens or a valid starting point for making human risk assessment decisions. However, compounds which test negative under the extreme conditions of the rodent bioassay are highly likely to not be capable of causing cancer in humans. Though I do not see a compelling reason to perform the rodent bioassay to focus on the negative data. In recent years there have been a number of proposals to move away from the standard bioassay. In particular, the decision tree devised by Cohen (2017), which places an emphasis on dose–response relationships and invites the use of MOA information, provides a sound basis for moving on from the bioassay and towards a rational approach to both identify chemicals which have the potential to cause cancer in humans and take dose–response relationships into consideration in order to place the extent, if any, of the risk they might pose into proper perspective.[17]

Conflicts of interest

The author has no conflict of interest to declare. The drafting of this paper was not funded by any agency in the government, private or not-for-profit sectors.

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Message

From: Dourson, Michael (doursoml) [doursoml@ucmail.uc.edu]
Sent: 8/2/2017 1:08:17 PM
To: Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]
Subject: FW: LNT
Attachments: Environ.Res. Flaws in LNT.pdf

Flag: Flag for follow up

Nancy

So what would the board certified toxicologists in Office of Chemical Safety and Pollution Prevention think about this?

Cheers!

Michael

-- If you can't explain it simply, you don't understand it well enough. Albert Einstein

Risk Science Center
Department of Environmental Health

From: Edward Calabrese <edwardc@schoolph.umass.edu>
Date: Wednesday, August 2, 2017 at 7:46 AM
To: Michael Dourson <doursoml@ucmail.uc.edu>
Subject: LNT

Mike:

See the attached....it is a new paper and the information is also new and potentially very significant.
It is worth reading....please share with your colleagues and students.

Ed

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Flaws in the LNT single-hit model for cancer risk: An historical assessment

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ABSTRACT

The LNT single-hit model was derived from the Nobel Prize-winning research of Herman J. Muller who showed that x-rays could induce gene mutations in *Drosophila* and that the dose response for these so-called mutational events was linear. Lewis J. Stadler, another well-known and respected geneticist at the time, strongly disagreed with and challenged Muller's claims. Detailed evaluations by Stadler over a prolonged series of investigations revealed that Muller's experiments had induced gross heritable chromosomal damage instead of specific gene mutations as had been claimed by Muller at his Nobel Lecture. These X-ray-induced alterations became progressively more frequent and were of larger magnitude (more destructive) with increasing doses. Thus, Muller's claim of having induced discrete gene mutations represented a substantial speculative overreach and was, in fact, without proof. The post hoc arguments of Muller to support his gene mutation hypothesis were significantly challenged and weakened by a series of new findings in the areas of cytogenetics, reverse mutation, adaptive and repair processes, and modern molecular methods for estimating induced genetic damage. These findings represented critical and substantial limitations to Muller's hypothesis of X-ray-induced gene mutations. Furthermore, they challenged the scientific foundations used in support of the LNT single-hit model by severing the logical nexus between Muller's data on radiation-induced inheritable alterations and the LNT single-hit model. These findings exposed fundamental scientific flaws that undermined not only the seminal recommendation of the 1956 BEAR I Genetics Panel to adopt the LNT single-hit Model for risk assessment but also any rationale for its continued use in the present day.

1. Introduction

On an August day in 1946 at the University of Rochester, the geneticist Ernst Caspari reported to his supervisor, Curt Stern, that his experiment on gamma-ray-induced mutations in *Drosophila* had produced results that neither scientist had anticipated. Caspari had fully expected to replicate the 1944 study of Ray-Chaudhuri who had shown that total X-ray/gamma-ray dose was the best predictor of mutational events in mature *Drosophila* spermatozoa. This study was important because it provided evidence that the dose response for ionizing radiation-induced mutations was cumulative, irreversible, independent of the dose rate, and linear at low doses. However, in contrast to Ray-Chaudhuri's earlier results, Caspari found that the dose rate rather than the total dose determined the dose response and that for a sufficiently low dose rate no response could be detected, revealing an apparent threshold response for gamma-ray-induced mutations. The Caspari studies were part of the Manhattan Project and as such were generally stronger scientifically than those of most independent, single researchers, including the studies of Ray-Chaudhuri. For example, as compared to the Ray-Chaudhuri study, the Caspari study had a more robust design, used larger numbers of flies, exerted superior oversight and guidance, applied better quality control, and assessed a notably lower dose and dose rate, amongst other factors (Calabrese, 2011; Caspari and Stern, 1948).

The Caspari data produced considerable alarm and concern throughout a radiation genetics community fearing that the LNT single-hit model might not now replace the currently accepted threshold dose-response model. The reactions of some leading radiation geneticists to the Caspari findings have been documented and published (Calabrese, 2011, 2015a). One especially succinct and notable response came from Milisav Demerec who asked of Caspari, "What can we do to save the hit model" (Calabrese, 2011). The above cited papers document prestigious members of the radiation geneticist community, including Nobel Prize recipients and an expert Genetics Panel of the U.S. National Academy of Sciences, who saved the "hit" model by obfuscating, deceiving and misrepresenting the scientific record. Sadly, these ideologically based and scandalous manipulations of the scientific record (Calabrese, 2015a, 2015b, 2017a) proved highly successful; they quickly infiltrated and affected the highest levels of regulatory policy in the US Government where they still remain in place today.

The present paper examines a surprisingly under-researched and critical aspect of the cancer risk assessment process by seeking answers to the

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following questions: (1) what was the genesis of the LNT Single-hit Hypothesis, (2) what was its scientific basis and (3) how and when was it validated? To address these questions it is necessary to re-examine the discovery of X-ray-induced heritable mutations by Hermann J. Muller, to assess the scientific validity of Muller's findings and interpretations and, finally, to understand how the scientific community reacted to and accepted Muller's personal account of his results. Acceptance by the scientific community essentially affirmed a biological basis for the Single-hit Model and, at the same time, reassured the public of its "legitimate" use in the assessment of cancer risk for decades to come.

Lewis J. Stadler, a plant geneticist of comparable standing to Muller within the radiation geneticist community, challenged Muller's 1927 claim of having induced gene mutations with X-rays and, as a result, initiated a fierce data-driven debate that only ended much later with the 1954 death of Stadler. Although Muller's views would eventually predominate, the experimental data and arguments offered by Stadler were formidable. The 25-year Muller-Stadler debate generated a unique scientific record that has never been used to re-evaluate the LNT single-hit model and, therefore, validate its application in the process of cancer risk assessment. This is quite astonishing considering that the LNT single-hit model would emerge primarily from the decades-long Muller-Stadler debate. Although Muller's views eventually prevailed, the questions raised herein are why they prevailed, should they have prevailed and, if not, then what does this mean for us today? This assessment concludes that the arguments of Stadler were based on stronger data and were more scientifically persuasive than were Muller's. Yet, in spite of Stadler's superior arguments, his views would show little penetration and have no impact on risk assessment policy.

The story of the LNT single-hit model is complicated and has numerous twists and turns. In the end, however, this new assessment enables a better clarification and understanding of the LNT single-hit model. It challenges the nearly universally accepted view that Muller, in his key groundbreaking experiments of 1927 (Muller, 1927a), had induced the "artificial transmutation of the gene" (i.e., induced "true" mutations) with high doses of X-rays. For it is now known that the vast majority, perhaps all, of the heritable phenotypic changes that Muller had observed were due to substantial non-gene alterations consisting mostly of small, large and massive chromosomal deletions. Furthermore, the LNT single-hit model made critical assumptions about mutations and their dose and dose-rate dependencies that were incorrect. The LNT single-hit cancer risk-assessment model, as it turns out, is the product of a sequence of historical myths about Muller's Nobel Prize findings. Amazingly, these myths mesmerized the scientific community even in the face of objective contemporary scientific challenges that have since been validated with modern analytic tools for mutation assessment. The historical findings presented herein demonstrate that the LNT single-hit model is scientifically flawed and also that the cancer risk assessment policy, which is based on the LNT single-hit model, is equally flawed.

2. Muller and the discovery of X-ray induced mutation

On July 22, 1927, Muller published a paper in the journal *Science* making the then striking claim that X-rays can induce heritable mutations in fruit fly gonads. However, because such a groundbreaking paper was published without data, some scientists, including his Ph.D. mentor T. H. Morgan (Carlson, 1981), were rightly skeptical and questioned whether Muller had the data he claimed.[1] Apparently, the extraordinary editorial decision at *Science* to publish such a significant paper without requiring data has yet to be explained some 90 years later. Two months after his *Science* publication, at the 5th International Genetics Congress in Berlin, Muller finally showed the world convincing evidence that X-rays induced heritable mutations in fruit fly gonads.

Muller (1927a, 1928a) used the method of sex-linked recessive lethality in the genomes of male and female fruit flies to demonstrate X-ray-induced mutations. To distinguish his findings from those of researchers who induced heritable "chromosomal" changes, including various types of aberrations (e.g., deletions, insertions), non-disjunction and other gross chromosomal alterations, Muller emphasized that he had artificially altered the "gene", a much smaller unit of inheritance than the "chromosome". In his first experiment Muller produced an apparent threshold dose response using four doses and a standard *Drosophila* strain. Switching to his newly developed C1B strain and reducing the number of doses to two (i.e., doses #2 and #4), Muller showed that mortality was enhanced by some 150 fold at the highest dose when results from the two experiments were combined. Although the findings were limited, the data suggested a non-linear (square root), dose-response function (Muller, 1927a). A broad spectrum of visual phenotypic changes was also reported, indicating that the X-ray treatment had affected many genes.

While it is generally believed that Muller had won the race to be first in inducing gene mutations by a scant three months,[2] narrowly beating several groups of plant geneticists from the US, including Lewis Stadler[3] from the University of Missouri and Thomas Goodspeed from the University of California at Berkeley, he may actually have come in second. Charles Stuart Gager (Brooklyn Botanical Gardens) and Albert Blakeslee (Carnegie Institute for Research) were the first researchers to report in the *Proceedings of the US National Academy of Sciences* in January of 1927 that ionizing radiation induced gene mutations in Jimson Weed (Datura).[4,5,6] Their paper revealed that radiation induced

[1] Carlson (1981) (pages 148–149) stated that T.H. Morgan believed that Muller had finally "hung himself", having recalled an earlier incident in which Muller overstated that the *Drosophila* spontaneous mutation rate for the X-chromosome was 1%. Morgan held there was little chance to escape the apparent exaggeration of the so-called 15,000% increase in X-ray induced mutation rate. The statement of Morgan reveals that he was reluctant to trust the assertions of Muller while also having reservations about his character. The lack of professional and perhaps personal regard of Muller may be seen when Morgan sponsored Sturtevant for membership in the NAS, rather than Muller, some five years after Muller's discovery of X-ray induced mutation (Carlson, 1981, page 174).

[2] Stadler presented his mutational evidence in Nashville, Tennessee, at the AAAS meeting in December of 1927. Stadler had treated barley seeds with X-rays in 1926, collecting the subsequent seeds later that season. These seeds were planted in the next growing season (1927). The findings revealed evidence of mutation. While 1300 tiller controls showed no trans-generational phenotypic changes, the X-ray treatment yielded 14 mutations in 1200 tillers (Carlson, 1981, page 151).

[3] Rhoades (1984) has emphasized that Muller and Stadler had a different goal/approach to their respective X-ray induced mutation research. In the case of Stadler, he employed X-rays to induce mutations to study gene structure and the nature of gene mutation alterations. Thus, Stadler focused on mutations at various loci that mediated specific phenotypic functions. In contrast, Muller focused on the spectrum of induced mutational changes.

[4] While Gager and Blakeslee would assert the primacy of their gene mutation discovery over the next 15 years (Blakeslee, 1940, 1942; Gager, 1931, 1936), they acknowledged that Muller had developed a method for generating large numbers of mutations. As primacy was a critical issue for Muller, he would routinely attempt to discredit the claims of others, including Gager and Blakeslee. In retrospect, the assertion of Gager and Blakeslee has withstood the criticism of Muller (Campos, 2015). However, the assertions and claims of Gager and Blakeslee would generally be ignored while Muller's findings would capture the excitement of the scientific community (Campos, 2015).

[5] Stadler (1936) would recognize the primacy of Gager and Blakeslee and their mutational findings. He specifically stated that: "Before the publication of Muller's results, several investigations of the genetic effects of penetrating inductions in plants were in progress and some positive results were obtained. The work of Gager and Blakeslee (1927) is especially noteworthy in this connection". Of the 113 progeny plants from parents treated with radium, 20 were chromosomal variants. Amongst these chromosomal variants two were heterozygous for new recessive genes. Even though the number of gene mutations was small, Stadler (1936) considered the findings of considerable importance. The publication of the Gager and Blakeslee (January 14, 1927) preceded the Muller note in *Science*, (i.e., July 22, 1927) which lacked any data, by more than six months. Despite his recognition and appreciation of the findings of Gager and Blakeslee (1927), Stadler (1954) would argue that they too fell short of "conclusive" proof due to inadequate statistical evidence and failure to show that the genetic changes reported were the result of the treatment and not to some type of genetic irregularity of the treated strains.

[6] Muller (1928a) would present his findings at the Fifth International Genetics Congress in Berlin (September 1927). At the same Congress Blakeslee (1928) would also

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a few apparent "real" gene mutations. Muller's principal achievement, on the other hand, was finding a methodology to enhance the production of what were believed to be unequivocal gene mutations. Conflating the methodology with the discovery helped create the notion and then the myth (Campos, 2015) that Muller produced the first artificial transmutation of a gene as well as the technique to vastly increase their production. More extensive assessments of the dose response would follow and expand Muller's basic observations in support of a linear dose response (Oliver, 1930; Hanson and Heys, 1929).

3. When is a mutation a "True" mutation?

Despite the consistently reproducible findings that X-rays could induce heritable mutations, questions began to arise as to whether Muller had actually induced "true" mutations.[7,8] Shades of doubt surfaced at the University of Missouri with Lewis J. Stadler's detailed cytogenetic evaluations of the effects of X-rays on maize. In a 1931 publication, Stadler began to draw a distinction between those changes that occur in the individual gene and those that relate to gross chromosomal alterations (e.g., deletions, reduplication or rearrangement of unchanged genes). Although this distinction, as Stadler saw it, was obviously important due to the role of gene mutation in evolution, gross alterations resulting from chromosomal irregularities were just as heritable[9] and therefore of no less importance.

Stadler had a preeminent scientific reputation; he was a highly regarded plant geneticist, a member of the U.S. NAS and the President of the Genetics Society of America (GSA) (1938). As such, his curiosity to differentiate between gene and chromosomal alterations was not only significant but also central to his research program on understanding the nature of the gene. He collaborated with the future Nobel Prize (1983) recipient Barbara McClintock and then recruited her to assess the effects of X-rays on maize chromosomes at the University of Missouri. McClintock's expertise proved immensely valuable, as she had just developed the capacity to markedly improve chromosomal staining of the pachytene stage of prophase.[10] So, McClintock began to assess Stadler's maize chromosomes in the summer of 1931. In her own words to the Nobel audience (McClintock, 1983) she described her research in Stadler's laboratory:

> "...It was to observe the effects of X-rays on chromosomes of maize that brought me to the University of Missouri in 1931.... Irradiated male gametes in Stadler's experiments carried wild-type alleles of known recessive mutants...An X-ray induced mutation altering the expression of the wild-type allele of one of these recessives should be identifiable in an individual plant derived from such a cross....-following my arrival at Columbia in June 1931, plants were selected where chromosomes were to be examined." (Meiotic stages were examined to look for certain kinds of events that might be responsible for the expression of recessives)...None of the recessive phenotypes in the examined plants arose from "gene mutation". Each reflected loss of a segment of a chromosome that carried the wild-type allele, and x-rays were responsible for inducing these deficiencies. They were also responsible for producing other types of chromosomal rearrangement, some of them unexpectedly complex...."

The collaboration between Stadler and McClintock affected Stadler's understanding of the problem almost immediately. His presentation in 1932 at the Sixth International Congress on Genetics at Cornell University challenged Muller's assertion of having induced gene mutation.[11] Furthermore, in his 1933 annual report (Stadler, 1933) to the National Research Council, Stadler stated that "investigation of the physical nature of the induced 'gene mutations' indicates that these are chiefly or wholly the result, not of changes within the gene, but of extragenic alterations of various kinds". Table 1 provides quotations from several of Stadler's publications (from 1932 to his final publication in 1954) that challenged Muller's assertions that he had observed "true" gene mutations. With the arrival of new analytic tools since the mid-1970's, such as the Southern blot (Southern, 1975), PCR (Mullis and Faloona, 1987) and precise DNA sequencing technologies, others in the research community extended this debate with much greater specificity and clarity.

Stadler's views on the nature of X-ray-induced genetic damage seemed to crystallize following new developments in cytological techniques that included Painter's salivary gland method (Painter, 1933, 1934a, 1934b, 1934c) in *Drosophila* and McClintock's earlier pachytene technique in maize (McClintock, 1929a, 1931) and their applications in the analysis of X-ray induced mutations.[12] As a result of the applications

(*footnote continued*)
summarize the mutation findings of Gager and himself as published earlier (Gager and Blakeslee, 1927). In the case of Muller, he seized the moment and captured the fascination of his audience; whereas Blakeslee would submerge/underplay his novel mutational findings in an article obscurely entitled "The Genetics of Datura". The failure of Gager and Blakeslee to promote their key findings was in striking contrast to the demonstrable Muller. Elof Carlson (1981), Muller's biographer (page 151), incorrectly stated that Blakeslee confirmed the X-ray-induced mutation results of Muller within a year after Muller's report. As documented here, Gager and Blakeslee (1927) published their data a year ahead of Muller's (1928a, 1928b) data in the conference proceedings and six months prior to Muller's data-free *Science* paper.

[7] A key aspect of the Muller story was his ability to redefine the contemporary view of what constituted a "real" mutation (Campos, 2015). Prior to Muller's engagement on this issue the generally accepted view was that inherited phenotypic changes were considered mutations. This could occur via alterations with the chromosome and/or gene. Muller would be unrelenting in his quest to drop chromosomal inheritance from the definition. So focused was Muller on winning this conceptual and terminological argument that he utilized the title "artificial transmutation of the gene" to describe the mutation he believed he had induced by X-ray treatment (Muller, 1927a).

[8] Prior to the discovery of McClintock, cytological evaluation of radiation-induced chromosome aberrations/changes were limited to the condensed metaphase chromosomes of somatic cells. In such a setting it is only possible to identify gross effects, including changes in chromosome number or large translocations or losses of chromosome sections. McClintock's contribution involved the capacity to study thin-stranded chromosomes of early prophase of meiosis, permitting a far more precise documentation of radiation-induced changes than had occurred previously. In maize, the chromosomes are approximately 10-fold longer during prophase than they are at metaphase and the homologous chromosomes are closely aligned. Thus, the loss of even a very small part of the chromosome is easily observed. Likewise, the nature of translocations/damage is therefore markedly more refined and could then be used with greater precision to assess chromosomal alterations at far higher resolution and at far lower doses of radiation.

[9] For Stadler (1954) the error of Muller in the debate was that he combined his observed effect (i.e., heritable phenotypic changes) (Step 1) with an interpretation that the observed effects were due to a specific mechanism (Step 2). Stadler (1954) emphasized that these two steps be kept distinct since Step 1 is a specific contribution to scientific knowledge, while the second is based on an inference/interpretation. By combining the two, the scientific community may confuse what it knows with what it thinks it knows. Stadler (1954) asserted that the belief that the frequency of gene mutation may be profoundly increased by X-ray treatment was "an illusion of this kind". The problem emerged from an insistence that X-rays induced gene mutations, since the mutants that are formed satisfy all the criteria of gene mutations (e.g., heritable, reversible) and that these mutants represent qualitative alterations in specific genes, since that is the definition of a gene mutation. In effect, Stadler argued that the error resides in using the same term for two concepts. This terminological combination then becomes a substitute for a bonafide scientific mechanism of proof - the trap to which the mutation field was vulnerable.

[10] The technique of McClintock (1931) for the assessment of maize chromosomes in prophase of meiosis made possible the cytological identification of minor structural chromosomal changes with a precision far exceeding that possible in studies using condensed chromosomes. The breakthrough methodological development of McClintock (1929a, 1929b, 1931) forced Stadler to realize that viable deficiencies were able to account for some of the apparent/presumed gene mutations induced by X-ray treatment. Thus, for Stadler (1931) it was "now clear that many of the variations identified by their genetic behaviors as gene mutations are due to mechanical alteration analogous to the grosser chromosomal aberrations.".

[11] Note that Stadler's presentation at the Sixth International Congress on Genetics was one of only three Plenary addresses on mutagenesis (Haynes, 1938). With Muller in the audience, Stadler had clearly offered a major public challenge to Muller's interpretation.

[12] Painter developed an application of the acetocarmine smear method that had been employed by cytogeneticists working on maize chromosomes (Glass, 1990). According to Carlson (1981), Muller first learned of Painter's (1933) advance while in Leningrad. He immediately understood its significance for the assessment of X-ray-induced small rearrangements and chromosome breaks.

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Table 1
Stadler's Challenge to Muller.

Stadler, L.J., 1932. Proc 6th Int. Cong Genet. 1, pp. 274–294 (Stadler, 1932)
"To state that an induced variation is a gene mutation is not to explain it but merely to label it."
Page 274–275
"We do not demonstrate that a chemical change has occurred; we simply infer, since no mechanical explanation can be found, that the variation must be due to this invisible mechanism."
Page 275
"We may define mutation as a transmissible change in the gene. But we identify mutation by experimental tests, and these tests are not such as to establish conclusively, in specific instances, that a change within the gene has occurred."
Page 275
"In effect, any Mendelizing variation which cannot be shown to be due to a change involving more than one gene is a mutation."
Page 275
"Thus the working definition of mutation necessarily differs somewhat from the ideal definition. It is this working definition which must be considered in generalizing from the experimental evidence. The mutations experimentally known may include not only variations due to alterations with the gene but also variations due to losses of genes or groups of genes, to additions of genes or groups of genes, and possibly also to changes in the spatial relationships of genes to one another."
Page 275–276
" ...the parallel between induced mutations and induced chromosome derangements is striking."
"The frequency of induced mutation is directly proportional to dosage (Stadler, 1928, 1930). So are the frequency of deficiencies induced by treatment of pollen (Stadler, 1931) and the frequency of endosperm mosaics induced by treatment shortly after fertilization (Goodsell, 1930).
Page 282
"The induced mutation also may involve various types of germinal change, for there are several conceivable ways in which irradiation may so modify a chromosome or to cause the appearance of a Mendelizing variation."
Page 291
" ....most of the induced mutations in plants are due to various extragenic alterations, chiefly non-lethal deficiencies. This is true in spite of the fact that the variation record as mutations in the experiments with plants are exclusively the "visible"...: "
Page 291
"The frequent occurrence in Drosophila of mutations at the points of breaks of the chromosomes suggests that the point mutations in this species as well as in plants may be largely of mechanical origin. This association of induced mutations with chromosome breakage does not necessarily exclude the possibility that the mutations are intra-genie changes, for it is conceivable that some change within the gene may be the cause of the break, and that in some instances the gene may continue to function as a gene after the change has occurred. But the possibility that some mutant characters may be due merely to the changed spatial relations of gene to another ("position effect") can not be disregarded."
Page 292
"....the occurrence of reversion is not proof that the original mutation could not have been due even to a deficiency."
Page 292
Source: Stadler, L.J., 1936. Duggar Editor, pp. 1263–1280 (Stadler, 1936)
"Mutation implies a hypothetical change within the individual gene, or at any rate a change affecting no than more than a single gene. But since the individual gene is invisible, the identification of a germinal variation as a mutation is a matter of inference we assume that it is due to a change in the gene."
Page1263
"Since chromosomal alterations (particularly small deficiencies) are known to simulate gene mutation in some instances, and since irradiation regularly induces chromosomal alterations (including deficiencies), there is obvious ground for the suspicion that the apparent gene mutations induced by irradiation are in fact due to extragenic alterations."
Page 1275
Source: Stadler, L.J., 1954. Science 120(3125), pp. 811–819 (Stadler, 1954)
"The purpose of experiments with gene mutation is to study the evolution of new gene forms. The techniques for studying gene mutation are, therefore, designed to measure the frequency of these changes in the genes. But a change in the gene may be recognized only by its effects, and it soon became clear that various extragenic alterations might produce the effects considered characteristic of typical gene mutation (10)".
Page 813
"But there was no test to identify mutations due to a change within the gene; it was simply inferred that the mutants that could not be identified as the result of specific mechanical causes were, in fact, due to gene mutation in the ideal sense (11)."
Page 813

Table 1 *(continued)*

"Another simplifying assumption was that mutant changes in gene effect must represent some transformation of the gene itself rather than some alteration affecting its expression. It was this assumption that made the demonstration of x-ray-induced mutation and reversion of the same gene seem critical proof of the induction of intragenic alterations. The assumption was definitely contradicted by the evidence of position effect. This evidence showed conclusively that a mutation did not necessarily represent a transformation or loss of the gene concerned; instead, it would be the result of a translocation affecting the expression of the unchanged gene."
Page 818

of such new cytological techniques in experimental studies, it was found that most of the X-ray-induced heritable changes produced at high doses were due to gross chromosomal aberrations or extragenic changes. In fact, the cytological advance by Painter (1934a, 1934b, 1934c) would profoundly affect the debate between "gene" and "chromosomal" mutations. So significant was this methodological development that *Science* published a brief summary note by Painter (1933) prior to his three papers in *Genetics* in 1934 (Painter, 1934a, 1934b, 1934c). Just as the earlier advances by McClintock (1929a, 1929b) had affected Stadler's view, so too did Painter's application influence the view of the *Drosophila* research community.

According to Carlson (1981), by the end of 1931 Stadler's criticisms of Muller's gene mutation claim seemed to be causing Muller some distress. Upon returning in December of 1931 from a conference of the American Society of Naturalists in New Orleans, Muller was showing stress on multiple fronts. His claims of radiation-induced mutations were being more broadly challenged and his once productive relationship with Patterson was now becoming one of confrontations and lack of trust. Likewise, his collaboration with the cytogeneticist Painter fell apart in 1929, again amidst distrust and professional jealousy. During this same period, Morgan would bypass Muller for an NAS nomination, favoring his former fellow graduate student, Sturtevant.

So penetrating and persuasive were the criticisms of Stadler that Muller's own convictions in the "artificial transmutation of the gene" were beginning to erode. Campos (2015) (see page 327, footnote 168) confirmed "it was Muller's discussions with Stadler and others that would ultimately cause Muller to begin to question the viability of a firm distinction between point mutations and exceptionally minor chromosomal rearrangements, as well as the nature of the position effects." Stadler's criticisms would follow Muller on his journeys from Texas to Germany to the Soviet Union and back to the U.S. Eventually Muller would recognize that ionizing radiation produced a vast abundance of major chromosomal aberrations at high doses. More importantly, his colleague Mark Belgovsky discovered that even small chromosomal rearrangements were readily induced by ionizing radiation and displayed a linear dose-response relationship (Belgovsky and Muller, 1938). These findings were of significance since they indicated that most of the induced gene (i.e., real) mutations at these lower doses were small-modest deletions or inversions.

Despite all the evidence that was being amassed against his 1927 "gene" mutation assertion in *Science*, Muller would cling to this idea because he thought that the mechanism of evolution could not involve heritable chromosomal mutations. Although the logic is sound that minor, intragenic mutations are the tiny engines of evolution, Muller was wrong in applying this logic to interpret the effects produced by high doses of X-rays.

In 1932, Muller left his position at the University of Texas (where Painter was a professor) to research with Timofeeff-Ressovsky in Berlin. At the end of about two years (and about the same time that Painter completed the development of his cytogenetic staining technique in Texas), Muller moved to the Soviet Union to direct a genetics research program. Muller continued to promote his arguments on gene mutation (Muller, 1935, 1939a, 1939b) in both Berlin and Leningrad/St. Petersburg and Moscow. Meanwhile, the cytogenetics tools developed by

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Painter, his former colleague, were being established and used to acquire evidence that challenged the acceptance of X-ray-induced gene mutations at high doses, representing a formidable obstacle to Muller. Painter's publications would also undermine the research of Timofeeff-Ressovsky et al. (1935) who were in the final stages of integrating two concepts: the LNT single-hit model and the mutation of "genes" with high doses of X-rays. Essentially, the new cytogenetics techniques of McClintock and Painter were yielding more refined data that was eroding support for the LNT single-hit model and, at the same time, shifting the discussion toward Ray-Chaudhuri's ideas of total-dose and linearity to dose rate and nonlinear threshold.

Meanwhile, Stadler undertook an extensive research program that evaluated X-ray-induced alterations at specific gene loci in maize, including possible changes within the gene itself. These experiments included visible assessments of chromosomal aberrations, transmission of chromosomal mediated changes through male/female germ cells, viability of the homozygote, and mutation reversion back to the non-mutated gene. Throughout his entire 25-year research effort, it is highly significant that Stadler would never find experimental evidence to support X-ray-induced gene (i.e., "real") mutations.

As research progressed, several developments emerged as significant. One involved the discovery of a position effect in *Drosophila*. This occurred when an X-ray-induced chromosomal aberration affected the translocation of a gene from one locus to another. With movement to the new locus, the transferred gene was found to affect activity in neighboring gene(s), even though the gene(s) itself was unaltered in its composition. If the transferred gene were physically returned to its original locus the organism would then revert back to its normal wild type. Interestingly, this genetic behavior was considered to have satisfied the criteria for reversibility, despite the fact that a "gene" had not been truly mutated, i.e., no change had occurred in its sequential nucleotide composition.

A second research development emerged as significant. It involved drawing the same conclusion (that X-rays induce gene mutations) from numerous studies based on an unproven and untested assumption (that phenotypic change equals compositional gene change). That is, it was assumed that an X-ray-induced phenotypic change could only result from the compositional alteration of a gene and that no verification of that assumption was apparently necessary. Missing was a detailed proof by the investigator that an X-ray-induced phenotypic change produced a corresponding alteration in the composition of a gene related to that specific phenotype. Also missing were more critical and detailed reviews of the publications to prevent uncontested and unproven assumptions from leading to false conclusions, wide acceptance and eventually erroneous dogma masquerading as theory. According to Nuffer (1957), a large number of such insufficiently assessed publications led to the belief that X-ray treatments were inducing gene mutations. However, when specific cases were examined more closely, some type of chromosomal aberration usually explained that which was assumed to be a gene mutation.

4. Muller's arguments to support the gene mutation hypothesis

To preserve the uniqueness and significance of his favored concept—the artificial transmutation of the gene, Muller published an 82-page paper in 1930 with his University of Texas colleague J.T. Patterson (Patterson and Muller, 1930). The assessment centered on whether the X-ray-induced trans-generational phenotypic changes in *Drosophila* were due either to losses (deletions), rearrangements of portions of chromosomes or to the so-called "progressive" point-like genetic changes that Muller assumed to drive evolution. This article reflected patterns in Muller's professional life; that is, he marshaled as much evidence as possible, presented it in excruciating detail, and never compromised on an essential point (i.e., classic example of a "Mullerism"). Also reflected in the paper was Muller's own perception of the fundamental weakness in his interpretation of a gene mutation, which occurred a year prior to the emergence of Stadler's (1931) questioning of this basic tenet.[13]

Patterson (Muller's Department Chair at UTexas) and Muller (1930) argued that the induced gene mutations are generally the direct result of relatively minor local electronic "hits" rather than more destructive processes (i.e., the gene versus chromosome mutation debate). The reasons for their stated beliefs included: (1) the general randomness and specificity of induced phenotypic changes (i.e., Muller's gene mutations); (2) identical phenotypes were independently affected; (3) phenotypic changes were dose dependent; (4) numerous toxic chemicals were not effective in producing such changes and (5) "most important of all, probably, is the fact that a direct and simple proportionality has been shown to exist between the frequency of the induced mutations and the amount (energy) of the radiation absorbed." They cited Hanson and Heys (1929) and Oliver (1930) to support this conclusion and then stated that "there is no indication in the results of any lower critical intensity, or threshold value, beneath which there is no (or a relatively lesser) effect." They concluded with "All these facts and considerations converge to indicate strongly that the mutations are rather direct effects of individual quantum-absorption electron hits…". Several years later, Oliver (1934), who received his Ph.D. in 1931 (Oliver, 1931a) while working under the direction of Muller, further expanded this list of arguments in favor of gene mutations. He indicated that X-ray-induced mutations often seem to appear similar to spontaneous mutations, an issue that would be re-examined at each stage of technological advance. Within this framework Oliver (1934) noted that the proportion of lethals to visibles or of dominants to recessives is similar to those reported for the spontaneous mutation rates (Morgan et al., 1932; Muller, 1930a, 1930b). Further supporting this perspective were observations that the frequently mutating genes in the spontaneous state are the same genes that frequently mutate when treated with X-rays.

In addition to this list of arguments in support of the gene mutation hypothesis, Muller would rely most heavily on two other linked arguments: (1) the occurrence of reverse mutations and (2) the multi-generational inheritability of mutations. Muller argued that if a mutation could be reversed then the gene had not been destroyed or deleted. Such reasoning led Muller and his supporters to conclude that "real" gene mutations are induced by the direct activity of X-rays.

Early reaction against such reasoning surprisingly came from one of Muller's own students. In the conclusion to his 1931 dissertation, Oliver (1931a) stated, "it is not possible to produce gene mutations without producing gene rearrangements". Later, Oliver also observed that X-ray-induced "inversions and translocations were produced in direct proportion to the intensity of treatment" (Oliver, 1934), a similar observation that had been previously used to support gene mutations. Oliver's proportionality observation for the induction of gross chromosomal damage proved to be consistent with later observations of other researchers (Timofeeff-Ressovsky, 1939; Demerec, 1937). Oliver (1934) also reported that translocations are induced with X-ray treatment of *Drosophila* sperm (Muller, 1928a, 1927a, 1927b; Weinstein,

[13] Within but three months of his momentous presentation in Berlin in September 1927 demonstrating X-ray induced heritable mutations, Muller (1927c) read a paper at the AAAS Conference in Nashville, Tennessee. During this presentation he addressed for the first time the possibility that his "gene mutations" were not gene mutations after all, but the results of chromosomal aberrations. He defended the gene mutation hypothesis by introducing the "reverse" mutation explanation, which he suggested provided a mechanism for evolution. Four months later Muller read a paper before the U.S. NAS (April 24, 1928) (Muller, 1928b) in which he raised the question "Are the X-rays merely punching holes in the chromosomes, or, more precisely stated, simply causing losses or inactivation of genes or gene parts? **If so, the usefulness of the X-ray, both from a practical and from a theoretical standpoint in biology, would be seriously limited** (emphasis added)." Muller therefore clearly understood that his X-ray-induced transgenerational phenotypic changes would only be significant when due to intra-genic/small point mutations. Muller stated that he was challenged to confront this issue by his former graduate student colleague and lifelong friend, Edgar Altenburg. Thus, the seeds of self-doubt on this issue preceded Stadler's own dissatisfactions with Muller's gene mutation interpretation by some three years.

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1928) with a frequency equal to that of "gene" mutations (Muller, 1928a; Muller and Altenburg, 1928; Muller and Altenburg, 1930; Oliver, 1931b; Whiting and Bostian, 1931). In his own research, Oliver (1934) recorded 21 lethals, 16 crossing-over conditions with most being inversions, and 17 translocations occurring in 105 subjects from the high dose parents (t_{16}, 4560 r), and 18 lethals, 13 crossing overs and 10 translocations, all with the X-chromosome in 400 offspring of parents treated with a lower dose (t_4, 1140 r). On page 391, Oliver (1934) stated, "it is probable that all lethal mutations are not point mutations, but that some are connected to, and due to chromosomal aberrations (Muller, 1927a; Weinstein, 1928) of definite deficiencies (Patterson, 1932)." These lethals, which are connected to chromosomal aberrations, increase rapidly with high doses (Oliver, 1930, 1932). On page 392, Oliver (1934) stated, "that X-rays increase the frequency of inversions as well as lethal mutations in *Drosophila*", as has been reported by Serebrovsky (1929), Muller (1927a, 1927b, 1928b), Oliver (1930) and others. The direct proportionality of the frequency of inductions of sex-linked inversions and translocations for *Drosophila* was determined by Oliver (1931b, 1932) and deficiencies in maize by Goodsell (1930) and Stadler (1931)" (page 392). It is curious that Oliver (1934)—Muller's former student—was the one who adopted such an active role in criticizing X-ray-induced gene mutations. It should be noted that Stadler (1932) also reported similar criticisms at the Sixth International Genetics Congress, suggesting that an alternative to Muller's view of discrete X-ray-induced "gene" mutations was emerging and growing stronger.

Oliver's dissertation (1931a) revealed that Muller's 150-fold increase in the frequency of lethal gene mutations at the highest dose was a significant overestimation. Oliver calculated that other damage-related endpoints, such as crossing over and translocations, could easily have accounted for a large proportion of the sex-linked lethality that had been attributed to gene mutation. Furthermore, Muller's estimation of "real mutations" would have decreased even more if Oliver (1931a) had included damage due to deletions and if Muller's control mutation rate had not been so low ($< 1/1000$). Finally, because the control background incidence of sex-linked recessive mortality ranged broadly over an order of magnitude (Muller, 1927a), it is possible that the variability of controls could have skewed Muller's estimation significantly upward.

Although sex-linked mortality probably increased, the estimated "massive" increase was probably not due to "real" gene mutations, as the scientific community was led to believe. This perspective is important because much of the excitement associated with Muller's findings was derived from the mistaken belief that Muller had produced large numbers of gene mutations.

5. The reverse mutation: a critical issue in the gene mutation debate

The debate between Stadler and Muller would continue with Stadler seeking the consultation of *Drosophila* experts to test the reverse mutation hypothesis as had been espoused by Patterson and Muller (1930). As a result of Stadler's inspiration, George Lefevre Jr. decided to dedicate his dissertation research to the task of testing Muller's "real" mutation theory. That is, he wanted to know whether Muller's research had produced "true" heritable mutations at the gene level or mostly a spectrum of modest to extremely large genetic lesions.

In reviewing the literature, Lefevre noted that other *Drosophila* researchers had also been intrigued by this question. Based on a series of experiments Timofeeff-Ressovsky (1939) reported 24 distinct reverse mutations at seven X-chromosomal loci (24 reverse mutations/343,183 genes) as well as 16 reverse mutations at six II-chromosomal loci. Earlier, Johnston and Winchester (1934) had induced 24 reverse mutations at eight X-chromosome loci (24 reverse mutations/713,001 genes). The combined rate constant for reverse mutations in these two papers is 1.3×10^{-8} at a dose of 4000-5000r. Amongst the reverse mutations noted by Timofeeff-Ressovsky (1939) and Johnston and Winchester (1934) are those which were affirmed by breeding confirmation tests, along with those mutations, based on phenotype evidence, that were thought to be reverse mutations but which could not be confirmed due to sterility induced by high X-ray doses. This mutation rate constant of ~ 1 mutation per 10^8 genes at 4000-5000r indicates that the incidence of such X-ray-induced phenotypic changes is extremely low. This X-ray inducing dose also frequently exceeded the maximum tolerated dose, with 60–70% sterility. If one assumes that a linear dose response occurs for each type of X-ray at a reverse mutation rate of 1×10^{-8} then a predicted genotoxic effect should decrease by about another order of magnitude at the lower dose of 250-300r. This would be similar to the lowest dose used by Oliver (1931a), but still several hundred-thousand-fold greater than the background radiation exposure.

Around the same time, several other reports also provided evidence of X-ray-induced reverse mutations. For example, Dubinin and Goldat (1936) reported seven reversals for yellow, schaete and scute characteristics, while Griffen and Stone (1939) and Glembozky (1936) also reported the occurrence of similar reverse mutations. Although results with *Drosophila* from the above studies were consistent with the Muller perspective, several other studies failed to induce reverse mutations via X-ray treatment. Kaufmann (1942) reported extensive negative data after radiating 25,000 forked and 12,000 white flies with 4000r. Raffel and Muller (1940) irradiated scute-4 scute-8 recombination chromosomes with 3000r and reported finding no non-scute among 50,000 progeny. Demerec (1938) also reported similar negative findings for reverse mutations of forked and white using approximately 100,000 offspring of recessive parents.[14] In addition to negative findings with respect to induced reverse mutation with X-rays, several effects were found to closely mimic such mutations, requiring enhanced precautions by researchers. For example, Lewis (1945) reported that crossing over between "pseudoalleles" might result in an apparent reverse mutational effect.

According to Lefevre (1949), reverse mutation studies need to preclude the possibility that crossing over could induce reverse mutations. Genetic suppressors can also affect the occurrence of reverse mutations and need to be evaluated as well. A third type of confounder could result from the "contamination" of several markers in the irradiated fly stock that might produce spurious reverse mutations. The presence and accidental co-mingling of other fly stocks possessing the same markers except one was possibly a source of this "contamination" and could lead to making incorrect conclusions about reverse mutations.

Given the above historical foundations, Lefevre offered the following perspective:

> "Because of the several mechanisms that can mimic reverse mutations, the early reports of successful induction of reverse mutation in Drosophila should not be accepted unqualifiedly as evidence of the similarity of induced spontaneous mutation."

For Lefevre, the key question had already been posed and tested by Patterson and Muller (1930). They reasoned that if radiation could induce a recessive mutation and also reverse it back to the normal dominant, then it must follow that the induced mutation could not result from a gross loss of genetic material. Patterson and Muller proceeded to test this argument by performing three successive irradiations and inducing the following three genetic changes: the first irradiation mutated a wild type allele (+) to its "forked" (f) mutant form; the second irradiation reverted the "forked" mutant back to the original wild type; and the third irradiation again mutated the wild type back to its "forked" mutation for a second time. The trans-generational

[14] The failure of Demerec to induce a reverse mutation in such a large number of *Drosophila* would soon lead him to switch to bacteria for his experimental model (Demerec, 1955).

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reversibility of such a specific phenotype (+→f→+→f) convinced Muller that these radiation-induced changes were qualitatively the same as those occurring spontaneously in nature and that they indeed represented "true" gene mutations and were not simply the result of gross chromosomal damage, as Stadler had been claiming.

Contrary to the results of Patterson and Muller, however, was the later dissertation research of Lefevre (1949) who found no evidence of reverse gene mutations when using 5000 r to examine 166,000 recessive X-chromosome loci. Follow up studies on somatic tissue also revealed no reverse mutations in experiments involving normalized 600,000 white loci exposed to 5000r. Together these findings led Lefevre (1950) to conclude that a "serious question is thus thrown on the reliability of the early reports of X-ray induced reverse mutation in *Drosophila*."

That a reverse mutation is proof of a "true" mutation was a debate that would last for nearly two decades in the published record of studies conducted with multiple biological models and inducing agents (De Serres, 1958; Lefevre and Green, 1959; Muller and Oster, 1957; Emmerling, 1955; Nuffer, 1957; Giles et al., 1955; Mottinger, 1970). Technological advancements would eventually supersede the debate and finally provide the insight and proof that Stadler (1932, 1954) was seeking (see the section entitled "The Modern Era"). The issue of reverse mutation would also play a central role in the research of Barbara McClintock who would propose an alternative to Muller's interpretation of X-ray-induced gene mutations.

6. Rejecting Muller: Mcclintock's alternative gene mutation mechanism

The research of Barbara McClintock significantly challenged Muller's (1927a) interpretation of X-ray-induced gene mutation in multiple ways. First, she developed a cytogenetic staining method that revealed detailed structures of maize chromosomes (McClintock, 1929b). In 1931 this new technology enabled Stadler and McClintock to evaluate research used to hypothesize the existence of X-ray-induced gene mutations in maize. Observations with the new cytogenetic techniques visually revealed that the phenotypic changes produced by the so-called gene mutations were actually due to genetic damage at the level of the chromosome (and not the gene) and consisted largely of deletions, translocations and other gross aberrations.

As a result, Stadler re-evaluated his previous findings in barley (Stadler, 1928) and realized he had been wrong in assuming that his observed heritable changes were due to gene mutations, i.e., Muller's so-called "point mutations". In fact, Stadler also soon came to reject Muller (1927a) evidence-free interpretation of his own *Drosophila* work that claimed a gene mutation mechanism was responsible for X-ray-induced heritable changes.

Later, McClintock (1938) would provide experimental evidence to support an indirect mechanism that could explain the trans-generational phenotypic changes induced by X-rays in Stadler's studies on maize. This mechanism became known as the break-fusion-bridge (BFB) process and it could induce massive chromosomal aberrations. It involved initial X-ray-induced chromosomal breakage, followed by the joining of the broken ends of two chromosomes, each with its own centromere. These joined/fused chromosomes would line up at metaphase and then break as their centromeres pulled in opposite directions, generating genetic damage.

While following up on the BFB discovery, McClintock detected another way to produce gene mutations. The newly proposed mechanism involved regulatory control over gene expression but did not involve altering the chemical composition of the gene (McClintock, 1953). She showed that specific genes could change location/position (i.e., transposition) within the genome and, in so doing, alter the expression of new neighboring genes (e.g., from dominant to recessive and vice versa). These observations, which would eventually result in McClintock receiving the Nobel Prize in 1983, provided a mechanism by which reverse phenotypic changes due to a "gene" (similar to those that Muller initially reported in his, 1930 paper with Patterson) could be obtained without altering the chemical nature of that "gene". These findings were significant because they presented a serious limitation to Muller's radiation-induced gene mutation model.

In October of 1948, Muller corresponded with McClintock and admitted to her that he thought her mutable-gene discovery involving transpositional elements (TEs) was a "magnificent achievement" (Comfort, 2001). He also wrote her stating "what a relief it is for us [I presume he actually meant himself] to know that mutable genes are a different class from the ordinary gene mutations after all." (Muller, 1948). Taken together, these statements affirmed that Muller recognized the significance and validity of McClintock's discovery but, at the same time, wanted his own hypothesis on radiation-induced gene mutations to be seen as separate and different from her discovery on mutable genes. There seems to be little question that Muller was concerned with how McClintock's discovery might be used to interpret his hypothesis of radiation-induced gene mutations. According to Comfort (2001), Muller had attended a seminar in October of 1948 on McClintock's work that was given by Salvadore Luria (1969 Nobel Prize recipient), a scientist who was greatly impressed with her work and thought it to be extremely important.[15] In his biography of McClintock, Comfort (2001) suggested that Muller had heard only what he wanted to hear at Luria's seminar because he was trying to "save" his gene mutation theory. However, McClintock, who saw her theory as an alternative to Muller's gene mutation model, would have none of it.[16] Muller's model assumed that a gene is an autonomous genetic unit in a string of genes and that a mutation was a discrete break occurring in a single gene on the string of genes. According to Comfort (1997, page 190), McClintock's "mutable genes, which mutate and then 'unmutate' again, are difficult to explain by this [i.e., Muller's] model." Although McClintock's mutable genes model could perhaps be seen as a complement to Muller's model, McClintock saw her model as superior in accuracy, scope, flexibility and integration and, therefore, as a compelling alternative to Muller's model. In fact, Comfort (1997), page 191) noted that McClintock was approaching the conclusion that "all mutations, including X-ray or chemically induced mutations, were the result of reversible alterations via the process of an integrative genome in which genes acted in suites, controlled by regulatory elements." In a "Memorandum" to Marcus Rhoades in January of 1949 (1949 – APS, see page 191, Comfort, 1997), McClintock would repeat this striking pronouncement and declare that all mutations may be explained by her transposable elements. She further proposed possible biochemical and physical mechanisms to explain her concept of gene mutation. In her 1948 annual Carnegie research report, McClintock (1948b) declared that her new findings "cast doubt on the interpretation that postulates a 'true' gene mutation, that is, a chemical change in a gene molecule". She argued instead that phenotypic changes were due to reversible inhibition and gene modulation. Such a remarkable statement essentially challenged the underlying premise of Muller's recent (1946a) Nobel Prize. McClintock's ideas on this topic would crystallize and were aptly expressed in the opening sentence of the Discussion in her seminal 1953 *Genetics* publication when she wrote, "Mutations need not express changes in genes but may be the result of changes affecting the control of gene action."

In the early 1950's McClintock would publish two major papers on

[15] Luria had obtained a 27-page letter of June 1948 that McClintock (1948a) had sent to S.C. Stephens, a former McClintock colleague, detailing her findings and interpretations.

[16] The "intercepted" 27-page letter to Stephens that Luria based his seminar on and that motivated Muller's congratulatory note to McClintock was superseded only one month later (July 1948b) with McClintock's further challenge to Muller's assumptions of gene mutation when she wrote; "Assumption that mutations occurring frequently are chromosomal changes is more reasonable than an assumption of a change in a gene in a certain direction." (McClintock, 1948c - Odd note September 1948, APS, Box 9, Series V.).

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transposable elements, mutable genes, and her concept of gene mutations (McClintock, 1950, 1951). These papers have become classics, being cited over 600 and 1000 times, respectively, in the Web of Science database, and would have a major impact on Muller's best friend and longtime *Drosophila* collaborator, Edgar Altenburg, at Rice University. Altenburg (1952a, 1952b, 1952c), who appeared to have been unaware of Muller's past communication with McClintock, wrote Muller on August 18 and 30 as well as on October 29, 1952, highlighting the significance of McClintock's findings and strongly encouraging Muller to study McClintock's papers. Altenburg would write Muller again on March 4, 1953, to repeat his previous suggestion and to give Muller a copy of his 25-page review manuscript on McClintock's research findings, a manuscript that had received McClintock's strong support/endorsement (Comfort, 2001). In this same letter Altenburg challenged Muller's two recently written chapters for a comprehensive genetics series edited by Alexander Hollaender. Altenburg stated to Muller, "In my opinion your Hollaender chapters suffer by lack of reference for Dr. McClintock's work. There are at least several places in your manuscript where it could have been profitably considered".

In his detailed 25-page review of McClintock's research on mutable loci in maize (January 1953), Altenburg concluded, "many of the mutants produced by X-rays undoubtedly represent position effects.[17] In this connection it is very significant that chromosome breakage produced by X-rays are in large measure in heterochromatin regions. Therefore, in X-ray treated material, it is not improbable that, in many cases at least, fragments of heterochromatin get inserted next to genes in other locations and suppress their phenotypic expression....". According to Altenburg's review, reverse mutations in *Drosophila* as well as maize, "are undoubtedly due in some cases at least to the release of normal genes from the suppressing effects of foreign heterochromatin adjacent to them, by deletion of the heterochromatin from a locus." This interpretation by Altenburg represented a unique challenge to Muller because his close colleague and collaborator (Altenburg) had apparently become convinced that Muller's interpretation was inadequate to account for the reverse mutation findings and needed to be modified and integrated into the McClintock framework, something that he probably knew would be very hard for Muller to accept (Altenburg, 1953a, 1953b).

Given that McClintock's findings on trans-positional mutator genes were highly relevant to the longstanding debate between Stadler and Muller over X-ray-induced gene mutations, one might have thought that Muller would have eagerly taken an interest in her challenging findings. A check of the Web of Science database on the key McClintock papers (1950, 1951, 1953) reveals that Muller never cited these papers. This lack of engagement with McClintock's findings was likely intentional, and suggests a strong element of strategic and tactical planning to avoid a direct conflict with McClintock.

In the previously mentioned 1948 letter to McClintock, Muller expressed his relief to have learned that her mutable genes were different from his proposed X-ray-induced gene-mutation model. At the time, McClintock emphatically disagreed with Muller's wishful and premature assumption and, ultimately, she would be proven correct. Over many years, McClintock's transposable elements (TEs) were shown to permeate organismal DNA (including Muller's *Drosophila* model) and to markedly affect mutation rates either in the absence or presence of exogenous agents such as ionizing radiation and chemical mutagens. Because TEs are powerful mutagenic factors that affect genomic instability, species have evolved highly diverse molecular strategies (e.g. RNA-induced posttranscriptional gene silencing to mediate the degradation of TE transcripts) to protect their respective genomes from the harmful activities of TEs. The fact that silencing of TEs profoundly affects background- and induced-mutation rates as well as cancer risks (Sturm et al., 2015) indicates that McClintock's model is superior to Muller's and therefore is a much better alternative (MacPhee, 1993; Sobels and Eeken, 1988; Velkov, 1999; Morales et al., 2015; Pesheva et al., 2005, 2008; Dimitrov et al., 2011). Likewise, in an integrated assessment of essential factors that are known to affect aging and lifespan, Sturm et al. (2015) concluded, "ageing is primarily caused by transposition–associated genomic instability."

Drosophila studies have now shown that X-rays can induce McClintock's mutator-gene system in a dose-dependent manner, with the upper part of the dose range overlapping with Muller (1927a) dose range (~1000–3500 r) (Handler and Gomez, 1997; Ratner et al., 2001). Furthermore, it was found that results from early *Drosophila* studies conducted by Timofeeff-Ressovsky et al. (1935) on radiation-induced mutations correlated amazingly well with results from studies conducted 66 years later by Ratner et al. (2001) on the induction of mobile genetic elements. This surprising correlation inspired Ratner's team (2001) to investigate a mechanism that may explain how mobile genetic elements could mediate X-ray-induced mutations. Other studies have shown that X-rays first activate mutator genes and then they, in turn, synergistically enhance the production of X-ray-induced mutations (Sobels and Eeken, 1981, 1987; Margulies et al., 1986, 1989; Balter et al., 1992; Zabanov et al., 1995; Ratner et al., 2001). Such results are particularly germane to the evaluation of the LNT-Single Hit model in that they strongly suggest interactions between the radiation dose, the transposable elements and the production of a range of genotoxic/mutational endpoints. Although the precise nature of such molecular and biophysical interactions still needs to be better defined, it is clear that McClintock's general concept has been strongly supported and that, as she predicted, there exists a mechanistic and sequential relationship between the radiating dose, the activation of TEs and, finally, the phenotypic display of a radiation-induced mutation.

In summary, the findings and interpretations of McClintock strongly challenged the Muller gene-mutation interpretation at multiple levels and in a manner that became progressively accepted by the research community for its scientific and technical accuracy and significance. Given the pristine reputation of McClintock within the genetics community for her scientific credibility, rigor, accuracy and intelligence, it appears that Muller chose not to challenge her alternative model for radiation-induced gene mutations, even when it conflicted directly with the argument on reverse mutations that he needed to defend his own model (Patterson and Muller, 1930).

During the summer of 1955 the NAS created the BEAR Committee, Genetics Panel. It was quite significant that neither McClintock nor anyone with her alternative perspective on gene mutation was invited to be a member of that panel.[18] A review of all transcripts, member reports and meeting summaries provided no evidence that the Genetics Panel discussed McClintock's alternative gene-mutation model. A review of the credentials of the geneticists on the panel revealed that

[17] In 1953 Peter Peterson, a maize researcher at the University of Iowa, reported the discovery of the mutable pale-green (pg) locus in strains of maize experimentally subjected to the atomic explosions at Bikini (Peterson, 1953, 1993). These findings supported the argument of McClintock/Altenburg.

[18] It should be noted that the Director of the Carnegie Institute, Milislav Demerec, McClintock's supervisor, was an invited member of the BEAR I Genetics Panel. In 1946, as mentioned in the Introduction of this paper, Demerec asked Ernst Caspari the key question of "what could be done to save the hit model?" following his striking findings. In addition, BF Kaufmann, the Assistant Director of the Carnegie Institute was also a member of the Genetics Panel. Calabrese (2015b) had earlier shown how Kaufmann misrepresented data that refuted a linear dose response in his oral presentation to the Genetics Panel. Kaufmann would replace Demerec as the Director of the Carnegie Institute in 1960. In a rather remarkable temporal coincidence, Caspari would spend 1947–1949 at the Carnegie Institute. Thus, three *Drosophila* geneticists associated with key aspects of the LNT model worked within the Department of Genetics at the Carnegie Institute with McClintock at the same time she was challenging Muller's gene mutation concept. It is not known whether and to what extent McClintock and these other individuals commented on this fundamental and very sensitive issue. It is also not known whether Demerec affected a possible appointment of McClintock to the Genetics Panel or whether he may have exerted pressure upon McClintock as she was challenging not only the basis of Muller's gene mutation model but also the fundamental underpinnings of the LNT model.

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seven members (Beadle, Cotterman, Crow, Little, Sonneborn, Sturtevant, Wright) had never published a paper on mutations, the key focus of the Panel. Glass had published only two papers on mutations over two decades. Likewise Kaufmann had a weak record of publishing on mutations (4 in total). Essentially all the academic geneticists had received Rockefeller Foundation funding. That the Rockefeller Foundation/NAS would have invited so many geneticists with no experience in the area of mutations and dose responses to serve on this BEAR I Genetics Panel is a striking observation that has not yet been noted in the literature. Yet, the Panel was portrayed as a team of all-stars with a high level of expertise and experience in the induction of mutations by radiation and in their dose-responses. Furthermore, the Panel actually served to validate and promote Muller's gene mutation model, which contrasted significantly with McClintock's gene mutation alternative. To make matters even more untenable, the evaluation by the Genetics Panel was undertaken in the presence of Muller, whose dominating personality, ideological conviction to his model and profound and obvious conflict of interest should have disqualified him as member of the Panel.

It's curious that two years later the NAS would constructively engage or, depending on your point of view, distract McClintock on a more than two-decade international involvement on the evolution of maize (Comfort, 2001). The activity demanded prolonged periods in South America and an intense amount of her time and energy, effectively deflecting her from valuable research needed to advance her model and debate Muller before his death in 1967. No matter how valid and important was McClintock's challenge to Muller's model, it would undoubtedly wane without her attention.

On the other hand, Muller would soon be confronted with a serious question, this time involving the LNT Single-hit Hypothesis. In a highlighted paper in *Science* on December 19, 1958, William L. Russell (Oakridge National Laboratory) reported the discovery of radiation-induced, dose-rate effects in the spermatogonia and oocytes of mice. These findings were deemed so significant and challenging to the LNT concept that Muller redirected his laboratory to the assessment of dose rates on radiation-induced mutations in *Drosophila*. Russell's findings would significantly challenge the prevailing mantra that declared radiation damage to be cumulative, irreversible and linear in its dose response. In contrast to the mantra, Russell showed that radiation-induced mutations in mouse oocytes yielded an unambiguous threshold response. A detailed historical assessment of the Russell-Muller dose-rate debate and its impact on radiation risk assessment was recently published (Calabrese, 2017a, 2017b).

Why did McClintock's alternative gene-mutation model fail to gain traction and why has it remained essentially unknown to the scientific community, never taking part in the cancer risk-assessment debate? Table 2 compares Muller and McClintock gene-mutation models for trans-generational phenotypic changes, and identifies reasons why the McClintock gene-mutation model was not adopted. Reasons for failure may be separated into two groups. The first group describes McClintock's failures and explains how they prevented her success (see Table 2, numbers 1–9). The second group lists the factors that enhanced the acceptance of Muller's model (see Table 2, numbers 10–14). Although the factors were many that contributed to Muller's success and McClintock's failure, in the end it boiled down to leadership. Muller grabbed for the leadership mantle while McClintock did not. Differences in their personal histories, goals and priorities, for example, probably all contributed to some extent to the leadership differences between them. Nonetheless, McClintock's scientific visibility quotient, in terms of published papers and conference presentations needed to advance her gene mutation model as a superior alternative, was so low compared to Muller's that her alternative model failed to become part of the risk assessment debate for the past nearly 70 years. In fact, her gene mutation theory/model was not mentioned once in the 250,000 pages of the official OSHA record of regulation hearings for major carcinogens during the 1978–1980 time period (OSHA, 1980). Despite this striking failure, the transposon rediscovery and "revival" of the past 40 years have had profound biological, biomedical and clinical significance, and yet it remains decoupled from the cancer risk assessment process.

7. The modern ERA

Numerous studies have been published depicting the influence that ionizing radiation has had on the spectrum of mutational types of damage, ranging from base substitutions to large deletions and including a broad range of biological treatment models and a number of doses and dose rates (Webber and de Serres, 1965; De Serres et al., 1967; Thacker, 1986, 1992; Thacker et al., 1990; De Serres, 1991; Fossett et al., 1994; Nelson et al., 1994, 1995; Park et al., 1995; Yamada et al., 1996; Colussi and Lohman, 1997; Colussi et al., 1998; Nohmi et al., 1999; Schwartz et al., 2000; Mognato et al., 2001; Furuno-Fukushi et al., 2003; Liu et al., 2003; Nakamura et al., 2005; Sudprasert et al., 2006; Toyokuni et al., 2009; Okudaira et al., 2010; Russell and Hunsicker, 2012; Asakawa et al., 2013).[19] Reliable general patterns of response have emerged indicating that the type of damage is highly dose and dose-rate dependent. Low doses/dose-rates are generally associated with different types and proportions of lesions than occur at high doses/dose rates. At low doses/dose-rates, various base changes (i.e., transversions, transitions, and tandem based substitutions), frameshifts, and small/modest sized deletions/insertions may occur. As the dose/dose rate increases there is a switch to progressively larger types of genetic damage, ranging from partial to complete gene to multi-gene lesions (e.g., deletions). In the case of mature spermatozoa, the proportion of large deletions is about 4–5 fold greater than seen in the spermatogonia stage, possibly due to DNA repair (Ward and Alexander, 1957; Russell et al., 1998; Russell, 2004). The results also imply that each type of genetic damage most likely has a unique/separate origin/mechanism. Such circumstances led Schwartz et al. (2000) to conclude: "risk from low-dose radiation exposure cannot be easily extrapolated from high-dose effects." Of particular note is that about 60 years after the report of Muller (1928a), Southern blot analysis of nucleotide sequence could not differentiate damage below 50 base pairs. The use of Southern blot therefore led to a practical definition of a "point" mutation (i.e., genetic damage $\leq$ 50 bp could include small deletions, frameshift mutations and a variety of base changes) (Grosovsky et al., 1988). Regardless of the lack of capacity in the mid-1970s to late 1980s to resolve the spectrum of point mutations, the dose-dependence of genotoxic endpoints was readily apparent.

In 1927 the information available on X-ray-induced genetic damage was literally a "black box" consisting of poor resolution of chromosome structure, inadequate cytogenetic capabilities, and a rudimentary understanding of the range of genetic damage. This knowledge void existed within the context of radiation exposures that at times included or bordered on induced sterility. In retrospect, what Muller called a "point" mutation was in fact a euphemism for an ambiguous type and imprecise amount of genetic damage rather than for a known discrete biochemical lesion with precise physical characteristics. Despite considerable uncertainty, massive interpretative overreach and apparent errors, Muller would convince the genetics community and regulatory agencies to accept his X-ray-induced gene mutation and dose-response

[19] In his 1927 paper Muller pointed out that X-ray-induced genetic damage was likely to be dose dependent. He stated that X-ray-induced chromosomal aberrations/rearrangements would be "less likely than point mutations to depend on single quanta." (Note that he did not define or reference "quanta". He then argued, "a re-examination of the effect of different dosages must therefore be carried out, in which different types of mutations are clearly distinguished from one another." (page 87, Column 1). When assessed for a broad range of X-ray dosages he argued that it might then be possible to determine whether low levels of background gamma radiation cause the type of "spontaneous" mutations observed in nature or without an experimental treatment. The Science paper therefore reveals that Muller was aware that his high-dose treatment had caused massive genetic damage. However, it is obvious that he was hoping that the dosing he used produced "point mutations".

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Table 2
Models of Transgenerational Phenotypic Changes (TPCs).

The Muller Gene Mutation Model	**The McClintock Gene Mutation Model**
Change in the Chemical Nature of the Gene	Gene Regulation/mutator Gene Concept/transposition Elements
TPCs are induced by direct hits of ionizing radiation on a gene, inducing mutation; indirectly via a host of possible secondary reactions.	TGCs are principally or possibly fully mediated by transpositional elements (TEs) via cellular regulatory processes. TPCs could be induced by agents that activate transposition elements.

Fate of the McClintock Gene Mutation Model: Why It Failed to Gain Traction for Cancer Risk Assessment During the 1950s and 1960s

1. McClintock's transposon concept was very complex and experimentally difficult to adopt for most contemporary labs; it was also not readily understood and adopted by the scientific community.
2. The death of Stadler in 1954 hurt McClintock's chances of success.
3. McClintock failed to get her supervisor, Milisav Demerec, to become a strong promoter of her scientific vision. In fact, McClintock resigned from the Carnegie Institute in 1953 due to frustration with the poor management/leadership qualities of Demerec; Vannevar Bush, the President of the Carnegie Institute for Scientific Research, interceded to get her to reconsider and stay.
4. The failure of McClintock to get appointed to the BEAR I Genetics Panel represented a significant lost opportunity to educate the Panel of her model and modify and possibly block the acceptance of the Muller model and LNT and to promote her own gene mutation model.
5. Demerec and Kaufmann, leaders from the Carnegie Institute, failed to promote her model at the BEAR I, Genetic Panel meetings. This failure was continued over to BEAR 2 and 3.
6. McClintock did not have graduate students that could have carried on her research/policy agenda. This was a double-edged sword as the seminal discoveries of McClintock may have been missed had her efforts had been delegated to others.
7. Failure of Altenburg to publish his comprehensive review of McClintock's transposon findings and their potential role in explaining radiation-induced mutation.
8. McClintock failed to address how her model related to medical /X-ray and environmental risk assessment.
9. McClintock failed to build an effective support group/organization to test, evaluate and promote her gene mutation model.
10. Muller's model received broadened application when it served as the basis of the LNT-single hit model. The promotion of the LNT-single hit model by several Nobel Prize winners represented a critical development.
11. Muller's receiving the Nobel Prize was essential for external validation of his gene mutation model and its risk assessment applications.
12. The recommendation to support LNT based on the Muller mutation model by the Manhattan Project facilitated its adoption by US government agencies.
13. Muller's testing methods were simple, quick and inexpensive. The interpretations were straight forward and easily understandable.
14. Muller and his radiation geneticist colleagues, typically with *Drosophila* research backgrounds, were organized, communicated often and had a strategy to promote the Muller gene mutation model.

interpretations as matters of scientific fact.

Although Muller attempted to rebut Stadler's (1954) posthumous challenge in *Science* (Muller, 1956; Muller and Oster, 1957) by claiming that "point" mutations were induced at various loci based on their capacity to reverse mutate to normal phenotypes, Muller's reverse mutation criteria was by no means a "gold" standard for small genetic changes (Thacker and Cox, 1983).[20] They noted that mutations caused by positional-effects (gene expression effects mediated by large genetic rearrangements) and those induced by transposable effects could affect gene reversions without gene mutations. Of significance here is that the gene loci frequently studied in *Drosophila* may display unusually high frequencies of reverse mutations, a situation that corresponds in magnitude to their frequencies for forward mutations. Thacker and Cox (1983) suggested that this phenomenon might be a component of the insertion/deletion cycle of a transposable element (Green, 1967) as seen in the use of the white apricot loss of *Drosophila* (Green, 1961).

Another severe blow to Muller's argument occurred when Voss and Falk (1973) used Muller's own biological model (*Drosophila* lethals) to examine about 600,000 gametes and found no evidence of "true" reverse mutations. In an earlier study from the same laboratory (Lifschytz and Falk, 1968), discrete and well-defined small zones within the chromosomes of *Drosophila* were occasionally found to have locus and region specificity for X-ray-induced mutations. More precisely, a small region around the maroon-like locus on the X chromosome demonstrated areas of large deletions that extended beyond a single locus. These same researchers also reported that all X-ray-induced post-meiotic lethals were due to chromosomal aberrations, further refuting Muller's argument.

In some studies, X-ray-induced mutations were frequently shown to involve a single locus. However, even in these cases, being "contained" within a single locus can still involve an extensive area of induced damage. For example, the size of one *Drosophila* gene locus is approximately 20,000 DNA base pairs (McGinnis et al., 1980) and therefore is theoretically large enough for extensive intra-gene damage to produce a mutation. Furthermore, large rearrangements or inversions can sometimes be misclassified as single gene changes even though one breakpoint of the inversion is located inside the zone of one gene and the other outside (McGinnis et al., 1980).

Bedford and Dewey (2002) published a paper on the "real" mutation controversy during and after the Muller era. In their summary they concluded, "no convincing evidence for the production of so-called point mutations was ever found for plants." Since Muller "found evidence for reversion of some X-ray induced mutations with *Drosophila*, he held the view that point mutations were in fact produced. In light of more recent information others have suggested that reversibility is by no means proof of a point mutation." Muller's argument equating "point" and reverse mutations in *Drosophila* may have been the key reason (along with a truly dominating leadership role in the field) that his gene mutation hypothesis prevailed (Stadler, 1932) for so long. Eventually, however, his hypothesis progressively succumbed to the combined effects of methodological advancements, invalidating experimental evidence, and the eroding support of his scientific community.[21]

[20] In this "rebuttal" Muller (1956) was forced to acknowledge that substantial findings on *Drosophila* had revealed that a very large proportion of what he called "point" mutations were now clear-cut deficiencies and other structural changes, a conclusion indistinguishable from that of Stadler. More specifically, Muller (1956), page 136) wrote that "…there is no doubt that in X-rayed *Drosophila* also, at least when the irradiation is applied to condensed chromosome stages, such as those of spermatozoa, deficiencies as well as other demonstrable structural changes arise with much higher frequency, relative to changes that appear to involve but one gene, than they do without treatment." This statement clearly reflects the eroding of Muller (1927a) position concerning the similarity of X-ray-induced vs spontaneous mutations and his statement that a high proportion of the gene changes were point mutations. It appears that Muller had given up considerable ground in the argument but still tried to retain some "wiggle room" so as not to raise the "white" flag of full intellectual surrender.

[21] In 1978 Charlette Auerbach, whose career was positively redirected by Muller in 1938 at Edinburgh, addressed the debate between Stadler and Muller over whether Muller had produced real gene mutations in his Nobel Prize research. For one with very close ties to Muller, it was very telling that she concluded that Stadler had firm experimental evidence with maize that confirmed his assertions, while Muller's evidence was suggestive but less direct. Such an assessment speaks highly for the intellectual penetration of the Stadler position. Auerbach (1978) then came to the defense of Muller (without providing a reference) stating that recent molecular analyses had "confirmed Muller's conclusions" that X-rays can produce true mutations. However, Muller was claiming that he had induced large numbers of mutations (i.e., "the artificial transmutation of the gene") but at the doses he used, the vast majority (perhaps all) were heritable chromosome aberrations. Many years later Muller's former post-doctoral colleague William R. Lee would report that "molecular analysis of X-ray-induced mutations in the germ line of *Drosophila* shows that nearly all are the result of X-ray-induced strand breakage" (Byrne and Lee, 1989; Batzer et al., 1988). The radiation doses studied by Lee were 5 Gy, 10 Gy, and 30 Gy with the doses reflecting those used by Muller. The findings of Lee and his colleagues strongly supported the position of Stadler in his debate with Muller. Thus, at the doses Muller tested he induced chromosomal aberrations and not "massive" new

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8. LNT single-HIT hypothesis formulation

The debate between Muller and Stadler was not simply about the mechanism of X-ray-induced mutations nor was it simply between Muller and Stadler. By the early 1940's, it had expanded significantly to include issues related to the linear dose-response and the single-hit models used to assess both genetic and cancer risks.[22] Furthermore, Stadler was not challenging only Muller but also other leading geneticists as well as a cadre of elite physicists, including the future Nobel Prize recipient Max Delbruck and other leading radiation geneticists (e.g., Timofeeff-Ressovsky).

Muller and Timofeeff-Ressovsky used X-ray-induced sex-linked recessive lethality in *Drosophila* to assess trans-generational phenotypic changes and, as a result, demonstrated a linear dose-response relationship. The fact that some somatic cells of *Drosophila* showed phenotypic trait reversal suggested the possibility of X-ray-induced gene mutations. Delbruck and others applied target theory to the linear dose-response results and, together with mathematical modeling by Zimmer (1941), indicated that only a single hit could yield a linear dose response (Fig. 1).

The major assumption of the LNT single-hit model is that low-dose effects are predictable from high-dose exposures. Such predictions based on this major assumption suggest that the damage produced at low doses is qualitatively the same as at high doses (i.e. same type of genotoxic damage and with similar spatial pattern distribution) and that quantitatively the amount of damage produced is directly proportionate to the dose.

Delbruck constructed a tentative "atomic physics" model of the gene, as inferred from the frequency of "point" mutations under varying physical conditions. The views of Delbruck were popularized and markedly advanced by the Nobel Prize-winning physicist Erwin Schrodinger (1944) in his book *What is Life*. The key features of the model were that the (1) gene is a molecule; (2) that a mutation of a gene is a result of the transition from one stable state to another; and (3) the effect was due to thermal agitation or the absorption of radiant energy (Stadler, 1954). The linear dose response and the constancy of mutation yield regardless of time (i.e., Delbruck's model negated the occurrence of dose rate) predicted that X-ray-induced mutations from single "hits". The constant proportionality of mutation yield to ionization, regardless of wavelength variation, indicated that the unit was a single ionization. The average size of a gene was estimated as well as a quantitative characterization of the volume within which the hits occurred. This estimate was used to account for the mutations reported. He deduced that the average gene was comprised of approximately 1000 atoms. Delbruck then estimated that 1.5 eV would be required to induce a mutation with this model. Since the energy released via a single ionization is about 30 eV (i.e. far more than the 1.5 eV), the ionizing radiation should be able to mutate essentially all genes regardless of their inherent stability.

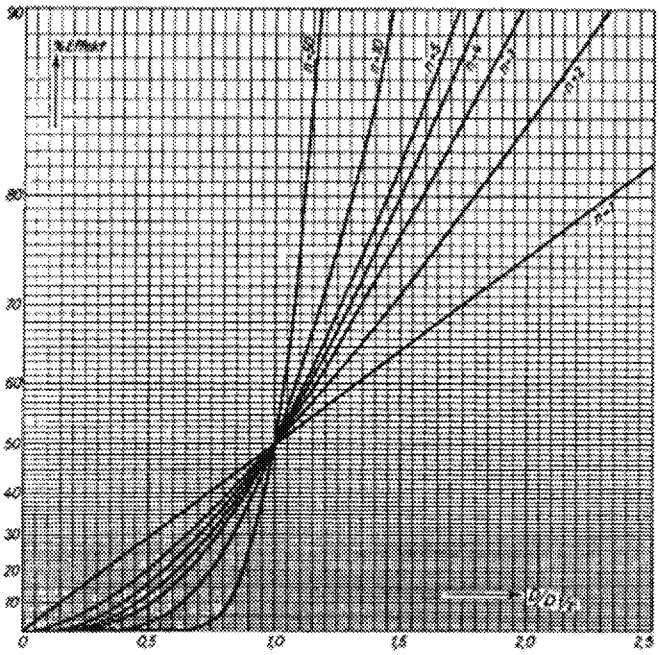

Fig. 1. Model dose-response curves, calculated theoretically for various number of "hits", n, on a single "target" assumed necessary to produce the effect. (From Zimmer (1941)).

9. Criticism of the LNT-single hit hypothesis

Stadler (1954) critiqued Delbruck's model and indicated "…it has been evident for many years that it (single hit theory) has no valid relationship to the experimental data from which it was derived." Furthermore, Stadler indicated that a non-gene alteration, such as a physical loss or rearrangement, represents a sound alternative to structurally altered genetic mutation and is strongly supported by evidence obtained in detailed assessments of X-ray-induced mutations. In concluding his critique, Stadler stated, "we have no basis for estimating the proportion of such extragenic mutations amongst the total of mutations observed and no grounds for assuming that this proportion is the same amongst the mutations observed under the various experiments."

The total dose and dose rate used during Muller's Nobel Prize research were extremely high. To provide a striking comparison, consider that Caspari and Stern (1948), who actually demonstrated an X-ray-induced threshold response in *Drosophila*, had to use a 50–60-thousand-fold **lower** dose rate than Muller did in his *Drosophila* studies. In addition to dose/dose rate concerns, the use of mature spermatozoa for cancer risk assessment was also problematic. Since mature haploid spermatozoa are more susceptible to X-ray-induced mutations than are cancer-forming diploid cells, it suggests that Muller's haploid spermatozoa are inappropriate for extrapolation to diploid somatic cells and, therefore, for cancer risk assessment. In contrast to spermatogonia and somatic cells, it was later discovered that DNA repair was absent in mature spermatozoa, offering a mechanistic explanation for the differential susceptibility to mutations. These concerns led the U.S. BEIR I Genetics Subcommittee (1972) to acknowledge errors made by the BEAR I (Anonymous, 1956) Genetics Panel on two issues: (1) using mutation data from mature spermatozoa to model risk of genetic damage and (2) considering dose rate as an irrelevant factor in cancer risk assessment (Calabrese, 2017b, 2017c).

It was not long after Muller's groundbreaking paper, according to Novitski (1976), before investigations began to counter Muller's gene-mutation hypothesis and reveal that chromosomal rearrangements were associated with sex-linked recessive lethals in *Drosophila* and that the proportion of these induced lethals increased with dose (Oliver, 1930; Demerec, 1937; Demerec and Fano, 1941). In fact, the general consensus was that lethal mutations were, as Novitski had noted, "a

(*footnote continued*)
"point mutations" as claimed.

[22] While the relationship between Muller and Stadler remains to be better clarified, there appears to have been a high degree of mutual respect (Muller, 1956). Of particular note is that from June 1948 until October 1949, Stadler was investigated by the FBI for concerns associated with linkages to communist groups within the US during the beginning of the Cold War. In fact, Stadler was denied a passport in June 1948 to attend the International Genetics Congress in Sweden. During this ordeal a number of leading geneticists came to the defense of Stadler. Based on obtained correspondence, Muller was strongly supportive of Stadler, showing remarkable leadership in this regard. While Stadler's answers to the FBI interrogatory were impressive in his defense so too were the actions of colleagues of very high stature such as Muller, Stern, Beadle, Demerec, and others. While Stadler was very grateful for the support of Muller, he and Muller would continue to scientifically debate on gene mutation until Stadler's death (May 1954) (Calabrese, 2017d).

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Table 3
The LNT Single-Hit Model Timeline.

Date	Topic	Context
1927	**X-Rays Induce Gene Mutations**	Muller wins race to be first or did he?
1929–1931	**Linear Dose Response**	Follow up experiments by Muller's students support linear interpretation
1929	**Improved Chromosome Cytology**	Barbara McClintock develops markedly improved cytogenetic method for maize chromosomes.
1930	**Muller Anticipates Challenges to Gene Mutation**	Tried to prove gene mutation interpretations; used reverse gene mutation concept (Patterson and Muller, 1930).
1931	**New Cytogenetic Methods Challenge Gene Mutation**	Stadler and McClintock determine that X-rays induced heritable chromosome (not gene) changes.
1932	**Stadler Challenges Muller's Gene Mutation Interpretation**	Plenary address at the Sixth International Genetics Congress
1935	**LNT Single-hit Model Formulation**	Leading physicists/radiation geneticists create new model (Timofeeff-Ressovsky et al., 1935)
1936	**Position Effect and Variegation/Mottling Effects**	New mechanism could challenge parts of Muller gene mutation model.
1936–1960	**Reverse Mutation (RM)**	Significant research initiated to assess the frequency of RM induced by radiation and spontaneously. Results are mixed; they do not provide confirmation of Muller's gene mutation interpretation.
1938	**McClintock Develops Procedure to Produce Mutations without X-Rays**	The BFB method could induce a large amount of mutations in a targeted manner.
1939	**Dose Rate and LNT Model**	Muller's graduate student, Ray-Chaudhuri finds no evidence of dose-rate in mature *Drosophila* spermatogonia
1944	**McClintock Discovers Transposable Genes**	This discovery integrated the position effect and reverse mutations along with a theory of regulatory control over gene expression.
1943–1946	**Manhattan Project Dose-Rate**	Ernst Caspari study supports a threshold and dose-rate, challenges Ray-Chaudhuri findings (Caspari and Stern, 1948).
1948	**McClintock's Alternative Gene Mutation Model**	Transposon-based model leads to mutation without need for direct hit on gene; challenges Muller's model (Muller and McClintock correspondence).
1958	**Dose-Rate Discovered in Mouse Model**	Russell discovers dose-rate for X-ray induced mutation in mouse spermatogonia and oocytes.

result of chromosome breakage (Herskowitz, 1946; Lea and Catcheside, 1945; Haldane and Lea, 1947; Lea, 1946)". In time, follow up investigations were conducted that combined Muller-like doses with molecular mapping techniques to detect deletion breakpoints. These radiation exposure studies revealed a very high proportion of large deletions (Aaron, 1979; Fossett et al., 1994). These findings also revealed that ionizing radiation induced sex-linked recessive lethals can quantitatively predict the occurrence of induced deletions, including large deletion frequencies (Byrne and Lee, 1989; Fossett et al., 1994). These results strongly support the hypothesis of Stadler (1932, 1954) that most, if not all, of Muller's reported "mutations" were not gene mutations but rather chromosomal aberrations of various types and magnitude.

When the reverse mutation argument of Muller began to lose traction, Muller would shift the debate to the dose-rate experiments of Ray-Chaudhuri (1939, 1944). These experiments on dose rate gave rise to what may have been a type of desperate hope for Muller and other radiation geneticists who believed that the LNT single-hit model still had a defensible basis. This shift in debate may have been an underlying factor in Muller's less-than-critical evaluation of Ray-Chaudhuri's dissertation and, subsequently, in Muller's repeatedly deliberate mischaracterizations of Caspari's dose-rate study, which did not support the findings of Ray-Chaudhuri (Calabrese, 2011).[23] In his Nobel Prize lecture (December 12, 1946) Muller would use the Ray-Chaudhuri dissertation to affirm that there is "no escape from the conclusion that there is no threshold dose…". Muller's extreme dependency on the findings of Ray-Chaudhuri to refute the threshold model while simultaneously affirming the LNT single-hit dose-response model may have motivated his direct, powerful, coordinated and often deceitful attacks on the Caspari study (Calabrese, 2011, 2015a).

The LNT single-hit theory also failed to provide validated risk estimates across X-ray induced doses that produced qualitatively different types of gross chromosomal damage with the expectation of predicting responses to doses many orders of magnitude below those tested and for which analytical methods would not be available for almost a half century. The LNT single-hit hypothesis became progressively and seriously flawed in multiple reinforcing ways, over the next four decades following its formulation in the mid-1930s. While the stage should have been set for model revision, the radiation geneticist community, as lead by Muller, Stern and other colleagues, would do what was necessary to "save the hit model", and so they did (Calabrese, 2015a, 2017a).

10. Discussion

This paper assesses the historical origins of the LNT single-hit model and its reliance upon data obtained from studies (especially those of Muller) of X-ray-induced trans-generational changes in *Drosophila* phenotypes (Table 3). Also explored is the intellectual struggle that existed between Muller and Stadler over the authenticity of "gene" mutations as a mechanism to explain X-ray-induced trans-generational phenotypic changes at high doses. Painter developed his advanced cytogenetic staining method for *Drosophila* chromosomes (1934c) after Muller had already formulated his hypothesis on "true" gene mutations. McClintock and Painter's new cytogenetic methods revealed that at high doses, similar to those used in Muller's Nobel Prize research, chromosomal aberrations and/or rearrangements of various types would strongly predominate, if not exclusively, over "gene" mutations. In time, newer methods would progressively improve and refine the assessment of such gross chromosomal alterations, affirming the position of Stadler. Other arguments used by Muller, such as the reverse mutation phenomenon, were often not supported in multiple large-scale studies prior to (e.g., Lefeve, 1949, 1950; Neuffer, 1957) and after Muller's death (Voss and Falk, 1973). As scientific support for the gene mutation hypothesis began to erode, Muller and others of the radiation genetics community would shift their support behind the findings of Ray-Chaudhuri (1939, 1944) in support of an LNT interpretation. Although the substantial and integrated Caspari-Spencer follow-up experiments (Caspari and Stern, 1948; Spencer and Stern, 1948) challenged the data of Ray-Chaudhuri, major efforts were made to discredit these threshold (i.e., Caspari and Stern, 1948) supportive findings by Muller, Stern and other members of the radiation geneticist community

[23] The acceptance of the Caspari data would hinge on the validity of his control group data. Curt Stern contacted Muller at Amherst College late in 1946 and in 1947 to obtain his findings since Muller had amassed voluminous data on spontaneous mutation in *Drosophila* in an attempt to address the many scientific challenges of Stadler. As a result of this situation, the Muller data fully supported the Caspari position and his claims that X-ray induced mutation display both dose-rate and a threshold response. This was of fundamental importance as it refuted the LNT Single-hit hypothesis. See Calabrese (2011, 2015a) for detailed discussions. Thus, it is highly ironic that Muller's research to refute Stadler led to a confirmation of the Caspari findings that challenged the LNT Single-hit model.

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(Calabrese, 2011, 2015a).

Muller and his colleagues were promoting the LNT single-hit model in the late 1940s and subsequent decades. However, had the cytogenetic advances of Painter (1934c) been available to Muller in 1927 (as McClintock's had become available to Stadler in, 1931), it is likely that Muller may have been more cautious in concluding that he had induced gene mutations. Nevertheless, Muller was clearly involved in a race to be the first to report that X-rays could induce gene mutations. Given all the publicity that Muller received, one can imagine that it would have been difficult for him to do anything but try to defend his interpretation.[24] Likewise, had this information been available prior to the formation of the LNT Single-hit model, this model may never have seen the light of day.

The findings herein complement a substantial series of papers on the history of LNT that started with the Manhattan Project (Calabrese, 2015a, 2015b, 2017a). These papers documented an era post-World War II that was dominated by strong ideological leadership in the community of radiation geneticists who managed to undermine the normal scientific practices of evaluation and review. During the period from 1946 to 1956, these leaders essentially directed a movement to promote and ensure the adoption of the LNT Single-hit model by regulatory agencies. It is interesting, however, that such widespread ideological behaviors were not evident prior to this period even though the same individuals dominated the field of traditional genetics. So then, what happened to change the traditionally accepted scientific practices of rigorous research, honest evaluation and unbiased debate to an ideologically driven ends-justify-the-means process entailing deceit, misdirection and harmful consequences?

The discovery of X-ray-induced mutation and its role in causing birth defects and cancer deaths stoked the fears of a public wary over nuclear holocaust in the immediate post-WW II era of the Cold War. X-rays used in newly developed medical techniques to diagnose and treat diseases as well as the possibility of significant human exposures to radioactive fallout from atomic bomb testing only heightened these fears. Society was ignorant, alarmed and fearful of radiation exposure and urgently demanded new policies from the federal government that could provide an assurance of safety and assuage public anxieties. The sense of urgency was palpable and driven largely by fear rather than the slow, deliberate and objective processes that are classic prerequisites to research, evaluation and debate. If not careful, however, this fear-driven sense of urgency could affect and skew the decision-making process of conscientious humans who are eager and feel pressured to quickly produce definitive results that protect the public and assuage anxieties while simultaneously satisfying their own needs as well as those of their superiors in the public and private sectors. A hurried and harried approach is antithetical to both good science and good management (decision-making) as is the failure to keep the functions and structures of science and management discrete and separate. Thus, keeping the science of risk assessment, for example, independent and separate from the human decision-making of risk management, which may be influenced by a fear-driven sense of urgency or other personal factors, would be necessary to avoid harmful and unintended consequences to society.

This potentially harmful mingling of science and management may well have been partially motivated by funding self-interest as well as a harried sense of urgency to fast-track a radiation health safety standard. The correspondence between members of the NAS BEAR Genetics Panels has shown that the members were not averse to exaggerating public health concerns in exchange for financial support for their research (Calabrese, 2014). Scientific self-interest has typically been attributed to scientists funded by industry, but the exchanges of letters among panelists make clear that they would deceive the scientific community and the public to obtain research funding. Likewise, their own words have been used to document that major funding groups—like the Rockefeller Foundation—(especially of the 1930–1960s) would manipulate these same panelists toward their scientific and policy interests (NAS/NRC, 1956 - see Weaver, page 35), an example of leveraging scientific funding that crossed ethical boundaries.

Lewis Stadler and others challenged Muller's gene mutation hypothesis at a time when it was sweeping the scientific and policy communities. As the data-driven challenges increased in strength over time, they began to weaken key aspects of Muller's high-dose gene-mutation model. This debate was protracted and profoundly significant, but finally reached a turning point with the findings of Caspari as they had the capacity to seriously undermine the adoption of the LNT Single-hit model. At this point, the tug-of-war between science and policy shifted and became an ideologically directed process led by scientists of great reputation, such as Stern and Muller, whose main goal was to ensure that the LNT Single-hit model would be adopted for cancer risk assessment. This story is now well documented (Calabrese, 2011, 2015a, 2015b).

The scientific foundations of the LNT single-hit model could not have been explored properly or completely without also examining the Nobel Prize research of Muller and the lifetime he spent defending it. As a result of this assessment, Muller's principal achievement may now be viewed from a slightly different perspective. Multiple leading geneticists (e.g. Timofeeff-Ressovsky, 1929; Crow and Abrahamson, 1997) of his era have claimed that Muller's importance was not so much that he was the first to produce gene mutations but rather that he showed how to produce copious mutations quickly (Campos, 2015). However, Muller's actions were focused almost entirely on the first question because he knew that this was the fundamental biological issue. How should one deal with the fact that 90-years of research have revealed that Muller did not produce the "gene" mutations that he had claimed in his 1927 *Science* article? Despite the historical significance given to both "gene" mutations and the LNT Single-hit model, the genetics field has remained essentially silent. Perhaps it is afraid to evaluate important aspects of Muller's story because he was an historical icon who had a major role in creating the field and establishing its centrality in biology. In fact, two esteemed geneticists and close allies with Muller had affirmed that Stadler was indeed correct in his claim that Muller produced mostly chromosomal deletions and not specific "gene" mutations (Crow and Abrahamson, 1997). In a review of the history of cytogenetics and genotoxicity, Garcia-Sagredi (2008) offered what seems to be an inspired Stadler-like remaking of the Muller discovery. He stated, "Muller (1927a) discovered that X-rays could cause mutations in *Drosophila*; specifically, that X-rays were able to produce rearrangements in the linear order of genes (for which he was awarded the Nobel Prize…"). However, rearrangement of the linear order of genes was not the goal Muller sought; it was the production of gene mutations. In the end, the greatest achievement of Muller was that he transformed the field of genetics with his exuberant, yet, incorrect claims. He inspired many laboratories to study radiation-induced mutations and was pivotal in influencing Charlotte Auerbach to assess chemical-induced mutagenicity, which opened up still another important new research area. He also awakened the scientific community and public to health concerns with excessive and unshielded radiation. Further, Muller's reporting of his mutation discovery in 1927 would inspire the creation of the Genetics Society of America during the December 1931 New Orleans meeting of the American Society of Zoologists (when the officers of the new Society were selected for 1932). The new society would play an important role in organizing the 6th International Genetics Congress at Cornell, an event that had highlighted Stadler's Plenary Address challenge to Muller's gene mutation claim. Muller did have an amazing impact on the field and society, but his 1927 paper should have an asterisk next to it.

[24] By not "winning" the race to first report X-ray-induced mutation, it provided Stadler with the opportunity to be more open to other possible data interpretations. Thus, even though his reports of X-ray-induced mutations in barley and maize seemed to fulfill all the standard features of gene mutations, Stadler would abandon this position after the cytogenetic findings of McClintock in the summer of 1931.

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11. Conclusion

1. The LNT Single-hit model was based on the assumption that Muller had induced the "artificial transmutation of the gene" by X-ray treatment of mature *Drosophila* spermatozoa.
2. Several years after Muller had created the LNT model (i.e., called Proportionality Rule), it was afforded an explanatory mechanism via target theory and integrated into the Single-hit model.
3. Research by Lewis Stadler challenged Muller's basic conclusion that he had observed X-ray-induced gene mutations. This challenge was based on cytogenetic advances and the evaluations of Barbara McClintock concerning the effects of X-rays on corn. These findings suggested that Muller had predominantly, if not exclusively, induced heritable chromosomal (not gene) mutations, challenging the significance of his findings on heritable mutations.
4. Numerous subsequent studies in multiple biological models, including *Drosophila*, strongly supported Stadler's interpretation. Muller's claim that he had demonstrated the "artificial transmutation of the gene" and greatly enhanced the production of gene mutations were gross overstatements at best, which profoundly misled the scientific community and policy-making in public health areas. The LNT Single-hit model was not supported by the very biological data upon which it was based.
5. In a remarkable reversal of scientific support, leaders of the radiation genetics community placed considerable reliance on the findings of Ray-Chaudhuri (1939, 1944), which supported LNT.
6. Since subsequent integrative studies by Caspari-Spencer did not support the LNT study conclusion of Ray-Chaudhuri, but rather that of a threshold, the Caspari-Spencer studies became the object of unfounded and deceptive criticisms by Muller, Stern and other leaders of the radiation genetics community in an attempt to preserve the LNT Single-hit model.
7. The findings presented herein reconstruct the early development of the LNT Single-hit model up to the post Manhattan Project/modern cancer risk assessment era, and complement the substantial, previously published assessments on the history of LNT starting with the Manhattan Project.
8. These integrated findings lead to the conclusion that the LNT Single-hit model should have been neither recommended by the BEAR I Genetics Panel nor adopted for cancer risk assessment, as was the case in the U.S. and most countries.

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Workshop Session: A Hitchhikers Guide to 21st Century Dose Response Assessment

Chairpersons:

Suzanne Fitzpatrick, US FDA, College Park, MD; and Michael Dourson, Risk Science Center, University of Cincinnati, Cincinnati, OH.

Endorser(s):

Regulatory and Safety Evaluation Specialty Section; Risk Assessment Specialty Section

Synopsis:

Dose response assessment techniques for the 21st Century will need to update older theoretical concepts and integrate new data steams in order to keep up with market demands and public dissemination of credible information. Several new methods or adaptations of existing methods, or commentaries on traditional, and some might say, out of date, methods, have been proposed. These 5 speakers, each with a unique viewpoint, offer some tantalizing options and commentaries.

Speakers:

Obituary notice: LNT dead at 89 years, a life in the spotlight. Ed Calabrese, University of Massachusetts

Abstract

Considerable recent findings have revealed that the linear dose response for cancer risk assessment has not only outlived its utility in predicting risk but is based on a flawed scientific foundation. The present article characterizes this demise of a key concept of environmental risk assessment, in the framework of a figurative obituary of a long-lived concept that has poorly served society. This obituary is intended to illustrate an integrated mix of poignant and improper historical judgments that led to both the acceptance and ultimately the demise of this once intellectually facile and nearly universally accepted concept.

Approaches for describing and communicating overall uncertainty in toxicity characterizations. Nancy Beck, U.S. Environmental Protection Agency (EPA)

Abstract

Single point estimates of human health hazard/toxicity values such as a reference dose (RfD) are generally used in chemical hazard and risk assessment programs for assessing potential risks associated with site- or use-specific exposures. The

resulting point estimates are often used by risk managers for regulatory decision-making, including standard setting, determination of emission controls, and mitigation of exposures to chemical substances. Risk managers, as well as stakeholders (interested and affected parties), often have limited information regarding assumptions and uncertainty factors in numerical estimates of both hazards and risks. Further, the use of different approaches for addressing uncertainty, which vary in transparency, can lead to a lack of confidence in the scientific underpinning of regulatory decision-making. The overarching goal of this paper, whichwas developed from an invited participant workshop, is to offer five approaches for presenting toxicity values in a transparent manner in order to improve the understanding, consideration, and informed use of uncertainty by risk assessors, risk managers, and stakeholders. The five approaches for improving the presentation and communication of uncertainty are described using U.S. Environmental Protection Agency's (EPA's) Integrated Risk Information System (IRIS) as a case study. These approaches will ensure transparency in the documentation, development, and use of toxicity values at EPA, the Agency for Toxic Substances and Disease Registry (ATSDR), and other similar assessment programs in the public and private sector. Further empirical testing will help to inform the approaches that will work best for specific audiences and situations.

Categorical regression: Characterizing a Toxicologist's Judgment in Noncancer Risk Assessment. Dan Krewski, University of Ottawa

Abstract

Noncancer health risk assessment involves the evaluation of multiple types of toxic effects. For regulatory recommendations such as the Reference Dose (RfD), the U.S. EPA relies heavily on expert judgment. This toxicological judgment mixes toxic impact with likelihood: what effects are adverse, which of these is critical, and which dose is the highest reliable No Observed Adverse Effect Level (NOAEL). Uncertainty is indicated by qualitative statements of confidence. Statistical regression using ordered categories of overall toxicity is proposed as a superior alternative: uncertainty and variability are represented by statistical models. All relevant data are used, not just the NOAEL for the critical effect, and health risk can be estimated at exposure levels above the RfD.

Quantitative Mode of Action Approach: How much data is enough? Mary K. Manibusan[1] and Richard A. Becker[2] 1. Exponent, Washington, D.C. and 2. American Chemistry Council, Washington, DC.

Abstract

To address the variability in interpretations of how much data is sufficient to support a mode of action, we have extended the evolved World Health Organization/International Programme on Chemical Safety mode of action (MOA) framework to enable quantitative scoring of the level of confidence in the weight of evidence supporting a hypothesized MOA. This quantitative approach

improves transparency in the scientific justification for use of MOA in characterizing hazards and selecting dose-response extrapolation methods for specific chemicals. The quantitative MOA confidence scoring method begins with selecting hypothesized MOAs, and for each hypothesized MOA, a score would be assigned to reflect the weight of evidence (WOE) in support of causality for each key event, using the evolved Bradford Hill causal considerations (biological plausibility, essentiality, dose-response and temporal concordance, consistency, and analogy). The calculated MOA score would be the summation of all the KE scores, which would be compared relative to other hypothesized MOA score(s). An initial proof of concept case study compared two potential MOAs (mutagenicity and peroxisome proliferator activated receptor-α) for clofibrate, a known rodent liver carcinogen. Quantitative confidence scoring of hypothesized MOAs can be very useful in communicating and characterizing the more likely operative MOA. While this case example demonstrates an initial proof of concept, additional chemical case examples representing a wider spectrum of MOAs are currently under development. Based on these additional case examples, specific lessons learned and opportunities to enhance and refine the methodology will be presented.

The Use of the TTC in Food Safety. George E.N. Kass, Daniela Maurici and Tobin Robinson, European Food Safety Authority, Parma, Italy

Abstract

Synthetic and naturally occurring substances present in food and feed, together with their possible breakdown or reaction products, represent a large number of substances, many of which require a risk assessment. The TTC approach is a screening and prioritization tool that can be used when hazard data are incomplete and human exposure can be estimated. However, the TTC is not applicable to the risk assessment of substances in food and feed when compound-specific assessment and toxicity data are available or are required under existing EU regulations. Consequently, substances like food additives for which a dossier or extensive toxicological data need to be submitted to support their evaluation cannot be assessed using the TTC approach. Instead, the use of the TTC can be applied to the risk assessment of unavoidable genotoxic residuals, for which carcinogenicity data are not available. Another sector where the TTC approach is applied by European Food Safety Authority (EFSA) is in the risk assessment of flavouring substances. This approach was initiated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in the early 1990's when flavouring substances were first evaluated and is currently also used by EFSA. More recently, the use of the TTC was proposed to support the safety evaluation of pesticide metabolites. Further potential applications of the TTC approach will be discussed.

Panel Discussion/Q&A.

Message

From: Beck, Nancy [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=168ECB5184AC44DE95A913297F353745-BECK, NANCY]
Sent: 6/6/2018 11:40:59 AM
To: Edward Calabrese [edwardc@schoolph.umass.edu]
Subject: RE: LNT

Thanks Ed.

Nancy B. Beck, Ph.D., DABT
Deputy Assistant Administrator, OCSPP
P: 202-564-1273
M: 202-731-9910
beck.nancy@epa.gov

From: Edward Calabrese [mailto:edwardc@schoolph.umass.edu]
Sent: Friday, June 1, 2018 3:42 PM
To: Beck, Nancy <Beck.Nancy@epa.gov>
Subject: LNT

Nancy:

Attached is my version on the history of LNT.
Please share with your colleagues.

Ed Calabrese

Message

From: Beck, Nancy [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=168ECB5184AC44DE95A913297F353745-BECK, NANCY]
Sent: 6/6/2018 11:40:41 AM
To: Clint Woods (woods.Clint@epa.gov) [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=bc65010f5c2e48f4bc2aa050db50d198-Woods, Clin]; Yamada, Richard (Yujiro) [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=4c34a1e0345e4d26b361b5031430639d-Yamada, Yuj]
Subject: FW: LNT
Attachments: Environ. Pollut. Muller-Mech-1.pdf

Yet another one from Ed. It amazes me how many times he has been able to publish the same story…
Yet not one paper suggests an alternative approach.

Nancy B. Beck, Ph.D., DABT
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From: Edward Calabrese [mailto:edwardc@schoolph.umass.edu]
Sent: Friday, June 1, 2018 3:42 PM
To: Beck, Nancy <Beck.Nancy@epa.gov>
Subject: LNT

Nancy:

Attached is my version on the history of LNT.
Please share with your colleagues.

Ed Calabrese

Message

From: Beck, Nancy [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=168ECB5184AC44DE95A913297F353745-BECK, NANCY]
Sent: 8/2/2018 12:06:21 AM
To: McClintic, Howard [McClintH@ctc.com]
Subject: RE: DRAFT Letter to Administrator Wheeler Our Science Committee's Report Is Pivotal to Eliminating the Linear No-Threshold (LNT) Methodology

Yes, you can send information to Ryan Jackson or to the Administrator directly. He should be in the EPA directory.

Regards,
Nancy

Nancy B. Beck, Ph.D., DABT
Deputy Assistant Administrator, OCSPP
P: 202-564-1273
M: Personal Matters / Ex. 6
beck.nancy@epa.gov

From: McClintic, Howard [mailto:McClintH@ctc.com]
Sent: Tuesday, July 24, 2018 4:39 PM
To: Beck, Nancy <Beck.Nancy@epa.gov>
Subject: DRAFT Letter to Administrator Wheeler Our Science Committee's Report Is Pivotal to Eliminating the Linear No-Threshold (LNT) Methodology

Good Day Dr. Beck,

Is Ryan Jackson the correct person to get this DRAFT email to Administrator Wheeler? I've asked some senior people at Murray Energy to direct it to him, if that's advisable? Please let me know if it is OR whatever is better?

Many thanks, most sincerely,

Howard
202 689 4586

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Good Day Mr. Jackson,

My colleague, Dr. Robert (Bob) Golden and I were in headquarters last month, presenting some of the highlights of our LNT (linear no threshold) Project that is all about ***"transparency"*** and using ***cost-benefit analyses***. I believe that Administrator Wheeler respects not only Dr. Michael Honeycutt of Texas (who added pages 9 to 11 of the attachment), but also Dr. Edward Calabrese of Univ. of Massachusetts, who is the Co-Chair of the Science Committee.

I am sure that the Administrator would want to know about our Science Committee's Goals and Objectives as well as the importance and purpose of our soon to be completed peer-reviewed Report. The attached PowerPoint Presentation visually depicts in slides 7 through 11 the distortive effects of the LNT (linear no-threshold) methodology, when sharply contrasted with (and compared to) science-based data points. These slides underscore EPA Administrator Pruitt's central point that the mathematical construct that the EPA has been using for assessing risk, the linear no-threshold (LNT) methodology, is of **MOST** questionable scientific validity. The LNT model was originally adopted by the National Academy of Sciences (NAS) in 1956 for radiation and in 1977 for chemicals. Because LNT-driven regulations, whether for chemicals or radiation, have, for many years, been claimed to be science-based (see (https://science.house.gov/sites/republicans.science.house.gov/files/documents/HHRG-113-SY-20131114-SD001%20.pdf as well as http://www.c-span.org/video/?327016-1/epa-administrator-gina-mccarthy-testimony-proposed-regulations), the underlying scientific foundation for such regulations, particularly the LNT model itself, should also, by definition, reflect empirical data. If such scientific data are lacking, as they are for

the LNT model, science-based regulatory methodologies (including benefit-cost analyses) for both chemicals and radiation should be updated to reflect significant advancements in scientific knowledge.

Dr. Robert (Bob) Golden, and I would like to have either a Conference Call or Meeting with Administrator Wheeler or whoever he and you might suggest. soon. I am very grateful for your time, and attention – Thank You.

Most sincerely yours,

Howard

Howard G. McClintic
Executive Director
202 689 4586

Comparison of the Scientific Basis of the Linear No Threshold (LNT) Model with Threshold Models for Cancer Risk Assessment of Radiation & Chemicals[a]

Chapters[a]	Author	Status
Prolog	Golden	Draft
Introduction	Bus	Draft
History of LNT	Calabrese	Final
The linear no-threshold model is less realistic than threshold or hormesis-based models: an evolutionary perspective	Costantini	Final
The LNT model for cancer induction is not supported by radiobiological data	Scott	Final
The impact of dose-rate on LNT hypothesis for radiation risk assessment	Brooks	Final
Re-evaluation of the linear no-threshold (LNT) model using new paradigms and modern molecular studies	Tharmalingam	Final
Threshold for low dose radon exposure	Brooks	Final
Review of the evidence for thresholds for DNA-reactive and epigenetic experimental chemical carcinogens	Williams & Kobets	Draft

Mechanistic aspects of chemical carcinogens demonstrating thresholds	H Clewell & R Clewell	In progress
Epidemiological analysis of low dose/dose rate radiation exposure data	Ricci	Final
Economic implication of LNT vs. threshold models for benefit-cost analyses; formaldehyde & radon	Williams	Draft
Discussion, conclusions & recommendations	All	To be drafted

a. Provisional titles

[1] https://www.nrc.gov/docs/ML1209/ML120970113.pdf

[1] Many areas in the world have natural background radiation levels higher than EPA's dose limits, natural background radiation in the United States is 30 times above the limit; Yangjiang, China, 60 times higher; Kerala coast, India, 100 times; and Ramsar, Iran, 2,000 times above the limit, without any adverse health effects. https://www.nrc.gov/docs/ML1209/ML120970113.pdf

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Message

From: Beck, Nancy [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=168ECB5184AC44DE95A913297F353745-BECK, NANCY]
Sent: 4/26/2018 11:42:20 PM
To: McClintic, Howard [McClintH@ctc.com]
Subject: Re: Your meeting request for EPA

I think Clint Woods is who you are referring to. He is in the air office.

Regards
Nancy.

———————
Nancy B. Beck, Ph.D. DABT
Deputy Assistant Administrator, OCSPP
P: 202-564-1273
M: Personal Matters / Ex. 6
Beck.Nancy@epa.gov

On Apr 26, 2018, at 3:44 PM, McClintic, Howard <McClintH@ctc.com> wrote:

Good Day Nancy,

Exciting times, right?

I had hoped to schedule a meeting with Assistant Administrator Bill Wehrum, whose reputation preceded him – see my comments about him vis-à-vis our shared law firm friend. When I was told that I was being re-directed from Air & Radiation to ORD, I looked at their Staff Directory. In our conversation, you referenced three names: Richard Yamada; Brittany Bolen and Clint ____.

The week of June 4th seems distant! In most ways, I have no choice, so I guess I'll agree, expecting that it will be changed. Can you organize another group any faster

and is more exposure of more people to our LNT Project better?

I value your advice.

Many thanks,

Howard
202 689 4586

From: Gentry, Nathan [mailto:Gentry.Nathan@epa.gov]
Sent: Thursday, April 26, 2018 3:07 PM
To: McClintic, Howard
Cc: rgolden124@aol.com
Subject: RE: Your meeting request for EPA

Howard,

I've been asked to set up a meeting for you with the folks listed below. Schedules are very tight over the next month, and these folks are going to be out of the office for most of the time. Could we look at something the week of **June 4**? I could set up some time the afternoon of June 5 or the morning of June 7.

EPA-ORD Participants
Richard Yamada, Deputy Assistant Administrator for Research and Development
Bruce Rodan, Associate Director for Science
Tina Bahadori, Director of the National Center for Environmental Assessment

Nathan Gentry

Scheduler for Jennifer Orme-Zavaleta, Richard Yamada, Chris Robbins and Bruce Rodan
Assistant Deputy Ethics Official
EPA Office of Research and Development
Phone: 202-564-9084
Fax: 202-565-2430

From: McClintic, Howard [mailto:McClintH@ctc.com]
Sent: Thursday, April 26, 2018 12:31 PM
To: Gentry, Nathan <Gentry.Nathan@epa.gov>
Cc: rgolden124@aol.com
Subject: FW: Your meeting request for EPA

Good Day Nathan,

Dr. Bob Golden and I are pleased that we will be working together.

I'm not certain that this is the most current list of ORD personnel https://www.ecos.org/wp-content/uploads/2017/09/EPA-ORD-Contacts-for-ECOS-August-2017.pdf, but maybe it is the most comprehensive? Who might you be thinking we should meet with at ORD? Also, with whom do you work?

I look forward to your response and am grateful for your guidance.

Most sincerely,

Howard
202 689 4586

From: McClintic, Howard
Sent: Thursday, April 26, 2018 8:56 AM
To: 'Lewis, Josh'; Bob Golden (RGolden124@aol.com)
Cc: Gentry, Nathan; Atkinson, Emily
Subject: RE: Your meeting request for EPA

Good Morning Emily, Josh and Nathan,

Thank you for your email.

Curiously, I was just reading the Federal Register Notice about ***Strengthening Transparency in Regulatory Science*** and found the LNT referenced on the bottom of page 8. The Federal Register item was shared with Dr. Ed Calabrese by Administrator Pruitt, who is well aware of almost all aspects of this Project.

Dr. Bob Golden and I will coordinate with Nathan at ORD, no problem, Thank You. I had wanted to meet with Assistant Administrator Wehrum because of his certain knowledge of the LNT per his work at Hunton Andrews Kurth LLP. Moreover, his colleague, Mark Bierbower, is a dear friend of mine and admirer of the Assistant Administrator – another time.

Anytime, starting next Tuesday, Bob and I are available for a Meeting.

Many thanks, most sincerely,

Howard
202 689 4586

Emily, thank you for your professionalism

From: Lewis, Josh [mailto:Lewis.Josh@epa.gov]
Sent: Thursday, April 26, 2018 8:18 AM
To: McClintic, Howard
Cc: Gentry, Nathan; Atkinson, Emily
Subject: Your meeting request for EPA

Hi Howard,

Following up on your meeting request below. In reviewing it, we think this meeting is more appropriate for our office of research and development. Nathan (cc'd) can be your point of contact there. Thank you.

Josh Lewis
Chief of Staff
Office of Air and Radiation
202 564 2095

From: McClintic, Howard [mailto:McClintH@ctc.com]
Sent: Tuesday, April 17, 2018 12:15 PM
To: Atkinson, Emily <Atkinson.Emily@epa.gov>
Cc: rgolden124@aol.com
Subject: RE: Please Give Me a Brief Call at Your Convenience

Good Day Emily,

Thank you for your response and responsiveness.

Here is one way to answer your question, I think. In advance of our Meeting, I will share some background material, but I assume that the Assistant Administrator knows the topic, the LNT, very well. For example, he may wish to know "who" is on the Science Committee, which will be furnished.

The week of April 23rd is an example – please suggest dates and times therein or thereafter. We probably **need 45 minutes** because the subject is "rich" as well as broad-and-deep, spanning 60 years.

Many thanks for your assistance with scheduling this appointment Emily.

Most sincerely yours,

Howard

202 689 4586

WHO: Dr. Robert (Bob) Golden, Toxicologist, Co-Chair of the LNT Science Committee

Howard McClintic, Executive Director of the ***CTC Foundation***

WHAT: A Discussion about the Need to Objectively Evaluate the Linear No-Threshold (LNT) Risk Assessment Methodology

- The Science Committee's Mission, Purpose and Progress

- Next Steps: Completion; Peer-Review; Publication; Release

WHY: The Importance and Priority of This Project Should be Fully Appreciated and Realized

WHERE: Assistant Administrator Wehrum's Office

WHEN: TBD – Week of April 23rd? Time period 10:30 AM to 2:30 PM **Duration:** 45 Minutes

---

From: Atkinson, Emily [mailto:Atkinson.Emily@epa.gov]
Sent: Tuesday, April 17, 2018 10:35 AM
To: McClintic, Howard
Subject: RE: Please Give Me a Brief Call at Your Convenience

Hi Howard,

I just heard your voice mail asking for a meeting with Bill Wehrum. Please send me the details of what topics you would like to discuss, who would attend and if you have a time frame in mind.

Once I have those details we will be able to look at scheduling options.

Thank you.

Emily

Emily Atkinson
Management Analyst/Office Manager

Immediate Office of the Assistant Administrator
Office of Air and Radiation, USEPA
Room 5412B, 1200 Pennsylvania Avenue NW
Washington, DC 20460
Voice: 202-564-1850
Email: atkinson.emily@epa.gov

From: McClintic, Howard [mailto:McClintH@ctc.com]
Sent: Tuesday, April 17, 2018 10:28 AM
To: Atkinson, Emily <Atkinson.Emily@epa.gov>
Subject: Please Give Me a Brief Call at Your Convenience

<image001.jpg>

Ensuring the Future Through Innovation, Science and Technology

2711 Jefferson Davis Hwy,, Suite 620

Arlington, VA 22202

(703) 310-5688 (703) 310-5655 FAX

Personal Matters / Ex. 6

E-Mail:
McClintH@ctc.com

Good Day Ms. Atkinson,

I have a quick question for you.

Thank you in advance.

Most sincerely yours,

Howard

Howard G. McClintic

Executive Director

202 689 4586

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Message

From: Beck, Nancy [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=168ECB5184AC44DE95A913297F353745-BECK, NANCY]
Sent: 3/13/2018 3:35:50 PM
To: Yamada, Richard (Yujiro) [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=4c34a1e0345e4d26b361b5031430639d-Yamada, Yuj]
Subject: Re: LNT and the recent Administrator meeting

I've never heard Ed articulate an alternative default approach. He does a great job articulating concerns w LNT.

Nancy B. Beck, Ph.D., DABT
Deputy Assistant Administrator, OCSPP
P: 202-564-1273
M: Personal Matters / Ex. 6
Beck.Nancy@epa.gov

On Mar 13, 2018, at 10:28 AM, Yamada, Richard (Yujiro) <yamada.richard@epa.gov> wrote:

FYI

Sent from my iPhone

Begin forwarded message:

From: "Jackson, Ryan" <jackson.ryan@epa.gov>
Date: March 13, 2018 at 11:11:27 AM EDT
To: Edward Calabrese <edwardc@schoolph.umass.edu>, "Yamada, Richard (Yujiro)" <yamada.richard@epa.gov>
Subject: RE: LNT and the recent Administrator meeting

Thank you Ed. It's something that Richard and I have talked a lot about.

From: Edward Calabrese [mailto:edwardc@schoolph.umass.edu]
Sent: Tuesday, March 13, 2018 9:42 AM
To: Jackson, Ryan <jackson.ryan@epa.gov>
Subject: LNT and the recent Administrator meeting

Ryan:

I received two emails today from people who were present at a meeting with Administrator Pruit yesterday. They noted that he indicated that
LNT was on his radar as something he wanted to fix.
If I can be of any use on this let me know as this is one of the areas I have been very active on.
I do have some very practical ideas that are based on very sound science that could drastically improve the area.

Ed Calabrese

Environ. Sci. Technol. **2010**, *44*, 3221–3231

Indoor Contamination with Hexabromocyclododecanes, Polybrominated Diphenyl Ethers, and Perfluoroalkyl Compounds: An Important Exposure Pathway for People?

STUART HARRAD,*,† CYNTHIA A. DE WIT,‡ MOHAMED ABOU-ELWAFA ABDALLAH,†,§ CAROLINE BERGH,∥ JUSTINA A. BJÖRKLUND,† ADRIAN COVACI,⊥ PER OLA DARNERUD,# JACOB DE BOER,∇ MIRIAM DIAMOND,○ SANDRA HUBER,◆ PIM LEONARDS,∇ MANOLIS MANDALAKIS,¶ CONNY OSTMAN,∥ LINE SMÅSTUEN HAUG,+ CATHRINE THOMSEN,+ AND THOMAS F. WEBSTER★

Division of Environmental Health and Risk Management, School of Geography, Earth, and Environmental Sciences, University of Birmingham, Birmingham B15 2TT, U.K., Department of Applied Environmental Science (ITM), Stockholm University, SE-106 91 Stockholm, Sweden, Department of Analytical Chemistry, Faculty of Pharmacy, Assiut University, 71526 Assiut, Egypt, Department of Analytical Chemistry, Stockholm University, SE-106 91 Stockholm, Sweden, Toxicological Centre, Department of Pharmaceutical Sciences, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium, National Food Administration, P.O. Box 622, SE-751 26 Uppsala, Sweden, Institute for Environmental Studies (IVM), VU University Amsterdam, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands, Department of Geography, 100 St George Street fifth floor, University of Toronto, Toronto, Canada M5S 3G3, Norwegian Institute for Air Research, The Polar Environmental Centre, NO-9296 Tromsø, Norway, Hellenic Center for Marine Research, Institute of Marine Biology and Genetics, GR 71003, Heraklion, Crete, Greece, Norwegian Institute of Public Health, P.O. Box 4404, Nydalen, N-0403 Oslo, Norway, and Department of Environmental Health, Boston University School of Public Health, Boston, Massachusetts 02118

Received November 16, 2009. Revised manuscript received March 17, 2010. Accepted March 31, 2010.

This review underlines the importance of indoor contamination as a pathway of human exposure to hexabromocyclododecanes (HBCDs), polybrominated diphenyl ethers (PBDEs), and perfluoroalkyl compounds (PFCs). There is ample evidence of substantial contamination of indoor dust with these chemicals and that their concentrations in indoor air exceed substantially those outdoors. Studies examining the relationship between body burden and exposure via indoor dust are inconsistent; while some indicate a link between body burdens and PBDE and HBCD exposure via dust ingestion, others find no correlation. Likewise, while concentrations in indoor dust and human tissues are both highly skewed, this does not necessarily imply causality. Evidence suggests exposure via dust ingestion is higher for toddlers than adults. Research priorities include identifying means of reducing indoor concentrations and indoor monitoring methods that provide the most "biologically-relevant" measures of exposure as well as monitoring a wider range of microenvironment categories. Other gaps include studies to improve understanding of the following: emission rates and mechanisms via which these contaminants migrate from products into indoor air and dust; relationships between indoor exposures and human body burdens; relevant physicochemical properties; the gastrointestinal uptake by humans of these chemicals from indoor dust; and human dust ingestion rates.

Introduction

This paper stems from the recent growth in studies that monitor, elucidate sources of, and evaluate potential human health impacts of human exposure to both brominated flame retardants (BFRs) and perfluoroalkyl compounds (PFCs) (*1–5*). Initial thinking about sources, fate, and human exposure pathways for these chemicals was informed by organochlorines such as dioxins for which sources are essentially outdoor and exposure primarily dietary. Recent research has challenged this paradigm for persistent organic pollutants (POPs) with significant indoor uses. For these, extensive indoor deployment contaminates indoor air and dust compounded by the high proportion of time spent indoors - an estimated 22 h per day for U.K. adults (*6*). Furthermore, while considerable uncertainty surrounds

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⊥ University of Antwerp.
National Food Administration.
∇ VU University Amsterdam.
○ University of Toronto.
◆ The Polar Environmental Centre.
¶ Institute of Marine Biology and Genetics.
+ Norwegian Institute of Public Health.
★ Boston University School of Public Health.

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Published on Web 04/13/2010

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human dust ingestion rates, the consensus is they are greater for young children (*7*).

While a wide range of POPs are present in indoor environments (*8*), this paper focuses on BFRs (polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs)) and PFCs (including perfluorocarboxylic acids/carboxylates (PFCAs, e.g. perfluorooctanoic acid (PFOA)), perfluoroalkylsulfonic acids/sulfonates (PFSAs, e.g. perfluorooctane sulfonate (PFOS)), and fluorotelomer alcohols (FTOHs)).

PBDEs are used in high impact polystyrene (HIPS) electronic housings, furniture foams, and fabrics at up to percent levels. The most recent figures for the three commercial formulations (Penta-BDE, Octa-BDE, and Deca-BDE) show their respective global production volumes in 2001 were 7500, 3790, and 56,100 t (*9*). Since PBDEs are blended physically within rather than bonded chemically to polymeric materials, they migrate into the environment where their persistence leads to contamination of humans (*10, 11*) that is of concern owing to their potential health risks (*12–16*). Such concerns have driven bans in several jurisdictions on manufacture and new use of all three formulations. Penta-BDE and Octa-BDE were listed recently under the Stockholm Convention on POPs (*17*) with some exposure guidelines proposed (*18, 19*).

Global production of HBCD in 2001 was 16,700 t (*9*). HBCD has found use as a flame retardant additive to expanded and extruded polystyrene foams for thermal insulation of buildings, back-coating of fabrics for furniture, and to a lesser extent in HIPS for electronic equipment like TVs (*3*). Like PBDEs, HBCD is not bound to polymeric products and is persistent and similarly ubiquitous in the environment and humans (*20, 21*). This has raised concerns because of its adverse health impacts in laboratory animals (*22–25*). Hence, while production continues, and no recognized health-based standard exists for HBCD, it is under active consideration for listing under the Stockholm Convention, and the European Chemicals Agency has declared it a priority substance under EU regulation that requires its associated risks to be controlled properly and its progressive replacement (*26*).

Industrial and consumer applications of PFCs are numerous owing to their unique properties (*27*). Examples include water-, soil-, and stain-resistant coatings for fabrics, oil-resistant coatings for paper products, fire fighting foams, paints, and floor polishes (*28–30*). Moreover, PFOA and other PFCAs are used as processing aids in production of fluoropolymers like polytetrafluoroethylene (*31*). Despite recent restrictions on production, they remain in humans (*32*), generating concerns about toxicity of some PFCs, with PFOS listed recently under the Stockholm Convention (*17*) and some exposure guidelines proposed (*33, 34*).

This paper addresses the following: strategies and methods for monitoring contamination and exposure; current evidence of contamination of indoor environments; source identification and attribution; causes of variability in contamination of indoor environments with BFRs and PFCs and the implications for source attribution and human exposure assessment and the contribution of indoor exposure to human body burdens.

The paper summarizes current knowledge, identifies gaps, and recommends research priorities.

Strategies for Monitoring BFRs and PFCs in Indoor Environments. *Indoor Air.* Sampling indoor air for BFRs, PFCs, and related POPs like polychlorinated biphenyls (PCBs) has been conducted via three approaches: (a) high-volume active sampling (*35*), (b) low-volume active sampling (*36*), and (c) passive air sampling (*37–40*). Each has benefits and disadvantages, but the principal concern is the comparability of data generated by different methods. High volume active air samplers underestimate concentrations if the volume of the microenvironment sampled is exceeded during sampling (*35*). This may also occur when deploying low volume active air samplers for extended periods in confined spaces like vehicles. Passive air samplers - the most common use polyurethane foam (PUF) disks (impregnated with XAD resin for PFCs (*41*)) as the sampling medium - avoid such problems, but semiquantitatively sample the vapor phase and a small but variable fraction of particulate-bound chemical. This is a significant limitation, as it precludes monitoring particulate-bound chemicals like BDE-209. Provided filter and vapor phase sorbents are used, this does not apply to active sampling. However, artifacts like volatilization of compounds from filter-collected particles, sorption of gas-phase compounds onto the filter, and reactions with oxidants during sampling may bias particle/gas partitioning estimates (*42*). Reports (*43*) of modified PUF disk-based samplers that sample quantitatively both particulate and vapor phases are thus timely. Moreover, notwithstanding a report of PBDE concentrations in different particle size fractions of outdoor air (*44*); given that finer airborne particles may more easily penetrate the lower respiratory tract, a potentially important data gap is the lack of knowledge regarding particle size distribution of BFRs and PFCs in indoor air.

The influence of sampling method is highlighted by a study where PBDE concentrations experienced by participants in their homes were significantly higher when using low volume active samplers worn by the participant (personal sampling), than when using low volume active samplers located at fixed points (static sampling) (*36*). Incremental exposure was greatest for congeners associated primarily with the particulate phase like BDE-209 and attributed to personal samplers capturing PBDEs associated with the "personal cloud" of particulates generated by participants.

Given elevated BFR concentrations in vehicle air, the location of samplers within the car is pertinent. Of the studies reporting airborne PBDEs in cars; while one deployed samplers in the trunk (*38*), the other sampled cabin air (*45*). The extent to which the former may fail to reflect accurately exposure of vehicle occupants is unknown, with studies required to establish whether significant differences exist in BFR contamination between the trunk and cabin. Moreover, such monitoring should ideally only be conducted during vehicle occupancy, reflecting only air to which occupants are exposed. This is especially pertinent in cars as the high in-vehicle temperatures and minimal ventilation that can occur during vehicle nonoccupancy can generate elevated BFR concentrations to which occupants are exposed only briefly.

Indoor Dust. A variety of approaches to sampling indoor dust exist. We stress the overriding objective of a sampling method in this context is to procure samples that reflect accurately the BFRs and PFCs to which an individual is exposed. Herein, we refer to such samples as "biologically-relevant" (*46*). With respect to "dust", we refer to settled dust for which exposure is presumed to occur via ingestion (usually accidental, but for some, particularly toddlers, deliberate). This is distinct from suspended dust, for which exposure occurs via inhalation. One approach is to take the contents of vacuum cleaners donated by householders (*38, 47*). Advantages are it provides an integrated measure of contamination and potential exposure from all rooms in which it is deployed. It is cost-effective and enhances donor compliance as it does not require the researcher to enter the home. However, such samples will not reflect accurately varying levels of contamination between different rooms. This may reduce the accuracy of exposure assessments if such between-room contamination differences are substantial. It seems unlikely that vacuum cleaners are used proportionally to the time spent—or more importantly, exposure—in each room. Other issues with participant-

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provided vacuum cleaner contents are as follows: variable vacuum cleaner sampling rates, the cleaner may be used in environments not frequented by the donor (e.g., lent to a friend), multiple uses of the same bag, and potential for post and during sampling contamination. Such issues introduce measurement error that will generally hide relationships to indicators of internal exposure.

A generally favored alternative is systematic procurement of samples by the research team using standardized procedures and equipment (*46, 48−54*). While possibly hindering donor compliance, this facilitates comparability between samples. Moreover, if pre-extracted sample receptacles (e.g., "socks"/Soxhlet thimbles) placed within the "sampling train" (furniture attachment) are used (*50, 54*), analyte loss and sample contamination (particularly from the vacuum cleaner) is minimized and sampling consistency maximized. The receptacles are replaced before taking each sample.

Even for researcher-procured samples, appreciable differences exist. While one approach samples the entire room surface until a sufficient mass of dust is collected (15−30 min) (*53*), another samples a standardized floor area for a standardized time period (*48−52*). Within-room variations exist in dust contamination with BFRs (*51, 52*). These mean vacuuming the entire room may oversample less-frequented parts; equally that sampling one specific area may not assess completely contamination within the room. This latter approach may afford a more biologically relevant dust sample provided the area sampled corresponds to that where exposure occurs.

Whether vacuum cleaner bags or researcher-collected samples are more suitable is unclear. In the only study of this issue, comparison of PBDEs in vacuum cleaner dust with researcher-collected samples for 20 homes found poor to moderate correlation between concentrations in the two sample types, with concentrations significantly lower in vacuum cleaner dust (*46*). The authors attributed this to the fact that researcher-collected dust was taken from rooms sampled specifically because of the likelihood of having sources of PBDEs. While confirming the influence of sampling method; without matching measurements of body burden, it does not indicate which is more biologically relevant (*46*). Similarly, no data exist comparing contamination in "whole-room" rather than "specific-area" dust samples. Combined with the absence of matched body burden measurements, assessment of the relative biological relevance of the two methods is not possible.

All the above studies sampled *floor* dust. The biological relevance of this is debatable. Instead, dust from elevated surfaces (e.g., bookshelves and tables) may reflect adult exposure better. While data on BFR concentrations in such samples exists (*55*), there is no systematic study of how concentrations in such samples compare with floor dust from identical microenvironments and their comparative biological-relevance.

Also pertinent is the upper and lower size fraction of dust collected. This is influenced by the pore size of the sampling receptacle (e.g., Soxhlet thimble) and the mesh size of any sieve employed postsampling. Assessment of the comparative biological relevance of different dust size fractions is required.

No universally agreed standard method exists for sampling indoor dust. Given the respective pitfalls/advantages of each method deployed, and uncertainty regarding their biological-relevance; there is insufficient information to allow development of a standardized method. Further research is required to identify the most biologically relevant approaches. Moreover, care should be taken when generalizing to larger populations from a relatively small number of samples, typically not randomly selected. It is recommended to provide as much detail as possible about sampling methods when reporting results.

Contamination of Indoor Environments. The database on indoor contamination with BFRs and PFCs was considered. We covered all indoor environments except occupational environments relating to BFR and PFC production or processes involving their incorporation into products and their dismantling. While the initial focus was on air, many recent papers report concentrations in dust. A comprehensive listing is not our aim, but Tables 1 and S1 and references therein illustrate the following key observations:

• While a substantial quantity of data for PBDEs exists, most relates to trihexabrominated congeners originating predominantly from the Penta-BDE product. The database for higher brominated congeners like BDE-209 in indoor dust is also substantial, but far less information is available for such congeners in air, as most studies have employed passive air samplers that sample mainly the vapor phase. More information is needed on HBCDs and PFCs in indoor air and dust. Additionally, while a past impediment to monitoring more volatile PFCs like FTOHs was the inability of conventional passive and active air sampling sorbents to retain such chemicals; recent development and application of XAD impregnated PUFs and alternative sorbent media should generate more data on such compounds (*41, 56−58*).

• Atmospheric concentrations of BFRs and PFCs indoors exceed substantially those outdoors.

• Distributions of BFR concentrations in indoor dust are highly skewed. Most studies report concentrations in a few samples that far exceed the median or geometric mean. This resembles the skewed pattern of human body burdens (*10, 59, 60*) and may indicate a causal link that would substantiate dust as the main source of exposure.

• The major PBDE in dust is usually BDE-209.

• Concentrations of Σtrihexa-BDEs in North American air and dust exceed those in Europe. This may explain higher body burdens in North Americans than in Europeans (*10, 59, 60*). However, those of BDE-209 in dust are highest in the U.K. and North America. The limited data suggest while international differences in indoor contamination with HBCDs exist, they are not substantial. There are too few data to evaluate whether such trends exist for PFCs.

• Most studies focus on homes, then offices; data exist that permit preliminary assessment of differences in contamination between microenvironment categories. However, we recognize the database is scant by comparison with that for outdoors and a more extensive database may reveal different trends. Current data suggest Σtrihexa-BDEs in offices exceed those in homes but there appears little difference between these microenvironment categories for other BFRs and PFCs. Far fewer data exist for cars, but vehicles in both the U.S.A. and Europe display measurable concentrations of PBDEs and HBCDs in air and dust with some samples displaying extremely high concentrations. Table 1 shows airplane dust can also display very high PBDE concentrations. In general, for HBCDs, cars, and for PBDEs, planes and cars appear to be heavily contaminated microenvironments. HBCDs and some PFCs are present in dust from primary school classrooms and child daycare centers in both Europe and the U.S.A. Too few data exist to permit meta-analysis of differences between microenvironment categories for PFCs, but differences between concentrations of PFOS and PFOA in different microenvironment categories do not appear significant.

Sources and Pathways of Contamination of Indoor Environments. The processes and rates via which BFRs and PFCs escape from treated products into the indoor environment remains poorly understood. Experimental evidence suggests some BFRs volatilize (*61−63*) before partitioning to dust, but this mechanism is not a convincing explanation of the elevated concentrations of BDE-209 in dust. While volatilization may explain low levels in air and dust of this

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TABLE 1. Summary of Median Concentrations (Range in Parentheses) of BFRs, PFOS, and PFOA in Indoor Dust (ng g^{-1}) and Indoor and Outdoor Air (pg m^{-3})

number of samples and location	target POPs	microenvironment category: homes	offices	daycare centres/ primary school classrooms	cars	outdoor	planes	ref
				Air				
n = 31, 33, 25, and 110 for U.K. homes, offices, cars, and outdoors, respectively	Σtrihexa-BDEs	24 (4–245)	71 (10–1416)	-	41 (11–8184)	8.7 (0.49–30)	-	(*38, 103*)
n = 74 and 7 for Ottawa homes and outdoors, respectively	Σtrihexa-BDEs	100 (2.0–3600)	-	-	-	2.6 (<dl-4.4)	-	(*40*)
n = 46, 24, and 14 for Kuwait homes, offices, and outdoors, respectively	Σtrihexa-BDEs	8.2 (2.5–136)	8.6 (2–385)	-	-	9.3[a] (2.5–32)	-	(*37, 104*)
n = 20, 41, and 35 for Boston homes, Greek cars, and Ontario, Canada for outdoors, respectively	BDE-209	94[b] (<48–651)	-	-	104 (nd-1,053)	19[a] (<dl-105)	-	(*36, 45, 105*)
n = 24 for U.S.A. (vapor phase)	Σtrihexa-BDEs	3200 (nr-15,000)	-	-	-	-	-	(*69*)
n = 33, 25, and 5 for U.K. homes, offices, and outdoors, respectively	ΣHBCDs	180 (67–1300)	170 (70–460)	-	-	37 (34–40)	-	(*48*)
				Dust				
n = 30, 18, and 20 for U.K. homes, offices, and cars, respectively	Σtrihexa-BDEs	46 (7.1–250)	100 (16–1100)	-	190 (54–22,000)	-	-	(*51*)
n = 46 and 60 for U.S. homes and cars, respectively	Σtrihexa-BDEs	1910 (590–34,400)	-	-	2,600 (436–59,800)	-	-	(*46, 106*)
n = 18, 15, and 9 for U.K. homes, offices, and cars, respectively	BDE-209	8100 (<dl-2,200,000)	6200 (620–280,000)	-	100,000 (12,000–2,600,000)	-	-	(*51*)
n = 46 and 60 for U.S. homes and cars, respectively	BDE-209	<dl (<dl-9600)	-	-	48,100 (4380–3,570,000)	-	-	(*46, 106*)
n = 24 for both U.S. homes and cars	ΣBDEs (inc. BDE-209)	21,000 (nr-290,000)	-	-	28,000 (nr-210,000,000)	-	-	(*69*)
n = 24 for both U.S. homes and cars	BDE-209	190 (<DL-66,000)	-	-	3,100 (<DL-210,000,000)	-	-	(*69*)
n = 20 in planes	Σtrihexa-BDEs	-	-	-	-	-	9,300 (270–760,000)	(*107*)
n = 20 in planes	BDE-209	-	-	-	-	-	17,700 (<DL-192,000)	(*107*)

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TABLE 1. Continued

number of samples and location	target POPs	microenvironment category: homes	offices	daycare centres/ primary school classrooms	cars	outdoor	planes	ref
n = 45, 28, 20, and 20 for U.K. homes, offices, primary school classrooms, and cars, respectively	ΣHBCDs	1,300 (140–140,000)	760 (90–6600)	5,200 (72–89,000)	13,000 (190–69,000)	-	-	(*48, 108*)
n = 13 for U.S. homes	ΣHBCDs	390 (110–4000)	-	-	-	-	-	(*49*)
n = 8 for Canadian homes	ΣHBCDs	640 (64–1300)	-	-	-	-	-	(*49*)
n = 20 for U.S. homes (main living area)	ΣHBCDs	354[b] (<4.5–130,200)	-	-	-	-		(*100*)
n = 10, 38, 10, 10, and 5 for Swedish houses, apartments, offices, daycare centers, and cars, respectively	PFOS	39 (15–120; houses); 19 (8–1100; apartments)	110 (29–490)	31 (23–65)	12 (8–33)	-		(*93*)
n = 20 for U.K. classrooms and child daycare centers	PFOS	-	-	1200 (85–3700)	-	-		(*108*)
n = 112 for U.S. homes and child daycare centers[c]	PFOS	142 (<dl-1960)	-	-	-	-		(*109*)
n = 67 for Ottawa homes	PFOS	38 (2.3–5065)	-	-	-	-		(*75*)
n = 10, 38, 10, 10, and 5 for Swedish houses, apartments, offices, daycare centers, and cars, respectively	PFOA	54 (15–98; houses); 93 (17–850; apartments)	70 (13–510)	41 (31–110)	33 (12–96)	-		(*93*)
n = 20 for U.K. classrooms	PFOA	-	-	220 (42–640)	-	-		(*108*)
n = 112 for U.S. homes and child daycare centers[c]	PFOA	201 (<dl-12,100)	-	-	-	-		(*109*)
n = 67 for Ottawa homes	PFOA	20 (1.2–1234)	-	-	-	-		(*75*)

[a] Average. [b] Geometric mean. [c] Concentrations for daycare centers not reported separately from those in homes; <dl = below detection limit; nr = not reported.

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highly involatile congener, recent studies exploiting the utility of forensic microscopy suggest strongly physical weathering occurs of particles or fibers from flame-retarded items (*64, 65*). These studies showed heterogeneous bromine distribution within dust containing very high BDE-209 concentrations, with samples dominated by a small number of particles/fibers highly enriched in bromine. While such techniques may offer insights into mechanisms via which BFRs and other trace contaminants transfer from treated items into dust, wider application of such techniques (e.g., elucidating sources of other BFRs in less contaminated dust samples) is needed to realize their potential.

Very few emission factors for BFRs from treated goods exist (*61, 62*), and controlled chamber studies to generate such data are required. A modeling study generated Σtrihexa-BDE emission factors from a personal computer (*66*). A tandem approach using both chamber and modeling studies is thought the most effective strategy to understanding indoor "effective" emissions and fate of BFRs and PFCs where "effective" refers to the emission rate that is a function of the receiving environment as well as the source.

Despite widespread BFR use in furniture, electronic goods, and construction materials, studies correlating BFR contamination in a microenvironment with numbers of potentially BFR-containing items have had mixed success owing to exposure misclassification. Use of hand-held X-ray fluorescence (XRF) instruments to determine the bromine content of household items appears promising (*67*). XRF is nondestructive, fast, and cost-effective. Correlations using XRF-determined bromine content as opposed to item counts were much stronger (*67*), and this approach offers potential for at least semiquantitative prediction of BFR contamination and human exposure. Indeed, XRF has been used to identify significant positive correlations between PBDE concentrations in human blood of 44 Americans and bromine content of their sleeping pillows and primary car seat cushions, suggesting proximity to such flame-retarded items may constitute a major source of exposure (*68*). A caveat is that use of XRF in this fashion does not eliminate exposure misclassification completely. It does not distinguish between bromine in PBDEs from that in other BFRs nor identify which PBDE formulation is present and can only detect bromine close to the surface of tested items, thereby misclassifying bromine-containing items where BFRs lie within a product (e.g., in printed circuit boards). Another complication is that XRF cannot predict the release rate of the contaminant from the treated item as this depends on e.g. the volatility of the BFR, the mode of incorporation (reactive or additive), the nature of the product itself (i.e., release of additive BFRs from a fabric is likely easier than from HIPS), and the driving force for emission (e.g., fugacity gradient driving volatilization). Such factors suggest relationships between contaminants in products and dust are stronger than suggested by current studies (*67*).

Sources of Variability in Concentrations. Understanding of how BFR and PFC concentrations vary over space (different rooms in a home, different parts of the same room) and time is limited. Such variations are important given their implications for source attribution and exposure. How representative is a single sample of air or dust from one room? Are there significant spatial variations in contamination within a room that impact on the accuracy of exposure assessment? Do concentrations in the most-frequented area of the room differ substantially from elsewhere?

A U.S. study reported no significant change in Penta- and Deca-BDE congeners but a statistically significant difference in Octa-BDE congeners in dust samples taken from the same rooms ($n = 40$) eight months apart (*46*). However, the latter were minor constituents in dust. More substantial temporal variability for PBDEs and HBCDs was observed in dust sampled monthly in U.K. homes over 9−10 months (*51, 52*). The maximum Σtrihexa-BDE concentration exceeded the minimum by a factor of ~50, 3.5, and 5.5 in the three homes, respectively; for BDE-209, these figures were 7.5, ~400, and ~35, and for ΣHBCDs 2.6, 224, and 40. In another study, PBDE concentrations in dust collected from 12 U.S. homes in two different seasons showed little consistency (*69*). While inconclusive owing to the very small number of environments studied, the data suggest substantial variation in estimates of exposure is possible, depending when a given room is sampled. Although temporal variation may not be hugely influential when considered alongside other potential influences on exposure; it provides one possible explanation for the order of magnitude differences in BDE-209 contamination of blood serum from members of one family sampled 90 days apart (*70*), particularly since the human half-life of BDE-209 is 15 days (*71*). In contrast, BDE-153 is estimated to require much longer (several years) to reach equilibrium in serum (*72*). Hence no significant change in body burden would be anticipated to arise from the reported temporal variations in dust contamination. It appears the influence of temporal variations should be considered when designing sampling strategies for monitoring both external exposure and body burdens for BFRs and PFCs with short human half-lives. In the U.K. studies, most of the temporal variability was attributable to changes in room contents, with removal and reintroduction to one room of a TV, coinciding with significant changes in HBCD concentrations (*52*).

With respect to within-building variations, while Penta- and Deca-BDE concentrations in 20 U.S. homes were significantly higher in the main living area than the bedroom, but there was no significant difference in Octa-BDE concentrations (*46*). Within-room spatial variability of HBCDs in dust appears related to proximity to sources. Declining ΣHBCDs concentrations were reported with distance from a TV in one U.K. home, with similar declines in a U.K. lecture hall with increasing distance from a PC and video projection console (*52*). In contrast, while within-room variability for PBDEs exceeds that attributable to sampling and analytical variation; its origins were not explicable (*51*).

While no data exist about within-room spatial variability in BFR and PFC concentrations in indoor air, variability between rooms in the same building and temporal/seasonal variability within the same room have been examined for trihexa-BDEs (*63*). This U.K. study reported appreciable (sometimes statistically significant) seasonal variation in trihexa-BDEs in 4 homes and 4 offices. Seasonal variability was less than that for PCBs in outdoor air (*73*). This was attributed to the narrower temperature range indoors and mitigation of summer peaks in concentration due to enhanced ventilation. In contrast, no seasonal variation in trihexa-BDE concentrations was found in 12 U.S. homes over two seasons (*69*). These data are consistent with those for PCBs (*74*). Concentrations of Penta-BDE and Deca-BDE in 20 Boston homes measured during winter (to control for ventilation) were correlated only moderately between the two rooms sampled in each home (*36*).

While a significant positive correlation between PFCs including PFOA and PFOS in indoor air and house dust and percentage of home carpeting was reported for 59 Ottawa homes (*75*), no correlation was found for perfluorooctanesulfonamides and sulfonamidoethanols (*76*). Using window films as a passive sampler of indoor concentrations, considerable differences were observed in PFC profiles in several buildings. The extent of carpeting and use of floor wax contributed to this variability (*77*). Within room and within building differences depended on the presence of a central building ventilation system and indoor-outdoor air exchange rates.

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Changes in room contents can influence airborne contamination. Within-room temporal variation offers insights into the validity of basing exposure assessments on a single spot measurement of contamination as well as source attribution. For example, monthly monitoring of office air over 10 months revealed an approximately 75% decrease in Σtrihexa-BDEs concentrations following replacement of a computer (*63*).

Indoor Exposure to and Its Influence on Human Body Burdens. A pivotal issue is the influence of indoor contamination on human body burdens. Understanding of this remains incomplete. Current thinking is that for BFRs indoor exposures may be for many individuals comparable to or greater than dietary intake. Ingestion of dust is considered the principal indoor exposure pathway, especially for young children (*78*). This is consistent with Penta-BDE congener concentrations in pooled Norwegian blood serum from 0−4 year-olds exceeding substantially those in adults (*79*). Likewise, in Australia, PBDE concentrations in pooled blood serum peaked at 2.6−3 years when breastfeeding has typically stopped (*80*). Similarly, a U.S. study showed higher PFOS and PFOA concentrations in pooled blood serum from 3−5 year olds compared to adults (*81*). Moreover, PFOA, perfluorohexanesulfonate (PFHxS), and perfluorononanoate concentrations in blood were highest in Australians <15 years, while PFOS levels peaked in adults >60 years (*32*). In contrast, another study found that while a subgroup of children displayed considerably higher PFHxS concentrations than adults, concentrations of other PFCs were similar in adults and children (*82*).

A recent study used a steady-state first order relationship (eq 1) to predict observed body burdens of PBDEs for Americans from observed intakes (*83*)

$$C = D/(k^{*}\,\mathrm{Vd}) \qquad (1)$$

where C = concentration in humans (mass per mass lipid for PBDEs/HBCDs or mass per blood volume for PFCs), D = intake from one or more pathways (mass per mass body weight per day), k = first order elimination rate constant (day^{-1}), and Vd = volume into which chemical is distributed in the body (mass of lipid for PBDEs/HBCDs or blood volume for PFCs).

Lorber (*83*) found substantial underprediction of body burdens when only dietary intake was considered but much closer agreement when exposure via ingestion of indoor dust was also considered. It was thus concluded that dust ingestion constitutes the principal exposure pathway to ΣPBDEs (BDEs 28, 47, 99, 100, 138, 153, 154, 183, and 209) for Americans (*83*). In addition, this pharmacokinetic modeling approach provides a conceptual framework for understanding relationships between exposure and body burden and for identifying knowledge gaps. While for PBDEs, parameters C, Vd, and D (for dietary intake) are well-characterized, aspects where understanding is incomplete are congener-specific human elimination rate constants (an overerestimate of the elimination rate constant for BDE-47 was identified as the likely cause of the substantial underprediction of this congener (*83*)), the scant database on human body burdens of BDE-209, and the reliance on the assumption that human body burdens are at steady state. Other uncertainties exist. While PBDE concentrations in indoor dust are well-established, intakes via ingestion of dust are influenced strongly by the dust ingestion rate. This is very uncertain, based on a very small number of primary studies designed to derive estimates of soil ingestion (*84, 85*). Better characterization of human dust ingestion rates constitutes a significant research gap. As it is thought dust ingestion occurs primarily via hand-to-mouth contact, a recently explored approach used contamination present in hand wipes to estimate exposure to PBDEs (*86*). However, uncertainties remain including the frequency and duration of hand-to-mouth events and efficiency of hand-to-mouth transfer. Furthermore, the bioavailability of BFRs and PFCs received by different pathways, i.e. inhalation, dust ingestion and diet, exerts a crucial influence on the intake (D) but remains currently little understood. The only peer-reviewed study reported the bioavailability to rats of PBDEs was similar regardless of whether the dose was administered as indoor dust or dissolved in corn oil, implying bioavailability of PBDEs from dust could be similar to that from diet (*87*). A pilot study of HBCD bioaccessibility from dust using an *in vitro* colon-enhanced physiologically based extraction test model found ΣHBCDs bioaccessibility was substantial. As with the PBDE bioavailability study in which the proportion retained varied between 69% (BDE-47) to 4% (BDE-209), substantial diastereomer-specific variation in bioaccessibility was observed (*88*). Such studies suggest human uptake of PBDEs and HBCDs from indoor dust is compound-specific and similar to that from diet.

The pharmacokinetic approach founded on eq 1 has also been applied to PFOS and PFOA. Several similar modeling approaches found dust and inhalation contribute to total intakes but that relative to diet (a major source) may not be as important as estimated for PBDEs (*89−92*). Specifically, indoor dust ingestion contributed ~5−10% of total PFOA intake when background contamination of drinking water was assumed (*92*). Drinking water was found to be an important pathway when water supplies were impacted by a point source of PFOA (*92*). However, hand-to-mouth contact with carpets was considered a major pathway of PFOS and PFOA exposure for infants, toddlers, and children (*89*). Moreover, while a Swedish study considered diet the most important exposure route, dust ingestion was significant under scenarios assuming a high dust ingestion rate (*93*). In addition to the uncertainties noted for PBDEs, additional research gaps exist for PFCs. Examples are the volume of blood assumed to be available for PFC distribution (substantial disagreement exists between otherwise similar studies (*92, 94*), the lack of spatially consistent estimates of intake from different exposure pathways (the study of Vestergren and Cousins (*92*) used U.S. dust intakes and German dietary intakes), and the infrequent detection of PFCs in the human diet (the German study (*94*) used by Vestergren and Cousins (*92*) could not detect PFOA in 117 and PFHxS in 208 out of the 214 diet samples analyzed).

A complementary approach to elucidating the influence of indoor exposures on human body burdens is regression of concentrations in dust and diet to which an individual is exposed with concentrations in blood, milk, or placental tissue. Six such studies exist for PBDEs (*11, 54, 95−98*) with one examining HBCDs (*21*). With two possible exceptions (*96, 97*), study power is limited by small participant numbers. A U.S. study of 12 participants detected significant positive correlations between Penta-BDE congeners in human milk and indoor dust and with exposure estimated via reported dietary habits from consumption of both dairy products and meat (*54*). A study of five Swedes reported a positive linear relationship between ΣBDE concentrations (including BDE-209) in house dust and plasma (*98*). However, the relationship was dependent strongly on one observation. Most recently, a Danish study of 47 volunteers detected a significant positive correlation between BDE-47 concentrations in indoor dust and placental tissue but not for other congeners (*96*). Neither the Belgian study of 19 individuals (*11*) nor that of 50 German subjects (*97*) could detect any correlation between Penta-BDE congener concentrations in human blood serum and in indoor dust and duplicate diets (and air for the German study). With two small exceptions (most notably a correlation between BDE-99 in air (but not dust) and milk), a similar

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lack of correlation between PBDEs in dust, air, and human milk was observed for the Australian study of 10 women (*95*). HBCD concentrations in dust but not diet were correlated positively with those in Belgian serum (*21*). In both the Belgian and U.S. studies (*11, 54*), difficulties in detecting BDE-209 in the milk or serum of participants meant the existence of a relationship between intake and body burden for BDE-209 could not be assessed. In contrast, BDE-209 was detected in the majority of Australian human milk samples but displayed no correlation with either air or dust (*95*). The absence of correlation between PBDEs in Belgian dust and diet and in matched serum samples was attributed to higher past and episodic current intakes being more important determinants of body burden than spot measurements of exposures made during the week-long study (*11*). Such findings underline the difficulties in obtaining biologically relevant measures of intake. Larger, more powerful, studies of the relationship between dust exposure and body burdens are required. As well as larger numbers of subjects, measurements of dietary and indoor intakes and body burdens should cover longer time periods to allow as far as possible for temporal fluctuations.

Research Priorities

This review underlines the importance of indoor contamination with BFRs and PFCs. While progress has been made, there are a number of areas that should form the focus of future research. One observation is that while significant knowledge gaps remain for PBDEs, we know much less about indoor exposure to PFCs and other BFRs. Specific gaps include studies that will

1. Evaluate interventions designed to reduce indoor exposures. These should range from immediate actions to enable individuals to reduce their likely burden (e.g., manipulate room ventilation, minimize carpeted areas and other chemical sinks) to longer term strategies (e.g., minimization of chemical migration from products by modifying product formulation and design).

2. Quantify emissions and elucidate pathways via which less volatile chemicals like BDE-209 migrate from products into and between air and dust, and other compartments of the indoor environment. This may be achieved via both experimental studies and mathematical modeling. Likewise, how are we primarily exposed to chemicals in dust: via inhalation of small particles, from hand-mouth contact, or dermal contact?

3. Characterize better the emission rates of BFRs and PFCs from treated goods.

4. Provide better data on BDE-209 in indoor air, diet, and human tissues. Combined, these will establish the relative influence of different exposure pathways on human body burdens particularly for toddlers/young children. A significant barrier for both BDE-209 and other BFRs and PFCs is the lack of validated, noninvasive indicators of body burden, and evaluation of alternatives like hair, saliva, and feces is required.

5. Improve understanding of the influence of different air and dust sampling strategies on interstudy comparability and the biological relevance of samples taken.

6. Monitor a fuller range of microenvironments. Most current data are for homes. While domestic environments are likely important vectors of exposure, data on cars and planes suggests they too may be important for some (e.g., taxi-drivers and aircrew) and require more extensive monitoring. Schools and child day-care centers, offices, and other transportation microenvironments like buses and trains are other microenvironments for which more data are required.

7. Widen the international coverage of current monitoring to facilitate better understanding of connections between production/use volumes in different countries and indoor concentrations and exposures.

8. Derive accurate dust ingestion rates and simultaneously improve our ability to procure "biologically relevant" measures of exposure. This includes improved characterization of the extent and causes of within-room spatial and temporal variability and within-building spatial variability in contamination. Such knowledge can be incorporated into exposure monitoring strategies - especially for BFRs and PFCs with short human half-lives.

9. Determine better the extent of and factors influencing human bioavailability and/or bioaccessibility of BFRs and PFCs in indoor dust.

10. Consolidate and improve the database on human half-lives and relevant physicochemical properties of BFRs and PFCs.

11. Conduct larger and more powerful studies of the relationship between concentrations of BFRs and PFCs in dust and body burden.

Most importantly, we need to utilize science to develop better policies to manage the past, current, and future reservoirs of BFRs and PFCs associated with indoor environments such that exposure is minimized. Such efforts require monitoring and comprehension of environmental behavior not only for those chemicals currently on the horizon but also those now emerging into the consciousness of exposure assessors. Examples include replacements for recently restricted BFRs, such as organophosphorus flame retardants (e.g., tris(1,3-dichloro-2-propyl)phosphate) and "new" BFRs like (2-ethylhexyl)tetrabromophthalate, 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE), and decabromodiphenyl ethane (DBDPE) (*51, 99–102*). All of this must be conducted alongside programs to comprehend better the human health impacts of BFRs and PFCs. Such programs should combine both experimental and pharmacokinetic assessment of multiple exposure pathways, their influence on body burdens, and epidemiology.

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Supporting Information Available

Summary of concentrations of selected PFCs in indoor and outdoor air and indoor dust (Table S1). This material is available free of charge via the Internet at http://pubs.acs.org.

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Recruitment Strategy
Toxicologist
18 February 2016

The appendices describe efforts on behalf of the Risk Assessment Division (RAD), and the Design for the Environment (DfE)/ Safer Choice strategy to broaden the pool of eligible candidates for Toxicologist positions in RAD and one position in DfE.

1. Notify Academic Institutions -- Appendix A & Appendix F
    a. Physical Science- School recruitment online websites (registration required) listed based on a combination of staff knowledge of quality of programs, school rankings (e.g., U.S. News and World Reports), and geographic criteria (metro Washington DC area).
    b. Toxicology – Schools with specific recognized toxicology programs.
    c. Posting job announcements.
    d. Draft RAD & DfE text for distribution and posting.
2. Notify Personal contacts -- Appendix B
3. Submit to Listservs and other job boards (e.g., professional organizations) -- Appendix C
4. Post Program narratives for outreach to social media: blogs, twitter, ACS, other public sites
    a. Appendix D: Safer Choice narrative that was published on the EPA blog.
    b. Appendix E: RAD functional statement -- distributed at the PMF Job Fair, August 14, 2015

5. Appendix F: Table of schools with hot links to relevant programs from "Eco/Human Health Outreach List (RAD 1/20/2016, Iris Camacho)

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Appendix A

School Recruitment, Career Portals, Professional Development Online Websites

Also - Appendix F: Eco/Human Health Outreach (RAD 1/20/2016, Iris Camacho)

a. Physical Science

DfEB registered with and will post an announcement on the websites of the following schools

School	State
Boston University	MA
Carnegie Mellon	PA
Columbia	NY
Emory	GA
George Washington	DC
Harvard	MA
Johns Hopkins	MD
Penn State	PA
Pittsburgh	PA
Stanford	CA
Texas A&M	TX
UC Berkeley	CA
UNC	NC
University of Maryland	MD
University of Texas – Austin	TX
University of Virginia	VA
UPenn	PA
Virginia Tech	VA
Yale	CT

b. Toxicology

The following table lists the U.S. News and World Reports best U.S. universities for Pharmacology and Toxicology: http://www.usnews.com/education/best-global-universities/pharmacology-toxicology).

U.S. News and World Report 2016: Top Universities for Pharmacology and Toxicology (U.S.) Based on Reputation and Research

Best Pharmacology & Toxicology Programs

Harvard University	Cambridge, MA
University of California--San Francisco	San Francisco, CA
University of North Carolina--Chapel Hill	Chapel Hill, NC
University of California--San Diego	La Jolla, CA
University of Washington	Seattle, WA
University of Michigan--Ann Arbor	Ann Arbor, MI
Duke University	Durham, NC
Johns Hopkins University	Baltimore, MD
Vanderbilt University	Nashville, TN
University of Pittsburgh	Pittsburgh, PA
Stanford University	Stanford, CA

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c. Posting on Academic Career Services/Professional Development and School websites

Web-based career portals (such as those above under Appendix A.i. & ii) offer services that range from posting job announcements, to Career Fairs, interviews, and customized recruiting

Although each web portal differs, the standard information required to post a job announcement is taken from the USAJobs announcement.

Job Posting Title	Job Location
Citizenship Requirements	Job function
Number of Openings	Position type
Days/Hours	Academic levels
Wage/ Salary	Degree levels
Employment start date	Majors
Job Description	Employer Name
Qualifications	Employer Contact information
Application Instructions	Posting date
Online Application Address or Link	Expiration data
Website link	Option to receive email when students apply

d. Draft RAD & DfE text for distribution and posting:

U.S. EPA's Risk Assessment Division (RAD) and Design for the Environment (DfE) Branch are announcing multiple vacancies to serve as toxicologist at EPA. The Risk Assessment Division leads all agency Risk Assessment under the Toxic Substances Control Act. The DfE Branch, which houses the Agency's Safer Choice program, uses multi-stakeholder engagement and technical tools to identify and encourage the use of safer chemicals in consumer products and industrial processes. Additional program information is available at http://www.epa.gov/aboutepa/about-office-chemical-safety-and-pollution-prevention-ocspp#oppt and http://www2.epa.gov/saferchoice. The positions are open to U.S. citizens through [Add date]. To review the Toxicologist position announcement and apply: [Add USAJobs Announcement link].

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Appendix B: Personal Staff Professional Contacts

Organization	Contact	DfE Staff
Emory	Email Ariadne Switchenberg	Aly
William and Mary	Email Adam Anthony	Aly
ASPPH Fellow Alums	Email	Aly
Carnegie Mellon	Email Terry Collins	Melanie
Juniata College	Email Richard Hark	Melanie
Virginia Tech	Email Sean McGinnis	Kathy
University of Washington	Email Nadine Gruhn	Bridget
AAAS	Web Listserv	Emma
SETAC	Web Listserv	Emma
Baylor	Email Bryan Brooks; George Cobb	Emma
N. Texas	Email Aaron Roberts	Emma
UCBerkeley	Email Arlene Blum	Emma
GW	Email Adelina Voutchkova-Kostal & Jakub Kostal	Emma
East Carolina	Email Brody School of Medicine Jamie DeWitt	Emma
U. Houston	Email V.Chancellor for Research Mary Ann Ottinger	Emma
		RAD Staff
UMass Amherst	Ed Calabrese	Gino
UNC – Chapel Hill	Alex Tropsha	Gino
North Carolina State	David Dorman	Gino
Rutgers	Hao Zhu	Gino

Example of email for personal contacts:

RAD:

I am writing to share with you the job announcement for Toxicologist vacancies in my office (the Risk Assessment Division]) at the U.S. EPA, Office of Pollution Prevention and Toxics. The Risk Assessment Division performs technical and science policy analyses to support regulatory and non-regulatory programs in OPPT. Specifically our group evaluates the health and environmental hazards, environmental releases, environmental fate, exposures (occupational, consumer and general population), engineering processes and risks of new and existing chemicals, microorganisms and applications of biotechnology.

Information on the program is available at http://www.epa.gov/assessing-and-managing-chemicals-under-tsca You may review the position and apply at [Add link]. The position is open to U.S. citizens through [Add date]. Please help us spread the word about the positions and encourage those you know to apply.

DfEB:

I am writing to share with you the job announcement for Toxicologist vacancies in my office (the Design for the Environment Branch) at the U.S. EPA, Office of Pollution Prevention and Toxics. The DfE Branch, which houses the Agency's Safer Choice program, uses multi-stakeholder engagement and technical tools to identify and encourage the use of safer chemicals in consumer products and industrial processes.

Information on the program is available at http://www2.epa.gov/saferchoice. You may review the position and apply at [Add link]. The position is open to U.S. citizens through [Add date]. Please help us spread the word about the positions and encourage those you know to apply.

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Appendix C

Sample Listservs and Other Job Boards

Organization	EPA Contact
Green Chemistry Listserv	Beth Drake
ACS	Carol Hetfield
Returned Peace Corps Volunteers	Bridget Williams

Example of text for listservs and job boards:

U.S. EPA's Risk Assessment Division (RAD) and Design for the Environment (DfE) Branch are announcing multiple vacancies to serve as toxicologist at EPA. The Risk Assessment Division leads all agency Risk Assessment under the Toxic Substances Control Act. The DfE Branch, which houses the Agency's Safer Choice program, uses multi-stakeholder engagement and technical tools to identify and encourage the use of safer chemicals in consumer products and industrial processes. Additional program information is available at:

- http://www.epa.gov/aboutepa/about-office-chemical-safety-and-pollution-prevention-ocspp#oppt
- http://www.epa.gov/assessing-and-managing-chemicals-under-tsca
- http://www2.epa.gov/saferchoice.

The positions are open to U.S. citizens through [Add date]. To review the Toxicologist position announcement and apply: [Add USAJobs link].

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Appendix D

EPA Blog Post

[Safer Choice narrative that was published on the EPA blog]

EPA Blog - Safer Choice Post

How you can help people make safer choices every day

How many people can say they really love their job? Lucky for me, I'm one of those people. As part of the Safer Choice Program the work I do helps people make safer choices for their families, pets, and the environment every day.

Safer Choice is EPA's label for safer chemical-based products, like all-purpose cleaners, laundry detergents, degreasers, and many others. Each day, consumers, custodians, cleaning staffs, and others use these products, and families, building occupants, and visitors are exposed to them. The Safer Choice program ensures that labeled products—and every ingredient in them—meet the program's stringent health and environmental criteria—and perform well, too.

Working in the Safer Choice Program, I have the privilege of seeing the results of our work in many tangible forms in real-time, every day. When I go to the grocery store and see a labeled product on the shelf, I know that the work I do helps protect people, animals and the environment from toxic chemicals.

So how can you help people make safer choices?

- First, look for products with the Safer Choice label in stores this summer. By choosing products with the Safer Choice label, you're driving the development of greener chemicals and supporting over 500 manufacturers and retailers that participate in our program.
- Spread the word about Safer Choice products to your friends and family. Let them know that over 600 consumer products qualify for the Safer Choice label. You can help them find Safer Choice products by searching our list of labeled products.
- Encourage the use of Safer Choice products in your community, at work, school and local businesses. Almost 2,000 products with the Safer Choice label are made specifically for industrial use. Check out our interactive graphic to find Safer Choice products for your community.

Also, if you're interested in helping people make safer choices across the country, take a look at two new Safer Choice job announcements: [link to announcements]. We're looking to build our team to take on the enormous opportunities in labeling safer personal care products. Perhaps we'll get to share the adventure.

Learn more about Safer Choice

Connect with us on Facebook: https://www.facebook.com/EPASaferChoice

About the Author: David DiFiore has worked for the Safer Choice Program since 1997. Before that, David worked in several other EPA programs, including the New Chemicals Program, where he learned the science and art of identifying and promoting safer chemicals and products.

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Appendix E

Source: RAD Functional Statement and Hiring Needs, PMF Hiring Fair, 8/11/2015

RISK ASSESSMENT DIVISION OF THE OFFICE OF POLLUTION PREVENTION AND TOXICS (OPPT)

The Risk Assessment Division (RAD), under the supervision of a Director, performs technical and science policy analyses to support OPPT regulatory and non-regulatory programs.

This Division's responsibilities include:

- Assessing and integrating the health and environmental hazards, environmental releases, environmental fate and transport, industrial hygiene, exposures, engineering processes and risks of new and existing chemicals, microorganisms and applications of biotechnology;
- Preparing hazard, fate, releases, exposure, engineering and risk characterizations, integrated and cross-cutting as necessary for the regulatory and non-regulatory programs they support;
- Reviewing and evaluating test data, predicted or modeled estimates or weight-of-evidence characterizations for health and environmental effects, fate and transport, as well as exposure and release related monitoring data/information, collected or submitted under TSCA or other authorities administered by OPPT or EPA;
- Developing and updating effects, environmental fate, release and exposure test methods, measurement techniques and assessment procedures, frameworks, and guidelines used to support OPPT programs and ensuring that procedures and criteria employed by OPPT are consistent with Agency guidelines and the current state-of-the-science;
- Developing, evaluating, managing and maintaining models, expert systems or other evaluation tools used by OPPT in any/all aforementioned activities;
- Collaborating with other OCSPP Offices (e.g., Office of Pesticides Programs & Office of Science Coordination & Policy) and EPA Offices (e.g., Office of Water, Office of Research and Development, Risk Assessment Forum) in defining and reviewing Agency science policies, scientific methods or approaches or research related to characterizing the health and environmental effects, engineering, environmental releases and fate and exposure of chemicals, microorganisms and applications of biotechnology;
- Participating and collaborating in international efforts related to health and environmental assessment and evaluation of chemicals and chemical safety; (8) providing expert scientific evaluation, analysis or other technical support to Agency reviews, regulations, audits, or enforcement activities;
- Contributing to and/or supporting OPPT's Pollution Prevention goals and activities (e.g. DfE, green chemistry and green engineering); and
- Managing the efficient flow of work and assignment of expertise necessary to accomplish the responsibilities previously described.

The Risk Assessment Division has the intention to hire PMF candidates grades 9/11/12 technical levels in multiple disciplines that would qualify to do the following:

Exposure and Fate assessment
Environmental engineering/ occupational exposure assessment
Microbiology
Epidemiology
Toxicology mechanistic and adverse outcome pathway analysis
Toxicology with experience in adsorption, distribution, metabolism and excretion (ADME) analysis.

Note that this covers multiple OPM jobs series including 400, 600 and 1300.
Please contact Stan Barone Jr, PhD. Deputy Director at barone.stan@epa.gov or 202.564.1169

Appendix F

ECO/Human health Outreach List (RAD 1/20/2016, Iris Camacho)

Professional Societies/Career sites:

Society of Toxicology (SOT) - http://www.toxicology.org/index.asp

- Job Bank-there is a fee of $110 to use the services, http://www.toxicology.org/application/jobbank/employerRecruiter/login.aspx
- Toxchange- you need to be a SOT member to post message to the community
- Special Interest Groups
  - American Association of Chinese inToxicology: Ching-Hung Hsu (president), chinghunghsu@yahoo.com
  - Association of Scientists of Indian Origin: Sudheer Beedanagari (president), beedanas@bms.com
  - Hispanic Organization of Toxicologists: Minerva Mercado (president), mercado.minerva@epa.gov
  - Korean Toxicologists Association in America: Christopher J. Choi (president), christopherchoi02@hotmail.com
  - Toxicologists of African Origin: Christopher W. Stewart (president), Christopher.stewart@mpiresearch.com
  - Women in Toxicology: Tao Wang (president), tao.wang@novartis.com
- Specialty Sections:
  - Immunotoxicology: Rafael A. Ponce (president), rponce@amgen.com
  - Inhalation and Respiratory: Jacob McDonald (president), jmcdonal@lrri.org
  - Metals: Walter T. Klimecki (president), klimecki@pharmacy.arizona.edu
  - Mixtures: David W. Herr (president), herr.david@epa.gov
  - Nanotoxicology: James C. Bonner (president), james_bonner@ncsu.edu
  - Neurotoxicology: William K. Boyes (president), boyes.william@epa.gov
  - Occupational and Public Health: Mary Ann Smith (president), mary.a.smith@uth.tmc.edu mary.a.smith@uth.tmc.edu
  - Reproductive and Developmental Toxicology: Jeffrey S. Moffit (president), jeffmoffit@outlook.com
  - Risk Assessment: Hugh A. Barton (president), hugh.barton@pfizer.com
- Ecological Society of America - http://www.esa.org/esa/careers-and-certification/job-board/
- American Chemical Society - http://www.acs.org/content/acs/en/careers.html
- Society of Environmental Toxicology and Chemistry (SETAC) – Career Center, https://www.setac.org/?page=careercenter
- Society for Risk Analysis (SRA) - http://www.sra.org/node/add/employment-posting
- Alliance for Risk Assessment- http://allianceforrisk.org/, Oliver Kroner, oliver.kroner@uc.edu
- Teratology Society's Job Bank - please send the posting information to tshq@teratology.org

Journals:

- AAAS (Science Careers) - http://www.sciencemag.org/careers
- Naturejobs for ads - http://www.nature.com/naturejobs/science/
- Environmental Science & Technology journal - http://pubs.acs.org/journal/esthag
- Annual Review of Pharmacology and Toxicology - http://www.annualreviews.org/journal/pharmtox
- Journal of Toxicology and Environmental Health - Part B: Critical Reviews - http://www.tandfonline.com/toc/uteb20/current
- Toxicological Sciences - http://toxsci.oxfordjournals.org/content/early/recent
- Critical Reviews in Toxicology - http://www.tandfonline.com/loi/itxc20#.VqY7DVX2ZD8
- Nanotoxicology - http://www.tandfonline.com/loi/inan20#.VqY7JVX2ZD8
- Journal of Exposure Science and Environmental Epidemiology - http://www.nature.com/jes/index.html
- Archives of Toxicology - http://link.springer.com/journal/204

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- Toxicology and Applied Pharmacology - http://www.journals.elsevier.com/toxicology-and-applied-pharmacology/
- Neurotoxicology and Teratology - http://www.journals.elsevier.com/neurotoxicology-and-teratology/
- Neurotoxicity Research - http://www.springer.com/biomed/neuroscience/journal/12640
- Ecotoxicology - http://link.springer.com/journal/10646
- Reproductive Toxicology - http://www.journals.elsevier.com/reproductive-toxicology/
- Toxicology - http://www.journals.elsevier.com/toxicology/
- Toxicology Letters - http://www.journals.elsevier.com/toxicology-letters/
- Journal of Applied Toxicology - http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1099-1263
- Inhalation Toxicology - http://www.tandfonline.com/toc/iiht20/current
- Journal of Toxicology and Environmental Health - Part A: Current Issues - http://www.tandfonline.com/loi/uteh20#.VqY8gFX2ZD8
- Journal of Pharmacological and Toxicological Methods - http://www.sciencedirect.com/science/journal/10568719
- Environmental Toxicology - http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1522-7278
- Regulatory Toxicology and Pharmacology - http://www.sciencedirect.com/science/journal/02732300
- Journal of Biochemical and Molecular Toxicology - http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1099-0461
- Journal of Immunotoxicology - http://www.tandfonline.com/loi/iimt20#.VqY8wVX2ZD8

- Nanotechnology
  - Center for Nanotechnology and Nanotoxicology - http://www.hsph.harvard.edu/nano/. I suggest sending postings to pdemokri@hsph.harvard.edu , brain@hsph.harvard.edu
  - University of Iowa, Nanotoxicology Research Program. Send posting to Dr. Peter Thorne, peter-thorne@uiowa.edu
  - NIEHS Center for Nanotoxicology – contact Dr. Joel G. Pounds at Pacific Northwest National Laboratory, joel.pounds@pnl.gov
  - University of Washington (Seattle), Nanotoxicology Center - http://depts.washington.edu/nanotox/investigators.html, send posting to Dr. Terrance Kavanagh, tjkav@u.washington.edu (center director)
  - Harper Nanotoxicology Laboratory at Oregon State University, send posting to Dr. Harper at stacey.harper@oregonstate.edu
  - Vince Castranova, NIOSH (retired), castranovav@gmail.com

Appendix F (continued**) Eco/Human Health Outreach List (RAD 1/20/2016, Iris Camacho)**

Universities and Other Programs

Table of schools with hot links to relevant program

Brown University	RI
CIIT Centers for Health Research (The Hamner Institutes)	NC
Clemson University	SC
Colorado State University	CO
Dartmouth College	NH
Duke University	NC
Florida A&M University	FL
Indiana University	IN
Iowa State University	IA
Johns Hopkins University School of Hygiene and Public Health	MD
Louisiana State University Medical Center	LA
Massachusetts Institute of Technology	MA
Michigan State University	MI
Mississippi State University	MS
New York University	NY
North Carolina State University	NC
The University of Louisiana at Monroe	LA
Northeastern University	MA
Oregon State University	OR
Purdue University	IN
Rutgers, The State University of New Jersey	NJ
San Diego State University	CA
St. John's University	NY
State University of New York at Buffalo	NY
Texas A&M University	TX
Texas Tech University	TX
University at Albany	NY
University of Alabama at Birmingham	AL
Graduate Training Program in Toxicology	AL
University of Arizona	AZ
University of Arkansas for Medical Sciences	AR
University of California, Berkeley	CA
University of California, Davis	CA
University of California, Irvine	CA
University of California, Los Angeles	CA
University of California, Riverside	CA
University of Cincinnati	OH
University of Colorado Health Sciences Center	CO
University of Connecticut	CT
University of Florida (PhD)	FL

University of Florida (MS)	FL
University of Georgia	GA
University of Illinois at Urbana—Champaign	IL
University of Iowa	IA
University of Kansas Medical Center	KS
University of Kentucky	KY
University of Louisville	KY
University of Maryland	MD
University of Michigan	MI
University of Mississippi	MS
University of Mississippi Medical Center	MS
University of Nebraska	NE
University of New Mexico	NM
University of North Carolina at Chapel Hill	NC
University of Oklahoma Health Sciences Center	OK
University of Pittsburgh Graduate School of Public Health	PA
University of Rochester School of Medicine and Dentistry	NY
University of Texas at Austin	TX
University of Texas at El Paso	TX
University of Texas Houston Health Science Center	TX
University of Texas Medical Branch	TX
University of the Sciences in Philadelphia	PA
University of Utah	UT
University of Washington	WA
University of Wisconsin–Madison	WI
Utah State University	UT
Vanderbilt University	TN
Virginia Commonwealth University	VA
Virginia/Maryland Regional College of Veterinary Medicine	VA
Wayne State University	MI
Wright State University	OH

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SHOSHONE-BANNOCK EXPOSURE SCENARIO FOR USE IN RISK ASSESSMENT:

TRADITIONAL SUBSISTENCE LIFEWAYS

SHOSHONE-BANNOCK TRIBES

ENVIRONMENTAL WASTE MANAGEMENT PROGRAM

FEBRUARY 2016

Shoshone-Bannock Tribes Exposure Scenario for Use in Risk Assessment: Traditional Subsistence Lifeways

Shoshone-Bannock Tribes
Environmental Waste Management Program

February 2016

Shoshone-Bannock Tribes
Environmental Waste Management Program
P. O. Box 306
Fort Hall, Idaho 83203

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Acknowledgements

Shoshone-Bannock Tribes and the Environmental Waste Management program would like to acknowledge and thank Dr. Barbara Harper of the Confederated Tribes of the Umatilla Indian Reservation (CTUIR), Department of Science and Engineering, and Stuart Harris, Director of the CTUIR Department of Science and Engineering, for their tremendous contribution and sharing that resulted in the *Provisional Shoshone-Bannock Risk Assessment Exposure Scenario* document back in 2009. Their 2009 document forms the backbone of the *Shoshone-Bannock Tribes Exposure Scenario for Use in Risk Assessment: Traditional Subsistence Lifeways, January 2016*. In addition, gratitude goes to Nathan Small, Shoshone-Bannock Tribal Elder, for assisting with the collection of information regarding the uses of flora and fauna here on the Fort Hall Reservation. Without their expertise, advice, and extensive research, this document would not have been possible.

We give you our thanks, blessings for good health and prosperity, and gratitude for your contributions to First Nation people.

ED_002221_00016420-00003

MEMORANDUM:

Date: May 8, 2009

To: Susan Hanson, Shoshone-Bannock Tribes

From: Barbara Harper, (CTUIR, OSU)

Subject: Provisional Shoshone-Bannock Risk Assessment Exposure Scenario

The intent of this memorandum is to provide a provisional 'exposure scenario' for evaluating risks at contaminated sites where natural resources appertaining to the Shoshone-Bannock peoples are affected.

This memo is not based on the anthropological research that is recommended[1]. Similarly, this memo includes some ecological information, but not in great detail with respect to identification of the species that are present. However, because the Snake River Basin is similar to the interior Columbia River Basin, information about the CTUIR diets is provided. The diet developed for the Washoe Tribe (Great Basin) is also provided because the southern Shoshone territories may have natural resources similar to those of the Washoe. These diets are provided with the intention that the Shoshone-Bannock Tribe modifies them based on their own knowledge. In the meantime, however, this scenario and diet will allow the Tribe and EPA to derive a much more accurate estimate of risks than a suburban scenario would allow.

Based on our years of research into direct exposure factors (inhalation, soil ingestion, and drinking water) as they apply to environmental lifestyles, we recommend using the following exposure factors:

- Soil ingestion = 400 mg/d for all age groups
- Inhalation rate = 25 m^3/d for adults, with children scaled from the adult value
- Drinking water = 3L/d for adults, with children scaled from the adult value; an additional 1L is ingested during each use of the sweat lodge.

For questions, please feel free to contact

me: Barbara Harper, PhD, DABT
Confederated Tribes of the Umatilla Indian Reservation
Department of Science and
Engineering bharper@amerion.com
541-966-2804

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A. Regulatory Context

Under CERCLA and other environmental statutes, EPA is required to identify populations who are more highly exposed; for example, subsistence populations and subsistence consumption of natural resources (Executive Order 12898[2]). EPA is also required to protect sensitive populations.[3]

> *"The Superfund law requires cleanup of the site to levels which are protective of human health and the environment, which will serve to minimize any disproportionately high and adverse environmental burdens impacting the EJ community".*[4]

Furthermore, EPA's American Indian Policy[5] sets forth EPA's principles as follows:

> *"The keynote of this effort will be to give special consideration to Tribal interests in making Agency policy and to insure the close involvement of Tribal Governments in making decisions and managing environmental programs affecting reservation lands."*

In particular, EPA

> "*will recognize Tribal governments as the primary parties for setting standards, making environmental policy decisions, and managing programs for reservations,*" and "*in keeping with the Federal Trust responsibility, will assure that Tribal concerns and interests are considered whenever EPA's actions and/or decisions may affect reservation environments."*

EPA then explains this principle as follows:

> "*EPA recognizes that trust responsibility derives from the historical relationship between the Federal government and Indian Tribes as expressed in certain Treaties and Federal Indian law. In keeping with that trust responsibility, the Agency will endeavor to protect the environmental interests of Indian Tribes when carrying out its responsibilities that may affect the reservations."*

---

[2] White House, 1994. Federal Actions To Address Environmental Justice In Minority Populations And Low income Populations: Feb. 11, 1994; 59 FR 7629, Feb. 16, 1994.

[3] Superfund Exposure Assessment Manual. EPA/540/1-88/001 OSWER directive 9285.5-1. U.S. Environmental Protection Agency Office of Remedial Response, U.S. Environmental Protection Agency, Washington, D.C. 1988.

[4] http://www.epa.gov/region02/community/ej/superfund.htm

[5] http://www.epa.gov/tribalportal/pdf/indian-policy-84.pdf

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EPA's OSWER Directive No. 9355.7-04 says that *"If the site is located in a community that is likely to have environmental justice concerns, extra efforts should be made to reach out to and consult with segments of the community that are not necessarily reached by conventional communication vehicles..."* One of the factors that must be considered is the presence of *"cultural factors (e.g., historical sites or Native American religious sites)."*

Because EPA guidance documents[6] do not include an exposure scenario for Native Americans, the CTUIR and Spokane Tribes developed their own scenarios in order to protect traditional subsistence lifeways[7]. These scenarios reflect specific traditional practices and foods that are unique to each Tribe and its local ecology. Subsequently, scenarios have been developed for several other Tribes across the US, including the Shoshone-Bannock Tribes. In each case the direct exposure factors are identical (inhalation rate, soil ingestion rate, drinking water rate) but each diet is tailored to the specific resources that are present in the ecology and documented in the anthropological literature and by tribal interviews where possible.

This document focuses only on the human exposure component of a risk assessment. The broader aspects of health and well-being that are at risk from contamination are clearly recognized, but are beyond the scope of this document. The shorthand label 'subsistence' is often used to refer solely to food and material acquisition. However, traditional cultural lifeways are more than simply obtaining calories. In the larger context, many other components of tribal health and well-being are also at risk from contamination of natural resources. Tribal members associate the loss of culture, language, education, identity, and heritage directly with the practices that are unavailable as a result of contamination. These cultural losses adversely affect all tribal members individually as well as affecting the community as a whole. A public health approach to risk assessment naturally integrates human, ecological, and cultural health into an overall definition of community health and well- being that is widely used in public health but not in risk assessment. The combination of subsistence lifeways and public health is adaptable to indigenous communities that, unlike westernized communities, turn to the local ecology for food, medicine, education, religion, occupation, income, and all aspects of a good life. This document, however, addresses only the methodology for evaluating exposures to contamination if information about contaminant concentrations is available.

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[6] U.S. EPA. Exposure Factors Handbook (1997) http://www.epa.gov/ncea/efh/ *and* Guidelines for Exposure Assessment. U.S. Environmental Protection Agency, Risk Assessment Forum, Washington, DC, 600Z-92/001 (1992) http://cfpub.epa.gov/ncea/raf/recordisplay.cfm?deid=15263; http://www.epa.gov/oswer/riskassessment/ragsa/pdf/preface.pdf

[7] Harris, S.G. and Harper, B.L. (1997) "A Native American Exposure Scenario." Risk Analysis, 17(6): 789-795; S.Harris and B Harper (2001) "Native American Diets and Exposure Factors." Environmental Research, Section A, 86:140-148; B Harper, B Flett, S Harris, C Abeyta, and F Kirschner (2002). "The Spokane Tribe's Multipathway Subsistence Exposure Scenario and Screening Level RME," Risk Analysis 22(3): 513-526.

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B. Methods

Several types of information are used to understand the degree of environmental contact and support the derivation of numerical exposure factors within traditional subsistence lifeways. This is an initial and abbreviated report, but it presents the findings of research regarding uses and consumption rates for the purpose of ongoing risk assessment activities. As funding and time permit, the depth of research will be expanded. But, the framework presented here will direct the Tribe as they continue this work.

The Natural Habitat (Environmental Setting) Framework

The historical and current ecological and climatological descriptions provide information about plants, animals, biodiversity, relative proportions of different habitat types, seasonality, and physiographic features of the environment. This information is needed to support estimates of dietary staples (the resources that are most abundant and reliable), and environmental characteristics that affect contact rates with soil, sediment, and water (for example, proportion of wetlands versus dry upland habitats).

The Ethno-Habitat (Natural Resource Use) Framework

Ethnobotanical and ethnohistorical literature describes the general diversity of plants used for food, medicine, or materials in various regional ecotypes and helps derive dietary intake values. Traditional ecological knowledge (TEK) combines anthropological and environmental knowledge with tribal knowledge, teaching, and observation. This information identifies the most important resources used by the Tribe, and the importance of various ecological functions and services, as well as refinement of estimates of frequency, intensity and duration of major activity types.

Diet Framework

In some cases, a complete diet may have been identified in the anthropological foraging theory literature, but more often the major dietary staples are identified but not fully quantified within a nutritionally complete diet. Information about natural resources and their uses is used to estimate relative importance of the major food categories. This is combined with nutritional information to estimate a nutritionally complete subsistence diet for as many major regional habitat types as are appropriate.

Direct exposure factors (soil, sediment, water, and air pathways) Framework

There is little data directly relevant to environmental contact rates with abiotic media for indigenous styles other than the foraging theory literature, which tends to be non-specific.

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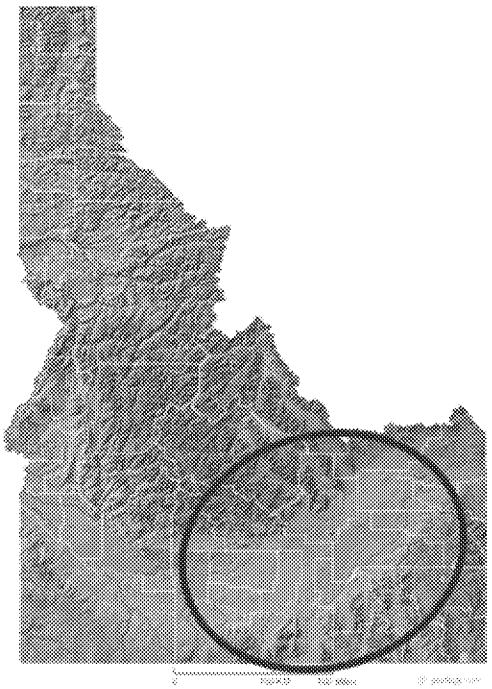
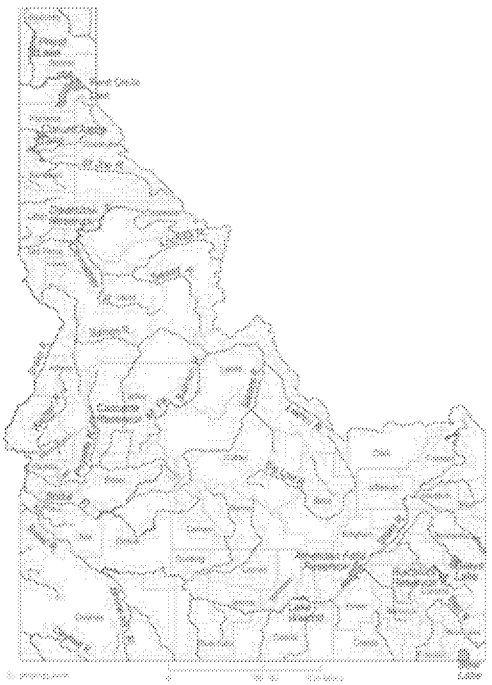
The approach taken to direct exposure factors begins with a description of the major activity categories. The crosswalk between major activities (hunting, fishing, gathering, and so on) and the abiotic exposure pathways (soil ingestion, sediment ingestion, water intake, and inhalation) is based on estimates of activity levels and the frequency, duration, and intensity of individual practices within each activity category. Physiological information adds knowledge of activity levels, the relation between inhalation rates and calorie needs, and other biomedical information provides some assurance that a reasonable and physiologically coherent set of exposure parameters had been developed.

C. The Natural Habitat (Environmental Setting)

The setting for this scenario is in the upper Snake River basin and nearby foothills and mountains. The purpose of the following general ecological description is to help identify the most prevalent staple edible resources that are provided by the natural habitat.





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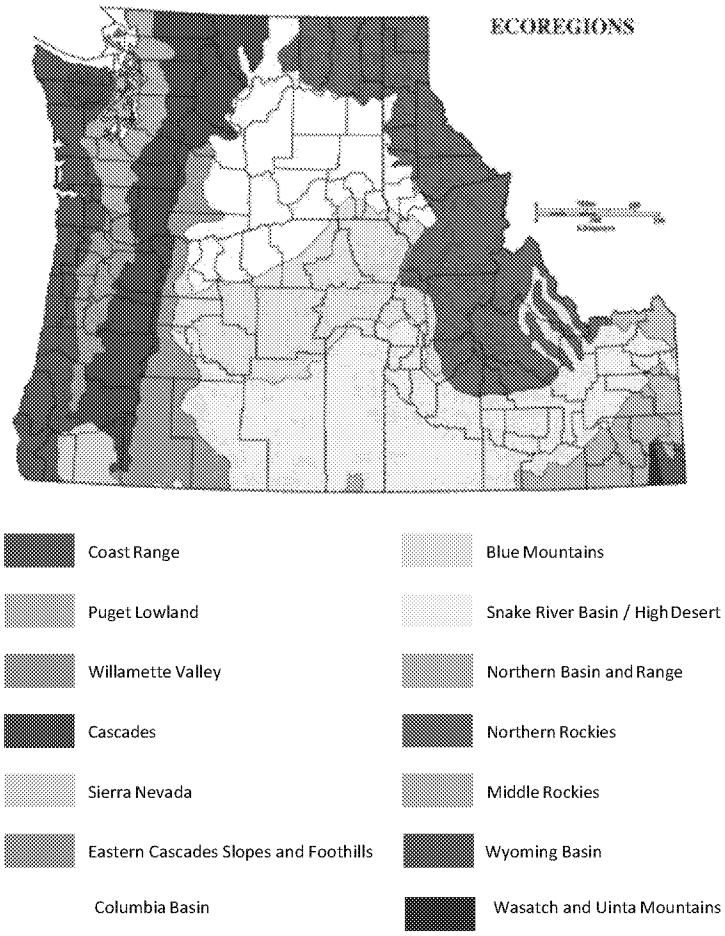

The broadest ecological description of the study area is the Snake River Basin/High Desert[8]. The Snake River Basin or Upper Snake River Plain forms part of the xeric intermontane basin and range area of the western United States. It is considerably lower and more gently sloping than the surrounding ecoregions. The ecoregion is characterized by nearly level river terraces, floodplains, and lake plains containing many canals and rivers. Elevation varies from 4,400 to 5,000 feet. Potential natural vegetation is sagebrush steppe, with saltbush-greasewood communities in the southwest. The region covers 1,463 square miles in eastern Idaho, mostly along the interstate corridor from American Falls to St. Anthony. It includes the Camas National Wildlife Refuge and most of the Fort Hall Indian Reservation.

The ecoregion is largely in the rain shadow of the Cascade Mountains and thus receives little precipitation. Latitude and physiography are influential factors in distinguishing this ecoregion from other similar ecoregions, such as the Wyoming Basin and Great Basin Shrub Steppes.

[8] This text was originally published in the book Terrestrial ecoregions of North America: a conservation assessment from Island Press. The terminology follows designations by Omernick, Bailey and Kuchler. Examples of ecosystem maps include:
http://academic.evergreen.edu/projects/virtualatlas/thematic/vegetation/veg_zone.htm;
http://en.wikipedia.org/wiki/Snake_River_Plain_(ecoregion)
http://www.worldwildlife.org/wildworld/profiles/terrestrial/na/na1309_full.html

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The Snake/Columbia Shrub Steppe is lower in elevation than the Wyoming Basin, and the two are separated by mountainous areas. The ecoregion exhibits more desert-like vegetation in southeastern Oregon, where elevation is considerably higher and precipitation lower.

The Northern Basin and Range ecoregion (just south of the Snake River Basin) consists of arid tablelands, intermontane basins, dissected lava plains, and widely scattered low mountains. The bulk of the region is covered by sagebrush steppe vegetation. The ecoregion is drier than the Columbia Plateau, it is higher and cooler than the Snake River Basin the north and east, and contains a lower density of mountain ranges than the adjacent ecoregion to the south.

The dominant vegetation in the Northern Basin and Range ecoregion is sagebrush (*Artemisia* spp.), typically associated with various wheatgrasses (*Agropyron* spp.), Idaho fescue (*Festuca idahoensis*), bluebunch wheatgrass, bluegrass, cheatgrass, rabbitbrush, squirreltail, needle-and- thread, Indian ricegrass, and fourwing saltbush. The ecoregion contains a number of isolated mountain ranges in the southern parts of Idaho and Oregon, and here the sagebrush shrublands grade into bunchgrasses and juniper woodlands. Some parts of these ranges contain areas of Douglas-fir (*Psuedotsuga menziesii*), subalpine fir (*Abies lasiocarpa*), and aspen (*Populus tremuloides*).).

Riparian areas in both the Snake Basin and Northern Basin feature sedges, perennial grasses, willows, and cottonwood. These habitats contain extensive wetlands, which provide vital waterfowl habitat in the Pacific Flyway.

D. The Ethno-Habitat (Natural Resource Use)

This section provides a summary of the initial description for the Shoshone-Bannock Tribes.

Prior to contact with Euro-Americans, the Shoshone and Bannock were hunters and gatherers, moving their camps on a regular basis to take advantage of plants and animals in season. This "annual round" was documented by early trappers in the area and by the information given to anthropologists in the first half of this century by Shoshone and Bannock. Each band had its winter base camps near the river or in the area indicated by the band name. With the arrival of spring and the depletion of stored food in the winter camps, the bands would move to seasonal camps as plants, salmon, or animals became available. Examples of seasonal camps include salmon-fishing camps along the forks of the Salmon River, and the Snake River and its tributaries below Shoshone Falls; Camas Prairie near present-day Fairfield where the roots of the camas were harvested; and the pine groves of the City of Rocks area where pine nuts could be gathered. With the acquisition of the horse in the 17th century and increased mobility, the annual round was able to expand to include excursions onto the Plains to hunt buffalo.[9]

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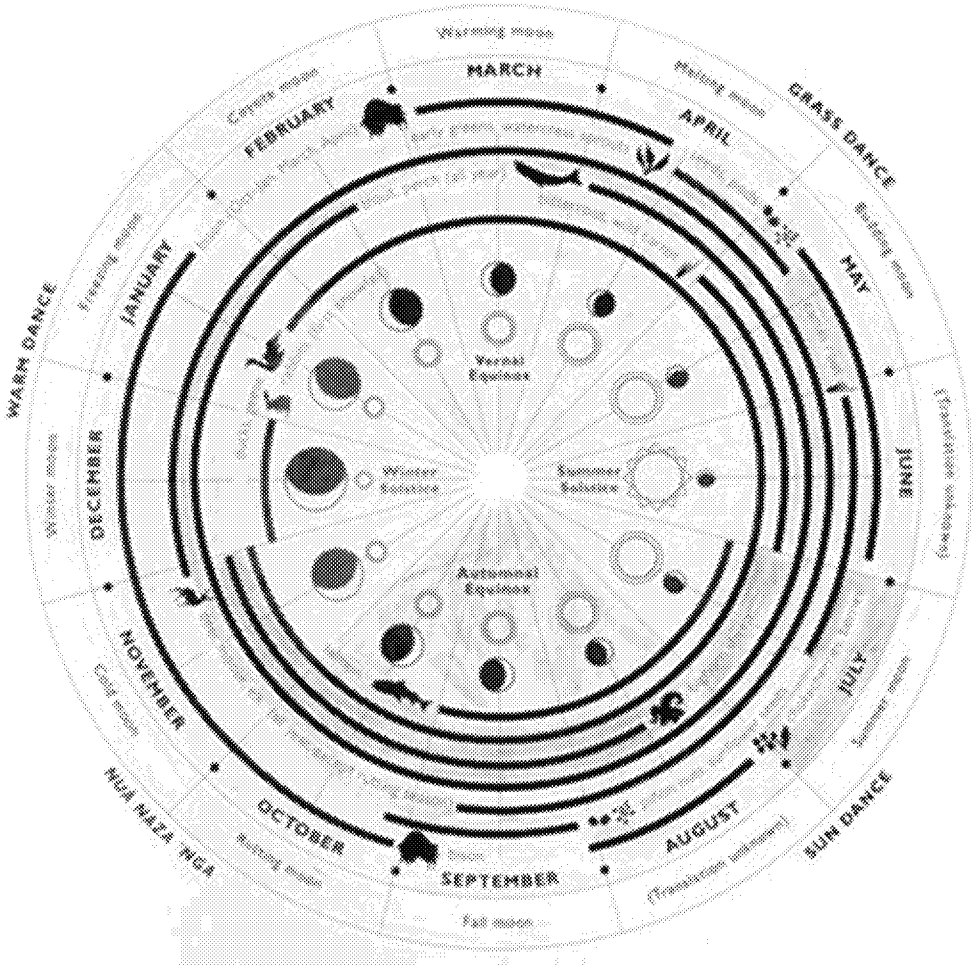

http://www.trailtribes.org/lemhi/great-circle.htm

Natural resources are integral to many traditional practices and celebrations throughout the year, many of which honor the traditional foods or First Foods. Based on the importance and many uses of the natural resources, an exposure scenario reflecting the underlying ethnohabitat was developed for use in dose and risk assessments at Hanford (Harper and Harris 1997; 2000; CTUIR 2004; OSU webpage[10]). Although the concept of ethnohabitat or ethnoecology has been used various forms in anthropological disciplines for many years, it had never been used in risk assessment.

[9] http://challenge.isu.edu/multicultural/Native AM/ShoBan/forthall.htm

[10] http://www.hhs.oregonstate.edu/ph/tribal-grant-main-page

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"An ethno-habitat can be defined as the set of cultural, religious, nutritional, educational, psychological and other services provided by intact, functioning ecosystems and landscapes. An ethno- habitat refers to the cultural survival of a people within its traditional homeland. A healthy ethno-habitat is one that supports its natural plant and animal communities and sustains the biophysical and spiritual health of its native peoples through time... These lands encompass traditional Indian homelands, places, habitats, resources, ancestral remains, cultural symbols, and cultural heritage. The presence of and access for traditional use to healthy habitats is fundamental to useable and harvestable levels of resources significant to Indian peoples as well as to healthy ecosystems."

--Stuart G. Harris of the Confederated Tribes of the Umatilla Indian Reservation

An initial gathering of natural resource use information for the Shoshone-Bannock Tribes has been completed using the interview process. Additional information in future efforts to expand and complete this scenario will include gleaning information from the Shoshone and Bannock literature, as well as, the journals of Lewis and Clark and other early explorers, anthropological literature, trading records, BIA records, and interviews.

E. Dietary Approach

Natural resources are gathered for food and medicine according to their seasonal availability. Many resources are both eaten fresh and stored for use throughout the year. There are clear seasonal variations in the diet, but the goal of most exposure scenarios is an annual average diet. This is because risks are calculated for annual and lifetime exposures. However, higher exposures might occur during seasonally-focused diets. For example, risks from eating fish are evaluated on a chronic (annual and multi-year) basis, but there may be health concerns from seasonally high (acute) exposures.

The general steps in describing a traditional diet are to:

1. Review information about ecological settings and natural resources, ethnohistorical and contemporary natural resource use, archaeological evidence, tribal history and interviews;
2. Review the seasonal aspects of resource availability – it is important to remember that the goal is an annual average diet.
3. Review databases of contemporary foods and diets for comparability, native foods currently eaten, ethnic data, regional data, and contemporary quantities;
4. Identify dietary staples and develop overall percentages of major food categories including medicinal plants for a completely subsistent diet(s);
5. Estimate total calories and quantities of food provided by the diets;
6. Refine estimates of major staples and food categories, macronutrients, and other factors. This is a reasonable estimate, not a statistical estimate with ranges.

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Major native vegetation food categories and representative foods were determined (Attachments 1 and 2), the representative species were identified and caloric information was retrieved from the USDA database[11] for either the exact species, a member of the same or nearest plant family, and the exact animal or other animal from a similar habitat and feeding guild (e.g., wetland omnivore or upland browser). The data for fresh or cooked foods matches the form of native foods eaten as closely as possible. The USDA data were derived from recent studies on contemporary domesticated and wild foods, including some information on different methods of food preparation. Some of the information was obtained by USDA in response to requests from Indian Tribes and is appropriate as to species and cooking method (e.g., roasted beaver). In other cases nutrient data are not available (such as for wild tubers or bulbs), so data for the nearest domesticated species was used. In most cases this would probably not alter the high-level food pyramid or food circle, even though native peoples recognize that flavor, texture, and "strength" differ between wild species and domesticated cultivars.

Each food category represents many other species. For example, in the discussions with the Tribes during the development of these diets, there was a general sense that tubers were always eaten, such as Jerusalem artichokes, and arrowroot. Bulbs were also mentioned frequently, including several kinds of lilies, as were greens and berries. These two categories, therefore, are accorded separate categories and a greater role than the archaeological information alone would indicate.

Additional studies will also describe some of the signature resources and the variety of preparation methods, storage forms, and other uses to which the resource is put. For example, the importance of buffalo to the community will be described, both in terms of food, but also the material and spiritual importance. A description of bannock bread will be included. The importance of salmon, pine nuts, or camas will be documented, with information about abundance, tribal stories, seasonal ceremonies will be described as information might be available and as the Tribe approves the release of the information. Although these sections rely on information that is already published, a review is recommended to prevent proprietary information from being inadvertently released.

Native plant species harvested and used by the Shoshone-Bannock Tribes, with associated validated consumption rates are presented in Attachment 1. Meat, wild and domestic, consumed by the Tribes are presented in Attachment 2 with associated consumption rates.

[11] http://www.ars.usda.gov/main/site_main.htm?modecode=12354500

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F. Direct Exposure Factors

The original CTUIR and other tribal scenarios are composite central-tendency scenarios based on a family or band that includes people who specialize in subsistence hunting, fishing, or gathering (i.e., there are some people who hunt all the time and provide all the meat for the family, some people who gather all the time, and some who fish all the time). Following CERCLA guidance, average representations, or 'Reference Indians' are constructed by averaging all activities across all members such that everyone does all activities daily. This approach underestimates the exposure to people who specialize in each activity, but is intended as a central tendency of the traditional lifestyle.

It is important to note that these are full-time scenarios. If other scenarios are developed to evaluate risks during day use only, or without groundwater access, these are no longer baseline scenarios, but "restricted" scenarios. We recommend additional discussions if such partial scenarios are developed. For example, we recommend that the soil ingestion rate should not be halved if day use is proposed (12 hours on site and 12 hours off-site).

We also recommend that careful consideration be given if only some of the traditional activity categories are proposed (e.g., a hunting-only scenario). The terms "fish, hunt or gather" are shorthand labels that identify some of the most visible activities within a traditional lifestyle, but they each include a wide range of associated activities such as preparation, processing, using or consuming, and various traditional and cultural activities. A subsistence economy includes people with a wide range of 'jobs' such as food procurement, processing, and distribution; transportation (pasturing and veterinary); botany/apothecary services; administration and coordination (chiefs); education (elders, linguists); governance (citizenship activities, conclaves); finance (trade, accumulation and discharge of obligations); spiritual health care; social gathering; and so on. The categories of 'fish, hunt, and gather' each include a full cross section of these activities. This is why 'hunting' is not just the act of shooting and eating an animal, but includes a full cross-section of all the activities that a hunter-specialist does as a full-time occupation.

The basic process for developing the direct exposure factors is to:

1. Start with an understanding of the major categories of traditional activities (such as hunting, fishing, gathering, basket making and so on),
2. Describe enough of the sub-activities that the complexity and interconnection of resources and activities can be appreciated,
3. Identify the major activities that contribute to exposure, and then
4. Iteratively crosswalk between activities, frequency, intensity, and duration of environmental contact to develop exposure factors.

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The scenario documentation then describes the activities that people undertake, including hunting, gathering foods and medicines, fishing, collecting firewood, making material items, and various other cultural and domestic activities. Once the activities comprising a particular lifestyle are known, they are translated into a form that is used for risk assessment. This translation captures the degree of environmental contact (frequency, duration, and intensity) that occurs through activities and diet, expressed as numerical "exposure factors." Exposure factors for direct exposure pathways allow the estimation of exposure to any contaminants in abiotic media (air, water, soil, and sediment), via inhalation, soil ingestion, water ingestion, and/or dermal exposure. Indirect pathways refer to contaminants that are incorporated into biota and may subsequently reach people who ingest or use them.

For the direct exposure factors, each of the major activity categories includes activities that result in exposure to each medium. For example, by estimating the relative amount of time spent in activities that result in high, medium, or low soil contact rates for each activity category, an overall soil ingestion rate was estimated. However, we recommend not attempting to be overly quantitative in enumerating the myriad of activities and resources in each category because this implies more precision than is warranted. Thus, each crosswalk is a systematic estimate but not a statistical exercise.

Signature activities may be described separately, such as basket making (gathering, preparing materials, making the basket, using the basket for food storage or cooking), or wood gathering (firewood, tipi poles, etc., with high-energy expenditures during felling, trimming, splitting, stripping, and so forth).

For each category of activities, the information needed for risk assessment is frequency of activity (e.g., daily), duration (e.g., 70 years), and intensity of environmental contact.

Activity Type	*General Description*
Hunting	Hunting includes a variety of preparation activities of low to moderate intensity. Hunting occurs in terrain ranging from flat and open to very steep and rugged. It may also include setting traplines, waiting in blinds, digging, climbing, etc. After the capture or kill, field dressing, packing or hauling, and other very strenuous activities occur, depending on the species. Subsequent activities include cutting, storing (e.g., smoking or drying), etc.
Fishing	Fishing includes building weirs and platforms, hauling in lines and nets, gaffing or gigging, wading (for shellfish), followed by cleaning the fish and carrying them to the place of use. Activities associated with smoking and constructing drying racks may be involved.
Gathering	A variety of activities is involved in gathering, such as hiking, bending, stooping, wading (marsh and water plants), digging, and carrying.
Sweatlodge Use	Sweatlodge building and repairing is intermittent, but collecting firewood is a constant activity.
Materials and Food Use	Many activities of varying intensity are involved in preparing materials for use or food storage. Some are quite vigorous such as pounding or grinding seeds and nuts into flour, preparing meat, and tanning hides, Many others are semi-active, such as basket making, flintknapping, construction of storage containers, cleaning village sites, sanitation activities, home repairs, and so on.

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Drinking Water

Harper et al. (2002) estimated an average water ingestion rate of 3 L/day for adults, based on total fluid intake for an arid climate, rather than the EPA default value of 2L/d. In addition, each use of the sweatlodge requires an additional 1L for rehydration, for a total of 4L per day.

For the sweatlodge, a steam model is presented in Section I. For CTUIR, daily use for 1 hour/day is assumed, based on interviews and documentation. For other Tribes, the frequency may vary.

G. Soil Ingestion

Ingestion of soil, sediment, or dust is the result of hand-to-mouth contact, swallowing inhaled dust, mouthing of objects, and ingestion of dirt or dust on food. The recommended subsistence soil ingestion rate of 400 mg/d is based on a review of EPA guidance, soil ingestion studies in suburban and indigenous populations, military, construction and utility worker studies, and local climatic, habitat, and geologic conditions. Components of the traditional lifestyle that contribute to soil ingestion include hunting, gathering, digging roots, processing and eating wild foods, preparing and using natural materials such as basket materials, tending livestock, building and repairing sweat lodges, tending cemeteries, and social gatherings. It also considers occupational activities such as wildlife field work, construction or road work, sample collection, and cultural resource field work.

EPA Guidance

EPA reviewed studies relevant to suburban populations and published summaries in its Exposure Factors Handbook (1989, 1991, and 1997). In the current iteration of the Exposure Factors Handbook[12], EPA recommends100 mg/d as a mean value for children in suburban settings, 200 mg/day as a conservative estimate of the mean, and a value of 400 mg/day as an "upper bound" value (exact percentile not specified). Most state and federal guidance uses 200 mg/d for children and 100 mg/d for adults in residential or agricultural settings.

A value for an ingestion rate for adult outdoor activities is no longer given in the 1997 Exposure Factors Handbook for adults as "too speculative." However, EPA's soil screening guidance recommends 330 mg/d for a construction or other outdoor worker. Risk assessments for construction workers typically use a rate of 480 mg/d. Some states recommend the use of 1 gram per acute soil ingestion event13 to approximate a non-average day for children, such as an outdoor day.

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[12] Environmental Protection Agency. 1997. Exposure Factors Handbook. Volumes I, II, III. U.S. Environmental Protection Agency, Office of Research and Development. EPA/600/P-95/002Fa.

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Military Guidance

The US military assumes 480 mg per exposure event[14] or per field day (Technical Guide 230).[15] Department Of Defense (2002)[16] recommendations for certain activities such as construction, landscaping, or other field activities is 480 mg/day. During deployment, DOD assumes that half of a soldier's time is spent in these higher-contact activities. The UN Balkans Task Force assumes that 1 gram of soil can be ingested per military field day[17].

Studies in suburban or urban populations

Written knowledge that humans often ingest soil dates back to the classical Greek era. Soil ingestion has been widely studied from a perspective of exposure to soil parasite eggs and other infections. More recently, soil ingestion was recognized to be a potentially significant pathway of exposure to contaminants. Several early studies estimated intakes by children. Estimates based on observation of 'sticky sweets' (Day et al., 1975), outdoor activities (Hawley, 1985), or camping (Van Wijnen et al., 1990). Other studies used tracer elements (Binder, et al., 1986; Clausing et al., 1987; Thompson and Burmaster, 1991; Calabrese et al., 1989; Stanek and Calabrese (1995a, 1997). These studies estimated a wide range of soil ingestion rates.

Pica (ingestion of more than 5000 mg/d) is generally thought of as a pediatric condition. ATSDR estimates that between 10 and 50% of children may exhibit pica behavior at some point. Regulatory guidance recommends using a soil ingestion rate of 5 or 10g/d for pica children. Some examples are:

(1) EPA (1997) recommends a value of 10g/d for a pica child.
(2) Florida recommends 10g per event for acute toxicity evaluation[18].
(3) ATSDR uses 5 g/day for a pica child[19]

[13] MADEP (1992). Background Documentation For The Development Of An "Available Cyanide" Benchmark Concentration. http://www.mass.gov/dep/ors/files/cn_soil.htm

[14] http://www.gulflink.osd.mil/pesto/pest_s22.htm, citing US Environmental Protection Agency, Office of Research and Development, Exposure Factors Handbook, Volume I, EPA/600/P-95/002a, August 1997 as the basis for the 480 mg/d.

[15] USACPPM TG 230A (1999). Short-Term Chemical Exposure Guidelines for Deployed Military Personnel. U.S. Army Center for Health Promotion and Preventive Medicine. Website: http://www.grid.unep.ch/btf/missions/september/dufinal.pdf

[16] Reference Document (RD) 230, "Exposure Guidelines for Deployed Military" A Companion Document to USACHPPM Technical Guide (TG) 230, "Chemical Exposure Guidelines for Deployed Military Personnel", January 2002. Website: http://chppm-www.apgea.army.mil/desp/; and http://books.nap.edu/books/0309092213/html/83.html#pagetop.

[17] UNEP/UNCHS Balkans Task Force (BTF) (1999). The potential effects on human health and the environment arising from possible use of depleted uranium during the 1999 Kosovo conflict. www.grid.unep.ch/btf/missions/september/dufinal.pdf

[18] Proposed Modifications To Identified Acute Toxicity-Based Soil Cleanup Target Level, December 1999, www.dep.state.fl.us/waste/quick_topics/ publications/wc/csf/focus/csf.pdf.

[19] For Example: El Paso Metals Survey, Appendix B, www.atsdr.cdc.gov/HAC/PHA/elpaso/epc_toc.html.

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Studies in Indigenous Populations

Studies of soil ingestion in indigenous populations have largely centered on estimates of past exposure (or dose reconstruction) of populations affected by atomic bomb tests. Haywood and Smith (1992) estimated potential doses to aboriginal inhabitants of the Maralinga and Emu areas of South Australia by considering the number of hours per week spent in sleeping, sitting, hunting or driving, cooking or butchering, and other activities. They noted that virtually all food, whether of local origin or purchased, has some dust content by the time of consumption due to methods of preparation and the nature of the environment. They recommend a soil intake of 1 to 10 gpd. Other authors have used estimates of 0.5 or 1 gpd in other indigenous populations such as the Marshall Islanders (Sun and Meinhold, 1997; LaGoy, 1987). Simon (1998) recommended using a soil ingestion rate for indigenous people in hunters/food gathering/nomadic societies of 1g/d in wet climates and 2 g/d in dry climates, and 3 g/d for all indigenous children, and 5 g/d if geophagia is common.

These estimates are supported by studies of human coprolites from archaeological sites. For instance, Nelson (1999) noted that human coprolites from a desert spring-fed aquatic system included obsidian chips (possibly from sharpening points with the teeth), grit (pumice and quartzite grains from grinding seeds and roots), and sand (from mussel and roots consumption). Her conclusions are based on finding grit in the same coprolites as seeds, and sand in the same coprolites as mussels and roots. She concludes that "the presence of sand in coprolites containing aquatic root fibers suggests that the roots were not well-cleaned prior to consumption.

Geophagia

Despite the limited awareness of geophagia in western countries, the deliberate consumption of dirt, usually clay, has been recorded in every region of the world both as idiosyncratic behavior of isolated individuals and as culturally prescribed behavior (Abrahams, 1997; Callahan, 2003; Johns and Duquette, 1991; Reid, 1992). It also routinely occurs in primates (Krishnamani and Mahaney (2000). Indigenous peoples have routinely used montmorillonite clays in food preparation to remove toxins (e.g., in acorn breads), as condiments or spices, or to aid digestion (e.g., kaolin clay in Kaopectate) (Reid, 1992; Krishnamani and Mahaney, 2000). Callahan (2003) also suggests that certain soils may reduce parasite loads (demonstrated in monkeys) through immune enhancement, and clays with aluminum salts may have an adjuvant effect as they do in commercial vaccines.

Pregnancy is the most common occasion for eating dirt in many societies, especially kaolin and montmorillonite clays in amounts of 30g to 50g a day. In some cultures, well-established trade routes and clay traders make rural clays available for geophagy even in urban settings. Clays from termite mounds are especially popular among traded clays, perhaps because they are rich in calcium (Callahan, 2003; Johns and Duquette, 1991). In countries such as Uganda where modern pharmaceuticals are either unobtainable or

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prohibitively expensive, ingested soils may be very important as a mineral supplement, particularly iron and calcium (Abrahams, 1997; Krishnamani and Mahaney, 2000; Johns and Duquette, 1991).

Finally, it has been suggested that B vitamins produced by soil fungi might have a nutritional benefit.

Data from dermal adherence

Dermal adherence of soil is generally studied in relation to dermal absorption of contaminants, but soil on the hands and face can be ingested, as well. Kissel, et al. (1996) included reed gatherers in tide flats. "Kids in mud" at a lakeshore had by far the highest skin loadings. Reed gatherers were next highest, followed by farmers and rugby players and irrigation installers. Holmes et al. (1999) studied a variety of occupations. Farmers, reed gatherers and kids in mud had the highest overall skin loadings, followed by equipment operators, gardeners, construction, and utility workers. Archaeologists and several other occupations had somewhat lower skin loadings.

Grain size affects adherence and tactile responses to ingested soil. Particles below the sand-silt size division (0.075 mm) adhering more than smaller sizes (see EPA, 1992[20] for more details). Sieving is recommended, and data for particle size <0.044 cm (RAGSe, App. C, Table C-4).

Data from washed or unwashed vegetation and vegetables.

Direct soil ingestion also occurs via food, for example from dust blowing onto food (Hinton, 1992), residual soil on garden produce or gathered native plants, particles on cooking utensils, and so on. Beresford and Howard (1991) found that soil adhesion to vegetation was highly seasonal, being highest in autumn and winter, and is important source of deposited radionuclides to grazing animals.

Subsistence lifestyles and rationale for soil ingestion rate

The derivation of the soil ingestion rate is based on the following points:

- The foraging-subsistence lifestyle is lived in close contact with the environment.
- Plateau winds and dust storms are fairly frequent. Incorporated into overall rate, rather than trying to segregate ingestion rates according to number of high-wind days per year because low-wind days are also spent in foraging activities.

[20] EPA (1992). Interim Report: Dermal Exposure Assessment: Principles And Applications. Office of Health and Environmental Assessment, Exposure Assessment Group. /600/8-91/011B

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- The original Plateau lifestyle – pit houses, caches, gathering tules and roots - includes processing and using foods, medicines, and materials. This is considered but not as today's living conditions.
- The house is assumed to have little landscaping other than the natural conditions or xeriscaping, some naturally bare soil, a gravel driveway, no air conditioning (more open windows), and a wood burning stove in the winter for heat.
- All persons participate in day-long outdoor group cultural activities at least once a month, such as pow-wows, horse races, and seasonal ceremonial as well as private family cultural activities. These activities tend to be large gatherings with a greater rate of dust resuspension and particulate inhalation. These are considered to be 1-gram events or greater.
- 400 mg/d is the upper bound for suburban children (EPA); traditional or subsistence activities are not suburban in environs or activities
- This rate is within the range of outdoor activity rates for adults (between 330 and 480); subsistence activities are more like the construction, utility worker or military soil contact levels. However, it is lower than 480 to allow for some low-contact days.
- The low soil-contact days are balanced with many 1-gram days and events (as suggested by Boyd et al., 1999) such as root gathering days, tule-camas-wapato gathering days, pow wows, rodeos, horse training and riding days, sweat lodge building or repair days, grave digging, and similar activities. There are also likely to be many high or intermediate-contact days, depending on the occupation (e.g., wildlife field work, construction or road work, cultural resource field work).
- This rate does not account for pica or geophagy
- Primary data is supported by dermal adherence data in gatherers and 'kids in mud'. Tule and wapato gathering are kid-in-mud activities
- This rate includes a consideration of residual soil on roots (a major food category) through observation and anecdote, but there is no quantitative data.

H. Inhalation Rate

An inhalation rate of 25m3/d is recommended for traditional (active outdoor) lifestyles. Many risk assessments use the EPA default value of 20m3/d (EPA 1997), which reflects contemporary lifestyles of the general population. However, EPA recognizes that inhalation rates may be higher in certain populations, such as athletes or outdoor workers, because levels of activity outdoors may be higher over long time periods. "If site-specific data are available to show that subsistence farmers and fishers have higher respiration rates due to rigorous physical activities than other receptors, that data may be appropriate."[21] Such subpopulation groups are considered 'high risk' subgroups.[22]

[21] EPA (OSWER) "Human Health Risk Assessment Protocol for Hazardous Waste Combustion Facilities, Support Materials Volume 1: Human Health Risk Assessment Protocol for Hazardous Waste Combustion Facilities" page 6-4, at (http://www.epa.gov/earth1r6/6pd/rcra_c/protocol/ volume_1/chpt6-hh.pdf)

[22] Exposure Factors Handbook, 1997, Volume 1. page 5-24

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In order to develop inhalation rates more appropriate to traditional lifestyles, we evaluated the approach that uses specific activity levels to estimate short-term and long-term inhalation rates. Several examples of this approach are:

- EPA's National Air Toxics Assessment (homepage: http://www.epa.gov/ttn/atw/nata/ natsa3.html) uses the CHAD database to estimate national average air toxics exposures by selecting a series of single day's patterns to represent an individual's annual activity pattern.
- The California Air Resources Board (CARB, 2000) reviewed ventilation rates for many activities in the CHAD database and concluded that 20 m^3/d represents an 85th percentile of typical adult activity lifestyles reflecting 8 hours sleeping and 16 hours of light activity with little moderate or heavy activity.
- In their technical guidance document, "Long-term Chemical Exposure Guidelines for Deployed Military Personnel," the US Army Center for Health Promotion and Preventive Medicine (USACHPPM) recommended an inhalation rate of 29.2 m^3/d for US Armed Service members that includes 8 hours of moderate duties.[23]
- EPA used 30 m^3/day for a year-long exposure estimate for the general public at the Hanford Superfund site in Washington state, based on a person doing 4 hours of heavy work, 8 hours of light activity, and 12 hours resting.[24]
- The DOE's Lawrence Berkeley Laboratory also used 30 m^3/d: "the working breathing rate is for 8 hours of work and, when combined with 8 hours of breathing at the active rate and 8 hours at the resting rate, gives a daily equivalent intake of 30 m^3 for an adult."[25]
- The Rocky Flats Oversight Panel recommended using 30 m^3/d.[26]

Using EPA guidance on hourly inhalation rates for different activity levels, a reasonable inhalation rate for an average tribal member's active lifestyle is an average rate of 26.2 m^3/d, based on 8 hours sleeping at 0.4 m^3/hr, 2 hours sedentary at 0.5 m^3/hr, 6 hours light activity at 1 m^3/hr, 6 hours moderate activity at 1.6 m^3/hr, and 2 hours heavy activity at 3.2 m^3/hr. Unlike most other exposure factors, which are upper bounds, the inhalation rate is an average rate, so to be consistent with national methodology, we have rounded the rate down to 25 m^3/day.

[23] http://www.gulflink.osd.mil/particulate_final/ particulate_final_s06.htm and http://www.gulflink.osd.mil/pm/pm_en.htm.

[24] "Report of Radiochemical Analyses for Air Filters from Hanford Area" Memorandum from Edwin L. Sensintaffar, Director of the National Air and Radiation Environmental Laboratory to Jerrold Leitch, Region 10 Radiation Program Manager (http://yosemite.epa.gov/R10/AIRPAGE.NSF/webpage/Hanford+Environmental+Perspective)

[25] (www.lbl.gov/ehs/epg/tritium/TritAppB.html)

[26] RAC (Risk Assessment Corporation). 1999. Task 1: Cleanup Levels at Other Sites. Rocky Flats Citizens Advisory Board, Rocky Flats Soil Action Level Oversight Panel. RAC Report No.

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The estimate of the activity levels associated with traditional lifestyles is based on anthropological studies, ethnographic literature on foraging theory and hunting-gathering lifestyles, and confirmatory interviews with Tribal members. The inhalation rate reflects a wide range of traditional indoor and outdoor activities, including (a) youth who are learning traditional subsistence skills, (b) adults who hunt, gather, fish, and work in environmental management occupations, and (c) elders who gather plants and medicines, prepare and use them, and teach traditional activities. At present, it is not possible to extrapolate directly from the CHAD database from window washing, for example, to hide scraping; research is underway to fill this data gap using heart rate monitors keyed to respiration rate during specific traditional activities.

Finally, there may be some ethnic specificity in the link between metabolic and inhalation rates such as thrifty genotype(s) and oxidation adiposity patterns (Goran, 2000; Fox et al., 1998; Muzzin et al., 1999; Rush et al., 1997; Saad et al., 1991; Kue Young et al., 2002), as well as ethnic differences in spirometry (Crapo et al., 1988; Lanese et al., 1978; Mapel et al., 1997; Aidaraliyev et al., 1993; Berman et al., 1994). There are several stress response genes that enable indigenous populations to respond to environmental stresses and to the rapid transition between extremes, including feast and famine, heat and cold, disruption in circadian rhythms, dehydration, seasonality, and explosive energy output or rapid transitions between minimum and maximum exercise and VO2max (Kimm et al., 2002; Snitker et al., 1998). This may affect inhalation rate, but at present this remains a testable hypothesis.

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I. Steam Model – Sweatlodge

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In this analysis it is assumed that the internal temperature of the sweat lodge is maintained at a constant 150 °F [339 °K] (personal communication). It is further assumed that the geometry of the lodge can be estimated as a hemisphere of radius *r* so that the internal volume is equal to:

$$V_{lodge} = \frac{2}{3} \cdot \pi \cdot r^3 \qquad (1)$$

where:

V_{lodge} = Internal volume of the sweat lodge (m^3)
r = radius of sweat lodge (m)
π = the constant *pi* (unitless); $\pi \approx 3.14159$

Finally, contaminants, termed Compounds of Potential Concern (COPC), are assumed to be introduced into the sweat lodge predominately through the water used to create steam.

Volatile and Semi-Volatile Compounds
For the purpose of the following analysis, volatile and semi-volatile compounds are defined as those with a boiling point less than, or equal to, 339 °K. Intake of COPCs via inhalation are typically estimated as:

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$$I_{inh} = \frac{C_v \cdot IR \cdot ET \cdot EF \cdot ED}{BW \cdot AT \cdot CF} \quad (2)$$

where:

I_{inh} = inhalation exposure to COPCs in the sweat lodge (mg/kg-day)

C_v = vapor phase COPC concentration (mg/m^3)

IR = inhalation rate (m^3/hr)

ET = exposure time (hr/event)

EF = exposure frequency (events/yr)

ED = exposure duration (yr)

BW = body weight (kg)

AT = averaging time for carcinogens (AT_C) or noncarcinogens (AT_N) (yr)

CF = units conversion factor of 365 (day/yr)

For compounds that preferential partition to the air phase it is assumed that a negligible quantity deposit on surfaces or partition into condensed liquid. Thus, the bulk of contaminants added in the water will remain in the vapor phase throughout the sweat and the vapor concentration of an individual COPC is given by:

$$C_v(t) = C_{dw}\left(\frac{V_w(t)}{V_{lodge}}\right) \quad (3)$$

where:

C_{dw} = dissolved surface water concentration of the COPC (mg/L); calculated according to EPA 1998a, Appendix B

$C_v(t)$ = time dependent vapor phase concentration of the COPC in the sweat lodge (mg/m^3)

$V_W(t)$ = cumulative volume of water used in the sweat at time t; see the discussion of $V_W(t)$ below (L)

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Combining Equations 1 through 3 and recognizing that the total inhalation exposure for a single sweat requires integration of the volume function over the duration of the sweat then the following equation for inhalation exposure results:

$$\int_0^{ET} I_{inh}(t) \cdot dt = I_{inh} = \frac{C_{dw} \cdot \left(\frac{1}{\frac{2}{3} \cdot \pi \cdot r^3} \right) \cdot IR \cdot EF \cdot ED}{BW \cdot AT \cdot CF} \cdot \int_0^{ET} V_w(t) \cdot dt \qquad (4)$$

If it is assumed that water is poured over heated rocks at a constant rate throughout the sweat, then the volume function would be described by the following linear equation:

$$V_w = \frac{V_{w,total}}{ET} \cdot t \qquad (5)$$

Where *Vw,total* is the total amount of water that will be used in the sweat to create steam in units of liters (L).

Noting that:

$$\int_0^{ET} V_w(t) \cdot dt = \frac{V_{w,total}}{ET} \cdot \int_0^{ET} t \cdot dt = \frac{V_{w,total}}{2} \cdot ET \qquad (6)$$

then the intake by inhalation is described by the following equation:

$$I_{inh} = \frac{C_{dw} \cdot \left(\frac{V_{w,total}}{2} \right) \cdot \left(\frac{1}{\frac{2}{3} \cdot \pi \cdot r^3} \right) \cdot IR \cdot ET \cdot EF \cdot ED}{BW \cdot AT \cdot CF} \qquad (7)$$

If more water is poured over the heated rocks during the first part of the sweat, then the following form would be more appropriate:

$$V_w(t) = \frac{V_{w,total} \cdot t}{k + t} \qquad (8)$$

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where *Vw,total* is the maximum amount of water poured over the heated rocks during a sweat and *k* indicates the time when half of the water has been used. Integration of the above equation between the limits of 0 and ET results in the following expression for intake via inhalation:

$$I_{inh} = \frac{C_{dw} \cdot \left(\frac{V_{w,total}}{\frac{2}{3} \cdot \pi \cdot r^3} \right) \cdot \left(ET + k \cdot \ln\left(\frac{k}{ET + k} \right) \right) \cdot IR \cdot EF \cdot ED}{BW \cdot AT \cdot CF} \qquad (9)$$

The assumptions regarding the mathematical representation of water volume in the sweat lodge are an uncertainty in estimating intake via inhalation for the Native American adult. For simplicity, the linear assumption represented by Equations 5 and 7 is a reasonable approximation for intake via inhalation of volatile and semivolatile compounds in the sweat lodge. Table 1 provides a list of typical values for the parameters used in Equation 7.

Table 1: Typical Parameter Values for Calculating I_{inh} for Volatile and Semi-volatile compounds

Parameter	Typical Value	Unit
Volume of water used in a sweat ($V_{w,total}$)	4	L
Radius of a hemispherical sweat lodge (*r*)	1	m
Inhalation rate (*IR*)	30	m^3/day
Length of a sweat event (*ET*)	1	hr/event
Number of sweats per year (*EF*)	365	events/yr
Number of years a person sweats in a life time (*ED*)	64	yr
Average body weight (*BW*)	70	kg
Averaging time (*AT*)	70 (carcinogen) ED (noncarcinogens)	yr
Conversion factor (*CF*)	365	day/yr

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Nonvolatile Compounds

For the purpose of the following analysis nonvolatile compounds are defined as those with a boiling point greater than 339 °K. The sweat lodge vapor concentration for nonvolatile compounds can be estimated by assuming that:

- Nonvolatile COPC become airborne as an aerosol as the water they were carried in vaporizes.
- Once airborne, nonvolatile compounds deposit onto solid surfaces with aqueous condensation.
- The ideal gas law can be applied to air and water vapor at the temperature and pressure of the sweat lodge.

With these assumptions the quantity of nonvolatile constituents in the air phase is limited to that which is carried into the air phase by the volume of liquid water needed to create saturated conditions in the lodge. Numerically this can be expressed as:

$$C_v = \left(\frac{V_{w,sat}}{V_{lodge}}\right) \cdot C_{dw} \qquad (10)$$

where *Vw,sat* represents the volume of liquid water needed to create a saturated vapor in the sweat lodge in units of liters (L). From the ideal gas law and the properties of liquid water, *Vw,sat* can be determined from:

$$V_{w,sat} = \left(\frac{p \cdot V_{w,air}}{R \cdot T}\right)\left(\frac{MW_w}{\rho_w}\right) \qquad (11)$$

where:

$V_{w,air}$ = volume of air space in sweat lodge occupied by water vapor (m^3)
p = ambient pressure (mmHg)
ρ_w = density of liquid water (g/L)
T = temperature of the sweat lodge (K)
R = ideal gas law constant (0.06237 (mmHg-m^3)/(gmole-K))
MW_w = molecular weight of water (AMU)

The volume of water vapor in the sweat lodge air can be estimated from the vapor pressure of water at the temperature of the sweat lodge (assumed constant at 339 °K), the ambient pressure, and the internal volume of the lodge.

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$$V_{w,air} = \left(\frac{p^*}{p}\right) \cdot V_{lodge} \tag{12}$$

where p^* represents the vapor pressure of water at temperature T (mmHg). The vapor pressure of water as a function of temperature is given by the Antoine equation as follows (Himmelblau, 1982):

$$\ln(p^*) = 18.3036 - \frac{3816.44}{T - 46.13} \tag{13}$$

Combining Equations 10 through 13 allows the concentration of nonvolatile COPC in the air to be determined as follows:

$$C_v = C_{dw} \cdot \left(\frac{MW_w}{R \cdot T \cdot \rho_w}\right) \cdot EXP\left(18.3036 - \frac{3816.44}{T - 46.13}\right) \tag{14}$$

Application of Equation 14 to the definition of vapor inhalation exposure given in Equation (2) yields the following result for nonvolatile compounds

$$I_{inh} = \left(\frac{IR \cdot ET \cdot EF \cdot ED}{BW \cdot AT \cdot CF}\right) \cdot C_{dw} \cdot \left(\frac{MW_w}{R \cdot T \cdot \rho_w}\right) \cdot EXP\left(18.3036 - \frac{3816.44}{T - 46.13}\right) \tag{15}$$

Table 2 provides a list of typical values for the parameters used in Equation 15.

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Table 2: Typical Parameter Values for Calculating I_{inh} for Nonvolatile compounds

Parameter	Typical Value	Unit
Temperature of the sweat lodge (T)	339 (150)	K (F)
Ideal gas law constant (R)	0.06237	$(mmHg \cdot m^3)/(gmole \cdot K)$
Inhalation rate (*IR*)	30	m^3/day
Length of a sweat event (*ET*)	1	hr/event
Number of sweats per year (*EF*)	365	events/yr
Number of years a person sweats in a life time (*ED*)	64	Yr
Average body weight (*BW*)	70	Kg
Averaging time (*AT*)	70 (carcinogen) ED (noncarcinogens)	Yr
Conversion factor (*CF*)	365	day/yr
Molecular weight of water (MW_w)	18	g/gmole
Density of liquid water (ρ_w)	1000	g/L

Dermal Exposure in Sweat Lodge

Dermal exposure to COPC in a sweat lodge can come from skin contact with contaminants in both the air and in water that condenses on the skin. Calculation of dermal exposure to COPC from water contacting the skin is typical represented by the following equations:

$$I_{d,j} = \frac{C_{dw} \cdot SA \cdot f_{SA,j} \cdot Kp_j \cdot ET \cdot EF \cdot ED \cdot CF_3}{BW \cdot AT \cdot CF_2} \quad (16)$$

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where:

$I_{d,l}$ = intake of COPCs from dermal absorption to liquid within the sweat lodge (mg/kg-day)

C_{dw} = dissolved-phase surface water concentration (mg/L); calculated according to EPA 1998a, Appendix B

SA_l = body surface area available for contact (m^2)

$f_{SA,l}$ = Fraction of skin area (SA) in contract with liquid (unitless)

Kp_l = COPC-specific water to skin permeability constant (cm/hr)

ET = exposure time (hr/event)

EF = exposure frequency (events/yr)

ED = exposure duration (yr)

CF_2 = units conversion factor of 365 (day/yr)

CF_3 = units conversion factor of 10 ($L/m^2 \cdot cm$)

BW = body weight (kg)

AT = averaging time for carcinogens (AT_C) or noncarcinogens (AT_N) (yr)

Dermal exposure resulting from skin contact with contaminants in the air is calculated as:

$$I_{d,v} = \frac{C_v \cdot SA \cdot f_{SA,v} \cdot Kp_v \cdot ET \cdot EF \cdot ED \cdot CF_1}{BW \cdot AT \cdot CF_2} \quad (17)$$

where:

$I_{d,v}$ = intake of COPCs from dermal absorption to vapor within the sweat lodge (mg/kg-day)

C_v = vapor-phase concentration for a COPC (mg/m^3)

$f_{SA,v}$ = Fraction of skin area (SA) in contract with vapor (unitless)

Kp_v = COPC-specific vapor to skin permeability constant (cm/hr)

CF_1 = units conversion factor of 0.01 (m/cm)

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Volatile and Semi-Volatile Compounds

Dermal exposure should be calculated using the same assumptions described for inhalation exposure. For volatile and semi volatile compounds (defined as those with a boiling point less than or equal to 339 °K), 100% volatilization with a hemispherical sweat lodge was assumed. Hence, the primary exposure pathway will be from vapor and exposure from condensed water can be neglected. The vapor concentration of COPC causing dermal exposure is identical to the inhalation concentration and is given by Equations 3 and 5. Combining Equations 3 and 5 with Equation 17 and integrating between the limits of 0 and ET results in the following prediction from dermal exposure to volatile and semi-volatile compounds.

$$I_{d,total} = I_{d,v} = \frac{C_{dw} \cdot \left(\frac{V_{w,total}}{2}\right) \cdot \left(\frac{1}{\frac{2}{3} \cdot \pi \cdot r^3}\right) \cdot SA \cdot f_{SA,v} \cdot Kp_v \cdot ET \cdot EF \cdot ED \cdot CF_1}{BW \cdot AT \cdot CF_2} \quad (18)$$

where *Id,total* is the total dermal exposure rate for volatile and semi-volatile compounds. Table 3 provides a list of typical values for the parameters used in Equation 18.

Table 3: Typical Parameter Values for Calculating $I_{d,total}$ for Volatile Semi-volatile compounds

Parameter	Typical Value	Unit
Volume of water used in a sweat ($V_{w,total}$)	4	L
Radius of a hemispherical sweat lodge (*r*)	1	m
Body surface area available for contact (*SA*)	1.8	m^2
Fraction of skin area (SA) in contact with vapor ($f_{SA,v}$)	0.0 - 1.0	unitless
COPC-specific permeability constant for vapor exposure (Kp_v)	1 to 1E-5	cm/hr
Length of a sweat event (*ET*)	1	hr/event
Number of sweats per year (*EF*)	365	events/yr
Number of years a person sweats in a life time (*ED*)	64	yr
Average body weight (*BW*)	70	kg
Averaging time (*AT*)	70 (carcinogen) ED	yr
	(noncarcinogens)	
Conversion factor (CF_1)	0.01	m/cm
Conversion factor (CF_2)	365	day/yr

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Nonvolatile Compounds

For non-volatile compounds (defined as those with a boiling point greater than 339 °K), the dermal exposure assumptions would result in a concentration in condensed water equal to that of the water added to the heated rocks and a vapor concentration as described by Equation 14. Thus, exposure through dermal contact resulting from water condensing on the skin would be calculated using the following equation:

$$I_{d,l} = \frac{C_{dw} \cdot SA \cdot f_{SA,l} \cdot Kp_l \cdot ET \cdot EF \cdot ED \cdot CF_3}{BW \cdot AT \cdot CF_2} \tag{19}$$

The dermal exposure to COCP in the vapor phase is represented by combining Equations 17 and 14 as follows:

$$I_{d,v} = \left(\frac{(SA \cdot f_{SA,v} \cdot Kp_v \cdot ET \cdot EF \cdot ED \cdot CF_1}{BW \cdot AT \cdot CF_2} \right) \cdot C_{dw} \cdot \left(\frac{MW_w}{R \cdot T \cdot \rho_w} \right) \cdot \ldots$$
$$\ldots EXP\left(18.3036 - \frac{3816.44}{T - 46.13} \right) \tag{20}$$

In this equation $f_{SA,v}$ and Kp_v represents the fraction of skin area (SA) in contact with vapor and the COPC-specific permeability coefficient for a contaminant from vapor to the skin.

The total dermal exposure for nonvolatile compounds is thus represented by the sum of $I_{d,v}$ and
$I_{d,l}$. That is:

$$I_{d,total} = I_{d,v} + I_{d,l} \tag{21}$$

It should be noted that at the temperature conditions assumed for a sweat lodge it is acceptable to neglect the vapor component of dermal exposure since it will always be less than 0.2% of the liquid exposure value. This can be demonstrated by computing the ratio of the vapor to liquid exposure rates. In this calculation it should be recognized that, in a sweat lodge, more of the body surface area will be covered with a layer of liquid water (condensation and perspiration) than would be dry and so available for only vapor contact (i.e. $f_{SA,l} > f_{SA,v}$). In addition the vapor permeability coefficient is generally within an order of magnitude of the corresponding liquid coefficient (EPA, 1992). With these assumptions the ratio of vapor to liquid dermal exposure is given by:

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$$\frac{I_{d,v}}{I_{d,l}} = \frac{CF_1 \cdot MW_w}{CF_3 \cdot \rho_w \cdot R \cdot T} \cdot \frac{f_{SA,v}}{f_{SA,l}} \cdot \frac{Kp_v}{Kp_l} \cdot EXP\left(18.3036 - \frac{3816.44}{T - 46.13}\right) \leq 1.6 \times 10^{-3} \quad (22)$$

Hence, for the purposes of this evaluation the dermal exposure to nonvolatile compounds can be represented by:

$$I_{d,max} = I_{d,l} \quad (23)$$

Table 4 provides a list of typical values for the parameters used in Equations 19 through 23.

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Table 4: Typical Parameter Values for Calculating $I_{d,total}$ for Nonvolatile compounds

Parameter	Typical Value	Unit
Volume of water used in a sweat ($V_{w,total}$)	4	L
Radius of a hemispherical sweat lodge (*r*)	1	m
Body surface area available for contact (*SA*)	1.8	m^2
Fraction of skin area (SA) in contact with liquid ($f_{SA,l}$)	0.0-1.0	unitless
Fraction of skin area (SA) in contact with vapor ($f_{SA,v}$)	1.0-$f_{SA,l}$	unitless
COPC-specific permeability constant from vapor contact with skin (Kp_v)	1 to 1E-5	cm/hr
COPC-specific permeability constant from water contact with skin (Kp_l)	1 to 1E-5	cm/hr
Length of a sweat event (*ET*)	1	hr
Number of sweats per year (*EF*)	365	events/yr
Number of years a person sweats in a life time (*ED*)	64	yr
Average body weight (*BW*)	70	kg
Averaging time (*AT*)	70 (carcinogen) ED (noncarcinogens)	yr

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Table 4, cont'd: Typical Parameter Values for Calculating $I_{d,total}$ for Nonvolatile compounds

Molecular weight of water (MW_w)	18	g/gmole
Density of liquid water (ρ_w)	1000	g/L
Temperature of the sweat lodge (T)	389 (150)	K (F)
Ideal gas law constant (R)	0.06237	$(mmHg \cdot m^3)/(gmole \cdot K)$
Conversion factor (CF_1)	0.01	m/cm
Conversion factor (CF_2)	365	day/yr
Conversion factor (CF_3)	10	L/m^2-cm

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M. ATTACHMENT 1

TABLE M-1 SHOSHONE-BANNOCK TRIBES VEGETATION TABLE

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		Exposure Pathway			Part of Plant													Gathered	Shared w/ family	Yearly Amount
Common Name	Scientific Name	Inhal.	Inges.	Abs.	Rts.	Lvs.	Bark	Bulb	Stem	Whole	Seeds	Berries	Fruit	Milk	Oil	Flower	Juice/sap		6-10 kids	ounces
Antelope brush	*Purshia tridentata*			X			X			X								Sp, Su, Fall		320
Arrowleaf balsamroot	*Balsmaorhiza sagittata* Nutt.		X	X	X						X							Sp, Su, Fall		320
Aspen	*Populus* sp.	X								X								Yearly		3,844,000
Aster (dwarf purple)	*Erigeron* spp.		X		X													Su, Fall		160
Broom snakeweed	*Gutierresia sarothrae* Britton		X							X								Sp, Su, Fall		320
Buckbrush	*Ceanothus velutinus* Douglas ex Hook.	X		X	X					X								Sp, Su, Fall		320
Buffalo berries	*Lepargyrea* spp.		X							X								Summer		704
Cat-Tail	*Typha latifolia*		X	X		X				X								Year round	per person	840
Cedar	*Cedrus*	X	X	X		X				X								Yearly		19,200
Chokecherry	*Prunus virginiana* L.	X	X	X		X	X					X						Su, Fall		7,744
Dandelion	*Taraxacum*		X		X											X		Sp, Su, Fall		720
Desert Parsley (fern)	*Lomatium dissectum*	X	X	X	X													Fall		720
Elderberry (red)	*Sambucus racemosa*		X	X	X							X						Summer		704
Golden currant	*Ribes aureum* Pursh		X							X		X						Sp, Su		320
Grasses	*Poaceae* (family)	X	X	X		X				X	X							Sp, Su, Fall		704
Honeysuckle	*Lonicera interrupta*			X	X													Summer		320
Huckleberry	*Vaccinium* sp.		X									X						Su, Fall		160
Indian Cabbage	*Stanleya pinnata*		X			X												Summer		100
Indian Paint Brush	*Castilleja* spp.		X	X		X										X		Sp, Su, Fall		320
Indian tea	*Ephedra* spp.		X						X		X							Sp, Su, Fall		380
Juniper	*Juniperus* spp	X	X	X		X			X	X								Yearly		9,600
Milkweed	*Asclepias speciosa*		X	X										X				Sp, Su, Fall		704
Oyster mushroom	*Pleurotus* sp.																	Sp, Su, Fall		160
Peppermint	*Mentha penardi*	X	X	X		X				X	X							Sp, Su, Fall		760
Prickly Pear	*Opuntia engelmanni*		X										X	X	X			Sp, Su		1,408
Puffballs	*Clavatia* sp.																	Sp, Su, Fall		320
Rabbit Brush (Gray)	*Chrysothamnus nauseosus*	X	X	X				X	X	X								Sp, Su, Fall		704
Russian Thistle	*Salsolala kali* L.		X						X									Summer		160
Sage (black)	*Artemisia nova*	X	X	X		X				X								Sp, Su, Fall		320
Sagebrush (big)	*Artemisia tridentata*	X	X			X	X			X								Yearly		7,744
Service berry	*Amelanchier alnifolia*		X	X			X					X						Su, Fall		704
Sneezeweed	*Helenium hoopseil*							X										Sp, Su		160
Spearmint	*Mentha spicata*		X								X							Sp, Su, Fall		760
Stinging nettle	*Urtica dioica*		X			X	X		X									Sp		160
Sunflower	*Wyethia longicaulis*		X	X	X						X							Su, Fall		704
Sunflower-Poor One	*Helianthus petioloaris*		X								X							Sp, Su		160
Sweet sage	*Artemisia dracunculoides*	X	X	X			X				X							Sp, Su, Fall		7,744
Sweetgrass	*Heirochloe odorata*	X	X	X						X								Sp, Su, Fall		704
Thistle	*Cirsium*		X	X	X				X									Summer		320
Three-lobed sumac	*Rhus tribobata*		X									X						Su, Fall		160
Watercress	*Nasturtium officinale*		X			X			X	X							X	Sp,Su, Fall		160
White Daisy	*Layia glandulosa*																	Su, Fall		160
Wild carrot	*Carum gairdoeri*		X		X					X								Summer		320
Wild Celery	*Apium* species		X							X								Su		160
Wild rose	*Rosacea* spn.	X	X	X	X	X	X		X	X								Summer	Ceremonial	704
Yarrow	*Achillea lamulosa*		X	X	X	X										X		Su, Fall		320

Inhal. = inhalation, Inges.=ingestion, Abs.=absorbtion, Rts.=roots, Lvs.=leaves

N. ATTACHMENT 2

TABLE N-2 SHOSHONE-BANNOCK TRIBES MEAT CONSUMPTION CALCULATIONS

Shoshone-Bannock Meat Consumption Calculations Table

Wet weight calculations

	# of tags per year	ave wt of meat	pounds harvested		
700 hunters					
Hunter gets:	**2 Mule Deer**	175.8	351.6		
	3 elk	350	1050.0		
	1 moose	950	950.0		
				Ounces	
Total per hunter			2351.6	37625.0	**Yearly Total**
			6.4	103.1	**Daily**
6 per household			1.1	17.2	**per person**
			0.0	0.0	**per day**
members			1646093.8	26337501.1	**total meat**
4200			391.9	6270.8	**per person**
			1.1	17.2	**daily**
Enrolled Members					
5400			304.8	4877.3	**per person**
			0.8	13.4	**per day**
700	**Cattle**	795.4	556801.0	8908816.0	**Total meat**
			103.1	1649.8	**per person**
			0.3	4.5	**daily**

Pounds	**15% fat calculation**	**Pounds**
209.5	**chuck**	4.7
155.8	**rounds**	23.4
134.6	**thin cuts**	20.2
115.7	**short loins**	17.4
66.6	**ribs**	10.0
32.7	**misc.**	4.9
714.9		80.5

one ounce equals the size of a golf ball.

Wet weight calculations				**Pounds**		**Ounces**	**Grams**
700	**Elk**		1050.0	735000.0	**Total meat**		
				175.0	**per person**		
				0.5	**per day**	7.7	**217.5**
				136.1	**Enrolled TM**		
				0.4	**per day**	6.0	**169.1**

3:1 ration difference from wet to dried

700	**Elk**		350.0	245000.0	**Total meat**		
				58.3	**per person**		
				0.2	**per day**	2.6	**72.5**
				45.4	**Enrolled TM**		
				0.1	**per day**	2.0	**56.4**
700	**Cattle**	265.1	185600.3	2969605.3	**Total meat**	296960.5	
			34.4	549.9	**per person**	549.9	**15590.1**
			0.1	1.5	**daily**	1.5	**42.7**

Message

From: Anitole, Katherine [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=4ACBA4F6BEF64933A73A201E54C8F92E-KANITOLE]
Sent: 8/1/2017 1:10:51 PM
To: OPPT RAD [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=f052c9d5c11b440b8393b73f0947a2d0-OPPT RAD]
Subject: FW: LNT
Attachments: Environ.Res. Flaws in LNT (1).pdf

From: Fitzpatrick, Julie
Sent: Tuesday, August 01, 2017 9:04 AM
To: RAF Members <RAF_Members@epa.gov>
Subject: FW: LNT

FYI

Julie W. Fitzpatrick
Human Health Science Coordinator
Risk Assessment Forum
U.S. Environmental Protection Agency
Office (202) 564-4212
Mobile (202) 288-2352
fitzpatrick.julie@epa.gov

From: Edward Calabrese [mailto:edwardc@schoolph.umass.edu]
Sent: Monday, July 31, 2017 10:00 PM
To: Fitzpatrick, Julie <Fitzpatrick.Julie@epa.gov>
Subject: LNT

Julie:

See the new paper on LNT....it took a lot of work to dig the many details out....
it is worth the read....please share with your colleagues.

Ed

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Flaws in the LNT single-hit model for cancer risk: An historical assessment

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ABSTRACT

The LNT single-hit model was derived from the Nobel Prize-winning research of Herman J. Muller who showed that x-rays could induce gene mutations in *Drosophila* and that the dose response for these so-called mutational events was linear. Lewis J. Stadler, another well-known and respected geneticist at the time, strongly disagreed with and challenged Muller's claims. Detailed evaluations by Stadler over a prolonged series of investigations revealed that Muller's experiments had induced gross heritable chromosomal damage instead of specific gene mutations as had been claimed by Muller at his Nobel Lecture. These X-ray-induced alterations became progressively more frequent and were of larger magnitude (more destructive) with increasing doses. Thus, Muller's claim of having induced discrete gene mutations represented a substantial speculative overreach and was, in fact, without proof. The post hoc arguments of Muller to support his gene mutation hypothesis were significantly challenged and weakened by a series of new findings in the areas of cytogenetics, reverse mutation, adaptive and repair processes, and modern molecular methods for estimating induced genetic damage. These findings represented critical and substantial limitations to Muller's hypothesis of X-ray-induced gene mutations. Furthermore, they challenged the scientific foundations used in support of the LNT single-hit model by severing the logical nexus between Muller's data on radiation-induced inheritable alterations and the LNT single-hit model. These findings exposed fundamental scientific flaws that undermined not only the seminal recommendation of the 1956 BEAR I Genetics Panel to adopt the LNT single-hit Model for risk assessment but also any rationale for its continued use in the present day.

1. Introduction

On an August day in 1946 at the University of Rochester, the geneticist Ernst Caspari reported to his supervisor, Curt Stern, that his experiment on gamma-ray-induced mutations in *Drosophila* had produced results that neither scientist had anticipated. Caspari had fully expected to replicate the 1944 study of Ray-Chaudhuri who had shown that total X-ray/gamma-ray dose was the best predictor of mutational events in mature *Drosophila* spermatozoa. This study was important because it provided evidence that the dose response for ionizing radiation-induced mutations was cumulative, irreversible, independent of the dose rate, and linear at low doses. However, in contrast to Ray-Chaudhuri's earlier results, Caspari found that the dose rate rather than the total dose determined the dose response and that for a sufficiently low dose rate no response could be detected, revealing an apparent threshold response for gamma-ray-induced mutations. The Caspari studies were part of the Manhattan Project and as such were generally stronger scientifically than those of most independent, single researchers, including the studies of Ray-Chaudhuri. For example, as compared to the Ray-Chaudhuri study, the Caspari study had a more robust design, used larger numbers of flies, exerted superior oversight and guidance, applied better quality control, and assessed a notably lower dose and dose rate, amongst other factors (Calabrese, 2011; Caspari and Stern, 1948).

The Caspari data produced considerable alarm and concern throughout a radiation genetics community fearing that the LNT single-hit model might not now replace the currently accepted threshold dose-response model. The reactions of some leading radiation geneticists to the Caspari findings have been documented and published (Calabrese, 2011, 2015a). One especially succinct and notable response came from Milisav Demerec who asked of Caspari, "What can we do to save the hit model" (Calabrese, 2011). The above cited papers document prestigious members of the radiation geneticist community, including Nobel Prize recipients and an expert Genetics Panel of the U.S. National Academy of Sciences, who saved the "hit" model by obfuscating, deceiving and misrepresenting the scientific record. Sadly, these ideologically based and scandalous manipulations of the scientific record (Calabrese, 2015a, 2015b, 2017a) proved highly successful; they quickly infiltrated and affected the highest levels of regulatory policy in the US Government where they still remain in place today.

The present paper examines a surprisingly under-researched and critical aspect of the cancer risk assessment process by seeking answers to the

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following questions: (1) what was the genesis of the LNT Single-hit Hypothesis, (2) what was its scientific basis and (3) how and when was it validated? To address these questions it is necessary to re-examine the discovery of X-ray-induced heritable mutations by Hermann J. Muller, to assess the scientific validity of Muller's findings and interpretations and, finally, to understand how the scientific community reacted to and accepted Muller's personal account of his results. Acceptance by the scientific community essentially affirmed a biological basis for the Single-hit Model and, at the same time, reassured the public of its "legitimate" use in the assessment of cancer risk for decades to come.

Lewis J. Stadler, a plant geneticist of comparable standing to Muller within the radiation geneticist community, challenged Muller's 1927 claim of having induced gene mutations with X-rays and, as a result, initiated a fierce data-driven debate that only ended much later with the 1954 death of Stadler. Although Muller's views would eventually predominate, the experimental data and arguments offered by Stadler were formidable. The 25-year Muller-Stadler debate generated a unique scientific record that has never been used to re-evaluate the LNT single-hit model and, therefore, validate its application in the process of cancer risk assessment. This is quite astonishing considering that the LNT single-hit model would emerge primarily from the decades-long Muller-Stadler debate. Although Muller's views eventually prevailed, the questions raised herein are why they prevailed, should they have prevailed and, if not, then what does this mean for us today? This assessment concludes that the arguments of Stadler were based on stronger data and were more scientifically persuasive than were Muller's. Yet, in spite of Stadler's superior arguments, his views would show little penetration and have no impact on risk assessment policy.

The story of the LNT single-hit model is complicated and has numerous twists and turns. In the end, however, this new assessment enables a better clarification and understanding of the LNT single-hit model. It challenges the nearly universally accepted view that Muller, in his key groundbreaking experiments of 1927 (Muller, 1927a), had induced the "artificial transmutation of the gene" (i.e., induced "true" mutations) with high doses of X-rays. For it is now known that the vast majority, perhaps all, of the heritable phenotypic changes that Muller had observed were due to substantial non-gene alterations consisting mostly of small, large and massive chromosomal deletions. Furthermore, the LNT single-hit model made critical assumptions about mutations and their dose and dose-rate dependencies that were incorrect. The LNT single-hit cancer risk-assessment model, as it turns out, is the product of a sequence of historical myths about Muller's Nobel Prize findings. Amazingly, these myths mesmerized the scientific community even in the face of objective contemporary scientific challenges that have since been validated with modern analytic tools for mutation assessment. The historical findings presented herein demonstrate that the LNT single-hit model is scientifically flawed and also that the cancer risk assessment policy, which is based on the LNT single-hit model, is equally flawed.

2. Muller and the discovery of X-ray induced mutation

On July 22, 1927, Muller published a paper in the journal *Science* making the then striking claim that X-rays can induce heritable mutations in fruit fly gonads. However, because such a groundbreaking paper was published without data, some scientists, including his Ph.D. mentor T. H. Morgan (Carlson, 1981), were rightly skeptical and questioned whether Muller had the data he claimed.[1] Apparently, the extraordinary editorial decision at *Science* to publish such a significant paper without requiring data has yet to be explained some 90 years later. Two months after his *Science* publication, at the 5th International Genetics Congress in Berlin, Muller finally showed the world convincing evidence that X-rays induced heritable mutations in fruit fly gonads.

Muller (1927a, 1928a) used the method of sex-linked recessive lethality in the genomes of male and female fruit flies to demonstrate X-ray-induced mutations. To distinguish his findings from those of researchers who induced heritable "chromosomal" changes, including various types of aberrations (e.g., deletions, insertions), non-disjunction and other gross chromosomal alterations, Muller emphasized that he had artificially altered the "gene", a much smaller unit of inheritance than the "chromosome". In his first experiment Muller produced an apparent threshold dose response using four doses and a standard *Drosophila* strain. Switching to his newly developed C1B strain and reducing the number of doses to two (i.e., doses #2 and #4), Muller showed that mortality was enhanced by some 150 fold at the highest dose when results from the two experiments were combined. Although the findings were limited, the data suggested a non-linear (square root), dose-response function (Muller, 1927a). A broad spectrum of visual phenotypic changes was also reported, indicating that the X-ray treatment had affected many genes.

While it is generally believed that Muller had won the race to be first in inducing gene mutations by a scant three months,[2] narrowly beating several groups of plant geneticists from the US, including Lewis Stadler[3] from the University of Missouri and Thomas Goodspeed from the University of California at Berkeley, he may actually have come in second. Charles Stuart Gager (Brooklyn Botanical Gardens) and Albert Blakeslee (Carnegie Institute for Research) were the first researchers to report in the *Proceedings of the US National Academy of Sciences* in January of 1927 that ionizing radiation induced gene mutations in Jimson Weed (Datura).[4,5,6] Their paper revealed that radiation induced

[1] Carlson (1981) (pages 148–149) stated that T.H. Morgan believed that Muller had finally "hung himself", having recalled an earlier incident in which Muller overstated that the *Drosophila* spontaneous mutation rate for the X-chromosome was 1%. Morgan held there was little chance to escape the apparent exaggeration of the so-called 15,000% increase in X-ray induced mutation rate. The statement of Morgan reveals that he was reluctant to trust the assertions of Muller while also having reservations about his character. The lack of professional and perhaps personal regard of Muller may be seen when Morgan sponsored Sturtevant for membership in the NAS, rather than Muller, some five years after Muller's discovery of X-ray induced mutation (Carlson, 1981, page 174).

[2] Stadler presented his mutational evidence in Nashville, Tennessee, at the AAAS meeting in December of 1927. Stadler had treated barley seeds with X-rays in 1926, collecting the subsequent seeds later that season. These seeds were planted in the next growing season (1927). The findings revealed evidence of mutation. While 1300 tiller controls showed no trans-generational phenotypic changes, the X-ray treatment yielded 14 mutations in 1200 tillers (Carlson, 1981, page 151).

[3] Rhoades (1984) has emphasized that Muller and Stadler had a different goal/approach to their respective X-ray induced mutation research. In the case of Stadler, he employed X-rays to induce mutations to study gene structure and the nature of gene mutation alterations. Thus, Stadler focused on mutations at various loci that mediated specific phenotypic functions. In contrast, Muller focused on the spectrum of induced mutational changes.

[4] While Gager and Blakeslee would assert the primacy of their gene mutation discovery over the next 15 years (Blakeslee, 1940, 1942; Gager, 1931, 1936), they acknowledged that Muller had developed a method for generating large numbers of mutations. As primacy was a critical issue for Muller, he would routinely attempt to discredit the claims of others, including Gager and Blakeslee. In retrospect, the assertion of Gager and Blakeslee has withstood the criticism of Muller (Campos, 2015). However, the assertions and claims of Gager and Blakeslee would generally be ignored while Muller's findings would capture the excitement of the scientific community (Campos, 2015).

[5] Stadler (1936) would recognize the primacy of Gager and Blakeslee and their mutational findings. He specifically stated that: "Before the publication of Muller's results, several investigations of the genetic effects of penetrating inductions in plants were in progress and some positive results were obtained. The work of Gager and Blakeslee (1927) is especially noteworthy in this connection". Of the 113 progeny plants from parents treated with radium, 20 were chromosomal variants. Amongst these chromosomal variants two were heterozygous for new recessive genes. Even though the number of gene mutations was small, Stadler (1936) considered the findings of considerable importance. The publication of the Gager and Blakeslee (January 14, 1927) preceded the Muller note in *Science*, (i.e., July 22, 1927) which lacked any data, by more than six months. Despite his recognition and appreciation of the findings of Gager and Blakeslee (1927), Stadler (1954) would argue that they too fell short of "conclusive" proof due to inadequate statistical evidence and failure to show that the genetic changes reported were the result of the treatment and not to some type of genetic irregularity of the treated strains.

[6] Muller (1928a) would present his findings at the Fifth International Genetics Congress in Berlin (September 1927). At the same Congress Blakeslee (1928) would also

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a few apparent "real" gene mutations. Muller's principal achievement, on the other hand, was finding a methodology to enhance the production of what were believed to be unequivocal gene mutations. Conflating the methodology with the discovery helped create the notion and then the myth (Campos, 2015) that Muller produced the first artificial transmutation of a gene as well as the technique to vastly increase their production. More extensive assessments of the dose response would follow and expand Muller's basic observations in support of a linear dose response (Oliver, 1930; Hanson and Heys, 1929).

3. When is a mutation a "True" mutation?

Despite the consistently reproducible findings that X-rays could induce heritable mutations, questions began to arise as to whether Muller had actually induced "true" mutations.[7,8] Shades of doubt surfaced at the University of Missouri with Lewis J. Stadler's detailed cytogenetic evaluations of the effects of X-rays on maize. In a 1931 publication, Stadler began to draw a distinction between those changes that occur in the individual gene and those that relate to gross chromosomal alterations (e.g., deletions, reduplication or rearrangement of unchanged genes). Although this distinction, as Stadler saw it, was obviously important due to the role of gene mutation in evolution, gross alterations resulting from chromosomal irregularities were just as heritable[9] and therefore of no less importance.

Stadler had a preeminent scientific reputation; he was a highly regarded plant geneticist, a member of the U.S. NAS and the President of the Genetics Society of America (GSA) (1938). As such, his curiosity to differentiate between gene and chromosomal alterations was not only significant but also central to his research program on understanding the nature of the gene. He collaborated with the future Nobel Prize (1983) recipient Barbara McClintock and then recruited her to assess the effects of X-rays on maize chromosomes at the University of Missouri. McClintock's expertise proved immensely valuable, as she had just developed the capacity to markedly improve chromosomal staining of the pachytene stage of prophase.[10] So, McClintock began to assess Stadler's maize chromosomes in the summer of 1931. In her own words to the Nobel audience (McClintock, 1983) she described her research in Stadler's laboratory:

> "...It was to observe the effects of X-rays on chromosomes of maize that brought me to the University of Missouri in 1931.... Irradiated male gametes in Stadler's experiments carried wild-type alleles of known recessive mutants...An X-ray induced mutation altering the expression of the wild-type allele of one of these recessives should be identifiable in an individual plant derived from such a cross....-following my arrival at Columbia in June 1931, plants were selected where chromosomes were to be examined." (Meiotic stages were examined to look for certain kinds of events that might be responsible for the expression of recessives)...None of the recessive phenotypes in the examined plants arose from "gene mutation". Each reflected loss of a segment of a chromosome that carried the wild-type allele, and x-rays were responsible for inducing these deficiencies. They were also responsible for producing other types of chromosomal rearrangement, some of them unexpectedly complex...."

The collaboration between Stadler and McClintock affected Stadler's understanding of the problem almost immediately. His presentation in 1932 at the Sixth International Congress on Genetics at Cornell University challenged Muller's assertion of having induced gene mutation.[11] Furthermore, in his 1933 annual report (Stadler, 1933) to the National Research Council, Stadler stated that "investigation of the physical nature of the induced 'gene mutations' indicates that these are chiefly or wholly the result, not of changes within the gene, but of extragenic alterations of various kinds". Table 1 provides quotations from several of Stadler's publications (from 1932 to his final publication in 1954) that challenged Muller's assertions that he had observed "true" gene mutations. With the arrival of new analytic tools since the mid-1970's, such as the Southern blot (Southern, 1975), PCR (Mullis and Faloona, 1987) and precise DNA sequencing technologies, others in the research community extended this debate with much greater specificity and clarity.

Stadler's views on the nature of X-ray-induced genetic damage seemed to crystallize following new developments in cytological techniques that included Painter's salivary gland method (Painter, 1933, 1934a, 1934b, 1934c) in *Drosophila* and McClintock's earlier pachytene technique in maize (McClintock, 1929a, 1931) and their applications in the analysis of X-ray induced mutations.[12] As a result of the applications

(*footnote continued*)
summarize the mutation findings of Gager and himself as published earlier (Gager and Blakeslee, 1927). In the case of Muller, he seized the moment and captured the fascination of his audience; whereas Blakeslee would submerge/underplay his novel mutational findings in an article obscurely entitled "The Genetics of Datura". The failure of Gager and Blakeslee to promote their key findings was in striking contrast to the demonstrable Muller. Elof Carlson (1981), Muller's biographer (page 151), incorrectly stated that Blakeslee confirmed the X-ray-induced mutation results of Muller within a year after Muller's report. As documented here, Gager and Blakeslee (1927) published their data a year ahead of Muller's (1928a, 1928b) data in the conference proceedings and six months prior to Muller's data-free *Science* paper.

[7] A key aspect of the Muller story was his ability to redefine the contemporary view of what constituted a "real" mutation (Campos, 2015). Prior to Muller's engagement on this issue the generally accepted view was that inherited phenotypic changes were considered mutations. This could occur via alterations with the chromosome and/or gene. Muller would be unrelenting in his quest to drop chromosomal inheritance from the definition. So focused was Muller on winning this conceptual and terminological argument that he utilized the title "artificial transmutation of the gene" to describe the mutation he believed he had induced by X-ray treatment (Muller, 1927a).

[8] Prior to the discovery of McClintock, cytological evaluation of radiation-induced chromosome aberrations/changes were limited to the condensed metaphase chromosomes of somatic cells. In such a setting it is only possible to identify gross effects, including changes in chromosome number or large translocations or losses of chromosome sections. McClintock's contribution involved the capacity to study thin-stranded chromosomes of early prophase of meiosis, permitting a far more precise documentation of radiation-induced changes than had occurred previously. In maize, the chromosomes are approximately 10-fold longer during prophase than they are at metaphase and the homologous chromosomes are closely aligned. Thus, the loss of even a very small part of the chromosome is easily observed. Likewise, the nature of translocations/damage is therefore markedly more refined and could then be used with greater precision to assess chromosomal alterations at far higher resolution and at far lower doses of radiation.

[9] For Stadler (1954) the error of Muller in the debate was that he combined his observed effect (i.e., heritable phenotypic changes) (Step 1) with an interpretation that the observed effects were due to a specific mechanism (Step 2). Stadler (1954) emphasized that these two steps be kept distinct since Step 1 is a specific contribution to scientific knowledge, while the second is based on an inference/interpretation. By combining the two, the scientific community may confuse what it knows with what it thinks it knows. Stadler (1954) asserted that the belief that the frequency of gene mutation may be profoundly increased by X-ray treatment was "an illusion of this kind". The problem emerged from an insistence that X-rays induced gene mutations, since the mutants that are formed satisfy all the criteria of gene mutations (e.g., heritable, reversible) and that these mutants represent qualitative alterations in specific genes, since that is the definition of a gene mutation. In effect, Stadler argued that the error resides in using the same term for two concepts. This terminological combination then becomes a substitute for a bonafide scientific mechanism of proof - the trap to which the mutation field was vulnerable.

[10] The technique of McClintock (1931) for the assessment of maize chromosomes in prophase of meiosis made possible the cytological identification of minor structural chromosomal changes with a precision far exceeding that possible in studies using condensed chromosomes. The breakthrough methodological development of McClintock (1929a, 1929b, 1931) forced Stadler to realize that viable deficiencies were able to account for some of the apparent/presumed gene mutations induced by X-ray treatment. Thus, for Stadler (1931) it was "now clear that many of the variations identified by their genetic behaviors as gene mutations are due to mechanical alteration analogous to the grosser chromosomal aberrations.".

[11] Note that Stadler's presentation at the Sixth International Congress on Genetics was one of only three Plenary addresses on mutagenesis (Haynes, 1938). With Muller in the audience, Stadler had clearly offered a major public challenge to Muller's interpretation.

[12] Painter developed an application of the acetocarmine smear method that had been employed by cytogeneticists working on maize chromosomes (Glass, 1990). According to Carlson (1981), Muller first learned of Painter's (1933) advance while in Leningrad. He immediately understood its significance for the assessment of X-ray-induced small rearrangements and chromosome breaks.

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Table 1
Stadler's Challenge to Muller.

Stadler, L.J., 1932. Proc 6th Int. Cong Genet. 1, pp. 274–294 (Stadler, 1932)
"To state that an induced variation is a gene mutation is not to explain it but merely to label it."
Page 274–275
"We do not demonstrate that a chemical change has occurred; we simply infer, since no mechanical explanation can be found, that the variation must be due to this invisible mechanism."
Page 275
"We may define mutation as a transmissible change in the gene. But we identify mutation by experimental tests, and these tests are not such as to establish conclusively, in specific instances, that a change within the gene has occurred."
Page 275
"In effect, any Mendelizing variation which cannot be shown to be due to a change involving more than one gene is a mutation."
Page 275
"Thus the working definition of mutation necessarily differs somewhat from the ideal definition. It is this working definition which must be considered in generalizing from the experimental evidence. The mutations experimentally known may include not only variations due to alterations with the gene but also variations due to losses of genes or groups of genes, to additions of genes or groups of genes, and possibly also to changes in the spatial relationships of genes to one another."
Page 275–276
" ...the parallel between induced mutations and induced chromosome derangements is striking."
"The frequency of induced mutation is directly proportional to dosage (Stadler, 1928, 1930). So are the frequency of deficiencies induced by treatment of pollen (Stadler, 1931) and the frequency of endosperm mosaics induced by treatment shortly after fertilization (Goodsell, 1930).
Page 282
"The induced mutation also may involve various types of germinal change, for there are several conceivable ways in which irradiation may so modify a chromosome or to cause the appearance of a Mendelizing variation."
Page 291
" ....most of the induced mutations in plants are due to various extragenic alterations, chiefly non-lethal deficiencies. This is true in spite of the fact that the variation record as mutations in the experiments with plants are exclusively the "visible"...: "
Page 291
"The frequent occurrence in Drosophila of mutations at the points of breaks of the chromosomes suggests that the point mutations in this species as well as in plants may be largely of mechanical origin. This association of induced mutations with chromosome breakage does not necessarily exclude the possibility that the mutations are intra-genie changes, for it is conceivable that some change within the gene may be the cause of the break, and that in some instances the gene may continue to function as a gene after the change has occurred. But the possibility that some mutant characters may be due merely to the changed spatial relations of gene to another ("position effect") can not be disregarded."
Page 292
"....the occurrence of reversion is not proof that the original mutation could not have been due even to a deficiency."
Page 292
Source: Stadler, L.J., 1936. Duggar Editor, pp. 1263–1280 (Stadler, 1936)
"Mutation implies a hypothetical change within the individual gene, or at any rate a change affecting no than more than a single gene. But since the individual gene is invisible, the identification of a germinal variation as a mutation is a matter of inference we assume that it is due to a change in the gene."
Page1263
"Since chromosomal alterations (particularly small deficiencies) are known to simulate gene mutation in some instances, and since irradiation regularly induces chromosomal alterations (including deficiencies), there is obvious ground for the suspicion that the apparent gene mutations induced by irradiation are in fact due to extragenic alterations."
Page 1275
Source: Stadler, L.J., 1954. Science 120(3125), pp. 811–819 (Stadler, 1954)
"The purpose of experiments with gene mutation is to study the evolution of new gene forms. The techniques for studying gene mutation are, therefore, designed to measure the frequency of these changes in the genes. But a change in the gene may be recognized only by its effects, and it soon became clear that various extragenic alterations might produce the effects considered characteristic of typical gene mutation (10)".
Page 813
"But there was no test to identify mutations due to a change within the gene; it was simply inferred that the mutants that could not be identified as the result of specific mechanical causes were, in fact, due to gene mutation in the ideal sense (11)."
Page 813

Table 1 *(continued)*

"Another simplifying assumption was that mutant changes in gene effect must represent some transformation of the gene itself rather than some alteration affecting its expression. It was this assumption that made the demonstration of x-ray-induced mutation and reversion of the same gene seem critical proof of the induction of intragenic alterations. The assumption was definitely contradicted by the evidence of position effect. This evidence showed conclusively that a mutation did not necessarily represent a transformation or loss of the gene concerned; instead, it would be the result of a translocation affecting the expression of the unchanged gene."
Page 818

of such new cytological techniques in experimental studies, it was found that most of the X-ray-induced heritable changes produced at high doses were due to gross chromosomal aberrations or extragenic changes. In fact, the cytological advance by Painter (1934a, 1934b, 1934c) would profoundly affect the debate between "gene" and "chromosomal" mutations. So significant was this methodological development that *Science* published a brief summary note by Painter (1933) prior to his three papers in *Genetics* in 1934 (Painter, 1934a, 1934b, 1934c). Just as the earlier advances by McClintock (1929a, 1929b) had affected Stadler's view, so too did Painter's application influence the view of the *Drosophila* research community.

According to Carlson (1981), by the end of 1931 Stadler's criticisms of Muller's gene mutation claim seemed to be causing Muller some distress. Upon returning in December of 1931 from a conference of the American Society of Naturalists in New Orleans, Muller was showing stress on multiple fronts. His claims of radiation-induced mutations were being more broadly challenged and his once productive relationship with Patterson was now becoming one of confrontations and lack of trust. Likewise, his collaboration with the cytogeneticist Painter fell apart in 1929, again amidst distrust and professional jealousy. During this same period, Morgan would bypass Muller for an NAS nomination, favoring his former fellow graduate student, Sturtevant.

So penetrating and persuasive were the criticisms of Stadler that Muller's own convictions in the "artificial transmutation of the gene" were beginning to erode. Campos (2015) (see page 327, footnote 168) confirmed "it was Muller's discussions with Stadler and others that would ultimately cause Muller to begin to question the viability of a firm distinction between point mutations and exceptionally minor chromosomal rearrangements, as well as the nature of the position effects." Stadler's criticisms would follow Muller on his journeys from Texas to Germany to the Soviet Union and back to the U.S. Eventually Muller would recognize that ionizing radiation produced a vast abundance of major chromosomal aberrations at high doses. More importantly, his colleague Mark Belgovsky discovered that even small chromosomal rearrangements were readily induced by ionizing radiation and displayed a linear dose-response relationship (Belgovsky and Muller, 1938). These findings were of significance since they indicated that most of the induced gene (i.e., real) mutations at these lower doses were small-modest deletions or inversions.

Despite all the evidence that was being amassed against his 1927 "gene" mutation assertion in *Science*, Muller would cling to this idea because he thought that the mechanism of evolution could not involve heritable chromosomal mutations. Although the logic is sound that minor, intragenic mutations are the tiny engines of evolution, Muller was wrong in applying this logic to interpret the effects produced by high doses of X-rays.

In 1932, Muller left his position at the University of Texas (where Painter was a professor) to research with Timofeeff-Ressovsky in Berlin. At the end of about two years (and about the same time that Painter completed the development of his cytogenetic staining technique in Texas), Muller moved to the Soviet Union to direct a genetics research program. Muller continued to promote his arguments on gene mutation (Muller, 1935, 1939a, 1939b) in both Berlin and Leningrad/St. Petersburg and Moscow. Meanwhile, the cytogenetics tools developed by

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Painter, his former colleague, were being established and used to acquire evidence that challenged the acceptance of X-ray-induced gene mutations at high doses, representing a formidable obstacle to Muller. Painter's publications would also undermine the research of Timofeeff-Ressovsky et al. (1935) who were in the final stages of integrating two concepts: the LNT single-hit model and the mutation of "genes" with high doses of X-rays. Essentially, the new cytogenetics techniques of McClintock and Painter were yielding more refined data that was eroding support for the LNT single-hit model and, at the same time, shifting the discussion toward Ray-Chaudhuri's ideas of total-dose and linearity to dose rate and nonlinear threshold.

Meanwhile, Stadler undertook an extensive research program that evaluated X-ray-induced alterations at specific gene loci in maize, including possible changes within the gene itself. These experiments included visible assessments of chromosomal aberrations, transmission of chromosomal mediated changes through male/female germ cells, viability of the homozygote, and mutation reversion back to the non-mutated gene. Throughout his entire 25-year research effort, it is highly significant that Stadler would never find experimental evidence to support X-ray-induced gene (i.e., "real") mutations.

As research progressed, several developments emerged as significant. One involved the discovery of a position effect in *Drosophila*. This occurred when an X-ray-induced chromosomal aberration affected the translocation of a gene from one locus to another. With movement to the new locus, the transferred gene was found to affect activity in neighboring gene(s), even though the gene(s) itself was unaltered in its composition. If the transferred gene were physically returned to its original locus the organism would then revert back to its normal wild type. Interestingly, this genetic behavior was considered to have satisfied the criteria for reversibility, despite the fact that a "gene" had not been truly mutated, i.e., no change had occurred in its sequential nucleotide composition.

A second research development emerged as significant. It involved drawing the same conclusion (that X-rays induce gene mutations) from numerous studies based on an unproven and untested assumption (that phenotypic change equals compositional gene change). That is, it was assumed that an X-ray-induced phenotypic change could only result from the compositional alteration of a gene and that no verification of that assumption was apparently necessary. Missing was a detailed proof by the investigator that an X-ray-induced phenotypic change produced a corresponding alteration in the composition of a gene related to that specific phenotype. Also missing were more critical and detailed reviews of the publications to prevent uncontested and unproven assumptions from leading to false conclusions, wide acceptance and eventually erroneous dogma masquerading as theory. According to Nuffer (1957), a large number of such insufficiently assessed publications led to the belief that X-ray treatments were inducing gene mutations. However, when specific cases were examined more closely, some type of chromosomal aberration usually explained that which was assumed to be a gene mutation.

4. Muller's arguments to support the gene mutation hypothesis

To preserve the uniqueness and significance of his favored concept—the artificial transmutation of the gene, Muller published an 82-page paper in 1930 with his University of Texas colleague J.T. Patterson (Patterson and Muller, 1930). The assessment centered on whether the X-ray-induced trans-generational phenotypic changes in *Drosophila* were due either to losses (deletions), rearrangements of portions of chromosomes or to the so-called "progressive" point-like genetic changes that Muller assumed to drive evolution. This article reflected patterns in Muller's professional life; that is, he marshaled as much evidence as possible, presented it in excruciating detail, and never compromised on an essential point (i.e., classic example of a "Mullerism"). Also reflected in the paper was Muller's own perception of the fundamental weakness in his interpretation of a gene mutation, which occurred a year prior to the emergence of Stadler's (1931) questioning of this basic tenet.[13]

Patterson (Muller's Department Chair at UTexas) and Muller (1930) argued that the induced gene mutations are generally the direct result of relatively minor local electronic "hits" rather than more destructive processes (i.e., the gene versus chromosome mutation debate). The reasons for their stated beliefs included: (1) the general randomness and specificity of induced phenotypic changes (i.e., Muller's gene mutations); (2) identical phenotypes were independently affected; (3) phenotypic changes were dose dependent; (4) numerous toxic chemicals were not effective in producing such changes and (5) "most important of all, probably, is the fact that a direct and simple proportionality has been shown to exist between the frequency of the induced mutations and the amount (energy) of the radiation absorbed." They cited Hanson and Heys (1929) and Oliver (1930) to support this conclusion and then stated that "there is no indication in the results of any lower critical intensity, or threshold value, beneath which there is no (or a relatively lesser) effect." They concluded with "All these facts and considerations converge to indicate strongly that the mutations are rather direct effects of individual quantum-absorption electron hits…". Several years later, Oliver (1934), who received his Ph.D. in 1931 (Oliver, 1931a) while working under the direction of Muller, further expanded this list of arguments in favor of gene mutations. He indicated that X-ray-induced mutations often seem to appear similar to spontaneous mutations, an issue that would be re-examined at each stage of technological advance. Within this framework Oliver (1934) noted that the proportion of lethals to visibles or of dominants to recessives is similar to those reported for the spontaneous mutation rates (Morgan et al., 1932; Muller, 1930a, 1930b). Further supporting this perspective were observations that the frequently mutating genes in the spontaneous state are the same genes that frequently mutate when treated with X-rays.

In addition to this list of arguments in support of the gene mutation hypothesis, Muller would rely most heavily on two other linked arguments: (1) the occurrence of reverse mutations and (2) the multi-generational inheritability of mutations. Muller argued that if a mutation could be reversed then the gene had not been destroyed or deleted. Such reasoning led Muller and his supporters to conclude that "real" gene mutations are induced by the direct activity of X-rays.

Early reaction against such reasoning surprisingly came from one of Muller's own students. In the conclusion to his 1931 dissertation, Oliver (1931a) stated, "it is not possible to produce gene mutations without producing gene rearrangements". Later, Oliver also observed that X-ray-induced "inversions and translocations were produced in direct proportion to the intensity of treatment" (Oliver, 1934), a similar observation that had been previously used to support gene mutations. Oliver's proportionality observation for the induction of gross chromosomal damage proved to be consistent with later observations of other researchers (Timofeeff-Ressovsky, 1939; Demerec, 1937). Oliver (1934) also reported that translocations are induced with X-ray treatment of *Drosophila* sperm (Muller, 1928a, 1927a, 1927b; Weinstein,

[13] Within but three months of his momentous presentation in Berlin in September 1927 demonstrating X-ray induced heritable mutations, Muller (1927c) read a paper at the AAAS Conference in Nashville, Tennessee. During this presentation he addressed for the first time the possibility that his "gene mutations" were not gene mutations after all, but the results of chromosomal aberrations. He defended the gene mutation hypothesis by introducing the "reverse" mutation explanation, which he suggested provided a mechanism for evolution. Four months later Muller read a paper before the U.S. NAS (April 24, 1928) (Muller, 1928b) in which he raised the question "Are the X-rays merely punching holes in the chromosomes, or, more precisely stated, simply causing losses or inactivation of genes or gene parts? **If so, the usefulness of the X-ray, both from a practical and from a theoretical standpoint in biology, would be seriously limited** (emphasis added)." Muller therefore clearly understood that his X-ray-induced transgenerational phenotypic changes would only be significant when due to intra-genic/small point mutations. Muller stated that he was challenged to confront this issue by his former graduate student colleague and lifelong friend, Edgar Altenburg. Thus, the seeds of self-doubt on this issue preceded Stadler's own dissatisfactions with Muller's gene mutation interpretation by some three years.

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1928) with a frequency equal to that of "gene" mutations (Muller, 1928a; Muller and Altenburg, 1928; Muller and Altenburg, 1930; Oliver, 1931b; Whiting and Bostian, 1931). In his own research, Oliver (1934) recorded 21 lethals, 16 crossing-over conditions with most being inversions, and 17 translocations occurring in 105 subjects from the high dose parents (t_{16}, 4560 r), and 18 lethals, 13 crossing overs and 10 translocations, all with the X-chromosome in 400 offspring of parents treated with a lower dose (t_4, 1140 r). On page 391, Oliver (1934) stated, "it is probable that all lethal mutations are not point mutations, but that some are connected to, and due to chromosomal aberrations (Muller, 1927a; Weinstein, 1928) of definite deficiencies (Patterson, 1932)." These lethals, which are connected to chromosomal aberrations, increase rapidly with high doses (Oliver, 1930, 1932). On page 392, Oliver (1934) stated, "that X-rays increase the frequency of inversions as well as lethal mutations in *Drosophila*", as has been reported by Serebrovsky (1929), Muller (1927a, 1927b, 1928b), Oliver (1930) and others. The direct proportionality of the frequency of inductions of sex-linked inversions and translocations for *Drosophila* was determined by Oliver (1931b, 1932) and deficiencies in maize by Goodsell (1930) and Stadler (1931)" (page 392). It is curious that Oliver (1934)—Muller's former student—was the one who adopted such an active role in criticizing X-ray-induced gene mutations. It should be noted that Stadler (1932) also reported similar criticisms at the Sixth International Genetics Congress, suggesting that an alternative to Muller's view of discrete X-ray-induced "gene" mutations was emerging and growing stronger.

Oliver's dissertation (1931a) revealed that Muller's 150-fold increase in the frequency of lethal gene mutations at the highest dose was a significant overestimation. Oliver calculated that other damage-related endpoints, such as crossing over and translocations, could easily have accounted for a large proportion of the sex-linked lethality that had been attributed to gene mutation. Furthermore, Muller's estimation of "real mutations" would have decreased even more if Oliver (1931a) had included damage due to deletions and if Muller's control mutation rate had not been so low ($< 1/1000$). Finally, because the control background incidence of sex-linked recessive mortality ranged broadly over an order of magnitude (Muller, 1927a), it is possible that the variability of controls could have skewed Muller's estimation significantly upward.

Although sex-linked mortality probably increased, the estimated "massive" increase was probably not due to "real" gene mutations, as the scientific community was led to believe. This perspective is important because much of the excitement associated with Muller's findings was derived from the mistaken belief that Muller had produced large numbers of gene mutations.

5. The reverse mutation: a critical issue in the gene mutation debate

The debate between Stadler and Muller would continue with Stadler seeking the consultation of *Drosophila* experts to test the reverse mutation hypothesis as had been espoused by Patterson and Muller (1930). As a result of Stadler's inspiration, George Lefevre Jr. decided to dedicate his dissertation research to the task of testing Muller's "real" mutation theory. That is, he wanted to know whether Muller's research had produced "true" heritable mutations at the gene level or mostly a spectrum of modest to extremely large genetic lesions.

In reviewing the literature, Lefevre noted that other *Drosophila* researchers had also been intrigued by this question. Based on a series of experiments Timofeeff-Ressovsky (1939) reported 24 distinct reverse mutations at seven X-chromosomal loci (24 reverse mutations/343,183 genes) as well as 16 reverse mutations at six II-chromosomal loci. Earlier, Johnston and Winchester (1934) had induced 24 reverse mutations at eight X-chromosome loci (24 reverse mutations/713,001 genes). The combined rate constant for reverse mutations in these two papers is 1.3×10^{-8} at a dose of 4000-5000r. Amongst the reverse mutations noted by Timofeeff-Ressovsky (1939) and Johnston and Winchester (1934) are those which were affirmed by breeding confirmation tests, along with those mutations, based on phenotype evidence, that were thought to be reverse mutations but which could not be confirmed due to sterility induced by high X-ray doses. This mutation rate constant of ~ 1 mutation per 10^8 genes at 4000-5000r indicates that the incidence of such X-ray-induced phenotypic changes is extremely low. This X-ray inducing dose also frequently exceeded the maximum tolerated dose, with 60–70% sterility. If one assumes that a linear dose response occurs for each type of X-ray at a reverse mutation rate of 1×10^{-8} then a predicted genotoxic effect should decrease by about another order of magnitude at the lower dose of 250-300r. This would be similar to the lowest dose used by Oliver (1931a), but still several hundred-thousand-fold greater than the background radiation exposure.

Around the same time, several other reports also provided evidence of X-ray-induced reverse mutations. For example, Dubinin and Goldat (1936) reported seven reversals for yellow, schaete and scute characteristics, while Griffen and Stone (1939) and Glembozky (1936) also reported the occurrence of similar reverse mutations. Although results with *Drosophila* from the above studies were consistent with the Muller perspective, several other studies failed to induce reverse mutations via X-ray treatment. Kaufmann (1942) reported extensive negative data after radiating 25,000 forked and 12,000 white flies with 4000r. Raffel and Muller (1940) irradiated scute-4 scute-8 recombination chromosomes with 3000r and reported finding no non-scute among 50,000 progeny. Demerec (1938) also reported similar negative findings for reverse mutations of forked and white using approximately 100,000 offspring of recessive parents.[14] In addition to negative findings with respect to induced reverse mutation with X-rays, several effects were found to closely mimic such mutations, requiring enhanced precautions by researchers. For example, Lewis (1945) reported that crossing over between "pseudoalleles" might result in an apparent reverse mutational effect.

According to Lefevre (1949), reverse mutation studies need to preclude the possibility that crossing over could induce reverse mutations. Genetic suppressors can also affect the occurrence of reverse mutations and need to be evaluated as well. A third type of confounder could result from the "contamination" of several markers in the irradiated fly stock that might produce spurious reverse mutations. The presence and accidental co-mingling of other fly stocks possessing the same markers except one was possibly a source of this "contamination" and could lead to making incorrect conclusions about reverse mutations.

Given the above historical foundations, Lefevre offered the following perspective:

> "Because of the several mechanisms that can mimic reverse mutations, the early reports of successful induction of reverse mutation in Drosophila should not be accepted unqualifiedly as evidence of the similarity of induced spontaneous mutation."

For Lefevre, the key question had already been posed and tested by Patterson and Muller (1930). They reasoned that if radiation could induce a recessive mutation and also reverse it back to the normal dominant, then it must follow that the induced mutation could not result from a gross loss of genetic material. Patterson and Muller proceeded to test this argument by performing three successive irradiations and inducing the following three genetic changes: the first irradiation mutated a wild type allele (+) to its "forked" (f) mutant form; the second irradiation reverted the "forked" mutant back to the original wild type; and the third irradiation again mutated the wild type back to its "forked" mutation for a second time. The trans-generational

[14] The failure of Demerec to induce a reverse mutation in such a large number of *Drosophila* would soon lead him to switch to bacteria for his experimental model (Demerec, 1955).

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reversibility of such a specific phenotype (+→f→+→f) convinced Muller that these radiation-induced changes were qualitatively the same as those occurring spontaneously in nature and that they indeed represented "true" gene mutations and were not simply the result of gross chromosomal damage, as Stadler had been claiming.

Contrary to the results of Patterson and Muller, however, was the later dissertation research of Lefevre (1949) who found no evidence of reverse gene mutations when using 5000 r to examine 166,000 recessive X-chromosome loci. Follow up studies on somatic tissue also revealed no reverse mutations in experiments involving normalized 600,000 white loci exposed to 5000r. Together these findings led Lefevre (1950) to conclude that a "serious question is thus thrown on the reliability of the early reports of X-ray induced reverse mutation in *Drosophila*."

That a reverse mutation is proof of a "true" mutation was a debate that would last for nearly two decades in the published record of studies conducted with multiple biological models and inducing agents (De Serres, 1958; Lefevre and Green, 1959; Muller and Oster, 1957; Emmerling, 1955; Nuffer, 1957; Giles et al., 1955; Mottinger, 1970). Technological advancements would eventually supersede the debate and finally provide the insight and proof that Stadler (1932, 1954) was seeking (see the section entitled "The Modern Era"). The issue of reverse mutation would also play a central role in the research of Barbara McClintock who would propose an alternative to Muller's interpretation of X-ray-induced gene mutations.

6. Rejecting Muller: Mcclintock's alternative gene mutation mechanism

The research of Barbara McClintock significantly challenged Muller's (1927a) interpretation of X-ray-induced gene mutation in multiple ways. First, she developed a cytogenetic staining method that revealed detailed structures of maize chromosomes (McClintock, 1929b). In 1931 this new technology enabled Stadler and McClintock to evaluate research used to hypothesize the existence of X-ray-induced gene mutations in maize. Observations with the new cytogenetic techniques visually revealed that the phenotypic changes produced by the so-called gene mutations were actually due to genetic damage at the level of the chromosome (and not the gene) and consisted largely of deletions, translocations and other gross aberrations.

As a result, Stadler re-evaluated his previous findings in barley (Stadler, 1928) and realized he had been wrong in assuming that his observed heritable changes were due to gene mutations, i.e., Muller's so-called "point mutations". In fact, Stadler also soon came to reject Muller (1927a) evidence-free interpretation of his own *Drosophila* work that claimed a gene mutation mechanism was responsible for X-ray-induced heritable changes.

Later, McClintock (1938) would provide experimental evidence to support an indirect mechanism that could explain the trans-generational phenotypic changes induced by X-rays in Stadler's studies on maize. This mechanism became known as the break-fusion-bridge (BFB) process and it could induce massive chromosomal aberrations. It involved initial X-ray-induced chromosomal breakage, followed by the joining of the broken ends of two chromosomes, each with its own centromere. These joined/fused chromosomes would line up at metaphase and then break as their centromeres pulled in opposite directions, generating genetic damage.

While following up on the BFB discovery, McClintock detected another way to produce gene mutations. The newly proposed mechanism involved regulatory control over gene expression but did not involve altering the chemical composition of the gene (McClintock, 1953). She showed that specific genes could change location/position (i.e., transposition) within the genome and, in so doing, alter the expression of new neighboring genes (e.g., from dominant to recessive and vice versa). These observations, which would eventually result in McClintock receiving the Nobel Prize in 1983, provided a mechanism by which reverse phenotypic changes due to a "gene" (similar to those that Muller initially reported in his, 1930 paper with Patterson) could be obtained without altering the chemical nature of that "gene". These findings were significant because they presented a serious limitation to Muller's radiation-induced gene mutation model.

In October of 1948, Muller corresponded with McClintock and admitted to her that he thought her mutable-gene discovery involving transpositional elements (TEs) was a "magnificent achievement" (Comfort, 2001). He also wrote her stating "what a relief it is for us [I presume he actually meant himself] to know that mutable genes are a different class from the ordinary gene mutations after all." (Muller, 1948). Taken together, these statements affirmed that Muller recognized the significance and validity of McClintock's discovery but, at the same time, wanted his own hypothesis on radiation-induced gene mutations to be seen as separate and different from her discovery on mutable genes. There seems to be little question that Muller was concerned with how McClintock's discovery might be used to interpret his hypothesis of radiation-induced gene mutations. According to Comfort (2001), Muller had attended a seminar in October of 1948 on McClintock's work that was given by Salvadore Luria (1969 Nobel Prize recipient), a scientist who was greatly impressed with her work and thought it to be extremely important.[15] In his biography of McClintock, Comfort (2001) suggested that Muller had heard only what he wanted to hear at Luria's seminar because he was trying to "save" his gene mutation theory. However, McClintock, who saw her theory as an alternative to Muller's gene mutation model, would have none of it.[16] Muller's model assumed that a gene is an autonomous genetic unit in a string of genes and that a mutation was a discrete break occurring in a single gene on the string of genes. According to Comfort (1997, page 190), McClintock's "mutable genes, which mutate and then 'unmutate' again, are difficult to explain by this [i.e., Muller's] model." Although McClintock's mutable genes model could perhaps be seen as a complement to Muller's model, McClintock saw her model as superior in accuracy, scope, flexibility and integration and, therefore, as a compelling alternative to Muller's model. In fact, Comfort (1997), page 191) noted that McClintock was approaching the conclusion that "all mutations, including X-ray or chemically induced mutations, were the result of reversible alterations via the process of an integrative genome in which genes acted in suites, controlled by regulatory elements." In a "Memorandum" to Marcus Rhoades in January of 1949 (1949 – APS, see page 191, Comfort, 1997), McClintock would repeat this striking pronouncement and declare that all mutations may be explained by her transposable elements. She further proposed possible biochemical and physical mechanisms to explain her concept of gene mutation. In her 1948 annual Carnegie research report, McClintock (1948b) declared that her new findings "cast doubt on the interpretation that postulates a 'true' gene mutation, that is, a chemical change in a gene molecule". She argued instead that phenotypic changes were due to reversible inhibition and gene modulation. Such a remarkable statement essentially challenged the underlying premise of Muller's recent (1946a) Nobel Prize. McClintock's ideas on this topic would crystallize and were aptly expressed in the opening sentence of the Discussion in her seminal 1953 *Genetics* publication when she wrote, "Mutations need not express changes in genes but may be the result of changes affecting the control of gene action."

In the early 1950's McClintock would publish two major papers on

[15] Luria had obtained a 27-page letter of June 1948 that McClintock (1948a) had sent to S.C. Stephens, a former McClintock colleague, detailing her findings and interpretations.

[16] The "intercepted" 27-page letter to Stephens that Luria based his seminar on and that motivated Muller's congratulatory note to McClintock was superseded only one month later (July 1948b) with McClintock's further challenge to Muller's assumptions of gene mutation when she wrote; "Assumption that mutations occurring frequently are chromosomal changes is more reasonable than an assumption of a change in a gene in a certain direction." (McClintock, 1948c - Odd note September 1948, APS, Box 9, Series V.).

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transposable elements, mutable genes, and her concept of gene mutations (McClintock, 1950, 1951). These papers have become classics, being cited over 600 and 1000 times, respectively, in the Web of Science database, and would have a major impact on Muller's best friend and longtime *Drosophila* collaborator, Edgar Altenburg, at Rice University. Altenburg (1952a, 1952b, 1952c), who appeared to have been unaware of Muller's past communication with McClintock, wrote Muller on August 18 and 30 as well as on October 29, 1952, highlighting the significance of McClintock's findings and strongly encouraging Muller to study McClintock's papers. Altenburg would write Muller again on March 4, 1953, to repeat his previous suggestion and to give Muller a copy of his 25-page review manuscript on McClintock's research findings, a manuscript that had received McClintock's strong support/endorsement (Comfort, 2001). In this same letter Altenburg challenged Muller's two recently written chapters for a comprehensive genetics series edited by Alexander Hollaender. Altenburg stated to Muller, "In my opinion your Hollaender chapters suffer by lack of reference for Dr. McClintock's work. There are at least several places in your manuscript where it could have been profitably considered".

In his detailed 25-page review of McClintock's research on mutable loci in maize (January 1953), Altenburg concluded, "many of the mutants produced by X-rays undoubtedly represent position effects.[17] In this connection it is very significant that chromosome breakage produced by X-rays are in large measure in heterochromatin regions. Therefore, in X-ray treated material, it is not improbable that, in many cases at least, fragments of heterochromatin get inserted next to genes in other locations and suppress their phenotypic expression....". According to Altenburg's review, reverse mutations in *Drosophila* as well as maize, "are undoubtedly due in some cases at least to the release of normal genes from the suppressing effects of foreign heterochromatin adjacent to them, by deletion of the heterochromatin from a locus." This interpretation by Altenburg represented a unique challenge to Muller because his close colleague and collaborator (Altenburg) had apparently become convinced that Muller's interpretation was inadequate to account for the reverse mutation findings and needed to be modified and integrated into the McClintock framework, something that he probably knew would be very hard for Muller to accept (Altenburg, 1953a, 1953b).

Given that McClintock's findings on trans-positional mutator genes were highly relevant to the longstanding debate between Stadler and Muller over X-ray-induced gene mutations, one might have thought that Muller would have eagerly taken an interest in her challenging findings. A check of the Web of Science database on the key McClintock papers (1950, 1951, 1953) reveals that Muller never cited these papers. This lack of engagement with McClintock's findings was likely intentional, and suggests a strong element of strategic and tactical planning to avoid a direct conflict with McClintock.

In the previously mentioned 1948 letter to McClintock, Muller expressed his relief to have learned that her mutable genes were different from his proposed X-ray-induced gene-mutation model. At the time, McClintock emphatically disagreed with Muller's wishful and premature assumption and, ultimately, she would be proven correct. Over many years, McClintock's transposable elements (TEs) were shown to permeate organismal DNA (including Muller's *Drosophila* model) and to markedly affect mutation rates either in the absence or presence of exogenous agents such as ionizing radiation and chemical mutagens. Because TEs are powerful mutagenic factors that affect genomic instability, species have evolved highly diverse molecular strategies (e.g. RNA-induced posttranscriptional gene silencing to mediate the degradation of TE transcripts) to protect their respective genomes from the harmful activities of TEs. The fact that silencing of TEs profoundly affects background- and induced-mutation rates as well as cancer risks (Sturm et al., 2015) indicates that McClintock's model is superior to Muller's and therefore is a much better alternative (MacPhee, 1993; Sobels and Eeken, 1988; Velkov, 1999; Morales et al., 2015; Pesheva et al., 2005, 2008; Dimitrov et al., 2011). Likewise, in an integrated assessment of essential factors that are known to affect aging and lifespan, Sturm et al. (2015) concluded, "ageing is primarily caused by transposition–associated genomic instability."

Drosophila studies have now shown that X-rays can induce McClintock's mutator-gene system in a dose-dependent manner, with the upper part of the dose range overlapping with Muller (1927a) dose range (~1000–3500 r) (Handler and Gomez, 1997; Ratner et al., 2001). Furthermore, it was found that results from early *Drosophila* studies conducted by Timofeeff-Ressovsky et al. (1935) on radiation-induced mutations correlated amazingly well with results from studies conducted 66 years later by Ratner et al. (2001) on the induction of mobile genetic elements. This surprising correlation inspired Ratner's team (2001) to investigate a mechanism that may explain how mobile genetic elements could mediate X-ray-induced mutations. Other studies have shown that X-rays first activate mutator genes and then they, in turn, synergistically enhance the production of X-ray-induced mutations (Sobels and Eeken, 1981, 1987; Margulies et al., 1986, 1989; Balter et al., 1992; Zabanov et al., 1995; Ratner et al., 2001). Such results are particularly germane to the evaluation of the LNT-Single Hit model in that they strongly suggest interactions between the radiation dose, the transposable elements and the production of a range of genotoxic/mutational endpoints. Although the precise nature of such molecular and biophysical interactions still needs to be better defined, it is clear that McClintock's general concept has been strongly supported and that, as she predicted, there exists a mechanistic and sequential relationship between the radiating dose, the activation of TEs and, finally, the phenotypic display of a radiation-induced mutation.

In summary, the findings and interpretations of McClintock strongly challenged the Muller gene-mutation interpretation at multiple levels and in a manner that became progressively accepted by the research community for its scientific and technical accuracy and significance. Given the pristine reputation of McClintock within the genetics community for her scientific credibility, rigor, accuracy and intelligence, it appears that Muller chose not to challenge her alternative model for radiation-induced gene mutations, even when it conflicted directly with the argument on reverse mutations that he needed to defend his own model (Patterson and Muller, 1930).

During the summer of 1955 the NAS created the BEAR Committee, Genetics Panel. It was quite significant that neither McClintock nor anyone with her alternative perspective on gene mutation was invited to be a member of that panel.[18] A review of all transcripts, member reports and meeting summaries provided no evidence that the Genetics Panel discussed McClintock's alternative gene-mutation model. A review of the credentials of the geneticists on the panel revealed that

[17] In 1953 Peter Peterson, a maize researcher at the University of Iowa, reported the discovery of the mutable pale-green (pg) locus in strains of maize experimentally subjected to the atomic explosions at Bikini (Peterson, 1953, 1993). These findings supported the argument of McClintock/Altenburg.

[18] It should be noted that the Director of the Carnegie Institute, Milislav Demerec, McClintock's supervisor, was an invited member of the BEAR I Genetics Panel. In 1946, as mentioned in the Introduction of this paper, Demerec asked Ernst Caspari the key question of "what could be done to save the hit model?" following his striking findings. In addition, BF Kaufmann, the Assistant Director of the Carnegie Institute was also a member of the Genetics Panel. Calabrese (2015b) had earlier shown how Kaufmann misrepresented data that refuted a linear dose response in his oral presentation to the Genetics Panel. Kaufmann would replace Demerec as the Director of the Carnegie Institute in 1960. In a rather remarkable temporal coincidence, Caspari would spend 1947–1949 at the Carnegie Institute. Thus, three *Drosophila* geneticists associated with key aspects of the LNT model worked within the Department of Genetics at the Carnegie Institute with McClintock at the same time she was challenging Muller's gene mutation concept. It is not known whether and to what extent McClintock and these other individuals commented on this fundamental and very sensitive issue. It is also not known whether Demerec affected a possible appointment of McClintock to the Genetics Panel or whether he may have exerted pressure upon McClintock as she was challenging not only the basis of Muller's gene mutation model but also the fundamental underpinnings of the LNT model.

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seven members (Beadle, Cotterman, Crow, Little, Sonneborn, Sturtevant, Wright) had never published a paper on mutations, the key focus of the Panel. Glass had published only two papers on mutations over two decades. Likewise Kaufmann had a weak record of publishing on mutations (4 in total). Essentially all the academic geneticists had received Rockefeller Foundation funding. That the Rockefeller Foundation/NAS would have invited so many geneticists with no experience in the area of mutations and dose responses to serve on this BEAR I Genetics Panel is a striking observation that has not yet been noted in the literature. Yet, the Panel was portrayed as a team of all-stars with a high level of expertise and experience in the induction of mutations by radiation and in their dose-responses. Furthermore, the Panel actually served to validate and promote Muller's gene mutation model, which contrasted significantly with McClintock's gene mutation alternative. To make matters even more untenable, the evaluation by the Genetics Panel was undertaken in the presence of Muller, whose dominating personality, ideological conviction to his model and profound and obvious conflict of interest should have disqualified him as member of the Panel.

It's curious that two years later the NAS would constructively engage or, depending on your point of view, distract McClintock on a more than two-decade international involvement on the evolution of maize (Comfort, 2001). The activity demanded prolonged periods in South America and an intense amount of her time and energy, effectively deflecting her from valuable research needed to advance her model and debate Muller before his death in 1967. No matter how valid and important was McClintock's challenge to Muller's model, it would undoubtedly wane without her attention.

On the other hand, Muller would soon be confronted with a serious question, this time involving the LNT Single-hit Hypothesis. In a highlighted paper in *Science* on December 19, 1958, William L. Russell (Oakridge National Laboratory) reported the discovery of radiation-induced, dose-rate effects in the spermatogonia and oocytes of mice. These findings were deemed so significant and challenging to the LNT concept that Muller redirected his laboratory to the assessment of dose rates on radiation-induced mutations in *Drosophila*. Russell's findings would significantly challenge the prevailing mantra that declared radiation damage to be cumulative, irreversible and linear in its dose response. In contrast to the mantra, Russell showed that radiation-induced mutations in mouse oocytes yielded an unambiguous threshold response. A detailed historical assessment of the Russell-Muller dose-rate debate and its impact on radiation risk assessment was recently published (Calabrese, 2017a, 2017b).

Why did McClintock's alternative gene-mutation model fail to gain traction and why has it remained essentially unknown to the scientific community, never taking part in the cancer risk-assessment debate? Table 2 compares Muller and McClintock gene-mutation models for trans-generational phenotypic changes, and identifies reasons why the McClintock gene-mutation model was not adopted. Reasons for failure may be separated into two groups. The first group describes McClintock's failures and explains how they prevented her success (see Table 2, numbers 1–9). The second group lists the factors that enhanced the acceptance of Muller's model (see Table 2, numbers 10–14). Although the factors were many that contributed to Muller's success and McClintock's failure, in the end it boiled down to leadership. Muller grabbed for the leadership mantle while McClintock did not. Differences in their personal histories, goals and priorities, for example, probably all contributed to some extent to the leadership differences between them. Nonetheless, McClintock's scientific visibility quotient, in terms of published papers and conference presentations needed to advance her gene mutation model as a superior alternative, was so low compared to Muller's that her alternative model failed to become part of the risk assessment debate for the past nearly 70 years. In fact, her gene mutation theory/model was not mentioned once in the 250,000 pages of the official OSHA record of regulation hearings for major carcinogens during the 1978–1980 time period (OSHA, 1980). Despite this striking failure, the transposon rediscovery and "revival" of the past 40 years have had profound biological, biomedical and clinical significance, and yet it remains decoupled from the cancer risk assessment process.

7. The modern ERA

Numerous studies have been published depicting the influence that ionizing radiation has had on the spectrum of mutational types of damage, ranging from base substitutions to large deletions and including a broad range of biological treatment models and a number of doses and dose rates (Webber and de Serres, 1965; De Serres et al., 1967; Thacker, 1986, 1992; Thacker et al., 1990; De Serres, 1991; Fossett et al., 1994; Nelson et al., 1994, 1995; Park et al., 1995; Yamada et al., 1996; Colussi and Lohman, 1997; Colussi et al., 1998; Nohmi et al., 1999; Schwartz et al., 2000; Mognato et al., 2001; Furuno-Fukushi et al., 2003; Liu et al., 2003; Nakamura et al., 2005; Sudprasert et al., 2006; Toyokuni et al., 2009; Okudaira et al., 2010; Russell and Hunsicker, 2012; Asakawa et al., 2013).[19] Reliable general patterns of response have emerged indicating that the type of damage is highly dose and dose-rate dependent. Low doses/dose-rates are generally associated with different types and proportions of lesions than occur at high doses/dose rates. At low doses/dose-rates, various base changes (i.e., transversions, transitions, and tandem based substitutions), frameshifts, and small/modest sized deletions/insertions may occur. As the dose/dose rate increases there is a switch to progressively larger types of genetic damage, ranging from partial to complete gene to multi-gene lesions (e.g., deletions). In the case of mature spermatozoa, the proportion of large deletions is about 4–5 fold greater than seen in the spermatogonia stage, possibly due to DNA repair (Ward and Alexander, 1957; Russell et al., 1998; Russell, 2004). The results also imply that each type of genetic damage most likely has a unique/separate origin/mechanism. Such circumstances led Schwartz et al. (2000) to conclude: "risk from low-dose radiation exposure cannot be easily extrapolated from high-dose effects." Of particular note is that about 60 years after the report of Muller (1928a), Southern blot analysis of nucleotide sequence could not differentiate damage below 50 base pairs. The use of Southern blot therefore led to a practical definition of a "point" mutation (i.e., genetic damage $\leq$ 50 bp could include small deletions, frameshift mutations and a variety of base changes) (Grosovsky et al., 1988). Regardless of the lack of capacity in the mid-1970s to late 1980s to resolve the spectrum of point mutations, the dose-dependence of genotoxic endpoints was readily apparent.

In 1927 the information available on X-ray-induced genetic damage was literally a "black box" consisting of poor resolution of chromosome structure, inadequate cytogenetic capabilities, and a rudimentary understanding of the range of genetic damage. This knowledge void existed within the context of radiation exposures that at times included or bordered on induced sterility. In retrospect, what Muller called a "point" mutation was in fact a euphemism for an ambiguous type and imprecise amount of genetic damage rather than for a known discrete biochemical lesion with precise physical characteristics. Despite considerable uncertainty, massive interpretative overreach and apparent errors, Muller would convince the genetics community and regulatory agencies to accept his X-ray-induced gene mutation and dose-response

[19] In his 1927 paper Muller pointed out that X-ray-induced genetic damage was likely to be dose dependent. He stated that X-ray-induced chromosomal aberrations/rearrangements would be "less likely than point mutations to depend on single quanta." (Note that he did not define or reference "quanta". He then argued, "a re-examination of the effect of different dosages must therefore be carried out, in which different types of mutations are clearly distinguished from one another." (page 87, Column 1). When assessed for a broad range of X-ray dosages he argued that it might then be possible to determine whether low levels of background gamma radiation cause the type of "spontaneous" mutations observed in nature or without an experimental treatment. The Science paper therefore reveals that Muller was aware that his high-dose treatment had caused massive genetic damage. However, it is obvious that he was hoping that the dosing he used produced "point mutations".

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Table 2
Models of Transgenerational Phenotypic Changes (TPCs).

The Muller Gene Mutation Model	**The McClintock Gene Mutation Model**
Change in the Chemical Nature of the Gene	Gene Regulation/mutator Gene Concept/transposition Elements
TPCs are induced by direct hits of ionizing radiation on a gene, inducing mutation; indirectly via a host of possible secondary reactions.	TGCs are principally or possibly fully mediated by transpositional elements (TEs) via cellular regulatory processes. TPCs could be induced by agents that activate transposition elements.

Fate of the McClintock Gene Mutation Model: Why It Failed to Gain Traction for Cancer Risk Assessment During the 1950s and 1960s

1. McClintock's transposon concept was very complex and experimentally difficult to adopt for most contemporary labs; it was also not readily understood and adopted by the scientific community.
2. The death of Stadler in 1954 hurt McClintock's chances of success.
3. McClintock failed to get her supervisor, Milisav Demerec, to become a strong promoter of her scientific vision. In fact, McClintock resigned from the Carnegie Institute in 1953 due to frustration with the poor management/leadership qualities of Demerec; Vannevar Bush, the President of the Carnegie Institute for Scientific Research, interceded to get her to reconsider and stay.
4. The failure of McClintock to get appointed to the BEAR I Genetics Panel represented a significant lost opportunity to educate the Panel of her model and modify and possibly block the acceptance of the Muller model and LNT and to promote her own gene mutation model.
5. Demerec and Kaufmann, leaders from the Carnegie Institute, failed to promote her model at the BEAR I, Genetic Panel meetings. This failure was continued over to BEAR 2 and 3.
6. McClintock did not have graduate students that could have carried on her research/policy agenda. This was a double-edged sword as the seminal discoveries of McClintock may have been missed had her efforts had been delegated to others.
7. Failure of Altenburg to publish his comprehensive review of McClintock's transposon findings and their potential role in explaining radiation-induced mutation.
8. McClintock failed to address how her model related to medical /X-ray and environmental risk assessment.
9. McClintock failed to build an effective support group/organization to test, evaluate and promote her gene mutation model.
10. Muller's model received broadened application when it served as the basis of the LNT-single hit model. The promotion of the LNT-single hit model by several Nobel Prize winners represented a critical development.
11. Muller's receiving the Nobel Prize was essential for external validation of his gene mutation model and its risk assessment applications.
12. The recommendation to support LNT based on the Muller mutation model by the Manhattan Project facilitated its adoption by US government agencies.
13. Muller's testing methods were simple, quick and inexpensive. The interpretations were straight forward and easily understandable.
14. Muller and his radiation geneticist colleagues, typically with *Drosophila* research backgrounds, were organized, communicated often and had a strategy to promote the Muller gene mutation model.

interpretations as matters of scientific fact.

Although Muller attempted to rebut Stadler's (1954) posthumous challenge in *Science* (Muller, 1956; Muller and Oster, 1957) by claiming that "point" mutations were induced at various loci based on their capacity to reverse mutate to normal phenotypes, Muller's reverse mutation criteria was by no means a "gold" standard for small genetic changes (Thacker and Cox, 1983).[20] They noted that mutations caused by positional-effects (gene expression effects mediated by large genetic rearrangements) and those induced by transposable effects could affect gene reversions without gene mutations. Of significance here is that the gene loci frequently studied in *Drosophila* may display unusually high frequencies of reverse mutations, a situation that corresponds in magnitude to their frequencies for forward mutations. Thacker and Cox (1983) suggested that this phenomenon might be a component of the insertion/deletion cycle of a transposable element (Green, 1967) as seen in the use of the white apricot loss of *Drosophila* (Green, 1961).

Another severe blow to Muller's argument occurred when Voss and Falk (1973) used Muller's own biological model (*Drosophila* lethals) to examine about 600,000 gametes and found no evidence of "true" reverse mutations. In an earlier study from the same laboratory (Lifschytz and Falk, 1968), discrete and well-defined small zones within the chromosomes of *Drosophila* were occasionally found to have locus and region specificity for X-ray-induced mutations. More precisely, a small region around the maroon-like locus on the X chromosome demonstrated areas of large deletions that extended beyond a single locus. These same researchers also reported that all X-ray-induced post-meiotic lethals were due to chromosomal aberrations, further refuting Muller's argument.

In some studies, X-ray-induced mutations were frequently shown to involve a single locus. However, even in these cases, being "contained" within a single locus can still involve an extensive area of induced damage. For example, the size of one *Drosophila* gene locus is approximately 20,000 DNA base pairs (McGinnis et al., 1980) and therefore is theoretically large enough for extensive intra-gene damage to produce a mutation. Furthermore, large rearrangements or inversions can sometimes be misclassified as single gene changes even though one breakpoint of the inversion is located inside the zone of one gene and the other outside (McGinnis et al., 1980).

Bedford and Dewey (2002) published a paper on the "real" mutation controversy during and after the Muller era. In their summary they concluded, "no convincing evidence for the production of so-called point mutations was ever found for plants." Since Muller "found evidence for reversion of some X-ray induced mutations with *Drosophila*, he held the view that point mutations were in fact produced. In light of more recent information others have suggested that reversibility is by no means proof of a point mutation." Muller's argument equating "point" and reverse mutations in *Drosophila* may have been the key reason (along with a truly dominating leadership role in the field) that his gene mutation hypothesis prevailed (Stadler, 1932) for so long. Eventually, however, his hypothesis progressively succumbed to the combined effects of methodological advancements, invalidating experimental evidence, and the eroding support of his scientific community.[21]

[20] In this "rebuttal" Muller (1956) was forced to acknowledge that substantial findings on *Drosophila* had revealed that a very large proportion of what he called "point" mutations were now clear-cut deficiencies and other structural changes, a conclusion indistinguishable from that of Stadler. More specifically, Muller (1956), page 136) wrote that "…there is no doubt that in X-rayed *Drosophila* also, at least when the irradiation is applied to condensed chromosome stages, such as those of spermatozoa, deficiencies as well as other demonstrable structural changes arise with much higher frequency, relative to changes that appear to involve but one gene, than they do without treatment." This statement clearly reflects the eroding of Muller (1927a) position concerning the similarity of X-ray-induced vs spontaneous mutations and his statement that a high proportion of the gene changes were point mutations. It appears that Muller had given up considerable ground in the argument but still tried to retain some "wiggle room" so as not to raise the "white" flag of full intellectual surrender.

[21] In 1978 Charlette Auerbach, whose career was positively redirected by Muller in 1938 at Edinburgh, addressed the debate between Stadler and Muller over whether Muller had produced real gene mutations in his Nobel Prize research. For one with very close ties to Muller, it was very telling that she concluded that Stadler had firm experimental evidence with maize that confirmed his assertions, while Muller's evidence was suggestive but less direct. Such an assessment speaks highly for the intellectual penetration of the Stadler position. Auerbach (1978) then came to the defense of Muller (without providing a reference) stating that recent molecular analyses had "confirmed Muller's conclusions" that X-rays can produce true mutations. However, Muller was claiming that he had induced large numbers of mutations (i.e., "the artificial transmutation of the gene") but at the doses he used, the vast majority (perhaps all) were heritable chromosome aberrations. Many years later Muller's former post-doctoral colleague William R. Lee would report that "molecular analysis of X-ray-induced mutations in the germ line of *Drosophila* shows that nearly all are the result of X-ray-induced strand breakage" (Byrne and Lee, 1989; Batzer et al., 1988). The radiation doses studied by Lee were 5 Gy, 10 Gy, and 30 Gy with the doses reflecting those used by Muller. The findings of Lee and his colleagues strongly supported the position of Stadler in his debate with Muller. Thus, at the doses Muller tested he induced chromosomal aberrations and not "massive" new

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8. LNT single-HIT hypothesis formulation

The debate between Muller and Stadler was not simply about the mechanism of X-ray-induced mutations nor was it simply between Muller and Stadler. By the early 1940's, it had expanded significantly to include issues related to the linear dose-response and the single-hit models used to assess both genetic and cancer risks.[22] Furthermore, Stadler was not challenging only Muller but also other leading geneticists as well as a cadre of elite physicists, including the future Nobel Prize recipient Max Delbruck and other leading radiation geneticists (e.g., Timofeeff-Ressovsky).

Muller and Timofeeff-Ressovsky used X-ray-induced sex-linked recessive lethality in *Drosophila* to assess trans-generational phenotypic changes and, as a result, demonstrated a linear dose-response relationship. The fact that some somatic cells of *Drosophila* showed phenotypic trait reversal suggested the possibility of X-ray-induced gene mutations. Delbruck and others applied target theory to the linear dose-response results and, together with mathematical modeling by Zimmer (1941), indicated that only a single hit could yield a linear dose response (Fig. 1).

The major assumption of the LNT single-hit model is that low-dose effects are predictable from high-dose exposures. Such predictions based on this major assumption suggest that the damage produced at low doses is qualitatively the same as at high doses (i.e. same type of genotoxic damage and with similar spatial pattern distribution) and that quantitatively the amount of damage produced is directly proportionate to the dose.

Delbruck constructed a tentative "atomic physics" model of the gene, as inferred from the frequency of "point" mutations under varying physical conditions. The views of Delbruck were popularized and markedly advanced by the Nobel Prize-winning physicist Erwin Schrodinger (1944) in his book *What is Life*. The key features of the model were that the (1) gene is a molecule; (2) that a mutation of a gene is a result of the transition from one stable state to another; and (3) the effect was due to thermal agitation or the absorption of radiant energy (Stadler, 1954). The linear dose response and the constancy of mutation yield regardless of time (i.e., Delbruck's model negated the occurrence of dose rate) predicted that X-ray-induced mutations from single "hits". The constant proportionality of mutation yield to ionization, regardless of wavelength variation, indicated that the unit was a single ionization. The average size of a gene was estimated as well as a quantitative characterization of the volume within which the hits occurred. This estimate was used to account for the mutations reported. He deduced that the average gene was comprised of approximately 1000 atoms. Delbruck then estimated that 1.5 eV would be required to induce a mutation with this model. Since the energy released via a single ionization is about 30 eV (i.e. far more than the 1.5 eV), the ionizing radiation should be able to mutate essentially all genes regardless of their inherent stability.

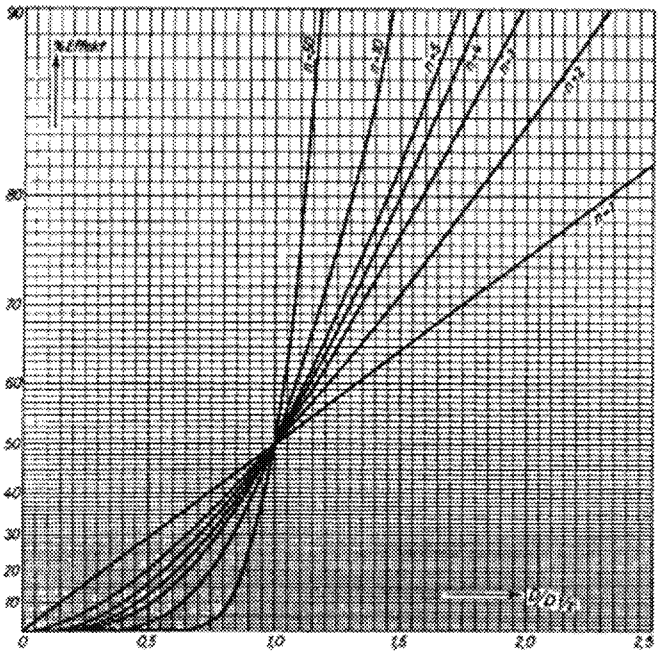

Fig. 1. Model dose-response curves, calculated theoretically for various number of "hits", n, on a single "target" assumed necessary to produce the effect. (From Zimmer (1941)).

9. Criticism of the LNT-single hit hypothesis

Stadler (1954) critiqued Delbruck's model and indicated "…it has been evident for many years that it (single hit theory) has no valid relationship to the experimental data from which it was derived." Furthermore, Stadler indicated that a non-gene alteration, such as a physical loss or rearrangement, represents a sound alternative to structurally altered genetic mutation and is strongly supported by evidence obtained in detailed assessments of X-ray-induced mutations. In concluding his critique, Stadler stated, "we have no basis for estimating the proportion of such extragenic mutations amongst the total of mutations observed and no grounds for assuming that this proportion is the same amongst the mutations observed under the various experiments."

The total dose and dose rate used during Muller's Nobel Prize research were extremely high. To provide a striking comparison, consider that Caspari and Stern (1948), who actually demonstrated an X-ray-induced threshold response in *Drosophila*, had to use a 50–60-thousand-fold **lower** dose rate than Muller did in his *Drosophila* studies. In addition to dose/dose rate concerns, the use of mature spermatozoa for cancer risk assessment was also problematic. Since mature haploid spermatozoa are more susceptible to X-ray-induced mutations than are cancer-forming diploid cells, it suggests that Muller's haploid spermatozoa are inappropriate for extrapolation to diploid somatic cells and, therefore, for cancer risk assessment. In contrast to spermatogonia and somatic cells, it was later discovered that DNA repair was absent in mature spermatozoa, offering a mechanistic explanation for the differential susceptibility to mutations. These concerns led the U.S. BEIR I Genetics Subcommittee (1972) to acknowledge errors made by the BEAR I (Anonymous, 1956) Genetics Panel on two issues: (1) using mutation data from mature spermatozoa to model risk of genetic damage and (2) considering dose rate as an irrelevant factor in cancer risk assessment (Calabrese, 2017b, 2017c).

It was not long after Muller's groundbreaking paper, according to Novitski (1976), before investigations began to counter Muller's gene-mutation hypothesis and reveal that chromosomal rearrangements were associated with sex-linked recessive lethals in *Drosophila* and that the proportion of these induced lethals increased with dose (Oliver, 1930; Demerec, 1937; Demerec and Fano, 1941). In fact, the general consensus was that lethal mutations were, as Novitski had noted, "a

(*footnote continued*)
"point mutations" as claimed.

[22] While the relationship between Muller and Stadler remains to be better clarified, there appears to have been a high degree of mutual respect (Muller, 1956). Of particular note is that from June 1948 until October 1949, Stadler was investigated by the FBI for concerns associated with linkages to communist groups within the US during the beginning of the Cold War. In fact, Stadler was denied a passport in June 1948 to attend the International Genetics Congress in Sweden. During this ordeal a number of leading geneticists came to the defense of Stadler. Based on obtained correspondence, Muller was strongly supportive of Stadler, showing remarkable leadership in this regard. While Stadler's answers to the FBI interrogatory were impressive in his defense so too were the actions of colleagues of very high stature such as Muller, Stern, Beadle, Demerec, and others. While Stadler was very grateful for the support of Muller, he and Muller would continue to scientifically debate on gene mutation until Stadler's death (May 1954) (Calabrese, 2017d).

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Table 3
The LNT Single-Hit Model Timeline.

Date	Topic	Context
1927	**X-Rays Induce Gene Mutations**	Muller wins race to be first or did he?
1929–1931	**Linear Dose Response**	Follow up experiments by Muller's students support linear interpretation
1929	**Improved Chromosome Cytology**	Barbara McClintock develops markedly improved cytogenetic method for maize chromosomes.
1930	**Muller Anticipates Challenges to Gene Mutation**	Tried to prove gene mutation interpretations; used reverse gene mutation concept (Patterson and Muller, 1930).
1931	**New Cytogenetic Methods Challenge Gene Mutation**	Stadler and McClintock determine that X-rays induced heritable chromosome (not gene) changes.
1932	**Stadler Challenges Muller's Gene Mutation Interpretation**	Plenary address at the Sixth International Genetics Congress
1935	**LNT Single-hit Model Formulation**	Leading physicists/radiation geneticists create new model (Timofeeff-Ressovsky et al., 1935)
1936	**Position Effect and Variegation/Mottling Effects**	New mechanism could challenge parts of Muller gene mutation model.
1936–1960	**Reverse Mutation (RM)**	Significant research initiated to assess the frequency of RM induced by radiation and spontaneously. Results are mixed; they do not provide confirmation of Muller's gene mutation interpretation.
1938	**McClintock Develops Procedure to Produce Mutations without X-Rays**	The BFB method could induce a large amount of mutations in a targeted manner.
1939	**Dose Rate and LNT Model**	Muller's graduate student, Ray-Chaudhuri finds no evidence of dose-rate in mature *Drosophila* spermatogonia
1944	**McClintock Discovers Transposable Genes**	This discovery integrated the position effect and reverse mutations along with a theory of regulatory control over gene expression.
1943–1946	**Manhattan Project Dose-Rate**	Ernst Caspari study supports a threshold and dose-rate, challenges Ray-Chaudhuri findings (Caspari and Stern, 1948).
1948	**McClintock's Alternative Gene Mutation Model**	Transposon-based model leads to mutation without need for direct hit on gene; challenges Muller's model (Muller and McClintock correspondence).
1958	**Dose-Rate Discovered in Mouse Model**	Russell discovers dose-rate for X-ray induced mutation in mouse spermatogonia and oocytes.

result of chromosome breakage (Herskowitz, 1946; Lea and Catcheside, 1945; Haldane and Lea, 1947; Lea, 1946)". In time, follow up investigations were conducted that combined Muller-like doses with molecular mapping techniques to detect deletion breakpoints. These radiation exposure studies revealed a very high proportion of large deletions (Aaron, 1979; Fossett et al., 1994). These findings also revealed that ionizing radiation induced sex-linked recessive lethals can quantitatively predict the occurrence of induced deletions, including large deletion frequencies (Byrne and Lee, 1989; Fossett et al., 1994). These results strongly support the hypothesis of Stadler (1932, 1954) that most, if not all, of Muller's reported "mutations" were not gene mutations but rather chromosomal aberrations of various types and magnitude.

When the reverse mutation argument of Muller began to lose traction, Muller would shift the debate to the dose-rate experiments of Ray-Chaudhuri (1939, 1944). These experiments on dose rate gave rise to what may have been a type of desperate hope for Muller and other radiation geneticists who believed that the LNT single-hit model still had a defensible basis. This shift in debate may have been an underlying factor in Muller's less-than-critical evaluation of Ray-Chaudhuri's dissertation and, subsequently, in Muller's repeatedly deliberate mischaracterizations of Caspari's dose-rate study, which did not support the findings of Ray-Chaudhuri (Calabrese, 2011).[23] In his Nobel Prize lecture (December 12, 1946) Muller would use the Ray-Chaudhuri dissertation to affirm that there is "no escape from the conclusion that there is no threshold dose…". Muller's extreme dependency on the findings of Ray-Chaudhuri to refute the threshold model while simultaneously affirming the LNT single-hit dose-response model may have motivated his direct, powerful, coordinated and often deceitful attacks on the Caspari study (Calabrese, 2011, 2015a).

The LNT single-hit theory also failed to provide validated risk estimates across X-ray induced doses that produced qualitatively different types of gross chromosomal damage with the expectation of predicting responses to doses many orders of magnitude below those tested and for which analytical methods would not be available for almost a half century. The LNT single-hit hypothesis became progressively and seriously flawed in multiple reinforcing ways, over the next four decades following its formulation in the mid-1930s. While the stage should have been set for model revision, the radiation geneticist community, as lead by Muller, Stern and other colleagues, would do what was necessary to "save the hit model", and so they did (Calabrese, 2015a, 2017a).

10. Discussion

This paper assesses the historical origins of the LNT single-hit model and its reliance upon data obtained from studies (especially those of Muller) of X-ray-induced trans-generational changes in *Drosophila* phenotypes (Table 3). Also explored is the intellectual struggle that existed between Muller and Stadler over the authenticity of "gene" mutations as a mechanism to explain X-ray-induced trans-generational phenotypic changes at high doses. Painter developed his advanced cytogenetic staining method for *Drosophila* chromosomes (1934c) after Muller had already formulated his hypothesis on "true" gene mutations. McClintock and Painter's new cytogenetic methods revealed that at high doses, similar to those used in Muller's Nobel Prize research, chromosomal aberrations and/or rearrangements of various types would strongly predominate, if not exclusively, over "gene" mutations. In time, newer methods would progressively improve and refine the assessment of such gross chromosomal alterations, affirming the position of Stadler. Other arguments used by Muller, such as the reverse mutation phenomenon, were often not supported in multiple large-scale studies prior to (e.g., Lefeve, 1949, 1950; Neuffer, 1957) and after Muller's death (Voss and Falk, 1973). As scientific support for the gene mutation hypothesis began to erode, Muller and others of the radiation genetics community would shift their support behind the findings of Ray-Chaudhuri (1939, 1944) in support of an LNT interpretation. Although the substantial and integrated Caspari-Spencer follow-up experiments (Caspari and Stern, 1948; Spencer and Stern, 1948) challenged the data of Ray-Chaudhuri, major efforts were made to discredit these threshold (i.e., Caspari and Stern, 1948) supportive findings by Muller, Stern and other members of the radiation geneticist community

[23] The acceptance of the Caspari data would hinge on the validity of his control group data. Curt Stern contacted Muller at Amherst College late in 1946 and in 1947 to obtain his findings since Muller had amassed voluminous data on spontaneous mutation in *Drosophila* in an attempt to address the many scientific challenges of Stadler. As a result of this situation, the Muller data fully supported the Caspari position and his claims that X-ray induced mutation display both dose-rate and a threshold response. This was of fundamental importance as it refuted the LNT Single-hit hypothesis. See Calabrese (2011, 2015a) for detailed discussions. Thus, it is highly ironic that Muller's research to refute Stadler led to a confirmation of the Caspari findings that challenged the LNT Single-hit model.

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(Calabrese, 2011, 2015a).

Muller and his colleagues were promoting the LNT single-hit model in the late 1940s and subsequent decades. However, had the cytogenetic advances of Painter (1934c) been available to Muller in 1927 (as McClintock's had become available to Stadler in, 1931), it is likely that Muller may have been more cautious in concluding that he had induced gene mutations. Nevertheless, Muller was clearly involved in a race to be the first to report that X-rays could induce gene mutations. Given all the publicity that Muller received, one can imagine that it would have been difficult for him to do anything but try to defend his interpretation.[24] Likewise, had this information been available prior to the formation of the LNT Single-hit model, this model may never have seen the light of day.

The findings herein complement a substantial series of papers on the history of LNT that started with the Manhattan Project (Calabrese, 2015a, 2015b, 2017a). These papers documented an era post-World War II that was dominated by strong ideological leadership in the community of radiation geneticists who managed to undermine the normal scientific practices of evaluation and review. During the period from 1946 to 1956, these leaders essentially directed a movement to promote and ensure the adoption of the LNT Single-hit model by regulatory agencies. It is interesting, however, that such widespread ideological behaviors were not evident prior to this period even though the same individuals dominated the field of traditional genetics. So then, what happened to change the traditionally accepted scientific practices of rigorous research, honest evaluation and unbiased debate to an ideologically driven ends-justify-the-means process entailing deceit, misdirection and harmful consequences?

The discovery of X-ray-induced mutation and its role in causing birth defects and cancer deaths stoked the fears of a public wary over nuclear holocaust in the immediate post-WW II era of the Cold War. X-rays used in newly developed medical techniques to diagnose and treat diseases as well as the possibility of significant human exposures to radioactive fallout from atomic bomb testing only heightened these fears. Society was ignorant, alarmed and fearful of radiation exposure and urgently demanded new policies from the federal government that could provide an assurance of safety and assuage public anxieties. The sense of urgency was palpable and driven largely by fear rather than the slow, deliberate and objective processes that are classic prerequisites to research, evaluation and debate. If not careful, however, this fear-driven sense of urgency could affect and skew the decision-making process of conscientious humans who are eager and feel pressured to quickly produce definitive results that protect the public and assuage anxieties while simultaneously satisfying their own needs as well as those of their superiors in the public and private sectors. A hurried and harried approach is antithetical to both good science and good management (decision-making) as is the failure to keep the functions and structures of science and management discrete and separate. Thus, keeping the science of risk assessment, for example, independent and separate from the human decision-making of risk management, which may be influenced by a fear-driven sense of urgency or other personal factors, would be necessary to avoid harmful and unintended consequences to society.

This potentially harmful mingling of science and management may well have been partially motivated by funding self-interest as well as a harried sense of urgency to fast-track a radiation health safety standard. The correspondence between members of the NAS BEAR Genetics Panels has shown that the members were not averse to exaggerating public health concerns in exchange for financial support for their research (Calabrese, 2014). Scientific self-interest has typically been attributed to scientists funded by industry, but the exchanges of letters among panelists make clear that they would deceive the scientific community and the public to obtain research funding. Likewise, their own words have been used to document that major funding groups—like the Rockefeller Foundation—(especially of the 1930–1960s) would manipulate these same panelists toward their scientific and policy interests (NAS/NRC, 1956 - see Weaver, page 35), an example of leveraging scientific funding that crossed ethical boundaries.

Lewis Stadler and others challenged Muller's gene mutation hypothesis at a time when it was sweeping the scientific and policy communities. As the data-driven challenges increased in strength over time, they began to weaken key aspects of Muller's high-dose gene-mutation model. This debate was protracted and profoundly significant, but finally reached a turning point with the findings of Caspari as they had the capacity to seriously undermine the adoption of the LNT Single-hit model. At this point, the tug-of-war between science and policy shifted and became an ideologically directed process led by scientists of great reputation, such as Stern and Muller, whose main goal was to ensure that the LNT Single-hit model would be adopted for cancer risk assessment. This story is now well documented (Calabrese, 2011, 2015a, 2015b).

The scientific foundations of the LNT single-hit model could not have been explored properly or completely without also examining the Nobel Prize research of Muller and the lifetime he spent defending it. As a result of this assessment, Muller's principal achievement may now be viewed from a slightly different perspective. Multiple leading geneticists (e.g. Timofeeff-Ressovsky, 1929; Crow and Abrahamson, 1997) of his era have claimed that Muller's importance was not so much that he was the first to produce gene mutations but rather that he showed how to produce copious mutations quickly (Campos, 2015). However, Muller's actions were focused almost entirely on the first question because he knew that this was the fundamental biological issue. How should one deal with the fact that 90-years of research have revealed that Muller did not produce the "gene" mutations that he had claimed in his 1927 *Science* article? Despite the historical significance given to both "gene" mutations and the LNT Single-hit model, the genetics field has remained essentially silent. Perhaps it is afraid to evaluate important aspects of Muller's story because he was an historical icon who had a major role in creating the field and establishing its centrality in biology. In fact, two esteemed geneticists and close allies with Muller had affirmed that Stadler was indeed correct in his claim that Muller produced mostly chromosomal deletions and not specific "gene" mutations (Crow and Abrahamson, 1997). In a review of the history of cytogenetics and genotoxicity, Garcia-Sagredi (2008) offered what seems to be an inspired Stadler-like remaking of the Muller discovery. He stated, "Muller (1927a) discovered that X-rays could cause mutations in *Drosophila*; specifically, that X-rays were able to produce rearrangements in the linear order of genes (for which he was awarded the Nobel Prize…"). However, rearrangement of the linear order of genes was not the goal Muller sought; it was the production of gene mutations. In the end, the greatest achievement of Muller was that he transformed the field of genetics with his exuberant, yet, incorrect claims. He inspired many laboratories to study radiation-induced mutations and was pivotal in influencing Charlotte Auerbach to assess chemical-induced mutagenicity, which opened up still another important new research area. He also awakened the scientific community and public to health concerns with excessive and unshielded radiation. Further, Muller's reporting of his mutation discovery in 1927 would inspire the creation of the Genetics Society of America during the December 1931 New Orleans meeting of the American Society of Zoologists (when the officers of the new Society were selected for 1932). The new society would play an important role in organizing the 6th International Genetics Congress at Cornell, an event that had highlighted Stadler's Plenary Address challenge to Muller's gene mutation claim. Muller did have an amazing impact on the field and society, but his 1927 paper should have an asterisk next to it.

[24] By not "winning" the race to first report X-ray-induced mutation, it provided Stadler with the opportunity to be more open to other possible data interpretations. Thus, even though his reports of X-ray-induced mutations in barley and maize seemed to fulfill all the standard features of gene mutations, Stadler would abandon this position after the cytogenetic findings of McClintock in the summer of 1931.

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11. Conclusion

1. The LNT Single-hit model was based on the assumption that Muller had induced the "artificial transmutation of the gene" by X-ray treatment of mature *Drosophila* spermatozoa.
2. Several years after Muller had created the LNT model (i.e., called Proportionality Rule), it was afforded an explanatory mechanism via target theory and integrated into the Single-hit model.
3. Research by Lewis Stadler challenged Muller's basic conclusion that he had observed X-ray-induced gene mutations. This challenge was based on cytogenetic advances and the evaluations of Barbara McClintock concerning the effects of X-rays on corn. These findings suggested that Muller had predominantly, if not exclusively, induced heritable chromosomal (not gene) mutations, challenging the significance of his findings on heritable mutations.
4. Numerous subsequent studies in multiple biological models, including *Drosophila*, strongly supported Stadler's interpretation. Muller's claim that he had demonstrated the "artificial transmutation of the gene" and greatly enhanced the production of gene mutations were gross overstatements at best, which profoundly misled the scientific community and policy-making in public health areas. The LNT Single-hit model was not supported by the very biological data upon which it was based.
5. In a remarkable reversal of scientific support, leaders of the radiation genetics community placed considerable reliance on the findings of Ray-Chaudhuri (1939, 1944), which supported LNT.
6. Since subsequent integrative studies by Caspari-Spencer did not support the LNT study conclusion of Ray-Chaudhuri, but rather that of a threshold, the Caspari-Spencer studies became the object of unfounded and deceptive criticisms by Muller, Stern and other leaders of the radiation genetics community in an attempt to preserve the LNT Single-hit model.
7. The findings presented herein reconstruct the early development of the LNT Single-hit model up to the post Manhattan Project/modern cancer risk assessment era, and complement the substantial, previously published assessments on the history of LNT starting with the Manhattan Project.
8. These integrated findings lead to the conclusion that the LNT Single-hit model should have been neither recommended by the BEAR I Genetics Panel nor adopted for cancer risk assessment, as was the case in the U.S. and most countries.

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Message

From: Anitole, Katherine [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=4ACBA4F6BEF64933A73A201E54C8F92E-KANITOLE]
Sent: 3/14/2017 2:20:27 PM
To: OPPT RAD [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=f052c9d5c11b440b8393b73f0947a2d0-OPPT RAD]
Subject: FW: LNT Obituary
Attachments: Environ. Res. LNT-Obit.-1.pdf

From: Fitzpatrick, Julie
Sent: Monday, March 13, 2017 12:43 PM
To: RAF Members <RAF_Members@epa.gov>; Behl, Betsy <Behl.Betsy@epa.gov>; Strong, Jamie <Strong.Jamie@epa.gov>
Subject: FW: LNT Obituary

RAF Members,

Creatively crafted introduction to Calabrese's latest papers.

Regards,
Julie

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From: Edward Calabrese [mailto:edwardc@schoolph.umass.edu]
Sent: Sunday, March 12, 2017 9:12 AM
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Subject: LNT Obituary

Julie:

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Obituary notice: LNT dead at 89 years, a life in the spotlight

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ABSTRACT

Considerable recent findings have revealed that the linear dose response for cancer risk assessment has not only outlived its utility in predicting risk but is based on a flawed scientific foundation. The present article characterizes this demise of a key concept of environmental risk assessment, in the framework of a figurative obituary of a long-lived concept that has poorly served society. This obituary is intended to illustrate an integrated mix of poignant and improper historical judgments that led to both the acceptance and ultimately the demise of this once intellectually facile and nearly universally accepted concept.

1. Introduction

The linear dose-response relationship for carcinogen risk assessment, otherwise known as the linear-no-threshold (nicknamed LNT) model died on January 10, 2017 due to an academic version of multiple system failure. This involved a poor theoretical basis, an incapacity for validation and a rejection by hundreds of studies, along with a striking ineptness for accurate predictions in the low dose zone (Calabrese, 2009, 2011a). Speeding its demise was a recently discovered series of epic scandals involving some of the best and brightest from the worlds of academia and government. Its final demise came with two recent publications in the journal *Environmental Research* (Calabrese, 2017a; Calabrese, 2017b) showing that LNT exhibited several serious academic mutations, which allowed it to be misapplied and to grow unchecked, a process that would ultimately lead to self-destruction. LNT was finally put to rest in a grave outside Washington, DC. The ceremony was surprisingly well attended by numerous inconsolable consultants and governmental regulators who made their economic livelihoods based on the tenets and applications of LNT in cancer risk assessment. The service was presided over by a former head of the US EPA, who had a difficult time keeping her composure, having lost a long time, but fundamentally misunderstood ideology, which harmed nearly everything it touched because of its blind acceptance and unproven character.

1.1. LNT: its life, significance and demise

LNT was always the star of the show, starting with its highly auspicious birth—an event that was heralded by some leaders of science as the key element in explaining life itself as well as the origins of man on earth. In 1928 LNT was born by the creativity of two physical chemists from no less than the University of California at Berkeley. One of these chemists, Professor Gilbert Lewis, an academic jack-of-all-trades and master of all, himself having been nominated for the Nobel Prize some 42 times, only to be repeatedly overlooked due to human jealousy born of rivalry. Despite his own death on a Saturday afternoon in March 1946 due to an apparent accidental release of cyanide during an experiment, Professor Lewis remains well known to all high school and college chemistry students for his famous Lewis acids and bases concepts. However, Lewis and his colleague Axel Olson decided to leave the comfort of their physical chemistry laboratory and proffer an explanation for a truly big question - how did we get here? Their answer to the evolutionary conundrum, they believed, was to be found in the recently published work of the radiation geneticist Hermann Muller, a professor at the University of Texas at Austin. Muller and many rivals long believed that unlocking the mechanism of evolution was the biggest question in biology and that whoever got there first would take home the Nobel Prize. Muller worked on this question for over 15 years, and then finally closed in on it late in 1926, a scant few months ahead of the competition. As he neared the research finish line in the spring of 1927, Muller used his contacts at the journal *Science* (Muller, 1927) to publish the first report of an exogenous agent, in this case X-rays, capable of producing mutations.

Lewis and Olsen did not hesitate to use Muller's discovery to declare that they had now figured out the mechanism of evolution. The engine of evolution, by their account, resulted from mutations occurring from continuous exposure to low levels of background radiation emanating from the cosmos and terrestrial earth. Their explanation, however, needed and therefore assumed a linear dose-response profile for radiation to insure that mutations could actually occur at background levels. Lewis and Olsen offered this explanation even though Muller's research at the time never revealed the true nature of the dose

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response for X-ray-induced mutations at low doses. Their explanation, based on an assumed extrapolation of Muller's dose response curve, was published in a 1928 article in the prestigious journal *Nature* (Olson and Lewis, 1928). Thus, the LNT was born, not in the service of and application for environmental risk assessment, but out of an unproven assumption to answer the most vexing question in life.

Intriguing though it was, the explanation of Lewis and Olson would fail to gain traction, eventually being discredited by Muller himself. Background radiation was so low or weak that it could only explain about 1/1300th the mutation rate in Muller's control group (Muller and Mott-Smith, 1930; Muller, 1930), making the LNT too inept for a plausible explanation. In a strange way, Lewis' mistake was compounded by the failure of Muller to present any data in the *Science* paper, forcing the overzealous Lewis to speculate.

Although Lewis and Olson receded from the messy world of evolutionary biology and their failed attempt to explain the mechanism of evolution was soon forgotten, the LNT model that they had fictionalized was not. In fact, Muller himself adopted it as his own model and he would soon become known as THE stepfather of LNT. A scant two years later Muller would reenergize the LNT concept, rename it, and then transform it into no less than a new universal biological law, calling it the Proportionality Rule (Muller, 1930). The Proportionality Rule, or LNT, was destined to become a central dogma of the radiation geneticist community.

Just as evolution needed a mechanism so did the Proportionality Rule; soon it would get one. The mechanism would emerge from a series of creative forays between big-time physicists and radiation biologists who met in Copenhagen during summers early in the 1930s—the likes of which involved Neils Bohr, Muller, and others with similar high standing. In a type of role reversal the physicists offered a mechanism that linked their concept of radiation target theory to several sets of new data from Muller's laboratory showing a linear dose response for radiation-induced germ cell mutation. The physicists proposed a single gene-single hit model that could reliably predict Muller's linear dose-response observations. The greater the number of gene hits beyond one, the more progressively the dose response resembled a threshold model. This enhanced version of LNT, now called the "single-hit" LNT model surfaced in 1935 in a newly created journal in the German language (Timofeeff-Ressovsky et al., 1935). However, because the journal never saw a second year of publication, the "single-hit" LNT model was never cited in leading scientific indices and spread among colleagues only by word-of-mouth and reprints.

To recount, an article in a prestigious journal literally fictionalized an unproven LNT into existence (born) in order to rationalize an evolutionary hypothesis that would soon be fully discredited by Muller, who surprisingly would then soon adopt the fictionalized LNT as his own after a theoretical physics' article in a short-lived and unknown journal was used to rationalize it back into existence (reborn). Thus, LNT not only was born and died, but, on the belief of one man and the mere musings of physical theory, also was reborn and even given a theoretical and intellectual foundation for the future—all without experimental proof of its existence. In fact, under such circumstances, it would be hard to imagine how the "single hit" version of LNT could have possibly failed. After all, it was surrounded, supported and collectively created by past, present and future Nobel Prize winners in physics and biology. But there was no getting around it, key experiments in support of LNT remained absent and the mutterings of support by prominent scientists would eventually ring hollow, and Muller knew it. Thus, Muller proposed an idea for a seminal experiment to calm the doubters and give LNT standing within the scientific world.

Muller knew that it was probably impossible to prove or validate the LNT in experimental systems. The dose of radiation would be so low that even theoretically small effects would be practically impossible to measure. LNT was, more than anything else, a belief rather than a science. That is, LNT really could not be directly tested. As an alternative, Muller proposed a dose-rate experiment to see if X-ray-induced mutations would be cumulative and irreversible. If this were the case, then the dose response should be linear even down to a single ionization. So, in the late 1930s Muller directed a project that, in effect, tried to determine whether the same number of mutations would occur when a dose was given all at once or spread over a number of smaller doses. If the amount of damage were the same in each case, LNT would be supported. In this key experiment Muller's scientific intuition proved correct and his belief in the Proportionality Rule or LNT—call it what you will—was sustained, at least for a while. At the time, however, this was an important moment for LNT, "proving" that it was not only real (Muller, 1951) but was also in the process of gaining many new converts from the field of radiation genetics.

Unfortunately, unforeseen problems soon emerged and the vindication of LNT proved to be short lived. Even though radiation geneticists had come to fully embrace LNT, it seems that Muller's dose-rate experiments had some important limitations such as his supervision of students and study design features. In addition, the Atomic Energy Commission was then seeking safety assurances for any personnel of the Manhattan Project who might be exposed to doses of radiation considerably lower than those used by Muller and his students in validating the LNT. In short, the U.S. Government needed to develop a safety program for radiation workers that would be both based on occupationally relevant doses and guided by the very best science. Thus, Muller needed to improve his approach to research on dose rates, this time with a greater degree of supervision, a stronger study design and a better quality control of laboratory procedures.

In concert with the Manhattan Project, a "dream team" of researchers from the fields of entomology and genetics was then organized at the University of Rochester to validate the LNT model at ever-lower doses of radiation. The research project was under the supervision of the outstanding geneticist Curt Stern who received assistance from Muller and other prominent scientists of the day. Anticipating straightforward confirmation of LNT, the team was more than surprised and disturbed when data emerged from high quality-controlled experiments that supported the well-entrenched threshold dose-response model instead of LNT. This sent the entire team into what could only be called "damage control". Deliberately violating scientific standards of unbiased objectivity, they sought unprincipled and frankly deceitful ways to preserve LNT and to discredit the findings. With a now well-known narrative, Stern and Muller connived to undermine the threshold model in deceptive ways, including Muller's comments at his Nobel Prize Lecture and repeatedly in the scientific literature, Stern's obfuscations, and staff members joining in with Stern either because of simply being afraid or refusing to speak up. They orchestrated a cover up that captured the field, eventually contaminating the US NAS BEAR Genetics Panel (1956), which committed scientific misconduct in an attempt to ensure that LNT would replace the threshold model for mutation and cancer risk assessment (Calabrese, 2011b, 2015). From the late 1950s until its recent demise, LNT would rule the risk assessment world. Even though it could not explain evolution, there was little else that was more significant in the environmental domain than LNT, with the possible exception of the new kid on the block by the name of climate change.

Despite their brilliance, however, Muller, Stern and colleagues would make two critical mistakes in judgment that would reveal the folly of their LNT recommendation. The first involved the use of the mature spermatozoa in mutation research. They incorrectly assumed that findings with the mature spermatozoa would apply to all cells—reproductive and somatic. However, before the decade of the 1950s ended, all signs suggested that this highly specialized reproductive cell was unlike all other cell types in that it lacked the capacity to repair genetic damage. By 1972, even members of Muller's own group of loyalists, such as Jim Crow the chair the US NAS BEIR Genetics Subcommittee, concluded that the US NAS Genetics Panel of 1956 got the key question of dose rate wrong and used the wrong cell type to

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estimate cancer risks. The second major problem was revealed a quarter century later when a senior geneticist at Oak Ridge National Labs found an error in the historical mutation rate used by the US NAS BEIR Genetics Subcommittee for the derivation and support of LNT. Recent publications in *Environmental Research* (Calabrese, 2017a; Calabrese, 2017b) showed that if this error had been detected in 1972 by the NAS Genetics Subcommittee then LNT would not have been adopted/affirmed and the threshold model would have been far more likely to be recommended for assessing radiation risks.

Although the rapid rise and success of LNT was due to plotted actions in the 1950s of formidable supporters (i.e., from the radiation genetics community and from the US National Academy of Sciences and its silent partner the Rockefeller Foundation), LNT was not without its formidable opponents as well. During the 1930s and 1940s, most opposition to LNT came from a medical community that had long maintained a loyal relationship with the threshold dose-response model. The medical community was afraid that LNT might limit the use of X-rays and radium in the treatments of cancer and other diseases because of possible risks to workers and patients. The medical community was smart and powerful and it controlled the agenda and the playing field. Nevertheless, it was still out maneuvered by key leaders of the Rockefeller Foundation who had a "Manchurian Candidate" by the name of Dr. Detlev Bronk, a simultaneous president of both the Rockefeller University and the US National Academy of Sciences. In an unprecedented move, the Foundation urged Bronk to organize the BEAR Committee and gave him the money and power to bestow opportunity and influence upon the dogma-driven community of radiation geneticists. This move diminished the medical community's hold over LNT and, at the same time, empowered LNT supporters, setting the stage for a major scientific revolution. With the medical community now neutralized and with Bronk in the driver's seat at the National Academy of Sciences, LNT simply had an easy ride to the top.

While the fledgling nuclear industry tried to fight back, it was simply outclassed by LNT, which could now easily frighten the public and politicians with warnings of cancer and images of deformed babies. This would be the principal modus operandi of LNT. Over the next 50 years LNT took on the chemical industry and set its sights on their universe of potentially harmful agents. The battles were long, hard and nasty, but in the end LNT whipped big industry. Even a series of Republican presidents could overcome neither LNT nor the stealthy long-term bureaucrats who frustrated their every move.

So, if the medical, nuclear and chemical communities could not disable LNT then what was the insidious disease that led to its demise? In such cases one nearly always looks for something else to blame. However, the truth of the matter is usually much closer to home. All LNT had to do was look into a mirror and see its weaknesses, which were always camouflaged by its handlers at the NAS, the Rockefeller Foundation, the EPA and other organizations, who benefited from LNT's outrageous scare tactics. LNT really knew that the discovery of dose rate effects and DNA repair was going to be a serious problem. LNT and its supporters did their best to deflect the impact of this discovery in multiple ways but it could not be denied. There were also many other adaptive responses that helped to ensure survival in a threatening world. These adaptive mechanisms could be turned on by very tiny doses of a plethora of agents and stresses, protecting all things biological. These observations led to the view that LNT could not make accurate predictions in the low-dose zone and, in fact, was only a player at high doses where relevance to human environmental exposures was almost non-existent. Over the past several decades thousands of studies have revealed that LNT could not be counted on to do the job as its predictions were routinely off by orders of magnitude. In fact, LNT had become an embarrassment to most toxicologists and radiation health scientists. The final straw for LNT occurred when it was revealed that the NAS BEIR I Genetics Subcommittee confirmed its existence because of an error which the recent papers in *Environmental Research* (Calabrese, 2017a; Calabrese, 2017b) finally corrected. When the error was fixed, LNT once again looked into the mirror and saw nothing. It knew that its time was up.

2. Conclusion

As the once-dominant dose-response model, LNT had many lessons to teach. Professors George and Draper wrote some 30 years ago, "all models are wrong, but some are useful" (George and Draper, 1987). Time has shown that LNT was indeed dreadfully wrong and not even particularly useful. For example, it significantly increases the cost of environmental clean ups without protective advantage and has denied people opportunities to receive novel and highly effective, low-dose radiation medical treatments. LNT is much like Swiss cheese; it has far too many holes to be useful and should certainly not guide policy. Unfortunately, LNT became a dogma, a belief that could not be tested, and had highly prestigious and deceptive proponents to ensure its survival. It was also used to frighten citizens and to intimidate politicians. However, LNT's nearly century-long life of ups and downs could end on a positive note if it were to symbolize how science becomes eroded when an ideologically-driven hypothesis is blindly followed, never proven and incuriously defended as central dogma—never in doubt but often wrong. Skepticism, objectivity and hypothesis testing are at the heart of science, not the ideological defense of dogma. If LNT's life and death can teach society this lesson, then its near century-long life may at least partially compensate for the innumerable disservices it has already dealt society.

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European Union Risk Assessment Report

TRIS (2-CHLOROETHYL) PHOSPHATE, TCEP

CAS-No.: 115-96-8

EINECS-No.: 204-118-5

RISK ASSESSMENT

July 2009

FINAL APPROVED VERSION

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This document is the last version of the Comprehensive Risk Assessment Report **Tris(2-chloroethyl) phosphate (TCEP)** a substance chosen from the EU 2nd Priority List.

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Foreword

We are pleased to present this Risk Assessment Report which is the result of in-depth work carried out by experts in one Member State, working in co-operation with their counterparts in the other Member States, the Commission Services, Industry and public interest groups.

The Risk Assessment was carried out in accordance with Council Regulation (EEC) 793/93[1] on the evaluation and control of the risks of "existing" substances. "Existing" substances are chemical substances in use within the European Community before September 1981 and listed in the European Inventory of Existing Commercial Chemical Substances. Regulation 793/93 provides a systematic framework for the evaluation of the risks to human health and the environment of these substances if they are produced or imported into the Community in volumes above 10 tonnes per year.

There are four overall stages in the Regulation for reducing the risks: data collection, priority setting, risk assessment and risk reduction. Data provided by Industry are used by Member States and the Commission services to determine the priority of the substances which need to be assessed. For each substance on a priority list, a Member State volunteers to act as "Rapporteur", undertaking the in-depth Risk Assessment and recommending a strategy to limit the risks of exposure to the substance, if necessary.

The methods for carrying out an in-depth Risk Assessment at Community level are laid down in Commission Regulation (EC) 1488/94[2], which is supported by a technical guidance document[3]. Normally, the "Rapporteur" and individual companies producing, importing and/or using the chemicals work closely together to develop a draft Risk Assessment Report, which is then presented at a meeting of Member State technical experts for endorsement. The Risk Assessment Report is then peer-reviewed by the Scientific Committee on Health and Environmental Risks (SCHER) which gives its opinion to the European Commission on the quality of the risk assessment.

If a Risk Assessment Report concludes that measures to reduce the risks of exposure to the substances are needed, beyond any measures which may already be in place, the next step in the process is for the "Rapporteur" to develop a proposal for a strategy to limit those risks.

The Risk Assessment Report is also presented to the Organisation for Economic Co-operation and Development as a contribution to the Chapter 19, Agenda 21 goals for evaluating chemicals, agreed at the United Nations Conference on Environment and Development, held in Rio de Janeiro in 1992 and confirmed in the Johannesburg Declaration on Sustainable Development at the World Summit on Sustainable Development, held in Johannesburg, South Africa in 2002.

This Risk Assessment improves our knowledge about the risks to human health and the environment from exposure to chemicals. We hope you will agree that the results of this in-depth study and intensive co-operation will make a worthwhile contribution to the Community objective of reducing the overall risks from exposure to chemicals.

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[1] O.J. No L 084, 05/04/199 p.0001 – 0075

[2] O.J. No L 161, 29/06/1994 p. 0003 – 0011

[3] Technical Guidance D!ocument, Part I – V, ISBN 92-827-801 [1234]

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0 OVERALL CONCLUSIONS/RESULTS OF THE RISK ASSESSMENT

CAS No.	115-96-8
EINECS No.	204-118-5
IUPAC Name	Phosphoric acid tris-(2-chlorethyl) ester
Synonym	Tris (2-chlorethyl) phosphate, TCEP

Overall results of the risk assessment:

( ) i) There is need for further information and/or testing

(X) ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

(X) iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account

Summary of conclusions:

Environment

( ) i) There is need for further information and/or testing

(X) ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

( ) iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account

ad ii)

This conclusion applies to all life cycle steps to all environmental compartments, to the function of waste water treatment plants and to secondary poisoning via the food chain.

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TCEP does not meet the PBT criteria.

A potential risk in future cannot be excluded if production and/or use volumes were to increase as a consequence of actions on other flame retardants. Therefore, it is recommended to monitor that the downtrend in use of TCEP is not reversed in future.

Human Health

Consumer

( ) i) There is need for further information and/or testing

(X) ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

(X) iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account

Ad iii) Risk reduction measures are required for babies with respect to the scenario sucking on toys taking into consideration the carcinogenic properties of the substance and the effects after repeated oral administration.

Workers

( ) i) There is need for further information and/or testing

( ) ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

(X) iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account

ad iii)

For TCEP three occupational exposure scenarios are evaluated. TCEP is produced (scenario 1) and is used for the production of formulations (scenario 2). The use of TCEP-containing formulations (scenario 3) includes spray application (scenario 3a) and applications without formation of aerosols (scenario 3b). The overall result of risk assessment indicates that

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current exposure levels (inhalation and dermal contact) are too high for all occupational exposure scenarios.

From the toxicological point of view, concern mainly derives from the carcinogenic properties of TCEP. In addition, chronic toxicity and partly fertility impairment gives reason for concern.

Measures selected for risk reduction should be able to substantially reduce TCEP exposure of workers. Special emphasis should be given to the "spray application" scenario (dermal contact and inhalation).

With respect to risk assessment for carcinogenicity inhalation exposure at the workplace should be reduced to a level of 0.2 mg/m^3 or below. It is recommended to establish an occupational exposure limit for TCEP.

Concerning skin contact, exposure should be controlled to levels in the range of 2 mg/person/day. On that background it needs to be carefully considered whether gloves could be able to sufficiently reduce the dermal risks from TCEP.

Human exposed via the environment

( ) i) There is need for further information and/or testing

(X) ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

( ) iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account

ad ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

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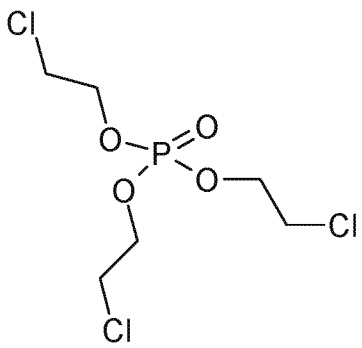
1 GENERAL SUBSTANCE INFORMATION

1.1 IDENTIFICATION OF THE SUBSTANCE

CAS No.:	115-96-8
EINECS No.:	204-118-5
IUPAC Name:	Tris(2-chloroethyl) phosphate
Synonyms	Tris(2-chlorethyl)phosphat Ethanol, 2-chloro-, phosphate (3:1) Tris(β-chloroethyl) phospahte Tris(chloroethyl) phosphate Tris(2-chloroethyl) orthophosphate Tri(2-chloroethyl) phosphate Tri(β-chloroethyl) phosphate Tris(.beta.-2-chloroethyl) phosphate
Molecular weight:	285.49 g/mol
Empirical formula:	$C_6H_{12}Cl_3O_4P$
Structural formula:	



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1.2 PURITY/IMPURITIES, ADDITIVES

Purity: 99.5 %

Impurities: water

1.3 PHYSICO-CHEMICAL PROPERTIES

Physical state	liquid at 20 °C	
Melting point	< -70 °C	Akzo Nobel (10.05.2000)
Boiling point	decomposition at 320 °C at 1013 hPa	Akzo Nobel (06.07.2000)
Density	1.4193 g/cm^{3}at 25 °C	Akzo Nobel (15.06.2000)
Vapour pressure	43 Pa at 136.9 °C 0.00114 Pa at 20 °C (extrapolated)	Akzo Nobel (06.07.2000)
Surface tension	not determined	
Water solubility	7820 mg/l at 20 °C	Hazelton Europe (18.04.1994)
Partition coefficient	logPow = 1.78	Hazleton Europe (20.04.1994)
Flash point	200 °C at 1013 hPa	Courtaulds Chemicals (1996)
Auto flammability	480 °C	Hoechst AG (1994)
Flammability	not extremely flammable not highly flammable not flammable	CHEMSAFE
Explosive properties	not explosive (structural reasons)	
Oxidizing properties	not oxidizing (structural reasons)	

Vapour Pressure

The vapour pressure of a substance is defined as the saturation pressure above a solid or liquid substance. At the thermodynamic equilibrium, the vapour pressure of a pure substance is a function of the temperature only.

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A vapour pressure of < 100 Pa at 20 °C was stated in the safety data sheets of the companies Bayer AG and Hoechst AG whereas Akzo Nobel give a value of < 40 Pa at 20 °C.

The value of 13,3 Pa at 25 °C was calculated by the Hoechst AG using the program "EPIWIN" version 2b of the Syracuse Research Corporation.

A value of $1.14 * 10^{-3}$ Pa at 20 °C was used for the risk assessment. This value is derived from the experimental determination of the vapour pressure at higher temperatures using the dynamic method. The measured values are in detail as follows:

0.43 hPa at 136.9 °C

0.99 hPa at 143.5 °C

2.03 hPa at 158.6 °C

5.00 hPa at 174.1 °C

15.03 hPa at 196.2 °C

The Clausius-Clapeyron equation was applied to these values for calculating the vapour pressure at 20 °C

Water Solubility

The water solubility of a substance is specified by its saturation concentration in pure water at a certain temperature, preferably at 20 °C.

The water solubility varies from 5 g/l to 7.8 g/l at 20 °C to in the safety data sheets of the companies Bayer AG, Hoechst AG, Akzo Nobel and Courtaulds Chemicals without giving further information on the test method or the testing procedure.

Moreover, the water solubility at 20 °C was determined experimentally using the flask method and result in a value of 7820 mg/l; the measured pH-values were in the range of 4.7 to 6.1. The test report was assessed as valid and plausible. Therefore the value of 7820 mg/l at 20 °C was used for the risk assessment.

Partition Coefficient

The partition coefficient (Pow) is defined as the ratio of the equilibrium concentrations (c) of a dissolved substance in a two phase system consisting of two largely immiscible solvents. In the case of n-octanol and water:

$$\text{Pow} = \text{equilibrium } c_{\text{n-octanol}} : \text{equilibrium } c_{\text{water}}$$

The partition coefficient therefore is the quotient of two concentrations and is usually given in the form of its logarithm to base ten (logPow).

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Hoechst AG gives a logPow-value of 0.544 in their safety data sheet without any further information. Beyond that, Hoechst AG calculated the logPow-value of 1.47 using the program "EPWIN" version 2.0b of Syracuse Research Corporation. The calculated value of the company Akzo Nobel was 1.49 without information on the applied method or program.

For the risk assessment the value of 1.78 was applied. This logPow was determined experimentally by Courtaulds Chemicals in a GLP-test using the flask-shaking-method.

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1.4 CLASSIFICATION

1.4.1 Current classification (Annex I of Directive 67/548/EEC)

Xn	Harmful
	Carc. Cat. 3
N	Dangerous for the environment
R40	Limited evidence of a carcinogenic effect
R22	Harmful if swallowed
R 51	Toxic to aquatic organisms
R 53	May cause long-term adverse effect in the aquatic environment

1.4.2 Proposed classification

In Germany TCEP is classified according to water-hazard class 2 (hazardous to water).

-
- (Working group (Human Health) on classification and labelling of dangerous substances under Directive 67/548/EEC)

At the meeting November 2005 the EU classification and labelling working group (Human Health) agreed finally upon the following classification for TCEP:

Class of danger:	T	**Toxic**
	Carcinogen	Cat. 3
	Reproductive toxicant	Cat. 2
	N	Dangerous for the environment
	R 22	Harmful if swallowed
	R 40	Limited evidence of a carcinogenic effect
	R 51	Toxic to aquatic organism
	R 53	May cause long term effect in the aquatic environment
	R 60	May impair fertility

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Currently tris(2-chloroethyl)phosphate is listed in Annex I to Commission for the 25th time Council Directive 67/548/EEC and has to be classified as Carcinogen Category 3 and labelled with Xn, (Harmful) and R 40 (Limited evidence of a carcinogenic effect).

The agreed classification proposal will be included in the next draft ATP.

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2 GENERAL INFORMATION ON EXPOSURE

The environmental part of this risk assessment was first drafted in 1998. At that time TCEP was produced in the EU in quantities of about 2000 t/a. However, the situation has changed recently. Up-to-date information given by industry revealed that there is no production in Europe[4] anymore and processing has been reduced, but marketing of TCEP-containing products is still relevant for the EU.

The risk assessment is performed using import data from 2002. All life cycles are calculated using generic scenarios since no formulator and processor is known to the rapporteur.

2.1 PRODUCTION

There is no production in Europe at present (2001/2002). According to IPCS (1998) all commercial TCEP is produced by the reaction of phosphorus oxychloride with ethylene oxide followed by subsequent purification.

Past trends

According to the maximum range of production/import for 1991/1992, given in the IUCLID-database, an amount of 10 500 t/a was relevant at that time for the European market. TCEP production and use has been in decline since the 1980s as its historic use in rigid and flexible polyurethane foams and systems has been substituted by other flame retardants. According to IPCS (1998) global consumption of TCEP peaked at over 9000 t in 1989 but had declined to below 4000 t by 1997. In 1998 the EU tonnage was 2040 t of which 1950 t were produced in the EU, 580 t imported and 490 t exported.

Present situation

Specific information given by industry revealed that there is no production of TCEP in the EU at present, i.e. in 2001/2002. There are 3 companies importing a total of 1150 t TCEP in the EU (partly from Russia and Poland[5]). All of these importers are exclusively traders of TCEP. No specific information on formulation or processing could be obtained. However, the importing companies provided information on fields of application of their sales. These data are used in the calculation of the environmental exposure.

A tonnage of 143 t was exported outside the EU in 2002. The total EU tonnage present can be estimated to be **1007 t/a**. This quantity is used further in the risk assessment.

---

[4] Referring to countries of the European Union before enlargement at 1st May 2004.

[5] In the context of this Risk Assessment, Russia and Poland were considered as being outside the EU.

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The rapporteur received information from a new EU member state[6] in 2005. This information has been voluntarily provided. It is used as additional information only. Production is estimated as 300 to 500 t for 2004. It is further stated that export to outside the EU is about 300 to 400 t for the same time period. The EU tonnage estimated above as 1007 t/a is used in the risk assessment.

TCEP is also formed as a reaction by-product in the manufacture of other commercial flame retardants in which TCEP has been declared an impurity. These flame retardants are currently undergoing ESR risk assessment (4th priority list, Rapporteur: UK/IRL). This additional amount of TCEP is considered only in the calculation of the regional background concentration (see Chapter 3.1.6).

2.2 PROCESSING / APPLICATION (CATEGORIES OF USE, AMOUNTS)

TCEP is used primarily as an additive plasticiser and viscosity regulator with flame-retarding properties for polyurethane, polyesters, polyvinyl chloride and other polymers.

Past trends

Historically the largest field of application of TCEP (80-90 % of the quantity produced) was that concerned with reducing the brittleness, and with the simultaneous flame-resistant finishing, of polyurethane in the production of celled, rigid or semi-rigid foam. The addition of 10 % TCEP relative to the finished foam is sufficient to achieve a clear flame retardant effect (GDCh, 1987).

On a small scale TCEP was also used as an intermediate for the production of wax additives (GDCh, 1987).

In GDCh (1987), further application fields of TCEP (10 – 20 % of total quantity) are given as follows[7]:

- Acetyl cellulose (10 - 70 % TCEP)
  - paints and varnishes
  - thermoplastics (foils, extrusion)
- Ethyl cellulose (foils)
- Nitrocellulose (paints and varnishes)
- Polyvinyl acetate (paints and varnishes)
- ,Polystyrole (adhesives for polyurethane foam)

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[6] Acession to EU in May 2004.

[7] This information is only given for illustrative purposes.

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- Polyvinyl chloride (20% TCEP at max.)

Present situation

There are indications that the market and respective application fields have changed over the past 15 years.

Currently TCEP is mainly used in the production of unsaturated polyester resins (~ 80 %). Other fields of application are acrylic resins, adhesives and coatings.

The main industrial branches to use TCEP as a flame-retardant plasticiser are the furniture, the textile and the building industry (roof insulation); it is also used in the manufacture of cars, railways and aircrafts.

Other utilisation of TCEP is represented by flame resistant paints and varnishes, e.g. for polyvinyl acetate or acetyl cellulose and the use as a secondary plasticiser for polyvinyl chloride to suppress the flammability resulting from plasticisers such as phthalates.

One company supplied quantitative information about the type of product and the application areas of their sales in 2002 (Table 2.1). However, these data are only representative for ~ 44 % of the total tonnage.

Table 2.1 Fields of application given by one company (representing 44 % of EU tonnage)

Type	Application are	%
Unsaturated polyester resin	Building industry, e.g. roofing	83
Acrylic resin	Roadside safety barriers	2
Adhesives	Building industry	5
Paints (wood and roofings)	Building industry, e.g. fire protection of wood	< 1
Polyurethane foam	Furniture	< 1
Others (unknown)	Unknown	9
Cellulose acetate	Transport	1
Textile coating	Upholstery	0

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One importer indicated unsaturated polyester resins (75 - 80 %) and flexible foam (20 – 25 %) as application fields of their sales whereas another company stated no TCEP was used in any foam anymore. More detailed information was not available.

The fraction of TCEP used in paints and varnishes is unclear. Industry stated that there has been a move by the paint industry away from that use since TCEP was classified as toxic several years ago. A survey by the European Council of Paints, Printing Inks and Artists' Colour Industry (CEPE, 2002) amongst its members showed that 3 out of 10 companies responding still use TCEP in paints in Europe, without specifying any quantities. An update of this information by CEPE (2004) states that the total volume of TCEP in paints in the EU amounted to 10 t in 2003. The representativeness of that statement could not be verified. It is further stated that no new company has started the use of TCEP in paints after 2002, leaving three companies continuing the use of TCEP in paint manufacturing in 2003/2004.

The above Table 2.1 lists a quantity of < 1 % in paints. There is no use of TCEP in paints and varnishes listed in the SPIN data base (see below).

The information given by a new EU member state specifies use of TCEP in polyurethane foams and unsaturated polyester resins. Uses of TCEP in paints are not known.

The lead company commented on the unresolved issue of use of TCEP in consumer paints (Akzo Nobel, 2004). It is said that there is no need to put TCEP into consumer paints since there is no regulation governing the flammability of domestic paints. Keeping in mind the higher costs of TCEP compared to other plasticisers like phthalates TCEP would not be used as plasticisers either in consumer paints. In contrast, professional paints need for certain uses flame retardant properties, however theses are specialised products. The largest coating manufacturer in the world confirmed that no flame retardants are formulated into domestic paints.

Due to these somehow contradicting statements it is difficult to determine a quantitative breakdown of usage reflecting the present situation in Europe.

Specific information is given for 44 % of the total tonnage specifying that 1 % of that tonnage goes into paints. 5 % is used for intermediates, 94 % in the polymer industry. Industry tried unsuccessfully to get specific use information on the remaining quantity (56 %).

It can be assumed that no TCEP is formulated into consumer paints. Furthermore, no TCEP use in paints is given either in the SPIN database or in the data provided by the new member state. CEPE stated a total use of about 10 t/a of TCEP in EU for industrial paints. This corresponds to 1 % of the total EU tonnage in accordance with the specific information given for the 44 % of the EU tonnage. Summarising all this information the Rapporteur proposes to carry over the information on 44 % of the EU tonnage to the total amount arriving at the following scenarios.

Table 2.2 Tonnages in various scenarios

	total tonnage in application
paints	1 % (10 t/a)

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polymers	94 % (947 t/a)
intermediate	5 % (50 t/a)

The resulting total mass balance and the respective industrial and use categories are shown in Table 2.3.

Table 2.3 Main, industrial and use category of TCEP

Main category (MC)	**Industrial category (IC)**	**Use category (UC)**	**Mass balance [in % of use]**
Use resulting in inclusion into or onto a matrix (II)	Polymers industry (11)	Flame retardants and fire preventing agents (22)	94
Use resulting in inclusion into or onto a matrix (II)	Paints and varnishes industry (14)	Flame retardants and fire preventing agents (22)	1
Non dispersive use (I)	Chemical industry (3)	Intermediate (33)	5

Product Register Data

The SPIN (Substances in Preparations In the Nordic countries) database was searched for information on TCEP in products on the national markets. The following data were found (Table 2.4):

Table 2.4 TCEP in products according to SPIN for 2001and 2003

country	2001		2003	
	number of preparations	tonnage	number of preparations	tonnage
Norway	13	1104	8	1285
Sweden	11	9	12	9
Finland	14	306	7	0.2
Denmark	25	190	13	4

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The above tonnages seem unrealistically high compared to the total identified EU tonnage. The reason is the way the data are recorded in SPIN:

The total amount of a substance in SPIN is the added quantity of the substance in all products the export amount subtracted. That is to say that if a substance is registered first as the imported raw material and then as part of the final preparation the quantity will be counted twice. Substances that are used for formulation of chemical products and that are imported, and most are in the Nordic countries, will thus be accounted for with maybe double the actual amount.

Another factor giving a distortion of the quantity value is when concentration has been registered as an interval. In such cases the upper limit has been chosen for calculations of the substance amount in Denmark, Finland and Norway. Depending on how wide the allowed interval is in the different countries the discrepancy between the given value and the true value will vary. For example the tonnage interval given for Norway in 2001 ranges from 61 t (min) to 1104 t (max).

Therefore, the tonnages in Table 2.4 have to be considered overestimations.

The tonnages notified in Denmark and Finland have gone down considerably between 2001 and 2003. Norway showing a notably high tonnage compared to the other countries in 2001 registered an even higher tonnage in 2003. Reasons may be due to the data recording explained above. However, it has to be noted that TCEP was notified as being present in consumer products in 2001 and 2002 but not in 2003.

Main industrial use categories (2001) are given as "Manufacture of rubber and plastic products" (Norway, 538 t, 5 preparations; Finland, 403 t, 5 preparations) and "Manufacture of chemicals and chemical products" (Norway, 41 t, 4 preparations). Specifying the technical use of these preparations, "Adhesives, binding agents" (Norway, 5 preparations) and "Flame retardants and extinguishing agents" (Denmark, 4 preparations) are identified as the main fields of application.

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3 ENVIRONMENT

3.1 Environmental exposure

3.1.1 Release into the environment

On the local scale releases of TCEP are expected during the industrial use of the polymer components as well as the formulation and industrial use (processing) of paints and varnishes. The less relevant use of TCEP as an intermediate should be considered additionally. Although there is no production in Europe a generic scenario for production is performed.

The flame retardant TCEP is physically combined with the polymer matrix. Therefore, TCEP could migrate to the surface. Releases might be expected during service life and disposal of products containing TCEP.

There is a recently published emission scenario document (ESD) on plastic additives: "Emission Scenario Document on Plastics Additives" (OECD, 2004). The document gives loss factors for flame retardants contained in plastic materials. However, these relate to solid flame retardants whereas TCEP is in the liquid state at room temperature. Although not appropriate for assessing losses for raw materials' handling and compounding, the ESD is used to estimate diffuse releases originating from service life and disposal. The ESD is also used to estimate diffuse losses from production and use of V6 where TCEP is present as impurity. These diffuse emissions are accounted for in the calculation of the regional background concentration. It has to be kept in mind that - in absence of further information - many of the assumptions made are "best guesses".

There is also an ESD for IC 14 Paints, Lacquers and Varnishes Industry available in Chapter 7 of the TGD (EC, 2003a).

A more elaborate coating emission scenario document "Chemicals used in the Coatings Industry: Paints, Lacquers and Varnishes" is currently being developed (EA, 2003). Difficulties in the application arise due to very limited information regarding the use of TCEP in the coating sector. Nevertheless, it is used to estimate the loss of TCEP to water and air during the formulation of paints (see 3.1.3).

ESDs provide very valuable information on branch and process specific emissions. However, difficulties in the application for this risk assessment arise due to limited information regarding the exact use of TCEP. If as a result too many (unconfirmed) assumptions had to be made, the ESD would not be any more realistic than generic calculations.

To nevertheless make the best use of the ESDs available, the Rapporteur decided on the following:

- ESD for Coating Industry (EA, 2003) is used to estimate the loss of TCEP to water and air during the formulation of paints, and compared with generic calculation using A- and B-Tables from the TGD (EC, 2003a) (see 3.1.3).

- ESD on Plastics Additives (OECD, 2004) is used to estimate emissions of TCEP during service life and disposal of polymers containing TCEP as well as diffuse releases of TCEP stemming from production, formulation (e.g. materials handling and

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compounding) and processing (e.g. conversion and moulding) including service life of V6. The estimated emissions are used for the calculation of the regional background concentration.

3.1.2 Environmental fate

Degradation

Biodegradation

Only a limited number of test results on aerobic and anaerobic biodegradability of TCEP are available.

In a modified Sturm test (OECD 301 B) on ready biodegradability with 10 mg/l and 20 mg/l TCEP a DOC removal of 13 % and 4 %, respectively, could be achieved after 28 days (Akzo, 1990a). However, cumulative CO_2 production by day 28 at either concentration was negligible with a maximum of 1 % of $ThCO_2$ at 20 mg/l.

In a MITI-I test (OECD 301C) employing sludge from different sewage treatment plants, rivers, bays and a lake as inoculum a biodegradation of 4 % after 28 days was obtained. Biodegradation was measured as BOD (CITI 1992). In a Zahn-Wellens test (OECD 302 B) with industrial activated sludge the elimination amounted to 15 % after 21 days. 11 % elimination after 1 h could be accounted to adsorption (Hoechst, 1978). In a second Zahn-Wellens test with industrial activated sludge the elimination after 27 days was < 10 % (Hoechst, 1985).

The anaerobic biodegradation of TCEP was tested according to ISO DIS 11734 with a concentration of 80 mg/l related to DOC. No degradation could be observed after 58 days (Noack, 1993).

The primary degradation of TCEP in a concentration of 5 mg/kg soil was investigated in a laboratory test system with standard soil for 100 days. The degradation kinetic curve could be fitted to a 2nd order square root function resulting in a DT_{50} and DT_{90} of 167 days and >>100 days, respectively. (Römbke et al., 1995)

Concluding from these results, TCEP must be considered as non biodegradable.

Although a certain low elimination may be expected under environmental conditions, the rate constant applied for the assessment of the elimination by biodegradation in municipal sewage treatment plants, surface waters, soils and sediments has to be set to zero as a realistic worst case (Table 3.1).

Table 3.1 Degradation constants of TCEP in different compartments

Compartment	degradation constant
Waste water treatment plant	$kbio_{WWTP} = 0\ h^{-1}$

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Aquatic environment	$kbio_{SW}$ = 0 d^{-1}
Sediment	$kbio_{SED}$ = 0 d^{-1}
Soil	$kbio_{SOIL}$ = 0 d^{-1}

Hydrolysis and Photolysis

Due to investigations by Brown et al. (1975) TCEP has a hydrolysis half-life of 3980 days (≈ 11 a) at pH = 7. Two further data at pH 3 (no hydrolysis) and at pH 10 ($t_{1/2}$ = 101 d) are given in the same reference. Therefore it can be concluded, that neither for the local nor for the regional scale hydrolysis contributes to the environmental degradation of TCEP.

A direct photolysis in water is not expected due to the molecular structure of TCEP, i.e. there is no relevant absorption above a wavelength of 290 nm.

Photooxidation in air

An estimation of the half-life for the atmospheric reaction of TCEP with hydroxyl radicals with the program AOP 1.65 yields a value of 17.5 h (24-h day, $5 \bullet 10^5$ OH/cm^3). Experimental studies are not available. With this estimated half-life it is expected that the photooxidation in air represents a major degradation path for TCEP in the environment.

Distribution

With a Henry's law constant of $4.155 \bullet 10^{-5}$ Pa • m^3 • mol^{-1}, TCEP has a low volatility.

Since no experimental results on the adsorption of TCEP to soil are available, the estimation of the adsorption coefficients to soil, sediment, suspended matter and sewage sludge are performed due to the TGD, using a log K_{ow} of 1.78 and calculating a K_{OC} of 110.2 l/kg with the appropriate SAR-equation (see Appendix 1).

The TGD does not differentiate between various types of phosphates. Basis is the K_{OW} since hydrophobic interactions are the most prominent type between non-polar chemicals and soil. However, more specific interactions of adsorption mechanisms are possible which might be better reflected in measurements.

Within the ESR assessment of other chloroalkyl phosphates (4th priority list; Rapp: UK) K_{OC} values were measured by HPLC according to OECD guideline 121. Results are listed in Table 3.2 and compared with K_{OC} values calculated according to TGD on the basis of log K_{OW} using QSAR for phosphates. In addition, there is a measured K_{OC} value for TDCP according to OECD guideline 106. An empirical relationship for dependence of log K_{OC} from log K_{OW} was derived and used to read across from TDCP to TCPP and V6. The resulting K_{OC} values are also listed in Table 3.2. (EC, 2006a).

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Table 3.2 Comparison between measured and calculated K_{OC} of some chloroalkyl phosphates

Substance (CAS)	K_{OC} derived from OECD 106 result for TDCP [l/kg]	K_{OC} measured by HPLC [l/kg]	K_{OC} estimated acc. to TGD (phosphates) [l/kg]
TCPP (13674-84-5)	174	576	304
TDCP (13674-87-8)	1780	12300	951
V6 (38051-10-4)	245	11000	360

Source: EU Risk Assessment Reports on TCPP, TDCP and V6 (Drafts, 2006)

It is apparent that all values estimated from HPLC are higher than the predicted values from the TGD and values derived from OECD 106 for TDCP. Both the latter are within the same order of magnitude. The 7-fold higher K_{OC} (TDCP) from the HPLC test suggests that some specific interaction with the HPLC column had occurred. (EC, 2006a). It was agreed to use the K_{OC} values derived from OECD 106 for TDCP for the risk assessments of these chloroalkyl phosphates.

Applying the same empirical relationship of $\log K_{OC}=-0.44+\log K_{OW}$ to read across to TCEP would result in a K_{OC} of 22 l/kg. This value is smaller than the one derived from TGD. However, uncertainty in reading across from TDCP to TCEP might be similar or higher than uncertainty in applying QSAR methods.

The estimated K_{OC} of 110.2 l/kg is used further in the risk assessment.

According to the classification scheme by Blume and Ahlsdorf (1993) the adsorption of TCEP is classified as being 'low'.

The partition coefficients are summarised in the following table:

Table 3.3 Partition coefficients of TCEP

Compartment	**Partition coefficient**
soil (2% OC)	K_{p_soil} = 2.2 l/kg
sediment (5 % OC)	K_{p_sed} = 5.5 l/kg
suspended matter (10 % OC)	K_{p_susp} = 11.0 l/kg
activated sludge (37 % OC)	K_{p_sludge} = 40.8 l/kg
raw sewage (30 % OC)	K_{p_sewage} = 33.1 l/kg

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Using the multimedia fugacity model EQC (level 1, type 1) for the equilibrium distribution of TCEP between the different environmental compartments, the following results are obtained:

Table 3.4 Distribution of TCEP according to EQC model

Compartment	Percentage
Air	<0.001
Water	94.8
Soil	5.06
Sediment	0.11

Based on the physiochemical properties of TCEP, the water compartment is the main target of distribution. Distribution into soil/sediment is much lower; partitioning into air can be neglected.

Bioaccumulation

The log K_{OW} of 1.78 indicates a low bioaccumulation potential. This is confirmed by experimentally determined bioaccumulation factors.

In a bioaccumulation study with *Cyprinus carpio* a BCF of 0.6 - 0.8 and < 1.2 - 5.1 was found after 42 days exposure to 1 mg/l and 0.1 mg/l, respectively (CITI, 1992).

In a static test with goldfish (*Carassius auratus*) at 0.9 mg/l (1 % of LC50) a BCF of 0.9 was achieved after 96 h. In the same test system at 2.1 mg/l with killifish (*Oryzias latipes*) a BCF of 2.2 was attained (Sasaki et al., 1981).

In a flow-through system with killifish at 2.1 - 2.4 mg/l uptake was rapid and maximum concentration was reached after 1 day. During 11 days concentration in fish remained constant resulting in BCFs of 1.2 - 1.4. At 12.7 mg/l during 5 days a BCF of 1.1 was recorded resulting in a tissue concentration of 14 mg/kg. Depuration was rapid with a half-life time of 0.7 h (Sasaki et al., 1982).

Elimination in waste water treatment plants

Based on the cited physical chemical properties in Chapter 1, as well as the biodegradation rate of 0 h^{-1} in the WWTP, the elimination through biodegradation, adsorption and volatilisation can be estimated with the model SimpleTreat 3.0. Due to this calculation model 98.6 % of TCEP remain in the water and 1.4 % is adsorbed to sludge. There is no elimination of TCEP by volatilisation into the atmosphere (Appendix 1).

Table 3.5 Elimination in WWTP

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Evaporation to air (%)	0
Release (dissolved) to water (%)	98.6
Adsorption to sewage sludge (%)	1.4
Degradation (%)	0
Total elimination from water (%)	**1.4**

3.1.3 Aquatic compartment

(incl. sediment)

3.1.3.1 Estimation of $Clocal_{water}$ / Generic approach for production and intermediate processing

For the industrial production of TCEP, ethylene oxide is added to phosphorous oxychloride in the presence of a catalyst. For purification and removal of the catalyst the crude TCEP is washed with aqueous-acid or aqueous-alkaline solutions and subsequently with water (GDCh, 1987).

The Technical Guidance Document (EC, 2002) proposes a generic (i.e. non site-specific) calculation for the release of chemicals to surface waters. Using the import/export data of 2002, 1007 t/a TCEP are relevant for the EU.

In a first approach it is assumed that 1 000 t/a are covered by one producer. An amount of 5 % of the regional tonnage of 503 t/a (see Chapter 2.2, Table 2.3 and Chapter 3.1.1.2, Figure 3.1) is assumed to be processed as intermediate at the same site. This quantity corresponds to 25 t/a.

A $Clocal_{water}$ of 28.76 µg/l is calculated for the generic production and processing of TCEP (Input: T_{prod} = 1000 t/a; f_{prod} = 0.3 %; T_{proc} = 25 t/a; f_{proc} = 2 %; $EFFLUENT_{STP}$=10000 m^3/d; DILUTION=40; Temission = 300 d/a; elimination 1.4 %).

Although standard procedure this approach has to be considered worst case since there is no production of TCEP in the EU anymore. For comparative purpose, a $Clocal_{water}$ of 4.11 µg/l was calculated for the processing only using the same input data as above.

3.1.3.2 Estimation of $Clocal_{water}$ / Generic approach: use

To calculate the generic, local concentrations in surface water resulting from the use of TCEP, the use pattern and percentages given in Chapter 2.2, Table 2.3 are used, i.e. 94 % polymers, 1 % paints and varnishes and 5 % intermediates.

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With the available information on the TCEP content in the respective formulations and products, a content of about 10 wt % for polymers and paints is assumed for this initial estimation.

It is further assumed that half of the EU continental use volumes are attributable to a highly industrialized region. The reasoning behind this assumption is firstly the 'low' consumption amount of 1007 t/a. Secondly, the industrial use of TCEP can be considered fairly specialised with industry indicating further reduction in use of TCEP in future.

The following scheme (Figure 3.1) summarises the proposed scenario for calculating the generic local concentrations for the use of TCEP, mainly using the A/B-tables from the TGD. The amount of TCEP contained in different applications is given in brackets. The respective tonnage of paints (formulation volume: 50 t/a) and polymers (formulation volume: 4730 t/a) is used to obtain the fraction of main source and the duration of the emission from the B-tables.

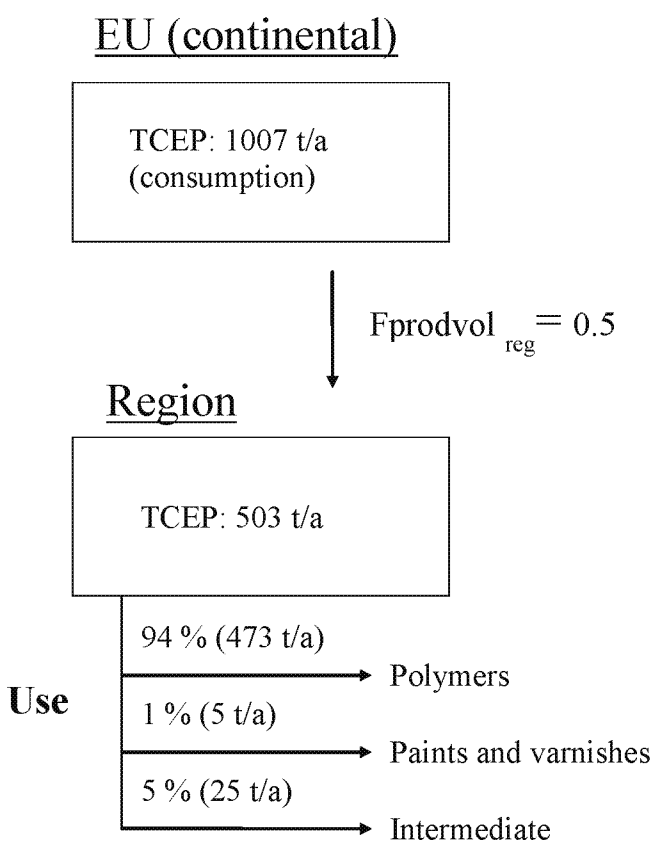

Figure 3.1 Schematic geographical representation of use of TCEP

An overview over the various use scenarios and the calculations of $Clocal_{water}$ is shown in Table 3.6 and a brief description is given subsequently.

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Table 3.6 Data used for aquatic exposure assessment from the use of TCEP

Use of TCEP	**Polymers industry**	**Paints and varnishes**	**Paints and varnishes**
Tonnage (t/a), regional	473	5	5
Industrial category Use category	11 (Polymers industry) 22 (Flame retardant)	14 (Paints,...) 22 (Flame retardant)	14 (Paints,...) 22 (Flame retardant)
Life cycle step	processing	formulation	industrial use (processing)
Release factor to water	0.0005 (A-table 3.11; I, A)	0.02 (A-table 2.1)	0.05 (A-table 3.15), water based
Fraction of main source	0.15 (B-table 3.9)	1 (B-table 2.10)	0.6 (B-table 3.13)
Number of days	284 (B-table 3.9)	300 (B-table 2.10)	200 (B-table 3.13)
Size of STP (m^3/d)	2 000	2 000	2 000
Dilution in receiving water	10	10	10
$Clocal_{effl.}$ (µg/l)	61.6	164	370
$Clocal_{water}$ (µg/l)	6.16	16.4	37.0

Processing of polymers

For the processing of polymers a generic calculation is performed using the A/B-tables for default emissions.

-- IC: 11 (Polymers industry); UC: 22 (Flame retardants), Category A, type I

The result of this estimation is $Clocal_{water}$ = 6.16 µg/l.

Formulation of paints and varnishes

For the formulation of paints and varnishes a generic calculation is performed using the A/B-tables for default emissions.

To derive the fraction of main source from the B tables, the tonnage is related to the formulated paints of 50 t/a.

-- IC: 14 (Paints and varnishes industry); UC: 22 (Flame retardants)

The result of this estimation is $Clocal_{water}$ = 16.4 µg/l.

There is an Emission Scenario Document (ESD) on "Chemicals used in the Coatings Industry: Paints, Lacquers and Varnishes" available (EA, 2003). In Chapter 3.1.3 this ESD is used to estimate the loss of TCEP to water and air during the formulation of paints.

Industrial use (processing) of paints and varnishes

For the industrial use of paints and varnishes a generic calculation is performed using the A/B-tables for default emissions. To derive the fraction of main source from the B tables, the tonnage is related to the formulated paints of 50 t/a.

-- IC: 14 (Paints and varnishes industry); UC: 22 (Flame retardants)

-- water based paints

The result of this estimation is $Clocal_{water}$ = 37.0 µg/l.

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3.1.3.3 Sediment

The calculation of the PEC_{local} for sediment is done using the highest local aquatic exposure of $PEClocal_{water}$ = 37.0 µg/l (industrial use of paints):

PEC_local_{sed} = 0.118 mg/kg (wet weight) = 0.306 mg/kg (dry weight)

Measured sediment concentrations are reported under „Monitoring data".

3.1.3.4 Monitoring data

Surface water

A synopsis of monitoring data for TCEP is given in GDCh (1987), concerning the river Rhine for German and Dutch locations. The reported values measured between 1972 and 1986 are generally in the range of 0.1 µg/l to 1 µg/l.

A detailed monitoring study for TCEP in the river Rhine and its tributaries was performed by Knepper and Karrenbrock (1996). With a detection limit of 0.01 µg/l, TCEP was found to be a permanent pollutant in surface waters. Concentrations at the location of Cologne were between 0.05 and 0.3 µg/l. Using the measured concentrations a load of 20 - 25 kg/d was calculated by the authors. For the tributaries of river Rhine, including the rivers Neckar, Main, Mosel, Lahn und Ruhr, concentrations ranging from 0.18 to 1.3 µg/l were determined, i.e. generally higher concentrations than had been measured in the Rhine.

A similar monitoring program was performed for the river Elbe and its tributaries (ARGE, 2000). TCEP was monitored in 1996 and 1998 at 7 sites along the river (6 measurements per year). Concentration of TCEP was found in ranges of 10 (min) – 220 (max) ng/l.

Spot tests on surface waters (i.e. 2 major rivers (Rhine and Neckar) as well as various smaller rivers and canals) above and below the discharge point of a municipal WWTP were performed in Baden-Wuerttemberg/Germany (Metzger and Möhle, 2001). TCEP was not at all detected at 5 out of 10 locations (detection limit: 0.1 µg/l); for a further 2 sites that was the case after the WWTP. Maximum concentration detected was 0.27 µg/l.

Fries and Püttmann (2003) monitored TCEP in the Oder river near the eastern border of Germany. At four different sampling times (March 2000, November 2000, March 2001, July 2001) nine river water samples were collected. Mean values ranged from 30 ng/l (March 2000) to 282 ng/l (March 2001). In July 2001 the measured concentrations were generally higher (554 – 1236 ng/l).

The same authors had monitored TCEP in various rivers in Germany (River Rhine at Rüsselsheim; R. Elbe at Hamburg and Dresden; R. Main at Frankfurt; R. Oder at Küstrin; R. Nidda at Frankfurt; R. Schwarzbach at Rüsselsheim) in an earlier study (Fries and Püttman, 2001). The concentrations ranged from non-detectable levels (< 1 ng/l) up to 220 ng/l (51 samples taken in March and November 2000). In comparison to concentrations of TCEP of 138 to 3000 ng/l measured previously in selected rivers (references cited therein from 1990-1999), the authors note a decreasing trend in environmental concentrations of TCEP.

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Concentrations of TCEP of <12 to 80 ng/l were measured in the River Ruhr basin/Germany (Bester et al., 2003)

Measurements during 1999-2000 of 95 organic wastewater contaminants in 139 streams across 30 U.S. states were recently published (Kolpin et al., 2002). The sampling sites focussed primarily on areas considered susceptible to contamination from human, industrial and agricultural waste water and represent a wide range of geography, hydrogeology, land use and climate. TCEP was detected in about 60 % of the samples. The maximum concentration was 0.54 µg/l.

Prösch et al. (2002) examined 29 bath lakes in Mecklenburg-Western Pomerania/Germany, a rural area with mainly agricultural utilisation. No TCEP was detectable in 18 of these randomly chosen lakes (detection limit: 0.01 µg/l; one-time sampling). The maximum measured concentration of 0.09 µg/l is assumed to stem from a contaminated site.

In Italy, Galassi (1991) reported concentrations ranging from < 10 ng/l up to 293 ng/l at one station at River Po and two marine stations in the Adriatic Sea within 4 sampling campaigns carried out from April to August 1988.

Monitoring data for the Midland region near Derby/UK was supplied for the years of 1990 to 2003 (Environment Agency, 2003). Measurements were taken around two locations presumably discharging TCEP. At one of the sites a company used to produce a flame retardant. The other location received effluent of a textile finishing company. Both companies have closed down in recent years however TCEP can still be detected. Whereas in early 1990s maximum values of up to 4 mg/l were measured, TCEP concentration has been constantly < 5 µg/l since around 1998 (90 percentile of all values: 3.55 µg/l). Values from January 2004 to July 2005 from the same region showed a 90 percentile of 1.03 µg/l (Environment Agency, 2005).

Precipitation

Chloroorganics were analysed in samples of rain (Ireland), snow (Poland, Southern Sweden, Antarctica) and glacier ice (Northern Sweden) (Laniewski et al., 1998). The sites were selected to represent both urban and remote areas. Apart from the surficial snow sample from Antarctica, TCEP was present in all other samples in concentrations ranging from 1 – 21 ng/l.

A TCEP concentration of 121 ng/l was detected in one sample of rain water from the Oderbruch area/Germany (Fries and Püttmann, 2003).

WWTP effluent

TCEP was measured in the effluent of 3 communal WWTP in Saxony-Anhalt and Saxony (Germany) in 1999 and found in concentrations between < 50 and 600 ng/l (ARGE, 2000).

Prösch et al. (2000) studied the occurrence of TCEP in effluents of 13 municipal WWTP in the German Baltic Sea catchment area. TCEP was detectable in all WWTPs in concentrations of 0.06 µg/l (min) to 1.66 µg/l (max), based on one measurement per month. The effluent discharge rate varied between 137 and 44380 m^3/d.

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Comparable results were found in a study in Baden-Wuerttemberg, another federal state of Germany (Metzger and Möhle, 2001). Values between < 0.1 µg/l (detection limit) and 1.88 µg/l (max) were detected in 22 WWTP in Spring/Summer 2002 (one-time sampling).

TCEP was measured in October 2002 (5 samples) and February/March 2003 (7 samples) in the influent and effluent of 2 communal WWTP in North Rhine Westphalia/Germany (MUNLV NRW, 2003). The measured values (median; influent/effluent) were 0.22/0.25 µg/l at Düsseldorf and 0.32/0.36 µg/l at Cologne, respectively. The authors could not explain the higher concentration of the effluent compared to the influent.

In July 2001, influent and effluent waste water samples (one-time sampling) were collected from three municipal WWTP and one industrial WWTP discharging their treated waste water into the Oder River (Fries and Püttmann, 2003). The mean concentrations of TCEP with regard to municipal WWTP were 986 ng/l (influent) and 352 ng/l (effluent), respectively. Interestingly, these concentrations are higher compared to the industrial WWTP (influent: 568 ng/l; effluent: < 1 ng/l (detection limit)) although representativeness is questionable.

A recent study determined the elimination efficiency with respect to various chlorinated and non-chlorinated organophosphorous flame retardants in two WWTP in the Ruhr/Rhine area in Germany (Meyer and Bester, 2004). Samples were taken in spring 2003 as 24-hour composite samples (automatic sampling; LOD: 6.1 ng TCEP/l. No elimination of TCEP was observed. Mean effluent concentrations were 350 ng/l and 370 ng/l, respectively.

Ground water

Ground water was investigated by Metzger and Möhle (2001) in Baden-Wuerttemberg/Germany (one-time sampling). One sample was taken at ca. 3 m depth at an uncontaminated sited and showed no TCEP (detection limit: 0.01 µg/l). At another site two samples were taken at 31 and 44 m below a landfill site. Originally a quarry this site was used as dumping ground between 1962 and 1977. About half of the waste originated from households (70 %) and industry (30 %). That was mixed with soil and construction waste. Concentrations of TCEP were found to be 0.37 µg/l (31 m depth) and 0.10 µg/l (44 m depth).

The same authors performed spot checks on landfill leachates originating from household landfills (3 sites) as well as from one hazardous waste site. The leachates stemming from household landfills showed TCEP concentrations of 0.17 and 0.19 µg/l. Two of the sites treat the leachates with activated charcoal before discharging them into municipal WWTPs. TCEP was not detectable in the effluent of the activated carbon treatment. TCEP concentration of the hazardous waste site leachate was 5.56 µg/l but again after treatment with activated charcoal no TCEP was detectable.

The Environment Agency (2005) of England and Wales analysed the concentration of TCEP in leachate from 22 landfills in southern England and Wales during Spring 2005. The data obtained are still being analysed. Nevertheless, these early data show that TCEP was only detected in one sample at 0.245 µg/l (detection limit of 10 µg/l for majority of measurements).

Fries and Püttmann (2003) collected 76 ground water samples at 3 different sampling times between March 2000 and March 2001 in the Oderbruch area located approximately 70 km

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east of Berlin. Around 25 monitoring wells (1.5 to 19 m depth) situated around 2 main sampling sites showed mean values of < 1 ng/l (detection limit) to 312 ng/l.

For 12 wells, located in a distance of 600 m from the bank of river Rhine, concentrations of about 0.17 µg/l at maximum were measured (Knepper and Karrenbrock, 1996). In a recent study 573 house wells in rural areas of Mecklenburg-Western Pomerania/Germany were investigated (Prösch et al., 2002). TCEP was only detected at 2 wells (detection limit: 0.01 µg/l) with a maximum concentration of 0.18 µg/l.

During 2004-2005 the Environment Agency (2006) conducted a survey on groundwater samples in England as part of a speculative monitoring to identify any contaminants. TCEP was measured in 61 samples. Concentrations ranged from 0.02 to 1.43 µg/l (90 percentile of all values: 0.33 µg/l). However, these results must be treated with caution since this was a screening exercise only using GCMS.

Sewage sludge

Sewage sludge samples from 10 German treatment plants were investigated by Weisser in 1992. Concentrations of < 0.5 mg/kg up to 11 mg/kg (dry weight) were found with a mean value of 3.1 mg/kg.

Sediment

In a paper published by the Niedersächsisches Landesamt für Ökologie (1997) TCEP concentrations for ten sediments from five German rivers (Elbe, Jeetzel, Leine, Oker and Aller) in Lower Saxony are reported. The measured concentrations ranged from 5.4 to 15.0 µg/kg (dry weight) with a median value for the ten sites measured of 8.3 µg/kg (dry weight). From this median value a concentration in surface water of 1.0 µg/l can be calculated at equilibrium according to the method described in the TGD. Concentrations of 0.5 – 100 µg/kg (dry weight) were monitored in the sediment of the river Elbe (ARGE, 2000).

In 1999 sediment samples of the 3 great rivers Rhine, Danube and Neckar in Baden-Wuerttemberg (Germany) were analysed for TCEP (Metzger and Möhle, 2001). Sampling was concentrated on freshly deposited sediment, i.e. the top layer, and took place only once. TCEP was not detectable in 6 out of 12 sites allocated at different spots of the rivers (detection limit: 20 µg/kg dry weight). The maximum concentration found was 188 µg/kg (dry weight).

3.1.3.5 Comparison between predicted and measured levels

Most of the available monitoring data were measured in Germany, partly in areas of small size and often done as one-time sampling. Representativeness might be questionable.

Most of the concentrations measured recently in surface waters (0.01 – 0.3 µg/l) are of comparable magnitude to the modelled $PECreg_{water}$ (0.087 µg/l).

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Measured sediment concentrations are scarce and a comparison with predicted concentrations cannot be made.

3.1.4 Atmosphere

3.1.4.1 Estimation of $Clocal_{air}$ / Generic approach: production and intermediate processing

No ESD for the release of chemicals into the atmosphere during production/processing is available at the moment. The emissions are therefore estimated with the emission tables presented in Appendix I of the Technical Guidance Document.

An emission factor of zero is given in Table A1.2 for all MC's for a vapour pressure < 1 Pa, i.e. default emission during production is zero.

The emission factor for the processing step is also zero (Table A3.3).

Therefore generic estimation results in zero atmospheric emissions during production/ processing.

3.1.4.2 Estimation of $Clocal_{air}$ / Generic approach: use

The following table summarizes the results of the calculations of $Clocal_{air}$ and $DEPtotal_{ann}$ for the use of TCEP performed according to the TGD.

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Table 3.7 Data used for atmospheric exposure assessment

Use of TCEP	**Polymers industry**	**Paints and varnishes**	**Paints and varnishes**
Tonnage (t/a), regional	473	5	5
Industrial category Use category	11 (Polymers industry) 22 (Flame retardant)	14 (Paints,...) 22 (Flame retardant)	14 (Paints,...) 22 (Flame retardant)
Life cycle step	processing	formulation	industrial use (processing)
Release factor to air	0.0005 (A-table 3.11, I, A)	0.001 (A-table 2.1; 1c)	0 (A-table 3.15), water based
Fraction of main source	0.15 (B-table 3.9)	1 (B-table 2.10)	--
Number of days	284 (B-table 3.9)	300 (B-table 2.10)	--
$Clocal_{air}$ (µg/m³)	0.035	0.005	0
$DEPtotal_{ann}$ (mg m^{-2} d^{-1})	1.23×10^{-4}	1.74×10^{-5}	0

3.1.4.3 Monitoring data

No monitoring data are available for the air compartment. Aston et al. (1996) detected TCEP in pine needle samples collected in the Sierra Nevada foothills/California (USA). Concentrations were up to 3 times higher (10-1950 ng/g_{wwt}) compared to those reported for aqueous matrices in other studies Aston et al. refer to.

The authors discuss their findings and suspect that aerial transport and deposition from nearby point sources may play a role. They argue that although organophosphate pesticides are used on California's orchards and volatilisation of these compounds has been demonstrated, the source of haloalkyl organophosphate vapours is unknown. They further discuss that it is possible that landfills or incineration sites of waste plastic articles emit TCEP into the air. Uptake into the plant might also occur via vapours volatilising from soil after being deposited there. Due to a general lack of data in that field it is not possible to broaden this discussion at this point.

3.1.5 Use of Emission Scenario Document for loss estimation to air and water (formulation of paints)

As mentioned in Chapter 3, there is an Emission Scenario Document (ESD) on "chemicals used in the coatings industry: paints, lacquers and varnishes" available (EA, 2003). Difficulties in the application arise due to limited information regarding the use of TCEP in the coating sector. Nevertheless, the ESD is used to estimate the loss of TCEP to water and air during the formulation of paints.

Since information is lacking regarding the exact conditions at formulation, the following assumptions are made:

Table 3.8 Assumptions made for application of ESD

condition	**value (unit)**
type of paint	water-based
tonnage TCEP used per site	5 t/a
tonnage paint used per site	50 t/a
number of batches	1 batch/day
number of days	300 days/a
loss to water per batch	0.5 %
loss to air per batch	0.164 %*

* for middle air speed, high boiling point substance, no lid fitted to vessel

Using the values above and standard WWTP with 1.4 % removal and standard dilution by 10, a $Clocal_{water}$ of 4.11 µg/l is calculated. For air, the $Clocal_{air}$ is 7.6 x 10^{-3} µg/m³.

These values compare very well with $Clocal_{water}$ of 16.4 µg/l and $Clocal_{air}$ of 5 x 10^{-3} µg/m³ derived for the formulation of paints using the A- and B-tables of the TGD (EC, 2003a), thereby backing these.

The application of the ESD is based on a number of assumptions. The calculations could be refined by obtaining specific information from the coating industry. This was in the course of the risk assessment not possible. In such cases it is recommended in the TGD to use the default emission tables of the TGD (EC, 2003a). The values derived from these tables are used in the risk characterisation.

3.1.6 Terrestrial compartment

A default calculation for the local soil concentration is performed, assuming a TCEP deposition from the generic paint formulation site as well as sewage sludge application due to sludge originating from the same site, representing the highest local TCEP concentration in the hydrosphere. This scenario is considered to represent a realistic worst case for the local terrestrial assessment. The following input data are used:

$DEPtotal_{ann}$ = 1.74 • 10^{-5} mg m^{-2} d^{-1}

Ksoil_water = 3.506

Csludge = 6.51 mg/kg

Kair_water = 1.75 • 10^{-8}

kbiosoil = 6.93 • 10^{-7} d^{-1}

The results for the local soil concentrations are listed below:

<u>exposure of the ecosystem</u>

bulk concentration:	$Clocal_{soil}$	= 0.039 mg/kg
porewater concentration:	$Clocal_{soil\_porew}$	= 0.019 mg/l

<u>exposure of the crops (human consumption)</u>

bulk concentration:	$Clocal_{agr.soil}$	= 0.037 mg/kg
porewater concentration:	$Clocal_{agr.soil\_porew}$	= 0.018 mg/l

<u>exposure of grass (cattle)</u>

bulk concentration:	$Clocal_{grassland}$ = 0.0086 mg/kg	
porewater concentration:	$Clocal_{grassland\_porew}$	= 0.0042 mg/l

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3.1.7 Non compartment specific exposure relevant to the food chain

Since there is no indication that TCEP may show a bioaccumulation potential, a risk characterization for exposure via the food chain is not necessary.

3.1.8 Regional exposure consideration

The calculations for the regional PECs are performed with SimpleBox 2.0. Since no information on the geographic localisation of the processing sites for TCEP and the respective quantities involved is available, the following assumptions are made:

- The local emissions for the formulation and industrial use of TCEP are summed up and distributed to the regional and continental area in a ratio of 50 % to 50 %. Considering the comparatively low total volume in Europe it can be assumed that only specialised companies at few sites work in this field. The default ratio of 10 % to 90 % is used only for the widely spread releases to the environment (diffuse releases through polymers during service life).

- All waste water is expected to be released via WWTP except for life cycle "diffuse releases from polymers" for which a connection rate to WWTP of 80 % is assumed.

- The diffuse releases from polymers and paints (covering 95 % of the total consumption amount) via migration are not yet included in the default emission tables of the TGD.

  Losses might occur during service life of the products (polymers and paints) and disposal. Difficulties in estimating these losses for TCEP arise due to very limited information regarding the actual use of TCEP in products and their application fields at present. Assumptions are made, however these are open to a certain degree of uncertainty.

  There is a recently revised emission scenario document (ESD) on plastic additives (OECD, 2004). The document gives loss factors for flame retardants. However, these relate to solid flame retardant whereas TCEP is in the liquid state at room temperature. Although not appropriate to assess losses for raw materials' handling and compounding, the ESD is applied to estimate diffuse releases originating from service life and disposal to be used in the regional background concentration.

  The total tonnage of TCEP used in polymers is 947 t/a. This tonnage is split 50:50 one half going into indoor service products and the other half into outdoor service. In a first approach it is further assumed that products have a service lifetime of 10 years.

  The waste could be recycled, remain in the environment, be put on landfill or be incinerated. The ESD gives general figures of disposal percentages for UK in the mid-1990s (OECD, 2004). Accordingly, about 8 % of plastics are recycled, 17 % are burned to generate heat and 75 % are incinerated or disposed of to landfill. Based on estimates for automotive and furniture foams from the draft V6 risk assessment (EC, 2003b) it is

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assumed that of these 75 % only 5 % is incinerated, the vast majority (95 %) ends up landfilled.

Organic substances are completely destroyed during incineration. Therefore emissions to water and to air are zero (OECD, 2004). In a first approach, no emissions are expected from the recycling process.

With respect to losses to water from landfill, the ESD states that the maximum potential loss could be calculated from the amount of additive remaining in the plastic at disposal, but that it is very unlikely that this amount would be released. Significant leaching of TCEP could be expected due to high solubility of TCEP (7.82 g/l at 20 °C) and the comparatively low adsorption potential. This assumption is fortified by monitoring data where TCEP was detected in the µg/l range in various ground water samples (see 3.1.2.4).

The Landfill Directive (1999/31/EC) calls for decreasing amounts of waste to be sent to landfill in all EU countries. Waste has to be used for energy recovery as much as possible with another potentially important route in the future being gasification of plastics including PUR (EC, 2006a).

There is a very limited amount of monitoring data on releases of TCEP from landfill sites in the EU (see Chapter 3.1.1.4). The Environment Agency (2005) of England and Wales analysed 22 landfill leachates. TCEP was detected in one out of 56 samples analysed (at a concentration of 0.245 µg/l). Metzger and Möhle (2001) investigated landfill leachates originating from household landfills (3 sites) as well as from one hazardous waste site. Concentrations were up to 0.19 µg/l (household) and 5.56 µg/l (hazardous waste site).

There is currently no guidance in the TGD on how to quantify landfill emissions. The data base on monitoring is too scarce to allow for extrapolation from the measurements to the EU. Emissions due to leaching from landfill are being researched at present but might not be available for some time (Pers comm., 2004).

The total tonnage ending up landfilled can be estimated to < 700 t/a. Although leaching can occur for significant time periods after discharge of the products to landfill site, the impact on the value of $PEC_{regional}$ is expected to be small. For now leaching is not taken into account further in the assessment. If in due time a model becomes available, the issue of leaching from landfill could be reconsidered.

Table 3.9 Loss factors for service life and disposal for use of TCEP in polymers according to OECD (2004)

	% loss to water over lifetime	% loss to air over lifetime
Indoor service, leaching to liquid waste	0.05	
Indoor service, volatility		0.05

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Outdoor service, leaching to environment	1.6	
Outdoor service, volatility		0.05
incineration	0	0
landfill	unknown	0

10 % of the total resulting emissions from service life and disposal are used as input for the region.

The possible diffuse releases from service life and waste disposal due to application of TCEP in paints are not considered due to a low total amount of TCEP present (5 t/a in region) and general lack of information.

- TCEP is present as an impurity in the substance V6. Production and use of V6 could therefore lead to environmental releases of TCEP. V6 (CAS: 38051-10-4) is currently undergoing ESR risk assessment (4th priority list, Rapporteur: UK). Since there is only one producer in the EU, the site-specific information is confidential.

  Diffuse releases of TCEP stemming from production, formulation (e.g. materials handling and compounding) and processing (e.g. conversion and moulding) including service life of V6 are estimated using the Emission Scenario Document on Plastic Additives (OECD, 2004). These are used for the calculation of the regional background concentration. Confidential site-specific information was provided to the Rapporteur.

  The release model of V6 containing TCEP as impurity was revised within the revision of the risk assessment of the ESR substance V6 (EC, 2006b). Refer to Chapter 3.1.8.1 for more information.

- No direct release to soil was identified. Diffuse release only occurs as a result of dispersive processes. Release is therefore to be expected as a result of deposition from air and sludge application (see Chapter 3.1.4).

The total environmental releases (continental and regional) are summarized in the following table:

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Table 3.10 Total releases of TCEP to different compartments (regional and continental)

field of application	ratio reg./cont.	total release to WWTPs (t/a)	total direct release to the surface water (t/a)	total release into the atmosphere (t/a)
processing of polymers	50/50	0.474	--	0.474
formulation of paints	50/50	0.2	--	0.01
industrial use (processing) of paints	50/50	0.5	--	0
use as intermediate	50/50	1.0	--	5.0×10^{-4}
service life of polymers	10/90	0.189	7.632	0.474
disposal	10/90	0	0	0
regional release from V6 production and use	-	0.451	0.113	0.051
total		**2.814**	**7.745**	**1.010**

The regional PECs resulting from the SimpleBox 2.0 calculations are given below (further details are presented in the Appendix 2):

$PECregional_{water}$ = 0.087 µg/l

$PECregional_{nat\ soil}$ = 0.049 µg/kg_{wwt}

$PECregional_{air}$ = 2.27×10^{-4} ng/m³

3.1.8.1 Update to release model of V6

TCEP is present as impurity in the ESR substance V6 (4.5 – 7.5 %; Rapporteur: UK/IRL). Production and use of V6 could therefore lead to environmental releases of TCEP which should be added to the regional background for the TCEP assessment.

Much of the V6 life cycle is confidential. In 2003, the Environment Agency made an estimate of the releases of TCEP caused by handling of V6 (Pers. comm., 2003). This estimation used a blanket correction factor to correct for the difference between release rates of "high" and "low" volatility plastic additives, as suggested in the ESD. TCEP is of "high" volatility while V6 is of "low" volatility. The resulting release rates were made available to the Rapporteur

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and used in the calculation of the regional background concentration of TCEP (cf. Table 3.10).

In the course of updating the exposure assessment of V6 (agreed at TCNES IV/2006) the release rates from foams had been subject to modelling and measurements. The physicochemical properties of TCEP are closer to TCPP than to V6. New release rates were applied using percentage rates of release of TCPP from equivalent life cycle stages. The resulting releases are shown in Table 3.11. It has to be noted that a change in the approach to modelling of waste remaining in the environment resulted in an overall increase in estimated release rate to air.

Table 3.11 Comparison of releases of TCEP from V6 handling between 2007 model and 2003 estimation

	to WWTPs (t/a)	**to surface water (directly) (t/a)**	**to atmosphere (t/a)**
regional release from V6 production and use (2007)	0.003	7.3×10^{-4}	0.089
total release (2007)	2.366	7.633	1.048
regional release from V6 production and use (2003)	*0.451*	*0.113*	*0.051*
total release (2003)	*2.814*	*7.745*	*1.010*

The respective regional PECs resulting from the SimpleBox 2.0 calculations using the total releases are given below (Table 3.12):

Table 3.12 Comparison of PECregional for various compartments using different V6 release rates

	2007 model	**2003 estimation**
$PECregional_{water}$	**0.071 µg/l**	**0.087 µg/l**
$PECregional_{nat\ soil}$	**0.055 $\mu g/kg_{wwt}$**	**0.049 $\mu g/kg_{wwt}$**
$PECregional_{air}$	**2.52×10^{-4} ng/m³**	**2.27×10^{-4} ng/m³**

It can be noted that regional PEC for water from 2007 model is smaller than from 2003 estimation. Both regional 2007-PECs for air and soil are higher than 2003-PECs. However, this change has no significance for the resulting local PECs and the consequent PEC/PNEC ratios (cf. Chapter 3.3).

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The refinements to the exposure model of V6 have changed the release rates of TCEP from V6 handling, and consequently the total release rates and the various PECregional. Although more accurate these changes have no significance for the PEC/PNEC ratios. For reasons of simplicity the PECregional as calculated from 2003 estimation are used in the risk assessment.

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3.2 EFFECTS ASSESSMENT: HAZARD IDENTIFICATION AND DOSE (CONCENTRATION) - RESPONSE (EFFECT) ASSESSMENT

3.2.1 Aquatic compartment

The available information about ecotoxic effects produced in various tests is summarized in the Table 3.11 below. The effects recorded refer almost exclusively to short-term exposure. All effect values refer to nominal concentrations. Only freshwater species have been tested.

3.2.1.1 Toxicity to algae

Noteworthy are striking differences between the NOEC/EC_{50} values determined in the tests conducted with *Scenedesmus subspicatus*.

An examination of the respective test protocols failed to establish the reasons for such large discrepancies. The differing algal strains and nutrient media used cannot be regarded as a plausible primary cause. The only difference between the test conditions of the two tests with *Scenedesmus subspicatus* was a difference in pH of about 0.5 unit. The test by Kühn et al. (1989) used at test start a pH of 8, while the study by Hoechst (1988) employed a pH of 7.5. However, it is not assumed that this difference in pH has such an influence on the test results.

As in the study by Kühn et al. (1989) a pH increase by 1.4 units (from 8.0 to 9.4) occurs between day 2 and 3 of exposure that may have influenced the toxicity, even if the validity criterion is fulfilled, it is proposed by Nyholm & Kusk (2003) to use the 48h-effect values from this study instead of the 72 h-values.

The test results cited in Akzo (1993) cannot be validated as neither a test report nor a reference is available. As one of the given values is in agreement with the results found by Kühn et al. (1989), it is assumed that the same test result is described.

In the 96 h test conducted with another green alga (*Selenastrum capricornutum*), considerable differences between the results of the range-finding test with no growth inhibition up to 100 mg/l and the outcome of the definitive test (NOEC = 5 mg/l) were found. In addition, a comparison of the growth characteristics given for both preliminary and definitive test reveals significantly stronger growth even in the controls in the former test, supplemented here by slight promotion of cell multiplication (compared to controls) at all concentrations tested (range: 0.1 - 100 mg/l). The authors cite the noticed instability of the TCEP dispersion as potential reason for the discrepancies in the effect values for both tests (Akzo, 1992). However, this observed instability cannot be explained by the physicochemical properties of the substance nor by any degradation mechanism.

3.2.1.2 Toxicity to aquatic invertebrates

On the whole the few available data indicate modest to low toxicity to the representatives of this group (although 48 h values for *Daphnia* are lacking) under conditions of short-term exposure. The results of the 7 d investigation with planarians collected in an unpolluted river should be considered as merely indicative values: Yoshioka et al. (1986) concluded from their correlation analysis including several test methods with various test species that the additional stress exerted by head cutting may influence the effect values. In this sense, planarian sensitivity may be lower than indicated by this head and eye regeneration study.

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Table 3.11: Data on ecotoxic effects of TCEP on aquatic plants (algae), invertebrates and fish

Taxon. group	Species	Exposure duration	Endpoint	Effect value (mg/l)	Reference/annotations
Green algae	*Scenedesmus subspicatus*	48 h	E_bC_{10} E_rC_{10} E_bC_{50} E_rC_{50}	0.29 0.65 2.0 5.0	Kühn et al., 1989 (WaBoLu res. Report N° 106 03 052/01)
		72 h	E_bC_{10} E_rC_{10} E_bC_{50} E_rC_{50}	0.2 0.55 1.1 3.6	
		96 h	E_bC_{10} EbC_{50}	0.3 1.2	
		72 h	NOEC E_bC_{50}	100 271, 278	Hoechst AG, 1988 Report N° OEK 88/082 E) two separate tests
			E_rC_{10} E_rC_{50}	148 522	Recalculation by Nyholm & Kusk (2003) from raw data
			EC_{10} EC_{50}	15.9 67.9	cited in Akzo, 1993, test report or reference not available (it is only stated that the value is from Akzo chemical database, containing various sources)
		72 h	EC_{10} EC_{50}	0.2 1.1	cited in Akzo, 1993, test report or reference not available (it is only stated that the value is from Akzo chemical database, containing various sources)
	Selenastrum capricornutum	96 h	NOEC LOEC E_bC_{50} E_rC_{50}	5 15 38 117	Akzo Chemicals, 1992 (CRL report F 92 016) in range finding test NOEC = 100 mg/l
Inverte-brates	Crustacea: *Daphnia magna*	24 h	EC_0 EC_{50} EC_{100}	100 340 1000	Hoechst AG, 1989
		24 h	EC_0 EC_{50}	186 451	Kühn et al., 1989 (WaBoLu res. Report

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		21 d	NOEC	13	N° 106 03 052/01) E = reproduction rate
		24 h	EC_{50}	235	Bayer AG, 1991
	Moina macrocopa	3 h	LC_{50}	1000	Yoshioka et al., 1986
	Planaria (flatworms): *Dugesia japonica*	7 d	EC_{50}/ LC_{50}	159	Yoshioka et al., 1986 head regeneration and mortality test

Table 3.11: continue

Taxon. Group	**Species**	**Exposure duration**	**Endpoint**	**Effect value (mg/l)**	**Reference/annotations**
Fish	*Oncorhynchus mykiss*	96 h	LC_0 LC_{50} LC_{100}	100 249 400	Akzo Chemicals, 1990b LSR report N° 90/AKL 021/0161)
	Oryzias latipes	48 h	LC_{50}	300	MITI, 1992
		96 h	LC_{50}	210	Sasaki et al., 1981
		96 h	LC_{50}	170	Yoshioka and Ose, 1993
		48 h	LC_{50}	230 (10 °C) 190 (20 °C) 66 (30 °C)	Tsuji et al., 1986
	Leuciscus idus melanotus	48 h 2 h	LC_0 EC_0 LC_{50} LC_{100}	ca. 100 10 ca. 200 300	Hoechst AG, 1978 (test report 6/78)
	Carassius auratus	96 h	LC_{50}	90	Sasaki et al., 1981
		7 d	EC_0/LC_0	5	Eldefrawi et al., 1977 (only one concentration tested)
	<u>dietary toxicity:</u> *Cyprinus carpio*	6 d	LD_0	up to 156 mg/kg food	Loeb and Kelly, 1963 (US Fish and Wildlife Serv.)

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3.2.1.3 Toxicity to fish

The data on the impact of short-term exposure indicate modest to low acute toxicity. As symptoms of progressive poisoning hyperventilation, loss of coordination in swimming, darkening pigmentation and apathy are mentioned in test protocols. At concentrations higher than 200 mg/l, 100 % mortality normally occurred within the first day of exposure. Sasaki et al. (1981) reported a deformation of the spinal column in 3 of 12 killifish after exposure to 200 mg/l for 24 - 72 h. Under these exposure conditions, protrusion of eyes was observed, too.

The 48 h LC_{50} values for killifish determined by Tsuji et al. (1986) in a static system illustrate the increase in toxic action resulting from rising temperature.

With regard to the few LC_0/NOEC values reported, some comments in the test protocols provide supplementary information about the range of the acute no-effect level in fish:

- in the 96 h test with juvenile trout (Akzo, 1990b) during the preliminary range finding investigation hyperventilation and darkening pigmentation were observed even at the lowest concentration tested (10 mg/l). These findings contrast considerably with the NOEC of 50 mg/l resulting from the definitive test. The differences in the results may be due to incomplete solution of TCEP as observed in the latter test only. However, this incomplete solution cannot be explained by the physicochemical properties of the test substance.

- during the 48 h test with golden orfe (Hoechst,1978) increasing disturbances in swimming at concentrations higher than 10 mg/l were recorded (at 25 mg/l in one, at 50 mg/l in two of 10 fish).

These auxiliary data appear to indicate that TCEP concentrations around 10 mg/l may represent a kind of threshold level for sublethal effects in sensitive fish (like trout) under conditions of short-term exposure.

The 7 d EC_0/LC_0 (5 mg/l) for goldfish determined by Eldefrawi et al. (1977) in a simple tank experiment is restricted in its indicative value regarding the NOEL to concentrations of 5 mg/l: since only one concentration (5 mg/l) was tested, higher EC_0 and LC_0 values cannot be excluded.

As mentioned in Chapter 3.1.1, TCEP bioconcentration by fish is low (Sasaki et al. 1981, 1982). Uptake and elimination take place rapidly as shown by a biological half-life value of 0.7 h in killifish (Sasaki et al., 1982). At test concentrations of 1 - 3 mg/l, the maximum bioconcentration ratio in killifish (1.2 - 1.4) was reached within the first day and remained practically constant until cessation of exposure after further 10 days. The former feature may at least partly explain the above mentioned early manifestation of mortality in fish lethality tests at higher concentration levels.

The analytical monitoring of TCEP carried out by Sasaki et al. (1982) did not trace metabolites of the test substance. Supplementary information about the potential extent of metabolic conversion was provided by Sasaki et al. (1985). They reported that in contrast to certain other phosphoric triesters the compound was only slightly affected when incubated with liver microsomes of goldfish in the presence of NADPH.

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3.2.1.4 Toxicity to microorganisms

In a respiration inhibition test according to OECD guideline 209 activated sludge of a predominantly domestic sewage treatment plant was exposed to TCEP for 3 h. The EC_{20}, EC_{50} and EC_{90} were 1.4 g/l, 3.2 g/l and 7.8 g/l, respectively (Akzo, 1990c).

The aerobic degradation of synthetic sewage by industrial activated sludge was inhibited at concentrations above 500 mg/l, up to 1000 mg/l inhibition was compensated after 1 day adaptation; below 500 mg/l degradation was rather enhanced (Hoechst, 1978).

In an inhibition test applying the ETAD fermentation tube method with anaerobic microorganisms gas evolution was inhibited at concentrations above 250 mg/l (Hoechst, 1978). In a second assay an EC_0 of 1500 mg/l after 24 h was reported (Hoechst, 1985). However, with the same method EC_{50} values of 10 - 100 mg/l were cited in an EU safety data sheet (Hoechst, 1994).

3.2.1.5 Determination of the $PNEC_{aqua}$

Despite remaining problems regarding the plausibility of widely differing effect values resulting from growth inhibition tests with algae, the lowest effect figures available refer to this group. As the available studies are regarded as valid and no reason for the conflicting results can be given, it is proposed to use the lowest effect value based on growth rate for the derivation of the PNECaqua. As explained above, the 48h-values are preferred to the 72h-values. Therefore, the 48h-ErC_{10} of 0.65 mg/l found by Kühn et al. for *Scenedesmus subspicatus* is used as basic value. Long-term tests with species from two trophic levels are available. Therefore an assessment factor of 50 can be regarded as suitable. However, as from the effect values for *Scenedesmus subspicatus* found by Kühn et al. it can be concluded, that algae are the most sensitive species to TCEP (EC50-value is a factor of 18 to 90 lower than EC50/LC50 values from fish and daphnids found in short-term tests), and it is therefore not expected that in a long-term test with fish an effect value below 0.65 mg/l will be found, an assessment factor of 10 is justified according to the TGD:

$$\mathbf{PNEC_{aqua}} = 0.65 \text{ mg/l} / 10 = \mathbf{65\ \mu g/l}$$

3.2.1.6 Determination of the $PNEC_{wwtp}$

For the effects assessment for microorganisms in sewage treatment plants the $PNEC_{wwtp}$ is calculated by applying an assessment factor of 100 on the EC_{50} from the OECD 209 respiration inhibition test (3.2 g/l) according to the TGD:

$$\mathbf{PNEC_{wwtp} = 32\ mg/l}$$

3.2.1.7 Sediment

No information about TCEP effects on sediment organisms could be found.

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Consequently, only a provisional $PNEC_{sed}$ can be determined based on equilibrium partitioning according to TGD using a $K_{susp\text{-}water}$ of 3.655:

$$\mathbf{PNEC_{sed}} = \frac{3.655 \bullet 0.065 \bullet 1000}{1150} = \mathbf{0.2\ mg/kg\ ww}$$

3.2.2 Atmosphere

No ecotoxicological data are available for this environmental compartment.

3.2.3 Terrestrial compartment

The taxon-related description and discussion of reported effects include soil organisms and species/groups inhabiting other terrestrial substrates.

With regard to soil, all relevant data available were generated by Römbke et al. (1995).

3.2.3.1 Toxicity to higher plants

The following results have been obtained for *Avena sativa* (oat) in a plant growth test conducted according to OECD 208 (slightly modified):

14 (16) d NOEC = 10 mg/kg (dw)

EC_{50} = 64 mg/kg (dw)

Römbke et al. (1995) mention that due to lacking data homogeneity, the estimated NOEC of 10 mg/kg could not be verified statistically.

At the lowest concentration within the range tested (1 - 1000 mg/kg) necrosis and chlorosis at leaf tips were observed. Though indicating that the overall no-effect-level for the ecotoxicological impact of TCEP on growth-related processes lies below 1 mg/kg, in the respective evaluation it has to be taken into account that at concentrations up to 10 mg/kg (dw) *Avena* plants were evidently able to recover from those injuries before the end of the test. The rapid loss of recovery potential at increasing concentrations is shown by a 76 % growth inhibition reported for 100 mg/kg (dw).

In contrast to the data for 14 (16) d exposure, in the preceding seed germination and seedling emergence test lasting 48 h no inhibition could be established at concentrations up to 1000 mg/kg (dw).

3.2.3.2 Toxicity to invertebrates

No mortality could be observed at concentrations of up to 1000 mg/kg in a 14 d earthworm toxicity test conducted according to a BBA guideline which (despite very slight

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modifications) corresponds to OECD 207. However, at that maximum concentration a significant weight reduction (> 10 %, compared to controls) in *Eisenia andrei* was determined. As NOEC for this endpoint a value of 580 mg/kg (resulting from the chosen spacing factor of $\sqrt{3}$) was reported (Römbke et al., 1995).

Among arthropods, a few insect species and a spider were investigated for their TCEP susceptibility:

The springtail *Folsomia candida* (3. - 5. larval stage at test start) was exposed in a 28 d reproduction test in accordance with a BBA guideline (Riepert, 1991) which corresponded to the analogous ISO draft. The following results were obtained (values in mg/kg (dw)):

mortality (adults):	LC_{10} = 19.3
	LC_{50} = 66.5
reproduction rate:	EC_{10} = 44.6
	EC_{50} = 131.9

The authors reported that a 30 % reduction in the reproduction rate was judged to be not statistically significant due to high rate variability observed when repeating the test, in contrast to the respective value of almost 90 % obtained for 125 mg/kg.

In the preceding 24 h test conducted with adult *Folsomia* specimens (≥ 3 mm in length) first signs of intoxication had been observed only at the highest concentration tested (1000 mg/kg).

As further insect species the carnivorous carabid beetle *Poecilus cupreus* representing the epigean fauna was subjected to a bait-lamina test (BBA guideline VI, 23-2.1.8, 1991). In this procedure, counting of traces of feeding activity on fly pupae *(Calliophora)* on soil (quartz sand, 70 % of maximum water capacity) is carried out. Groups of adults were exposed to 5 mg/kg (dw) for two weeks. No mortality or any behavioural effects were observed for two weeks in 5 replicates, but a mean reduction of 28 % in the feeding rate was determined. This effect, though not designating the chosen TCEP concentration as precise LOEC, may be seen as quite indicative: according to an EC proposal (1991) a rate of > 30 % should be used as qualifying criterion within the regulation process for plant protection agents.

Supplementary information about TCEP toxicity to insects refers to species which are not or only occasionally exposed to pollutants in soil: Ludvik and Decker (1947) reported a 48 h mortality rate of 51.3 % in aphids *(Myzus persicae)* after spraying with 2000 mg/l. No mortality was observed after 72 h when adult houseflies (*Musca domestica*) were fed a mixture of 1 % TCEP in sugar and dried milk. Topical application in this species led to the same result (Eldefrawi et al., 1977).

On the whole, the cited results point to low TCEP impact on insects under conditions of 1 - 3 d exposure. Regarding the mechanism of action, this view is affirmed in part by research of Eldefrawi et al. (1977) in which TCEP turned out to be a weak enzyme inhibitor in the midgut system of larval armyworm *(Spodoptera sp.)*.

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The only available information on TCEP toxicity to spiders was provided by Römbke et al. (1995) who conducted a substrate contact test according to a respective BBA guideline (draft, 1992). Subadult females of the epigean genus *Pardosa* were exposed to 5 mg/kg (dw) contained in moistened quartz sand for two weeks. A 14 d mortality rate of 41.7 % was established, supplemented by behavioural effects (disturbances in locomotion) starting on day 9 of exposure. No effect on the feeding rate could be determined. The authors mentioned that the cited mortality rate due to the low number of test organisms (groups of 4 spiders in 3 replicates) could not be statistically verified. However, the observed sublethal effect on the motility should be evaluated as being ecotoxicologically relevant.

3.2.3.3 Toxicity to soil microorganisms

The impact of TCEP on soil microflora was investigated in two natural soils under aerobic conditions with 5 mg/kg and 50 mg/kg soil, resp., for 28 days (Römbke et al., 1995). In the sandy soil (C_{org} < 1 %) with 5 mg/kg the dehydrogenase activity, after an initial increase of 21 %, was inhibited by 15 % after 28 days. With 50 mg/kg, after an initial increase of 16 %, the inhibition increased up to 42 %. In the loamy soil (C_{org} = 2.2 %) only the high concentration caused an inhibition > 15 % (20 % inhibition after 28 d).

3.2.3.4 Toxicity to birds

Sprague et al. (1981) tested TCEP for delayed neurotoxicity on 12- to 14-month-old white Leghorn hens. 24 h after a single oral application of undiluted TCEP at a dose of 10 ml/kg (= 14.2 g/kg), or undiluted corn oil (2 ml/kg) as a control, they investigated the inhibition of a protein processing esterase activity in vitro (referred to as "neurotoxic esterase"). Marked inhibition of this enzyme (> 75 %) is regarded as an indicator of delayed neurotoxicity.

A 30 % inhibition of this enzyme was observed, which was statistically significant ($p < 0.05$). At the same time the plasma-cholinesterase activity was inhibited by 87.1 % ($p < 0.05$). Since the inhibition of the "neurotoxic esterase" of the brain was not greater than 75 % the authors postulated that TCEP cannot cause delayed neurotoxicity.

This view was corroborated in a second study in which 10 ml/kg (= 14.2 g/kg) undiluted TCEP or 10 ml/kg undiluted corn oil, were administered orally twice, with an interval of 3 weeks. During the three weeks after the last application, and at the end of this period, no group showed any indications of delayed neurotoxicity either behaviourally or histopathologically. In particular, no axonal degeneration was observed.

Treatment with TCEP did, nevertheless, coincide with a decrease in body weight, a transient decrease in food uptake immediately after the applications, a cessation of egg production, and the induction of moulting. Four out of 18 animals died during the study.

In another study on hens, TCEP again failed to show neurotoxic effects following oral application of 2.5 g/kg or intraperitoneal application of 1.0 g/kg body weight (Bayer, 1986).

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3.2.3.5 $PNEC_{soil}$ calculation

On the basis of the various effect values reported (supplemented by information of merely indicative value) higher plants may be regarded as being somewhat more sensitive to TCEP than susceptible invertebrates. Regarding invertebrates, the available information points to notably higher susceptibility of arthropods compared to earthworms. The poor data on *Pardosa* do not allow to draw further detailed conclusions with respect to a higher sensitivity of insects or spiders. On the whole, the data point to similar susceptibility.

Long-term tests are available for springtails and soil microorganisms. No significant differences in sensitivity between *Folsomia* and bacteria can be derived from the respective test results for 28 d exposure.

However, since the available information on *Folsomia* covers a broader spectrum of effects, the lowest effect value reported for this species is chosen as reference value for the $PNEC_{soil}$ derivation (28 d LC_{10} = 19.3 mg/kg dw for adults, assumed to represent a NOEC).

According to the TGD an assessment factor of 50 has to be applied to this value.

$PNEC_{soil}$ = 19.3 mg/kg (dry weight)/ 50 = 0.386 mg/kg (dry weight)

$PNEC_{soil}$ = 0.341 mg/kg (wet weight)

3.2.4 Non compartment specific effects relevant to the food chain (secondary poisoning)

Since TCEP does not possess a bioaccumulation potential, the derivation of a $PNEC_{oral}$ is not necessary.

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3.3 RISK CHARACTERISATION

3.3.1 Aquatic compartment

(incl. sediment)

Waste water treatment plants

The highest discharge concentration for waste water treatment plants was calculated as **0.370 mg/l** for the industrial use of paints and varnishes. Generic model is used here for the calculation of the $Clocal_{effl}$. No specific information is available for this area of use of the substance or for the exposure in the environment. Consequently, standard scenarios had to be used for the calculation of the concentration in the waste water treatment plant.

Taking into consideration a $\mathbf{PNEC_{WWTP}}$ of **32 mg/l**, a $Clocal_{effl}$/PNEC ratio of **0.01** results for the industrial use of paints and varnishes. Since the $Clocal_{effl}$/PNEC ratio < **1**, there is no risk expected to the microorganism population in the WWTP.

Conclusion (ii)

Aquatic environments

The PEC/PNEC ratios for all of the areas of production, formulation and processing are summarized in the following table. The ratios are calculated using a $PECregional_{water}$ of 8.71 x 10^{-5} mg/l and a **PNEC** of **65 µg/l**.

Table 3.12 Risk characterisation for aquatic compartment

Company/area of use	PEC_{local} (µg/l)	$PEC/PNEC_{aqua}$
Production and processing*	28.9	0.44
Processing only	4.20	0.06
Processing of polymers	6.25	0.10
Formulation of paints and varnishes	16.52	0.25
Processing of paints and varnishes (industrial use)	37.1	0.57

* for information only, since no production of TCEP in EU anymore

PEC/PNEC < 1 could be derived for all life cycle steps using generic calculations.

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Conclusion (ii)

Sediment

No effect values on sediment organisms could be found. This allowed only for the derivation of a provisional $PNEC_{sed}$ using the equilibrium partitioning method.

A $PEClocal_{sed}$ based on the $PEClocal_{water}$ was calculated in Chapter 3.1.1.3, but hereof no information beyond those obtained from the risk characterisation for the water phase can be derived.

Some monitoring data from sediments of different rivers in Germany are available. However, the geographical area concerned is small and no information regarding the representativeness of the data is available. The data basis is therefore regarded not sufficient for risk characterisation.

3.3.2 Atmosphere

Due to the atmospheric half-life ($t_{1/2}$ = 17.5 h), abiotic effects on the atmosphere, such as global warming and ozone depletion, are not to be expected in connection with TCEP. The highest calculated air concentration is around 0.035 $\mu g/m^3$ for processing of polymers. Since no data are available on the ecotoxicological effect of the substance in connection with this environmental compartment, it is not possible to perform a quantitative assessment of this environmental compartment. On the basis of the available information on the substance, further testing seems not necessary.

Conclusion (ii)

3.3.3 Terrestrial compartment

Releases into the terrestrial compartment as a result of deposition from the atmosphere and sewage sludge application are to be expected. The highest soil concentration for TCEP amounting to 0.039 mg/kg (wet weight) results from the formulation of paints and varnishes giving a PEC_{soil} of **0.039 mg/kg** (wet weight).

Based on the $PNEC_{soil}$ of **341 µg/kg** (wet weight) a PEC/PNEC ratio of **0.12** is calculated.

There is at present no indication of a risk to the local terrestrial environment.

Conclusion (ii)

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3.3.4 PBT-assessment

The following table shows the criteria as defined in the TGD to identify PBT/vPvB substances, and the values relevant for TCEP. The description of the relevant tests can be found in Chapter 3 (**P** and **B**) and in Chapter 3.2 (**T**).

Table 3.13 Data for TCEP and PBT/vPvB criteria according to TGD

Criterion	PBT-criteria	vPvB-criteria	TCEP
P	Half-life > 60 d in marine water or > 40 d in freshwater or half-life > 180 d in marine sediment or > 120 d in freshwater sediment	Half-life > 60 d in marine- or freshwater or half-life > 180 d in marine or freshwater sediment	non biodegradable (surface water) DT_{50} (soil): 167 d
B	BCF > 2000	BCF > 5000	BCF (fish): < 1.2 – 5.1
T	Chronic NOEC < 0.01 mg/l or CMR or endocrine disrupting effects	Not applicable	E_rC_{10} (algae): 0.65 mg/l Carcinogenic Cat. 2 Toxic for Reproduction Cat. 2

TCEP has to be considered as non biodegradable (DT_{50}: ∞ days). An investigation on primary degradation of TCEP in soil showed a half life of 167 days.

The highest measured BCF in fish are <1.2 – 5.1.

The lowest long-term effect value of 0.65 mg/l was found for *Scenedesmus subspicatus*.

TCEP has been proposed to be classified as Carcinogenic (Cat. 2). There is evidence of chronic toxicity (T, R45).

It can be concluded that TCEP meets the **P/vP**- and the **T**-criteria. The **B**-criteria is not fulfilled. Overall TCEP does not meet the PBT criteria.

There is not enough information available to exclude the possibility of sites being located at the sea. TCEP is not degradable and shows limited sorption (98.6 % released to water from WWTP). The concentration in seawater can be estimated to be about 10 % of that in freshwater. As the marine PNEC will be 10 % of the freshwater PNEC, the overall marine PEC/PNEC ratios will be similar to those for freshwater. Due to the low BCF values bioaccumulation is not expected and the assessment of secondary poisoning is not considered necessary. In view of all arguments above, there is no need for a marine risk assessment.

3.3.5 Non compartment specific effects relevant to the food chain (secondary poisoning)

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Since there is no indication of bioaccumulation of TCEP, a risk characterisation for exposure via the food chain is not necessary.

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4 HUMAN HEALTH

4.1 HUMAN HEALTH (TOXICITY)

4.1.1 Exposure assessment

4.1.1.1 General discussion

In the EU, Poland is at present the only country producing Tris(2-chlorethyl)phosphate (TCEP, amount and use pattern confidential). The production and import of TCEP has declined during the past years. In the year 2001, there was no production within the EU, but marketing of TCEP-containing products was and is still relevant (app.1000 t of TCEP in 2002).

The main use of TCEP is the production of unsaturated polyester resins. One company supplied quantitative information about the application areas of their sales in 2001:

Unsaturated polyester resins	83 %
Acrylic resins	2 %
Adhesives	5 %
Coatings (wood and roofings)	<1 %
Polyurethane	<1 %
Others (not known)	9 %
Cellulose acetate	1 %
Textile	0 %

The company states, that the unknown applications (2.3 % corresponding to 10 – 15 tons) might eventually be used in paints and varnishes. Three companies give information on the use of TCEP in

Marine and protective coatings
Industrial paints
Fire retarding lacquers and paints (2.5 – 10 %)
Plasticiser with flame retarding properties in water based intumescent material (up to 7%)
Polyurethane industry
Flame retardant for flexible slabstock polyether and moulded foams (automotive industry, furniture industry).

According to information from 1987, the main industrial branches using formed products containing TCEP as a flame-retardant plasticizer are the furniture, the textile and the building industry (roof insulation); TCEP is also used in the manufacture of cars, railways and aeroplanes (GDCh, 1987).

Information on the concentrations of TCEP are not the same: due to information from two producers the concentration of TCEP in products amounts to 5 - 12 % (w/w). In addition in one product (cellulose acetate) a concentration of up to 70 % TCEP is possible. According to literature data concentrations up to 40 % are used.

Industry informed the Rapporteur, that less than 10 t/y TCEP are applied in paints.

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According to information from the producers, since its classification as a carcinogenic (category 3 a), the use of TCEP in polyurethane foams has declined to a large extent and is currently limited to special products.

For further detailed information on the uses of TCEP see chapter 2.

For workers the inhalation and dermal exposure routes are the most likely.

4.1.1.2 Occupational exposure

The exposure assessment generally aims at assessing exposure levels representing the reasonable worst case situation. The reasonable worst case is regarded as the level of exposure which is exceeded in a small percentage of cases over the whole spectrum of likely circumstances of use for a specific scenario.

The assessment of inhalation exposure is mainly based on measured exposure levels from which – if possible – 90th or 95th percentiles are derived as representing reasonable worst case situations. For the purpose of exposure assessment only data measured later than 1990, if available, are taken. Scenarios are clustered as far as possible to make the description transparent. If quantitative exposure data is not available, model estimates are taken forward to the risk characterisation. Short term exposure levels are given on the basis of measurement data. In lack of such data short term exposure levels are only calculated when they are needed for the risk characterisation. This is only rarely the case. The procedure is applied because there is no common understanding on deriving short term exposure levels on the basis of modelled 8 h TWA.

Beside inhalation exposure, dermal exposure is assessed for each scenario. Two terms can be used to describe dermal exposure:
Potential dermal exposure is an estimate of the amount of a substance landing on the outside of work wear and on the exposed skin.
Actual dermal exposure is an estimate of the amount of a substance actually reaching the skin.
There is an agreement between the EU member states, within the framework of existing substance, to assess - as a rule - dermal exposure as exposure to hands and parts of the forearms. In this, the main difference between both terms – potential and actual - is the protection of hands and forearms by work wear and – more important – the protection by gloves. Within this exposure assessment, the exposure reducing effect achievable by gloves is only considered if information is provided, that for a certain scenario gloves are a widely accepted protective measure and that the gloves are fundamentally suitable for protection against the substance under consideration. As a measure for the latter, tests according to DIN EN 374 are taken as criteria. For most down stream uses it is commonly known, that gloves are not generally worn. In these cases, dermal exposure is assessed as actual dermal exposure for the unprotected worker. Since often quantitative information on dermal exposure is not available, the EASE model is used for assessing dermal exposure, at the most.

Industrial activities using TCEP present opportunities for exposure. Exposure ranges depend on the physico-chemical properties of the substance, the particular operation and the risk reduction measures in use. TCEP is a liquid with a vapour pressure of 0.00114 Pa at 20 °C (extrapolated). Inhalation exposure is to be expected if processes are performed at elevated temperatures or if dusts containing TCEP are formed. Industry states that processes at

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elevated temperature do not occur because of the degradation of TCEP at elevated temperatures and that the substance or products containing the substance do not occur in a powdery state.

The following scenarios are regarded to be relevant for occupational exposure:

Scenario 1: Production of TCEP

Scenario 2: Use of TCEP at ambient temperatures for the production of polymers and formulations

Scenario 3: Use of formulations and products containing TCEP

The flame retardant TCEP is physically bound within the polymer matrix and, therefore, TCEP could migrate to the surface especially during processing steps especially those performed at high temperatures. Therefore release of TCEP from plastic products may be a potential way of exposure. In this, it should be considered, that not only plastic products produced in Europe but also products imported are concerned. The very low vapour pressure at room temperature and the high molecular weight lead to negligible migration. A prediction of consumer exposure caused by the release of TCEP from plastic products is made in section 4.1.1.3 "consumer exposure". In conclusion, exposure to TCEP during subsequent use of flame retarded equipment is likely to be negligible.

Since no information on exposure levels are available, the assessment of inhalation and dermal exposure is based on model estimates (according to the EASE model). Because the composition of the applied formulations (paints, adhesives etc.) is generally not known, it is not possible to correct the inhalation EASE estimate for the pure substance in consideration of the percentage of TCEP.

The number of exposed workers is not known for any of these scenarios.

OELs are not established in the EU.

Recycling:

There is no information concerning the exposure during recycling of plastic waste. Generally, the recycling of halogenated flame retardants is problematic, because of the possible release of halogenated compounds into the environment. There are two possibilities in the work up of plastic waste: thermic and shredding. It is supposed that mixtures of different plastics are recycled together.

4.1.1.2.1 Production of TCEP (scenario 1)

At present, TCEP is not produced in the EU but in the new EU member state Poland. Limited confidential information is available from the polish company. To some extend, information provided by companies which terminated the production is taken into account.

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TCEP is produced by the catalytic addition of ethylene oxide and phoshoryl chloride in closed systems. The pure product is obtained after cleaning steps, removal of the catalyst and drying in vacuum. The production is described to be continuously or batch-wise. The liquid product is filled in tanks via closed pipelines or in drums using local exhaust ventilation (Hoechst, 1994).

For the large-scale chemical industry high standards of control at the workplaces are assumed to be practised even if the containment is breached, e.g. during filling. Exposure may occur during coupling and uncoupling of transfer lines, drumming, cleaning, maintenance, repair works and the taking of process samples. It is assumed, that such activities are predominantly performed with local exhaust ventilation (LEV). According to information from the producers, personal protective equipment (gloves, eye protection and protective clothes) are worn.

Inhalation exposure

Workplace measurements

A limited number of confidential workplace measurement results were provided by one producer, which terminated the production.

EASE estimation

EASE for Windows 2.0, Aug. 1997

EASE estimation for the production and further processing of TCEP in the large-scale chemical industry:

Input parameters:	T = 20 °C, closed system, significant breaching, LEV present
	(vapour pressure < 1 Pa)
Level of exposure:	0 – 1.2 mg/m^3 (0 – 0.1 ml/m^3).

Due to the low vapour pressure of TCEP, the same exposure level is predicted for workplaces without LEV.

Conclusions

Since representative workplace measurement results are not available, exposure levels of 0 – 1.2 mg/m^3 (8-h TWA) predicted by the EASE model are used to assess the risks of inhalative exposure. 1.2 mg/m^3 should be taken as representing the reasonable worst case situation.

It is to be assumed that the substance is processed daily. Consequently, the duration and the frequency of exposure to TCEP are assumed to be daily and for the entire length of the shift.

The assessed exposure level (incl. duration of exposure) is representative for the Polish company.

Dermal exposure

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When producing and further processing dermal exposure could occur during activities like drumming, coupling and uncoupling transfer lines, sampling, cleaning, maintenance and repair work. For the unprotected worker, according to the EASE model, potential dermal exposure is assessed as follows:

Input parameters:	Non dispersive use, direct handling, intermittent
Level of exposure:	0.1 – 1 mg/cm^2/day.

Considering an exposed area of 420 cm^2 (palms of hands) the model yields an exposure level of 42 - 420 mg/person/day. For assessing actual dermal exposure levels, it has to be considered that the substance is manufactured and further processed primarily in closed systems and that the use of PPE (here gloves and eye protection) is highly accepted. The extent of protection of the personal protective equipment (here gloves) depends inter alia on the suitability of the recommended material with regard to the permeation properties of the substance. Beside this fundamental suitability, the level of protection depends on the real working conditions, i.e. the working behavior, the information of the workers etc.

Three producers and importers submitted information about the used glove material (rubber). But there is a lack of information with regard to the suitability of the recommended materials. For the use of unsuitable gloves, dermal exposure is assessed as worst case estimation applying the EASE model, estimating a dermal exposure level for immediate dermal contact without gloves. It is not possible to consider the limited protection provided by unsuitable glove material.

Since the suitability of the gloves cannot be presupposed, the model estimates of 42 – 420 mg/person/day for the unprotected worker should be taken.

Conclusions

For assessing the health risks from daily dermal exposure in the area of production and further processing (scenario 1), an exposure level of 42 – 420 mg/person/day should be taken. This exposure assessment is based on information provided by the companies, that unsuitable gloves are worn and takes into account the possible dermal exposure under actual workplace conditions.

Exposure to the eyes is largely avoided by using eye protection.

4.1.1.2.2 Use of TCEP at ambient temperatures for the production of polymers and formulations (scenario 2)

TCEP is used as a plasticizer in different formulations (e.g. paints, lacquers) containing cellulose derivatives, polyvinyl acetate etc. (Baumann, 1997; Biethan et al., 1979) in different resins (for glues and adhesives) and in polymers (polyurethane, cellulose acetate). According to the available information the concentration of TCEP in these formulations amounts to 5 to 16 %. In the starting polymer mixture (liquid, dispersion) higher concentrations of up to 40 % are possible. It is to be expected, that the formulation processes often have several steps in common, e.g. transporting and filling, mixing on site with additives and the base polymers or

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resins, adjusting and filling the products into drums or other containers. Information on the formulation of products containing TCEP is provided by one company (Hoechst, 1994).

There is no detailed information which processing steps are applied in case of TCEP. Therefore, only a very general description can be given. According to information from the representative company, TCEP is neither handled in powdery formulations nor at elevated temperature.

According to information provided by one company incompany transfer of TCEP occurs via closed pipelines. TCEP is brought to the customer sites mainly in tanks, to a less extent in drums. The substance is filled into storage tanks equipped with fixed pipelines to the location of further processing. The further processing is performed in closed systems and filling of the final product (here containing 16 % TCEP) is done with a local exhaust ventilation being present. Duration and frequency of exposure are assumed to be full shift and daily, although transfer at beginning of the process and drumming may be done only during part of the day, especially if production is discontinuously. In this case, duration of inhalation and dermal exposure may be shorter than the shift length.

Inhalation Exposure

Workplace measurements

Workplace measurement results are not available.

EASE estimation

Input parameters:	T = 20°C, closed system breached or non-dispersive use (vapour pressure < 1 Pa).
Level of exposure:	0 – 0.1 ppm (0 – 1.2 mg/m³)

Conclusions

Because no monitoring data are available, the EASE model was used to estimate the potential exposure at the workplace. In general for vapour pressures below 1 Pa the result of the estimate is 0 – 1.2 mg/m^3 (0 – 0.1 ppm), independent of the use pattern "closed system" (with the possibility to be breached), "non-dispersive use", or "wide dispersive use". It is to be assumed that the substance is processed daily, so the duration and the frequency of exposure to TCEP are assumed to be daily and for the entire length of the shift.

Dermal exposure

For the further processing of TCEP in the production of polymers and of formulations, (e.g. plastics manufacturing industry) it cannot be excluded that gloves are not regularly worn and

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that immediate dermal contacts occur during activities like drumming, filling, cleaning, repair and maintenance. The corresponding exposure for the unprotected worker is assessed in application of the EASE model:

Input parameters:	Direct handling, non dispersive use, intermittent
Exposure levels:	0.1 - 1 mg/cm^2/day.

Considering an exposed area of 420 cm^2 (palms of two hands) the exposure level of daily dermal exposure amounts to 42 - 420 mg/person/day.

Conclusions

For assessing the health risks of daily dermal exposure in the area of production of polymers and formiulations (scenario 2), an exposure level of 42 – 420 mg/person/day should be taken. This exposure assessment is based on the assumption that suitable gloves are not worn. It cannot be presupposed, that eye protection is regularly used.

4.1.1.2.3 Use of formulations and products containing TCEP (scenario 3)

In general, TCEP can be a component of e.g. lacquers, paints, glues, adhesives and formulations which are used in e.g. the textile, building and construction and furniture (upholstery) industry. It is to be assumed, that the use of TCEP is restricted to applications which require flame-retarding properties. Industry states, that less than 10 t/y TCEP are applied in paints.

Spraying of paints, lacquers and adhesives is performed in many different industrial and skilled-trade sectors, e.g. vehicle production and repair, treatment and processing of metal and wood and the furniture industry. Lacquers and paints are applied by brushing, rolling, spraying, dipping or covering by pouring. Spraying may be performed manually or automatically. Inhalation exposure caused by droplet aerosols may be a source of exposure. Industry states that lacquers containing TCEP are used in the building industry and textile coating.

In case of mechanical treatment of foams containing TCEP, exposure to TCEP is possible.

In the view of occupational exposure, the application of paints, lacquers, glues, adhesives and flame-retardant formulations are relevant as well as the preparation of paints and cleaning after finishing work. During using formulations the use of PPE (here respiratory protection, gloves and eye protection) is not regarded to be a general measure to reduce exposure.

The concentration of TCEP in end products is assumed to be ≤ 25 %.

Inhalation Exposure

Workplace measurements

Workplace measurement results are not available.

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As analogous data for spraying measurement results described in the revised TGD are used (see Appendix IC). Based on measured exposure levels on polyisocyanates, NL derived the following relationship:

Exposure level = 10*fs/30

Fs: percentage of the relevant substance in the paint
30: percentage of polyisocyanate in total paint
10: estimated reasonable worst case exposure level for isocyanates).

Taking a percental content of 25 % TCEP into account, exposure amounts to 8.3 mg/m³. For mechanical or thermal treatment of foams containing TCEP, as a rough estimation, exposure levels are assumed to be below the level assessed for spray applications.

EASE estimations

If no droplet aerosols are produced, inhalation exposure is lower due to the low vapour pressure of TCEP.

Input parameters: T = 20°C, non-dispersive use (vapour pressure < 1 Pa).

Level of exposure: 0 – 0.1 ppm (0 – 1.2 mg/m³)

Conclusions

Inhalation exposure has to be assessed for the use of formulations containing TCEP (e.g. paints, flame-retardant formulations, glues) in fields with lower protection levels, e.g. in small and medium sized companies. The concentration of TCEP is assumed to be ≤ 25 %. For assessing the risks of inhalation exposure during spray application, 8.3 mg/m³ should be taken if spraying techniques are applied (scenario 3a). In case of activities without the formation of droplet aerosols, 1.2 mg/m³ should be taken as representing the reasonable worst case situation (scenario 3b). The duration and frequency of exposure to TCEP are assumed to be daily and for the entire length of the shift.

Dermal exposure

For the use of formulations containing TCEP in small and medium sized chemical enterprises and the skilled-trade area as well as in enterprises belonging to other industrial areas it cannot be excluded that gloves are not regularly worn and that immediate dermal contacts occur during activities like preparation of paints and flame-retardant coatings, filling and cleaning activities. Taking into consideration that personal protective equipment is not generally worn during painting works, the estimation of dermal exposure levels is performed for the unprotected worker.

Spraying (scenario 3a)

The corresponding exposure for spraying techniques is assessed in application of the EASE model:

Input parameters: T = 20°C, direct handling, wide dispersive use, intermittent

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Exposure levels: 1 - 5 mg/cm^2/day.

Considering a TCEP content of ≤ 25 % and an exposed area of 840 cm^2 (hands) an exposure level of 210 - 1050 mg/person/day is obtained.

Dermal exposure during spray painting is due to the deposition of spray mist, back bouncing, contact with contaminated spray gun and possibly also with freshly painted surfaces. The estimates are based on an experimental study in 3 off-shore facilities where containers were painted (Lansink *et al.*, 1998) and on studies by HSE and IOM on airless spray application of antifouling paint (HSE, 1999). The Lansink *et al.* (1998) study involved 12 painters, using 3 - 13l of paint with a duration of 4 -21 minutes. A fluorescent tracer was added at 0.0074% (w/w). Exposure levels were presented based on the tracer and a linear extrapolation of exposure related to duration (3 hours, in which 150-200l could have been applied) was done to compare the study with the other studies. The HSE compilation included a total of 70 exposure data provided by 18 separate surveys. The amounts of paint used during spray sessions in the HSE document ranged between 25 and 800l and the spray session ranged from 40 to 360 minutes (median about 180 minutes). On the basis of the 90th percentile of the extrapolated results of Lansink *et al.* (1998) and the 95th percentile of the HSE data a reasonable worst case estimate of 10000 mg on an exposed area of 840 cm^2 was derived. In consideration of a concentration in formulations of ≤ 25 % dermal exposure through direct skin contact during spraying of the formulations is estimated to < 2500 mg/person/day.

Without the formation of droplet aerosols (scenario 3b)

Input parameters: T = 20°C, direct handling, non dispersive use, intermittent

Exposure levels: 0.1 - 1 mg/cm^2/day.

Considering a TCEP content of ≤ 25 % and an exposed area of 840 cm^2 (hands) an exposure level of 21 - 210 mg/person/day is obtained. The upper level is regarded to represent the reasonable worst case situation.

It cannot be presupposed that eye protection is regularly used. For assessing the risks, hand eye contacts as well as possible splashes to the eye should be considered.

Conclusions

For assessing the risk of daily dermal exposure during painting works and use of glues and adhesives, an exposure level of < 2500 mg/person/day should be taken (analogous data, scenario 3a). For uses without the formation of aerosols, dermal exposure is considerably lower: 210 mg/person/day (scenario 3b).

4.1.1.2.4 Summary

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In the EU, the production and import of Tris(2-chlorethyl)phosphate (TCEP) has declined in the past years. In the year 2001, there is no production within the EU, but marketing of TCEP-containing products is still relevant (app.1000 t of TCEP in 2002). The substance is currently produced Poland (amount and use pattern confidential). This is considered in scenario 1.

TCEP is used as a plasticizer with flame-retarding properties in polyurethane, polyester, polyvinyl chloride and other polymers as well as in formulations like paints, lacquers, glues, adhesives and flame-retardant coatings for textiles. The main use of TCEP is the production of unsaturated polyester resins. The use pattern has changed during the last years. One company supplied quantitative information about the application areas of their sales in 2001:

Unsaturated polyester resins	83 %
Acrylic resins	2 %
Adhesives	5 %
Coatings (wood and roofings)	<1 %
Polyurethane	<1 %
Others (not known)	9 %
Cellulose acetate	1 %
Textile	0 %

Due to the low vapour pressure of the substance (< 1 Pa at 20°C), relevant inhalation exposure is likely to occur only if the plastics are handled at elevated temperatures. Industry described, that the substance is neither handled at elevated temperatures nor is used in a powdery state.

For occupational exposure there are 3 main scenarios:

Scenario 1: Production of TECP

Scenario 2: Use of TCEP at ambient temperatures for the production of polymers and formulations

Scenario 3: Use of paints, lacquers, glues, adhesives and flame-retardant coatings.

Relevant inhalation and dermal exposure levels are given in table 4.1.1.6 A and 4.1.1.6 B, respectively. Since no measurement data are available EASE estimates are taken for exposure assessment.

TCEP is a component of different formulations like paints, lacquers, glues and adhesives (scenario 2). For the formulation processes, exposure relevant activities are filling, charging, cleaning, sampling, repair and maintenance activities as well as possibly mixing.

Exposure is to be expected when using paints, lacquers, glues, adhesives and flame-retardant coatings (scenario 3 a - c). The formulations are used in different industrial and skilled-trade areas. Industry states, that less than 10 t/y are applied in paints. During spray application of paints, adhesives and other formulations the formation of droplet aerosols is possible. This is considered in scenario 3a. Scenario 3b represents exposure for activities without the formation of aerosols.If foams containing TCEP are mechanically or thermally treated (e.g.

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cutting using hot wires), exposure is assumed to be below the level predicted for spray applications.

Dermal exposure is assessed for the unprotected worker in application of the EASE model or by taking analogous data into account (table 4.2).

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Table 4.1.1.6.A Summary of inhalation exposure levels (reasonable worst case) of tris(2-chlorethyl)phosphat which are relevant for occupational risk assessment

Inhalation exposure								
Exposure scenario	**Form of exposure**	**Activity**	**Duration [hs/day]**	**Frequency [days/year]**	**Shift average [mg/m³]**	**Method**	**Short-term exposure[mg/m³]**	**Method**
1. Production	Vapour	drumming, (de)coupling of transfer lines, cleaning, repair	shift length	daily	**1.2** [1)]	EASE	-	-
2. Formulation of paints, lacquers, glues and adhesives (resins), use of TCEP in the production of polymer formulations and products (polyurethane, cellulose acetate).	Vapour	charging, (de)coupling of transfer lines sampling, cleaning, repair, maintenance	shift length	daily	**1.2**	EASE	-	-
4. Use of paints, glues lacquers, adhesives, flame-retardant coatings (25 % TCEP)								
a) spray application	Droplet aerosol	spray application	shift length	daily	**8.3 (3a)**	analogous data	-	-
b) without formation of aerosols	vapour	different techniques, e.g. brushing, rolling	shift length	daily	**1.2 (3b)**	EASE	-	-

LEV: Local Exhaust Ventilation
[1)] The same exposure level is predeicted for workplaces without LEV (due to the low vapour pressure of the substance)

Table 4.1.1.6 B Summary of dermal exposure levels (reasonable worst case) of tris(2-chlorethyl)phosphate which are relevant for occupational risk assessment

Dermal exposure								
Exposure scenario	**Form of exposure**	**Activity**	**Frequency [days/year]**	**Contact level (1)**	**Level of exposure [mg/cm²/day]**	**Exposed area [cm²]**	**Shift average [mg/p/day]**	**Method (use of gloves)**
1. Production	Liquid	drumming, (de)coupling of transfer lines, cleaning, repair	daily	intermittent	1	420	**420**	EASE (unsuitable gloves)
2. Formulation of paints, lacquers, glues and adhesives (resins), use of TCEP in the production of polymer formulations and products (polyurethane, cellulose acetate).	Liquid	charging, (de)coupling of transfer lines sampling, cleaning, repair, maintenance	daily	intermittent	1	420	**420**	EASE (irregular use of gloves)
3. Use of resins, paints, glues lacquers, adhesives, flame-retardant coatings (25 % TCEP)								
a) spray application	Droplet aerosol	spray application	daily	intermittent	12 (3a)	840	**< 2500**	analogous data
b) without formation of aerosols	liquid, sticky material	different techniques, e.g. brushing, rolling	daily	intermittent	1 (3b)	840	**210**	EASE

(1) Contact level according to the EASE model

.

However, it has to be noted, that the applied model calculations are of preliminary nature and have to be revised as soon as further information becomes available.

4.1.1.3 Consumer exposure

The total scenario of TCEP consumer exposure is depicted in figure 4.1.1.3-1. According to several reports (Pardemann et al., 2000, Sagunski & Roßkamp, 2002, Kersten & Reich, 2003, Becker et al., 2002, Nagorka & Ullrich, 2003, Ingerowski et al., 2001), it is shown, that TCEP will be released from a number of sources which have been treated with flame retardant, namely timber, foam rubber, carpets, plastic materials (e.g. electronic devices, TV, car interior etc.), glues, and lacquers. Upholstery may sometimes be proved with flame retardant. Although there are measurements available of TCEP inside these materials, the degree of migration from the materials is not known.

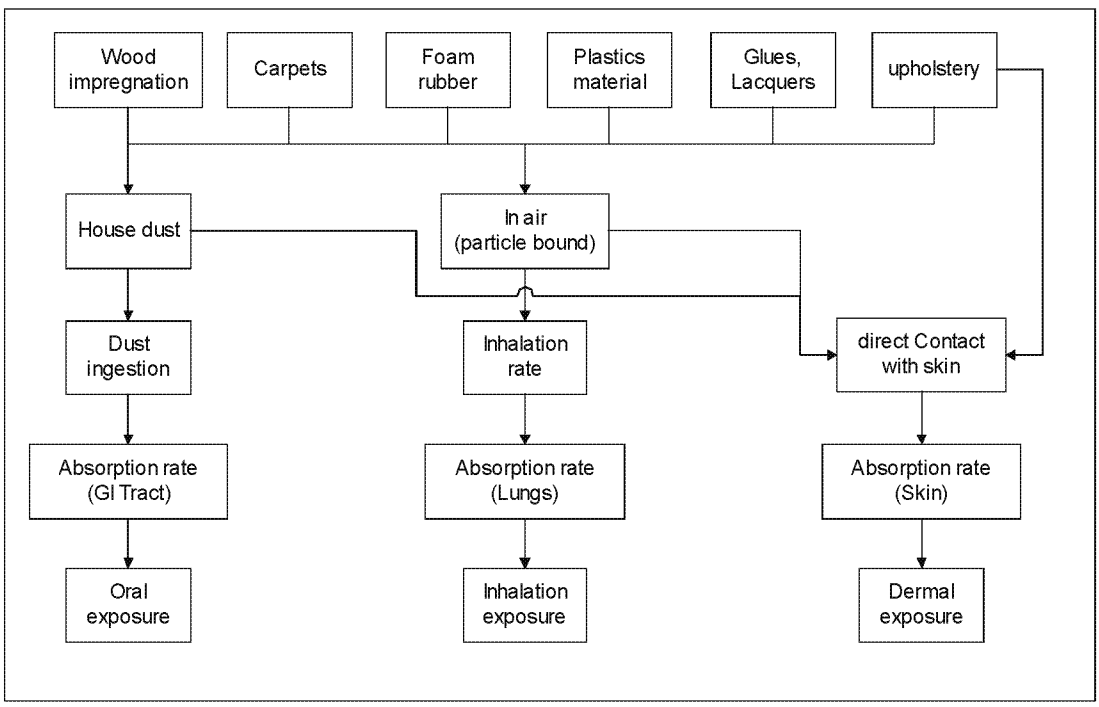

Fig 4.1.1.3-1: Scenario of TCEP exposure for reasonable worst case estimation, according to the above mentioned publications.

TCEP is a non-volatile substance, which does not appear in its gaseous form under normal conditions. Therefore, it is released primarily by abrasion and becomes part of the dust fraction. The latter is divided into two parts, house dust and airborne dust. Dust burden therefore reflects the sum of all the sources (Sagunski & Roßkamp, 2002).

Oral exposure can be referred to dust intake, due to hand-to-mouth behaviour, contamination of articles for daily use, e.g. toys which can be put into the mouth. This pathway of exposure may play a particular role for children and is covered by the hand-to-mouth activities. Inhalation exposure takes place by inhaling airborne particles, and dermal exposure can occur from direct contact with e.g. furniture coverings, as well as with house dust and airborne dust.

Absorption rates in this approach include the desorption of TCEP from dust and the subsequent absorption in the GI-tract or in the lungs and were set to 100% as a worst case approach.

Data on which this assessment is based on

First of all, a study performed by Sagunski et al. (1997) gives an overview on the exposure paths "house dust" and "airborne particles". In this study, house dust was sampled by typical sampling methods taking vacuum cleaners, and the airborne particles were sampled by adsorbing it to polyurethane by drawing air through a sampling advice. This method samples particles as well as possible gaseous TCEP.

House dust:

In an interlaboratory comparative study published by Ingerowski et al. (2001), TCEP dust concentrations have been measured in appr. 1000 German households by three different laboratories using identical methodology (Fig. 4.1.1.3-2). These data and the data from Sagunski (1997) correspond to each other. The range of dust concentration (between zero and 121 µg/kg) is also in agreement with a number of other - smaller - studies (Becker et al. (2002), Bürgi (2002), Hansen et al. (2000), Kersten & Reich (2003), Marklund et al., 2003, Salthammer & Wensing (2002)).

The data published by Ingerowski et al. were taken to evaluate of their distribution for further probabilistic exposure assessment by using the @RISK 4.5 professional tool and MS-EXCEL. The distribution function for TCEP in house dust was best fitted by a log-logistic distribution[8] as shown in Fig. 4.1.1.3-2. The 95th percentile of this distribution is 11.9 mg/kg, and the median 0.6 mg/kg. This distribution covers also the highest values reported by Marklund et al. (2003) in libraries and the other studies mentioned.

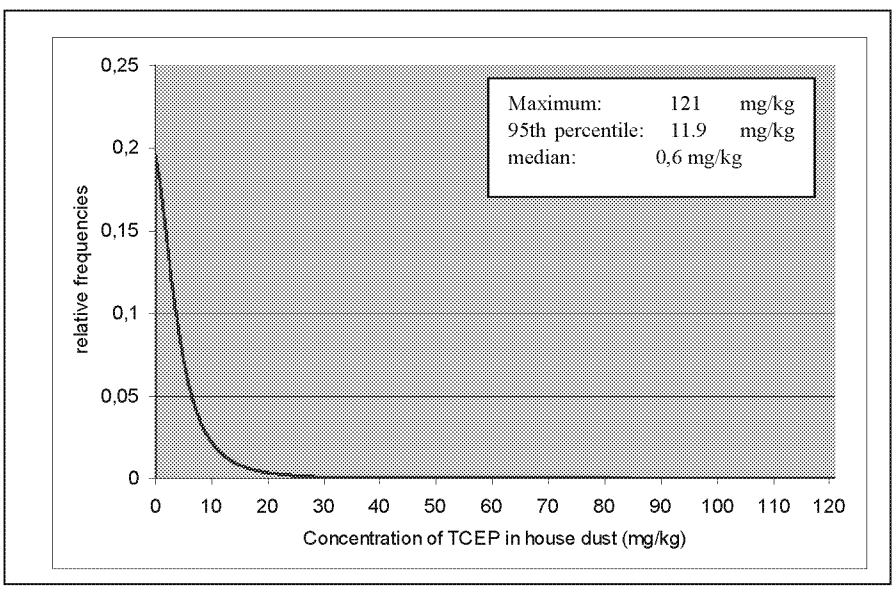

Fig 4.1.1.3-2: Distribution of TCEP concentration results in 983 dust samples taken in German households and analysed in three different labs (Ingerowski, 2001).

It can be assumed that the data give a sound overview about TCEP dust contamination, which can be used for oral exposure assessment by taking commonly accepted dust uptake data.

Airborne dust:

The range of airborne dust concentrations of TCEP as determined in the same study lies between zero and a maximum of 6000 ng/m³, which is in agreement with other authors (Hansen et al., 2000, Bürgi, 2002).

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[8] RiskLoglogisticAlt(50%;0,61;90%;8,02; 95%;11,86; RiskTruncate(0;121))

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The check for best fit of the data revealed a log-normal distribution[9] with a 95th percentile of 134 ng/m³, and a median of 10 ng/m³. This distribution covers also room air concentrations of max. 30 ng/m³ published by Otake et al. (2001), without specification of dust adsorption, as well as those measured in cars by Wensing et al. (2003).

Toys: From Danish EPA the content of TCEP in a cube for babies was analysed. The cube is intended as a toy for babies (recommended for 0 months and above) to stimulate their senses and their exploring behaviour.This cube is 10 x 10 x 10 cm and weight 100 g. The core of the cube consists of PUR foam. The foam is covered with coloured textile with child motives and at the edges of the cube there are five textile handles. At the handles there are three small plastic rings attached for rattling. A little bell is placed in the middle of the cube imbedded in the PUR foam. About half of the weight of the total cube is estimated to be PUR foam.

Analytical data:
The constituents of the cube were analysed separately. PUR foam had a content of 3300 and 5200 mg TCEP/kg, whereas the textile covering the cube had a content of 160 mg TCEP/kg. A migration test in an aqueous medium has shown that the TCEP is easily dissolved and migrates into the solution.

Exposure assessment:

TCEP exposure was assessed using a probabilistic approach based on the studies mentioned above. Calculations have been performed taking the @RISK-4.5-professional software tool in combination with MS EXCEL.

Oral exposure

Uptake from house dust

TCEP uptake was calculated by the formula

$$E_{TCEP(oral)} = \frac{C_{TCEP,\ dust} * I_{orl,\ dust}}{BW}$$

where $C_{TCEP,\ dust}$ is the dust concentration, $I_{orl,dust}$ is the uptake of dust, and BW is the body weight. According to the age categories of the AUH Report (1995), the oral exposure was estimated for a 1-3 year old child and for a female adult (> 20 years). The dust uptake and body weight[10] data (normal distribution, weighted for 1 to 3 year of age) are taken from the AUH Report (1995). According to these data, the values for this assessment were set as follows: normal dust uptake is set to 20 mg/d and 100 mg/d as upper limit of normal uptake of dust by children. For the adult, the respective values are 2 and 10 mg/d.

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[9] @RISK formula: RiskLogNormalAlt(10%;5; 50%;10; 90%;40; RiskTruncate(0;6000))

[10] body weight: RiskNormalAlt(50%;9,1;5%;7,6;RiskTruncate(0;)) (children)
RiskNormalAlt(50%;58;5%;47;RiskTruncate(0;)) (adults)

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This estimation of uptake includes soil uptake and therefore leads to a slight overestimate of exposure via dust. It should be mentioned that the upper range of the uptake determined by Calabrese is in agreement with newer data obtained by Freeman & Adgate (2003) who found a daily dust uptake of 100 mg in small children.

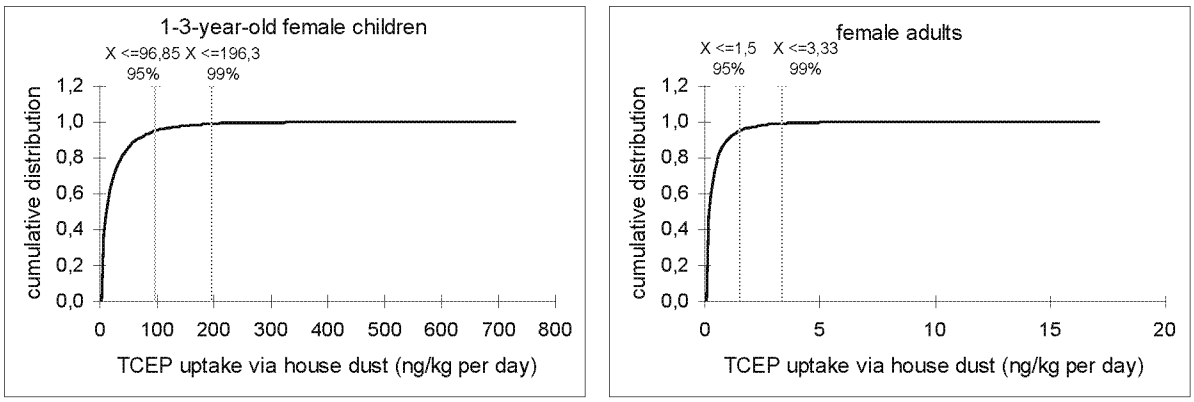

Fig. 4.1.1.3-3: Distributions of oral TCEP uptake from house dust ingestion in three-year-old children and female adults. The results are obtained by taking distribution-based analysis.

As shown in Fig. 4.1.1.3-3, the distribution of TCEP oral uptake is characterised by an extended slope on the right. The 95th and 99th percentile and the maximum are 0.0015, 0.0033 and 0.017 µg/kg per day for female adults. The respective values for children, representing a vulnerable population due to their specific hand-mouth-behaviour are 0.1, 0.2, and 0.7 µg/kg and day, respectively.

Uptake by sucking on toy

It is difficult to estimate the TCEP exposure of children sucking on toys containing TCEP. The rate of migration is affected by factors such as the relative solubility of TCEP in PUR foam and textile and in saliva, the temperature, thickness of textile or foam layer and the physical forces acting on the foam and textile. From a migration test in aqueous media (artificial sweat) it has been found that TCEP easily migrates from the foam cube into the solution. But there are no data available.

Danish calculation (Danish EPA, 2004)

As an exposure scenario it is assumed that a baby of about 3 months plays with a cube during 3 months. Due to mouthing behaviour the cube is sucked intensively and during the 90 days of play about 50% of the total amount of the TCEP -as a worst case scenario- is anticipated to be dissolved and swallowed by the baby. Anticipating an average weight of the baby/ infant of 6 kg during the three month and a total TCEP content of 260 mg (50 g PUR foam with a content of 5200 mg TCEP/kg) a daily dose of 0.24 mg TCEP/ kg bw/d can be calculated (0.5 (total release factor) x 260 mg TCEP / (90 d x 6 kg)).

Discussion

The Danish calculation considers that the TCEP from the foam will migrate through the textile layer during sucking. If this migration is not considered and is only taken the measured TCEP of 8 mg in the textile a daily dose of 7.4 µg TCEP/ kg bw/d can be calculated.

Inhalation exposure

Inhalation exposure to TCEP was calculated by the formula

$$E_{TCEP(inh)} = \frac{C_{air} * Q_{inh}}{BW}$$

where C_{air} is the distribution of the concentration of TCEP in airborne dust, obtained from the Ingerowski et al. study, and Q_{inh} is the respiration volume. The distribution of Q_{inh} was set according to the data given in the AUH report which are based primarily on data published by Finley et al. and in the EPA-Exposure factors handbook.

The evaluation of TCEP uptake by inhalation taking the same approach as described above for dust uptake, revealed a 95th, 99th percentile and a maximum value of 0.07, 0.96, and 3.9 µg/kg per day TCEP for a 3 year old child, and 0.03, 0.4, and 1.4 µg/kg per day for the adult person, respectively. These estimations are in agreement with estimations published elsewhere (Salthammer & Wensing, 2002). Assuming that TCEP will be desorbed totally from the particles and absorbed in the lung alveoles the upper range of this concentration should be taken for characterising a possible risk from inhalation.

Dermal exposure

Textiles (upholstered furniture)

According to measurements which have been performed by Bruckert et al. (1990) an amount of ≈ 130 µg/25 cm² per 24 hours (= 0.217 µg/cm²/h) of tris(2-chloroethyl)phosphate can be released from upholstery containing an amount of 8 mg/cm².

The area of a person sitting in an armchair covered by textiles accounts for about 1000 cm² (half of both forearms and hands). A four hour contact (e.g. during watching TV) would lead to an amount of ≈ 870 µg/event (= 0.217 µg/cm²/h * 4 h * 1000 cm²). Taking 100 events into account, the yearly total amount of TCEP migrating from an upholstered armchair to the skin accounts for 87000 µg, resulting in an average exposure of ~ 3.9 µg/kg of BW per day, under consideration of a body weight of 60 kg.

For 1-3 year old children, the dermal external contact reveals an amount of 330 µg/event, taking the same conditions as for female adults (0.217 µg/cm²/h * 4 h *380 cm²). Under these conditions, the dermal exposure would account for 10 µg/kg per day (body weight 9.1 kg).

Dermal exposure by airborne dust

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The dermal exposure to airborne dust can be neglected due to the very low concentration of 0.6 µg/m³ (the 98th percentile for air concentrations of TCEP), with a contact volume of max. 184 cm³ (calculated by multiplying the total body surface of 18.400 cm² (TGD default) with thickness on skin of 0.01 cm (also TGD default). Under these conditions the amount of TCEP in contact with skin is below the nanogram-range.

Dermal exposure by house dust contact

Freeman & Adgate (2003) have estimated a maximum load of ~ 5.0 mg of dust per hand in 1 - 4 year old children. Taking the 98th percentile of TCEP dust concentration reported by Ingerowski et al. (18 ng/mg of house dust), than the total dermal exposure via this pathway would account for ~ 0.18 µg per day (both hands). This value is only applicable for children; the burden resulting from this estimation is 0.018 µg/kg of body weight, considering a child having a bodyweight of 10 kg.

Conclusions

Inhalation:

Taken from the measurements performed by Ingerowski et al. (2001) the 98th percentile for air concentrations of TCEP is 0.6 µg/m³. This value lies at the extreme upper range of a huge number of measurements. Although other studies have revealed lower values, due to the high number of measurements, this value is proposed to be taken as a reasonable worst case estimate. For risk characterisation it should be taken into account that the major part is bound to dust and the degree of desorption is unknown.

The model estimate revealed an uptake by inhalation (99th percentiles) of TCEP accounts for 0.4 for adults and 0.96 µg/kg bw and day for children, under consideration of the above mentioned precautions (100% absorption).

Dermal exposure:

The worst case estimation of TCEP by exposure from migration from upholstery accounts for 3.9 µg/kg bw and day. Dermal exposure from airborne dust can be neglected due to the low concentrations of TCEP in dust (max. 0.1 µg/m³). Dermal exposure from house dust accounts for a maximum of 0.02 µg/kg bw per day (in children).

Dermal exposure to TCPE from different sources can be estimated to a total of about ~ 4 µg/kg bw per day. Dermal exposure of children (10 µg/kg bw/day) as related to bodyweight can exceed that in adults.

Oral exposure:

Oral exposure of TCEP is characterised by dust uptake. The estimated amount accounts for 0.0033 µg/kg bw/day for an adult, representing the 99th percentile. Because this pathway may represent a significant source of exposure for children, the 99th percentile representing daily uptake was also estimated for a three year old child and revealed 0.2 µg/kg bw/day.

For babies of about 3 months the significant source could be sucking at a toy. Under worst worst case assumptions values can be achieved up to 240 µg/kg bw per day (Danish calculation).

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Total body burdens:

For female adults, a body burden would account for ~ 4.5 µg/kg bw/day, under reasonable worst case conditions, and taking all paths into consideration. The respective value for 1 - 3 year-old children is then 11 µg/kg bw/day. However, for babies of about 3 months a body burden would account up to 240 µg/kg bw per day by sucking on toys, the other paths can be neglected.

4.1.1.4 Indirect exposure via the environment

According to Appendix VII of Chapter 2 of the TGD, the indirect exposure to humans via the environment through food, drinking water and air is estimated for a local and a regional approach. For the local concentrations the default scenario for the formulation of paints is used, representing the local worst case. This scenario is compared to an average intake due to exposure via the regional background concentration. In the Appendix 3, the input data and results of the calculations are presented.

The following input parameters were selected:

annual average local PEC in surface water:	13.59 µg/l
annual average local PEC in air:	0.0038 µg/m³
local PEC in grassland:	8.66 µg/kg
local PEC in porewater of agricultural soil:	18.1 µg/l
local PEC in porewater of grassland:	4.18 µg/l
local PEC in groundwater under agricultural soil:	18.1 µg/l
regional PEC in surface water:	0.0871 µg/l
regional PEC in air: ng/m³	2.27×10^{-4}
regional PEC in agricultural soil:	0.061 µg/kg
regional PEC in pore water of agriculture soil:	0.0295 µg/l

The resulting total daily doses are:

$$\mathbf{DOSE_{tot\_local} = 5.842\ \mu g \cdot kg_{b.w.}^{-1} \cdot d^{-1}}$$

$$\mathbf{DOSE_{tot\_regional} = 0.0111\ \mu g \cdot kg_{b.w.}^{-1} \cdot d^{-1}}$$

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The calculated doses comprise the following routes:

Table 4.6

Route	regional model, percentage of total dose	local source model; percentage of total dose
drinking water	22.4	8.85
Air	<0.01	0.01
Stem	66.8	85.9
Root	2.38	2.77
Meat	<0.01	<0.01
Milk	0.02	<0.01
Fish	8.38	2.48

The main route of exposure is the stem for the regional and local approach.

However, it has to be noted, that the applied model calculations are of preliminary nature (i.e. according to TGD "state of the art" methods serving screening purposes) and have to be revised as soon as further information becomes available.

4.1.1.5 (Combined exposure)

4.1.2 Effects assessment: Hazard identification and Dose (concentration) - response (effect) assessment

4.1.2.1 Toxico-kinetics, metabolism and distribution

4.1.2.1.1 Studies in animals

<u>In vivo studies</u>

Oral

Greater than 90% of the orally administered radioactivity (88 mg C14-tris(2-chlorethyl)phosphate (TCEP)kg bw,) in rats were excreted in the urine within 72 hours; 7% via faeces and 1% as carbon dioxide. Elimination from plasma and red blood cells occurred biphasic (half life 3 and 3.4 hours and 1.8 and 10.8 days, respectively). At 0.5 and 4 hours radioactivity was evenly distributed throughout the tissues. Higher concentrations were found in liver, kidney, fat and GI contents (Chadwick et al., 1989).

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In an other study in male Wistar rats (5 animals per group) 93% excretion of the orally applied dose (14 mg C14-TCEP/kg bw) in urine, within 168 hours was observed. 6% of the dose was recovered in the feces and 1% in the expired air. The distribution of radioactivity in the tissues was measured 24h after dosing. Lower concentrations of radioactivity were found in brain, muscle, testis, spleen and lung, higher concentrations in liver and kidney. The biliary/fecal ratio was 4.62 at 48 hours. This suggests reabsorption of radioactivity from the gastrointestinal tract. It was supposed that enterohepatic circulation of TCEP occurs (Minegishi et al., 1988).

In a further study on regional brain levels of TCEP male and female F344 rats (3 rats per group) were orally administered 0; 175; 350 or 700 mg/kg bw of the C14-labeled substance as single dosage or 0; 175 and 350 mg/kg bw/d daily within 14 days in females only. The substance was well absorbed from the gastrointestinal tract and distributed in all cerebral regions. Metabolism and excretion nearly totally completed within 72 hours following single or repeated doses. As single dosage (175 mg/kg bw) applied radioactivity was excreted with urine (85%), faeces (less than 10%) and as carbon dioxide. Accumulation of radioactivity was not detected in any cerebral region. After dosage of 350 mg/kg bw acceleration of metabolism and elimination was observed in males whilst in females slightly enhanced plasma concentrations were detected within first minutes post-dosing (Herr et al., 1991).

In a metabolic study male and female B6C3F1 mice and F344 rats were dosed (175 mg/kg bw) with the C14-labeled TCEP either as single dosage or daily within 9 days in rats only (3 mice and 4 rats per group, respectively). In both species excretion within 24 hours was via urine (more than 75%) and faeces (less than 10%). Sex differences in the elimination rate of rats were not detected. Elimination within the first 4 to 8 hours occurred three times faster in mice compared to rats (>70% versus about 40%). Repeated application did not result in a change of metabolism and elimination rate. Metabolites in urine were identical in both species. Main metabolites were bis(2-chloroethyl)carboxymethylphosphate, bis(2-chloroethyl)hydrogen phosphate and bis(2-chloroethyl-2-hydroxyethyl-phosphate glucuronide (Sanders et al., 1990; Burka et al., 1991).

Inhalation

No animal data available.

Dermal

No animal data available.

<u>In vitro studies</u>

In an in vitro study on the metabolism of C14-labeled TCEP in liver slices and liver microsomes by humans and rats liver preparations bis(2-chloroethyl)hydrogen phosphate, 2-chloroethanol were identified as main metabolites. Three further compounds could be found, their structure was not identified. The most important finding of this study is a marked difference in the rate of metabolism and the metabolic pattern between male and female rat microsomes, i.e. male rat liver microsomes metabolized TCEP at a greater rate than did female rat liver microsomes. This difference was not observed in liver slices which gave rise to the assumption of an extramicrosomal metabolism. As in human liver slices and in human

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liver microsomes no gender differences in metabolism were observed the finding of gender related differences in rat seems not to have taken into consideration for the risk assessment (Chapman et al., 1991).

4.1.2.1.2 Human data

oral, inhalativ, dermal

No human data available.

4.1.2.1.3 Conclusion

No toxicokinetic data on TCEP have been reported for humans. The substance is well absorbed (> 90% of the dose) and distributed in rats after oral administration. Higher concentrations were found in liver and kidney up to 24h after administration. An enterohepatic circulation is supposed to occur. Elimination from plasma and red blood cells occurred biphasic with a half-life of 3 and 3.4 hours in the beginning and 1.8 and 10.8 days in the second phase. Metabolism and elimination are the same after single and repeated application. Metabolites in urine were identical in rats and mice. Main metabolites were bis(2-chloroethyl) carboxymethylphosphate, bis (2-chloroethyl)hydrogen phosphate and bis(2-chloroethyl) -2-hydroxyethyl-phosphate glucuronide. In the risk characterisation, the rates of oral, dermal, and inhalation absorption are assumed to be 100%.

4.1.2.2 Acute toxicity

4.1.2.2.1 Studies in animals

Oral

Tris(2-chloroethyl)phosphate exhibits moderate acute oral toxicity, with oral LD50 values for female and male rats detected within the range of 430 mg/kg bw (Stauffer Chemical Company, unpublished report 1972) and 1230 mg/kg bw (Ulsamer et al., 1980): The older studies used different lots of a test substance called "Fyrol CEF" of undefined purity. In a study performed according to GLP and to current international guidelines an oral LD50 of 1182 mg/kg body weight resulted for male rats and an oral LD50 of 1123 mg/kg for females. Doses of 800, 1000, and 1260 mg/kg bw were administered orally to groups of 5 male and 5 female rats/dose, using water as vehicle. Deaths (one female at 1000 mg/kg and 4 females and 4 males at 1260 mg/kg) occurred on days 2-4. As clinical signs, pilo-erection and increased salivation were observed in all dose groups; hunced posture, abnormal gait, lethargy, decreased respiratory rate, ptosis, pallor of the extremities were observed at 1000 and 1260 mg/kg bw only. Survivors recovered within 4 days. Necropsy of the rats that died during the study revealed a very pale cortex in the left kidney of one female dosed at 1260 mg/kg bw; no other macroscopic abnormalities were detected (Huntingdon Research Center, unpublished report 1990).

Inhalation

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In a test with rats exposed to an atmosphere saturated with tris(2-chloroethyl)phosphate aerosols (creation of a mist by bubbling air through the sample held at the arbitrary temperature of 170°C, cooling the air, and furnishing it to animals in a small chamber), no rats died after an 8-hours inhalation time (Smyth et al., 1951). In a limit test, five male and five female rats were exposed to a nominal concentration of 25.7 mg/L for 1 hour in a 32 L positive pressure inhalation chamber. The test material was generated by means of a midget impinger. The test animals were observed for 14 days. No mortality resulted, rats revealed moderate lacrymation and salivation, all animals appeared normal within 3 hours (Stauffer Chemical Company, unpublished report 1974).

Dermal

In a limit test using three different lots of undiluted tris(2-chloroethyl)phosphate (no information on purity), four rabbits were each exposed dermally to 2150 mg/kg bw for 24 hours, using occlusive patches. The animals were observed 14 days for mortalities and signs of toxicity. No apparent signs of toxicity were observed. No cholinesterase depression was observed in any of the rabbits 72 hours after treatment (Stauffer Chemical Company, unpublished report 1972).

4.1.2.2.2 Human data

No human data available.

4.1.2.2.3 Conclusion

Tris(2-chloroethyl)phosphate has demonstrated moderate toxicity after oral application, with oral LD50 for rats in the range of 430-1230 mg/kg bw. The inhalation toxicity seems to be low as judged on the basis of test results with rats that survived an 8-hours exposure to saturated substance aerosols or an 1-hour exposure to a nominal concentration of 25.7 mg/L. Acute dermal toxicity in the rabbit is low, the dermal LD50 value was detected to be > 2150 mg/kg bw.

Information on human experience with tris(2-chloroethyl)phosphate is not available. The substance is to be classified as "harmful" according to EEC classification guidelines, labeling with "R 22, Harmful if swallowed" is appropriate.

4.1.2.3 Irritation

4.1.2.3.1 Skin

Animal studies

Abraded and intact skin of 9 albino rabbits was occlusively exposed to 0.5 ml of three lots of unknown purity of the undiluted substance/animal for 24 hours. Results for intact skin: No edema, but erythema grade 1 after 24 hours for all three lots. Results for abraded skin: None of the rabbits demonstrated edema after 24 hours, but all rabbits revealed edema after 72 hours; erythema grad 1-2 were seen in nearly all of the animals at 24 and 72 hours observation. No data on reversibility of these effects and no information on the duration of the study are mentioned (Stauffer Chemical Company, unpublished report 1972).

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The skin of three albino rabbits was semi-occlusively exposed to 0.5 ml of the undiluted substance (purity > 99%) per animal for 4 hours in a Draize test performed according to GLP and to international test guidelines (EECB.4/OECD 404). Result: All rabbits revealed slight erythema that reversed within 24 hours (Huntingdon Research Center, unpublished report 1991; Hoechst AG, unpublished report 1988a). In a second test according to test guidelines EECB.4/OECD 404 the skin of 3 albino rabbits was semi-occlusively exposed to 0.5 ml of the undiluted substance (purity 99.5%) per animal for 4 hours. Result: 1/3 rabbits revealed slight erythema grade 1 that reversed within 24 hours (Hoechst AG, unpublished report 1988a).

Human data

No human data are available.

4.1.2.3.2 Eye

Studies in animals

In a Draize test according to GLP and to international test guidelines (EECB.5) instillation of 0.1 ml of undiluted tris(2-chloroethyl)phosphate (purity > 99%) into the eyes of each of three rabbits resulted in mild conjunctival irritation (edema grade 1 in all animals on day 1, conjunctival redness grade 1 in all animals for a period of 1 or 2 days). Three days after instillation all effects had reversed. No corneal damage or iridial inflammation was seen (Huntingdon Research Center, unpublished report 1991b). Similar results demonstrated a second study according to GLP and to international guidelines (EECB.5/OECD TG 405): The installation of 0.1 ml of the undiluted substance (purity 99.5%) resulted in weak conjunctival irritation (oedema grade 1 in 2/3 animals on day 1, conjunctival redness grade 2 in all rabbits on day 1). Twenty four hours after instillation all effects had reversed (Hoechst AG, unpublished report 1988b). In a study performed in 1972 with three different lots of unknown purity, no local effects on rabbit eyes were detected (Stauffer Chemical Company, unpublished report 1972).

Human data

No human data available.

4.1.2.3.3 Conclusion

Human data on local irritant substance properties are not available. Tris(2-chloroethyl)phosphate exhibits only weak local irritation to skin and to the conjunctivae of the eyes of rabbits. Thus, TCEP is not considered to be a skin and eye irritant.

4.1.2.4 Corrosivity

Animal studies

From the data presented in the preceding text it is evident that tris(2-chloroethyl)phosphate is not a corrosive substance.

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Human data

No human data available.

Conclusion

Tris(2-chloroethyl)phosphate is not a corrosive substance, as judged on the basis of animal studies presented in the preceding text.

4.1.2.5 Sensitisation

4.1.2.5.1 Studies in animals

The skin sensitisation potential of TCEP was evaluated with the Buehler method (Mobil (1983) as cited by BG Chemie, 1995). Guinea pigs (5 animals each per sex) were induced once per week with the undiluted substance over three weeks. Two weeks after the 3rd induction the animals were challenged with the undiluted substance. No allergic reactions were seen (oberservation time 24 or 48 h). No further data are available.

4.1.2.5.2 Human data

No human data available.

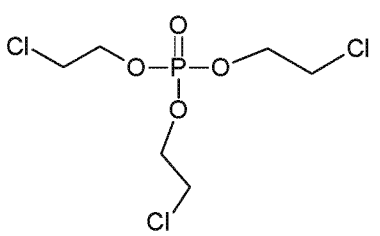
4.1.2.5.3 Data available on structurally related chloroalkyl phosphates

Tris(2-chloroethyl) phosphate shows structural analogy to the two priority substances Tris(2-chloro-1-methylethyl) phosphate and Tris[2-chloro-1-(chloromethyl)ethyl] phosphate being assessed by Ireland/UK (cf. EU RAR TCPP (2006), EU RAR TDCP (2006). Therefore, information about these chloroalkyl phosphate esters is included here and will be used for a read-across approach to assess the skin sensitisation potential of TCEP.

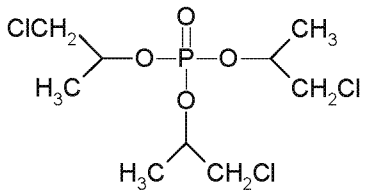
4.1.2.5.3.1 Chemical structures

The substance structures are shown below.

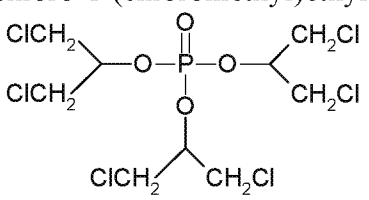
Tris(2-chloroethyl) phosphate (TCEP)



Tris(2-chloro-1-methylethyl) phosphate (TCPP)



Tris[2-chloro-1-(chloromethyl)ethyl] phosphate (TDCP)



4.1.2.5.3.2 Physico-chemical and alkylating properties

Selected physico-chemical properties of the three chloroalkyl phosphates used for the read-across are listed in table 4.7. The molecular weights and logPow values are in the same range, whereas solubility decreases in the order from TCEP to TDCP.

Table 4.7

Selected physico-chemical properties of TCEP, TCPP and TDCP

	TCEP	TCPP	TDCP
Molecular weight (g/mol)	285	327	430
Water solubility (mg/l, 20°C)	7820	1080	18.1
Partition coefficient (LogPow)	1.78	2.68	3.69

The alkylating properties of chloroalkyl phoshates have been investigated by several authors, e.g. Crook and Haggis (1969), Bissell (1977), Levchik et al. (2005). The results show that TCEP, TCPP and TDCP tend to N-alkylate aromatic amines and the urethane structure of PU foams at higher temperatures (> 140°C) under special reaction conditions. But it is unlikely that such reactions will occur with primary aliphatic amines at lower temperatures in aqueous media (Belke et al., 2003). Thus, it can be concluded that N-alkylation reactions at the nitrogen atoms of the polypeptide backbone or of ε-amino groups of proteins will not occur at body temperature in physiological media. Therefore, the potential of TCEP and analogues to be bound covalently to proteins via alkylation can be regarded as negligible.

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4.1.2.5.3.3 Animal data

Tris(2-chloro-1-methylethyl)phosphate (TCPP)

No evidence of skin sensitisation was found in a 1979 study (SafePharm, 1979). Following a range-finding test, 0.1 mls of a 5% solution of TCPP was selected for intradermal induction followed after 24 hours, by application of undiluted test substance for 48 hours, as the topical induction. 10% sodium lauryl sulphate was applied 24 hours prior to the topical induction. 10 guinea pigs were treated with TCPP and 4 remained untreated as controls. Two weeks after topical induction, the neat test substance was applied for 24 hours under occlusive dressing. There was no significant response after challenge. While this study is not GLP compliant (performed in 1979), its result is considered to be acceptable as a negative result.

In a GLP-compliant Local Lymph Node Assay (LLNA) conducted in accordance with OECD Guideline No. 429 TCPP was considered to be a non-sensitiser (EU RAR TCPP, 2006). Groups of four CBA/Ca mice were treated with 25 µl undiluted TCPP or concentrations of 50% or 25% v/v in acetone:olive oil 4:1 for three days. A further group of four mice received the vehicle alone. Five days following the first topical application, all mice were injected via the tail vein with 250 µl of phosphate buffered saline (PBS) containing a total of 20 µCi ^{3}H-methyl thymidine (specific activity 2.0 Ci/mmol). All mice were terminated five hours after injection. Stimulation indices of 1.55, 1.97 and 1.56 were recorded for concentrations of 25, 50 and 100% (v/v), respectively.

In two personal communications from occupational physicians from two EU TCPP manufacturing sites, the Irish rapporteur was informed that there has been no evidence of skin sensitisation among workers.

Also, in a personal communication from the regulatory affairs manager of a US-based TCPP manufacturing site, the Irish rapporteur was informed that worker medical records at the site for 13 years preceeding 2001 show no evidence of health effects, including skin sensitisation, associated with exposure to and handling of TCPP.

In conclusion, evidence from a guinea pig study as well as from a local lymph node assay indicates that TCPP does not possess significant skin sensitisation potential. No information is available on the respiratory sensitisation potential of TCPP.

Tris[2-chloro-1-(chloromethyl)ethyl]phosphate (TDCP)

In a well-reported guinea pig maximisation test conducted to OECD guidelines, TDCP showed no evidence of dermal sensitisation (Manciaux, 2001, EU RAR TDCP, 2006). A group of 20 test animals received an intradermal injection of 25% TDCP in corn oil and a topical application of 100% TDCP, preceded by a topical application of 10% sodium lauryl sulphate (administered on induction day 7). 10 control animals received vehicle only. Subsequent dermal challenge with 100% TDCP resulted in no signs of erythema or oedema in any of the test or control animals. A positive control study was included using mercaptobenzothiazole, which gave appropriate responses.

In conclusion, evidence from a study in guinea pigs indicates that TDCP does not possess significant skin sensitisation potential. No information is available on the respiratory sensitisation potential of TDCP.

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4.1.2.5.4 Conclusion

Human data on sensitizing properties of tris(2-chloroethyl)phosphate are not available. An animal skin sensitisation study (Buehler Test) showed no skin sensitizing potential of TCEP. No further testing is recommended based on read across from sensitisation data of the two other chloroalkyl phosphate esters TCPP and TDCP (substances of the 4th EU Priority list) which represent structurally related substances and have been tested in guinea pigs and in a local lymph node assay. The results indicate that these substances do not possess significant skin sensitisation potential.

Based on all information on the three structurally related chloroalkyl phosphates (results of animal testing, similarity in physicochemical data and chemical structures, as well as alkylating properties of TCEP, TCPP and TDCP) it is concluded that TCEP should be non-sensitizing to humans.

No information is available on the respiratory sensitisation potential of TCEP and the other two chloroalkyl phosphates.

4.1.2.6 Repeated dose toxicity

4.1.2.6.1 Animal data

The most reliable repeated dose toxicity studies used the oral route (gavage and feeding) of exposure to tris(2-chloroethyl)phosphate. Results of these studies give the indication that brain and kidney are the main target organs of toxicity, although there is a species and sex-related variability. There is also clear evidence that mice were less sensitive to the effects of tris(2-chloroethyl)phosphate than rats. Results of studies with repeated application of tris(2-chloroethyl)phosphate are summarized in Table 4.11 Several experimental investigations are long-term/long-life studies designed for examination of carcinogenicity. Therefore, for long-term studies see also 4.1.2.8.

4.1.2.6.1.1 General endpoints

- ***Oral***

Gavage studies (rat and mouse)

In order to evaluate toxicological effects occurring as a result of repeated daily dosing with tris(2-chloroethyl)phosphate for expected lifespan, and to determine the concentrations for subsequent carcinogenicity trials 14-day and 16-week studies in rats and mice were performed. Since many organophosphorous chemicals demonstrate neurotoxic activity by inhibiting the enzyme cholinesterase, the ability of tris(2-chloroethyl)phosphate to inhibit this enzyme was also studied (Matthews 1990, NTP 1991). In these studies tris(2-chloroethyl)phosphate was administered once per day, 5 days/week by gavage in corn oil.

14-day studies

<u>Rats</u>

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Groups of 5 male and 5 female F344/N rats (8-9 weeks old) received tris(2-chloroethyl)phosphate (purity 98%) in doses of 0, 22, 44, 88, 175, and 350 mg/kg bw/d for 12 doses over 16 days. No mortality due to tris(2-chloroethyl)phosphate, no changes of body weight gain and no clinical signs of toxicity and/or neurotoxicity were observed in both sexes up to 350 mg/kg bw/d. At 175 and 350 mg/kg bw/d the mean absolute and relative kidney weights of male rats were 10 and 12% higher than those of the controls, respectively. Liver weights of females in the 350 mg/kg bw/d dose group were significantly (17%) higher than those of the controls. In addition a significant decrease of absolute and relative lung weights in female rats receiving 88 to 350 mg/kg bw/d ($p \leq 0.05$) were seen. Nevertheless all organ weight changes were observed without further corroborating findings, therefore these findings were not considered to be of toxicological significance. No other organ weight variations attributable to tris(2-chloroethyl)phosphate were observed in rats of both sexes. Cholinesterase activity determined on fresh sera collected at necropsy was not reduced in dosed male rats, but was decreased by 18% and 20% in females at 175 ($p \leq 0.01$) and 350 mg/kg bw/d ($p \leq 0.05$) tris(2-chloroethyl)phosphate. This finding was only considered as a biomarker of exposure, but not considered as toxicologically relevant because statistically significant inhibition of plasma ChE of ≥20% is regarded as toxicologically adverse. (Based on the published policies on cholinesterase inhibition from WHO/UNEP (1990), U.S.EPA (Sette, 1997; Dorsey, 1997) and JMPR (Reprt, 1998) a ststistical significant AchE inhibition of ≥ 20% is regarded as toxicologically relevant ("adverse"). . No gross or histopathologic lesions were observed at any dose level.

The NOAEL for male and for female rats was 350 mg/kg bw/d (NTP 1991, Matthews 1990).

Mice

Groups of five B6C3F1 mice (9-10 weeks old) per sex were treated by gavage with 0, 44, 88, 175, 350, and 700 mg/kg bw/d tris(2-chloroethyl)phosphate (purity 98%) for 12 doses over 16 days. There were no treatment-related effects on mortality, body weight gain, absolute and relative organ weight or histopathological abnormalities up to 700 mg/kg bw/d. Gavage trauma accounted for the deaths of two males (each one at 175 mg/kg bw/d and 350 mg/kg bw/d) and of one female at 700 mg/kg bw/d. Mice of both sexes given 350 and 700 mg/kg bw/d tris(2-chloroethyl)phosphate exhibited ataxia and convulsive movements during the first 3 days of dosing. These symptoms did not persist after the first three days (no more data). In treated mice of both sexes serum cholinesterase activity was similar to that of controls.

The overall NOAEL for male and female mice for convulsion and ataxia was 175 mg/kg bw/d (NTP 1991; Matthews, 1990).

16-week studies

During week 4, the two highest doses were incorrectly prepared and administered for the first three days of the week. Rats and mice of these two highest dose levels received a double dose during these three days in week 4.

Rats

Groups of 10 male and 10 female F344/N rats received tris(2-chloroethyl)phosphate (purity 98%) in doses of 0, 22, 44, 88, 175, and 350 mg/kg bw/d for 16 weeks (female) or 18 weeks (male). During the course of the subchronic toxicity study (4th week) two females each died

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at 350 and 175 mg/kg bw as a result of the reported error in test substance preparation. Female rats receiving overdoses exhibited signs of toxicity, including ataxia, excessive salivation, gasping, and convulsion. The overdosed male rats showed no signs of toxicity, and none died. During the 16-week study, there were additional deaths of rats, one male receiving 175 mg/kg bw/d, 5 males receiving 350 mg/kg bw/d, and three females receiving 350 mg/kg bw/d that were not associated with the overdosing. The deaths of one male rat in the 350 mg/kg bw/d group and one male and two female rats in the 22 mg/kg bw/d dose group occurred as a result of gavage trauma. Female rats receiving 175 and 350 mg/kg bw/d experienced occasional periods of hyperactivity after dosing. Periodic convulsions were noted in 350 mg/kg bw/d dosed females but not in males during week 12. Final body weights of female rats receiving 350 mg/kg bw/d were 20% greater than those of controls; final mean body weights of the remaining groups of dosed female rats and dosed male rats were similar. The relative liver and kidney weights were significantly ($p \leq 0.01$) increased in males receiving 350 mg/kg bw/d and in females receiving 44 to 350 mg/kg bw/d. In males given 350 mg/kg bw/d the increases in relative liver and kidney weights were 22% and 26%, respectively. In females the increases in relative liver weights were 13%, 13%, 19%, and 50% in the 44, 88, 175, and 350 mg/kg bw/d groups, respectively; the increases in relative kidney weight were 8%, 11%, 11% and 22% in the 44, 88, 175 and 350 mg/kg bw/d groups, respectively. These effects occurred in the absence of any apparent histopathological lesions in either of these tissues. At 350 mg/kg bw/d, female rats showed decreases in absolute brain (11%) and thymus (19%) weights. Microscopy revealed significantly increased lesions in the brains of male and female rats. Tris(2-chloroethyl)phosphate-related neuronal necrosis occurred in the hippocampus and thalamus of female rats at 175 and 350 mg/kg bw/d and, to a lesser extent, in male rats at 350 mg/kg bw/d (mild damage, not significant). These effects were more pronounced in female rats than in males. Loss of neurons in the area of the hippocampus was observed in 10/10 females and 2/10 males of the 350 mg/kg bw/d group, and in 8/10 females of the 175 mg/kg bw/d dose group. There was a significant sex-related difference in dose-response ($p=0.001$). The hippocampal damage was significant in female rats receiving 350 and 175 mg/kg bw/d, and their severity increased with dose in female rats ($p=0.0001$). The affected neurons were noted predominantly in the dorsomedial portion of the pyramidal row of the hippocampus. Necrosis and loss of thalamic nuclei were also observed in two female rats receiving 350 mg/kg bw/d. In some cases, there was tissue mineralization and microgliosis associated with loss of pyramidal cells in the hippocampus. Cholinesterase activity determined in serum at necropsy was 75% and 59% of the control value in female rats receiving 175 or 350 mg/kg bw/d tris(2-chloroethyl)phosphate, respectively ($p \leq 0.01$), but was not reduced in male rats.

Overall, significant effects on organ weights were seen from increased relative liver and kidney weights of males receiving 350 mg/kg bw/d and in females receiving 44 to 350 mg/kg bw/d. Since the increases in relative liver and kidney weights in males and in females could not be correlated to histopathologic effects, these findings in organ weights were not considered to be toxicologically adverse in this study. The most relevant toxic effects observed in this study were mortality in rats receiving 350 mg/kg bw/d and brain lesions seen in the hippocampal region in rats receiving 175 or 350 mg/kg bw/d. The NOAEL for neuronal effects in the hippocampus in female rats was 88 mg/kg bw/d and in male rats 175 mg/kg bw/d, respectively (NTP 1991, Matthews 1990).

Mice

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Groups of 10 B6C3F1 mice of each sex were treated with 0, 44, 88, 175, 350, and 700 mg/kg bw/d tris(2-chloroethyl)phosphate (purity 98%) for 16 weeks. There were no treatment-related deaths, although gavage trauma caused the deaths of three males (each at 175, 350, and 700 mg/kg bw/d) and of two females (each at 175, and 350 mg/kg bw/d) before the end of the study. No differences in body weight gain and final mean body weight, or differences in cholinesterase activity were noted in mice receiving 44 to 700 mg/kg bw/d tris(2-chloroethyl)phosphate. The mean absolute liver weights were significantly ($p \leq 0.01$) increased in females receiving 175 to 700 mg/kg bw/d (14%, 20%, and 13%, respectively) and in male mice receiving 700 mg/kg bw/d (5%). The liver-to-body weight ratios, however, were not increased. The increased absolute liver weights were seen without corroborative findings in morphology. In male mice in the 175 to 700 mg/kg bw/d dose groups the absolute kidney weights were significantly ($p \leq 0.01$) decreased (5%, 10%, and 20%, respectively). The kidney-to-body weight ratios, however, were not affected. Histopathologically examinations of the kidneys showed epithelial cells with enlarged nuclei (mild cytomegaly and karyomegaly) in all animals of both sexes given 700 mg/kg bw/d. Lesions were observed primarily in the proximal convoluted tubules of the inner cortex and outer strip of the outer medulla and to a lesser extent in the straight portion of the loops of Henle in the medulla. The hippocampal and thalamal lesion like in rats was not observed in either sex of mice. Sperm count in mice was slightly decreased ($p = 0.05$) in animals receiving 700 mg/kg bw compared to that of controls.

Organ weight determinations revealed statistically significant increased livers in females given 175 to 700 mg/kg bw/d and in males given 700 mg/kg bw/d without further corroborating findings, so these findings were not considered to be toxicologically adverse. Relevant toxic effects were present in male and female mice receiving 700 mg/kg bw/d. There were renal lesions (mild cytomegaly and karyomegaly) and significantly reduced mean absolute kidney weights.

A NOAEL for renal effects for mice of both sexes was estimated at 350 mg/kg bw/d (NTP 1991, Matthews 1990).

Adverse effects on the brain in rats and mice after repeated exposure to tris(2-chloroethyl)phosphate will be discussed under chapter 4.1.2.6.1.2 Specific endpoints - neurotoxicity

103-week studies

Information on neoplastic findings of tris(2-chloroethyl)phosphate in experimental animals obtained from these carcinogenicity studies (NTP 1991, Matthews 1993) are described in detail in section 4.1.2.8.

Rats

Groups of 60 F344/N rats per sex received 0, 44, or 88 mg/kg bw/d tris(2-chloroethyl)phosphate (purity 98%) in corn oil by gavage, once per day, 5 days per week, for up to 103 weeks. An interim evaluation (necropsy, histopathology, hematology, and clinical chemistry) was performed on ten animals per sex per group after a treatment period of 66 weeks. At 66 weeks, one female receiving 88 mg/kg bw/d dies on day 261, and one vehicle control male died on day 408; the remaining lived until interim sacrifice on day 458 and 459. There were no adverse effects seen on body weight gain or hematology. There were

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significantly decreases in serum alkaline phosphatase and alanine aminotransferase in females receiving 88 mg/kg bw/d ($p \leq 0.01$). Slight increases in mean absolute liver and kidney weights were recorded in males at the same dose level; for relative liver and kidney weights statistically significant increases of 14% and 20%, respectively, were observed ($p \leq 0.01$). At this time point there were renal tubule adenomas in one male receiving 88 mg/kg bw/d. Focal brain lesions were located in the cerebrum and thalamus. In cerebrum and thalamus, local necrosis and accumulation of inflammatory cells, reactive gliosis, and endothelial hypertrophy and hyperplasia were observed in 3/10 females given 88 mg/kg bw/d.

At 103 weeks the final mean body weights of test and control groups were similar. There were no clinical signs of toxicity. Reduced survival was noted in male and female rats at 88 mg/kg bw/d (males: 51% survival compared to 78% in controls; females 37% compared to 66% in controls $p \leq 0.01$). Female rats dying early or sacrificed while moribund frequently had brain lesions, by contrast male rats did not. The mean treatment-related effects of tris(2-chloroethyl)phosphate occurred in the kidney and the brain. A significant increase of focal hyperplasia of the renal tubule epithelium was recorded in both treated male and female rats (in males: 0/50, 2/50; and 24/50 and in females: 0/50, 3/50, and 16/50). Hyperplasia of the renal tubule epithelium occurred in the convoluted tubules of the cortex and were characterized by stratification of the epithelial cells with partial to complete obliteration of the tubule lumens. A summary of the incidence of hyperplasia in the renal tubules in rats in the 2-year study of tris(2-chloroethyl)phosphate is given in the following table 4.8

Table 4.8 Incidence of hyperplasia in the renal tubules of F344/N rats in the 2-year study of tris(2-chloroethyl)phosphate (NTP 1991, Matthews 1993)

	Vehicle control	**44 mg/kg bw/d**	**88 mg/kg bw/d**
male (number examined)	50	50	50
Hyperplasia, focal		1 (2%)	1(2%)
Mineralization		1 (2%)	
Epithelium, hyperplasia		1 (2%)	1 (2%)
Epithelium, hyperplasia, focal			18 (36%)
Epithelium, hyperplasia, multifocal			4 (8%)
female (number examined)	50	50	50
Epithelium, hyperplasia			4 (8%)
Epithelium, hyperplasia, focal		3 (6%)	11 (22%)
Epithelium, hyperplasia, multifocal			1 (2%)

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For this relevant toxic effect there was a clear dose-response relationship in the kidneys in male and female rats at both treatment groups, 44 and 88 mg/kg bw/d, thus, a NOAEL for kidney lesions could not be estimated in this study. So, 44 mg/kg bw/d is considered to be the LOAEL for kidney lesions in animals of both sexes.

A marked increase in the incidence of degenerative lesions of the brain stem and cerebrum (thalamus, hypothalamus, and basal ganglia) was noted in over 44% of female rats receiving 88 mg/kg bw/d. In contrast to the findings in the 16-week studies, degenerative lesions were widely distributed in the gray and white matter of the brain stem and cerebral cortex of high-dose females and, to a lesser extent in male rats. Degenerative lesions consisting of gliosis, mineralization, hemorrhage, and/or hemosiderin accumulation occurred in the cerebrum and brain stem of more than 50% of female rats receiving 88 mg/kg bw/d tris(2-chloroethyl)phosphate; whereas similar lesions were seen in only a few dosed males, however, they were assessed as toxicologically relevant, although these findings were not significantly increased in incidence and severity, nevertheless they were increased in relation to treatment with tris(2-chloroethyl)phosphate. Accordingly, the NOAEL for brain lesions is 44 mg/kg bw/d for male and female F344/N rats (NTP 1991, Matthews 1993). A summary of non-neoplastic brain lesions in rats in the 2-year study of tris(2-chloroethyl)phosphate is given in the special part: "Other special studies – Neurotoxicity", where more details can be found.

Mice

Groups of 60 B6C3F1 mice of each sex received 0, 175, or 350 mg/kg bw/d tris(2-chloroethyl)phosphate (purity 98%) by oral gavage on the same dosing schedule. Ten animals per sex per group species were predetermined for interim evaluation (necropsy, histopathology, hematology, and clinical chemistry) after 66 weeks. At 66 weeks, there were no effects seen on mortality, body weight gain, hematology or clinical chemistry. Microscopy of the kidneys revealed hyperplasia of tubule epithelial cells in two males receiving 350 mg/kg bw/d.

At 103 weeks mortality and body weight gain were similar among dosed groups of male and female mice and their respective controls. The main target organ for toxicity was the kidney, in which nuclear enlargement (karyomegaly) of tubule epithelial cells was present in tris(2-chloroethyl)phosphate treated male and female mice given 175 or 350 mg/kg bw/d (16/50, and 39/50 males; and 5/49 and 44/50 females). This finding was present in approximately 80% of mice receiving 350 mg/kg bw/d and less frequently in mice receiving 175 mg/kg bw/d, while no signs of cellular necrosis were reported from both dose levels. The affected cells were in the proximal convoluted tubules of the inner cortex and outer stripe of the outer medulla and, to a lesser extent, in the pars recta of the loops of Henle in the outer medulla. The lesion was minimal in most mice and consisted of only a few widely scattered tubule epithelial cells with enlarged hyperchromatic single nuclei. In concurrent controls, karyomegaly were observed in 2/50 males and in 0/50 females. An overview of selected renal tubule cell lesions in B6C3F1 mice in the 2-year study of tris(2-chloroethyl)phosphate is given in the following table 4.9.

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Table 4.9 Selected renal tubule cell lesions in B6C3F1mice in the 2-year study of tris(2-chloroethyl)phosphate (NTP 1991, Matthews 1993)

	Vehicle control	**175 mg/kg bw/d**	**350 mg/kg bw/d**
male (number examined)	50	50	50
Karyomegaly	2	16	39**
Hyperplasia (original + step sections)	1	0	3
female (number examined)	50	49	50
Karyomegaly	0	5*	44**
Hyperplasia (original + step sections)	0	1	2

* significantly different (p≤0.05); ** significantly different (p≤0.01) from the control group by logistic regression tests

There was a clear dose-response relationship for this relevant toxic effect in the kidneys in male and female mice at both treatment groups. The NOAEL for kidney lesions for mice could not be estimated in this study. Therefore, 175 mg/kg bw/d is considered to be the LOAEL for changes in kidney morphology in B6C3F1 mice of both sexes. In the liver, there was an increased incidence of foci of cytologic alteration, particularly eosinophilic foci in males (control: 0/50, 0%; 175 mg/kg bw/d: 3/50, 6%; 350 mg/kg bw/d: 8/50, 16%), although the incidence of basophilic (control: 1/50, 2%; 175 mg/kg bw/d: 2/50, 4%; 350 mg/kg bw/d: 1/50, 2%) or clear cell foci (control: 4/50, 8%; 175 mg/kg bw/d: 1/50, 2%; 350 mg/kg bw/d: 5/50, 10%) were not. Eosinophilic, basophilic, and clear cell foci comprise a morphological continuum with hepatocellular adenoma and are believed to be precursors of hepatocellular neoplasms. In female mice there was no increase in the incidence of hepatocellular foci. An overall NOAEL for non-neoplastic lesions for male and female mice could not be determined (NTP 1991, Matthews 1993).

- *Diet studies (rat and mouse)*

28-day study

Rats

In a 28-day dietary range-finding study (Stauffer Chemical Company, unpublished report 1980a), groups of 10 male and 10 female Sprague-Dawley CD rats were administered at dose levels of 0, 500, 850, 1500, and 2000 ppm (equivalent to 0, 42, 72, 125, and 163 mg/kg bw/d in males, and 0, 50, 88, 144, and 191 mg/kg bw/d in females, calculation on nominal dietary levels) of tris(2-chloroethyl)phosphate in the diet. The mean weekly test substance consumption values reported in the study report were calculated to mean values for the period

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of 12 weeks. Additionally two weeks after initiation of dosing, the 200 ppm dose level (equivalent to 19 mg/kg bw/d in males, and 20 mg/kg bw/d in females) was increased to 4000 ppm (equivalent to 293 mg/kg bw/d in males and 334 mg/kg bw/d in females), and in another dose group receiving 350 ppm (equivalent to 30 mg/kg bw/d in males and 38 mg/kg bw/d in females) for three weeks the dose level was increased to 8000 ppm (equivalent to 495 mg/kg bw/d in males and 508 mg/kg bw/d in females) for one week. This study was not conducted in line with the requirements of the guideline testing protocols of B.7/ OECD TG 407; it differs in some respects from the published guidelines; following significant deficiencies exist: only a small selection of parameters in clinical biochemistry; no organ weight assessment, no histopathology. However, basic data are given in this range finding study, and they were used as supporting information. There were no test substance-related deaths. The mean body weights of treated rats at all dose levels were comparable to the control rats. A statistically significant decrease in food consumption after one week of treatment was observed in males and females at 8000 ppm (equivalent to 495 mg/kg bw/d in males and 508 mg/kg bw/d in females, $p \leq 0.05$). Hematology and clinical chemistry examinations showed no biologically significant treatment-related alterations. The seminal vesicles and/or prostate were smaller than normal in one rat in the dose group of 1500 ppm (equivalent to 125 mg/kg bw/d) and in three males receiving 8000 ppm (equivalent to 495 mg/kg bw/d). The testes of one male were dissimilar in size and weight, which may have been related to ingestion of the test substance.

The results of this study provided the basis for selection of appropriate dose levels for the following 3-month study.

3-month study

Rats

In a three month dietary toxicity study mostly according to OECD test guideline 408 (no microscopy of the brain) five groups of Sprague-Dawley CD rats (20/sex/group) were fed 0, 400, 1000, 3000, and 8000 ppm (equivalent to 0, 26, 65, 192, and 506 mg/kg bw/d in males; and 0, 30, 75, 215, and 586 mg/kg bw/d in females, calculation of intake based on nominal concentrations) tris(2-chloroethyl)phosphate (purity commercial grade) in a commercial diet (Stauffer Chemical Company, unpublished report 1980b).

No treatment-related deaths and clinical signs were seen in rats of both sexes. Body weights and the mean weekly food consumption values of males and females consuming 8000 ppm tris(2-chloroethyl)phosphate were significant ($p \leq 0.05$) lower (13-16%; 11-18% respectively) than controls. Food consumption was also depressed in females of the 3000 ppm dose group, however, the difference was only 5% and may be biologically significant. Final body weights were lower in males and females at 3000 ppm (-7% and -8%, resp.) and at 8000 ppm (-18% and -17%, resp.). Analysis of clinical chemistry, hematology, urinalysis, and cholinesterase revealed no compound-related effects. The mean relative liver and kidney weights were significantly increased in both sexes of 3000 ppm and 8000 ppm (equivalent to 506.42 mg/kg bw/d in males, and 586.22 mg/kg bw/d in females, $p \leq 0.05$) dose groups. In male rats mean relative liver weight was 19% higher at 3000 ppm and 22% at 8000 ppm when compared to the concurrent controls. In females increases of 9% and 30% were noted, respectively. For mean relative kidney weights the following significant increases were determined: in males: 9% at 1000 ppm, 15% at 3000 ppm and 22% at 8000 ppm; and in females: 12% at 3000 ppm and 13% at 8000 ppm, respectively. There were a number of tubular hyperplasias in control and treatment groups with a tendency to increase in incidence at higher dosages (total

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incidences 6/20, 4/20, 7/20, 8/20 and 11/20 at 0, 400, 1000, 3000 and 8000 ppm). The attribution of the small raise in numbers at 1000 and 3000 ppm compared to the control level to the treatment may be questionable, but the incidence at 8000 ppm was markedly above that of the control group. The mean severity seems to be elevated at 400 ppm compared to the control level, but no dose-dependent increase in severity was seen at higher dose levels (mean severity: 1.3, 2.75, 1.1, 1.0 and 1.2 at 0, 400, 1000, 3000 and 8000 ppm). In the light of the mean severity scores at higher dosages, the two males out of 4 affected at 400 ppm, which showed marked or severe regenerative hyperplasia should be considered as incidental. There were no other gross or histological lesions attributed to tris(2-chloroethyl)phosphate treatment. The relative mean weights of the gonads and the brain were also reduced in dose groups at 3000 and/or 8000 ppm. Except for absolute heart effects that were lower in high dose males and females, the absolute mean weights of other organs were similar to those of the controls. This and the absence of other treatment-related gross or histological lesions support that the relative increases in organ weights might be due to the lower body weight gain at 3000 and 8000 ppm. This reduction in growth could be attributed to lower food consumption. The increase in incidence in regnerative hyperplasia in the renal cortex at the 8000 ppm is considered to be related to the treatment. Therefore 3000 ppm is the NOAEL (equivalent to 192 mg/kg bw/d in males; and 215 mg/kg bw in females).

Little information was obtained from an early diet study in rats (Stauffer Chemical Company, unpublished report 1975, cited as summary without detailed information in Ulsamer et al. 1980). Groups of 10 male and 10 female rats (strain not specified) were administered up to 0.5% (250 mg/kg bw/d) tris(2-chloroethyl)phosphate for a period of 30 days in the diet. No deaths occurred. At the end of the treatment no adverse effects on growth, appearance and behavior, liver and kidney weights, or changes in pathological examinations of survivors were seen.

Overall, no treatment-related effects in rats was shown up to 0.5% (approximately 250 mg/kg bw/d) tris(2-chloroethyl)phosphate in the diet for 30 days. The NOAEL was considered to be 250 mg/kg bw/d. Because of limitations in testing and reporting, this study should to be not useful for risk assessment.

18 months study

Information on neoplastic findings of tris(2-chloroethyl)phosphate in mice obtained from the following chronic toxicity study are described in detail in section 4.1.2.8. This 18 months dietary study in Slc:ddY mice was conducted as a part of a safety evaluation program on substitutes for fire proofing agents, especially to evaluate possible carcinogenicity. There were basic data comparable to the guideline OECD TG 451 with acceptable restrictions. The test parameters documented do not totally comply with this testing guideline for carcinogenicity. However, there was a well-documented study report which meets basic scientific principles. The supplied data are considered as valid for use in risk assessment.

Mice

Groups of 50 male and 50 female Scl:ddY mice received 0, 0.012, 0.06, 0.3, and 1.5% (equivalent to 0, 12, 60, 300, and 1500 mg/kg bw/d, calculated on an assumed body weight of

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20 g and food consumption of 10% body weight/day) tris(2-chloroethyl)phosphate (purity 98%) by dietary administration for 18 months (Takada et al., 1989). Reduced survival was reported in male mice from week 70 and in female mice from week 60 compared to control groups. In both sexes fed 1500 mg/kg bw/d tris(2-chloroethyl)phosphate the final survival was approximately 40% compared to around 65% survival in controls. A marked reduction in body weight gain was noted in males and females receiving 1500 mg/kg bw/d (approximately 60% lower than the control value). No differences in food consumption were found between the groups for both sexes. In hematology, no changes were found apart from significant elevation in platelets in males of the 300 mg/kg bw/d dose group. Significant declines were found in the weights of the heart and testes in males and kidneys in females from the 1500 mg/kg bw/d dose group (no more data). At microscopy, various changes were observed in the different organs of all groups. The kidney and the liver were identified as target organs. There were predominate neoplastic changes in these organs (for details see 4.1.2.8 Carcinogenicity). Furthermore tris(2-chloroethyl)phosphate related non-neoplastic changes were reported from the liver and predominant from the kidney. In the liver, focal necrosis, vacuolation of the liver cells and extramedullary hematopoiesis were observed in animals of all groups including the control group. The most relevant effects were seen in the kidneys. In males and females of all treatment groups, hyperplasia and hypertrophy of the urinary tubule epithelium together with enlargement of the nuclei were observed. These nuclei were polymorphic, and several times abnormal division, degeneration and necrosis were observed. Unfortunately, no incidences of these findings were reported. However, such findings were not seen in the concurrent controls. In addition, cysts, necrosis of the urinary tubule epithelium and interstitial fibrosis were observed in animals receiving 1500 mg/kg bw/d.

In summary, the data reported for the highest dose level of 1500 mg/kg bw/d, e.g. reduced survival, reduction in body weight gain (>10%) and severe toxicity indicate that the top dose exceeds the MTD (maximum tolerated dose) for this mouse strain. However, relevant changes in tissue morphology were not only observed in male and female mice in the kidneys of the top dose level. Signs of prolonged regenerative cell proliferation, which occurs possibly after renal tubule damage were reported from all treatment groups. There were hyperplasia and hypertrophy of the urinary tubule epithelium together with enlargement of the nuclei, which showed abnormal division, degeneration and necrosis at several times. Although no quantitative information on these microscopic findings in the kidney of treated groups were reported they were assessed as relevant toxic effects, also seen from this point of view that no such kidney findings were observed in the concurrent controls. Thus, a NOAEL for kidney effects (hyperplasia and hypertrophy of the urinary tubule epithelium together with enlargement of the nuclei) could not be established in this study. Therefore, 12 mg/kg bw/d is considered to be the LOAEL for kidney effects in male and female Scl:ddY mice in the 18 months diet study.

- *Inhalation*

No information available.

- *Dermal*

No information available.

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4.1.2.6.1.2 Specific endpoint studies:

- *Neurotoxicity*

The neurotoxicity effects of tris(2-chloroethyl)phosphate were evaluated in White Leghorn hens only.

Hens

In an acute delayed neurotoxicity study according to OECD test guidelines 418 (no data on clinical biochemistry) the acute delayed neurotoxic potential of tris(2-chloroethyl)phosphate was determined in adult (12-14 months old), White Leghorn hens. 18 hens received two oral doses of 10 ml/kg (approximately 14200 mg/kg bw) tris(2-chloroethyl)phosphate (purity: commercial grade) with a three week interval (Stauffer Chemical Company, unpublished report 1979). Two additional groups, 10 hens per group, served as controls. A positive control group was treated with 500 mg/kg bw tri-o-cresyl phosphate, an organophosphorus neurotoxin. The negative control group received corn oil. Four out of 18 tris(2-chloroethyl)phosphate-treated hens died during the study. Predominant observed signs of toxicity were severe feather loss, cessation of egg production, reduced food consumption, and loss of body weight. Walking behavior of the tris(2-chloroethyl)phosphate-treated hens were identical to those seen in the corn oil group. There were no microscopical changes in brain, spinal cord or sciatic nerve. None of the 14/18 surviving hens receiving tris(2-chloroethyl)phosphate showed nerve fiber degeneration similar to that seen in tri-o-cresyl phosphate-treated positive controls.

No evidence of neurotoxicity was seen following two oral administrations (on Day 1 and again 3 weeks later) of 14.2 g/kg bw/d tris(2-chloroethyl)phosphate in hens.

- *Adverse effects on the brain in rats and mice after repeated exposure to tris(2-chloroethyl)phosphate*

Rats and mice

Clinical signs of neurotoxicity were observed in female F344/N rats during an oral 16-week gavage study and in B6C3F1 mice in the course of an oral range-finding toxicity study of 16 days. Clinical signs of toxicity, including ataxia, excessive salivation, gasping, and convulsion were noted in those female F344/N rats receiving double the target levels of 175 and 350 mg/kg bw/d tris(2-chloroethyl)phosphate for 3 days in week four during the 16-week toxicity study due to an incorrectly test material preparation. In addition in this subchronic study female rats receiving 175 and 350 mg/kg bw/d tris(2-chloroethyl)phosphate experienced occasional periods of hyperactivity after dosing. Periodic convulsions were noted in 350 mg/kg bw/d dosed females but not in males during week 12, and also not in the overdosed male rats in this study (NTP 1991, Matthews 1990). Male and female B6C3F1 mice given 350 and 700 mg/kg bw/d tris(2-chloroethyl)phosphate for 16 days exhibited ataxia and convulsive movements during the first 3 days of dosing (NTP 1991, Matthews 1990).

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No adverse effects on growth, appearance and behavior were seen in the 2-year gavage studies with rats given 44 and 88 mg/kg bw/d of tris(2-chloroethyl)-phosphate and in the mice given up to 350 mg/kg bw/d (NTP 1991, Matthews 1993).

- *Determination of the acetylcholinesterase activity*

Since it is known that most organophosphates cause neurotoxic effects in humans through the inhibition of the enzyme acetylcholinesterase (AChE) in the central and peripheral nervous system, measurements of a possible inhibition of that enzyme as consequence to tris(2-chloroethyl)phosphate treatment in experimental animals were investigated in 14-day and 16-week oral studies in F344/N rats and B6C3F1mice (NTP 1991, Matthews 1990), and also in a three months dietary subchronic study in Sprague-Dawley CD rats (Stauffer Chemical Company, unpublished report 1980b). To assess the AChE inhibition in toxicity studies a multi-stage approach is indispensable. However, there are no data available for measurements the AChE inhibition in the CNS and PNS, in erythrocytes or whole blood. Only results of cholinesterase (ChE) measurements in serum are available. It has to be taken into consideration that testing serum cholinesterase is not very useful as the neuronal enzyme has different structural requirements for inhibition by organophosphates. Rats

Relevant treatment-related changes in serum cholinesterase activity were noted in female F344/N rats given 175 and 350 mg/kg bw/d tris(2-chloroethyl) phosphate for 16 weeks. Cholinesterase activity determined in serum at necropsy was down to 75% and 59% of the control value at 175 and 350 mg/kg bw/d, respectively ($p \leq 0.01$). No changes in activity were observed in treated male F344/N rats. The observed inhibition of cholinesterase activity is considered only as indication for exposure to TCEP.

- *Histologically examinations of the brain*

Reports in the literature indicate that most organophosphorous compounds induce neurotoxic effects. Therefore, the NTP data were included in a further investigation in order to characterize the effect of tris(2-chloroethyl)-phosphate on the nervous system. In these examinations additionally sections of forebrain, midbrain, brain stem and cerebellum from each animal were examined by light microscopy (Matthews 1990).

Rats and mice

In a 16-week subchronic oral (gavage) toxicity study in F344/N rats, tris(2-chloroethyl)phosphate-related neuronal necrosis occurred in the hippocampus and thalamus of female rats at 175 (8/10) and 350 mg/kg bw/d (10/10) and, to a lesser extent, in male rats treated at 350 mg/kg bw/d (2/10) (NTP 1991; Matthews 1990). At interim evaluation after 66 weeks of the 2-year study on F344/N rats, brain lesions were focally located in the cerebrum and thalamus. They were characterized by necrosis with accumulation of inflammatory cells, reactive gliosis, and endothelial hypertrophy and hyperplasia. Such lesions were observed in 3/10 females given 88 mg/kg bw/d. A marked increase in the incidence of degenerative lesions of the brain stem and cerebrum (thalamus, hypothalamus, and basal ganglia) such as

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gliosis, hemorrhage, necrosis, and mineralization was noted in female F344/N rats at 88 mg/kg bw/d after oral long-term/long-life exposure (103 weeks). In contrast to the findings in the 16-week study, degenerative lesions were widely distributed in the gray and white matter of the brain stem and cerebral cortex of females receiving 88 mg/kg bw/d. Degenerative lesions of the brain occurred in the cerebrum and brain stem of more than 50% of female rats receiving 88 mg/kg bw/d tris(2-chloroethyl)phosphate; similar lesions were seen in only a few dosed male rats. In both sexes the brain lesions varied in severity from minimal to marked and often involved extensive areas. In some animals, the lesions were bilateral and symmetrical; in others, they were bilateral and asymmetrical or unilateral. The active lesions were characterized by degeneration and necrosis with hemorrhage, while resolving lesions exhibited loss of neurons and neutrophil, proliferation of glial cells, capillary hyperplasia, hypertrophy of the tunica media of small vessels, and hemosiderin-laden macrophages. Deposits of minerals occurred in some of these foci (NTP 1991, Matthews 1993). An overview of non-neoplastic brain lesions in rats in the 2-year study of tris(2-chloroethyl)phosphate is given in the following table 4.10.

Table 4.10 Selected brain lesions in F344/N rats in the 2-year study of tris(2-chloroethyl)phosphate (NTP 1991, Matthews 1993)

	Vehicle control	**44 mg/kg bw/d**	**88 mg/kg bw/d**
male (number examined)	50	49	50
Brain stem, hemorrhage			1(2%)
Brain stem, pigmentation, hemosiderin	1 (2%)		
Cerebrum, gliosis, focal			1 (2%)
Cerebrum, hemorrhage		1 (2%)	1 (2%)
Cerbrum, pigmentation, hemosiderin			1 (2%)
Pons, hemorrhage			3 (6%)
female (number examined)	50	50	50
Brain stem, gliosis	1 (2%)		15 (30%)**
Brain stem, hemorrhage	1 (2%)		12 (24%)**

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Brain stem, mineralization			7 (14%)**
Brain stem, necrosis			1 (2%)
Brain stem, pigmentation, hemosiderin	1 (2%)		17 (34%)**
Cerebellum, hemorrhage	1 (2%)		2 (4%)
Cerebellum, necrosis			1 (2%)
Cerebellum, pigmentation, hemosiderin	1 (2%)		
Cerebrum, gliosis			19 (38%)**
Cerebrum, hemorrhage	1(2%)		17 (34%)**
Cerebrum, mineralization			15 (30%)**
Cerebrum, pigmentation, hemosiderin			22 (44%)**
Pons, hemorrhage		1 (2%)	

** significantly different ($p \leq 0.01$) from the control group by logistic regression tests

Mice were less sensitive to these effects to tris(2-chloroethyl)phosphate than rats. In B6C3F1 mice of both sexes no tris(2-chloroethyl)phosphate induced lesions of the brain were recorded at 44 to 700 mg/kg bw/d after repeated oral treatment by gavage for 16 weeks and up to and involving 350 mg/kg bw/d for 103 weeks (NTP 1991, Matthews 1993).

In summary no relevant toxic effects on the brain were observed following subchronic oral treatment of 175 mg/kg bw/d tris(2-chloroethyl)phosphate in male F344/N rats and of 88 mg/kg bw/d in females (NTP 1991; Matthews 1990). However, at the interim sacrifice after 66 weeks of the 2-year study F344/N rats, focal brain lesions in the cerebrum and thalamus were seen in females receiving 88 mg/kg bw/d, but not in males. After 103 treatment weeks such lesions in the brain were observed in over 50% of female F344/N rats receiving 88 mg/kg bw/d tris(2-chloroethyl)phosphate. Similar lesions occurred only in a few males, however, they were assessed as toxicologically relevant, although these findings were not significant increased in incidence and severity, but they were increased in relation to treatment with tris(2-chloroethyl)phosphate. Therefore, the NOAEL for brain lesions is 44 mg/kg bw/d for male and female F344/N rats (NTP 1991; Matthews 1993). There were no tris(2-chloroethyl)phosphate related microscopic changes in the brain of B6C3F1 mice treated up to and involving 350 mg/kg bw/d for 103 weeks when compared with controls. Therefore, the NOAEL for brain lesions is 350 mg/kg bw/d for B6C3F1 mice of both sexes (NTP 1991; Matthews 1993).

4.1.2.6.2 Human data

Ingerowski & Ingerowski (1997) reported a case of exposure to TCEP by a 5 year old child showing neurotoxic signs after a long term contact. The same case has been reported to the

Case Register of Federal Institute for Risk Assessment (formerly BgVV) (Case Register BgVV 1061/97), too.

The girl developed a progredient paresis. She slept in a room which was equipped with wood panellings treated with wood preserver containing 3% of TCEP. Measurements of TCEP in the timber work revealed 600 mg/kg of wood. 9 further chemicals were also analysed but below the detection limit. EMG and nerve conduction velocity testing showed generalized neurogenic defects, whereas the clinical findings could not be clearly classified as "spinal muscle dystrophy". In the further clinical course a diagnosis of "spinal muscle dystrophy of Kugelberg-Welander type" was established with tetraparesis. After renovation of the house (removal of all timber panels), the clinical status improved. Two years after exposure no functional deficits are present.

Because TCEP was not determined in house dust, an exposure analysis based on the concentration in the wood panel only identifies the possible source, but does not help for quantifying the exposure. In this case, oral exposure is possible by uptake of dust and inhalation exposure inhaling particles containing TCEP. Dermal contact should also be taken into account by contact with dust and touching the panels. The symptoms of illness increased with increasing time of exposure, and ceased after discontinuation (dechallenge reaction). From these reasons, a relationship between TCEP exposure and the neurotoxic signs cannot be neglected.

4.1.2.6.3 Summary of animal toxicity data after repeated exposure to tris(2-chloroethyl)phosphate

The main toxic effects of tris(2-chloroethyl)phosphate from animal toxicity studies were summarized in the following table, Table 4.11.

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Table 4.11:

Summary table: Animal toxicity data after repeated exposure to tris(2-chlorethyl)phosphate

Study design: Species, strain (male/female) Exposure route Exposure duration Dose	Non-neoplastic effects (selected) at LOAEL NOAEL	Reference
F344/N rat (5m/5f) Oral Gavage 16 days, 5 d/wk (12 doses) 0, 22, 44, 88, 175, 350 mg/kg bw/d	350 mg/kg bw: ↑** liver weight (f) ≥175 mg/kg bw/d: ↑** kidney weight, abs/rel (m) ↓** serum cholinesterase activity (f) ≥88 mg/kg bw/d: ↓* lung weight, abs/rel (f) $NOAEL_{sys}$ (m/f): 350 mg/kg bw/d	NTP 1991 Matthews 1990
F344/N rat (10m/10f) Oral Gavage 16/18 weeks (f/m), 5 d/wk 0, 22, 44, 88, 175, 350 mg/kg bw/d	350 mg/kg bw/d: mortality: 4/10 (m), 3/10 (f) periodic convulsion during week 12 (f) ↑** liver and kidney weights, rel (m) ↓ brain, thymus, abs (f) neuronal necrosis, loss of neurons in the brain (f:10/10; m: 2/10) ≥175 mg/kg bw/d: in the brain: neuronal necrosis (10/10 f) loss of neurons (8/10 f) ↓** serum cholinesterase activity (f) ≥44 mg/kg bw/d: ↑** liver and kidney weights, rel (f) $NOAEL_{sys}$ for brain lesions: (m): 175 mg/kg bw/d (f): 88 mg/kg bw/d	NTP 1991 Matthews 1990
F344/N rat (10m/10f) Oral Gavage 66 weeks (interim sacrifice), 5 d/wk 0, 44, 88 mg/kg bw/d rat	88 mg/kg bw/d: ↓** AP (f), ↓** ALAT (f) ↑** liver and kidney weights, rel (m) renal tubule adenoma (1/10m) brain: local necrosis, accumulation of inflammatory cells, reactive gliosis, endothelial hypertrophy (3/10 f) $NOAEL_{sys}$ for brain lesions (f): 44 mg/kg bw/d	NTP 1991 Matthews 1993

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F344/N rat (60m/60f) Oral Gavage 103 weeks, 5 d/wk 0, 44, 88 mg/kg bw/d rat	88 mg/kg bw/d: ↓** survival (m/f); ↑** focal hyperplasia of tubule epithelium of the kidney (m:24/50; f: 16/50) ↑** degenerative lesions in the brain (f) ↑ lesions in the brain (m) 44 mg/kg bw/d: ↑** focal hyperplasia of tubule epithelium of the kidney (m:2/50; f: 3/50) $LOAEL_{sys}$ for kidney lesions (m/f): 44 mg/kg bw/d $NOAEL_{sys}$ for brain lesions (m/f): 44 mg/kg bw/d	

For key to symbols, see at the end of the table

Table 4.11 (continued):

Summary table: Animal toxicity data after repeated exposure to tris(2-chlorethyl)phosphate

Study design: Species, strain (male/female) Exposure route Exposure duration Dose	Non-neoplastic effects (selected) at LOAEL NOAEL	Reference
Sprague-Dawley CD Rat (10m/10f) Oral In feed 28 days, daily 0, 500, 850, 1500, or 2000 ppm (m: 0, 41.75, 71.5, 125 or 163 mg/kg bw/d; f: 0, 50, 88, 144 or 191 mg/kg bw/d); 200→4000 ppm (m: 293 mg/kg bw/d; f: 334 mg/kg bw/d); 350 ppm (m: 30 mg/kg bw/d; f: 38 mg/kg bw/d)→8000 ppm (m: 495 mg/kg bw/d; f: 508 mg/kg bw/d)	8000 ppm (m: 495 mg/kg bw/d; f: 508 mg/kg bw/d): ↓** food consumption (after 1 week) smaller seminal vesicles and/or prostate (m: 3/10) ↓** testes weight, size (m: 1/10) 1500 ppm (m: 125 mg/kg bw/d): smaller seminal vesicles and/or prostate (m: 1/10) $NOAEL_{sys}$ (m): 850 ppm (71.5 mg/kg bw/d) $NOAEL_{sys}$ (f): 8000 ppm (508 mg/kg bw/d)	Stauffer Chemical Company 1980a
Sprague-Dawley CD Rat (20m/20f) Oral in feed 3 months, daily 0, 400, 1000, 3000, or 8000 ppm (m: 0, 25.96, 65.43, 192, or 506 mg/kg bw/d; f: 0, 30.4, 75.15, 215, or 586 mg/kg bw/d)	8000 ppm (m: 506 mg/kg bw/d; f: 586 mg/kg bw/d): ↓* food consumption (m/f) ↓* body weight (m/f) ↑* relative liver and kidney weights (m/f) ↑ incidence in regenerative hyperplasia in the renal cortex 3000 ppm (m: 192 mg/kg bw/d; f: 215 mg/kg bw/d): ↓ food consumption (f) ↓ body weight (m/f) ↑* relative liver and kidney weights (m/f) 1000 ppm (75 mg/kg bw/d): ↑* relative kidney weights (m) $NOAEL_{sys}$: (m/f): 3000 ppm (m: 192 mg/kg bw/d; f: 215 mg/kg bw/d) for kidney effects	Stauffer Chemical Company 1980b
Sprague-Dawley Rat (10m/10f) Oral In feed 30 days, daily 0.5% (250 mg/kg bw/d)	5000 ppm (appr. 250 mg/kg bw): no treatment-related effects $NOAEL_{sys}$ (m/f): 5000 ppm (appr. 250 mg/kg bw)	Ulsamer 1980

For key to symbols, see at the end of the table

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Table 4.11 (continued):
Summary table: Animal toxicity data after repeated exposure to tris(2-chlorethyl)phosphate

Study design: Species, strain (male/female) Exposure route Exposure duration Dose	Non-neoplastic effects (selected) at LOAEL NOAEL	Reference
B6C3F1 Mice (5m/5f) Oral Gavage 16 days, 5 d/wk (12 doses) 0, 44, 88, 175, 350, or 700 mg/kg bw/d	≥350 mg/kg bw: ataxia, convulsive movements during the first three days of dosing (m/f) $NOAEL_{sys}$ (m/f): 175 mg/kg bw/d	NTP 1991 Matthews 1990
B6C3F1 Mice (10m/10f) Oral Gavage 16 weeks (f), 18 weeks (m), 5 d/wk 0, 44, 88, 175, 350, or 700 mg/kg bw/d	700 mg/kg bw/d: ↑** liver weights, abs (m) ↑** nuclear enlargement of tubule epithelial cells of the kidney (10/10 m/f) ↓* sperm count (10/10 m) ≥175 mg/kg bw/d: ↑** absolute liver weights (f) ↓** absolute kidney weights (m) $NOAEL_{sys}$ for renal lesions (m/f): 350 mg/kg bw/d	NTP 1991 Matthews 1990
B6C3F1 Mice (10m/10f) Oral Gavage 66 weeks (interim sacrifice), 5 d/wk 0, 175, 350 mg/kg bw/d	350 mg/kg bw/d: hyperplasia of tubule epithelial cells of the kidney (2/10 m) $NOAEL_{sys}$ (m/f): 175 mg/kg bw/d	NTP 1991 Matthews 1993
B6C3F1 Mice (60m/60f) Oral Gavage 103 weeks, 5 d/wk 0, 175, 350 mg/kg bw/d rat	350 mg/kg bw/d: ↑** nuclear enlargement of tubule epithelial cells of the kidney (39/50 m; 44/50 f) 175 mg/kg bw/d: ↑** nuclear enlargement of tubule epithelial cells of the kidney (16/50 m; 5/49 f) $LOAEL_{sys}$ for kidney lesions (m/f): 175 mg/kg bw/d $NOAEL_{sys}$ not derived for kidney lesions	
Scl:ddY mice (50m/50f) Oral in feed 18 months, daily 0, 12, 60, 300, 1500 mg/kg bw/d	1500 mg/kg bw/d: cysts, necrosis of the urinary tubule epithelium and interstitial fibrosis ≥12 mg/kg bw/d: hyperplasia and hypertrophy of the urinary tubule epithelium together with enlargement of the nuclei $LOAEL_{sys}$ for kidney effects (m/f): 12 mg/kg bw/d $NOAEL_{sys}$ not derived for kidney lesions	Takada et al. 1989

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White Leghorn Hens (18 test animals, 10/negativ and 10/positiv control group) Oral by stomach tube 2 treatments (on day 1 and again 3 weeks later) 0, 14200 mg/kg bw	14200 mg/kg bw: mortality (4/18) ↓** body weight cessation of egg production feather loss $NOAEL_{sys}$: not derived	Stauffer Chemical Company 1979

↑**: statistically significant increase compared with controls (p<0.01); ↑*: statistically significant increase compared with controls (p<0.05);↑ increase compared with controls , no statistically significant but possibly of toxicological relevance; ↓**: statistically significant decrease compared with controls (p<0.01); ↓*: statistically significant decrease compared with controls (p<0.05); ↓: decrease compared with controls, no statistically significant but possibly of toxicological relevance; →: during the study dose level was increased to; m: male; f: female; AP: Alkaline phosphatase; ALAT: Alanine aminotransferase; abs: absolute; rel: relative; $LOAEL_{sys}$: lowest observed adverse effect level for systemic effects; $NOAEL_{sys}$: no observed adverse effect level for systemic effects

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In experimental animals, the primary target organs after short- and long-term oral exposure to tris(2-chloroethyl)phosphate were the brain, the kidneys, and the liver.

Effects in the brain:

In rats, but not in mice, long-term oral exposure (subchronic and chronic) of tris(2-chloroethyl)phosphate caused degenerative lesions consisting of gliosis, mineralization, hemorrhage, and pigmentation (hemosiderin accumulation) in the cerebrum and brain stem. These degenerative lesions in the brain of rats occurred in a dose related pattern, and in addition, in a clear time-response relationship in frequency, intensity and severity. Male rats are less sensitive to adverse brain effects than female rats. Degenerative lesions in the brain were observed in female F344/N rats at ≥175 mg/kg bw/d after a treatment period of 16 weeks and after a long-term exposure in more than 40% of female F344/N rats receiving 88 mg/kg bw/d tris(2-chloroethyl)phosphate. Similar lesions were seen in male F344/N rats at 350 mg/kg bw/d after treatment for 16 weeks and in only a few long-term dosed male F344/N rats at 88 mg/kg bw/d. Although these findings in the brain of long-term dosed male F344/N rats were not significant increased in incidence and severity, they were assessed as toxicologically relevant, because they were elevated in a small number in relation to treatment with tris(2-chloroethyl)phosphate when compared with control data (NTP 1991, Matthews 1990). In B6C3F1 mice treated at 44 mg/kg bw/d to 700 mg/kg bw/d tris(2-chloroethyl)phosphate by gavage for 16 weeks and up to and involving 350 mg/kg bw/d for 103 weeks no such microscopic changes in the brain were observed (NTP 1991, Matthews 1993).

A relevant reduction of serum cholinesterase activity was determined only in female F344/N rats receiving ≥175 mg/kg bw/d tris(2-chloroethyl)phosphate by gavage for 16 weeks (NTP 1991, Matthews 1990). No inhibition of cholinesterase activity were determined in tris(2-chloroethyl)phosphate-treated male F344/N rats (NTP 1991, Matthews 1990), and also not in male and female Sprague-Dawley CD rats receiving dietary doses of up to 8000 ppm (equivalent to about 506 mg/kg bw/d in males and 586 mg/kg bw/d in females) for three months (Stauffer Chemical Company, unpublished report 1980). In B6C3F1 mice of both sexes receiving each up to 700 mg/kg bw/d tris(2-chloroethyl)phosphate serum cholinesterase activity was similar to that of controls both after subacute and subchronic oral administration (NTP 1991, Matthews 1990). Since no measurements to AChE inhibition in the CNS, PNS, and in erythrocytes are carried out, it is difficult to assess the results of ChE measurements in plasma. For this reason the observed inhibition of plasma ChE is considered only as indicator for exposure to tris(2-chloroethyl)phosphate.

A very high oral dose of tris(2-chloroethyl)phosphate (14200 mg/kg bw) did not cause behavioral effects or nerve damage suggestive of neurotoxicity in White Leghorn hens (Stauffer Chem. Company 1979).

Effects in the kidneys:

Differences in kidney weights when compared with that of controls were presented in studies on rats and mice after repeated exposure to tris(2-chloroethyl)phosphate. In Sprague-Dawley CD rats repeated oral administration via the diet for three months of ≥1000 ppm (equivalent to 65.43 mg/kg bw/d) in males and of ≥3000 ppm (equivalent to 214.62 mg/kg bw/d) in females led to a significant increase of the mean relative kidney weights (Stauffer Chem. Company 1980). Increased relative kidney weights were also observed in female F344/N rats

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receiving ≥44 mg/kg bw/d by gavage for 16 weeks (NTP 1991, Matthews 1990). In male F344/N rats the relative kidney weights were significantly increased at 350 mg/kg bw/d after gavage for 16 weeks (NTP 1991, Matthews 1990) and of both relative and absolute kidney weights at 88 mg/kg bw/d after 66 weeks (NTP 1991, Matthews 1993). Histopathologic examination of these kidneys showed an increase in incidence in regenerative hyperplasia in the renal cortex for male Sprague-Dawley CD rats receiving 8000 ppm (approximately 506 mg/kg bw/d) tris(2-chloroethyl)phosphate in the diet for three months (Stauffer Chem. Company 1980b). Markedly increase of focal or multifocal hyperplasia of the renal tubule epithelium was observed in male and female F344/N rats receiving 44 or 88 mg/kg bw/d for 103 weeks (NTP 1991; Matthews 1993). In B6C3F1 mice, reduced absolute kidney weights were determined in males receiving ≥175 mg/kg bw/d after repeated oral administration by gavage of tris(2-chloroethyl)phosphate for 16 weeks. At 700 mg/kg bw/d mild cytomegaly of the epithelium of the proximal tubules and loops of Henle was observed in kidneys of B6C3F1 mice of both sexes (NTP 1991, Matthews 1990). After long-life exposure, karyomegaly of renal tubule epithelial cells was seen in male and female B6C3F1 mice treated with ≥175 mg/kg bw/d and was present in approximately 80% of the 350 mg/kg bw dosed B6C3F1 mice (NTP 1991; Matthews 1993). In a further study in mice performed to determine the carcinogenic potential of tris(2-chloroethyl)phosphate relevant changes in the tissue morphology of kidneys were observed. There were hyperplasia and hypertrophy of the urinary tubule epithelium together with enlargement of the nuclei. Those findings were also seen in the kidneys of male and female Scl:ddY mice fed ≥12 mg/kg bw/d tris(2-chloroethyl)phosphate in the diet for 18 months, but were not reported from the concurrent control groups. In mice receiving 1500 mg/kg bw/d, additionally necrosis of the urinary tubule epithelium and interstitial fibrosis were observed (Takada et al., 1989).

Effects in the liver:

In female F344/N rats receiving 350 mg/kg bw/d by gavage for 16 days relative and absolute liver weights were significantly (17%) increased when compared to controls (Matthews 1990; NTP 1991). At the same dose level increased relative liver weights were observed in male F344/N rats treated for 16 weeks and in females at ≥44 mg/kg bw/d, respectively. After 66 weeks mean absolute and relative liver weights were significantly increased in male F344/N rats receiving 88 mg/kg bw/d (Matthews 1990, NTP 1991). In male and female Sprague-Dawley CD rats repeated oral administration of ≥3000 ppm (equivalent to ≥191.87 mg/kg bw/d in males and ≥214.62 mg/kg bw/d in females) via the diet for three months showed a significant increase of the mean relative liver weights (Stauffer Chem. Company 1980). In B6C3F1 mice the mean absolute liver weights were significantly ($p \leq 0.01$) increased in females receiving 175 to 700 mg/kg bw/d and in male mice receiving 700 mg/kg bw/d (Matthews 1990, NTP 1991). Changes in liver morphology were noted in Scl:ddY mice of all dose groups (≥12 mg/kg bw/d). There were focal necrosis, vacuolation of hepatocytes and extramedullary hematopoiesis. However, such findings were also observed in controls.

Effects in the liver observed in F344/N rats and B6C3F1 mice after treatment with tris(2-chloroethyl)phosphate were limited to increased organ weights, without other corroborating findings (morphology or biochemistry). Therefore, these findings in organ weights were not considered to be toxicologically adverse.

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No tests for repeated dose toxicity by inhalation on experimental animals are available. There is no toxicity study in conformity to the current requirements of the current repeated dose toxicity testing protocols by the dermal application route. No firm conclusion could be drawn from short and long-term skin tests for initiating/promoting activity in Swiss mice described in section 4.1.2.8.

No/Lowest observed adverse effect level (N/LOAEL)

The data submitted are considered acceptable with regard to the basic requirements as specified in Annex VIIA of Directive 67/548/EEC. The available data permit the derivation of $NOAEL_{sys}$ for repeated-dose oral toxicity. No studies are available to assess toxicity after repeated inhalation and dermal exposure.

Oral exposure:

In experimental animals, the principal affected organs observed following repeated oral administration are CNS, kidney and liver. However, relevant toxic effects were observed in the brain and kidneys.

The $NOAEL_{sys}$ values deriving from animal studies to tris(2-chloroethyl)phosphate after oral administration for brain and kidney findings after different exposure duration are summarized in Table 4.12.

Table 4.12

Summary table: $NOAEL_{sys}$ values for relevant non-neoplastic effects in the brain and kidneys derived from animal studies to tris(2-chlorethyl)phosphate

Species, strain (m/f)	Exposure route	Exposure duration	$NOAEL_{sys}$ for relevant non-neoplastic effects	Reference
F344/N rat (5m/5f)	Oral by gavage	16 days 5 d/wk	m/f: 350 mg/kg bw/d m: for brain and kidney effects	NTP 1991 Matthews 1990
F344/N rat (10m/10f)	Oral by gavage	16 weeks 5 d/wk	f: 88 mg/kg bw/d m: 175 mg/kg bw/d for neurotoxicological effects	NTP 1991 Matthews 1990
Sprague-Dawley CD rat (10m/10f)	Oral in feed	3 months daily	m/f: 3000 ppm (m: 192 mg/kg bw/d; f: 215 mg/kg bw/d) for kidney effects	Stauffer Chemical Company 1980b
F344/N rat (60m/60f)	Oral by gavage	103 weeks 5 d/wk	m/f: 44 mg/kg bw/d for brain lesions m/f: not established for kidney effects	NTP 1991 Matthews 1993
B6C3F1 mouse (5m/5f)	Oral by gavage	16 days 5 d/wk	m/f: 175 mg/kg bw/d for convulsion and ataxia	NTP 1991 Matthews 1990
B6C3F1 mouse	Oral by gavage	16 weeks 5 d/wk	m/f: 350 mg/kg bw/d for kidney effects	NTP 1991 Matthews

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Species, strain (m/f)	Exposure route	Exposure duration	$NOAEL_{sys}$ for relevant non-neoplastic effects	Reference
(10m/10f)				1990
B6C3F1 mouse (10m/10f)	Oral by gavage	66 weeks 5 d/wk	m/f: 350 mg/kg bw/d for brain lesions m/f: 175 mg/kg bw/d for kidney lesions	NTP 1991 Matthews 1993
B6C3F1 mouse (60m/60f)	Oral by gavage	103 weeks 5 d/wk	m/f: 350 mg/kg bw/d for brain lesions m/f: not established for kidney effects	NTP 1991 Matthews 1993
Scl:ddY mice (50m/50f)	Oral in feed	18 months, daily	m/f: not established for kidney effects	Takada et al. 1989

m: male; f: female; $NOAEL_{sys}$: No observed adverse effect level for systemic effects

Derivation of a NOAEL for brain effects:

Tris(2-chloroethyl)phosphate produces brain lesions in female F344/N rats and, to a lesser extent in male rats, but not in mice (NTP 1991, Matthews 1990). They were observed in several guideline studies with different duration. In female F344/N rats, neurotoxicity was induced at dosages of ≥175 mg/kg bw/d tris(2-chloroethyl)phosphate after an exposure period of 16 weeks and the absence of these lesions was found at 88 mg/kg bw/d. However, 88 mg/kg bw/d tris(2-chloroethyl)phosphate induced degenerative lesions in the brain of female rats after a life-time exposure. In male F344/N rats similar lesions were seen after treatment for 16 weeks at 350 mg/kg bw/d, and in very few cases at 88 mg/kg bw/d after 103 treatment weeks. Although these findings in the brain on male F344/N rats at 88 mg/kg bw/d were not significant increased in incidence and severity, they were assessed as toxicologically relevant, because they were increased in relation to treatment with tris(2-chloroethyl)phosphate. Therefore, a no observed adverse effect level (NOAEL) of 44 mg/kg bw/d for brain lesions could be derived for male and female F344/N rats from the 103-week (gavage) rat study (NTP 1991, Matthews 1993). Mice were less sensitive for brain effects to tris(2-chloroethyl)phosphate than rats. There were no tris(2-chloroethyl)phosphate related microscopic changes in the brain of B6C3F1 mice treated up to and involving 350 mg/kg bw/d for 103 weeks when compared with controls. Therefore, the NOAEL for brain lesions is 350 mg/kg bw/d for B6C3F1 mice of both sexes (NTP 1991, Matthews 1993). A very high oral dose of tris(2-chloroethyl)phosphate (14200 mg/kg bw) did not cause behavioral effect or nerve damage suggestive of neurotoxicity in White Leghorn hens (Stauffer Chem. Company 1979).

For adverse effects on the brain (hippocampal lesions):

103-week oral (gavage)/ F344/N rat

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$NOAEL_{sys}$ 44 mg/kg bw/d (NTP 1991; Matthews 1993)

103-week oral (gavage)/ B6C3F1 mice

$NOAEL_{sys}$ 350 mg/kg bw/d (NTP 1991; Matthews 1993)

Derivation of a N/LOAEL for kidney effects:

Kidney effects were observed in rats and mice each of both sexes in several studies with different duration. The studies were guideline studies or comparable to guideline studies with acceptable restrictions. Changes in the tissue morphology of the kidneys appear to be the most sensitive endpoint for repeated exposure of tris(2-chloroethyl)phosphate in rats and mice, especially in context with carcinogenesis. A NOAEL for these kidney findings could not be derived for male and female F344/N rats and for B6C3F1 mice of both sexes in chronic toxicity studies. In F344/N rats, mostly findings in the kidneys appeared at doses ≥44 mg/kg bw/d tris(2-chloroethyl)phosphate. At this dosage an increase of relative kidney weights was observed in female F344/N rats after a 16-week repeated oral administration by gavage, and focal hyperplasia of tubule epithelium of the kidney in male and female F344/N rats after life-time oral (gavage) administration (NTP 1991, Matthews 1990). In a feeding study of 3 months statistically significant increase in relative kidney weight was noted in male Sprague-Dawley CD rats at doses of 1000 ppm (approx. 65 mg/kg bw) and above and in female rats at 3000 ppm (approx. 215 mg/kg bw/d) and above. In male rats treated with 8000 ppm (approximately 506 mg/kg bw/d) tris(2-chloroethyl)phosphate an increased incidence in regenerative hyperplasia in the renal cortex was observed and a NOAEL for kidney effects for male and female Sprague-Dawley CD rats at 3000 ppm (equivalent to 192 mg/kg bw/d in males; and 215 mg/kg bw/d in females) (Stauffer Chemical Company 1980b). In a well-conducted 16-week study by gavage in B6C3F1 mice, a no observed adverse effect level of 350 mg/kg bw/d for mice of both sexes was derived (NTP 1991, Matthews 1990). However, after long-term exposure to 175 mg/kg bw/d and 350 mg/kg bw/d tris(2-chloroethyl)phosphate, karyomegaly of renal tubule epithelial cells was noted in male and female B6C3F1 mice given in the low dose 175 mg/kg bw/d and was present in approximately 80% of the 350 mg/kg bw/d dosed male and female mice. Because a NOAEL for kidney lesions could not be established for male and female B6C3F1mice in the 2-year study, 175 mg/kg bw/d is considered to be the LOAEL for kidney lesions in B6C3F1 mice of both sexes (NTP 1991, Matthews 1993). Kidney lesions observed as hyperplasia and hypertrophy of the urinary tubule epithelium together with enlargement of the nuclei were noted in male and female Scl:ddY mice fed at ≥12 mg/kg bw/d tris(2-chloroethyl)phosphate in the diet for 18 months. Unfortunately, no incidences on the microscopic findings in the kidneys of treated groups were reported. There are no published historical control data for the Scl:ddYY mouse strain. However, there are data from concurrent control groups of a 2-year study investigated the chronic toxicity and the carcinogenicity of 1,2,4-trichlorbenzene on the skin using the Scl:ddYY mouse strain (Yamamoto, 1982). In the control groups of 50 male and 50 female mice skin-painting was carried out with acetone alone. No findings of cellular necrosis with a regenerative hyerplasia of the urinary tubule epithelium or enlargement of the nuclei were reported in this study from the concurrent controls. As main microscopic findings in the kidneys inflammation and amylolidosis were observed. The following incidences were noted: inflammation in 12/50 males and in 2/50 females; amyloidosis in 4/50 males and in 5/50 females.

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In addition in the 18-month dietary study, changes in tissue morphology of the kidneys were not only observed in male and female mice at the top dose level of 1500 mg/kg bw/d, which clear exceeded the MTD (maximum tolerated dose) for this mouse strain. Sign of cytotoxicity leading to cellular necrosis with a regenerative hyperplasia were reported from all treatment groups. There were hyperplasia and hypertrophy of the urinary tubule epithelium together with enlargement of the nuclei, which showed abnormal division, degeneration and necrosis at several times. Although no quantitative information on these microscopic findings in the kidney of treated groups were reported they were assessed as relevant toxic effects, also seen from this point of view that no such kidney findings were observed in the concurrent controls. Thus, 12 mg/kg bw/d is considered to be the LOAEL for kidney lesions in Scl:ddY mice of both sexes (Takada et al., 1989).

For kidney lesions:
103-week oral (gavage)/ F344/N rat

$LOAEL_{sys}$ 44 mg/kg bw/d (NTP 1991, Matthews 1993)

3-months oral (feed)/male Sprague-Dawley CD rat
$NOAEL_{sys}$ 3000 ppm (192 mg/kg bw/d) (Stauffer Company 1980b)

103-week oral (gavage)/ B6C3F1 mice
$LOAEL_{sys}$ 175 mg/kg bw/d (NTP 1991, Matthews 1993)

18 months oral (feed)/ Scl:ddY mice
$LOAEL_{sys}$ 12 mg/kg bw/d (Takada et al., 1989)

Most of the available studies in rats and mice also reported effects in the liver. These findings observed in F344/N rats and B6C3F1 mice were limited to increased organ weights, without other corroborating findings in morphology. Therefore, these findings in organ weights were not considered to be toxicologically relevant. However, changes in liver morphology were reported in Scl:ddY mice of all dose groups (≥12 mg/kg bw/d). There were focal necrosis, vacuolation of hepatocytes and extramedullary hematopoiesis. However, such findings were also observed in controls.

In conclusion, all listed studies were of sufficient data quality. Degenerative lesions in the brain occurred in rats in a dose related pattern, and in addition, in a clear time-response relationship in frequency, intensity and severity. Male rats were less sensitive to adverse brain effects than female rats. The lowest NOAELs for systemic effects/brain effects were derived in each case from subchronic and chronic oral toxicity studies for rats at dose levels between 44 to 88/175 mg/kg bw/d. Mice were less sensitive to tris(2-chloroethyl)phosphate than rats with respect to brain effects. NOAELs for brain effects were established between 175 to 350 mg/kg bw/d in mice of both sexes.

However, the most sensitive NOAEL/LOAEL was derived for kidney lesions. Because kidney effects appear to be the most sensitive endpoint for repeated exposure of tris(2-chloroethyl)phosphate and both the rat and the mouse are the species most sensitive to tris(2-chloroethyl)phosphate. Kidney effects observed in rats and mice were dose- and time-related

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with respect to incidence and severity. Changes in kidney morphology were noted in Sprague-Dawley CD rats and F344/N rats and in B6C3F1 and Scl:ddY mice. There were hyperplasia, and karyomegaly in the cortical tubule epithelium in the kidneys combined with signs of cellular necrosis. In both rat strains and both mouse strains kidney lesions of this same kind were observed. Only in subchronic toxicity studies a no-observed-adverse-effect-level (NOAEL) for kidney lesions was estimated; for male and female Sprague-Dawley CD rats at 3000 ppm (m: 192 mg/kg bw/d; f: 215 mg/kg bw/d) (Stauffer Chemical Company 1980b) and for B6C3F1 mice at 350 mg/kg bw/d (NTP 1991, Matthews 1990). However, a NOAEL for tissue changes in the kidney could not be derived for male and female F344/N rats after chronic exposure to ≥44 mg/kg bw/d tris(2-chloroethyl)phosphate, furthermore, for B6C3F1 mice after long-life exposure to ≥175 mg/kg bw/d (NTP 1991, Matthews 1993), and also not for Scl:ddY mice after feeding of ≥12 mg/kg bw/d in the diet for 18 months (Takada et al. 1989). This carcinogenicity study in Scl:ddY mice (cf. 4.1.2.8.1) differs in some respects from the published guidelines, however, the study is accepted due to the fact that the test procedure described is comparable to the guideline study with acceptable restrictions and is performed in accordance with generally accepted scientific standard. Therefore, the lowest LOAEL of 12 mg/kg bw/d for kidney lesions in Scl:ddY mice will be chosen as the basis for risk characterisation.

No data on local effects after repeated exposure to TCEP are available.

Conclusion on classification for repeated dose studies:

On the basis of the available data, classification of tris(2-chloroethyl)phosphate as harmful and labelling with Xn, R 48/22 (Harmful: danger of serious damage to health by prolonged exposure if swallowed) according to the criteria given in Directive 67/548/EEC is not necessary.

4.1.2.7 Mutagenicity

4.1.2.7.1 Studies in vitro

Bacterial assays (table 4.13)

In bacterial gene mutation tests there were negative results in Salmonella typhimurium tester strains TA 100, TA 1535 and TA 1538 up to approximately 10,000 µg/plate and in TA 98, TA 1537, up to 3333 µg/plate (Stauffer Chemical Company 1976; Prival et al. 1977; Haworth et al. 1983; NTP 1991).
Only Nakamura et al. (1979) reported on a positive finding with S-9 mix in one tester strain (TA 1535) for doses from 280 µg/plate up to 2800 µg/plate; higher doses had strong toxic effects.

Assays with mammalian cells (tables 4.14-4.17)

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A mammalian cell mutation assay with mouse lymphoma cells was negative with and without S-9 mix up to a cytotoxic dose of 1.07 µl/ml (Stauffer Chem. 1978). A gene mutation assay with V79 cells, only done without S-9 mix, was negative for concentrations up to 2000 µg/ml; no informations about cytotoxicity were given (Sala et al. 1982).

A chromosomal aberration assays with Chinese hamster ovary cells were negative for concentrations up to 1600 µg/ml with and without S-9 mix. No informations about cytotoxic effects were given (Galloway et al. 1987: also citated in NTP 1991).

A SCE test with V79 cells (Sala et al., 1982) was marginally positive with S-9 mix at the highest tested dose of 700 µg/ml and without S-9 mix from 700 µg/ml up to 3000 µg/ml (with S-9 mix: 9.7 SCE per cell as compared to 7.1 in the control; without S-9 mix: up to 9.0 SCE per cell as compared to 5.5 in the control); without S-9 mix the highest tested dose was strongly toxic. Another SCE test, also done with and without S-9 mix, had an equivocal result. Only in one out of two experiments there was a slight increase of SCE frequencies with S-9 mix from 500 µg/ml up to 1600 µg/ml; without S-9 mix concentrations up to 160 µg/ml were negative (Galloway et al. 1987). No informations about toxic effects were given.

An UDS test (liquid scintillation counting) with human WI-38 cells was negative with S-9 mix for concentrations up to 0.5 µl/ml and without S-9 mix for concentrations up to 0.1 µl/ml (Stauffer Chem. 1979). No informations about toxic effects were given.

4.1.2.7.2 Studies in vivo

Rodent bone marrow micronucleus tests (table 4.18)

In two in vivo micronucleus tests negative results were observed in bone marrow cells of mice after oral administration or intraperitoneal injection of the tested substance up to doses which correspond to the maximum tolerated dose.
Otto (1984) reported on a negative finding after oral administration of 1000 mg/kg bodyweight. The tested dose induced clinical signs and lethal effects. There are no data on local toxicity (P/N ratio). In an IRI study (1993) a negative result was obtained after intraperitoneal injection of doses up to 700 mg/kg bodyweight. Toxic signs were observed from 350 mg/kg bodyweight upwards. The highest tested dose of 700 mg/kg bodyweight induced lethal effects and at the 48 h-sampling also local cytotoxic effects (decrease of P/N ratio).

Furthermore, Sala et al. (1982) reported on an in vivo micronucleus assay with Chinese hamsters. After single intraperitoneal injection of doses up to 250 mg/kg bodyweight approximately a doubling of the spontaneous frequency of micronucleated polychromatic erythrocytes was found at 125 and 250 mg/kg bodyweight. Although a slight increase of micronucleated cells was observed the overall result was characterized as questionable by the authors. The authors also concluded that further data in the same or in other species are needed.

Drosophila melanogaster (table 4.19)

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Vogel and Nirvard (1991) reported on a negative test for mitotic recombination in somatic cells of Drosophila (test solutions up to 40 mmol/l were dropped on the surface of the food).

4.1.2.7.3 Conclusion

In general, bacterial gene mutation tests were negative. In vitro genotoxicity tests with mammalian cells were negative for gene and chromosome mutations, in a mouse-lymphoma-assay and in a UDS-test. Very weak effects in in vitro SCE tests are considered to be without relevance for mutagenicity. Two in vivo mice micronucleus tests were negative for application up to maximum tolerated doses, whereas a positive result of questionable validity was observed in another test. Also a Drosophila test was negative.
Overall, it can be concluded that there is no relevant evidence for mutagenicity of tris(2-chloroethyl)phosphate.

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Table 4.13 In vitro tests: Bacterial genotoxicity

Test system	Concentration range		Result	Toxicity	Remarks	Reference
	with S-9 mix	without S-9 mix				
Gene mutation; TA,98, TA 100, TA 1535, TA 1537, TA 1538	0.001 - 1.0 µl/plate (1.0 - 1000 µg/plate)	0.001 - 1.0 µl/plate (1.0 - 1000 µg/plate)	negative	no toxic effects	plate incorporation method purity: no data	Stauffer Chemical Company, 1976
Gene mutation; TA 100, TA 1535, TA 1538	1.0 - 10 µl/plate (1000 - 10'000 µg/plate)	1.0 - 10 µl/plate (1000 - 10'000 µg/plate)	negative	no toxic effects	plate incorporation method purity: no data	Prival et al., 1977
Gene mutation; TA 98, TA 100, TA 1535, TA 1537	33 - 3333 µg/plate	33 - 3333 µg/plate	negative	with and without S-9 mix at the highest tested dose	preincubation method purity: 99.5 %	Haworth et al., 1983
Gene mutation; TA 98, TA 100, TA 1537, TA 1538	330 - 3330 µg/plate	33 - 3333 µg/plate	negative	with and without S-9 mix at the highest tested dose	preincubation method purity: 98.0 %	NTP, 1991
Gene mutation; TA 98, TA 100, TA 1535, TA 1537, TA 1538	1.0 - 30 µmol/plate (285.5 - 8565 µg/plate)	1.0 - 30 µmol/plate (285.5 - 8565 µg/plate)	positive	with and without S-9 mix at the highest tested dose	strongly positive only in TA 1535 from 1.0 µmol/plate up to 10 µmol/plate (higher doses were strongly toxic); dose-dependent effect purity: no data	Nakamura et al., 1979

Table 4.14 In vitro tests: Mammalian cell gene mutations or Mouse-lymphoma-assay

Test system	Concentration range		Result	Remarks	Reference
	with S-9 mix	without S-9 mix			

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mouse lymphoma assay; L5178Y cells; tk locus	0,09 - 1.07 µl/ml (90 - 1070 µg/ml)	0,09 - 1.07 µl/ml (90 - 1070 µg/ml)	negative	toxicity: with S-9 mix clear effect at the highest tested dose; without S-9 mix clear effects from 0.8 µl/ml upwards purity: no data	Stauffer Chemical Company, 1978
HPRT locus; V79 cells	no tested	500 - 2000 µg/ml	negative	no information on toxicity purity: substance was purchased from Hoechst	Sala et al., 1982

Table 4.15 In vitro tests: Chromosomal aberrations

Test system	Concentration range		Result	Remarks	Reference
	with S-9 mix	without S-9 mix			
chromosomal aberrations; CHO cells	160 - 1600 µg/ml	160 - 1600 µg/ml	negative	treatment / sampling time: 2/14 h (with S-9 mix) and 12/14 h (without S-9 mix) no information on toxicity purity: 98 %	Galloway et al., 1987 (also citated in NTP, 1991)

Table 4.16 In vitro tests: sister chromatid exchanges (SCE)

Test system	Concentration range		Result	Remarks	Reference
	with S-9 mix	without S-9 mix			

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V79 cells	490 - 700 µg/ml	343 - 3000 µg/ml	positive	marginally positive with and without S-9 mix: 1. experiment/without S-9 mix dose (µg/ml) SCE/cell neg.co. 5.6 343 6.8 490 7.0 700 **8.2** 1000 **7.4** 1. experiment/with S-9 mix dose (µg/ml) SCE/cell neg.co. 7.1 490 8.5 700 9.7 2. experiment/without S-9 mix dose (µg/ml) SCE/cell neg.co. 4.6 2000 7.0 3000 9.0 with and without S-9 mix no positive controls without S-9 mix strongly toxic at the highest tested dose purity: substance was purchased from Hoechst	Sala et al., 1982

CHO cells	160 - 1600 µg/ml	5.0 - 160 µg/ml	equivocal	slight increase of SCE in one out of two experiments with S-9 mix; negative without S-9 mix 1. experiment/with S-9 mix dose (µg/ml) SCE/cell neg.co. 8.3 160 9.2 500 10.1 1600 9.9 po.co. 22.6 2. experiment/with S-9 mix dose (µg/ml) SCE/cell neg.co. 9.2 1200 9.3 1400 10.1 1600 10.6 po.co. 21.2 no informations on toxicity purity: substance was purchased from NTP chemical repository	Galloway et al., 1987 (also cited in NTP, 1991)

Table 4.17 In vitro tests: unscheduled DNA syntheses (UDS)

Test system	Concentration range		Result	Remarks	Reference
	with S-9 mix	without S-9 mix			
human WI-38 cells	0.0005 - 0.5 µl/ml (0.5 - 500 µg/ml)	0.001 - 0.1 µl/ml (1.0 - 100 µg/ml)	negative	liquid scintillation counting toxicity: no data purity: no data	Stauffer Chemical Company, 1979

Table 4.18 In vivo tests: Rodent bone marrow tests with mice

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Test system	Doses (mg/kg bw)	Expos. regimen	Sampl. Times	Result	Local cytotox.	General toxicity	Remarks	Reference
CD-1 mice; bone marrow erythrocytes	175 - 700	1 x i.p.	16, 24 and 48 h	negative	decre-ased P/N ratio at highest tested dose at 48 h - sampling	lethal effects at the highest tested dose clinical signs from 350 mg/kg upwards	highest tested dose corresponds to the maximum tolerated dose 5 males and 5 females per group purity: 99.2 %	IRI, 1993
NMRI mice; bone marrow erythrocytes	1000	1 x p.o.	24, 48 and 72 h	negative	no data	toxic signs lethal effects (3/30)	tested dose corresponds to the maximum tolerated dose 5 males and 5 females per group purity: 99.0 %	Otto, 1984
Chinese hamsters; bone marrow erythrocytes	62.5 - 250	1 x i.p.	24 h	equivocal	no data	no data	although a slight increase of micronucleated cells was observed the overall result was characterized as questionable by the authors dose MN cells (%) neg. co. 0.35 62.5 0.52 125 0.66 250 0.70 po.co. 7.06 2 males and 2 females per group purity: no data	Sala et al., 1982

Table 4.19 In vivo tests: Tests with Drosophila melanogaster

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Test system	Exposure	Result	Remarks	Reference
mitotic recombination	feeding: 2.5 - 40 mmol/l were dropped on the surface of the food	negative	purity - the only information: substance was commercially available	Vogel and Nirvard, 1991

4.1.2.8 Carcinogenicity

Carcinogenicity studies by gavage conducted to modern regulatory standards are available in rats and mice. These studies provide clear evidence that tris(2-chloroethyl)phosphate is carcinogenic in two animal species. Tris(2-chloroethyl)phosphate causes benign and malignant tumors at various organ sites in rats and mice.

4.1.2.8.1 Studies in animals

In vivo studies

- ***Oral***

Gavage studies (rat and mouse)

Rats

Groups of 60 male and 60 female F344/N rats were administered by gavage to tris(2-chloroethyl)phosphate in corn oil for 5 days per week for up to 103 weeks (NTP 1991; Matthews 1993). Ten animals per sex per group from each species were designed for interim evaluation (necropsy, hematology, and clinical chemistry) at 66 weeks.

Rats of each sex received 0, 44, or 88 mg/kg bw /d tris(2-chloroethyl)phosphate (purity 98%). Results of non-neoplastic findings at interim sacrifice (66 weeks) and at 103 weeks were described in detail in section 4.1.2.6.
The survival of female rats receiving 88 mg/kg bw /d and, to a lesser extent, male rats was reduced in relation to controls. The reduced survival of females was attributed, in part, to the neurotoxicity of tris(2-chloroethyl)phosphate and to a marginally increased incidence of mononuclear cell leukemia. Final mean body weights of surviving rats were similar to those of controls. The principal tris(2-chloroethyl)phosphate-related effects occurred in the kidney and brain of dosed rats (for brain lesions see section 4.1.2.6).Oral administration of tris(2-chloroethyl)phosphate to rats for up to two years was associated with a marked increase in the incidence of neoplastic lesions in the kidney, mainly proliferative lesions and adenomas of the renal tubule. They occurred with a dose-related increased incidence in both males and females. Hyperplasia and adenomas occurred in nearly 50% of the 88 mg-dose males and in 10% of the 44 mg-dose males. In male rats receiving 88 mg/kg bw tris(2-chloroethyl)phosphate renal tubule hyperplasia were seen in: control 0/50; low-dose 2/50; high-dose 24/50; renal tubule adenoma in: control 1/50; low-dose 5/50; high-dose 24/50 and, to a lesser extent, in female rats renal tubule hyperplasia was observed in: control 0/50; low-dose 3/50; high-dose 16/50, and renal tubule adenoma: control 0/50; low-dose 2/50; high-dose 5/50. The increased incidences in the 88 and 44 mg/kg bw/d groups for adenomas in the

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kidneys exceeded the NTP historical control range for this lesion. The historical incidence of renal tubule adenoma of the kidney in control gavage (corn oil) F344/N rats from previous 2-year studies up to 1989 is 0.2% (0-2% range) in males and 0.1% (0-2% range) in females (NTP 1991; Matthews 1993). The same range for the overall mean historical incidences of renal tubule adenoma of the kidneys in vehicle control F344/N rats from recent 2-year carcinogenicity studies by the NTP was reported in another review (Eustis 1994). There was also less than 1% [0.75% (0-2% range) in males and 0.19% (0-2% range) in females]. The renal tubule proliferating lesions were small. The historical incidence of hyperplasia in the kidney in control gavage F344/N rats is 1.18% (0-6% range) in males and 0.10% (0-2% range) in females (Eustis 1994). Renal tubule carcinoma occurred in one control and one high-dose male rat. The overall rate of carcinomas in the NTP historical control gavage F344/N rats was also very small, 4/1,069 (0.37%) for males and 0/1,068 (0.00%) for females (Eustis 1994). Thus, the renal response in rats appears to be restricted to hyperplasia and benign tumors. Adenoma and carcinoma of the renal tubule constitute a morphological continuum, and there are no cytologic features that clearly and unambiguously distinguish the more benign neoplasms from those with the ability to metastasize. It is assumed that the renal adenomas represent an early stage of the development of a carcinoma. Therefore, the marked increase in the incidence of renal tubule neoplasms in F344/N rats gavaged with tris(2-chloroethyl)phosphate is considered to represent reasonable clear evidence of carcinogenic activity.
Thyroid follicular cell neoplasms in male and female rats may have been related to tris(2-chloroethyl)phosphate administration. The incidence of thyroid gland follicular cell neoplasms (adenomas) was slightly increased (male rats: control 1/50; low-dose 2/48; high-dose 3/50; female rats: control 0/50; low-dose 1/50; high-dose 1/50). Thyroid follicular cell neoplasms occurred with a significant positive trend in female rats, and the incidence of follicular cell adenoma or carcinoma combined was significantly greater in the 88 mg/kg bw/d females than in controls. The incidence of follicular cell neoplasms was also increased in the 88 mg/kg bw/d males, but this increase was not statistically significant. The combined incidence of follicular cell neoplasms in the 88 mg/kg bw/d male and female rats equals or exceeds the upper rates for NTP historical controls from previous 2-year studies up to 1989 (males: 51/2,106, 2.4, range 0-10%; females: 34/2,107, 1.6%, range 0-6%). However, the low incidence of follicular cell hyperplasia did not support an effect of tris(2-chloroethyl)phosphate on the thyroid gland; no hyperplasia was seen in males, and only one dosed and one control female had follicular cell hyperplasia. The lack of hyperplasia in rats argues against considering the follicular cell neoplasms as related to tris(2-chloroethyl)phosphate since most thyroid carcinogens also induce hyperplasia. Therefore, it is uncertain whether the thyroid follicular cell neoplasms in the F344/N rats are related to the administration of tris(2-chloroethyl)phosphate.

Mononuclear cell leukemia in male and female rats may have been related to tris(2-chloroethyl)phosphate administration. The incidence of mononuclear cell leukemia was increased in both dosed male and female rats (male rats: control 5/50; low-dose 14/50; high-dose 13/50 and female rats: control 14/50; low-dose 16/50; high-dose 20/50). There were significant positive trends in both sexes, and the incidence in males receiving 44 or 88 mg/kg bw/d and females receiving 88 mg/kg bw/d was significantly greater than in their respective controls, but it is uncertain whether these were related to tris(2-chloroethyl)phosphate administration. Since the frequency of mononuclear cell leukemia in dosed male and female rats was within in the range of historical controls from previous 2-year studies up to 1989 (males:2-44%, females: 0-33%, s. key at the end of the Table 4.19). Thus, for both males and females the highest rate is within the historical control range. Therefore, these marginal

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increases in leukemia in male and female F344/N rats were not considered to be clearly related to administration of tris(2-chloroethyl)phosphate. In the brain, benign granular cell tumors were observed in 3/50 males at 88 mg/kg bw/d only. There were no treatment-related increases in the incidence of tumors in the brain of females. The incidence of the mostly observed neoplastic lesions in F344/N rats in the 2-year gavage study of tris(2-chloroethyl)phosphate was given in the following table, Table 4.20.

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Table 4.20

Summary table: Incidence of neoplastic lesions in F344/N rats after life-time exposure to tris(2-chloroethyl)phosphate (NTP 1991, Matthews 1993)

Doses	0	44 mg/kg bw/d	88 mg/kg bw/d
Male			
Kidney: Renal tubule			
Hyperplasia, overall rates[a]	0/50 (0%)	2/50 (4%)	24/50 (48%)
Historical control data[b]:	12/1,019 (1.18%); range 0%-6%		
Adenoma, overall rates[a]	1/50 (2%)	5/50 (10%)	24/50 (48%)
Carcinoma, overall rates[a]	1/50 (2%)	0/50 (0%)	1/50 (2%)
Adenoma or Carcinoma, overall rates	2/50 (4%)	5/50 (10%)	25/50 (50%)
Historical control data[b]:	12/2,142 (0.6% ± 0.9%); range 0%-2%		
Thyroid Gland: Follicular Cell			
Hyperplasia, overall rates[a]	0/50 (0%)	0/50 (0%)	0/50 (0%)
Adenoma, overall rates[a]	1/50 (2%)	2/48 (4%)	3/50 (6%)
Carcinoma, overall rates[a]	0/50 (0%)	0/50 (0%)	2/50 (4%)
Adenoma or Carcinoma, overall rates	1/50 (2%)	2/50 (4%)	5/50 (10%)
Historical control data[c]:	51/2,106 (2.4% ± 2.3%); range 0%-10%		
Hematopoietic System: Mononuclear Cell Leukemia			
Overall rates[a]	5/50 (10%)	14/50 (28%)	13/50 (26%)
Historical control data[d]:	321/2,149 (14.9% ± 10.8%); range 0%-44%		
Female			
Kidney: Renal tubule			
Hyperplasia, overall rates[a]	0/50 (0%)	3/50 (6%)	16/50 (32%)
Historical control data[b]:	1/1,019 (0.10%); range 0%-2%		
Adenoma, overall rates[a]	0/50 (0%)	2/50 (4%)	5/50 (10%)
Historical control data[e]:	1/2,144 (0.1% ± 0.3%); range 0%-2%		
Thyroid Gland: Follicular Cell			
Hyperplasia, overall rates[a]	1/50 (2%)	0/50 (0%)	1/50 (2%)
Adenoma, overall rates[a]	0/50 (0%)	1/50 (2%)	1/50 (2%)
Carcinoma, overall rates[a]	0/50 (0%)	2/50 (4%)	3/50 (6%)
Adenoma or Carcinoma, overall rates[a]	0/50 (0%)	3/50 (6%)	4/50 (8%)
Historical control data[f]:	341/2,107 (1.6% ± 1.6%); range 0%-6%		
Hematopoietic System: Mononuclear Cell Leukemia			
Overall rates[a]	14/50 (28%)	16/50 (32%)	20/50 (40%)

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Historical control data[g]:	329/2,150 (15.3% ± 10.6%); range 0%-33%

[a] Number of tumor-bearing animals/number of animals examined at site; [b] 2-year historical incidence for vehicle control groups in NTP corn oil gavage studies (mean ± standard deviation); [c] 2-year historical incidence for vehicle control groups in NTP corn oil gavage studies (mean ± standard deviation); [d] 2-year historical incidence for vehicle control groups in NTP corn oil gavage studies (mean ± standard deviation); [e] 2-year historical incidence for vehicle control groups in NTP corn oil gavage studies (mean ± standard deviation); [f] 2-year historical incidence for vehicle control groups in NTP corn oil gavage studies (mean ± standard deviation); [g] 2-year historical incidence for vehicle control groups in NTP corn oil gavage studies (mean ± standard deviation)

Overall, in a standard carcinogenicity study (2-year gavage study) in F344/N rats, there was clear evidence of carcinogenic activity for males and females receiving tris(2-chloroethyl)phosphate as shown by increased incidences of renal tubule adenomas at ≥44 mg/kg bw /d (below the maximum tolerated dose level, MTD), and statistically significantly high incidences at 88 mg/kg bw/d. Moreover, focal renal tubule hyperplasia, a possible precursor of adenoma, was also seen in about half the 88 mg/kg bw/d males.

Mouse

The 2-year carcinogenicity study in B6C3F1 mice was conducted by administering (gavage) 0, 175, or 350 mg/kg bw tris(2-chloroethyl)phosphate (purity 98%) in corn oil to groups of 60 male and 60 female mice, 5 days per week for up to 103 weeks (NTP 1991, Matthews 1993). Ten animals per sex per group from each species were designed for interim evaluation (necropsy, hematology, and clinical chemistry) at 66 weeks. Results of non-neoplastic findings at interim sacrifice (66 weeks) and at 103 weeks were described in detail in section 4.1.2.6.

There were no significant differences in survival between dosed and control groups of either sex, and final mean body weights of mice were similar among all groups. The principal tris(2-chloroethyl)phosphate-related effects occurred in the kidney. In the original diagnosis, in which single sections of left (through the longitudinal plane) and right (through the transverse plane) kidneys were examined microscopically, the following neoplastic lesions were observed: tubule cell adenomas in one control male, one low-dose female, and one high-dose male, and an adenocarcinoma was seen in another high-dose male. Karyomegaly (nuclear enlargements) in the proximal convoluted tubules was noted in the kidneys of appr. 80% of mice receiving 350 mg/kg bw/d and less frequently in mice receiving 175 mg/kg bw/d. Focal hyperplasia, a potentially preneoplastic lesion, was seen in a high-dose male. Thus, it has to operate on the assumption that long-life administration of tris(2-chloroethyl)phosphate may lead to marginal increase in renal tubule neoplasms, and increased incidences of renal tubule hyperplasia also in mice. However, the interpretation of animal carcinogenicity tests traditionally rely almost exclusively upon a comparison of specific tumor rates in treated vs. matched and, perhaps, historical control animals. Another approach to aid interpretation of the data was to examine multiple sections of the kidney. Because of the occurrence of tris(2-chloroethyl)phosphate-related kidney neoplasms in F344/N rats, the association of karyomegaly with kidney neoplasms known from other chemicals, and the low spontaneous incidence of kidney neoplasms in historical control, additional sections of the kidney were prepared from each control and treated mice, examined microscopically, and evaluated to provide a clearer indication of the potential effect of tris(2-chloroethyl)phosphate in this

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organ and to give more data for comparison of the dosed and control groups, and therefore, to allow a more rigorous statistical comparison with concurrent controls. In this subsequent examination of step-sections of all the mouse kidneys, two additional adenomas were observed in high-dose males and one in a low-dose female. Focal hyperplasia was observed in one additional control male and four additional high-dose males (two from the 66-week interim and two from the 2-year group).

A survey of the incidence of selected renal tubule cell lesions and neoplastic findings in the Harderian gland in B6C3F1 mice in the 2-year gavage study of tris(2-chloroethyl)phosphate (NTP 1991, Matthews 1993) was given in the following table, Table 4.21.

Table 4.21

Summary table: Incidence of selected renal tubule cell lesions and neoplastic findings in the Harderian gland in B6C3F1 mice after life-time exposure to tris(2-chloroethyl)phosphate (NTP 1991, Matthews 1993)

Doses	0	175 mg/kg bw/d	350 mg/kg bw/d
Male			
Kidney: Renal tubule			
Karyomegaly, overall rates[a]	2/50 (4%)	16/50 (32%)	39/50 (78%)
Hyperplasia, original and step sections combined[a]	1/50 (2%)	0/50 (0%)	3/50 (6%)
Adenoma, original and step sections combined[a]	1/50 (2%)	1/50 (2%)	3/50 (6%)
Adenocarcinoma, original and step sections combined[a]	0/50 (0%)	0/50 (0%)	1/50 (2%)
Historical control data[c]:	5/2,183 (0.2%), 0-2% range		
Historical control data[d]:	4/949 (0.42%), 0-2% range)		
Female			
Kidney: Renal tubule			
Karyomegaly, overall rates[a]	0/50 (0%)	5/49 (10%)	44/50 (88%)
Hyperplasia, original and step sections combined[a]	0/50 (0%)	1/49 (2%)	2/50 (4%)
Adenoma, original and step sections combined[a, e]	0/50 (0%)	1/49 (2%)	0/50 (0%)

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Historical control data[d]:	0/949 (0.00%)		
Harderian Gland			
Hyperplasia, combined[a, b]	1/58 (2%)	4/59 (7%)	2/59 (3%)
Adenoma, combined[a, b]	3/59 (5%)	7/60 (12%)	9/60 (15%)
Adenoma or carcinoma, combined[a, b]	3/59 (5%)	8/60 (13%)	10/60 (17%)
Historical control data[e]:	53/2,193 (2.4%), 0-10% range		
Historical control data[f]:	18/609 (3.0%)		
Historical control data[g]:	(2.8%)		
Historical control data[h]:	(5.1%)		

[a] Number of tumor-bearing animals/number of animals examined at site; [b] combined data from the 66-week interim evaluation and 104-week terminal evaluation; [c] 2-year historical incidence of renal tubule adenomas for vehicle control groups in NTP corn oil gavage studies up to 1989 (NTP 1991); [d] 2-year historical incidence of renal tubule adenomas for vehicle control groups data, analyzed from 16 recent NTP studies (Eustis et al. 1994); [e] 2-year historical incidence of Harderian gland tumors for vehicle control groups in NTP corn oil gavage studies up to 1989 (NTP 1991); [f] Morphology of spontaneous Harderian gland tumors in aged B6C3F1 mice (Ihara M et al., 1994); [g] Spontaneous tumors in aging (C57BL/6N X C3H/HeN)F1 (B6C3F1) mice (Tamano et al., 1988); [h] Frequency and time of spontaneous tumors found in B6C3F1 mice oncogenicity studies over 10 years (Eiben R, 2001)

In male B6C3F1 mice a further evaluation of renal tumors using the results of original single sections combined with those of step-sections revealed the following incidences of renal tubule neoplasms 1/50 (2%), 1/50 (2%), and 4/50 (8%) for males. In female mice, during the initial examination of single sections, one adenoma was observed in the low-dose group. The step-sections did not identify any additional neoplasm in females, although it did identify hyperplasia in one low- and two high-dose female mice. In male mice, the observed incidences for adenoma in the kidneys in the 350 mg/kg bw/d group exceeded the NTP historical control range for this lesion. Renal tubule neoplasms have been observed infrequently in gavage vehicle (corn oil) control B6C3F1 mice. The historical incidence of renal tubule adenoma of the kidney in control gavage (corn oil) male B6C3F1 mice from previous 2-year studies up to 1989 reported in the presented NTP study is 0.2% (5/2,183; 0-2% range)(NTP 1991). In another report examined 16 recent NTP studies the historical incidences of renal tubule adenoma of the kidney in control gavage (corn oil) B6C3F1 mice were 4/949 (0.42%; 0-2% range) in males and 0/943 (0.00%) in females (Eustis et al. 1994). Although the slight increase in tubule cell neoplasms in male B6C3F1 mice was not statistically significant, the marginal increase in both tubule cell hyperplasia and tubule cell neoplasms is suggestive of a substance-related effect. The 8% incidence of renal tubule neoplasms in the 350 mg/kg bw/d males is greater than the overall rate of 0.2-0.42% for the groups of historical control gavage male mice for which multiple sections were evaluated. In addition to these observations, the incidences of renal tubule hyperplasia and the rates of atypical renal tubule epithelial cells were also increased. The nucleoli were increased in size and number or were too large for size of the cell (increased nuclear: cytoplasmic ratio). Dose-related increased rates of karyomegaly in cells of renal tubule epithelium were noted in both male and female mice given ≥175 mg/kg bw/d when compared to controls. Based on the additional results received from the resection examination and the observations of increased renal proliferative lesions and also of cell atypia in renal tubule epithelium in mice treated

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with tris(2-chloroethyl)phosphate, and because renal tubule neoplasms are uncommon in control B6C3F1 mice, thus, the data in the kidneys were considered to provide a clear evidence of carcinogenic activity of tris(2-chloroethyl)phosphate for male B6C3F1 mice.

Furthermore, there was equivocal evidence of carcinogenic activity for female B6C3F1 mice as shown by a marginally increased incidence of Harderian gland adenomas. In females receiving 350 mg/kg bw/d tris(2-chloroethyl)phosphate the incidence of Harderian gland neoplasms, primarily adenomas, was marginally increased relative to concurrent controls. There were adenoma or carcinoma: control 3/50, low-dose 8/50, and high-dose 7/50; in addition, three Harderian gland neoplasms occurred in the 350 mg-dose female mice evaluated after 66 weeks. If the incidence rates for the interim sacrifice groups and 2-year animals are combined, there is a significant trend, and the incidence in the 350 mg/kg bw/d group is significantly greater than that in controls (adenoma or carcinoma: 3/59, 8/60, 10/60). Few reports are available regarding the incidence of spontaneous Harderian gland tumors in mice. However, the frequency in all strains is low. The historical incidence of Harderian gland tumors in control gavage (corn oil) female B6C3F1 mice from previous 2-year studies up to 1989 reported in the presented NTP study is 2.4% (53/2,193; 0-10% range) (NTP 1991). In another survive spontaneous Harderian gland tumors in B6C3F1 mice were found in 18 (3.0%) of 609 females (Ihara et al. 1994), and in 2.8% females sacrificed in studies between weeks 79 to 104 (Tamano et al., 1988). In a further study evaluating frequency and time of spontaneous tumors in B6C3F1 mice in oncogenicity studies over 10 years, Harderian gland adenomas were found in 5.1% females (Eiben 2001). In this view the incidences of Harderian gland tumors in both dose groups of females exceeded the range of the concurrent controls and also of the historical controls. There were no corresponding tris(2-chloroethyl)phosphate-related increase incidence of hyperplasia of the Harderian gland in female mice. Therefore, this marginal increase was considered as equivocal evidence of carcinogenic activity to tris(2-chloroethyl)phosphate.

Overall, in a standard carcinogenicity study (2-year gavage study) in B6C3F1 mice, some evidence of carcinogenic activity for male mice was obtained by a marginally increased incidence of renal tubule cell neoplasms at 350 mg/kg bw/d tris(2-chloroethyl)phosphate, and for females as shown by a markedly increased incidence of Harderian gland adenomas at the lowest tested dose of 175 mg/kg bw/d tris(2-chloroethyl)phosphate and above.

Diet study (mouse)

Groups of 50 male and 50 female Scl:ddY mice were fed a commercial diet containing approximately 0, 0.012, 0.06, 0.3, and 1.5% (equivalent to 0, 12, 60, 300, and 1500 mg/kg bw/d, calculated on an assumed body weight of 20 g and food consumption of 10% body weight/day) tris(2-chloroethyl)phosphate (purity 98%) over a period of 18 months (Takada et al., 1989). This study differs in some respects from the published guideline OECD TG 451. There are basic data comparable to this guideline with respectable restrictions. The test parameter documented do not totally comply with this testing guideline for carcinogenicity, however, there was a well-documented study reports which meets basic scientific standards. The supplied data are considered as valid for use in risk assessment.

In both sexes fed 1500 mg/kg bw/d tris(2-chloroethyl)phosphate, mortality rates were clearly higher (approximately 40% survival compared to around 65% survival in controls) and body

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weight gain was apparently suppressed compared to other groups (approximately 60% lower than the control value). Survival of groups dosed ≤300 mg/kg bw/d was not significantly different than that of controls, and no symptoms of toxicity were observed in mice of either sex. No differences in food consumption were found between the groups for both sexes. In hematology, no changes were found apart from significant elevation in platelets in males of the 300 mg/kg bw/d dose group. Significant declines were found in the weights of the heart and testes in males and kidneys in females from the 1500 mg/kg bw/d dose group. At histopathological examinations, hyperplasia, and hypertrophy of the urinary tubule epithelium together with karyomegaly were noted in the kidney of all treated animals. These nuclei were polymorphic, and several times abnormal division, degeneration and necrosis were observed. Unfortunately, no incidences of these findings were reported. In addition, cysts of the kidneys, necrosis and interstitial fibrosis were observed only in animals of the 1500 mg/kg bw/d dose group. No such findings in the kidney were reported in controls.

Increased tumor incidence was observed in the kidney when compared to concurrent controls. The renal tumors included renal cell adenomas with proliferation of tumor cells into tubules, cell heteromorphy and poor cell division, and renal cell carcinomas in which the tumor cells reproduced substantially, were polymorphic and showed cell division. An increased incidence of renal adenomas was reported in males at 300 mg/kg bw/d and above, and in females at the top dose. The following incidences of adenomas were observed: in males (0/50, 0/49, 0/49, 2/47, 9/50), and in females (0/49, 0/49, 0/50, 0/49, 2/50). Furthermore there was an increased incidence of renal carcinomas in males (2/20, 0/49, 2/49, 3/47, 32/50) and females (1/50 at 1500 mg/kg bw/d and zero in all other groups). Although the number of kidney tumors observed in male mice at dose of 300 mg/kg bw/d was small compared to concurrent controls, this finding was assessed as relevant, especially in terms of spontaneous renal neoplasms in mice occur infrequently. When they do develop, the affected mice are generally at least at the age of 12 months and are usually older than 18 months (Cohen and Friedell, 1982). Spontaneous occurring renal adenomas and carcinomas are rare in all mice strains with exception of strain BALB/cf/Cd and various lines of Swiss mice (CD-1, CF-1, Swiss-Webster, Swiss-E1). Males seem to be more frequently affected than females. The following frequencies of spontaneous epithelial tumors of male mouse kidney cortex are reported: B6C3F1: 0.1%, 3/2543 (Ward et al., 1979); C57BL/6CrSlc: 0.4%, 2/463 (Nakamura et al., 1992); SWR/J: 0.1%, 1/671 (Rabstein et al., 1973); CD1 Ha M/ICR: 0.2%, 2/1000 (Percy and Jonas, 1971); SPF Swiss Webster derived: 1.9%, 2/101 (Sher, 1974); CF-1 Swiss: 3/55, 5.4% (Tomatis et al., 1972); BALB/cf/Cd: 60-70%, 215-251/358 (Rabstein and Peters, 1973). It is even more rare in females, with reported incidences in B6C3F1: 0.08%, 2/2522 (Ward et al., 1979); C57BL/6CrSlc: 0.2%, 1/463 (Nakamura et al., 1992); SWR/J: 0 (Rabstein et al., 1973); CD1 Ha M/ICR: 0 (Percy and Jonas, 1971); SPF Swiss Webster derived: 0 (Sher, 1974); CF-1 Swiss: 1/56, 1.8% (Tomatis et al., 1972); BALB/c: 0.5%, 4/791 (Frith, 1986); BALB/cf/Cd: 40%, - (Claude, 1958). Unfortunately, there are no published historical control data for the ScI:ddYY mouse strain available. However, there are data from concurrent control groups of a 2-year study investigating the chronic toxicity and the carcinogenicity of 1,2,4-trichlorbenzene on the skin using the ScI:ddYY mouse strain (Yamamoto, 1982). In the control group of 50 male and 50 female mice skin-painting was carried out with acetone alone. The incidence of kidney tumors was zero in animals of both sexes. Further data from concurrent controls were found in a study which examined the induction of renal and liver tumors in male ddY mice by Ochratoxin A, a mycotoxin (Kanisawa and Shigetoshi, 1978). In the control group of 10 male mice fed basal died for 50 weeks no renal cell tumors and no atypical tubule cells were observed. The data presented showed that the very low incidence of neoplasms in the kidneys of the concurrent control groups of ScI:ddYY mice in the described

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study and the zero incidence of the concurrent control groups in the 2-year study of 1,2,4-trichlorobenzene corresponds with the low frequency for spontaneous kidney tumors in several mouse strains reported in the literature.

Increased tumor incidences were also observed in the liver of male mice when compared to concurrent controls. There were adenomas in which the tumor cells proliferated and compressed the surrounding liver cells. They showed enlarged cytoplasm and poor cell division, together with carcinomas in which the tumor cells were heteromorphic with atypical-sized or contained multiple nuclei presenting a funicular structure with two or more cell layers. The incidence of benign hepatocellular adenomas was significantly increased in the 300 mg/kg bw/d male dose group and above. In addition there was a slight , but not dose-related, increase in the incidence of liver carcinomas in males (1/50, 1/49, 4/49, 2/47, 3/50). The liver is the most common target site in the mouse, and among mutagens as well as nonmutagens. Hepatocellular adenoma and hepatocellular carcinoma occur spontaneously in many strains. The incidence of spontaneous liver tumors in mice varies greatly among strains. In common, in a given strain, spontaneous hepatocellular neoplasms are much more frequent in males than in females. The following frequencies of spontaneous liver tumors were reported: B6C3F1: 7-58% in males, 0-21% in females (Tarone et al., 1980); C57BL/6CrSlc: 9.5% in males, 1.8% in females (Nakamura et al., 1992); CF-1: 21-39% in males (Turusov et al., 1973). In the above cited 2-year study of 1,2,4-trichlorobenzene on the skin using the ScI:ddYY mouse strain no liver tumors were noted in male and female mice of the concurrent control groups (Yamamoto, 1982). In the other long-term study of ochratoxin A using male ddY mice hyperplastic nodules in the liver were found in two of 10 male control mice fed only basal diet for 50 weeks (Kanisawa and Shigetoshi, 1978). Thus, there was stated that the liver tumor incidences for the concurrent controls reported in the described study with ScI:ddYY mice confirmed these published data. In view of this results, it was concluded that tris(2-chloroethyl)phosphate is an oncogenic substance which may induce tumors in the liver of +ScI:ddYY mice.

In females, significant increases were found in the incidence of papillomas and squamous cell carcinomas (combined) in the forestomach of the 1500 mg/kg bw/d dose group, and in those of leukemia of 300 mg/kg bw/d and 1500 mg/kg bw/d dose groups. However, the relevance of these findings in the forestomach for human health risk assessment is questionable, since humans have no forestomach. However, rodent forestomach carcinogens may promote human carcinogenesis in other, different anatomical sites. In addition, some other tumors, like lung adenomas and mammary carcinomas, and leukemia in females, occurred in greater number in the treated groups but with no significant difference in incidence between tris(2-chloroethyl)phosphate treated groups and controls. A survey of tumor incidences in the kidneys and liver in Scl:ddY mice exposed to tris(2-chloroethyl)phosphate via the diet over a period of 18 months was given in the following table, Table 4.22.

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Table 4.22

Summary table: Incidence of renal cell tumors, and hepatocellular tumors seen in Scl:ddY male mice after an 18 months exposure period via the diet to tris(2-chloroethyl)phosphate (Takada et al., 1989)

Doses (mg/kg bw/d)	0	12	60	300	1500
			Male		
Kidney					
Adenoma, overall rates[a]	0/50 (0%)	0/49 (0%)	0/49 (0%)	2/47 (4.3%)	9/50 (18%)
Carcinoma, overall rates[a]	2/50 (4.1%)	0/49 (0%)	2/49 (4.1%)	3/47 (6.4%)	32/50 (64%)
Adenoma and Carcinoma, combined[a]	2/50 (4%)	0/49 (0%)	2/49 (4.1%)	5/47 (10.6%)	41/50 (82%)
Liver					
Adenoma, overall rates[a]	3/50 (6%)	4/49 (8.2%)	3/49 (6.1%)	10/47 (21.3%)	16/50 (32%)
Carcinoma, overall rates[a]	1/50 (2%)	1/49 (2%)	4/49 (8.2%)	2/47 (4.3%)	3/50 (6%)
Adenoma and Carcinoma, combined[a]	4/50 (8%)	5/49 (10.3%)	7/49 (14.3%)	12/47 (25.5%)	19/38 (82%)

[a] Number of tumor-bearing animals/number of animals examined at site

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Overall, tris(2-chloroethyl)phosphate was clearly carcinogenic following oral administration in male Scl:ddY mice. There were increased tumor incidences in the kidney and liver when compared to the concurrent controls. Clear evidence of carcinogenic activity of tris(2-chloroethyl)phosphate for male Scl:ddY mice was observed by a dose-related increased incidence of tumors in the kidneys at ≥300 mg/kg bw/d (statistically significantly high incidences of tumors at 1500 mg/kg bw/d); and by an increased incidence of tumors in the liver at ≥60 mg/kg bw/d (statistically significantly high incidences of tumors at ≥300 mg/kg bw/d).

In summary, the data of the highest dose level of 1500 mg/kg bw/d were excluded from further viewing because the top dose exceeded the MTD (maximum tolerated dose) for this mouse strain shown by reduced survival and reduction in body weight gain (>10%). However, based on the results presented in the employed dose range it was concluded that increased incidence of kidney and liver tumors in Scl:ddY mice occurred at ≥300 mg/kg bw/d in the diet for 18 months. At this dose range increased tumor rates were also determined in the study with B6C3F1. In addition, the tumor incidences of the concurrent controls reported in this mouse study confirmed the published data for spontaneous kidney and liver tumors of other mouse strains. From the data presented it appears that Scl:ddY mice were not particularly more susceptible to renal cell and liver tumors than other mouse strains tested. In consideration of the concurrent controls and control data obtained with the same mouse strain in two other bioassays it is quite evident that the incidences of kidney tumors and hepatocellular tumors of animals of the 300 mg/kg bw/d dose group lie above the frame of the historical data. The data in the kidneys and liver were considered to provide a clear evidence of tris(2-chloroethyl)phosphate induced carcinogenic activity in male Scl:ddY mice. Because of increased rates of renal proliferative lesions and of cell atypia in renal tubule epithelium which were observed in animals treated at ≥12 mg/kg bw/d, a NOAEL for kidney tumor formation could not established. However, 60 mg/kg bw/d is considered to be the NOAEL for liver tumor formation in this study.

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- ***Dermal***

Mice

Groups of 25 Swiss mice at 45 days of age were used in an *in vivo* short-term skin test for studying the sebaceous gland suppression and for induction of epidermal hyperplasia. Animals received dorsal applications on days 1, 3 and 5 of tris(2-chloroethyl)phosphate (purity unspecified) at 0, 31.9, 53.2, 74.5 mg in an acetone solution (total dose applied in three applications). Benzo(a)pyrene was used as positive control. The treated skin areas were removed on day 8 and the number of sebaceous glands and the thickness of epidermis were measured by standard procedures. No suppression of the sebaceous glands and no hypoplasia were induced in the skin of mice treated with tris(2-chloroethyl)phosphate. However, data are without relevance within the overall database for the test substance (Sala et al., 1982).

In a short reported study, groups of 32 female mice received skin applications of tris(2-chloroethyl)phosphate (purity unspecified), each of 21 mg per day, in acetone, 2 times/week on shaved skin for 78 weeks. No skin tumors were obtained. One hepatoma and one endometrial sarcoma were found, and an increased incidence of lung adenomas (12/32) was reported. Thus, the test produced no convincing evidence of carcinogenic potential for tris(2-chloroethyl)phosphate by the dermal route. Due to limitations of experimental design (lack of controls) and reporting, no firm conclusion can be made (Sala et al., 1982).

Tumor promotion

In addition, tris(2-chloroethyl)phosphate was tested for initiating/promoting activity in Swiss mice by skin application (Sala et al., 1982). Groups of 35 female Swiss mice, nine weeks of age, received skin applications of tris(2-chloroethyl)phosphate (purity unspecified) in acetone to examine its initiating potential. One group of mice received a single application of 71 mg/mouse tris(2-chloroethyl)phosphate followed by applications of 1 µg/mouse tetradecanoyl phorbol acetate (TPA) twice a week for 78 weeks. A control group was treated with TPA alone. The animals were examined regularly and the time of appearance of papillomas and of malignant neoplasms on skin were recorded. Systematic post-mortem and histological examinations were performed on all animals. The incidence of squamous-cell papillomas of the skin was 17/33 (52%) tris(2-chloroethyl)phosphate plus TPA-treated animals and 12/28 (48%) TPA-treated mice; squamous-cell carcinomas of the skin developed in 2/33 tris(2-chloroethyl)phosphate plus TPA-treated animals but none of the TPA-treated controls. The incidences of lung adenomas were 7/33 and 5/28 in tris(2-chloroethyl)phosphate plus TPA-treated and in TPA-treated groups, respectively. Tris(2-chloroethyl)phosphate showed no significant complete carcinogenic, initiating or promoting activity on mouse skin and lung. Nevertheless, the promoting activity and complete carcinogenicity of tris(2-chloroethyl)phosphate in this study could not be evaluated because of the lack of negative controls.

Overall, there are no dermal carcinogenicity studies according to modern test design to tris(2-chloroethyl)phosphate. In the skin test for sebaceous gland suppression and for the induction of epidermal hyperplasia, there was neither a decrease in the number of sebaceous glands nor an increase in the thickness of the epidermis. The findings of the initiation/promotion studies with tris(2-chloroethyl)phosphate showed no significant carcinogenic, promoting or initiating potential on mouse skin and lung. But limitations in study design and reporting do not allow firm conclusions to be drawn.

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- ***Inhalation***

No information available.

Summary of animal in vivo data on carcinogenicity to tris(2-chloroethyl)phosphate

There is sufficient evidence for the carcinogenicity of tris(2-chloroethyl)phosphate in experimental animals. There are positive results in two animal species. There were similarities in the target organs. Evidence of carcinogenicity in the kidneys has been observed in both rats and mice. Carcinogenic potential in rats and mice was demonstrated for the oral route. There are standard carcinogenicity oral (gavage) studies using F344/N rats and B6C3F1 mice (NTP 1991, Matthews 1993). There was clear evidence of carcinogenic activity in male and female F344/N rats. In the F344/N rat, tris(2-chloroethyl)phosphate caused an increased incidence of renal tubule adenomas in both males and females after oral administration of 44 mg/kg bw/d and above for up to two years. These adenomas occurred in nearly 50% of male F344/N rats receiving 88 mg/kg bw/d and in 10% of the F344/N males receiving 44 mg/kg bw/d (below the maximum tolerated dose level, MTD). To a lesser extent a statistically significant increase was observed in female F344/N rats (88 mg/kg bw/d: 10%, 44 mg/kg bw/d: 4%). In view of the results obtained in a similar study with B6C3F1 mice it seemed mice are less sensitive than rats to the effects of tris(2-chloroethyl)phosphate, because doses in the carcinogenicity study were about four times greater than those administered to F344/N rats. In the one carcinogenicity study in B6C3F1 mice, evidence of carcinogenic activity for male B6C3F1 mice was obtained by a marginally increased incidence of renal tubule cell neoplasms at 350 mg/kg bw/d (below the MTD). The evidence for carcinogenicity was shown by the increased incidence of tubule cell neoplasms (2 additional adenomas and two hyperplasias in 350 mg/kg bw/d dose male mice) obtained in supplemental evaluation of additional kidney sections. There was an increased incidence of tubule cell neoplasms of 8%, which was outside the overall rates of 0.2-0.42% for the groups of control gavage male mice and the historical control range of 0-2% (NTP 1991). Although the slight increase in tubule cell neoplasms was not statistically significant, the markedly increase in both tubule cell hyperplasia together with cell atypia in renal tubule epithelium in both male and female mice given ≥175 mg/kg bw/d and tubule cell neoplasms are suggestive of a treatment-related effect and are considered to present evidence of carcinogenic activity for male B6C3F1 mice. For female B6C3F1 mice there was an equivocal evidence of carcinogenic activity as shown by a marginal increased incidence of Harderian gland adenomas at the lowest tested doses of 175 mg/kg bw/d and above. The incidences of Harderian gland tumors in both dose groups were greater than those in the concurrent controls and also in historical controls (2.4% NTP 1991; 3.0%, Ihara et al., 1994, 2.8%, Tamano et al., 1988, and 5.1%, Eiben, 2001).

A further carcinogenicity study revealed significant increases in the incidence of tumors in the kidney and liver of male Scl:ddY mice after administration of tris(2-chloroethyl)phosphate in the diet for 18 months. However, this study differs in some respect from published guidelines, but is comparable to OECD TG 451 with acceptable restrictions. Clear evidence of carcinogenic activity of tris(2-chloroethyl)phosphate for male Scl:ddY mice was observed by a dose-related increased incidence of tumors of the kidneys at ≥300 mg/kg bw/d and above; and by an increased incidence of tumors in the livers at ≥60 mg/kg bw/d (statistically significantly high incidences of tumors at 300 mg/kg bw/d (below the MTD), which were each outside the range of concurrent controls and the historical range of other mouse strains (Takada et al., 1989).

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There is no report available on the carcinogenicity of tris(2-chloroethyl)phosphate after inhalation.

There are no dermal carcinogenicity studies according to guideline study (OECD, etc.) to tris(2-chloroethyl)phosphate available. The findings that are available are negative, but limitations in study design and reporting do not allow firm conclusions to be drawn.

In vitro studies

Cell transformation assays

There are two cell transformation tests to tris(2-chloroethyl)phosphate available.

In a test using SHE cells (without S-9 mix) an increase in the transformation rate was observed, however, without dose-response relationship. Increases over control of 3.3% (1.4 transformed colonies/plate) at 400 µg/ml tris(2-chloroethyl)phosphate and of 1.2% (0.6 transformed colonies/plate) at 500 µg/ml tris(2-chloroethyl)phosphate were measured. At 600 µg/ml tris(2-chloroethyl)phosphate a negative response was observed. Toxic effects were induced at 800 µg/ml (Sala et al., 1982).

Another cell transformation assay (type III foci) using C3H10T1/2 cells was negative with and without S-9 mix up to 1500 µg/ml tris(2-chloroethyl)phosphate. However, the methodology of the test and the results are insufficiently documented (Sala et al., 1982).

Summary on cell transformation tests:

Results of the two cell transformation tests showed controversial findings of tris(2-chloroethyl)phosphate. In the test system with SHE cells (without S-9 mix) an increased transformation rate was noted, however, negative data were described in another test using C3H10T1/2 cells with and without S-9 mix. Nevertheless, these very weak effects in in vitro SCE tests are considered to be without relevance for mutagenicity.

Concern from mutagenicity data:

The conclusion is that there is no relevant evidence for mutagenicity of tris(2-chloroethyl)phosphate as bacterial gene mutation tests were negative, *in vitro* genotoxicity tests with mammalian cells were negative for gene and chromosome mutations, and two valid *in vivo* mammalian micronucleus tests and a Drosophila test were negative.

Discussion on carcinogenicity

There is no indication to assume that the tumors induced in rats and mice may be related to primary genotoxic effects. The existence of other/alternative (non genotoxic) mechanisms is assumed.

Kidney tumors

Evidence in the rat:	*F344/N rats (male and female)*
Evidence in the mouse:	*B6C3F1 mice (male), Scl:ddY mice (male)*

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In rodent bioassays, the kidney is one of the most frequent sites for the induction of cancer by chemicals, contrasting with the situation in humans where the kidney is an infrequent site of primary cancer development. The main portion of these studies shows positive evidence for male rat kidney tumors; renal tumor induction is a much less frequent response in mice. Whereas, results of the 2-year rat study showed evidence of kidney tumors not only in male F344/N rats but also in females, and in addition, treatment-related kidney tumors were also observed in males of two mouse strains, after treatment for two years in B6C3F1 mice and after 18 months in Scl:ddY mice.

Not all rodent renal carcinogens (or their reactive metabolites) are genotoxic, acting by way of direct adduct formation with kidney DNA. There are also examples of secondary mechanisms of chemical carcinogenesis in rat kidney. It is known that tubules of the renal cortex are particularly vulnerable because this region received about 80% of the renal blood flow. In addition, the proximal tubule cells of the kidney have a high capacity for transporting organic ions and certain other substrates directly from the peritubular circulation into the tubular fluid and vice versa. This process can lead to significant levels of nephrotoxicants within the tubule cell. Next to the liver, the kidney is critical site for biotransformation of many different chemicals, being endowed with enzymatic pathways for carrying out extensive oxidative, reductive, hydrolytic, and conjugative processes. Information from metabolic studies on rats and mice give clues to conditions of action and possible mechanism of action to tris(2-chloroethyl)phosphate showing the kidney as target organ. So, it is assumed that the kidneys were preferentially exposed to large quantities of tris(2-chloroethyl)phosphate distributed by the circulatory system. Most chemicals with tumorgenic activity in rodent kidney require metabolic activation to a reactive species termed the ultimate carcinogen. The knowledge of the biochemical and molecular mechanisms of renal carcinogenesis in rodents is far from complete. The kidney possesses a number of enzymatic functions, which not only represent detoxification or synthetic mechanisms, but are also capable of metabolic activation of chemicals. One of the metabolic pathways involved in the proximal tubules is the cytochrome P450 monooxygenase system.

It has been argued that sustained tissue damage and subsequent cell proliferation is a common predisposing factor for cancer development in rodents, that is dependent on a specific metabolic process, e.g. halogenated alkenes. These chemicals are biotransformed to nephrotoxic metabolites via a multistep pathway involving the cytosolic enzyme β-lyase. The selective renal action of these compounds commences with glutathione S-conjugate formation in the liver, transport of the S-conjugate to the kidney where it is metabolized to a cysteine S-conjugate by one of several brush border enzymes, mainly γ-glutamyltranspeptidase, and finally renal bioactivation by cysteine conjugate β-lyase to yield cytotoxic or mutagenic metabolites. The constant stimulus of cytotoxicity leads to compensatory tissue repair and cell proliferation which in time ends up in the formation of benign renal tumors.

Although there may be interspecies differences at the level of specific enzymes, these various predisposing factors apply equally to humans and to rodents. However, one physiological peculiarity that would predispose the male rat kidney, but not the human kidney, to the carcinogenic action of certain chemicals, is the unique presence in male rats of high urinary levels of a specific protein, namely $\alpha_{2\mu}$-globulin. This protein belongs to the lipocailin family, is synthesized in high levels in the male liver, and is involved in an independent secondary tumor induction mechanism in the rat kidney identified in various chemicals that can induce $\alpha_{2\mu}$-globulin nephropathy. These renal carcinogens include a diverse group of chemicals, mainly light hydrocarbons used as fuels and the food constituent *d*-limonene, that are known

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to induce a renal syndrome termed hyaline droplet nephropathy involving accumulation of male rat-specific urinary protein, $\alpha_{2\mu}$-globulin. The induction of renal tubule tumors by this diverse group of chemicals only in male rats correlates with the absence of high circulating levels of $\alpha_{2\mu}$-globulin in female rats and the complete absence of $\alpha_{2\mu}$-globulin in male and female mice. In this context, there are no indications of renal tumor development caused by tris(2-chloroethyl)phosphate interactions with $\alpha_{2\mu}$-globulin.

Another factor that may predispose the male rat to renal tumor formation is the spontaneous age-related condition encountered in virtually all carcinogenicity bioassays, chronic progessive nephropathy (CPN). CPN is a degenerative condition which affects male rats more severely than females. There is also a sustained regenerative component to CPN because many affected tubules are characterized by a marked increase in proliferative activity, and atypical tubule hyperplasia (a preneoplastic lesion) has been reported to occur in very advanced stages of the disease, e.g. hydroquinone.

In general, humans and rodents develop a similar spectrum of tumor types in the kidney. The main renal tumor types in rats and mice were renal tubule adenoma and carcinoma. Tubule tumors are also the most important kidney tumors in humans. In the kidneys of tris(2-chloroethyl)phosphate exposed F344/N rats and both B6C3F1 and Scl:ddY mice increased tumor rates of the same anatomical site were observed. There were primarily renal tubule adenoma, but carcinoma occurred also. Nevertheless, the mechanism of tumor development in the kidneys of tris(2-chloroethyl)phosphate treated rats and mice remains unclear. Data of gene mutation tests and genotoxicity tests are suggestive to assume that tris(2-chloroethyl)phosphate appears to have no genotoxic potential. A skin-painting carcinogenicity study with one of the possible toxic metabolite 2-chloroethanol failed to demonstrate carcinogenicity. However, renal carcinogens acting through those causing secondary or indirect DNA effects can be assumed to represent equivalent risks for humans. There are no data on the mechanism(s) of toxicity and carcinogenicity action of tris(2-chloroethyl)phosphate. It is likely that carcinogenicity would be mediated by non-genotoxic (epigenetic) mechanisms. However, a species-specific mechanism of tumor formation in the kidney was not identified. Perhaps the major factor underlying the renal carcinogenesis is the rate of blood flow through the kidney. This combined with a reactively high metabolic capability suggests a possible step of action in tumor response to tris(2-chloroethyl)-phosphate. There is postulated that tris(2-chloroethyl)-phosphate may have induced renal tumors in the F344/N rat secondary via cytotoxicity followed by cell proliferation mechanism e.g. hydroquinone, halogenated alkenes. But, there are data suggestive of evidence of carcinogenicity also in female F344/N rats and in male mice of two mouse strains following chronic exposure to tris(2-chloroethyl)-phosphate. So, the mechanism of tumor formation following tris(2-chloroethyl)-phosphate exposure is not definitively established.

Renal carcinogens acting via a secondary or indirect mechanism involve threshold phenomena for their tumor-inducing or nephrotoxic effects. However, the problem is that there are possibly many different modes of action thought to be involved in the renal tumor formation in tris(2-chloroethyl)-phosphate administered rats and mice. To date, there is a uncertainty identification of the crucial event, especially on the primary toxic effects of concern for tris(2-chloroethyl)-phosphate following development of tumors in animal cancer bioassays. However, the difficulty is that the determination of a threshold dose for non-genotoxic carcinogens is based by fully knowledge of the cascade of effects following interaction of the substance with its biological target(s). For this reason, at present it may be extremely difficult or impossible to identify a threshold for the underlying toxicity of kidney

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tumor development to tris(2-chloroethyl)-phosphate. In F344/N rats tumor formation was observed in animals of both sexes at all dose tested, ≥44 mg/kg bw/d. Based on this data, the NOAEL for kidney tumor formation could not established for male and female F344/N rats (NTP 1991; Matthews 1993). An increased kidney tumor rate and increased incidences of renal tubule hyperplasia were noted in male B6C3F1 mice after treatment with the high dose of 350 mg/kg bw/d for two years whereas increased rates of cellular atypia of renal tubule epithelial cells such as karyomegaly were seen in both male and female mice given ≥175 mg/kg bw/d (NTP 1991, Matthews 1993). At 300 mg/kg bw/d, which presented the same dose range seen adenomas in the kidneys in male B6C3F1 mice, an increased incidence of renal tubule adenomas were noted in male Scl:ddY mice after treatment with tris(2-chloroethyl)-phosphate in the diet for 18 months. However, findings in the kidneys observed as hyperplasia and hypertrophy of the urinary tubule epithelium together with nuclei enlargement were determined in male Scl:ddY mice fed at ≥12 mg/kg bw/d when compared to controls (Takada et al., 1989). Therefore, a NOAEL for tumor formation in the kidney could not be established. Overall, no conclusion can be drawn about the relevance of these tumors to humans due to the lack of knowledge of the possible mode of action. However, considering all available data, the relevance to humans is probably very significant.

Liver tumors
Evidence in the mouse: Scl:ddY mice (male)

Due to its anatomical position and its enzymatic content, the liver is the organ most frequently affected in toxicological studies on rodents and is also the organ most frequently affected in carcinogenicity studies in rats and mice. The mechanism of tumor development in the liver of tris(2-chloroethyl)phosphate treated Scl:ddY mice was not studied.

Non-genotoxic mouse liver carcinogens are known to have different mode of action. In the literature a link between persistent liver damage and the development of liver tumors was discussed for non-genotoxic carcinogens. This phenomenon is not unique to the liver since necrosis and regeneration are associated with a high incidence of tumors in other organs, notably urothelium (Grasso 1987a, 1987b). Special tests examining the effect of subcellular injury on the turnover rate of liver cells were not done in the presented mouse study.

In addition, there are also some non-genotoxic compounds inducing liver enlargement and mitogenesis without unequivocal signs of hepatotoxicity. There was discussed that the early phases of hepatomegaly are associated with mitogenic effects that can be measured as cell in S-phase within the first few days of administration. The later stages of hepatomegaly appear to be associated more with cellular hypertrophy. Further, sustained hepatomegaly induced by chemicals is associated with proliferation of the smooth endoplasmatic reticulum (SER), peroxisomes and the induction of a range of liver enzymes. There were no results available that such mode of action or potential interaction of modes of action may operate in tris(2-chloroethyl)phosphate treated mice.

In summary, at present there is no clear data on the mechanism of liver carcinogenesis by tris(2-chloroethyl)phosphate in the male Scl:ddY mice. However, it is conceivable that liver cell damage and feature of the hepatic reparative response may be responsible for tumor production to tris(2-chloroethyl)phosphate. In the study with Scl:ddY mice local necrosis, and vacuolation of liver cells were observed in all treatment groups (≥12 mg/kg bw/d) but including the controls whereas no such findings were reported from the other mouse study.

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Based on the results presented there was stated that tris(2-chloroethyl)phosphate is an oncogenic substance which may induce tumors in the liver of male ScI:ddYY mice. The dose level of 60 mg/kg bw/d is considered to be the NOAEL for tumor formation in the liver. Overall, no conclusion can be drawn about the relevance of this tumors to humans due to the lack of knowledge of the possible mode of action. However, considering all available data, the relevance to humans is probably significant.

Harderian gland tumors
Evidence in the mouse: B6C3F1 mice (female)

The Harderian gland is one of the few organs that is unique to a limited number of animal species; for example, primates do not have Harderian glands. Harderian gland neoplasms are not an unusual finding in mice; the incidence of this tumor, as with most other neoplasms of mice, increases with age. Because of the post-orbital location of the gland, large tumors can cause exophthalmos. In the 2-year study on B6C3F1 mice, an equivocal evidence of carcinogenic activity as shown by a marginal increased incidence of Harderian gland adenomas in females was noted at the lowest tested doses of 175 mg/kg bw/d tris(2-chloroethyl)phosphate and above. However, the actual incidence of neoplasms of this gland in this study was under-reported because microscopy was performed only on those glands which had visible gross lesions (NTP 1991, Matthews 1993). In conclusion, the incidences of Harderian gland tumors in both dose groups of females were greater than those in the concurrent controls and also in historical controls (2.4%, NTP 1991; 3.0%, Ihara et al., 1994, 2.8% Tamano et al., 1988, and 5.1% Eiben, 2001).

Due to the fact that the Harderian gland has no human counterpart and in absence further relevant information of the possible mode of action these tumors are considered of no relevance to humans.

4.1.2.8.2 Human data

No data are available.

4.1.2.8.3 Summary and conclusion on carcinogenicity

From animal data it is obvious that there is a cancerogenic potential of tris(2-chloroethyl)phosphate. There are relevant guideline cancer studies using F344/N rats and B6C3F1 mice available (NTP 1991, Matthews 1993). In addition data of a diet study for 18 months using Scl:ddY mice comparable to guideline study with acceptable restrictions is available (Takada et al., 1989).

Carcinogenic potential of tris(2-chloroethyl)phosphate in rats and mice was demonstrated for the oral route.

Tris(2-chloroethyl)phosphate caused primarily benign tumors but also and malignant tumors in the kidney in F344/N rats (males and females) and also in male mice of two mouse strains (B6C3F1, Scl:ddY). Rats and mice of both strains developed a similar spectrum of tumor

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types in the kidneys. Additionally tumor development after tris(2-chloroethyl)phosphate treatment was seen in the liver of male in Scl:ddY mice, and in Harderian gland of B6C3F1 female mice, respectively. The data in the kidneys were considered to provide a clear evidence of tris(2-chloroethyl)phosphate induced carcinogenic activity in male Scl:ddY mice. Because of increased rates of renal proliferative lesions and of cell atypia in renal tubule epithelium which were observed in animals treated at ≥12 mg/kg bw/d, a NOAEL for kidney tumor formation could not established. Thus, for risk characterisation purposes a LOAEL of 12 mg/kg bw/d is brought forward for tumor formation.

An increased incidence of renal tubular cell adenomas and carcinomas was observed in male and female F344/N rats at ≥44 mg/kg bw/d (below MTD); statistically significantly high incidences of tumors at 88 mg/kg bw/d (NTP 1991, Matthews 1993). In male B6C3F1 mice increased incidences of renal tubule cell neoplasms and of renal tubule cell hyperplasia were reported at 350 mg/kg bw/d (below MTD). In addition, increased rates of cellular atypia of renal tubule epithelium cells such as karyomegaly were noted in both male and female mice given ≥175 mg/kg bw/d (NTP 1991, Matthews 1993). Dose-related increased incidence of tumors in the kidneys were also seen in male Scl:ddY mice fed diet concentrations of 300 mg/kg bw/d (below MTD) and above for 18 months. Also in male Scl:ddY mice statistically significantly high incidences of tumors (adenomas and carcinomas) in the liver were noted at 300 mg/kg bw/d (below MTD) and above. In female B6C3F1 mice marginally increased incidence of Harderian gland adenomas was seen at the lowest tested doses of 175 mg/kg bw/d and above (NTP 1991, Matthews 1993).

No species-specific mode of action for tris(2-chloroethyl)phosphate carcinogenesis was identified.

A reasonable threshold mechanism could not be identified for all tumors and tumor sites. Cytotoxicity was assumed as underlying mode of carcinogenesis in the kidney although indications on cytotoxicity, inflammation or the involvement of apoptosis are presently absent or not available. However, signs of increased proliferation and karyomegaly of renal tubule epithelial cells were seen in F344 rats of both sexes at ≥44 mg/kg bw/d and in male B6C3F1 mice at ≥175 mg/kg bw/d (NTP 1991, Matthews 1993) as hyperplasia and hypertrophy of the urinary tubule epithelium together with nuclei enlargement in male Scl:ddY mice fed at ≥12 mg/kg bw/d for 18 months (Takada et al., 1989). No other non-genotoxic mode of action was identified.

Persistent cell damage and development of tumors was also discussed for non-genotoxic modes in the liver. In the study with Scl:ddY mice local necrosis, and vacuolation of liver cells were observed in all treatment groups (≥12 mg/kg bw/d). However, other non-genotoxic modes might be suspected, but not yet verified. A NOAEL for the cytotoxic effects was not established, and also not for cell proliferation mechanism.

The carcinogenic effect of tris(2-chloroethyl)phosphate is thought to be related to non-genotoxic (epigenetic) mechanisms.

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According to the decision of the EU C&L WG tris(2-chloroethyl)phosphate will be classified as a carcinogen, category 3 and labelled as Harmful, Xn, R 40.

4.1.2.9 Toxicity for reproduction

4.1.2.9.1 Fertility impairment

Animal studies

Tris(2-chloroethyl)-phosphate (purity ≥ 98 %) was investigated for possible impairment of reproduction and fertility in CD-1 mice in a study using the reproductive assessment continuous breeding protocol (RACB) by oral administration (gavage) at dose levels of 175, 350 and 700 mg/kg bw/d (Gulati et al., 1991). The dose levels used during this study had been obtained from a preceding 14-day treatment dose finding study. At the beginning of the continuous breeding phase, groups of 40 pairs were used for the control (corn oil) and of 20 pairs for the treated groups. Animals were treated for a total of 18 weeks (1 week before mating, 14 weeks held as breeding pairs and three weeks thereafter). The last litters born during the holding period following the continuous breeding phase were reared by their dams until weaning, after which treatment of these F1 animals was initiated by the same route and at the same concentration as F0 parental animals. At the age of 74 ± 10 days 20 non-sibling F1 animals per treatment group were mated for 1 week and held until delivery. In addition, a separate one-week crossover mating trial had been performed to determine the affected sex. This mating trial consisted of three groups of 20 pairs each: control males x control females, control males x 700 mg/kg bw/d treated females, 700 mg/kg bw/d treated males x control females. Endpoints evaluated during the continuous breeding study were clinical signs of toxicity, parental body weight, parental average water consumption during representative weeks, fertility (number of pairs producing a litter/number of breeding pairs), number of litters/pair, number of live pups/litter, proportion of pups born alive, sex of live pups and the pup body weight immediately after birth. At necropsy of the animals the livers, kidneys, testes, epididymides, prostate, seminal vesicles with coagulating glands and ovaries were weighed and fixed for histopathological evaluation. The endpoints evaluated for the F1 animals and for those of the crossover mating trial were the same as for the continuous breeding part of the study. In addition, for these two latter parts of the study also epididymal sperm motility, sperm morphology, sperm count and estrual cyclicity were evaluated.

F0 animals (continuous breeding phase):

No treatment related mortalities were observed and no clinical signs were reported. Water consumption in the treated groups was similar to that of the controls. Mean body weight of the animals (combined males and females) was similar in comparison to the controls during the mating and holding period. All pairs were fertile in terms of delivering at least one litter. However, while there was no difference between the 175 mg/kg dose group and the control in the number of pairs producing five consecutive litters (17/19 versus 35/38 in the controls) during the continuous breeding phase, the number of pairs producing a 5th litter was statistically significantly reduced in the 350 mg/kg dose group (13/18 versus 35/38 in the controls). This effect was even more pronounced in the high dose treated groups. No 4th or 5th litter at all was produced in the 700 mg/kg dose group, only 2 out of 18 pairs delivered a 3rd litter and also the number of pairs producing a 2nd litter was statistically significantly reduced (12/18 versus 37/38 in the controls). TCEP treatment also revealed a dose related decrease in the average numbers of live pups/litter which was statistically significantly reduced at the 350

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mg/kg dose group for the 3rd (9.3 ± 0.9 pups/litter versus 13.1 ± 0.7 pups/litter in the control), 4th and 5th litter and at the 700 mg/kg dose group for the 2nd (4.3 ± 1.1 pups/litter versus 13.1 ± 0.6 pups/litter in the control) and 3rd litter. Absolute pup birth weights were increased in the mid and high dose groups, however, the differences were not statistically significant when adjusted for litter size. The cumulative days-to-litter values were significantly greater for the second litter in the 700 mg/kg/d dose group (65.9 ± 6.4 days) in comparison to the control group (40.8 ± 0.3 days).

F1 generation (last litters from continuous breeding phase):

Since poor fertility and pup survival in the 700 mg/kg/d group left insufficient animals for the second generation only the 175 and 350 mg/kg/day treated dose groups were performed. No treatment related effects were seen for the body weights of both male and female parental animals and for their water consumption data. Percentage of pregnant (90%) and fertile (90%) pairs in the 175 mg/kg dose group were comparable to that of the controls (85%, resp. 89%). In the 350 mg/kg dose group percentages of pregnant (70%) and fertile (70%) pairs were decreased, but ratios were not statistically significantly different from the controls. The average number of live pups/litter was statistically significantly reduced in the 350 mg/kg dose group (7.6 ± 1.1 pups/litter versus 11.4 ± 0.5 pups/litter in the control) and less male pups were found in both of the treated groups when compared to the controls.

Cross over mating trial:

Pregnancy and fertility indices were statistically significantly lower in the high dose treated male/control female group in comparison to both the control male/control female group or the control male/high dose treated female group. Only one out of 18 pairs of the high dose treated male/control female group produced a litter. Average number of live pups/litter were statistically significantly lower in both of the treated groups (from treated males: 3.0 pups/litter; from treated females: 7.2 ± 0.9 pups/litter) in comparison to the control (10.3 ± 0.7 pups/litter).

Necropsy results on organ evaluation:

At daily doses of 700 mg/kg bw the males of the cross over mating trial revealed statistically significantly higher absolute and relative liver weights, statistically significantly lower absolute and relative kidney/adrenal and right testis weights and statistically significantly lower absolute right epididymides weights. At daily doses of 350 mg/kg bw the parental F1 males showed statistically significantly lower absolute and relative right epididymides weights. At 175 mg/kg/d no differences were observed in the parental F1 males for right caudal, right epididymal and right testes weights in comparison to the controls. At daily doses of 700 mg/kg bw the females of the cross over mating trial revealed statistically significantly lower absolute and relative kidney/adrenal weights. At daily doses of 350 mg/kg bw the parental F1 females showed statistically significantly increased absolute kidney/adrenal weights. No effects on the evaluated organ weights were observed at the 175 mg/kg/d dose level.

Sperm evaluations:

At daily doses of 700 mg/kg bw evaluated from the cross over mating trial epididymal sperm density per gramme caudal tissue was statistically significantly reduced to about 810 x 10^6 in comparison to controls (about 1223 x 10^6), percentage epididymal sperm motility was

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statistically significantly reduced to about 35% in comparison to the controls (about 78%) and percentage abnormal sperm was statistically significantly increased to about 32 % (controls: 9 %). Also, testicular spermatid head count data revealed statistically significant reduction in average spermatid count and total spermatid heads per gramme testis tissue. At daily doses of 350 and 175 mg/kg bw evaluated in the parental F1 animals no differences were observed for percentage epididymal sperm motility, epididymal sperm density and percentage abnormal sperm in comparison to the control groups

Vaginal cytology evaluations:

Neither for the 700 mg/kg/d dose level evaluated in females during the cross over mating trial nor for the 350 and 175 mg/kg/d dose level evaluated in the F1 females any effects on average estrous cycle length or estrual cyclicity had been revealed.

In a further study (Gulati and Russel, 1985, Morrissey et al. 1988) tris(2-chloroethyl)-phosphate was evaluated for effects on sperm parameters and on vaginal cytology (SMVC evaluations) in F-344 rats and in B6C3F1 mice. This study is indicated to have been part of NTP-sponsored 13-week subchronic tests in rodents. Groups of ten male and female animals were treated by gavage with doses of 44, 175, and 700 mg/kg/d (mice) and with 22, 88, and 175 mg/kg/d (rats) for approximately 18 weeks. Endpoints determined for the males at necropsy included right testis weight, right epididymal weight, right caudal weight, sperm motility, sperm number per gramme caudal tissue and sperm morphology, and for the females estrual cyclicity . In male B6C3F1 mice tris(2-chloroethyl)-phosphate administered at doses of 44 and 175 mg/kg bw/d had no significant effect on whole body weight, right caudal, right epididymal, or right testis weights and on sperm motility, sperm count, or on the incidence of abnormal sperm in comparison to controls. At 700 mg/kg bw/d a significant decrease was noted with respect to right epididymal and right testicular weights. Furthermore, at this dose level the average sperm count per gramme caudal tissue was statistically significantly decreased and the incidence of abnormal sperm was statistically significantly elevated relative to the control values. In male F-344 rats tris(2-chloroethyl)-phosphate administered at doses of up to and including 175 mg/kg bw/d had no significant effect on whole body weight, right caudal, right epididymal, or right testis weights, or on the incidence of abnormal sperm. At 175 mg/kg bw/d sperm count was significantly higher in comparison to the controls, whereas percent sperm motility as slightly decreased (77% versus control value of 89%). In female F-344 rats tris(2-chloroethyl)-phosphate treatment had no apparent effect on estrual cyclicity. In female B6C3F1 mice an increased length of the estrous cycle and variations in the relative frequencies of estrous stages were found for the 44 and 175 mg/kg bw/d groups, however, not at the high dose group.

Three studies with repeat administration of TCEP (Stauffer 1980a, Takada et al., 1989 and NTP 1991) gave some further hint that the male reproductive system might be a target (cf. chapter 4.1.2.6, Table 4.11).

4.1.2.9.2 Developmental toxicity

Animal studies

Teratogenic effects of tris-(2-chloroethyl)phosphate were investigated in fetuses and offspring of Wistar rats (Kawashima et al., 1983, summary available only). The substance (suspended in olive oil) was given by gavage to pregnant dams day during days 7 to 15 of gestation. Each

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group consisted of 23-30 animals and received doses of 50, 100, and 200 mg/kg bw/d. No changes in maternal body weight gain, food consumption and general appearance were found in the groups of 50 and 100 mg/kg. At the highest dose level of 200 mg/kg 7 out of 30 dams died. Also maternal food consumption was markedly depressed and clinical symptoms such as piloerection and general weakness were recorded. It is reported that on the 20th day of gestation there was no increase in embryonic/fetal death and/or gross malformations attributable to treatment with tris(2-chloroethyl)phosphate at any dose level examined. There was a small increase in skeletal variations (cervical ribs, varied sternebrae, lumbar ribs) at the two higher dose levels. In the postnatal examination, the development of the offspring in all groups examined was well maintained without any disorders attributable to the treatment in morphological examination and in some functional tests such as open field, water maze, rota rod, slop test, pain reflex and Peyer´s reflex examinations. The authors concluded that tris(2-chloroethyl)phosphate had been revealed not to be teratogenic in rats even at maternally toxic doses.

With regard to the developmental study of Kawashima et al. (1983) death of 7 dams out of 30 was reported to have occurred during gestational days 10 to 14. For the latter ones, slight piloerection and weakness and explicitely no signs of intoxication were reported. Animals had not been necropsied or further investigated. Maternal death, however, was obviously of no impact, since development of the conceptus as well as postnatal development of the offspring of the 23 dams that were carried on from the 200 mg/kg dose group remained unaffected.

Tris(2-chloroethyl)phosphate was also included in the evaluation of a total of 60 chemicals within a Chernoff-Kavlock screening assay on CD-1 mice (Hardin et al., 1987). Dams had been treated by gavage during g.d. 6 to 13 with 940 mg/kg bw/d. Maternal weight gain was significantly less in treated dams in comparison to controls. However, there were no effects on the number of viable litters, live born/litter, percentage survival, birth weight and weight gain of the pubs.

Human data

No data available.

Other information

From a very poorly documented study (Shepel´skaja and Dyschinewitsch, 1981) it is reported, that male rats (strain and group size not specified, details of the test protocol not provided) were kept in inhalation chambers with a volume of 200 l and during a course of 4 months were exposed to TCEP concentrations (no further data on chemical identity/purity or vapour generation provided) of 0.5 or 1.5 mg/m^3. During the course of the study, males were mated (no further information provided) to check reproductive function, and pregnant females investigated on day 21 of gestation. As a result, it is reported that spermatology on males from the 1.5 mg/m^3 exposure group revealed no change in the number of sperm, however a diminution in the number of actively motile sperm, yet with unimpaired duration of motility. No changes were reported for the 0.5 mg/m^3 exposure group. Indication on some abnormal sperm morphology were reported, however sperm head morphology was unaffected. Histology of the testes reported a slight increase in the spermatogenesis index in both

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exposure groups, increases in number of spermatogonia in seminiferous tubules, increased incidences of desquamated epithelial cells in the tubular interstitium and a decrease in number of tubules with cells in meiotic stages. In females mated to males from the 0.5 or 1.5 mg/m^3 exposure group an increase in pre- and postimplantation embryonic death and a lower number of progeny was reported. In females mated to males from the 0.5 mg/m^3 exposure group an increase in postimplantation embryonic death was reported as well as lower weight and size of the foetuses in comparison to controls. Due to very poor documentation and reporting the study is not considered sufficient for the purpose of risk assessment.

4.1.2.9.3 Summary and conclusion on reproductive toxicity

Tris(2-chloroethyl)phosphate treatment revealed significant impairment of fertility for both sexes during continuous breeding and for two successive generations in mice. Reproductive failure was observed at daily doses of 700 mg/kg bw with at best and no more than 3 litters produced and with no pups surviving from the last litter produced. The findings were essentially confirmed from the results of a separate cross over mating trial in mice at the same dose level. The reproductive system of male mice appeared to be more sensitive to tris(2-chloroethyl)phosphate treatment as evidenced by less successive reproduction of treated males in comparison to treated females and further by significant male reproductive organ weight reduction and sperm parameter impairment in mice of two different strains. Based on a statistically significant reduction of the number of litters produced by the F0 generation, reduced pregnancy and fertility indices in the F1 generation, and statistically significantly reduced litter size in both the F0 and the F1 generations a NOAEL/fertility of 175 mg/kg bw/d was derived from the study in CD-1 mice with oral administration (Gulati and Chapin, 1991).

A firm conclusion on developmental toxicity is hampered by poor reporting of rather old data as only a summary report and a reporting from a screening assay are available. However, it appears, that tris(2-chloroethyl)phosphate has no embryo-/fetotoxic or specific teratogenic properties in mice and rats even at maternally toxic doses. A NOAEL/developmental toxicity of 200 mg/kg bw/d and a NOAEL/maternal toxicity of 100 mg/kg bw/d was derived from a study with rats with oral administration (Kawashima et al., 1983).

Based on the available animal data tris(2-chloroethyl)phosphate is identified as a reproductive toxicant with a significant toxic potential adverse to fertility. Treatment of mice resulted in significant impairment of reproductive success of both sexes and of male reproductive organs and of sperm parameters. Therefore, tris(2-chloroethyl)phosphate will be classified and labeled as reproductive toxicant Cat. 2, R60. No significant toxicity to embryo-/fetal development has been revealed from tris(2-chloroethyl)phosphate treatment of pregnant rats.

4.1.3 Risk characterisation

4.1.3.1 General aspects

Data on human experience with tris(2-chloroethyl) phosphate (TCEP) are not available. TCEP is well absorbed (> 90% of the dose) and distributed in rats after oral administration.

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Higher concentrations were found in liver and kidney up to 24h after administration. An enterohepatic circulation is supposed to occur. Metabolism and elimination are the same after single and repeated application. Metabolites in urine were identical in rats and mice. Main metabolites were bis(2-chloroethyl) carboxymethylphosphate, bis(2-chloroethyl)hydrogen phosphate and bis(2-chloroethyl)-2-hydroxyethyl-phosphate glucuronide. Data are not available for inhalation and dermal exposure. For risk characterisation purposes, the rates of oral, dermal, and inhalation absorption are assumed to be 100%.

TCEP demonstrated moderate toxicity after oral application (LD50 for rats in the range of 430-1230 mg/kg bw). In an experiment with rabbits, the substance has demonstrated low acute dermal toxicity (LD50 > 2150 mg/kg bw). In experiments with rats low acute inhalation toxicity was detected.

Draize tests with rabbits revealed weak irritation of the skin and mild irritation of the conjunctivae. TCEP is not considered to be a skin and eye irritant.

Human data on skin sensitisation potential of the substance are not available. An animal study (Buehler Test) showed no skin sensitizing potential of TCEP. Sensitisation data of the two other chloroalkyl phosphates TCPP and TDCP which are structurally related to TCEP indicate that these substances do not possess significant skin sensitisation potential. Taking into consideration all information on the three chloroalkyl phosphates TCEP, TCPP and TDCP including alkylating properties of these substances it is concluded that TCEP should be non-sensitizing to humans.

No information is available on the respiratory sensitisation potential of TCEP and the two other chloroalkyl phosphates.

Kidneys, brain and liver appeared to be the main sites of the toxic attack in experimental animals after repeated oral application of tris(2-chloroethyl) phosphate (dose ranges from 22 to 700 mg/kg bw/d in rats and up to 1500 mg/kg bw/d in mice).

Kidneys appear to be the most sensitive organ for repeated exposure of tris(2-chloroethyl) phosphate. Kidney effects observed in rats and mice were dose- and time-related with respect to incidence and severity. Kidney findings were noted in Sprague-Dawley and F344/N rats and in B6C3F1 and Scl:ddY mice. In both rat strains and both mouse strains kidney lesions of the same kind were observed. In subchronic toxicity studies a NOAEL for kidney lesions was estimated (for male and female Sprague-Dawley rats at 3000 ppm, 192 and 215 mg/kg bw/d, resp., and for B6C3F1 mice at 350 mg/kg bw/d. (Stauffer Chemical Company 1980b). However, a NOAEL for kidney effects could not be derived for male and female F344/N rats after chronic exposure to ≥44 mg/kg bw/d tris(2-chloroethyl) phosphate, furthermore, for B6C3F1 mice after long-life exposure to ≥175 mg/kg bw/d (NTP 1991), and for Scl:ddY mice after feeding of ≥12 mg/kg bw/d in the diet for 18 months (Takada et al. 1989). 12 mg/kg bw/d is considered as LOAEL for kidney lesions and will be used for risk characterisation.

Brain effects following repeated oral application of TCEP were found in rats, but not in mice. A dose- and sex-dependent neuronal necrosis in the hippocampal and thalamal region of the brain was observed. This lesion is more common and more severe in female than in male rats. In addition, gliosis, hemorrhage, pigmentation (hemosiderin accumulation), and mineralization in the brains of some female rats were found. Degenerative lesions in the brain occurred in rats in a dose related pattern, and in addition, in a clear time-response relationship

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in frequency, intensity and severity. Male rats were less sensitive to adverse brain effects than female rats. The lowest NOAELs for systemic brain effects were derived in each case from subchronic and chronic oral toxicity studies for rats at dose levels between 44 to 175 mg/kg bw/d. With respect to brain effects mice were less sensitive to TCEP than rats. The NOAEL for brain effects was established to be 44 mg/kg bw/d in F344 rats.

Liver effects observed in F344/N rats and B6C3F1 mice after treatment with TCEP were limited to increased organ weights without other corroborating findings. The increase in liver weights was not considered to be toxicologically adverse.

No data on local effects after repeated exposure to TCEP are available.

Serum cholinesterase activity was inhibited in female F344/N rats receiving 175 and 350 mg/kg bw/d tris(2-chloroethyl) phosphate by gavage for 16 weeks, but not in male F344/N rats. There were no such tris(2-chloroethyl) phosphate-related differences in cholinesterase activity in B6C3F1 mice of both sexes in subacute as well as subchronic studies up to 700 mg/kg bw/d. The inhibition of serum cholinesterase activity can only be considered as an indication for exposure to TCEP, but not for prediction of ill-health effects.

Although several chloroalkyl phosphates have shown to produce delayed neurotoxicity in hens, a very high oral dose of tris(2-chloroethyl) phosphate (14200 mg/kg bw) did not cause behavioral effects or nerve damage suggestive of neurotoxicity in White Leghorn hens. Clinical signs of neurotoxicity, including ataxia, excessive salivation, gasping, and convulsion were noted in female F344/N rats receiving double the target levels of 175 and 350 mg/kg bw/d TCEP for 3 days in week four during an oral 16-week gavage study. In addition, female rats dosed with 175 and 350 mg/kg bw/d TCEP experienced occasional periods of hyperactivity after dosing. Male and female B6C3F1 mice given 350 and 700 mg/kg bw/d TCEP for 16 days exhibited ataxia and convulsive movements during the first 3 days of dosing.

In general, bacterial gene mutation tests were negative. In vitro genotoxicity tests with mammalian cells were negative for gene and chromosome mutations, in a mouse-lymphoma-assay and in a UDS-test. Very weak effects in in vitro SCE tests are considered to be without relevance for mutagenicity. Two in vivo mice micronucleus tests were negative for application up to maximum tolerated doses, while a positive result of questionable validity was observed in another test. Also a Drosophila test was negative. Overall, it can be concluded that there is no relevant evidence for mutagenicity of tris(2-chloroethyl) phosphate.

There are no human data on carcinogenicity. Tris(2-chloroethyl) phosphate is carcinogenic in both sexes of rats and mice. It causes the formation of predominantly benign tumors in the kidney, but also benign and malignant tumors at various sites in experimental animals. It induces kidney tumors in F344/N rats at doses ≥44 mg/kg bw/d, in male B6C3F1 mice at 350 mg/kg bw/d; and in male Scl:ddY mice at diet concentrations of 300 mg/kg bw/d and above. In addition, dose-related increased incidences of hyperplasia and hypertrophy of the urinary tubule epithelium together with karyomegaly were also observed in these animals. Such findings were noted in male and female F344/N rats at doses ≥44 mg/kg bw/d, in both male and female B6C3F1 mice given ≥175 mg/kg bw/d, and in male Scl:ddY mice fed at ≥12 mg/kg bw/d tris(2-chloroethyl) phosphate. The value of 12 mg/kg bw/d is considered as LOAEL for tumor formation. In addition, TCEP induces tumors in the liver of male Scl:ddY mice at 300 mg/kg bw/d and above, and in the Harderian gland of female B6C3F1 mice at

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≥175 mg/kg bw/d. Since there is no evidence of a direct genotoxic mode of action, it was assumed that carcinogenicity would be mediated by non-genotoxic (epigenetic) mechanisms.

There are no human data on reproductive toxicity. Tris(2-chloroethyl) phosphate treatment revealed significant impairment of reproductive capacity and fertility during continuous breeding and for two successive generations in mice for both sexes (dose levels of 175, 300, and 700 mg/kg bw/d). The reproductive system of male mice appeared to be more sensitive to tris(2-chloroethyl) phosphate treatment than that of females. An oral NOAEL/fertility of 175 mg/kg bw/d was derived from the studies with mice. With respect to developmental toxicity, it appears on the basis of the available data, that tris(2-chloroethyl) phosphate has no embryo-/fetotoxic or specific teratogenic properties even at maternally toxic doses. An oral NOAEL/developmental toxicity of 200 mg/kg bw/d (NOAEL/maternal toxicity = 100 mg/kg bw/d) was derived from studies with rats.

4.1.3.2 Workers

4.1.3.2.1 General aspects of occupational risk assessment

Tris(2-chloroethyl)phosphate (TCEP) is a liquid substance with a vapour pressure of 10^{-3} Pa at 20°C which is soluble in water and organic solvents. It is mainly used as plasticizer for polymers with flame-retarding properties. The occupational exposure scenarios have been described and discussed in section 4.1.1.2. Exposure routes to be considered at the workplace are inhalation against TCEP vapours, inhalative exposure to aerosols and skin contact with the liquid substance and its formulations.

The toxicological data of TCEP have been described and discussed in section 4.1.2. For each endpoint the specific experimental threshold level identified during hazard assessment will be taken forward for occupational risk assessment. Quantitative human toxicity data are not available, therefore risk considerations and estimations have to be based on animal data which have to be extrapolated accordingly. Default values concerning physiological parameters used during this procedure are listed below. From the toxicity profile of TCEP the most prominent effects seem to be carcinogenicity and repeated dose toxicity.

body weight, rat	250 g
body weight, human	70 kg
respiratory rate, rat at rest	0.8 l/min/kg
respiratory volume worker during 8h of light activity)	10 m^3

Systemic availability for different routes of exposure

For the majority of toxicological endpoints TCEP data originate from oral studies. Since workers are exposed either by inhalation or by skin contact, route to route transformation is essential for worker risk assessment. It is recognized though, that route to route extrapolation is associated with a high degree of uncertainty.

According to toxicokinetic data TCEP seems to be readily absorbed via the oral route. No data is available concerning absorption after inhalation or dermal application. For TCEP,

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acute oral, dermal and inhalative studies are available, indicating a relatively low toxic potency following acute dermal application. Because comparison of results from acute toxicity studies generally gives very limited information concerning the systemic availability at relatively low doses (ECB, 2000), acute toxicity studies are not used to conclude on route-specific absorption for other toxicological endpoints. As a default assumption a complete absorption (100%) at all routes is assumed which is to some extent consistent with the physicochemical properties of the substance (MW < 500, solubility in water and organic solvents, $Log_{pow} = 1.7$).

Occupational exposure and internal body burden

In order to assess risks by combined exposure a socalled "internal body burden" is identified. This parameter combines the different contributions of dermal and inhalative exposures of workers to a total effective dose. For TCEP an approach weighed by absorption is not necessary because systemic availability is assumed to be similar at both routes (see above).

In table 4.1.3.2.A the route specific exposure values are listed and the internal body burdens of workers as result of repeated combined exposure via inhalation and dermal exposure are identified. The highest exposure level is obtained for scenario 3a, with a total internal body burden of up to about 2500 mg/person/day.

Short term exposure levels are not available. A calculation of short term exposure levels is not considered necessary, because there are no data which characterise TCEP as respiratory irritant. Inhalation toxicity of TCEP is assumed to depend rather on dose than on concentration.

Table 4.1.3.2.A: Occupational exposure levels and internal body burden of workers

Exposure scenario		Inhalation shift average (mg/m^3)	Dermal contact shift average (mg/p/d)	Internal body burden of workers after repeated exposure (mg/p/d)		
				Inhalation[(1)]	Dermal[(2)]	Combined
1	Production	1.2	420	12	420	432
2	Processing to formulations	1.2	420	12	420	432
3a	Use of formulations with spray application	8.3	2500	83	2500	2583
3b	Use of formulations without aerosol formation	1.2	210	12	210	222

[(1)] based on the assumption of 100% inhalative absorption; breathing volume of 10 m^3 per shift
[(2)] based on the assumption of 100% systemic availability of TCEP after dermal contact

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Calculation of MOS values

For toxicological endpoints with quantitative data available, MOS values are calculated as quotient of experimental NOAEL (or LOAEL) from animal studies and workplace exposure assessments. For TCEP, oral doses from experimental studies are converted to air concentrations or dermal exposure levels before calculation of scenario-specific MOS values. For this procedure the physiological default values from above are used to modify the dose unit of effects data. As result a socalled "starting point" for risk assessment is identified.

MOS values for inhalative and dermal route are considered separately. The combined MOS value is calculated as quotient of the internal NAEL (or LAEL) and internal body burden of workers. Because 100 % absorption at all routes is assumed for TCEP (see above) the internal NAEL is supposed to be similar to the external NOAEL. In case of acute toxicity, different NOAELs for different application routes are available for TCEP.

With respect to the possible outcome of an assessment for combined risks, interest focusses on scenarios with conclusion ii at both exposure routes. By theoretical considerations combined exposure will not increase the most critical route-specific risk component more than twice. It is recognized on that background, that combined risks only rarely will decide concern. For matters of completeness however, all combined MOS values are given in this report on TCEP.

Evaluation of MOS values

Risk assessment based on MOS values implies the identification of a minimal MOS as decision mark between conclusion ii and iii. To obtain this, assessment factors are identified for TCEP, which vary depending on data availability and the specific toxicological endpoint to be evaluated. Scientifically based adjustment factors describe the extrapolation of animal data to the worker population. The uncertainties in the specific calculations are weighed by expert judgement and expressed as an additional "uncertainty factor". The value of the minimal MOS results from the multiplicative combination of the different assessment factors. For carcinogenic risk assessment of TCEP, a modified MOS approach is used.

If the MOS value for a certain exposure scenario is below the minimal MOS for a specific endpoint, the corresponding risk situation is considered to be of concern. A MOS value higher than the minimal MOS indicates no concern.

In a parallel procedure, which gives identical but more direct results, the toxicological starting point taken forward to risk characterisation may be divided by the endpoint-specific assessment factors. As result an exposure level is identified for TCEP which by direct comparison with the occupational exposure levels may serve as trigger for decisions. In the context of this risk assessment report it will be called "critical exposure level". Concern will be expressed for scenarios above this trigger value.

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Interspecies differences

No information is available which allows to describe human susceptibility in comparison to that of experimental animals. As default approach worker risk assessment will therefore be based on the data from the most sensitive experimental species for a certain endpoint. Interspecies extrapolation is then performed using the concept of metabolic rate scaling.

For interspecies extrapolation of oral or dermal data metabolic rate scaling results in lower effective dose levels in mg per kg bodyweight for humans compared to experimental animals. For mice the scaling factor is 7, for rats 4 and for rabbits 2.2 (NO_NL, 1999). The scaling factor is calculated by the formula $(BW_{human}/BW_{animal})^{0.25}$.

For inhalation the principle of metabolic rate scaling implies that a specific inhalation exposure level (in mg/m^3) is toxicologically equivalent in experimental animals and humans. However, care has to be taken to rely the extrapolation between species on directly comparable conditions: under study conditions rats are thought to be at a state of reduced activity; the according human breathing volume in 8 hours is 6.7 m^3 (0.2 l/min/kg x 60min/h x 8h x 70 kg). Workers, however, are assumed to breathe 10 m^3 during a normal working day under conditions of light to moderate activity. Thus for workers the amount of substance inhaled must be spread over a 1.5 times higher breathing volume. Maintaining toxicological equivalence means that compared to the experimental levels the according occupational air concentrations will be 1.5 times lower.

Intraspecies differences

Humans differ in sensitivity due to biological factors. The actual risks for a single person may either be less or more pronounced than estimated for the average human. It is recognised that in order to cover the most sensitive person a very high default assessment factor would be required.

Based on an evaluation of empirical data by Schneider et al. (2004) it is anticipated that a factor of 5 will be sufficient to protect the major part of the worker population (about 95%). Using a lower factor of 3, for instance, would be protective for about 90% of the population whereas a factor of 10 would include 99% of the population. The empirical data do not allow to decide if a lower factor would be sufficient for certain toxicological effects, like for instance local effects in the airways. In the absence of further specific information the factor of 5 is used as default for all toxicological endpoints.

Duration adjustment

According to the fact that studies with suitable experimental design are available for TCEP there is no need for a specific duration adjustment step in extrapolation. Where adaptation of daily or weekly doses is necessary, adjustment is based on linearity.

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Uncertainty considerations

The default adjustment factors outlined above are based upon evaluation of literature data for different chemicals. From a statistical point of view the individual parameters have to be understood as point estimates belonging to probability density distributions. Each factor is taken as geometric mean (point near the maximum) from its density function. The multiplicative combination of all factors is therefore supposed to result in a central tendency estimate. It addresses a likely situation for that percentile of the population reflected by the intraspecies factor.

To complete the assessment, the uncertainty included in the procedure outlined above should be addressed and, if necessary, used to modify the minimal MOS in terms of precaution. On that purpose several aspects should be taken into account, which by their nature are not easy to quantify. Examples are the reliability of the data base, the variability in assessment factors, the different steps necessary to bridge data gaps, the biological relevance of the observed effects.

4.1.3.2.2 Endpoint-specific risk assessment for worker

Acute Toxicity

Acute toxicity by inhalation

In an acute inhalation limit test rats were exposed to a nominal concentration of 25.7 mg/l for 1 hour, generated by means of a midget impinger. Rats showed moderate lacrymation and salivation without mortality. As starting point for MOS calculation the air concentration of 25700 mg/m^3 is chosen which did not lead to severe acute effects in rats.

Evaluation of the MOS values has to account for the following aspects: (a) study duration was 1 hour compared to occupational exposure of 8 hours, (b) physiological differences between humans at rest and workers account for a factor of 1.5, (c) the starting point for risk characterisation is some sort of an acute LOAEC which is transfered to an acute NOAEC with a factor of 3, (d) human intraspecies variation is accounted for by a factor of 5, (e) a further uncertainty factor of 2 is proposed because the LOAEL is based on a nominal (not measured) air concentration which gives indication that the risk situation might be more critical than estimated. Altogether the minimal MOS calculates to 360 (8/1 x 1.5 x 3 x 5 x 2). The critical exposure level is identified as 70 mg/m^3 (25700 mg/m^3 / 360). It is recognized that the quality of the acute inhalation study is rather limited. Nevertheless, this assessment of acute inhalation toxicity to some degree is consistent with the results of oral toxicity testing (see below in the chapter on acute toxicity by combined exposure).

The highest shift average value for inhalation is 8.3 mg/m^3 for spray application (scenario 3a). The according MOS value calculates to 3096 (25700 / 8.3), which, in comparison to the minimal MOS of 360, does not give reason for concern.

Acute toxicity by dermal contact

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In a limit test in rabbits with occlusive exposure for 24 hours a dose of 2150 mg/kg did not result in apparent signs of toxicity. As starting point for MOS calculation the human dose corresponding to the dermal NOAEL in rabbits is calculated to 150500 mg/person (2150 mg/kg x 70 kg).

Evaluation of the MOS values has to account for the following aspects: (a) metabolic rate scaling from rabbits to humans reveals a factor of about 2, (b) human intraspecies variation is accounted for by a factor of 5, (c) against the background of rather stringent exposure conditions in the dermal toxicity study a further uncertainty factor is not considered necessary. Altogether the minimal MOS calculates to 10 (2 x 5). Based on the result of acute dermal toxicity testing the critical exposure level is identified as about 15000 mg/person (150500 mg/person / 10).

The highest dermal exposure level is reported to be up to 2500 mg/person for spray application (scenario 3a). The according MOS value calculates to 60 (150500 / 2500) which, in comparison to the minimal MOS of about 10, does not give reason for concern (compare table 4.1.3.2.B).

Acute toxicity by combined exposure

There may be different starting points for characterisation of acute toxicity by combined exposure. As well the quality of the diverse acute toxicity data for the different application ways as the relationship in potency of the different routes of exposure should be taken into account: Acute dermal toxicity is clearly lower (dermal limit test in rabbits, exposure for 24 hours of about 2000 mg/kg without apparent sings of toxicity, see above) than acute oral toxicity (LD50 in rats is about 1000 mg/kg, 800 mg/kg are without lethality) and inhalative toxicity (moderate lacrymation and salivation without lethality at 1,200 mg/kg in rats (calculation: with a breathing volume of 0.8 l/min/kg a rat breathes 48 l in 1 hour. If 25 mg/l TCEP are in the air, the rat breathes 1,200 mg/kg (25mg/l x 48 l/hour)).

Risk assessment for acute toxicity by combined exposure starts with oral toxicity studies keeping in mind, that the resulting calculation might be probably conservative. In a rat study a LD50 between 1000 and 1260 mg/kg was calculated. At 800 mg/kg clinical signs as pilo-erection and salivation are reported, but no mortality. It might be additionally helpful to have a look at the results of the developmental oral toxicity study by Kawashima et al. (1983). Pregnant dams were orally exposed (by gavage) from day 7 to 15 of pregnancy. While 200 mg/kg/d led to mortality, no changes in maternal body weight gain, food consumption and general appearance resulted from oral application up to 100 mg/kg/d. For oral application, 100% absorption is assumed.

Based on this oral (internal) starting point of 100 mg/kg/d (7000 mg/p/d) the evaluation of scenario-specific MOS values has to account for the following aspects: (a) metabolic rate scaling is reflected by a factor of 4, human intraspecies variation is described by a factor of 5, the acute NOAEL is considered to be higher than the subacute NOAEL used as starting point (factor 1/3). Altogether, the minimal MOS calculates to about 7 (4 x 5 x 1/3).

There is only one occupational scenario (spray application, scenario 3a) for which the scenario-specific MOS (of 3) is lower than the minimal MOS (of 7). Comparison of the results of acute toxicity for the various routes of application shows that acute oral toxicity is much more pronounced than acute dermal toxicity (see above). Recognizing that the internal exposure in scenario 3a is nearly totally governed by dermal contact, conclusion ii for

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scenario 3a for combined exposure is warranted. This decision is consistent with the decision for scenario 3a for dermal contact alone which is based on an acute dermal toxicity study rather than on acute oral testing (table 4.1.3.2.B).

Conclusion: ii

Table 4.1.3.2.B: MOS values for acute toxicity of TCEP, systemic effects

	Inhalation			Dermal			Combined		
Starting point for MOS calculation	25700 mg/m^3			150500 mg/p/d			7000 mg/p/d		
Minimal MOS	360			10			7		
Critical exposure level	70 mg/m^3			15000 mg/p/d			1000 mg/p/d		
	Exposure (mg/m^3)	MOS	Conclusions	Exposure (mg/p/d)	MOS	Conclusions	Internal body burden (mg/p/d)	MOS	Conclusions
1 Production	1.2	21417	ii	420	358	ii	432	16	ii
2 Processing to formulations	1.2	21417	ii	420	358	ii	432	16	ii
3a Use of formulations with spray application	8.3	3096	ii	2500	60	ii	2583	3	ii[(1)]
3b Use of formulations without aerosol formation	1.2	21417	ii	210	717	ii	222	32	ii

(1) conclusion ii was reached although MOS is lower than minMOS; explanation see text

Irritation/Corrosivity

Dermal and eye irritation

In studies with rabbits slight dermal erythema and weak irritation of the conjunctivae was observed for TCEP. The effects are not sufficient for classification. There is no concern from dermal or eye irritation at the workplace.

Conclusion: ii

Acute respiratory irritation

No studies are available concerning the irritation potential of TCEP after inhalation. From irritation studies at skin or eyes comes no indication that the substance may cause serious effects at the site of initial contact. A risk relevant damage of the airways by acute irritation properties is therefore not anticipated. There is no reason for concern.

Conclusion: ii

Sensitisation

Skin sensitisation

Human data on sensitizing properties of the substance are not available.

The skin sensitisation potential of TCEP was evaluated with the Buehler method at Guinea pigs (Mobil (1983). No allergic reactions were seen. Additionally the test results of two other structurally related chloroalkyl phosphate esters (guinea pig maximisation tests) gave no evidence to skin sensitising properties. Based on all information on the structurally related chloroalkyl phosphates (results of animal testing, similarity in physicochemical data and chemical structures, as well as alkylating properties of TCEP, TCPP and TDCP) it is concluded that TCEP should be non-sensitizing to humans. There is no reason for concern.

Conclusion: ii

Respiratory sensitisation

No information is available on the respiratory sensitisation potential of TCEP. For the time being a valid study to investigate respiratory sensitisation in experimental animals cannot be recommended. Considering that the production of TCEP is performed in closed systems and that TCEP has a low vapour pressure one would expect that exposure of the respiratory tract is low. However, TCEP is not suspected to be a potent respiratory sensitiser in humans according to the fact that during all the years of use no notice of specific case reports has been given. There is no concern from respiratory sensitisation at the workplace.

Conclusion: ii

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Repeated dose toxicity

Local effects (RDT) by inhalation or dermal contact

In a rabbit study TCEP caused slight irritation (dermal, eye). There is no information on irritation potency following acute or repeated exposure and there are no experimental studies or reported experiences concerning respiratory tract irritation following acute or repeated exposure. Against that background, conclusion ii is reached. However, it should be mentioned, that on the available basis of acute irritation data no valid predictions can be made about the irritation potency following repeated exposure (Rennen et al. 2002).

Conclusion: ii

Systemic effects (RDT) by inhalation, dermal contact and combined exposure

Several studies with repeated application, mainly by the oral route, have been performed in mice and rats. Primary target organs turned out to be the kidneys, the brain and the liver. The effects are expressed to a different degree in mice and rats, additionally male and female animal appear to be differently susceptible.

The LOAEL of 12 mg/kg bw/d for kidney lesions in Scl:ddY mice is chosen as the basis for risk assessment. Mice fed ≥12 mg/kg bw/d tris(2-chloroethyl)phosphate in the diet for 18 months developed hyperplasia and hypertrophy of the urinary tubule epithelium together with enlargement of the nuclei cysts (Takada et al. 1989). This LOAEL is the lowest LOAEL from different oral chronic toxicity studies with TCEP. The according human dose calculates to 840 mg/person/day (12 mg/kg/d x 70 kg), the corresponding occupational air concentration for 8-hour inhalation is identified as 84 mg/m^3 (840 mg/person/day / 10 m^3/day). These values are used as starting points for MOS calculations.

Evaluation of the MOS values has to account for the following aspects: (a) a LOAEL-to-NAEL extrapolation factor of 3 is proposed, (b) assessment starts from a mouse dose scaled per body weight, thus for species extrapolation from mice to humans a factor of 7 is introduced, (c) the human intraspecies variation is accounted for by a factor of 5, (d) experimental dosing was 7 days per week compared to occupational exposure of 5 days per week, (e) no furhter uncertainty factor is proposed. Altogether the minimal MOS for systemic effects after repeated exposure calculates to 75 (3 x 7 x 5 x 5/7). The according critical exposure level is 10 mg/person/day (840 mg/person/day / 75) which is used for the assessment of dermal contact (external dose) and combined exposure (internal dose). The corresponding critical exposure level for inhalation is 1 mg/m^3 (84 mg/m^3 / 75).

As can be derived from the data in table 4.1.3.2.C conclusion iii is reached for all occupational exposure scenarios for all routes of exposure. However, those occupational scenarios with inhalation exposure levels of 1.2 mg/m^3 are considered to be borderline situations.

The most critical risk situation seems to be dermal exposure during spray application (scenario 3a). For dermal scenarios 1, 2, 3a and 3b, the scenario-specific MOS values for RDT are less than 1/10 of the minimal MOS. This clear difference should be taken into account, when considering the consequence of a dermal absorption of possibly somewhat less

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than 100% on conclusions for dermal risk assessment. Handling sticky resins (scenario 3c) leads to concern as well, but is considered less critical compared to the other dermal scenarios.

Conclusion: iii

Table 4.1.3.2.C: MOS values for repeated dose toxicity of TCEP, systemic effects

	Inhalation			Dermal			Combined		
Starting point for MOS calculation	84 mg/m^3			840 mg/p/d			840 mg/p/d		
Minimal MOS	75			75			75		
Critical exposure level	1 mg/m^3			10 mg/p/d			10 mg/p/d		
	Exposure (mg/m^3)	MOS	Conclusion	Exposure (mg/p/d)	MOS	Conclusion	Internal body burden (mg/p/d)	MOS	Conclusion
1 Production	1.2	70	iii	420	2	iii	432	2	iii[1)]
2 Processing to formulations	1.2	70	iii	420	2	iii	432	2	iii[1)]
3a Use of formulations with spray application	8.3	10	iii	2500	0.3	iii	2583	0.3	iii[1)]
3b Use of formulations without aerosol formation	1.2	70	iii	210	4	iii	222	4	iii[1)]

1) conclusion iii already results from a single exposure component (inhalation or dermal), therefore it does not seem specific for combined exposure scenarios

Mutagenicity

In vitro and in vivo tests have been performed to investigate the genotoxic properties of TCEP. In summary there is no relevant evidence for mutagenicity of TCEP. There is no reason for concern.

Conclusion: ii

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Carcinogenicity

Carcinogenicity by inhalative, dermal and combined exposure

Carcinogenicity studies by gavage conducted to modern regulatory standards are available in rats and mice. These studies provide clear evidence that TCEP is carcinogenic in experimental animals. In the rat, TCEP caused an increased incidence of renal tubule adenomas in males and females at 44 mg/kg/day and above. In addition, thyroid follicular cell neoplasms and mononuclear cell leukemia may have been related to TCEP administration. In a comparable study with B6C3F1 mice, evidence for carcinogenicity was shown by a slightly increased incidence of tubule cell neoplasms obtained in supplemental evaluation of additional kidney sections. In a further experiment with Scl:ddY mice a significant increase of kidney and liver tumours occurred at 300 mg/kg/day and above. The detailed tumour incidences are summarized in chapter 4.1.2.8.

From dermal short-term tests there is no indication for a carcinogenic, promoting or initiating potential of TCEP on mouse skin. However limitations in study design and reporting do not allow firm conclusions to be drawn from the dermal studies. There is no inhalative study. Thus for the both exposure routes which are relevant at the workplace no route-specific reliable information is available concerning the endpoint carcinogenicity. As default approach the oral data are used for risk assessment at the workplace, which clearly demonstrate the potential of TCEP to induce neoplasm.

There is no evidence of a direct genotoxic mode of action. For carcinogenic risk assessment it is assumed that carcinogenicity is mediated by a non-genotoxic (epigenetic) mechanism. At present available data on TCEP do not allow for a scientifically-based identification of a threshold level for TCEP carcinogenicity. Basically, there is no valid information on the specific thresholded mode-of-action that results in secondary formation of tumours (see discussion in chapter 4.1.2.8). Recognizing the scientific difficulties of establishing a threshold level for the carcinogenicity of TCEP, for occupational risk assessment it is nevertheless considered adequate and justifiable to give risk managers some additional practical guidance on the carcinogenic potency of TCEP. It seems possible, that one of the adverse effects described in the TCEP reports on chronic toxicity might be responsible for the induction of tumour development. Thus, risk assessment for repeated dose toxicity might give some additional guidance. If any of the reported chronic effects of TCEP is responsible as starting point for TCEP tumour development, an increase of tumour incidence is not anticipated to occur up to the critical exposure levels for repeated dose toxicity (see preceding chapter). This approach implies that the LOAEL for kidney lesions in mice is used as starting point for carcinogenic risk assessment as well.

The MOS approach for repeated dose toxicity is proposed to be modified for carcinogenic risk assessment. The main reason is, that the degree of adversity of neoplasms is considered to be much more pronounced than the degree of adversity of non-neoplastic adverse effects. This consideration supports the introduction of an additional extrapolation factor for carcinogenity in order to increase the level of protection.

On a practical basis, it is proposed to introduce an additional adjustment factor of 5. This approach results in a minimal MOS for carcinogenicity of 375 (75 x 5) and the critical exposure levels of 0.2 mg/m^3 (inhalation) and 2 mg/p/d (dermal contact).

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A sound justification of the amount of this additional adjustment factor of 5 is difficult. However, it might be possible to evaluate the consequences of the proposed approach by the following theoretical considerations. According to Dybing et al. (1977) a T25 value for TCEP is calculated. Based on the overall rates for kidney adenomas and carcinomas in the rat carcinogenicity study a T25 of 130 mg/kg/day is obtained (44 mg/kg/d x 25% / 6% x 5d/7d x 103wk/104wk). In case of a linear dose-response relationship (which is not valid for TCEP) the corresponding level of risk for the proposed critical exposure level of 2 mg/p/d (or 0.029 mg/kg/d) can be easily calculated (0.25 x 0.029/130). According to this calculation, the proposed critical exposure level of 0.2 mg/m³ (inhalation) and 2 mg/p/d (dermal contact) would be connected with a risk level of about 6:100.000 in case of (non-existing) linearity. Accounting for species extrapolation and differences in exposure schedule does not substantially modify this dimension of risk. Against that background of pragmatic considerations, being aware of the scientific uncertainties involved, carcinogenic risk assessment for TCEP is proposed to be based on that modified MOS approach for repeated dose toxicity (see table 4.1.3.2.D).

As can be derived from the data in table 4.1.3.2.D for carcinogenicity conclusion iii is reached for all occupational exposure scenarios for all routes of exposure. The MOS approach used for carcinogenic risk assessment for TCEP indicates that occupational exposure levels in all three scenarios have to be reduced substantially. The most critical risk situation seems to be dermal exposure during spray application (scenario 3a). Assuming limited protection from wearing suitable gloves (e.g. 90% protection), it needs to be carefully considered whether gloves could be able to sufficiently reduce the dermal risks from TCEP.

Conclusion: iii

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Table 4.1.3.2.D: Estimation of MOS values for cancer risks by TCEP

	Inhalation			Dermal			Combined		
Starting point for MOS calculation	84 mg/m^3			840 mg/p/d			840 mg/p/d		
Minimal MOS	375			375			375		
Critical exposure level	0.2 mg/m^3			2 mg/p/d			2 mg/p/d		
	Exposure (mg/m^3)	MOS	Conclusion	Exposure (mg/p/d)	MOS	Conclusion	Internal body burden (mg/p/d)	MOS	Conclusion
1 Production	1.2	70	iii	420	2	iii	432	2	iii[1)]
2 Processing to formulations	1.2	70	iii	420	2	iii	432	2	iii[1)]
3a Use of formulations with spray application	8.3	10	iii	2500	0.3	iii	2583	0.3	iii[1)]
3b Use of formulations without aerosol formation	1.2	70	iii	210	4	iii	222	4	iii[1)]

(1) conclusion iii already results from a single exposure component (inhalation or dermal), therefore it does not seem specific for combined exposure scenarios

Reproductive toxicity, fertility impairment

Fertility impairment by inhalation, dermal and combined exposure

In an oral study with a continuous breeding protocol TCEP treatment revealed impairment of fertility in mice. At 700 mg/kg/day significant reduction of the number of litters produced by the F0 generation was observed. The findings were confirmed with a separate cross over mating trial at the same dose level. The reproductive system of male mice seemed to be more sensitive to the TCEP treatment than that of female mice. A NOAEL of 175 mg/kg/day is derived from the study (see chapter 4.1.2.9). As starting point for MOS calculation the human dose according to the experimental NOAEL is identified as 12250 mg/person/day (175 mg/kg/d x 70 kg), the corresponding occupational air concentration for 8 hours inhalation calculates to 1225 mg/m^3.

In evaluation of the MOS values the following aspects have to be considered: (a) metabolic rate scaling from mice to humans yields a factor of 7, (b) for human intraspecies variation a factor of 5 is used, (c) dosing was 7 days per week in the study compared to occupational exposure of 5 days per week, which should be accounted for, (d) an uncertainty factor of 3 is proposed because the nature of the effects is judged to be severe and there is indication for a

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steep dose response relationship. Together the minimal MOS calculates to 75 (7 x 5 x 5/7 x 3). The according critical exposure level is 160 mg/person/day (12250 mg/person/day / 75) or 16 mg/m^3 (1225 mg/m^3 / 75). It has to be noticed that these values lie above the critical exposure level for repeated dose toxicity.

In table 4.1.3.2.E MOS values and conclusions for fertility impairment of TCEP are listed in detail. Concern is expressed for dermal exposure scenarios (scenarios 1, 2, 3a and 3b). Handling sticky resins (scenario 3c) results in conclusion ii.

With respect to fertility impairment risk reduction measures at the workplace appear to be necessary. However, it should be kept in mind, that as a consequence of risk assessment with respect to repeated dose toxicity and carcinogenicity, risk reduction measures will have to be requested, which efficiently reduce occupational exposures to a level preventing workers from the according effects. If these control measures are implemented and complied with, exposures will be brought to a level which is below concern with respect to fertility impairment too.

Conclusion: iii

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Table 4.1.3.2.E: MOS values for fertility impairment of TCEP

	Inhalation			Dermal			Combined		
Starting point for MOS calculation	1225 mg/m^3			12250 mg/p/d			12250 mg/p/d		
Minimal MOS	75			75			75		
Critical exposure level	16 mg/m^3			160 mg/p/d			160 mg/p/d		
	Exposure (mg/m^3)	MOS	Conclusion	Exposure (mg/p/d)	MOS	Conclusion	Internal body burden (mg/p/d)	MOS	Conclusion
1 Production	1.2	1021	ii	420	29	iii	432	28	iii[1)]
2 Processing to formulations	1.2	1021	ii	420	29	iii	432	28	iii[1)]
3a Use of formulations with spray application	8.3	148	ii	2500	5	iii	2583	5	iii[1)]
3b Use of formulations without aerosol formation	1.2	1021	ii	210	58	iii	222	55	iii[1)]

[1)] conclusion iii already results from a single exposure component (inhalation or dermal), therefore it does not seem specific for combined exposure scenarios

Reproductive toxicity, developmental effects

Developmental effects by inhalative, dermal and combined exposure

No significant toxicity to embryo or fetal development has been observed after oral TCEP treatment of pregnant rats. Concerning developmental toxicity the NOAEL from this study is reported as 200 mg/kg/day, a dose which clearly induced maternal toxicity. Based on the available data, the results are interpreted as giving no indication for developmental toxicity of TCEP up to doses which induce chronic toxic effects and/or carcinogenicity. There is no concern with respect to this endpoint for workers.

Conclusion: ii

4.1.3.2.3 Summary of occupational risk assessment

Table 4.1.3.2.F indicates the toxicological endpoints of concern for TCEP. There is concern for fertility impairment, repeated dose toxicity and for carcinogenicity.

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Table 4.1.3.2.F: Endpoint-specific overall conclusions

Toxicological endpoints		general conclusion
Acute toxicity	inhalation	ii
	dermal	ii
	combined	ii
Irritation/ Corrosivity	dermal	ii
	eye	ii
	acute respiratory tract	ii
Sensitisation	skin	ii
	respiratory	ii
Repeated dose toxicity	inhalation, local	ii
	inhalation, systemic	iii
	dermal, local	ii
	dermal, systemic	iii
	combined, systemic	iii[1)]
Mutagenicity		ii
Carcinogenicity	inhalation	iii
	dermal	iii
	combined	iii[1)]
Fertility impairment	inhalation	ii
	dermal	iii
	combined	iii[1)]
Developmental toxicity	inhalation	ii
	dermal	ii
	combined	ii

1) conclusion iii already results from from a single exposure component (inhalation or dermal), therefore it does not seem specific for combined exposure scenarios

In table 4.1.3.2.G occupational exposure scenarios are listed in the order of scenario numbers to give an overview for all exposure situations with concern. All toxicological endpoints are listed, which at least in one case give reason for conclusion iii. Under this aspect acute toxicity, irritation, dermal and respiratory sensitisation, and developmental toxicity are not included in table 4.1.3.2.G. For TCEP concern in general results from inhalation and from dermal contact to the substance.

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Table 4.1.3.2.G: Summary of exposure scenarios with concern for TCEP [1]

Exposure scenarios		Repeated dose toxicity			Carcino-genicity			Fertility		
		inhalation	dermal	combined [2]	inhalation	dermal	combined [2]	inhalation	dermal	combined [2]
1	Production	iii	iii	iii	iii	iii	iii		iii	iii
2	Processing to formulations	iii	iii	iii	iii	iii	iii		iii	iii
3a	Use of formulations with spray application	iii	iii	iii	iii	iii	iii		iii	iii
3b	Use of formulations without aerosol formation	iii	iii	iii	iii	iii	iii		iii	iii

[1] blanc fields: conclusion ii

[2] conclusion iii already results from a single exposure component (inhalation or dermal), therefore it does not seem specific for combined exposure scenarios

Risk estimation is mainly based on oral studies. Because no data are available concerning absorption after inhalation or skin contact, route to route extrapolation is based on the default assumption of 100 % absorption at all routes.

The most important toxicological endpoint is TCEP carcinogenicity in combination with repeated dose toxicity. On the background of the exposure assessment and the proposed critical exposure levels, the according health risks are comparably high. This evaluation of risk is based on the assumption that a thresholded mode-of-action might be possible for TCEP tumour induction.

Tables 4.1.3.2.H (inhalation) and 4.1.3.2.I (dermal contact) try to visualize the risk profile of TCEP. According to the specific arrangement of exposure scenarios and critical exposure levels for different toxicological endpoints you will find the relatively high risks in the left upper corner, the relatively low risks in the bottom right corner of the tables.

On the background of cancer risks, air concentrations of TCEP at the workplace should be controlled to a level in the range of 0.2 mg/m^3 (critical exposure level for carcinogenicity). By this exposure reduction inhalative risks from other endpoints, as repeated dose toxicity and fertility impairment would similarly and effectively be mitigated too. Special emphasis should be given to reduce skin contact with TCEP (even if dermal absorption proved to be somewhat lower than 100%). Based on cancer risk assessment, dermal exposure should be controlled to levels in the range of 2 mg/person/day. On that background it needs to be carefully considered whether gloves could be able to sufficiently reduce dermal exposure.

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Table 4.1.3.2.H: Ranking of the critical exposure levels for TCEP with respect to inhalative exposure at the workplace

Exposure scenario		Exposure level in mg/m³	Carcino-genicity	Repeated dose toxicity, systemic	Fertility	Acute toxicity
			Critical exposure level in mg/m³			
			0.2 mg/m³	1 mg/m³	16 mg/m³	70 mg/m³
3a	Use of formulations with spray application	8.3	iii	iii		
1	Production	1.2	iii	iii		
2	Processing to formulations	1.2	iii	iii		
3b	Use of formulations without aerosol formation	1.2	iii	iii		

(1) blank fields: conclusion ii

Table 4.1.3.2.I: Ranking of the critical exposure levels for TCEP with respect to dermal exposure at the workplace

Exposure scenario		Exposure level in mg/p/d	Carcino-genicity	Repeated dose toxicity, systemic	Fertility	Acute toxicity
			Critical exposure level in mg/p/d			
			2 mg/p/d	10 mg/p/d	160 mg/p/d	15000 mg/p/d
3a	Use of formulations with spray application	2500	iii	iii	iii	
1	Production	420	iii	iii	iii	
2	Processing to formulations	420	iii	iii	iii	
3b	Use of formulations without aerosol formation	210	iii	iii	iii	

(1) blank fields: conclusion ii

4.1.3.3 Consumers

Exposure

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Tris(2-chloroethyl) phosphate (TCEP) is used as a flame retardant in materials that may come in contact with consumers. Several reports have shown that TCEP will be released from a number of sources which have been treated with flame retardants, namely timber, foam rubber, carpets, plastic materials (e.g. electronic devices, TV, car interior etc.), glues, and lacquers. Upholstery may sometimes be proved with flame retardants. It is released primarily by abrasion and becomes part of the dust fraction. Dust burden therefore reflects the sum of all the sources house dust and airborne dust.

Oral exposure can be referred to dust intake, due to hand-to-mouth behaviour, contamination of articles for daily use, e.g. toys. This pathway of exposure may play a particular role for children. Inhalation exposure takes place by inhaling airborne particles, and dermal exposure can occur from direct contact with e.g. furniture coverings as well as with house dust and airborne dust.

Inhalation

The measurements performed by Ingerowski et al. (2001) resulted in a 98th percentile for air concentrations of TCEP of 0.6 µg/m³. This value lies at the extreme upper range of a huge number of measurements. Although other studies have revealed lower values, this value was taken as a reasonable worst case for calculating the body burden.

The model estimate revealed an uptake by inhalation (99th percentiles) of TCEP of 0.4 and 0.96 µg/kg bw/d for adults and for children, respectively, under consideration of 100% absorption.

Dermal

Dermal exposure due to migration of tris(2-chloroethyl) phosphate from upholstery accounts for 3.9 µg/kg bw/d under worst case conditions (100% dermal absorption). Dermal exposure from airborne dust can be neglected due to the low concentrations of TCEP in dust (max. 0.1 µg/m³). Dermal exposure from house dust was calculated to amount to 0.02 µg/kg bw/d for children.

Dermal exposure from different sources can be estimated to a total of about 4 µg/kg bw/d for adults. Dermal exposure of children as related to bodyweight (10 µg/kg bw/d) can exceed that in adults.

Oral

Oral exposure of TCEP is characterised by dust uptake. The estimated amount accounts 0.0033 µg/kg bw/d for an adult, representing the 99th percentile. This pathway may represent a significant source of exposure for children, the 99th percentile representing daily uptake for a three year old child was estimated to be 0.2 µg/kg bw/d.

For babies of about 3 months a significant source could be sucking at a toy. Under worst case assumptions exposure values can be estimated up to 240 µg/kg bw/d.

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Total body burdens

For female adults, a body burden would account for ~ 4.5 µg/kg bw/d, under reasonable worst case conditions and taking all paths into consideration. The respective value for 1 - 3 year-old children is 11 µg/kg bw/d. For babies of about 3 months a body burden would account up to 240 µg/kg bw/d by sucking on toys, the other paths can be neglected.

Effects

Acute Toxicity

Following exposure assessment, consumers are expected to be exposed to tris(2-chloroethyl) phosphate several orders of magnitude lower than the range of doses which can be derived from acute oral toxicity based on animal LD50 values (oral, rats: 430-1230 mg/kg bw). In an experiment with rabbits, the substance has demonstrated low dermal toxicity, in experiments with rats, low acute inhalation toxicity. Therefore, the substance is of no concern for the consumer in relation to acute oral, inhalation or dermal toxicity.

Conclusion: ii

Irritation

Data on human experience with tris(2-chloroethyl) phosphate are not available.

In experiments with rabbits, the substance demonstrated weak skin irritation after occlusive or semi-occlusive patch testing for 4 to 24 hours exposure times. After instillation into the eyes of rabbits, mild conjunctival irritation was detected; this irritation healed within 2 days.

Conclusion: ii

Corrosivity

Information on local effects on skin and eyes of humans is not available. Tris(2-chloroethyl) phosphate is not a corrosive substance, as judged on the basis of Draize tests with rabbits.

Conclusion: ii

Sensitisation

Human data on sensitizing properties of tris(2-chloroethyl) phosphate are not available. An animal study (Buehler Test) showed no skin sensitizing potential of TCEP. The read across from sensitisation data of the two other chloroalkyl phosphates TCPP and TDCP indicated that these substances do not possess significant skin sensitisation potential. Based on data on TCEP and structurally related substances it is concluded that there is no concern.

Conclusion: ii

Repeated dose toxicity

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NOAELs for systemic effects on the brain were derived in each case from subchronic oral toxicity studies for rats at dose levels between 44 to 75 mg/kg bw/d. A NOAEL for kidney lesions was estimated only in subchronic toxicity studies for female Sprague-Dawley rats at 215 mg/kg bw/d and for B6C3F1 mice at 350 mg/kg bw/d. No NOAEL for kidney effects could be derived for male Sprague-Dawley rats fed ≥26 mg/kg bw/d in the diet for three months, and for male and female F344/N rats after chronic exposure to ≥44 mg/kg bw/d. For mice the LOAELs for kidney lesions range between 12 to 175 mg/kg bw/d (for Scl:ddY mice after feeding in the diet for 18 months and for B6C3F1 mice after long-life exposure, respectively). Because kidney effects appear to be the most sensitive endpoint for repeated exposure of tris(2-chloroethyl) phosphate the LOAEL for kidney lesions in Scl:ddY mice (12 mg/kg bw/d) was chosen as the basis for risk assessment.

Margin of safety (MOS)

For the decision on the MOS, the following aspects have been considered and taken into account:

- Uncertainty arising from the variability in the experimental data

  There are no reasons to assume an important uncertainty.

- Overall confidence in the database

  There are no contradiction results. All listed studies were of sufficient data quality.

- Intra- and interspecies variation

  Results of studies give clear evidence that mice were more sensitive to the kidney effects than rats. Thus, the interspecies variation seems to be considerable. There is a single report in humans after high inhalation exposure of a child aged five years. An extremely rare genetic susceptibility may be discussed as the underlying cause for the neuromuscular disease which became apparent and disappeared after cessation of exposure.

- Dose response relationship

  There is no reason to assume a special concern.

- Nature and severity of the effect

  The adverse effects seen at the lowest observed adverse effect level are in kidneys and these effects are considered to be severe (irreversible).

  There are no reasons to assume that the effects shown in the animal experiments are limited to the species tested, thus being not of relevance for humans.

- Differences in exposure (route, duration, frequency and pattern)

  The estimated body burdens (with an assumed absorption of 100%) are compared with an oral LOAEL from an 18 month study.

  There are no reasons to assume that special concern can be derived neither from this procedure. Toxicokinetic information are not available.

- The human population to which the quantitative and/or qualitative information on exposure applies.

  Following the exposure considerations there is reason to assume a special risk for babies sucking a TCEP containing textile cube and for children due to TCEP exposure from house dust.

- Other factors

  There are no other factors known requiring a peculiar margin of safety.

MOS for exposure scenarios via different routes

Table 4.1.3.3
Repeated dose toxicity of TCEP:
Margins of Safety for consumer exposure via different routes

Route	Exposure (µg/kg bw/d)	MOS *)	Conclusion
Inhalation			
Adults	0.4	30000	(ii)
Children	0.96	12500	(ii)
Dermal			
Adults	~ 4	3000	(ii)
Children	10	1200	(ii)
Oral			
Adults	0.0033	$>3 \cdot 10^6$	(ii)
Children	0.2	60000	(ii)
Babies	240	50	(iii)

*) The MOS was derived by using the oral LOAEL of 12 mg/kg bw/d (kidney effects)

Repeated dose toxicity - Adults

For all exposure scenarios via different routes the margins of safety are judged to be sufficient taking into account that the absorption figures are reasonable worse case estimates and even that a LOAEL has been used.

Conclusion: ii

Repeated dose toxicity - Children

The margins of safety for children specific exposure via different routes are judged to be sufficient taking into account that the absorption figures are reasonable worse case estimates and even that a LOAEL has been used.

Conclusion: ii

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Repeated dose toxicity - Babies

The margin of safety for babies due to sucking on toys is judged to be not sufficient to cover all uncertainties. It has to be taken into account that a NOAEL for kidney effects is not available. The use of a LOAEL for risk characterisation requires a higher margin of safety. Even in case of an underlying worst case exposure estimate the MOS of 50 is considered to be not sufficient thus leading to concern for repeated dose toxicity.

Conclusion: iii

Mutagenicity

In general, bacterial gene mutation tests were negative. In vitro genotoxicity tests with mammalian cells were negative for gene and chromosome mutations. Two in vivo mammalian micronucleus tests and a Drosophila test were negative. Very weak effects in in vitro SCE tests are considered to be without relevance for mutagenicity. There is the overall conclusion of no relevant evidence for mutagenicity of tris(2-chloroethyl)phosphate.

Conclusion: ii

Carcinogenicity

Tris(2-chloroethyl)phosphate induces the formation of benign and malignant tumors at various sites in experimental animals. Renal tubule cell neoplasms were found in rats and mice each in both sexes. At present the mechanism of tumor development in the kidneys of tris(2-chloroethyl) phosphate treated rats and mice remains unclear. Data of gene mutation tests and genotoxicity tests are suggestive to assume that TCEP appears to have no genotoxic potential. Thus it is likely that the tumor formation in the kidney would be mediated by non-genotoxic (epigenetic) mechanisms. A species specific mechanism of tumor formation in the kidney was not identified.

At present available data on TCEP do not allow for a scientifically-based identification of a threshold level for TCEP carcinogenicity. Basically, there is no valid information on the specific thresholded mode-of-action that results in formation of kidney tumours (see discussion in chapter 4.1.2.8). It seems possible, that any of the adverse effects described in the TCEP studies on chronic toxicity might be responsible for the induction of tumour development.

Although it is considered likely that tumour formation especially in the kidney would be mediated by a non-genotoxic (epigenetic) mechanism, a threshold level for carcinogenicity has not been established due to the lack of relevant data. Renal tumors in male and female F344/N rats, in male B6C3F1 mice and in male Scl:ddY mice may be induced secondary via cytotoxicity followed by cell proliferation. However, a NOAEL for the cytotoxic effects and also for a cell proliferation mechanism could not be derived.

The scientific difficulties of establishing a threshold level for the carcinogenicity of TCEP have to be recognized. However, one could imagine that any of the adverse effects described in the studies on chronic toxicity of TCEP might be responsible for the induction of tumour development. Given this, the LOAEL for kidney lesions obtained in Scl:ddY mice after feeding of 12 mg/kg bw/d in the diet for 18 months might be used as a surrogate LOAEL for

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risk assessment purposes on carcinogenicity using a MOS approach. Thus, it is therefore considered justified to perform the risk assessment of consumers on the assumption that a thresholded mode-of-action might be possible for TCEP tumour induction.

The total body burden for female adults estimated for reasonable worst case conditions and taking into account all paths of exposure (inhalation, dermal, and oral) would account for ~ 4.5 µg/kg bw/d. The respective value for 1 - 3 year-old children is calculated to be 11 µg/kg bw/d. Taking into account the underlying worst case exposure scenarios with assumed bioavailabilities of 100% for all exposure routes the margin of safety between the assumed oral LOAEL of 12 mg/kg bw/d (kidney lesions) and the estimated exposure levels is judged to be sufficient to conclude on no concern for tumor formation in consumers (MOS 2660) and children (MOS 1090), too.

Conclusion: ii

An oral body burden for babies (3 months) for worst case conditions was calculated to be 240 µg/kg bw/d. It has to be taken into account that a NOAEL for for tumor formation is not available. The use of a LOAEL for risk characterisation requires a higher margin of safety. Even taking into account the underlying worst case exposure scenario with an assumed bioavailability of 100% the margin of safety between the assumed LOAEL of 12 mg/kg bw/d and the exposure level is judged to be not sufficient with respect to a possibility of tumor formation (MOS 50).

Conclusion: iii

Toxicity for reproduction

- Fertility

Tris(2-chloroethyl) phosphate treatment revealed significant impairment of reproductive capacity and fertility during continuous breeding and for two successive generations in mice for both sexes. An oral NOAEL/fertility of 175 mg/kg bw/d was derived from the studies with mice. Reproductive failure was observed at daily doses of 700 mg/kg bw with at best and no more than 3 litters produced and with no pups surviving from the last litter produced. At 700 mg/kg bw/d a significant decrease was noted with respect to right epididymal and right testicular weights. Furthermore, at this dose level the average sperm count per gramme caudal tissue was statistically significantly decreased and the incidence of abnormal sperm was statistically significantly elevated relative to the control values.

Margin of safety (MOS)

For the decision on the MOS, the following aspects have been considered and taken into account:

- Uncertainty arising from the variability in the experimental data

There are no reasons to assume an important uncertainty.

- Overall confidence in the database

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There are no reasons to assume no confidence.

- Intra- and interspecies variation

The reproductive system of male mice appeared to be more sensitive to tris(2-chloroethyl) phosphate treatment as evidenced by less successive reproduction of treated males in comparison to treated females and further by significant male reproductive organ weight reduction and sperm parameter impairment in mice of two different strains.

- Dose response relationship

There is no reason to assume a special concern.

- Nature and severity of the effect

Tris(2-chloroethyl) phosphate treatment revealed significant impairment of fertility for both sexes during continuous breeding and for two successive generations in mice.

- Differences in exposure (route, duration, frequency and pattern)

The estimated total body burden with an assumed oral and dermal absorption and absorption by inhalation of 100% is compared with an oral NOAEL.

There are no reasons to assume that special concern can be derived from this procedure. Toxicokinetic information are not available.

- The human population to which the quantitative and/or qualitative information on exposure applies

Following the exposure scenarios there might be a special risk for babies and children due to group-specific exposure behavior.

- Other factors

There are no other factors known requiring a peculiar margin of safety.

MOS for exposure scenarios for different routes

The total body burden for female adults estimated for reasonable worst case conditions and taking into account all paths of exposure (inhalation, dermal, and oral) would account for ~ 4.5 µg/kg bw/d. The respective value for 1 - 3 year-old children is calculated to be 11 µg/kg bw/d. The margin of safety between the oral NOAEL (fertility) of 175 mg/kg bw/d and the estimated exposure levels in the µg range is judged to be sufficient to conclude on no concern for fertility to consumers (MOS 39000) and children (MOS 16000), too.

Conclusion: ii

A body burden for babies (3 months) for worst case conditions was calculated to be 240 µg/kg bw/d orally. The margin of safety between the oral NOAEL of 175 mg/kg bw/d and the estimated exposure level is judged to be sufficient to conclude on no concern for fertility to babies (MOS 730).

Conclusion: ii

- Developmental toxicity

Teratogenic effects of tris(2-chloroethyl) phosphate were investigated up to doses of 200 mg/kg bw/d. No embryo-/fetotoxic or specific teratogenic properties were observed even at maternally toxic doses (NOAEL/maternal toxicity = 100 mg/kgbw/d). Therefore, developmental toxicity is not considered to be a relevant endpoint.

Conclusion: ii

4.1.3.4 Man exposed via the environment

Exposure

Indirect exposure via the environment is calculated using data for intake via food, air and drinking water. Following the local scenario data (cf. 4.1.1.4) a total daily intake of TCEP for humans of 5.8 µg/kg bw/d is calculated. Calculations for the regional scenario resulted in a total daily dose of 1.1×10^{-5} mg/kg bw/d. The main route of exposure is the stem for both approaches.

Effects

Acute Toxicity

Following exposure assessment, exposure of humans via the environment is expected to be several orders of magnitude lower than the range of doses which can be derived from acute oral toxicity based on animal LD50 values (oral, rats: 430-1230 mg/kg bw). Therefore, the substance is of no concern for the consumer in relation to acute oral toxicity.

Conclusion: ii

Irritation

Data on human experience with tris(2-chloroethyl) phosphate are not available.

In experiments with rabbits, the substance demonstrated weak skin irritation after occlusive or semi-occlusive patch testing. After instillation into the eyes of rabbits, mild conjunctival irritation was detected. Given the oral route as predominant way of uptake there is no concern.

Conclusion: ii

Corrosivity

Information on local effects on skin and eyes of humans is not available. Tris(2-chloroethyl) phosphate is not a corrosive substance. Given the oral route as predominant way of uptake there is no concern.

Conclusion: ii

Sensitisation

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There are no human data on sensitisation available. Based on available data on TCEP and structurally related substances which have been tested in animal tests and the fact that the oral uptake is the main route it is concluded that there is no concern.

Conclusion: ii

Repeated dose toxicity

NOAELs for systemic effects on the brain were derived subchronic oral toxicity studies for rats at dose levels between 44 to 75 mg/kg bw/d. A NOAEL for kidney lesions was estimated only for female SD rats at 215 mg/kg bw/d and B6C3F1 mice at 350 mg/kg bw/d. No NOAEL could be derived for male SD and for male and female F344/N rats (cf. 4.1.1.3). Because kidney effects appear to be the most sensitive endpoint for repeated exposure of tris(2-chloroethyl) phosphate the lowest LOAEL for kidney lesions in Scl:ddY mice (12 mg/kg bw/d) was chosen as the basis for risk assessment.

Margin of safety (MOS)

For the decision on the MOS, the following aspects have been considered and taken into account:

- Uncertainty arising from the variability in the experimental data

  There are no reasons to assume an important uncertainty.

- Overall confidence in the database

  There are no contradiction results. All listed studies were of sufficient data quality.

- Intra- and interspecies variation

  Results of studies give clear evidence that mice were more sensitive to the kidney effects than rats. Thus, the interspecies variation seems to be considerable. There is a single report in humans after high inhalation exposure of a child aged five years.

- Dose response relationship

  There is no reason to assume a special concern.

- Nature and severity of the effect

  The effects described as low observed adverse effect are effects in kidneys, these effects are considered to be severe (irreversible).

  There are no reasons to assume that the effects shown in the animal experiments are limited to the species tested, thus being not of relevance for humans.

- Differences in exposure (route, duration, frequency and pattern)

  The calculated total daily doses are compared with an oral LOAEL from an 18 month study.

There are no reasons to assume that special concern can be derived neither from this procedure. Toxicokinetic information are not available.

- The human population to which the quantitative and/or qualitative information on exposure applies.

There is no reason to assume a special risk for elderly or children.

- Other factors

There are no other factors known requiring a peculiar margin of safety.

MOS for exposure scenario:

Man exposed indirectly via the environment

Local scenario

The calculated total daily intake 0.0058 mg/kg bw/d is compared with an oral LOAEL of 12 mg/kg bw/d. The margin of safety of 2069 is judged to be sufficient taking into account considerations on intra- and interspecies variation, nature and severity of the effects and that a LOAEL is used for derivation of the MOS. Thus, the substance is considered to be of no concern in relation to local indirect exposure via the environment.

Conclusion: ii

Regional scenario

The regional total daily intake was calculated to be 1.1×10^{-5} mg/kg bw/d. The margin of safety of about 10^6 is judged to be sufficient. Thus, there is no concern in relation to regional exposure via the environment.

Conclusion: ii

Mutagenicity

In general, bacterial gene mutation tests were negative. In vitro genotoxicity tests with mammalian cells were negative for gene and chromosome mutations. Two in vivo mammalian micronucleus tests and a Drosophila test were negative. Very weak effects in in vitro SCE tests are considered to be without relevance for mutagenicity. Thus, there is no concern in relation to exposure of man via the environment.

Conclusion: ii

Carcinogenicity

Tris(2-chloroethyl)phosphate induces the formation of benign and malignant tumors at various sites in experimental animals. Renal tubule cell neoplasms were found in rats and mice each in both sexes. At present the mechanism of tumor development in the kidneys of tris(2-chloroethyl)phosphate treated rats and mice remains unclear. Data of gene mutation tests and genotoxicity tests are suggestive to assume that tris(2-chloroethyl) phosphate appears to have no genotoxic potential. Thus it is likely that the tumor formation in the kidney

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would be mediated by non-genotoxic (epigenetic) mechanisms. A species specific mechanism of tumor formation in the kidney was not identified.

At present available data on TCEP do not allow for a scientifically-based identification of a threshold level for TCEP carcinogenicity. Basically, there is no valid information on the specific thresholded mode-of-action that results in formation of kidney tumours (see discussion in chapter 4.1.2.8). It seems possible, that any of the adverse effects described in the TCEP studies on chronic toxicity might be responsible for the induction of tumour development.

Although it is considered likely that tumour formation especially in the kidney would be mediated by a non-genotoxic (epigenetic) mechanism, a threshold level for carcinogenicity has not been established due to the lack of relevant data. Renal tumors in male and female F344/N rats, in male B6C3F1 mice and in male Scl:ddY mice may be induced secondary via cytotoxicity followed by cell proliferation. However, a NOAEL for the cytotoxic effects and also for a cell proliferation mechanism could not be derived.

The scientific difficulties of establishing a threshold level for the carcinogenicity of TCEP have to be recognized. However, one could imagine that any of the adverse effects described in the studies on chronic toxicity of TCEP might be responsible for the induction of tumour development. Given this, the LOAEL for kidney lesions obtained in Scl:ddY mice after feeding of 12 mg/kg bw/d in the diet for 18 months might be used as a surrogate LOAEL for risk assessment purposes on carcinogenicity using a MOS approach. Thus, it is therefore considered justified to perform the risk assessment of man via the environment on the assumption that a thresholded mode-of-action might be possible for TCEP tumour induction.

Local scenario

The calculated total daily intake 0.0058 mg/kg bw/d is compared with an oral LOAEL of 12 mg/kg bw/d (kidney lesions). The margin of safety of 2069 is judged to be sufficient taking into account the assumptions used in derivation of the LOAEL. Thus, the substance is considered to be of no concern in relation to local indirect exposure via the environment.

Conclusion: ii

Regional scenario

The regional total daily intake was calculated to be 1.1×10^{-5} mg/kg bw/d. The margin of safety of about 10^6 is judged to be sufficient. Thus, the substance is no concern for tumor formation in relation to regional exposure via the environment.

Conclusion: ii

Toxicity for reproduction

- Fertility

Tris(2-chloroethyl) phosphate treatment revealed significant impairment of reproductive capacity and fertility during continuous breeding and for two successive generations in mice for both sexes. An oral NOAEL/fertility of 175 mg/kg bw/d was derived from the studies with mice. Reproductive failure was observed at daily doses of 700 mg/kg bw (cf. 4.1.3.3).

Margin of safety (MOS)

For the decision on the MOS, the following aspects have been considered and taken into account:

- Uncertainty arising from the variability in the experimental data

There are no reasons to assume an important uncertainty.

- Overall confidence in the database

There are no reasons to assume no confidence.

- Intra- and interspecies variation

The reproductive system of male mice appeared to be more sensitive to tris(2-chloroethyl) phosphate treatment as evidenced by less successive reproduction of treated males in comparison to treated females and further by significant male reproductive organ weight reduction and sperm parameter impairment in mice of two different strains.

- Dose response relationship

There is no reason to assume a special concern.

- Nature and severity of the effect

Tris(2-chloroethyl) phosphate treatment revealed significant impairment of fertility for both sexes during continuous breeding and for two successive generations in mice.

- Differences in exposure (route, duration, frequency and pattern)

The calculated total daily doses were compared with an oral NOAEL.

There are no reasons to assume that special concern can be derived from this procedure. Toxicokinetic information are not available.

- The human population to which the quantitative and/or qualitative information on exposure applies

There is no reason to assume a special risk for elderly or children.

- Other factors

There are no other factors known requiring a peculiar margin of safety.

MOS for exposure scenario:

Man exposed indirectly via the environment

Local scenario

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The total daily intake was calculated to be 0.0058 mg/kg bw/d. The margin of safety of about 30000 is judged to be sufficient. Thus, the substance is of no concern in relation to local indirect exposure via the environment.

Conclusion: ii

Regional scenario

The total daily intake was calculated to be 1.1×10^{-5} mg/kg bw/d. The margin of safety in the range of 10^7 is judged to be sufficient for regional indirect exposure.

Conclusion: ii

- Developmental toxicity

Teratogenic effects of tris(2-chloroethyl) phosphate were investigated up to doses of 200 mg/kg bw/d. No embryo-/fetotoxic or specific teratogenic properties were observed even at maternally toxic doses (NOAEL/maternal toxicity = 100 mg/kg bw/d). Therefore, developmental toxicity is not considered to be of concern in relation to indirect exposure via the environment

Conclusion: ii

4.2 HUMAN HEALTH (PHYSICO-CHEMICAL PROPERTIES)

4.2.1 Exposure assessment

4.2.1.1 Occupational exposure

4.2.1.2 Consumer exposure

4.2.1.3 Indirect exposure via the environment

4.2.2 Effects assessment: Hazard identification and Dose (concentration) - response (effect) assessment

4.2.2.1 Explosivity

Tris(2-chloroethyl)phosphate is not explosive.

4.2.2.2 Flammability

Tris(2-chloroethyl)phosphate is not flammable.

4.2.2.3 Oxidising potential

Due to its chemical structure, Tris(2-chloroethyl)phosphate is not expected to possess any oxidizing properties.

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4.2.3 Risk characterisation

4.2.3.1 Workers

Not applicable

4.2.3.2 Consumers

Not applicable

ED_002221_00023974-00180

5 CONCLUSIONS / RESULTS

Overall results of the risk assessment:

( ) i) There is need for further information and/or testing

(X) ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

(X) iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account

Summary of conclusions:

Environment

( ) i) There is need for further information and/or testing

(X) ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

( ) iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account

ad ii)

This conclusion applies to all life cycle steps to all environmental compartments, to the function of waste water treatment plants and to secondary poisoning via the food chain.

TCEP does not meet the PBT criteria.

A potential risk in future cannot be excluded if production and/or use volumes were to increase as a consequence of actions on other flame retardants. Therefore, it is recommended to monitor that the downtrend in use of TCEP is not reversed in future.

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Human Health

Consumer

( ) i) There is need for further information and/or testing

(X) ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

(X) iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account

Ad iii) Risk reduction measures are required for babies with respect to the scenario sucking on toys taking into consideration the carcinogenic properties of the substance and the effects after repeated oral administration.

Workers

( ) i) There is need for further information and/or testing

( ) ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

(X) iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account

ad iii)

For TCEP three occupational exposure scenarios are evaluated. TCEP is produced (scenario 1) and is used for the production of formulations (scenario 2). The use of TCEP-containing formulations (scenario 3) includes spray application (scenario 3a) and applications without formation of aerosols (scenario 3b). The overall result of risk assessment indicates that current exposure levels (inhalation and dermal contact) are too high for all occupational exposure scenarios.

From the toxicological point of view, concern mainly derives from the carcinogenic properties of TCEP. In addition, chronic toxicity and partly fertility impairment give reason for concern.

Measures selected for risk reduction should be able to substantially reduce TCEP exposure of workers. Special emphasis should be given to the "spray application" scenario (dermal contact and inhalation).

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With respect to risk assessment for carcinogenicity inhalation exposure at the workplace should be reduced to a level of 0.2 mg/m^3 or below. It is recommended to establish an occupational exposure limit for TCEP.

Concerning skin contact, exposure should be controlled to levels in the range of 2 mg/person/day. On that background it needs to be carefully considered whether gloves could be able to sufficiently reduce the dermal risks from TCEP.

Human exposed via the environment

( ) i) There is need for further information and/or testing

(X) ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

( ) iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account

ad ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

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Appendix I

Distribution and fate

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Distribution and Fate

$d := Tag$

Substance: TCEP

melting point:	$MP := 219 \cdot K$
vapour pressure:	$VP := 13.33 \cdot Pa$
water solubility:	$SOL := 7820 \cdot mg \cdot l^{-1}$
part. coefficient octanol/water:	$LOGP_{OW} := 1.78$
molecular weight:	$MOLW := 0.285 \cdot kg \cdot Mol^{-1}$
gas constant:	$R := 8.3143 \cdot J \cdot Mol^{-1} \cdot K^{-1}$
temperature:	$T := 285 \cdot K$
conc. of suspended matter in the river:	$SUSP_{water} := 15 \cdot mg \cdot l^{-1}$
density of the solid phase:	$RHO_{solid} := 2500 \cdot kg \cdot m^{-3}$
volume fraction water in susp. matter:	$Fwater_{susp} := 0.9$
volume fraction solids in susp.matter:	$Fsolid_{susp} := 0.1$
volume fraction of water in sediment:	$Fwater_{sed} := 0.8$
volume fraction of solids in sediment:	$Fsolid_{sed} := 0.2$
volume fraction of air in soil:	$Fair_{soil} := 0.2$
volume fraction of water in soil:	$Fwater_{soil} := 0.2$
volume fraction of solids in soil:	$Fsolid_{soil} := 0.6$
aerobic fraction of the sediment comp.:	$Faer_{sed} := 0.1$
product of CONjunge and $SURF_{air}$:	$product := 10^{-4} \cdot Pa$

distribution air/water: Henry-constant

$$HENRY := \frac{VP \cdot MOLW}{SOL} \qquad HENRY = 0.486 Pa \cdot m^3 \cdot Mol^{-1}$$

$$\log\left(\frac{HENRY}{Pa \cdot m^3 \cdot Mol^{-1}}\right) = -0.314$$

$K_{air\_water} := \frac{HENRY}{R \cdot T}$ $K_{air\_water} = 2.0502 \times 10^{-4}$

solid/water-partition ceefficient Kp_{comp} and total compartment/water-partition coefficient K_{comp_water}

$a := 0.49$ (a,b from TGD, p. 539, equ. for phosphates)

$b := 1.17$ $K_{OC} := 10^{a \cdot LOGP_{OW} + b} \cdot l \cdot kg^{-1}$ $K_{OC} = 110.205 l \cdot kg^{-1}$

Suspended matter

$Kp_{susp} := 0.1 \cdot K_{OC}$ $Kp_{susp} = 11.02 l \cdot kg^{-1}$

$K_{susp\_water} := Fwater_{susp} + Fsolid_{susp} \cdot Kp_{susp} \cdot RHO_{solid}$ $K_{susp\_water} = 3.655$

factor for the calculation of $Clocal_{water}$:

$faktor := 1 + Kp_{susp} \cdot SUSP_{water}$ $faktor = 1$

Sediment

$Kp_{sed} := 0.05 \cdot K_{OC}$ $Kp_{sed} = 5.51 l \cdot kg^{-1}$

$K_{sed\_water} := Fwater_{sed} + Fsolid_{sed} \cdot Kp_{sed} \cdot RHO_{solid}$ $K_{sed\_water} = 3.555$

Soil

$Kp_{soil} := 0.02 \cdot K_{OC}$ $Kp_{soil} = 2.204 l \cdot kg^{-1}$

$K_{soil\_water} := Fair_{soil} \cdot K_{air\_water} + Fwater_{soil} + Fsolid_{soil} \cdot Kp_{soil} \cdot RHO_{solid}$

$K_{soil\_water} = 3.506$

Sludge (activated sludge)

$K_{p\_sludge} := 0.37 \cdot K_{OC}$

$K_{p\_sludge} = 40.776 l \cdot kg^{-1}$

Raw sewage

$K_{p\_sewage} := 0.30 \cdot K_{OC}$

$K_{p\_sewage} = 33.061 l \cdot kg^{-1}$

Elimination in STPs

rate constant in STP: k = 0 d^{-1}

elimination P = f (k, logpow, logH) = 2.3 %

fraction directed to surface water $Fstp_{water}$ = 97.7

biodegradation in different compartments

surface water

$kbio_{water} := 0 \cdot d^{-1}$ (cTGD, table 5)

soil

$DT50bio_{soil} := 1 \cdot 10^{6} \cdot d$ (cTGD, table 6)

$kbio_{soil} := \frac{\ln(2)}{DT50bio_{soil}}$ $kbio_{soil} = 2.888 \times 10^{-8} h^{-1}$ $kbio_{soil} = 6.931 \times 10^{-7} d^{-1}$

sediment

$kbio_{sed} := \frac{\ln(2)}{DT50bio_{soil}} \cdot Faer_{sed}$ $kbio_{sed} = 2.888 \times 10^{-9} h^{-1}$

degradation in surface waters

$khydr_{water} := 1.74 \cdot 10^{-4} \cdot d^{-1}$ (t 1/2 = 3980 d)

$kphoto_{water} := 1 \cdot 10^{-10} \cdot d^{-1}$

$kdeg_{water} := khydr_{water} + kphoto_{water} + kbio_{water}$

$kdeg_{water} = 1.74 \times 10^{-4} d^{-1}$

$kdeg_{water} = 7.25 \times 10^{-6} h^{-1}$

Atmosphere

calculation of CONjunge * SURFaer for the OPS-model

$$VPL := \frac{VP}{\exp\left[6.79\left(1 - \frac{MP}{285 \cdot K}\right)\right]}$$

$$VP := \text{wenn}(MP > 285 \cdot K, VPL, VP)$$

$$VP = 13.33 Pa$$

$$Fass_{aer} := \frac{product}{VP + product}$$

$$Fass_{aer} = 7.502 \times 10^{-6}$$

degradation in the atmosphere

$kdeg_{air}$ = 3.96 * 10^{-2} h^{-1} (see AOP-calculation)

Appendix II

Continental and regional exposure

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SimpleBox2.0a - Berechnung regionaler + kontinentaler PEC's

- Anpassung an TGD (1996) / EUSES 1.00: Michael Feibicke (06/98)

INPUT - TCEP

Parameter names acc. SimpleBox20	Unit	Input	Parameter names according Euses
Physicochemical properties			
COMPOUND NAME	[-]	TCEP	Substance
MOL WEIGHT	[g.mol^{-1}]	285	Molecular weight
MELTING POINT	[° C]	-70	Melting Point
VAPOR PRESSURE(25)	[Pa]	0,00114	Vapour pressure at 25°C
log Kow	[log10]	1,78	Octanol-water partition coefficient
SOLUBILITY(25)	[mg.l^{-1}]	7820	Water solubility
Distribution - Partition coefficients			
- Solids water partitioning (derived from K_{oc})			
Kp(soil)	[l.kg$_d^{-1}$]	2,204	Solids-water partitioning in soil
Kp(sed)	[l.kg$_d^{-1}$]	5,51	Solids-water partitioning in sediment
Kp(susp)	[l.kg$_d^{-1}$]	11,02	Solids-water partitioning in sudpended matter
- Biota-water			
BCF(fish)	[l.kg$_w^{-1}$]	6,5	Biocentration factor for aquatic biota
Degradation and Transfromation rates			
- Characterisation and STP			
PASSreadytest	[y / n]	n	Characterization of biodegradability
- Environmental Total Degradation			
kdeg(air)	[d^{-1}]	9,50E-01	Rate constant for degradation in air
kdeg(water)	[d^{-1}]	1,74E-04	Rate constant for degradation in bulk surface water
kdeg(soil)	[d^{-1}]	6,93E-07	Rate constant for degradation in bulk soil
kdeg(sed)	[d^{-1}]	6,93E-08	Rate constant for degradation in bulk sediment
Sewage treatment (e.g. calculated by SimpleTreat)			
- Continental			
FR(volatstp) [C]	[-]	0,00E+00	Fraction of emission directed to air (STPcont)
FR(effstp) [C]	[-]	9,86E-01	Fraction of emission directed to water (STPcont)
FR(sludgestp) [C]	[-]	1,40E-02	Fraction of emission directed to sludge (STPcont)
- Regional			
FR(volatstp) [R]	[-]	0,00E+00	Fraction of emission directed to air (STPreg)
FR(effstp) [R]	[-]	9,86E-01	Fraction of emission directed to water (STPreg)
FR(sludgestp) [R]	[-]	1,40E-02	Fraction of emission directed to sludge (STPreg)
Release estimation			
- Continental			
Edirect(air) [C]	[t.y^{-1}]	0,6692	Total continental emission to air
STPload [C]	[t.y^{-1}]	1,258	Total continental emission to wastewater
Edirect(water1) [C]	[t.y^{-1}]	6,869	Total continental emission to surface water
Edirect(soil3) [C]	[t.y^{-1}]	0	Total continental emission to industrial soil
Edirect(soil2) [C]	[t.y^{-1}]	0	Total continental emission to agricultural soil
- Regional			
Edirect(air) [R]	[t.y^{-1}]	0,341	Total regional emission to air
STPload [R]	[t.y^{-1}]	1,558	Total regional emission to wastewater
Edirect(water1) [R]	[t.y^{-1}]	0,876	Total regional emission to surface water
Edirect(soil3) [R]	[t.y^{-1}]	0	Total regional emission to industrial soil
Edirect(soil2) [R]	[t.y^{-1}]	0	Total regional emission to agricultural soil

OUTPUT - TCEP

Zur Neuberechnung der Daten: ->Extras ->Optionen ->Berechnen -> Datei_berechnen -> F9 drücken, sonst keine komplette Neuberechnung aller Bezüge!!

Parameter names acc. SimpleBox20	Unit	Output	Parameter names according Euses
Physicochemical properties			
COMPOUND NAME	[-]	TCEP	Substance
Output			
- Continental			
PECsurfacewater (total)	[mg.l^{-1}]	1,18E-05	Continental PEC in surface water (total)
PECsurfacewater (dissolved)	[mg.l^{-1}]	1,18E-05	Continental PEC in surface water (dissolved)
PECair	[mg.m^{-3}]	5,17E-12	Continental PEC in air (total)
PECagr.soil	[mg.kg$_{wwt}^{-1}$]	1,23E-06	Continental PEC in agricultural soil (total)
PECporewater agr.soil	[mg.l^{-1}]	5,94E-07	Continental PEC in pore water of agricultural soils
PECnat.soil	[mg.kg$_{wwt}^{-1}$]	1,12E-06	Continental PEC in natural soil (total)
PECind.soil	[mg.kg$_{wwt}^{-1}$]	1,12E-06	Continental PEC in industrial soil (total)
PECsediment	[mg.kg$_{wwt}^{-1}$]	3,50E-05	Continental PEC in sediment (total)
- Regional			
PECsurfacewater (total)	[mg.l^{-1}]	8,71E-05	Regional PEC in surface water (total)
PECsurfacewater (dissolved)	[mg.l^{-1}]	8,70E-05	Regional PEC in surface water (dissolved)
PECair	[mg.m^{-3}]	2,27E-10	Regional PEC in air (total)
PECagr.soil	[mg.kg$_{wwt}^{-1}$]	6,09E-05	Regional PEC in agricultural soil (total)
PECporewater agr.soil	[mg.l^{-1}]	2,95E-05	Regional PEC in pore water of agricultural soils
PECnat.soil	[mg.kg$_{wwt}^{-1}$]	4,91E-05	Regional PEC in natural soil (total)
PECind.soil	[mg.kg$_{wwt}^{-1}$]	4,91E-05	Regional PEC in industrial soil (total)
PECsediment	[mg.kg$_{wwt}^{-1}$]	2,51E-04	Regional PEC in sediment (total)

Appendix III

Indirect exposure via the environment

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INDIRECT EXPOSURE VIA THE ENVIRONMENT

(TGD On New and Existing Chemicals, chapter 2)

Parameter [Unit]	*Symbol*
Definitions (for the use in this document)	
definition of the unit 'kg$_{bw}$' for body weight	$kg_{bw} := 1 \cdot kg$
definition of the unit 'd' for day	$d := 1 \cdot Tag$
	$\mu g := 10^{-3} \cdot mg$
	$scenario := 1..2$
	$local := 1$
	$regional := 2$
Constants	
gas - constant R	$R := 8.314 J \cdot K^{-1} \cdot Mol^{-1}$
Defaults	
volumefraction air in plant tissue [-]	$Fair_{plant} := 0.3$
volumefraction water in plant tissue [-]	$Fwater_{plant} := 0.65$
volumefraction lipids in plant tissue [-]	$Flipid_{plant} := 0.01$
bulk density of plant tissue [kg$_{wet\ plant}$ *m$_{plant}$$^{-3}$]	$RHO_{plant} := 700 \cdot kg \cdot m^{-3}$
leaf surface area [m^2]	$AREA_{plant} := 5 \cdot m^2$
conductance (0.001 m*s^{-1}) [m*d^{-1}]	$g_{plant} := 0.001 \cdot m \cdot s^{-1}$
shoot volume [m^3]	$V_{leaf} := 0.002 \cdot m^3$
transpiration stream [m^3*d^{-1}]	$Q_{transp} := 1 \cdot 10^{-3} \cdot m^3 \cdot d^{-1}$
correction exponent for differences between plant lipids and octanol [-]	$b := 0.95$
growth rate constant for dilution by growth [d^{-1}]	$kgrowth_{plant} := 0.035 d^{-1}$
pseudo-first order rate constant for metabolism in plants [d^{-1}]	$kmetab_{plant} := 0 \cdot d^{-1}$
pseudo-first order rate constant for photolysis in plants [d^{-1}]	$kphoto_{plant} := 0 \cdot d^{-1}$

concentration in meat and milk

daily intake of grass [$kg_{wetgrass}$*d^{-1}]	$IC_{grass} := 67.6 \cdot kg \cdot d^{-1}$
daily intake of soil [$kg_{wet\ soil}$*d^{-1}]	$IC_{soil} := 0.46 \cdot kg \cdot d^{-1}$
daily intake of air [m_{air}^3*d^{-1}]	$IC_{air} := 122 \cdot m^3 \cdot d^{-1}$
daily intake of drinkingwater [l*d^{-1}]	$IC_{drw} := 55 \cdot l \cdot d^{-1}$

daily intake for human

daily intake for the several pathways [kg_{chem}*d^{-1}] or [m^3*d^{-1}]	$IH_{drw} := 2 \cdot l \cdot d^{-1}$
	$IH_{fish} := 0.115 \cdot kg \cdot d^{-1}$
	$IH_{stem} := 1.2 \cdot kg \cdot d^{-1}$
	$IH_{root} := 0.384 \cdot kg \cdot d^{-1}$
	$IH_{meat} := 0.301 \cdot kg \cdot d^{-1}$
	$IH_{milk} := 0.561 \cdot kg \cdot d^{-1}$
	$IH_{air} := 20 \cdot m^3 \cdot d^{-1}$
bioavailability through route of intake [-]	$BIO_{inh} := 0.75$
	$BIO_{oral} := 1.0$
average body weight of human [kg]	$BW := 70 \cdot kg_{bw}$

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Input

chemical properties

	$\log K_{OW} := 1.78$
octanol-water partitioning coefficient [-]	$K_{OW} := 10^{\log K_{OW}}$
Henry - partitioning coefficient [Pa*m³*mol⁻¹]	$HENRY := 4.155 \cdot 10^{-5} \cdot Pa \cdot m^3 \cdot Mol^{-1}$
air-water partitioning coefficient [-]	$K_{air\_water} := 1.753 \cdot 10^{-8}$
fraction of the chemical associated with aerosol particles [-]	$F_{ass\_aer} := 0.081$
half-life for biodegration in surface water [d]	$DT_{50\_bio\_water} := 1 \cdot 10^{6} \cdot d$

environmental concentrations

annual average local PEC in surface water (dissolved) [mg_{chem} * l_{water}^{-1}]	$PEClocal_{water\_ann} := 13.59 \cdot \mu g \cdot l^{-1}$
annual average local PEC in air (total) [mg_{chem} * m_{air}^{-3}]	$PEClocal_{air\_ann} := 0.0038 \cdot \mu g \cdot m^{-3}$
local PEC in grassland (total), averaged over 180 days [mg_{chem} * kg_{soil}^{-1}]	$PEClocal_{grassland} := 8.66 \cdot \mu g \cdot kg^{-1}$
local PEC in porewater of agriculture soil [mg_{chem} * $l_{porewater}^{-1}$]	$PEClocal_{agr\_soil\_porew} := 18.1 \cdot \mu g \cdot l^{-1}$
local PEC in porewater of grassland [mg_{chem} * $l_{porewater}^{-1}$]	$PEClocal_{grassland\_porew} := 4.18 \cdot \mu g \cdot l^{-1}$
local PEC in groundwater under agriculture soil [mg_{chem} * l_{water}^{-1}]	$PEClocal_{grw} := 18.1 \cdot \mu g \cdot l^{-1}$
regional PEC in surface water (dissolved) [mg_{chem} * l_{water}^{-1}]	$PECregional_{water} := 0.0871 \cdot \mu g \cdot l^{-1}$
regional PEC in air (total) [mg_{chem} * m_{air}^{-3}]	$PECregional_{air} := 2.27 \cdot 10^{-10} \cdot mg \cdot m^{-3}$
regional PEC in agriculture soil (total) [mg_{chem}*kg_{soil}^{-1}	$PECregional_{agr\_soil} := 6.09 \cdot 10^{-5} \cdot mg \cdot kg^{-1}$
regional PEC in porewater of agriculture soils [mg_{chem}*l_{water}^{-1}	$PECregional_{agr\_soil\_porew} := 2.95 \cdot 10^{-5} \cdot mg \cdot l^{-1}$

Definition of the concentrations used for indirect exposure

$C_{water_{local}} := PEClocal_{water\_ann}$

$C_{air_{local}} := PEClocal_{air\_ann}$

$C_{grassland_{local}} := PEClocal_{grassland}$

$C_{agr\_porew_{local}} := PEClocal_{agr\_soil\_porew}$

$C_{grass\_porew_{local}} := PEClocal_{grassland\_porew}$

$C_{grw_{local}} := PEClocal_{grw}$

$C_{water_{regional}} := PECregional_{water}$

$C_{air_{regional}} := PECregional_{air}$

$C_{grassland_{regional}} := PECregional_{agr\_soil}$

$C_{agr\_porew_{regional}} := PECregional_{agr\_soil\_porew}$

$C_{grass\_porew_{regional}} := PECregional_{agr\_soil\_porew}$

$C_{grw_{regional}} := PECregional_{agr\_soil\_porew}$

bioconcentration in fish

bioconcentration factor for fish
[$m_{water}^{3}*kg_{chem}^{-1}$]

$$BCF_{fish} := 10^{0.85 \cdot \log K_{OW} - 0.7} \cdot l \cdot kg^{-1}$$

modified equation for logKow > 6

$$BCF_{fish} := wenn\left[\log K_{OW} > 6, \left(-0.278 \log K_{OW}^{2} + 3.38 \log K_{OW} - 5.94\right) \cdot l \cdot kg^{-1}, BCF_{fish}\right]$$

$$C_{fish_{scenario}} := BCF_{fish} \cdot C_{water_{scenario}}$$

bioconcentration in plants

$$K_{plant\_water} := Fwater_{plant} + Flipid_{plant} \cdot K_{OW}^{b}$$

$$Croot_{agr\_plant_{scenario}} := \frac{K_{plant\_water} \cdot C_{agr\_porew_{scenario}}}{RHO_{plant}}$$

$$TSCF := 0.784 e^{\frac{-\left(\log K_{OW} - 1.78\right)^{2}}{2.44}}$$

remark: for $\log K_{OW}$ out of the range from -0.5 to 4.5
the TSCF is limited by the values for $\log K_{OW}$ = -0.5 resp. 4.5

$$TSCF := wenn\left(\log K_{OW} < -0.5, 0.903, TSCF\right)$$

$$TSCF := wenn\left(\log K_{OW} > 4.5, 0.832, TSCF\right)$$

$$K_{leaf\_air} := Fair_{plant} + \frac{K_{plant\_water}}{K_{air\_water}}$$

$$kelim_{plant} := kmetab_{plant} + kphoto_{plant}$$

$$\alpha := \frac{AREA_{plant} \cdot g_{plant}}{K_{leaf\_air} \cdot V_{leaf}} + kelim_{plant} + kgrowth_{plant}$$

$$\beta_{agr\_plant_{scenario}} := C_{agr\_porew_{scenario}} \cdot TSCF \cdot \frac{Q_{transp}}{V_{leaf}} + \left(1 - F_{ass\_aer}\right) \cdot C_{air_{scenario}} \cdot g_{plant} \cdot \frac{AREA_{plant}}{V_{leaf}}$$

$$C_{leaf\_crops_{scenario}} := \frac{\beta_{agr\_plant_{scenario}}}{\alpha \cdot RHO_{plant}}$$

$$\beta_{grass\_plant_{scenario}} := C_{grass\_porew_{scenario}} \cdot TSCF \cdot \frac{Q_{transp}}{V_{leaf}} + \left(1 - F_{ass\_aer}\right) \cdot C_{air_{scenario}} \cdot g_{plant} \cdot \frac{AREA_{plant}}{V_{leaf}}$$

$$C_{leaf\_grass_{scenario}} := \frac{\beta_{grass\_plant_{scenario}}}{\alpha \cdot RHO_{plant}}$$

purification of drinking water

system may defined dependent from the aerobic biodegradation

$$system := wenn\left(DT_{50\_bio\_water} < 10 \cdot d, 0, 1\right)$$

select a column on dependence from log K_{OW}

$$FIndex := wenn\left(logK_{OW} < 4, 0, wenn\left(logK_{OW} > 5, 2, 1\right)\right)$$

$$Fpur_{logKow} := \begin{pmatrix} 1 & \frac{1}{4} & \frac{1}{16} \\ 1 & \frac{1}{2} & \frac{1}{4} \end{pmatrix}$$

$$Fpur := \frac{Fpur_{logKow_{system,FIndex}}}{wenn\left(HENRY > 100 \cdot Pa \cdot m^3 \cdot Mol^{-1}, 2, 1\right)}$$

$$C_{drw_{scenario}} := wenn\left[C_{grw_{scenario}} > \left(C_{water_{scenario}} \cdot Fpur\right), C_{grw_{scenario}}, C_{water_{scenario}} \cdot Fpur\right]$$

Biotransfer to meat and milk

$$BTF_{meat} := 10^{-7.6 + logK_{OW}} \cdot kg^{-1} \cdot d$$

remark: for $logK_{OW}$ out of the range from 1.5 to 6.5

the BTF_{meat} is limited by the values for $logK_{OW}$ = 1.5 resp. 6.5

$$BTF_{meat} := wenn\left(logK_{OW} < 1.5, 7.943 \cdot 10^{-7} \cdot kg^{-1} \cdot d, BTF_{meat}\right)$$

ED_002221_00023974-00209

$$BTF_{meat} := wenn\left(logK_{OW} > 6.5, 0.07943kg^{-1}\cdot d, BTF_{meat}\right)$$

$$C_{meat_{scenario}} := BTF_{meat}\cdot\left(\begin{array}{l} C_{leaf\_grass_{scenario}}\cdot IC_{grass} + C_{grassland_{scenario}}\cdot IC_{soil} \ldots \\ + C_{air_{scenario}}\cdot IC_{air} + C_{drw_{scenario}}\cdot IC_{drw} \end{array}\right)$$

$$BTF_{milk} := 10^{-8.1+logK_{OW}}\cdot kg^{-1}\cdot d$$

remark: for $logK_{OW}$ out of the range from 3 to 6.5

the BTF_{milk} is limited by the values for $logK_{OW}$ = 1.5 resp. 6.5

$$BTF_{milk} := wenn\left(logK_{OW} < 3, 7.943\,10^{-6}\cdot kg^{-1}\cdot d, BTF_{milk}\right)$$

$$BTF_{milk} := wenn\left(logK_{OW} > 6.5, 0.02512kg^{-1}\cdot d, BTF_{milk}\right)$$

$$C_{milk_{scenario}} := BTF_{milk}\cdot\left(\begin{array}{l} C_{leaf\_grass_{scenario}}\cdot IC_{grass} + C_{grassland_{scenario}}\cdot IC_{soil} \ldots \\ + C_{air_{scenario}}\cdot IC_{air} + C_{drw_{scenario}}\cdot IC_{drw} \end{array}\right)$$

total daily intake for human

daily dose through intake of several pathways
[$kg_{chem}*kg_{bw}^{-1}*d^{-1}$]

$$DOSE_{drw_{scenario}} := \frac{C_{drw_{scenario}} \cdot IH_{drw}}{BW}$$

$$DOSE_{air_{scenario}} := \frac{C_{air_{scenario}} \cdot IH_{air} \cdot BIO_{inh}}{BW \cdot BIO_{oral}}$$

$$DOSE_{stem_{scenario}} := \frac{C_{leaf\_crops_{scenario}} \cdot IH_{stem}}{BW}$$

$$DOSE_{root_{scenario}} := \frac{Croot_{agr\_plant_{scenario}} \cdot IH_{root}}{BW}$$

$$DOSE_{meat_{scenario}} := \frac{C_{meat_{scenario}} \cdot IH_{meat}}{BW}$$

$$DOSE_{milk_{scenario}} := \frac{C_{milk_{scenario}} \cdot IH_{milk}}{BW}$$

$$DOSE_{fish_{scenario}} := \frac{C_{fish_{scenario}} \cdot IH_{fish}}{BW}$$

total daily intake for human

total daily intake for human as sum of each pathway
[$kg_{chem}*kg_{bw}^{-1}*d^{-1}$]

$$DOSE_{tot_{scenario}} := DOSE_{drw_{scenario}} + DOSE_{fish_{scenario}} + DOSE_{stem_{scenario}} + DOSE_{root_{scenario}} \ldots + DOSE_{meat_{scenario}} + DOSE_{milk_{scenario}} + DOSE_{air_{scenario}}$$

relative doses of specific different pathway (%)

$$RDOSE_{drw_{scenario}} := \frac{DOSE_{drw_{scenario}} \cdot 100\%}{DOSE_{tot_{scenario}}}$$

$$RDOSE_{air_{scenario}} := \frac{DOSE_{air_{scenario}} \cdot 100\%}{DOSE_{tot_{scenario}}}$$

$$RDOSE_{stem_{scenario}} := \frac{DOSE_{stem_{scenario}} \cdot 100\%}{DOSE_{tot_{scenario}}}$$

$$RDOSE_{root_{scenario}} := \frac{DOSE_{root_{scenario}} \cdot 100\%}{DOSE_{tot_{scenario}}}$$

$$RDOSE_{meat_{scenario}} := \frac{DOSE_{meat_{scenario}} \cdot 100\%}{DOSE_{tot_{scenario}}}$$

$$RDOSE_{milk_{scenario}} := \frac{DOSE_{milk_{scenario}} \cdot 100\%}{DOSE_{tot_{scenario}}}$$

$$RDOSE_{fish_{scenario}} := \frac{DOSE_{fish_{scenario}} \cdot 100\%}{DOSE_{tot_{scenario}}}$$

Results of calculation

$DOSE_{tot_{local}} = 5.842007 \times 10^{-3} \frac{mg}{kg_{bw} \cdot d}$

$DOSE_{tot_{regional}} = 1.110433 \times 10^{-5} \frac{mg}{kg_{bw} \cdot d}$

$RDOSE_{drw_{local}} = 8.852144\%$

$RDOSE_{drw_{regional}} = 22.410813\%$

$RDOSE_{air_{local}} = 0.013938\%$

$RDOSE_{air_{regional}} = 4.380529 \times 10^{-4}\%$

$RDOSE_{stem_{local}} = 85.870726\%$

$RDOSE_{stem_{regional}} = 66.814222\%$

$RDOSE_{root_{local}} = 2.770143\%$

$RDOSE_{root_{regional}} = 2.375282\%$

$RDOSE_{meat_{local}} = 7.832217 \times 10^{-4}\%$

$RDOSE_{meat_{regional}} = 1.997168 \times 10^{-3}\%$

$RDOSE_{milk_{local}} = 7.66065 \times 10^{-3}\%$

$RDOSE_{milk_{regional}} = 0.019534\%$

$RDOSE_{fish_{local}} = 2.484604\%$

$RDOSE_{fish_{regional}} = 8.377713\%$

European Commission

EUR [ECB: click here to insert EUR No.] - European Union Risk Assessment Report [ECB: click here to insert SUBSTANCE NAME, and volume no.]

Editors: (keep this updated)

Luxembourg: Office for Official Publications of the European Communities

[ECB: insert year] – VIII pp., [ECB: insert number of pages] pp. – 17.0 x 24.0 cm

Environment and quality of life series

ISBN [ECB: insert ISBN No.]

Price (excluding VAT) in Luxembourg: EUR [ECB:insert price]

The report provides the comprehensive risk assessment of the substance Tris (2-chloroethyl) phosphate. It has been prepared by Germany in the frame of Council Regulation (EEC) No. 793/93 on the evaluation and control of the risks of existing substances, following the principles for assessment of the risks to man and the environment, laid down in Commission Regulation (EC) No. 1488/94.

The evaluation considers the emissions and the resulting exposure to the environment and the human populations in all life cycle steps. Following the exposure assessment, the environmental risk characterisation for each protection goal in the aquatic, terrestrial and atmospheric compartment has been determined.
There is no concern identified for the environment from the production and use of the substance.

For human health the scenarios for occupational exposure, consumer exposure and humans exposed via the environment have been examined and the possible risks have been identified.The human health risk assessment concludes that there is concern for workers with regard to carcinogenicity, repeated dose toxicity and impairment of fertility. For consumers (babies) there is concern for repeated oral exposure. There is no concern for humans exposed via the environment and for human health (physico-chemical properties).

Message

From: Henry, Tala [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=8BFC0A617A4A43BAA8856541C70622BE-THENRY02]
Sent: 3/13/2017 8:36:09 PM
To: Camacho, Iris (Camacho.Iris@epa.gov) [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=5598d2cc8e3c4302aff255a840a991dc-Camacho, Iris]
Subject: FW: LNT Obituary
Attachments: Environ. Res. LNT-Obit.-1.pdf

FYI

Tala R. Henry, Ph.D.
Director, Risk Assessment Division
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency

T: 202-564-2959
E: henry.tala@epa.gov

From: Fitzpatrick, Julie
Sent: Monday, March 13, 2017 12:43 PM
To: RAF Members <RAF_Members@epa.gov>; Behl, Betsy <Behl.Betsy@epa.gov>; Strong, Jamie <Strong.Jamie@epa.gov>
Subject: FW: LNT Obituary

RAF Members,

Creatively crafted introduction to Calabrese's latest papers.

Regards,
Julie

Julie W. Fitzpatrick
Human Health Science Coordinator
Risk Assessment Forum
U.S. Environmental Protection Agency
Office (202) 564-4212
Mobile [Personal Matters / Ex. 6]
fitzpatrick.julie@epa.gov

From: Edward Calabrese [mailto:edwardc@schoolph.umass.edu]
Sent: Sunday, March 12, 2017 9:12 AM
To: Fitzpatrick, Julie <Fitzpatrick.Julie@epa.gov>
Subject: LNT Obituary

Julie:

See the attached....

Ed

Message

From: Morris, Jeff [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=55C34872E6EA40CAB78BE910AEC63321-MORRIS, JEFF]
Sent: 2/17/2017 12:33:38 AM
To: Cleland-Hamnett, Wendy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=b84439fcdf02426abd539d8bb6c9ef6f-Cleland-Hamnett, Wendy]
Subject: Re: What do you think of hormesis?

So can you say who's asking what we think of hormesis? Just curious.

Jeff Morris
Office of Pollution Prevention & Toxics
US EPA

On Feb 16, 2017, at 7:20 PM, Cleland-Hamnett, Wendy <Cleland-Hamnett.Wendy@epa.gov> wrote:

> This guy rumored to be nominee for ord.
>
> https://www.umass.edu/sphhs/person/faculty/edward-j-calabrese
>
> Shared via the Google app
>
> Wendy Cleland-Hamnett
> Acting Assistant Administrator
> Principal Deputy Assistant Administrator
> Office of Chemical Safety & Pollution Prevention
> U.S. EPA

Message

From: EPA Alumni Association [newsletter@epaalumni.org]
Sent: 10/21/2018 2:55:30 PM
To: Wise, Louise [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=cf7be035da4b45a3a7d45c84c9f4b4a3-LWise]
Subject: Transition Update #92, Week of October 14-20, 2018

Transition Update #92
Week of October 14-20, 2018

Nominees and Appointments

No change from last week, and no action is expected until after the November elections.

The nominations of Peter Wright (Land and Emergency Management), and Chad McIntosh (International and Tribal Affairs) are still waiting for a vote by the full Senate. The Environment and Public Works Committee held a hearing for them on August 1st, and they were placed on the Senate Executive Calendar on that same date. No votes have been scheduled.

An Environment and Public Works Committee hearing on the nomination of Alexandra Dunn to be the Assistant Administrator for Chemical Safety and Pollution Prevention has not yet been scheduled.

Open Positions

The Washington Post Appointment Tracker shows the following positions at EPA with no one currently nominated or confirmed.

- Administrator
- Assistant Administrator for Environmental Information
- Assistant Administrator for Research and Development
- Assistant Administrator for Administration and Resources Management

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October

The paragraphs

October 15, 2015
Sidelined children's health official says EPA inaction means "kids are disposable"

As the director of the Office of Children's Health Protection (OCHP), it was Dr, Ruth Etzel's job to determine the impacts of regulations on children.

In an interview not authorized by EPA, she said "I often think of the Office of Children's Health as the conscience of EPA, because, you know, we're kind of nagging at them: 'Is this okay for children? Are you sure this is okay for children?'"

CBS News reports that her monthly meetings with the Administrator cease with the beginning of the Trump administration. New regulations on lead were put on hold and one administration official told her that anything involving new regulation "wouldn't fly."

> "My sense is that the government has absolutely no intention of taking any action toward seriously changing lead in children's environments," Dr. Etzel said.
>
> ABC Correspondent Anna Werner asked, "What does that mean for the kids?"
>
> "It basically means that our kids will continue to be poisoned," Dr. Etzel replied. "It basically means that kids are disposable, they don't matter."

The video can be found at the link below.

In the video, Dr. Etzel does say she is on leave with pay, but that she has not been told of any reason for her administrative leave other than it is for an investigation.
The following statement from Chief of Staff Ryan Jackson:

In a statement to CBS News, EPA Chief of Staff Ryan Jackson said,

> "Although EPA does not customarily comment on personnel matters, due to circulating misinformation, the Director of EPA's Office of Children's Health

Protection was placed on leave to give the Agency the opportunity to review allegations about the Director's leadership of the office."

CBS News: Sidelined children's health official says EPA inaction means "kids are disposable"

October 15, 2018
EPA tells air pollution scientists 'your service on the panel has concluded'

Under President Trump, the EPA has tried to limit the outside scientific research it takes into account when it writes new regulations. In the latest example, EPA is dismissing dozens of outside scientists who were charged with advising the agency on how to set air pollution standards -- or being considered for that role.

The Washington Post last week reported that EPA is disbanding two panels charged with reviewing data on soot and ozone. Instead of the two panels of about two dozen researchers each, EPA is now going to rely on the seven person Clean Air Scientific Advisory Committee (CASAC) alone perform those assessments and make recommendations to the agency's political leaders. Previously, CASAC and the now-scrapped panels worked together to craft findings.

Further, EPA has packed the CASAC with state and local regulators from conservative states, rather than with academics with relevant credentials. New members include a biochemist from the Texas Commission on Environmental Quality; an air pollution control engineer with the Jefferson County, Ala., Department of Health; a toxicologist with the Utah Department of Environmental Quality; and a pulmonary doctor and professor emeritus from the University of Rochester Medical Center. Other members include a consultant from Denver who clients have included the American Chemistry Council, the American Petroleum Institute, and European petroleum consortium CONCAWE, an air modeling expert from Georgia Environmental Protection Division, and a research ecologist from the Corps of Engineers in Vicksburg.

Washington Post: The Energy 202: EPA tells air pollution scientists 'your service on the panel has concluded'

October 15, 2015
'Hyperalarming' study shows massive insect loss

The study reported on in this Washington Post article ties the loss of insects in a protected area in Puerto Rico to climate change. They looked at other possible causes (pesticides in particular) and note that pesticide use has fallen by 80 percent since 1969, the period studied.

Another study of the early impacts of climate change.

Washington Post: 'Hyperalarming' study shows massive insect loss

October 15, 2018
EPA weighs allowing oil companies to pump wastewater into rivers, streams

In producing oil, about 4 times as much water comes to the surface as oil. For many years, after separating the oil, the wastewater, still contaminated with salts, heavy metals, and organic contaminants, was injected deep underground into non-potable aquifers. The problem is that the Permian Basin (West Texas) is reaching capacity for injection, and drillers, are looking for other places to dispose of their wastewater.

Faced with this issue, the Houston Chronicle reports that the Trump administration is examining whether to adjust federal clean water regulations to allow drillers to discharge wastewater directly into rivers and streams from which communities draw their water supplies.

The article notes that from a regulatory standpoint, drillers are allowed to do this in limited circumstances now. However, the treatment process to remove the salt-, heavy metal- and chemical contaminants enough to meet state or federal water standards is so costly that it's rarely done, experts say.

The article also notes that technology is improving, and while the alternative of trucking the wastewater hundreds of miles may make more sense now, it may not in the near future.

Another option being investigated is treatment and use in irrigation. It is not clear if the standards would be less stringent than for discharge into rivers.

Houston Chronicle: EPA weighs allowing oil companies to pump wastewater into rivers, streams

October 16, 2018
Trump's Top Economic Aide On Dire UN Climate Change Report: No Need To 'Panic'

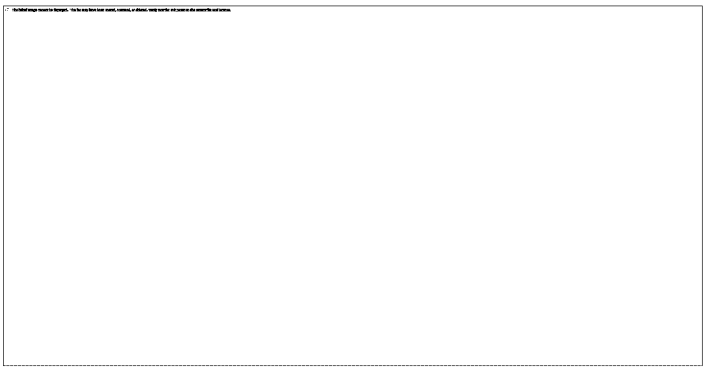
Larry Kudlow thinks that the dire UN IPCC report on global warming "overestimates" the impacts.

When will someone ask him, or any other climate deniers, what the basis for their statements is? Kudlow, the head of Trump's National Economic Council, has a BA in history from the University of Rochester, and even less qualifications to support his comments on climate change than he does for his job heading the National Economic Council (where at least he does have some work and TV commentating experience).

When asked for the basis for Pruitt's claims on climate change, EPA came up empty. If there is a FOIA request for the information underlying Kudlow's statements, the same result is highly probable.

Huffington Post: Trump's Top Economic Aide On Dire UN Climate Change Report: No Need To 'Panic'

October 16, 2018
'Poorly articulated' DOE grid plan stalls - source



E&E News reports that the plan was sent to the White House by Energy Secretary Rick Perry in response to direct instructions from Trump to slow the retirement of coal and nuclear plants.

"Efforts to ensure resilience of the grid continue while DOE's poorly articulated policy flounders in the National Economic Council," said an official, who requested anonymity.

A "pre-decisional" document leaked earlier this year proposed purchasing electricity from unidentified U.S. coal and nuclear power plants to prevent their "premature" retirement, citing heightened threats of cyberattacks and natural disasters. conservative free market think tanks, and oil, gas, wind, and renewable power interests as a "bailout."\

The "pre-decisional" draft has faced a slew of questions about legal authority from Don McGahn, the outgoing White House Counsel,and pushback from the National Economic Council, led by Washington insider Larry Kudlow.

E&E News: 'Poorly articulated' DOE grid plan stalls - source

October 16, 2018
Did Trump create a new talking point for skeptics?

On Sunday, Trump said something new (for him) about climate change. The question is will it become a talking point.

"I don't think it's a hoax. I think there's probably a difference; I think something's happening," Trump said. "Something's changing, and it'll change back again. I don't think it's a hoax; I think there's probably a difference, but I don't know that it's man-made. I'm not denying climate change. But it could very well go back."

Climate skeptics have long downplayed the role of human activity, saying saying that Earth's climate system has always fluctuated naturally. Trump flipped that around. He looked to the future instead of the past by suggesting that today's higher temperatures could decrease on their own - or "change back."

The president has a knack for boiling big ideas into manageable phrases that can be repeated, even if they're misleading.

"Unfortunately, when the president says something, or when any leader says something, it has the potential to spark it being a new talking point," said Susan Joy Hassol, director of the science outreach nonprofit group Climate Communication and a senior science writer on the National Climate Assessment.

Hassol said Trump's claims are a variation of the most popular talking point for climate contrarians, the notion that the climate is always changing and therefore humans can't do anything about it, like curb their use of fossil fuels.

"This is the No. 1 myth, it's a reflection of ignorance; climate has changed before, climate scientists are quite aware of that," she said***. "We also know why it's changed before and the long time scales on which it has changed before, and we know how different current global warming is from all of those."***

E&E News: Did Trump create a new talking point for skeptics?

October 16, 2018
Standards too weak - evidence in draft EPA report

In a draft report apparently put online yesterday, EPA acknowledged evidence that its current standards for airborne fine particulates are not tight enough to adequately protect public health.

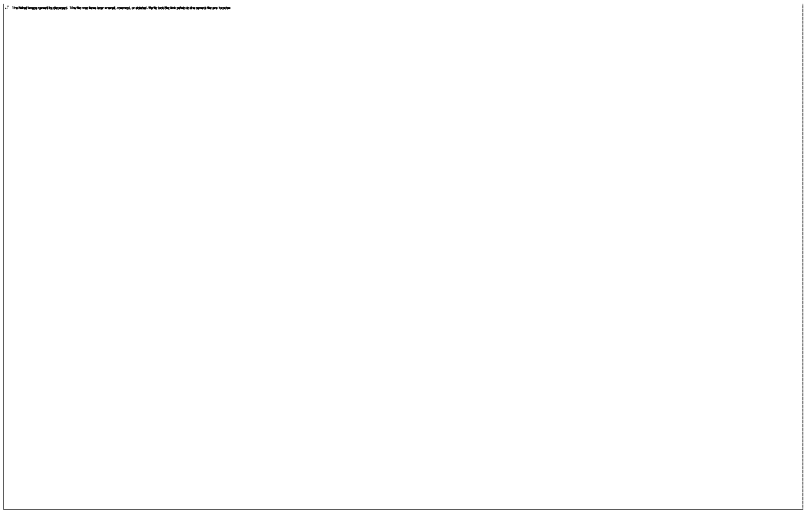
The draft report, entitled *Integrated Science Assessment for Particulate Matter*, EPA/600/R-18/179 October 2018 says that a"causal relationship" exists between short-term exposure to fine particulates and the risk of premature death.

EPA's current annual standard, set in 2012, is 12 micrograms per cubic meter of air. But the report notes that U.S. studies "indicate a linear relationship at levels as low as" 5 micrograms per cubic meter. Particularly vulnerable are children, based on "strong evidence of impaired lung function growth," and minorities, the draft adds.

E&E News: Standards too weak - evidence in draft EPA report

Draft EPA Report: Integrated Science Assessment for Particulate Matter, EPA/600/R-18/179 October 2018

October 16, 2018
The latest thing climate change is threatening is our history



Dubrovnik, Croatia

According to this story in the Post, the old city of Dubrovnik is one major storm away from a flood that could cover 10 percent of a medieval city long known as the "Pearl of the Adriatic," or more currently, as the main setting for HBO's "Game of Thrones."

Dubrovnik is just one of about 40 treasured historical sites across the Mediterranean, including the winding canals of Venice and the ancient city of Carthage, at risk from rising seas, according to a study published Tuesday in the journal Nature Communications.

The study shows that of 49 cultural World Heritage Sites located in low-lying coastal areas of the Mediterranean, 37 are at risk from a 100-year flood and 42 from coastal erosion, already today. Until 2100, flood risk may increase by 50% and erosion risk by 13% across the region, with considerably higher increases at individual World Heritage Sites.

Washington Post: The latest thing climate change is threatening is our history

Nature Communications: Mediterranean UNESCO World Heritage at risk from coastal flooding and erosion due to sea-level rise

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October 17, 2018
Trump's claim about climate scientists is 'misleading and very damaging,' weather group says

After President Trump said that climate scientists who find that human activities are driving climate change have a "very big political agenda," the American Meteorological Society (AMS), pushed back forcefully on that assertion.

In a letter to Trump published Tuesday, the AMS called Trump's claim "misleading and very damaging."

The letter took issue not only with Trump's statement about the motivations of climate scientists but also with his reluctance to accept their conclusions.

Trump conceded that "something's changing" with the climate but that it would "change back." He would not say humans are the cause of climate change. "I don't know that it's man-made." When asked about the human role in climate change, and the "huge chunks of ice just falling into the ocean" in Greenland, Trump doubled down: "And you don't know whether or not that would have happened with or without man. You don't know. "

In its letter, the AMS countered that multiple lines of evidence indicate that such changes are primarily caused by human activity. "Each of these lines of evidence has undergone rigorous testing and has overcome all credible challenges," the letter said. "They reinforce one another and there are no contradictory lines of evidence that withstand scientific scrutiny."

Wasnington Post: Trump's claim about climate scientists is 'misleading and very damaging,' weather group says

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October 17, 2018
EPA puts off final say on science transparency rule

The EPA Wednesday it is putting off for at least a year any final announcement on a controversial proposal overhauling how the agency evaluates science.

Supporters say the rule would help the public understand the science behind EPA regulations, by requiring scientists to disclose more details of the individual cases underlying public-health studies. Critics disagree, saying that the rule could force regulators to ignore the findings of major public-health studies, since much of the individual information on patients in those studies is confidential.

EPA public hearings on the proposal drew mainly throngs of critics- health officials, academics, researchers and others, who said the proposed rule would give the administration an excuse to not consider some studies showing impacts.

The EPA says it received almost 600,000 public comments on the proposal.

AP: EPA puts off final say on science transparency rule

October 17, 2018
EPA touts falling emissions during first year of Trump administration

U.S. greenhouse gas emissions from the power sector and large manufacturing facilities fell 2.7 in 2017, the first year of the Trump administration, even as it has pursued a deregulatory agenda.

> "Thanks to President Trump's regulatory reform agenda, the economy is booming, energy production is surging, and we are reducing greenhouse gas emissions from major industrial sources," EPA Acting Administrator Andrew Wheeler said in a statement. "These achievements flow largely from technological breakthroughs in the private sector, not the heavy hand of government. The Trump Administration has proven that federal regulations are not necessary to drive CO2 reductions. While many around the world are talking about reducing greenhouse gases, the U.S. continues to deliver, and today's report is further evidence of our action-oriented approach."

Washington Examiner: EPA touts falling emissions during first year of Trump administration

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October 17, 2018
Scientists bet $10K on the climate. Guess who lost

In 2005, James Annan, a climate scientist at the Japan Agency for Marine-Earth Science and Technology, was fed up with researchers and conservative thinkers who reject mainstream climate science and were arguing that climate models were wrong or that Earth would enter a cooler period after solar flares faded.

So he offered them a bet. The wager was $10,000 that the Earth would continue warming through 2017, with the winner decided decided by comparing global surface temperatures from 1998 to 2003 with those between 2012 to 2017.

Seven prominent climate contrarians refused to bet. Among them was Richard Lindzen, a physicist associated with the Massachusetts Institute of Technology who has rejected mainstream climate science.

"They didn't believe what they were saying; this was the whole point to the betting," Annan said. "It has a serious scientific point to it. ... It's one way of making the point that they're playing debating games."

Eventually, two Russian scientists, Galina Mashnich and Vladimir Bashkirtsev, agreed to the wager. This week, Annan declared that the contest was over and that Mashnich and Bashkirtsev had lost. It comes as NASA said this week that 2018 could be the fourth-warmest year on record and the fourth year in a row that is 1.8 degrees Fahrenheit above the 19th-century average.

The researchers won't pay, and Bashkirtsev wants a new bet. It would raise the stakes to $100,000 and cover another eight-year period.

E&E News: Scientists bet $10K on the climate. Guess who lost

October 17, 2018
EPA Signals Andrew Wheeler Could Stay In Place Until 2020 Without Senate Confirmation

This week, the EPA press office put out two minor updates on the trip "Administrator Wheeler" took to Louisiana.

Why is this important? Wheeler is not the EPA Administrator. He has not even been nominated to the job. He is the acting EPA administrator. The agency had been noting the distinction until this week.

With 101 days as acting administrator, Wheeler is fifth in time as acting Administrator. On Dec. 9th, he will pass Bob Perciasepe's roughly five-month stint as acting administrator in 2013.

The White House could keep Wheeler as the EPA's boss for the remaining 748 days of the president's term - nearly six times the current record for an acting administrator.

The White House also did not respond to questions about its plan for Wheeler.

Huffington Post: EPA Signals Andrew Wheeler Could Stay In Place Until 2020 Without Senate Confirmation

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October 17, 2018
Federal judge orders EPA to protect salmon from warm temps in Columbia River basin

The U.S. District Court for the Western District at Seattle in a 16-page ruling Wednesday ordered the U.S. Environmental Protection Agency (EPA) to protect Columbia Basin salmon and steelhead from dangerously warm water temperatures in the Columbia and Snake Rivers.

Climate change has exacerbated a long standing problem with water temperature in reservoirs behind hydropower dams on the rivers, increasing the number days in which temperatures exceed what can be tolerated by salmon and steelhead, which are cold-water species. In 2015, 250,000 adult sockeye salmon died when the Columbia and Snake rivers became too warm. Hot water pushed survival rates for critically endangered Snake River sockeye to only 4 percent in 2015.

"Because of today's victory, EPA will finally write a comprehensive plan to deal with dams' impact on water temperature and salmon survival," said Brett VandenHeuvel, executive director of Columbia Riverkeeper, one of the plaintiffs in the suit.

The court found that the EPA has failed to undertake its mandatory duty to enforce and ensure a temperature daily maximum, just as it must also enforce other types of water-quality parameters under the Clean Water Act. Federal Judge Ricardo Martinez ordered the agency to issue a temperature standard for the river.

Some reports say that the only way to meet the water temperature maximums is to remove dams.

Seattle Times: Federal judge orders EPA to protect salmon from warm temps in Columbia River basin

The Ruling: COLUMBIA RIVERKEEPER, et al., Plaintiffs, v. SCOTT PRUITT, et al.,

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October 18, 2018
EPA to unveil plans to weaken rule limiting toxic mercury pollution

The Guardian reports that the EPA will unveil plans next month to start weakening the economic justification for a rule limiting toxic mercury pollution from coal plants.

The EPA isn't rescinding the standard as of yet but has finished deciding to reconsider the underlying analysis for the 2011 rule, according to the government's newly published agenda. Conservatives say EPA shouldn't have counted health improvements from the mercury rule that would have come from eliminating other kinds of pollution from coal plants, including soot and nitrogen oxide linked to respiratory illnesses and early deaths.

The logic behind this argument is unfathomable, but perhaps will be made more clear next month.

The Guardian: EPA to unveil plans to weaken rule limiting toxic mercury pollution

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October 18, 2018
EPA taking UN climate report 'very seriously,' agency chief says

Acting EPA Administrator Andrew Wheeler said Thursday the agency is taking a recent United Nations report on the dire future of climate change "very seriously" while touting a number of new EPA rule changes that critics say will only worsen emissions.

The Hill: EPA taking UN climate report 'very seriously,' agency chief says

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October 18, 2018
Skeptics named as finalists for Science Advisory Board

E&E News reports that 174 finalists for EPA's Science Advisory Board include researchers who reject mainstream climate science and who have fought against environmental regulations for years.

Among them is an economist from the conservative Heritage Foundation whose work was cited by President Trump as a justification for withdrawing from the Paris climate agreement. Another downplays the dangers of air pollution. Several scientists are from energy companies like Exxon Mobil Corp. and Chevron Corp., and the list includes a researcher who argues that more carbon dioxide is good for the planet.

A few are associated with the Heartland Institute, which has advocated for the rejection of climate science to lawmakers, teachers and voters. Among its efforts is the publication of books, like the "Roosters of the Apocalypse," which describes climate change as an "apocalyptic prophecy."

EPA will accept public comment until Nov. 7.

Former Administrator Scott Pruitt sought to reshape the SAB by forcing off academic researchers who received agency grants, while elevating those who were funded by industry. The released list seems consistent with this policy.

E&E News: Skeptics named as finalists for Science Advisory Board

List of Candidates with "biosketches"

October 18, 2018
Emissions Reductions Touted by EPA Are at Odds with Its Policies

On Wednesday, EPA yesterday touted the United States' progress in lowering greenhouse gas emissions, tying the reduction to "President Trump's regulatory reform agenda."

"Thanks to President Trump's regulatory reform agenda, the economy is booming, energy production is surging, and we are reducing greenhouse gas emissions from major industrial sources," acting EPA Administrator Andrew Wheeler said in a statement. He was referring to a report released by EPA showing total reported U.S. greenhouse gas emissions decreased by 2.7 percent from 2016 to 2017. Reported emissions from large power plants also fell 4.5 percent since 2016 and 19.7 percent since 2011.

The problem, according to experts, is that EPA is patting itself on the back for a decline that's largely due to the rise of natural gas and renewable energy. And that rise is sharply at odds with Trump's vows to save the struggling domestic coal industry.

"EPA is taking credit for emission reductions that are a direct result of the decline of coal in America," said John Larsen, an analyst with the Rhodium Group, an economic research firm.

"The president has said he wants to bring back coal in America and put the miners back to work," Larsen said. "But EPA is taking credit for the opposite."

Scientific American: Emissions Reductions Touted by EPA Are at Odds with Its Policies

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October 18, 2018
Rule to roll back glider kit regs removed from EPA's regulatory calendar

The rule that would have repealed Obama-era limitations on production of glider kit trucks has been stripped from the Environmental Protection Agency's regulatory calendar, casting doubt on whether the rule will be finalized. The rule would have removed the 300-truck annual production cap placed on glider kit builders by 2016's Phase 2 tractor-trailer emissions regs.

Commercial Carrier Journal: Rule to roll back glider kit regs removed from EPAâ€™s regulatory calendar

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October 19, 2018
Democrats demand to know why EPA children's health official was abruptly put on leave

A number of Democratic senators are demanding an explanation from the EPA about why it put a leading children's health expert on paid leave without warning.

The director of EPA's Office of Children's Health Protection, Dr. Ruth Etzel, spoke out only on "CBS This Morning" on Monday. At least eight U.S. senators sent letters criticizing the EPA's move and said it raises serious concerns about the agency's commitment to children's health.

CBS News: Democrats demand to know why EPA children's health official was abruptly put on leave

See also 1st story in this issue - ***Sidelined children's health official says EPA inaction means "kids are disposable"***

October 19, 2018
EPA Withdraws Proposed Uranium Mining Rules

EPA on Friday announced that it was withdrawing the proposed *Health and Environmental Protection Standards for Uranium and Thorium Mill Tailings* that were proposed on January 19, 2017. The rules would have regulated byproduct materials produced by uranium in-situ recovery (ISR) activities, with a primary focus on groundwater protection and restoration.

The EPA notice says that EPA believes that the legal authority for the regulations is questionable, that current regulations adequately protect human health and the environment, and that the increased ISR activity is unlikely to materialize.

EPA Announcement

October 20, 2018
Why radiation protection experts are concerned over EPA proposal

Interesting article on EPA's decision to solicit the advice of controversial toxicologist, Edward Calabrese, to consider changes to how it regulates radiation. Calabrese is well known for his unconventional and outlying view that low-dose radiation is not dangerous.

He is also a proponent of hormesis, a theory that says that low exposures to toxins can be beneficial by stimulating the immune system. Hormesis is not widely accepted in the scientific community, but underlies Dr. Calabrese's opinion that radiation protection standards ought to be significantly raised.

PBS: Why radiation protection experts are concerned over EPA proposal

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New Jersey Department of Environmental Protection Jobs

If anyone you know MAY be interested in joining NJDEP over the next year or so, they should apply now for EVERY position for which THEY qualify.

https://www.nj.gov/dep/jobs/

In fact, many NJ agencies are now hiring - we finally have a budget & all Cabinet officers have finally been confirmed. State jobs are posted on the NJ job website. I know that BPU & EDA have postings & ALL of the application deadline are VERY SOON!

Jeanne Fox, Region II RA 1994-01
jeanne.fox52@gmail.com
973-271-0500

EPA Alumni Association Job Center - Our new Job Center (linked below) features jobs that employers have listed that are specifically aimed at current EPA employees and alumni. In addition, we provide links to over 1000 external job banks that are offering environmentally-relevant jobs. Please help us make our Job Center a success by:

- Telling prospective employers about the opportunity to list their jobs for free,
- Telling current employees and alumni about the Job Center and asking them to help spread the word.

Link to Job Center Web Site

Major EPA Rules Open for Comment

Save EPA (http://www.saveepaalums.info/) is tracking significant rules open for public comment. They also have, for each of the rules, prepared suggested comments to help a commenter prepare their own comments and submit them to EPA. They also have prepared a "A PRACTICAL GUIDE FOR RESISTING THE TRUMP DE-REGULATORY AGENDA," that can be found at at this link:
A PRACTICAL GUIDE FOR RESISTING THE TRUMP DE-REGULATORY AGENDA

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Coming soon: Oil and Natural Gas Sector: Emission Standards for New, Reconstructed, and Modified Sources Reconsideration

Andrew Wheeler signed the rules on September 11th, but they have not yet been published in the Federal Register. The proposed rule was published in the Federal Register on Monday, October 15th, and the 60 day public comment period will close on 12/17/2018.

The rule that this proposes to modify would have forced oil and gas operations to inspect for methane leaks more frequently, fix the issues more quickly, and with fixes approved by professional engineers. Methane is a major greenhouse gas, and methane leaks like this have been postulated to nearly offset the emissions advantage of natural gas over has over coal when it comes to impacts on global warming.

Link to copy of regulations on EPA website.

Link to Proposed Rule in Federal Register
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Vehicle Standards Rollback Proposal

Open for Comment through October 23, 2018

Reversing EPA's January 2017 finding, Pruitt signed a new determination that the existing model year 2022-2025 greenhouse gas standards for cars, SUVs and light trucks should be revised. Pruitt concluded that the standards "may be too stringent."

In a second step, the Trump Administration August 2 announced a proposed rule to weaken the greenhouse gas (GHG) emissions standards and fuel economy standards. Rather than tightening standards over time like the existing rule, the proposal would freeze standards at model year 2020 levels through model year 2026. The effect would be to would roll back stronger vehicle standards that are already on the books -- EPA's GHG emissions standards for 2021-2025 and NHTSA's fuel economy standards for 2021. The Trump Administration bases the proposed rollback on a new economic analysis purporting to show that the rollback would reduce projected traffic fatalities and be good for the country's overall welfare. The new analysis contradicts the earlier economic analyses of the existing standards and, as detailed below, has been disavowed by EPA's career technical experts. The Trump Administration also suggests that the existing standards would do little to reduce climate change.

In addition to rolling back existing standards, the proposal would withdraw a 2013 EPA waiver that allows California to have stronger vehicle emissions standards than the federal government under three state programs -- the Advanced Clean Car program, Zero Emissions Vehicle mandate, and GHG emission standards for 2021-2025. Federal law requires EPA to grant California waivers

if certain criteria are met, in recognition of California's longstanding need for and leadership in reducing vehicle pollution. EPA has granted California waivers many times over the last 40 years. Federal law also gives other states the ability to adopt California's vehicle standards, and many states have done so. If EPA withdraws California's waiver for greenhouse gas standards and two other programs, other states will no longer be able to adopt them.

Proposed Rule

Docket, including links to Federal Register Notices, primary and secondary supporting documents, and comments submitted to date.
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Affordable Clean Energy Rule (proposed rule)

Comments due by October 30, 2018.

On August 31st, the EPA published the Federal Register notice on the Affordable Clean Energy proposed rule (83 FR 44746). According to Regulations.gov, the EPA' proposed rule contains three distinct actions, including:

- First, EPA is proposing to replace the Clean Power Plan (CPP) with revised emissions guidelines (the Affordable Clean Energy (ACE) rule) that inform the development, submittal, and implementation of state plans to reduce greenhouse gas (GHG) emission from certain EGUs. In the proposed emissions guidelines, consistent with the interpretation described in the proposed repeal of the CPP, the Agency is proposing to determine that heat rate improvement (HRI) measures are the best system of emission reduction (BSER) for existing coal-fired EGUs.
- Second, EPA is proposing new regulations that provide direction to both EPA and the states on the implementation of emission guidelines. The new proposed implementing regulations would apply to this action and any future emission guideline issued under section 111(d) of the Clean Air Act (CAA).
- Third, the Agency is proposing revisions to the New Source Review (NSR) program that will help prevent NSR from being a barrier to the implementation of efficiency projects at EGUs.

Proposed Rule

Docket

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Changes to the Endangered Species Act (Not from the SaveEPAAlums.com web site.

Below is an excerpt from the usfws site,
https://www.fws.gov/endangered/improving_ESA/regulation-revisions.html

The U.S. Fish and Wildlife Service (Service) and the National Oceanic and Atmospheric Administration's National Marine Fisheries Service (NOAA Fisheries) jointly propose revisions to regulations that implement portions of the Endangered Species Act (ESA).

View the proposed Section 4 (listing and critical habitat) rule: Federal Register notice.
View the proposed 4(d) (protective regulations) rule: Federal Register notice .
View the proposed Section 7 (interagency cooperation) rule: Federal Register Notice.

The proposed rules will publish in the Federal Register on July 25, 2018, and will provide detailed information on how the public can submit written comments and information concerning these provisions.

Comments for each notice must be received within 60 days, by September 24, 2018.

All comments will be posted on http://www.regulations.gov. This generally means any personal information provided through the process will be posted.

Similar information can be found on the NOAA site, https://www.fisheries.noaa.gov/action/proposed-rules-revise-endangered-species-act-regulations .

This weekly summary of news about the transition at EPA is sent only to Alumni Association members who have subscribed. If you decide you no longer wish to receive these newsletters, please drop us a note at newsletter@epaalumni.org.
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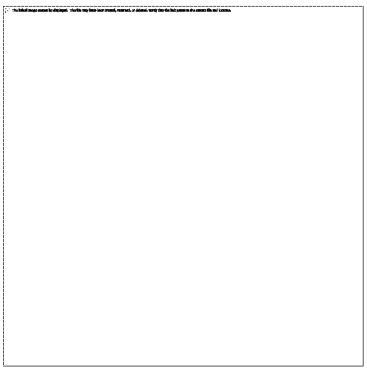

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Message

From: EPA Alumni Association [newsletter@epaalumni.org]
Sent: 10/7/2018 5:42:01 PM
To: Wise, Louise [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=cf7be035da4b45a3a7d45c84c9f4b4a3-LWise]
Subject: Transition Update #90, Week of September 30 - October 6, 2018

Transition Update #90
Week of September 30 - October 6, 2018

Nominees and Appointments

The nominations of Peter Wright (Land and Emergency Management), and Chad McIntosh (International and Tribal Affairs) are still waiting for a vote by the full Senate. The Environment and Public Works Committee held a hearing for them on August 1st, and they were placed on the Senate Executive Calendar on that same date. No votes have been scheduled.

An Environment and Public Works Committee hearing on the nomination of Alexandra Dunn to be the Assistant Administrator for Chemical Safety and Pollution Prevention has not yet been scheduled.

Open Positions

The Washington Post Appointment Tracker shows the following positions at EPA with no one currently nominated or confirmed.

- Administrator
- Assistant Administrator for Environmental Information
- Assistant Administrator for Research and Development
- Assistant Administrator for Administration and Resources Management

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October 1, 2018
EPA chief defends rule rollbacks while at children's health event

Andrew Wheeler defended EPA's environmental policy rollbacks as he argued that the EPA is significantly contributing to children's health at an event celebrating Child Health Day.

He maintained that EPA isn't touching "health-based" standards. He said the proposals to roll back greenhouse gas emissions standards for cars and power plants pertain to energy efficiency.

Wheeler said that the EPA's own figures showing health impacts for the rollbacks - such as that the proposed replacement for the Obama administration's Clean Power Plan would cause 1,400 additional deaths - are based on "co-benefits," or the benefits obtained from reducing pollutants that are not directly regulated, like nitrogen oxides and particulate matter.

The Washington Post report on the roll back of the emission standards discusses them as part of larger effort to narrow what the government counts as benefits when crafting air rules. If adopted, the change would prevent the office from calculating positive health effects -- known as "co-benefits" -- that come from reducing pollutants other than those being targeted.

It is not hard to imagine that if the consideration of co-benefits is not allowed in air rulemaking that it will be far behind in other areas where co-benefits are part of the analysis of the costs and benefits of the rules. This is a least the third rule that is being proposed for rollback based on removing consideration of co-benefits. The Clean Power Rule and the regulation of hydrofluorocarbons (HFCs), a potent greenhouse gas.

It is difficult to understand EPA's decision not to consider "co-benefits." The Trump administration certainly seems willing to consider "co-costs." They want to include additional deaths associated with newer lighter more fuel efficient cars on the road in a rule aimed a gas mileage and climate change (a finding that in and of itself is not well supported).

The Hill: EPA chief defends rule rollbacks while at children's health event

NY Times: Trump Administration Prepares a Major Weakening of Mercury Emissions Rules

Washington Post: In rollback of mercury rule, Trump could revamp how government values human health

Huffington Post: Mercury Can Cause Brain Damage In Kids. The EPA Wants To Weaken Rules On Its Emissions

The Hill: Trump moves to target EPA mercury regulation

October 1, 2018
Even the Trump Administration Acknowledges That Global Temperatures Are Rising to Catastrophic Levels

Mother Jones' article begins with the following paragraph:

> Predictions of climate change's devastating impact-coastal flooding, demonstrable sea level rise, and more extreme weather events, to name a few-are so ubiquitous it becomes nearly impossible to fully understand their potentially catastrophic implications. ***But an acknowledgement last week that global temperatures may rise by a shocking 7 degrees by 2100 was startling for many reasons, especially because the Trump administration was the source of the estimate.***

This forecast was buried in a report by the National Highway Traffic Safety Administration to justify Trumpp's decision to roll back fuel efficiency standards. Using a scenario in which the government never adjusted the original fuel efficiency benchmarks set by Congress in 1975, global temperatures would rise on average by 6.271 degrees Fahrenheit.

The article goes on to say that the estimate was not included to justify the importance of fighting climate change, but rather ***"Just the opposite: The analysis assumes the planet's fate is already sealed."***

Mother Jones: Even the Trump Administration Acknowledges That Global Temperatures Are Rising to Catastrophic Levels

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October 1, 2018
Citizens Have Put Renewable Energy on This Year's Ballots

The administration has been rolling back environmental regulations and opening millions of acres of public land to oil and gas drilling.The Interior Department has announced plans to

gut an Obama-era methane pollution rules, as has EPA. giving natural gas producers more leeway to emit the powerful greenhouse gas.

However, twenty-four states, including most Western ones, ballot initiatives, which allow citizens new laws and regulations to a vote in general elections. Nationwide, 64 citizen-driven initiatives will appear on state ballots in November, and in the West, many aim to encourage renewable energy development-and reduce reliance on fossil fuels. Here are some of them.

Arizona: Proposition 127, known as the Renewable Energy Standards Initiative, would require electric utilities to get half of their power from renewable sources like wind and solar-though not nuclear-by 2030.

Colorado: Proposition 112-the Safer Setbacks for Fracking Initiative-to a vote this year. It would prohibit new oil and gas wells and production facilities within 2,500 feet of schools, houses, playgrounds, parks, drinking water sources and more.

Nevada: Question 6, the Renewable Energy Promotion Initiative, would require utilities to get 50 percent of their electricity from renewable sources by 2030, faces little formal opposition.

Question 3, The Energy Choice Initiative, which is on the ballot for the second time because it amends the constitution, is more controversial. It would allow consumers to choose who they buy power from. Big energy consumers want the freedom to buy cheaper power on the open market without penalty and support it. Environmental organizations say the initiative threatens clean energy development. NV Energy, the regulated monopoly that provides 90 percent of Nevada's electricity, has several solar projects planned but has said it would abandon some of these projects if the initiative passes due to costs.

Washington: Initiative 1631 would create funding for investments in clean energy and pollution programs through a "carbon fee" paid for by high carbon emitters like utilities and oil companies. If it passes, Washington would be the first state with such a fee.

Mother Jones: Citizens Have Put Renewable Energy on This Yearâ€™s Ballots

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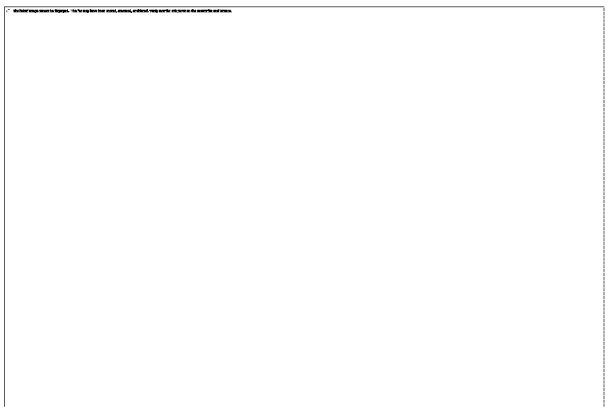
October 1, 2018
More Proof Trump EPA 'Working for Coal Millionaires' as EPA Plan to Weaken Mercury Regulations Revealed



The subtitle of the Common Dreams article is ***"If anyone needed further proof that Wheeler and Wehrum are still working for coal millionaires, this is it."***

The rollback was included on a "wish list" that coal CEO Robert Murray passed along to Energy Secretary Rick Perry shortly after President Donald Trump took office in 2017, due to the costs of compliance for the coal industry.

The article also notes the commonly reported fact that Andrew Wheeler worked for Murray's company as a lobbyist, and Bill Wehrum, EPA's AA for Air and Radiation, represented energy companies as a lawyer.

The existing rule costs the $386 billion electric power industry about $9.6 billion annually (less than 2.5% of revenues), but it saves a total of $80 billion per year in healthcare costs associated with health conditions caused by pollution. Additionally, the rules saved another $6 billion in health benefits associated with the reduction of mercury. According to the New York Times, the existing rule has cut mercury emissions by 70% since

EPA's rationale for rolling back the existing rule is their decision not to consider "co-benefits."

Common Dreams: More Proof Trump EPA 'Working for Coal Millionaires' as EPA Plan to Weaken Mercury Regulations Revealed

EWG: Trump's EPA Plans to Gut Rules That Have Sharply Cut Mercury Pollution

NY Times: Trump Administration Prepares a Major Weakening of Mercury Emissions Rules

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October 1, 2018
NGOs petition US EPA to require asbestos reporting

Six NGOs have petitioned the US EPA to amend its TSCA chemical data reporting rule (CDR) to

- require that asbestos be reported under the CDR rule, in order to collect information on its current import and use. The substance is currently exempted from reporting as it is 'naturally occurring';
- lower the reporting threshold, eliminate exemptions for impurities and its presence in articles, and require processors to report "in order to assure that EPA has the information on asbestos necessary to meet its TSCA responsibilities";
- block such reports from being protected as confidential business information.

The TSCA section 21 petition says that additional data is necessary to support the substance's ongoing risk evaluation. "Without adequate information on ongoing importation and use of asbestos and asbestos-containing products, the risk evaluation will fail to provide a meaningful picture of the threat that asbestos poses to public health and citizens will be in the dark about exposure to asbestos in their communities and places of employment."

Chemical Watch: NGOs petition US EPA to require asbestos reporting

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October 1, 2018

Interior Department follows EPA lead on 'open science'

E&E News carried an article on the Department of Interior's decision to implement an 'open science' policy similar to that being proposed for use at EPA. The policy, Departmemt of Interior Order 3369, was signed by Deputy Secretary David Bernhardt on September 28th. The arguments for and against the Interior Department order seem similar to those for and against the EPA order.

However, the impacts might seem wider for the EPA. The example for the interior department is the exactly location of native american sites generally not made available to the public, and if a decision affects a site, there cannot be an independent review if it requires that the data be made available to the public. In the EPA case, it is personal information on patients or individuals involved in the studies that might be the basis for disqualifying the study from use as the information cannot be release.

E&E News: Agency follows EPA lead on 'open science'

The Scientist: Interior Department Wants Stricter Data Standards in Conservation Decisions

The Hill: Interior Dept. implements new science policy
DOI Order 3369: Promoting Open Science

October 2, 2018
Trump Administration working to weaken radiation regulations

The subheader for this NBC News article is "As recently as this March, the EPA's online guidelines for radiation effects advised: "Current science suggests there is some cancer risk from any exposure to radiation."

The government's current, decades-old guidance says that any exposure to harmful radiation is a cancer risk. And critics say the proposed change could lead to higher levels of exposure for workers at nuclear installations and oil and gas drilling sites, medical workers doing X-rays and CT scans, people living next to Superfund sites and any members of the public who one day might find themselves exposed to a radiation release.

EPA's online guidance was edited in July to emphasize the low individual odds of cancer: "According to radiation safety experts, radiation exposures of ...100 millisieverts usually result in no harmful health effects, because radiation below these levels is a minor contributor to our overall cancer risk," the revised policy says.

Edward Calabrese, a toxicologist at the University of Massachusetts argues that smaller exposures of cell-damaging radiation and other carcinogens can serve as stressors that activate the body's repair mechanisms and can make people healthier. They compare it to physical exercise or sunlight.

The U.S. Food and Drug Administration, which regulates electronic devices that emit radiation, advises, broadly, that a single CT scan with a dose of 10 millisieverts may increase risks of a fatal cancer by about 1 chance in 2,000.

Note: 100 millisievert is equal to 10 rem. Background radiation in the United States is about 300 millirem (0.3 rem) per year. Currently, the average person in the United States gets

about an additional 300 millirem from medical sources (xrays, nuclear medicine, etc.). The Department of Energy (DOE) has a total effective dose limit for workers of 5 rem per year, and most contractors at DOE sites have limits that are about 25% of that limit. From 2012 to 2016, none of the over 120,000 monitored individuals (with over 10,000 with a measurable dose) at DOE sites nationwide received more than 2 rem (20 millisieverts). The TOTAL dose to all workers runs around 700 person rem, or the dose that would be allowed to 70 people under the proposed EPA standard.

The DOE limit for dose to members of the public per DOE Order 458.1, Radiation Protection of the Public and the Environment, is just 100 millirem per year (one millisievert). A bit lower that 10 Sievert, like one 10,000th of what EPA is considering.

NBC News: Trump administration working to weaken EPA radiation regulations

AP: EPA-Radiation Rollback story

NY Magazine's Daily Intelligencer: EPA: Actually, a Little Radiation Might Be Good For You

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October 2, 2018
Getting Radiation Poisoning to Own the Libs
Radiation Is Good For You may be the hottest take of all time.

In a somewhat snarky article, Esquire takes on the Trump Administration's attitude towards science.

> It is a vile calumny to call the current administration "anti-science." A low slander, it is-a naked libel and an outright asparagus, as Louie Gohmert would put it. This administration is all about science, especially when that science can be cited to make a case for deregulating something that the administration* wants to deregulate. Then, it's all about science. **Of course, the science it is all about is not considered valid by, you know, scientists,** but, hey, how do you think The Most Awesome Man Of Television makes a living, anyway?

The article ends noting that the new rules are supported by Steven Milloy, who is known for challenging widely accepted ideas about anthropogenic climate change and the health risks of tobacco. Of course, Milloy also attacked the Pope (over climate change).

Read at your own risk.

Ok, maybe it wasn't "somewhat snarky." "Full on snarky" is more accurate.

Esquire: Getting Radiation Poisoning to Own the LibsRadiation Is Good For You may be the hottest take of all time.

October 2, 2018
Ex-Koch engineer to lead EPA office on scientific research

No, the Trump administration has not nominated an Assistant Administrator for Research and Development. ORD has been without a permanent Assistant Administrator for 6 years now.

However, The Hill reports that EPA has confirmed that David Dunlap will head the EPA's Office of Research and Development (ORD) as a "deputy chief", a role that will not need Senate confirmation'

Dunlap worked for Koch Industries for more than eight years, with his employment there ending in August. He was the Director of Policy and Regulatory affairs at Koch Industries according to his LinkedIn page.

His LinkedIn page also says he started the new role at EPA in September. The EPA's web site shows him as the Deputy Assistant Administrator for Research & Development. Dunlap replaces Richard Yamada, who was appointed to the role in June 2017. The EPA would not comment on the reason for Yamada's departure.

The Hill: Ex-Koch engineer to lead EPA office on scientific research

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October 3, 2018
Making 'Fringe' Scientist Who Argues Exposure Good for People a Key Witness, Trump's EPA Moves to Roll Back Radiation Safety Rules

EPA sent toxicologist Edward Calabrese-who has argued that loosening radiation regulations could have positive health effects on humans while also saving money for businesses that currently work to limit exposure, as its lead witness to testify before the Senate Environment and Public Works Committee.

Cindy Folkers, a radiation and health hazard specialist at Beyond Nuclear, said in a statement that "The agency is ignoring scientific evidence by instead claiming a little radiation is good for you. This is clearly an attempt to save industry money at the expense of women and children's health."

Calabrese's views have been "generally dismissed by the great bulk of scientists," physicist Jan Beyea told the Associated Press.

Commondreams.org: Making 'Fringe' Scientist Who Argues Exposure Good for People a Key Witness, Trump's EPA Moves to Roll Back Radiation Safety Rules

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October 3, 2018
Oversight of the Environmental Protection Agency's Implementation of Sound and Transparent Science in Regulation

The Senate Committee on Environment and Public Works held a hearing on the "secret science" rule implementation on October 3rd (Wednesday) at 2:15 pm Washington DC time.

The link below leads to a video recording of the hearing for anyone interested. The actual hearing begins at 22:30.

Another article below (Is a little radiation good for you? . . .) has a little on the content of their testimony.

Link to video of hearing:

October 3, 2018
Climate scientists are struggling to find the right words for very bad news

In Incheon, South Korea, this week, representatives of over 130 countries and about 50 scientists are trying to reach agreement on an all-important report on this question: ***What chance does the planet have of keeping climate change to a moderate, controllable level?***

In a huge peer review process, they are trying to reach consensus on what it would mean - and what it would take - to limit the warming of the planet to just 1.5 degrees Celsius, or 2.7 degrees Fahrenheit, when 1 degree Celsius has already occurred and greenhouse gas emissions remain at record highs.

The new report will feed into a process called the "Talanoa Dialogue," in which parties to the Paris agreement begin to consider the large gap between what they say they want to achieve and what they are actually doing.

From what this article says, and it is worth reading carefully, there is only a very small chance that the world can keep warming below 1.5 degree C, and the prospects for the world are pretty dire if we don't. Maybe even if we do.

Washington Post: Climate scientists are struggling to find the right words for very bad news

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October 4, 2018

Wheeler to staff: Sound alarms about health risks

In an article still behind their firewall, E&E News reported on Acting Administrator Andrew Wheeler's "elevation memo" urging agency staff to alert senior leaders when they come across grave environmental and public health risks. Wheeler's memo (linked below) says that all EPA employees have an important role in identifying and addressing those risks under the agency's authority.

"I am affirming, as past Administrators have, the importance of elevating human health and environmental risks so that we can properly assess and respond at appropriate policy and government levels in a timely and effective manner,"

Wheeler noted that EPA set up its original "elevation" policy in January 2016. Then-Administrator Gina McCarthy drafted that memo in the aftermath of EPA's sluggish response to the drinking water crisis in Flint, Mich

E&E News: Wheeler "Elevation Memo"

October 4, 2018
Ex-EPA chief: Trump will pollute your air and make you pay for it

CNN published an article by Christine Todd Whitman that says that with the Trump administration taking steps to roll back America's clean-cars standards, states are preparing for battle over states' authority to protect their citizens from dangerous pollution, and California regulators have approved new measures to defend the state's vehicle emissions standards.

Ms. Whitman writes that we should all be rooting for these regulators. She further writes that "Since the election of Donald Trump, the Environmental Protection Agency (EPA) has been guided by appointed officials who have fundamentally failed to uphold its mission: to protect public health and the environment. From undermining and ignoring established science, to rolling back lifesaving public health protections, it's become abundantly clear that this administration has no intention of upholding the core mission of the EPA."

She ends with this:

> While the public hearings may be over, the public can still submit comments into the federal register until October 23, and I'd encourage all to do so. My hope is that with this public input fresh in their minds, Trump and EPA officials will reverse their attempt to roll back these lifesaving standards.
>
> After all, who really benefits from weakening our clean-car standards? The answer is certainly not our environment or American families.

CNN: Ex-EPA chief: Trump will pollute your air and make you pay for it

---

October 4, 2018
Trump likely to make pipeline push next year, aide says

The Hill reports that the the Director of the President's National Economic Council, Larry Kudlow, says that Trump is likely to make a renewed push to permit and build oil and natural gas pipelines next year.

New York and other northeastern states have been some of the most active in working to block pipeline infrastructure, arguing that construction and the lines themselves would be environmentally disastrous.

The Hill: Trump likely to make pipeline push next year, aide says

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October 4, 2018
Is a little radiation good for you? Controversial theory pops up in Senate hearing on EPA transparency plan

In Senate hearings on the the EPA's transparency rule, on of the three witnesses was University of Massachusetts Amherst toxicologist Edward Calabrese, who is a promoter of the controversial theory of hormesis - that small doses of toxic agents can be healthful. It is a theory that is not supported by a wide majority of scientists in the world.

Calabrese was not a vocal proponent of the transparency rule, saying "It's very difficult to ever define what is a truly replicated study."

The second witness was not a scientist but an economist, Robert Hahn of Georgetown University in Washington, D.C., who argued the agency should be more transparent about how it uses scientific studies.

The third witness was Rush Holt, CEO of the American Association for the Advancement of Science, which publishes *Science*Insider, was critical of the EPA proposal, which is opposed by a wide range of science groups. It represents an "insidious dodge," he said, and if adopted will weaken the agency's ability to protect people from the risk of toxic pollutants.

Sciencemag: Is a little radiation good for you? Controversial theory pops up in Senate hearing on EPA transparency plan

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October 5, 2018
PFAS Hearings in Kalamazoo

On Tuesday, October 2nd, EPA announce that they would hold a public hearing on Friday, October 5th in Kalamazoo that would be led Cathy Stepp, EPA Region 5 RA, Peter Grevatt, director of the EPA's Office of Ground Water and Drinking Water, and Carol Isaacs, director of Michigan's PFAS Action Response Team. The hearing was
will be limited to input from 18 invited local officials , with no place for public input on the agenda.

A search on Google did not turn up any articles written after the hearing. However, E&E News had an article behind their firewall that noted that the public was angry that they were not allowed any opportunity to give input at this EPA "roundtable."

MLive: EPA sets short-notice meeting on PFAS in Michigan

September 5, 2018
GOP senators ask EPA to block states that have 'hijacked' rule to stop fossil fuel production

In five Republican senators asked EPA Administrator Andrew Wheeler to issue revised guidance on the use of Section 401 of the Clean Water Act.

Section 401 requires that any industry that is applying for a federal permit to allow them to discharge into a water system must also obtain a certification from the state in which the discharge is coming from to ensure they are complying with water quality standards.

The lawmakers letter said the current statute is being used to "fight" fossil fuel projects rather than protect water quality. They did not offer examples of projects that might have been unfairly struck down under the provision.

This is not the first example of Barrasso going after this provision of the Clean Water Act. The second link below is to a story in the Casper (Wy) Star Tribune from August 16th about Barrasso's attempt to amend the CWA because of the us by states of the Section 401 requirements to block coal development projects.

So is the Republican party in favor of states rights? Or are they only in favor it states rights when they are used as an argument to block Federal requirements that they don't like?

The Hill: GOP senators ask EPA to block states that have 'hijacked' rule to stop fossil fuel production

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October 5, 2018
To topple climate rules, officials erase air pollutants

EPA is considering whether it will count the co-benefits that come along with cutting greenhouse gases, like the health gains from reducing particulate matter. Narrowing the scope of health benefits achieved in climate-focused regulations would sharply curb the public health estimates used to justify their existence, according to experts.

It may also complicate future efforts to craft climate policy. "It is just a way to make these rules look less cost-effective," said Janet McCabe, acting EPA air chief under former President Obama.

Using co-benefits to craft regulations has been an important part of EPA's job of protecting public health for decades, said William Reilly, the EPA administrator under former President George H.W. Bush. Considering co-benefits can dramatically expand the scope of public health protections included in a regulation, he said.

The example used in the article is smoking. If you quit smoking to avoid getting lung cancer, should you ignore the other benefits you get such as reductions in risk of heart attacks and stroke, reduced risk of other cancers (colon, bladder, cervix, etc.), and more?

E&E News: To topple climate rules, officials erase air pollutants

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October 5, 2018
Kids' case tests 'Hail Mary' climate argument

The "Hail Mary" is that twenty-one young Americans will go to trial later this month to argue that the government has infringed on their constitutional right to a safe climate. Their argument draws in part from the idea of public trust, a centuries-old principle dating to ancient Rome and British common law. The plaintiffs say that the governments reaction to climate change is eroding their constitutionally protected rights, and also the safeguards of the public trust doctrine.

arguing that the government's reaction to and fueling of climate change has eroded both rights of theirs protected under the Constitution and the safeguards of the public trust doctrine. The climate, they argue, must be protected for current and future generations.

The trial in Juliana v. United States slated to begin Oct. 29 in federal court in Oregon could be a milestone in climate law, if if they are successful, it could be a milestone in environmental regulation.

Mary Wood, a University of Oregon law professor, took up the issue in the wake of Hurricane Katrina, wondering whether the public trust doctrine would be useful in addressing man-made climate change.

"When I actually read the science that emerged around the time of Katrina, especially the science of Dr. James Hansen, I found it mind-blowing," she said. "I thought, ***'There's so little time left before irreversible tipping points, statutory law is never going to work in time.'"***

She set out to draft a legal plan that would force the government to act ***swiftly*** to curb heat-trapping emissions, directly because of its obligation to protect the atmosphere as a shared public resource.

Under the concept of "atmospheric trust," the United States and other governments would be required to act as trustees of the atmosphere, with members of the public as beneficiaries.

Bound to prevent major harm to the resource, government officials would use the best available science to define those limits and then work to meet the obligation.

E&E News: Kids' case tests 'Hail Mary' climate argument

October 5, 2018
Former EPA official Richard Yamada lands at Pentagon

The Pentagon has confirmed that Dr. Richard Yamada has joined the Strategic Capabilities Office.

At EPA, Yamada was deputy assistant administrator in its Office of Research and Development, where he worked on several of the agency's more controversial moves under the Trump administration, including EPA's proposal to limit what scientific data the agency uses to develop its regulations and former Administrator Scott Pruitt's now-defunct "red-team, blue-team" exercise to debate climate science.

E& Former Trump EPA official lands at Pentagon

New Jersey Department of Environmental Protection Jobs

If anyone you know MAY be interested in joining NJDEP over the next year or so, they should apply now for EVERY position for which THEY qualify.

https://www.nj.gov/dep/jobs/

In fact, many NJ agencies are now hiring - we finally have a budget & all Cabinet officers have finally been confirmed. State jobs are posted on the NJ job website. I know that BPU & EDA have postings & ALL of the application deadline are VERY SOON!

Jeanne Fox, Region II RA 1994-01
jeanne.fox52@gmail.com
973-271-0500

EPA Alumni Association Job Center - Our new Job Center (linked below) features jobs that employers have listed that are specifically aimed at current EPA employees and alumni. In addition, we provide links to over 1000 external job banks that are offering environmentally-relevant jobs. Please help us make our Job Center a success by:

- Telling prospective employers about the opportunity to list their jobs for free,
- Telling current employees and alumni about the Job Center and asking them to help spread the word.

Link to Job Center Web Site

Major EPA Rules Open for Comment

Save EPA (http://www.saveepaalums.info/) is tracking significant rules open for public comment. They also have, for each of the rules, prepared suggested comments to help a commenter prepare their own comments and submit them to EPA. They also have prepared a "A PRACTICAL GUIDE FOR RESISTING THE TRUMP DE-REGULATORY AGENDA," that can be found at at this link:
A PRACTICAL GUIDE FOR RESISTING THE TRUMP DE-REGULATORY AGENDA

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Coming soon: Oil and Natural Gas Sector: Emission Standards for New, Reconstructed, and Modified Sources Reconsideration

Andrew Wheeler signed the rules on September 11th, but they have not yet been published in the Federal Register. The public comment period will be 60 days after publication.

Link to copy of regulations on EPA website.
==========
Vehicle Standards Rollback Proposal

Open for Comment through October 23, 2018

Reversing EPA's January 2017 finding, Pruitt signed a new determination that the existing model year 2022-2025 greenhouse gas standards for cars, SUVs and light trucks should be revised. Pruitt concluded that the standards "may be too stringent."

In a second step, the Trump Administration August 2 announced a proposed rule to weaken the greenhouse gas (GHG) emissions standards and fuel economy standards. Rather than tightening standards over time like the existing rule, the proposal would freeze standards at model year 2020 levels through model year 2026. The effect would be to would roll back stronger vehicle standards that are already on the books -- EPA's GHG emissions standards for 2021-2025 and NHTSA's fuel economy standards for 2021. The Trump Administration bases the proposed rollback on a new economic analysis purporting to show that the rollback would reduce projected traffic fatalities and be good for the country's overall welfare. The new analysis contradicts the earlier economic analyses of the existing standards and, as detailed below, has been disavowed by EPA's career technical experts. The Trump Administration also suggests that the existing standards would do little to reduce climate change.

In addition to rolling back existing standards, the proposal would withdraw a 2013 EPA waiver that allows California to have stronger vehicle emissions standards than the federal government under three state programs -- the Advanced Clean Car program, Zero Emissions Vehicle mandate, and GHG emission standards for 2021-2025. Federal law requires EPA to grant California waivers if certain criteria are met, in recognition of California's longstanding need for and leadership in reducing vehicle pollution. EPA has granted California waivers many times over the last 40 years. Federal law also gives other states the ability to adopt California's vehicle standards, and many states have done so. If EPA withdraws California's waiver for greenhouse gas standards and two other programs, other states will no longer be able to adopt them.

Proposed Rule

Docket, including links to Federal Register Notices, primary and secondary supporting documents, and comments submitted to date.
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Affordable Clean Energy Rule (proposed rule)

Comments due by October 30, 2018.

On August 31st, the EPA published the Federal Register notice on the Affordable Clean Energy proposed rule (83 FR 44746). According to Regulations.gov, the EPA' proposed rule contains three distinct actions, including:

- First, EPA is proposing to replace the Clean Power Plan (CPP) with revised emissions guidelines (the Affordable Clean Energy (ACE) rule) that inform the development, submittal, and implementation of state plans to reduce greenhouse gas (GHG) emission from certain EGUs. In the proposed emissions guidelines, consistent with the interpretation described in the proposed repeal of the CPP, the Agency is proposing to determine that heat rate improvement (HRI) measures are the best system of emission reduction (BSER) for existing coal-fired EGUs.
- Second, EPA is proposing new regulations that provide direction to both EPA and the states on the implementation of emission guidelines. The new proposed implementing regulations would apply to this action and any future emission guideline issued under section 111(d) of the Clean Air Act (CAA).
- Third, the Agency is proposing revisions to the New Source Review (NSR) program that will help prevent NSR from being a barrier to the implementation of efficiency projects at EGUs.

Proposed Rule

Docket

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Changes to the Endangered Species Act (Not from the SaveEPAAlums.com web site.

Below is an excerpt from the usfws site,
https://www.fws.gov/endangered/improving_ESA/regulation-revisions.html

The U.S. Fish and Wildlife Service (Service) and the National Oceanic and Atmospheric Administration's National Marine Fisheries Service (NOAA Fisheries) jointly propose revisions to regulations that implement portions of the Endangered Species Act (ESA).

View the proposed Section 4 (listing and critical habitat) rule: Federal Register notice.
View the proposed 4(d) (protective regulations) rule: Federal Register notice .
View the proposed Section 7 (interagency cooperation) rule: Federal Register Notice.
The proposed rules will publish in the Federal Register on July 25, 2018, and will provide detailed information on how the public can submit written comments and information concerning these provisions.

Comments for each notice must be received within 60 days, by September 24, 2018.

All comments will be posted on http://www.regulations.gov. This generally means any personal information provided through the process will be posted.

Similar information can be found on the NOAA site, https://www.fisheries.noaa.gov/action/proposed-rules-revise-endangered-species-act-regulations .

This weekly summary of news about the transition at EPA is sent only to Alumni Association members who have subscribed. If you decide you no longer wish to receive these newsletters, please drop us a note at newsletter@epaalumni.org.
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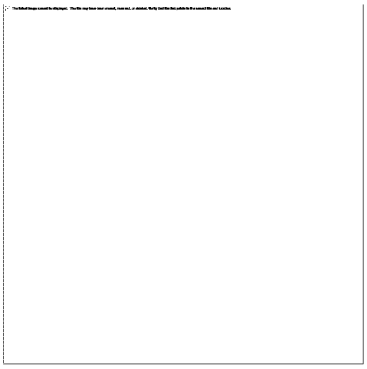

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Message

From: Jackson, Ryan [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=38BC8E18791A47D88A279DB2FEC8BD60-JACKSON, RY]
Sent: 10/19/2018 5:28:53 PM
To: Dunlap, David [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=591eb15a268249dda0c05a7451f765c3-Dunlap, Dav]; Bolen, Brittany [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=31e872a691114372b5a6a88482a66e48-Bolen, Brit]; Woods, Clint [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=bc65010f5c2e48f4bc2aa050db50d198-Woods, Clin]
Subject: FW: new paper
Attachments: Muller-Nobel Prize-1.pdf

From: Edward Calabrese [mailto:edwardc@schoolph.umass.edu]
Sent: Friday, October 19, 2018 12:35 PM
To: Jackson, Ryan <jackson.ryan@epa.gov>
Subject: new paper

Ryan:

See this new paper.....it gets to the heart of the start of the LNT model. It is a short and interesting read.
Please share with your colleagues.

Ed

Message

From: Konkus, John [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=555471B2BAA6419E8E141696F4577062-KONKUS, JOH]
Sent: 9/14/2018 3:08:47 PM
To: Woods, Clint [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=bc65010f5c2e48f4bc2aa050db50d198-Woods, Clin]
Subject: RE: AP requesting interview with EPA radiation-protection officials regarding LNT standards vs. threshold, hormetic standards

She has this now and seems satisfied with the answer.

From: Woods, Clint
Sent: Friday, September 14, 2018 10:32 AM
To: Konkus, John <konkus.john@epa.gov>
Subject: RE: AP requesting interview with EPA radiation-protection officials regarding LNT standards vs. threshold, hormetic standards

Here's my contribution:

Deliberative Process / Ex. 5

From: Konkus, John
Sent: Friday, September 14, 2018 9:27 AM
To: Woods, Clint <woods.clint@epa.gov>
Subject: FW: AP requesting interview with EPA radiation-protection officials regarding LNT standards vs. threshold, hormetic standards

See below...

From: Jones, Enesta
Sent: Friday, September 14, 2018 8:45 AM
To: Konkus, John <konkus.john@epa.gov>
Cc: Press <Press@epa.gov>
Subject: Re: AP requesting interview with EPA radiation-protection officials regarding LNT standards vs. threshold, hormetic standards

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

On Sep 14, 2018, at 8:30 AM, Konkus, John <konkus.john@epa.gov> wrote:

Ellen: We're working on something more robust for you. Should have it this morning.

John

Sent from my iPhone

On Sep 14, 2018, at 5:52 AM, Knickmeyer, Ellen <EKnickmeyer@ap.org> wrote:

> Hi, Enesta,
>
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>
> When does that entry date from? When was it revised and what part was revised?
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> Your other statement provides no information at all, except to indicate the EPA may decide to reverse its longstanding policy on radlation exposure without any advance notice to the public it is considering doing that.
>
> Can you answer my questions and provide an EPA expert to speak to? These are non-answers and public silence on important public health questions for Americans.
>
> Best,
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>
> Sent from my iPhone
>
> On Sep 13, 2018, at 6:01 PM, Jones, Enesta <Jones.Enesta@epa.gov> wrote:
>
>> Ellen,
>> I inadvertently cut off the end of our response. Here it is:
>>
>> Find more information here: https://www.epa.gov/radiation/radiation-health-effects
>>
>> Ellen,
>>
>> "EPA is currently reviewing the more than 590,000 comments on the Strengthening Transparency in Regulatory Sciences proposal. The EPA will determine a timeline for a decision after it has more fully assessed the comments." — **EPA spokesperson**

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Hello – wanted to also ask, is this still EPA policy?
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"Radiation protection, like the regulation of other carcinogenic agents, is—in the absence of compelling evidence to the contrary—predicated on the linear, no-threshold (LNT) hypothesis, which assumes that the risk of cancer due to a low dose exposure is proportional to dose, with no threshold."

"Given the current state of the science, the consensus positions of key scientific and governmental bodies, as well as the conservatism and calculational convenience of the LNT assumption, it is unlikely that EPA will modify this approach in the near future."

Best,
Ellen

From: Knickmeyer, Ellen
Sent: Thursday, September 13, 2018 10:06 AM
To: 'press@epa.gov' <press@epa.gov>; 'Konkus, John' <konkus.john@epa.gov>
Cc: 'edwards.jonathan@epa.gov' <edwards.jonathan@epa.gov>; 'veal.lee@epa.gov' <veal.lee@epa.gov>; 'griggs.john@epa.gov' <griggs.john@epa.gov>
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Dr. Edward J. Calabrese, Professor, Environmental Health Sciences, University of Massachusetts: "The proposal represents a major scientific step forward by recognizing the widespread occurrence of non-linear dose responses in toxicology and epidemiology for chemicals and radiation and the need to incorporate such data in the risk assessment process

And as with last year's EPA guidance last year that emergency responders can safely tolerate "low doses" of radiation

https://www.epa.gov/sites/production/files/2017-07/documents/pags_comm_tool_p9.pdf

I need to finish the story by Tuesday afternoon. Can you please make an EPA radiation-protection or analytics senior official available to talk to me by Monday on the topic? Where has the EPA moved since April on the LNT vs. threshold vs. hormesis argument,

have there been any more moves within EPA away from LNT, and what are EPA radiation officials' thoughts on the topic?

All best,
Ellen Knickmeyer

Ex. 6

eknickmeyer@ap.org
ellen.knickmeyer@gmail.com

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Sent: 9/14/2018 2:33:03 PM
To: Woods, Clint [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=bc65010f5c2e48f4bc2aa050db50d198-Woods, Clin]
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From: Jackson, Ryan [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=38BC8E18791A47D88A279DB2FEC8BD60-JACKSON, RY]
Sent: 5/28/2018 1:46:27 PM
To: Woods, Clint [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=bc65010f5c2e48f4bc2aa050db50d198-Woods, Clin]; Yamada, Richard (Yujiro) [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=4c34a1e0345e4d26b361b5031430639d-Yamada, Yuj]; Bolen, Brittany [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=31e872a691114372b5a6a88482a66e48-Bolen, Brit]
Subject: Fwd: LNT
Attachments: Environ. Pollut. Muller-Mech-1.pdf; ATT00001.htm

Ryan Jackson
Chief of Staff
U.S. EPA
Personal Phone / Ex. 6

Begin forwarded message:

From: Edward Calabrese <edwardc@schoolph.umass.edu>
Date: May 28, 2018 at 5:28:48 AM EDT
To: "jackson.ryan@epa.gov" <jackson.ryan@epa.gov>
Subject: LNT

Ryan:

See the attached new paper on LNT....it tells the entire historical story in this one integrated paper. It represents an amazing historical unfolding, yet with enormous implications for EPA risk assessment. It shows that the LNT that is used all over the world and led by the US is based on a myth, no, actually a hoax. This story needs to be read and understood by the Agency, by Congress and the media. Please share this paper widely within the Agency and elsewhere.

By the way, I did send in my Comments to the EPA proposal last week, two days before the paper was finally published.

Ed Calabrese

Message

From: Konkus, John [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=555471B2BAA6419E8E141696F4577062-KONKUS, JOH]
Sent: 10/3/2018 9:00:33 PM
To: Abboud, Michael [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=b6f5af791a1842f1adcc088cbf9ed3ce-Abboud, Mic]
CC: Woods, Clint [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=bc65010f5c2e48f4bc2aa050db50d198-Woods, Clin]
Subject: Re: Questions on risk models and EPA's proposal on scientific studies-on deadline

Ok

Sent from my iPhone

On Oct 3, 2018, at 4:55 PM, Abboud, Michael <abboud.michael@epa.gov> wrote:

I mean, I think this really speaks for itself we should just send it.

Deliberative Process / Ex. 5

From: Konkus, John
Sent: Wednesday, October 3, 2018 4:52 PM
To: Woods, Clint <woods.clint@epa.gov>
Cc: Abboud, Michael <abboud.michael@epa.gov>
Subject: Re: Questions on risk models and EPA's proposal on scientific studies-on deadline

Michael can you package this for Ellen?

Sent from my iPhone

On Oct 3, 2018, at 4:47 PM, Woods, Clint <woods.clint@epa.gov> wrote:

Deliberative Process / Ex. 5

On Oct 3, 2018, at 4:36 PM, Konkus, John <konkus.john@epa.gov> wrote:

Thoughts?

From: Knickmeyer, Ellen [mailto:EKnickmeyer@ap.org]
Sent: Wednesday, October 3, 2018 4:25 PM
To: Press <Press@epa.gov>; Konkus, John <konkus.john@epa.gov>
Subject: RE: Questions on risk models and EPA's proposal on scientific studies-on deadline

Hi, John,
Dr. Calabrese just now and the independent physicists I talked to said that the J and U shaped models that the EPA proposal cites refers only to hormesis, which is the idea that cancer-causing agents are actually beneficial at lower doses.

Can you comment on that, specifically? Would the proposal require regulators to consider that hazardous substances at low doses are healthy, and if not, what does the reference to U and J shaped models mean?
Thanks,
Ellen

From: Knickmeyer, Ellen
Sent: Wednesday, October 03, 2018 8:25 AM
To: Press <Press@epa.gov>; Konkus, John <konkus.john@epa.gov>
Subject: Questions on risk models and EPA's proposal on scientific studies

(Sent a few minutes ago but it looks like it may have hung up in my email – repeating again just in case.)

Hi, John.
I'm sure we will be writing more on the EPA's proposal on scientific studies and wanted to nail down a couple points from the proposal. Please bear with me, because this is technical, and we have to be able to phrase it for readers in the simplest way possible.

One question – the EPA proposal says there is "growing empirical evidence of non-linearity in the concentration response function for specific pollutants and health effects."

Can you please send along and specify what studies, pollutants, and health effects are being cited there?

Second question – the EPA proposal below says in considering risks of low doses the EPA should look not just at linear models, but threshold, U-shaped, and bell-shaped models, and consider "model uncertainty" as a default.

The physics academics I talk to, and Dr. Calabrese in his writing, say U-shaped and J-shaped curves apply only to models showing beneficial effects from lower doses of harmful substances, or the "hormesis" that Calabrese writes about for radiation and toxins. Can you confirm that, or if not what does that refer to, specifically?

EPA Proposal:
In addition, this proposed regulation is designed to increase transparency of the assumptions underlying dose response models. As a case in point, there is growing empirical evidence of non-linearity in the concentrationresponse function for specific pollutants and health effects. The use of default models, without consideration of alternatives or model uncertainty, can obscure the scientific justification for EPA actions. To be even more transparent about these complex relationships, EPA should give appropriate consideration to high quality studies that explore: A broad class of parametric concentration-response models with a robust set of potential confounding variables; nonparametric models that incorporate fewer assumptions; various threshold models across the exposure range; and spatial heterogeneity. EPA should also incorporate the concept of model uncertainty when needed as a default to optimize low dose risk estimation based on major competing models, including linear, threshold, and U-shaped, Jshaped, and bell-shaped models.

Calabrese on "hormesis" in radiation and chemicals:
Enter an alternative model, which claims that the fundamental shape of the dose–response curve is neither linear nor threshold, but rather U-shaped. This so-called hormesis model—after the Greek word 'to excite'—was first applied to describe dose–response relationships

by Southam & Ehrlich (1943) more than 60 years ago. A typical hormetic curve is either Ushaped or has an inverted U-shaped dose–response, depending on the endpoint measured. If the endpoint is growth or longevity, the dose–response would be that of an inverted U-shape; if the endpoint is disease incidence, then the dose–response would be described as U- or J-shaped (Fig 1).

Thank you,
Ellen

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Message

From: Konkus, John [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=555471B2BAA6419E8E141696F4577062-KONKUS, JOH]
Sent: 10/3/2018 6:46:02 PM
To: Woods, Clint [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=bc65010f5c2e48f4bc2aa050db50d198-Woods, Clin]
Subject: RE: URGENT: Radiation regulation clarification

Yup. Watching now. I told her it's a Congressional hearing not an EPA hearing.

From: Woods, Clint
Sent: Wednesday, October 3, 2018 2:45 PM
To: Konkus, John <konkus.john@epa.gov>
Subject: RE: URGENT: Radiation regulation clarification

Senate EPW: https://www.epw.senate.gov/public/index.cfm/hearings?ID=AA9CF4BB-B5FB-4BF6-B0E4-E33BDE0FF26A

From: Konkus, John
Sent: Wednesday, October 3, 2018 1:39 PM
To: Woods, Clint <woods.clint@epa.gov>
Subject: FW: URGENT: Radiation regulation clarification

This isn't our hearing is it?

From: Yessenia Funes [mailto:yessenia.funes@earther.com]
Sent: Wednesday, October 3, 2018 1:38 PM
To: Konkus, John <konkus.john@epa.gov>
Cc: Press <Press@epa.gov>; Lynn, Tricia <lynn.tricia@epa.gov>
Subject: Re: URGENT: Radiation regulation clarification

Thanks! I saw their correction but still was hoping to get some clarity.

What is the point of having Edward Calabrese speak at today's hearing? Was there any intention? Do you all expect he will talk about radiation?

Thanks.

Yessenia Funes

Environmental Justice Reporter, Earther
Gizmodo Media Group
2 West 17th Street
New York, NY 10011
@yessfun
Ex. 6
yessenia.funes@earther.com

On Wed, Oct 3, 2018 at 1:30 PM Konkus, John <konkus.john@epa.gov> wrote:

1. AP ran a correction to their story: Associated Press Issues Correction to False Reporting: http://createsend.com/t/d-7B1B1A763AA87F9B2540EF23F30FEDED
2. Here's a statement for the record: "The proposed regulation doesn't talk about radiation or any particular chemicals. EPA's policy is to continue to use the linear-no-threshold model for population-level radiation protection purposes which would not – under the proposed regulation that has not been finalized – trigger any change in that policy." – EPA Spokesperson

From: Yessenia Funes [mailto:yessenia.funes@earther.com]
Sent: Wednesday, October 3, 2018 1:26 PM
To: Press <Press@epa.gov>; Lynn, Tricia <lynn.tricia@epa.gov>; john.konkus@gmail.com
Subject: URGENT: Radiation regulation clarification

Good afternoon,

My name is Yessenia Funes. I'm a reporter at Earther, a national environmental news site with the Gizmodo Media Group. I'm reaching out in the hopes of getting clarification on this AP report on the administration weakening radiation regulation.

I'm trying to get a sense of what the EPA has planned for regulating radiation under its proposal to increase transparency around science. The EPA told the AP that this will not involve altering regulations around radiation, so I'm confused on the purpose of Edward Calabrese speaking on this rule given this is his area of expertise.

I'd love to speak to someone on the phone asap to clarify what exactly the EPA is planning, why it invited this researcher to testify on the science transparency rule, and the purpose of weakening the language online related to radiation exposure. If the AP has gotten it wrong, I think it's important to inform readers given how other outlets are picking this up and running with it.

Hoping to connect soon. Thank you.

Yessenia Funes

Environmental Justice Reporter, Earther

Gizmodo Media Group

2 West 17th Street

New York, NY 10011

@yessfun

Ex. 6

yessenia.funes@earther.com

Message

From: Konkus, John [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=555471B2BAA6419E8E141696F4577062-KONKUS, JOH]
Sent: 9/14/2018 12:11:57 PM
To: Woods, Clint [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=bc65010f5c2e48f4bc2aa050db50d198-Woods, Clin]
Subject: RE: AP requesting interview with EPA radiation-protection officials regarding LNT standards vs. threshold, hormetic standards

OK tell me who would be best.

From: Woods, Clint
Sent: Friday, September 14, 2018 8:10 AM
To: Konkus, John <konkus.john@epa.gov>
Subject: Re: AP requesting interview with EPA radiation-protection officials regarding LNT standards vs. threshold, hormetic standards

Oh, and I missed one of Ellen's emails with requests yesterday so it makes sense to maybe provide more info and/or a background interview with oar/ord

On Sep 14, 2018, at 6:32 AM, Konkus, John <konkus.john@epa.gov> wrote:

> FYI I'm going to have to deal with this today. I'll call you.
>
> Sent from my iPhone
>
> Begin forwarded message:
>
> > **Resent-From:** <Press@epa.gov>
> > **From:** "Knickmeyer, Ellen" <EKnickmeyer@ap.org>
> > **Date:** September 14, 2018 at 5:52:06 AM EDT
> > **To:** "Jones, Enesta" <Jones.Enesta@epa.gov>
> > **Cc:** Press <Press@epa.gov>, "Konkus, John" <konkus.john@epa.gov>, "Edwards, Jonathan" <Edwards.Jonathan@epa.gov>, "Veal, Lee" <Veal.Lee@epa.gov>, "Griggs, John" <Griggs.John@epa.gov>
> > **Subject: Re: AP requesting interview with EPA radiation-protection officials regarding LNT standards vs. threshold, hormetic standards**
> >
> > Hi, Enesta,
> >
> > That EPA website entry you sent me a link to seems to be a contradictory statements saying exposures below 10 rem usually have no harmful health effects but the EPA still follows the guideline that no exposure is safe.
> >
> > When does that entry date from? When was it revised and what part was revised?
> >
> > Your other statement provides no information at all, except to indicate the EPA may decide to reverse its longstanding policy on radiation exposure without any advance notice to the public it is considering doing that.
> >
> > Can you answer my questions and provide an EPA expert to speak to? These are non-answers and public silence on important public health questions for Americans.

Best,
Ellen

Sent from my iPhone

On Sep 13, 2018, at 6:01 PM, Jones, Enesta <Jones.Enesta@epa.gov> wrote:

> Ellen,
> I inadvertently cut off the end of our response. Here it is:
>
> Find more information here: https://www.epa.gov/radiation/radiation-health-effects
>
> Ellen,
>
> "EPA is currently reviewing the more than 590,000 comments on the Strengthening Transparency in Regulatory Sciences proposal. The EPA will determine a timeline for a decision after it has more fully assessed the comments." — **EPA spokesperson**
>
> On Sep 13, 2018, at 10:32 AM, Knickmeyer, Ellen <EKnickmeyer@ap.org> wrote:
>
> Hello – wanted to also ask, is this still EPA policy?
> https://www.epa.gov/sites/production/files/2015-05/documents/low-dose-284-291.pdf
> "Radiation protection, like the regulation of other carcinogenic agents, is—in the absence of compelling evidence to the contrary—predicated on the linear, no-threshold (LNT) hypothesis, which assumes that the risk of cancer due to a low dose exposure is proportional to dose, with no threshold."
>
> "Given the current state of the science, the consensus positions of key scientific and governmental bodies, as well as the conservatism and calculational convenience of the LNT assumption, it is unlikely that EPA will modify this approach in the near future."
>
> Best,
> Ellen
>
> ---
>
> **From:** Knickmeyer, Ellen
> **Sent:** Thursday, September 13, 2018 10:06 AM
> **To:** 'press@epa.gov' <press@epa.gov>; 'Konkus, John' <konkus.john@epa.gov>
> **Cc:** 'edwards.jonathan@epa.gov' <edwards.jonathan@epa.gov>; 'veal.lee@epa.gov' <veal.lee@epa.gov>; 'griggs.john@epa.gov' <griggs.john@epa.gov>
> **Subject:** AP requesting interview with EPA radiation-protection officials regarding LNT standards vs. threshold, hormetic standards

Hi, all,
I'm doing a story on the current EPA expressing openness to moving away from the linear non-threshold standard for radiation protection and toward standards that maintain lower doses of radiation and other carcinogens can be of acceptably low risk or beneficial. That's as with Dr. Calabrese's comments in April on Mr. Pruitt's proposed science "transparency" rule, which the EPA cited in announcing the proposal:

Dr. Edward J. Calabrese, Professor, Environmental Health Sciences, University of Massachusetts: "The proposal represents a major scientific step forward by recognizing the widespread occurrence of non-linear dose responses in toxicology and epidemiology for chemicals and radiation and the need to incorporate such data in the risk assessment process

And as with last year's EPA guidance last year that emergency responders can safely tolerate "low doses" of radiation

https://www.epa.gov/sites/production/files/2017-07/documents/pags_comm_tool_p9.pdf

I need to finish the story by Tuesday afternoon. Can you please make an EPA radiation-protection or analytics senior official available to talk to me by Monday on the topic? Where has the EPA moved since April on the LNT vs. threshold vs. hormesis argument, have there been any more moves within EPA away from LNT, and what are EPA radiation officials' thoughts on the topic?

All best,
Ellen Knickmeyer

Ex. 6

eknickmeyer@ap.org
ellen.knickmeyer@gmail.com

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Message

From: Konkus, John [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=555471B2BAA6419E8E141696F4577062-KONKUS, JOH]
Sent: 10/3/2018 5:38:34 PM
To: Abboud, Michael [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=b6f5af791a1842f1adcc088cbf9ed3ce-Abboud, Mic]; Woods, Clint [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=bc65010f5c2e48f4bc2aa050db50d198-Woods, Clin]; Block, Molly [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=60d0c681a16441a0b4fa16aa2dd4b9c5-Block, Moll]; Hewitt, James [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=41b19dd598d340bb8032923d902d4bd1-Hewitt, Jam]; Beach, Christopher [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=6b124299bb6f46a39aa5d84519f25d5d-Beach, Chri]; Jackson, Ryan [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=38bc8e18791a47d88a279db2fec8bd60-Jackson, Ry]; Gunasekara, Mandy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=53d1a3caa8bb4ebab8a2d28ca59b6f45-Gunasekara,]; Molina, Michael [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=d19c1d68da1a4587866e1850f22a6ae5-Molina, Mic]
Subject: AP Forced To Issue A Major Correction For Its Latest Article On Trump's EPA | The Daily Caller

https://dailycaller.com/2018/10/03/ap-major-correction-epa/

AP Forced To Issue A Major Correction For Its Latest Article On Trump's EPA

1:26 PM 10/03/2018

- **AP reported Tuesday that EPA was weakening radiation regulations.**
- **AP then backpedaled on the claim and issued a major correction.**
- **'[S]top the spread of alarming misinformation," EPA said in a statement on the correction.**

The Associated Press issued a major correction Wednesday to a story claiming the Environmental Protection Agency (EPA) said "a little radiation may be healthy."

EPA officials said The AP's story "was not only wrong, but contrary to information provided to the reporter by the EPA press office in response to her inquiries." EPA officials welcomed the correction and said media outlets who parroted The AP should do the same.

"If you used AP's inaccurate reporting from October 2nd on EPA's radiation standards you should want to immediately correct your stories to stop the spread of alarming misinformation," EPA said in an emailed statement sent Wednesday.

The AP published a story Tuesday claiming EPA was moving to "weaken U.S. radiation regulations" by changing a "decades-old guidance says that any exposure to harmful radiation is a cancer risk."

> AP NewsBreak: Can a little radiation damage be good for you? EPA proposal says yes. http://apne.ws/6oHcQdb
>
> — The Associated Press (@AP) 4:07 PM - Oct 2, 2018

This change, The AP noted, "could lead to higher levels of exposure for workers at nuclear installations." The news organization admitted that while the policy change did not explicitly mention "radiation," it quoted University of Massachusetts toxicologist Edward Calabrese. **(RELATED: Trump Officials Say There's 'A Major Problem' With The UN's Upcoming Climate Report, Leaked Comments Show)**

Calabrese is a critic of EPA's use of the linear no-threshold model championed by environmental interests. Calabrese's research has called into question this modeling when it comes to radiation, and he's suggested that low levels of radiation may even bring some health benefits.

The AP, in part, based its story on an EPA news release that quoted Calabrese. However, 20 hours after initially publishing its piece it issued a correction:

> *"In an early version of a story Oct. 2 about EPA regulation of radiation, The Associated Press reported erroneously in a headline that EPA says a little radiation may be good for you. As the story made clear, that assessment came from scientific outliers, including one quoted by EPA in a news release. The headline was changed in later versions of the story."*

But that was after it made waves across the media.

The AP story was picked up by NBC News, which still has the uncorrected version on its website as of Wednesday afternoon. PBS News also re-published the AP article, but still hadn't corrected it.

Esquire magazine repackaged The AP story with the sassy headline: "Getting Radiation Poisoning to Own the Libs." The magazine's summary of The AP's article still has no correction.

EPA said it "provided numerous statements to the AP reporter regarding her inquiries on the subject and made abundantly clear that the regulation she was referencing did not even include the word 'radiation' in its almost 5,000 words."

The AP's report is actually referring to a transparency rule EPA proposed earlier in 2018 that would require the agency to include a wider range of public health models when crafting regulations. It would not eliminate linear no-threshold modeling as The AP's initial reporting implied.

This isn't the first time media outlets claimed EPA was weakening radiation standards. Bloomberg News reported in 2017 EPA was "weakening radiation safety levels" based on documents unearthed by an environmental group.

However, the document Bloomberg relied on in its report was a planning document, not a regulatory one, that drew on guidance released in the final days of the Obama administration.

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Sent from my iPhone

Message

From: Konkus, John [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=555471B2BAA6419E8E141696F4577062-KONKUS, JOH]
Sent: 10/3/2018 12:32:36 PM
To: Block, Molly [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=60d0c681a16441a0b4fa16aa2dd4b9c5-Block, Moll]
CC: Woods, Clint [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=bc65010f5c2e48f4bc2aa050db50d198-Woods, Clin]; Abboud, Michael [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=b6f5af791a1842f1adcc088cbf9ed3ce-Abboud, Mic]
Subject: Re: Questions on dose responses and the EPA proposal on scientific studies

Deliberative Process / Ex. 5

Sent from my iPhone

On Oct 3, 2018, at 8:30 AM, Block, Molly <block.molly@epa.gov> wrote:

We made it in the Skimm this am:

What to say when you can't stop eating candy...
A little is good. Which is what the EPA thinks...about radiation. The Trump admin is apparently moving to weaken radiation regulations. Radiation is everywhere (think: potassium in bananas, microwaves heating your food). And can often be harmless. But the danger is in higher-energy, shorter-wave radiation which can sometimes cause cancer. Previous admins have said that any exposure to harmful radiation is a cancer risk. Now the EPA is apparently coming in with some proposed edits. And would reportedly require that different info be considered to figure out what exposure level is acceptable. Supporters say this would be a huge money saver. Critics say this would be a huge exposure risk. Today, the proposal will be discussed at a congressional hearing.

From: Konkus, John
Sent: Wednesday, October 3, 2018 8:28 AM
To: Woods, Clint <woods.clint@epa.gov>
Cc: Abboud, Michael <abboud.michael@epa.gov>; Block, Molly <block.molly@epa.gov>
Subject: Re: Questions on dose responses and the EPA proposal on scientific studies

Agree. Thanks.

Sent from my iPhone

On Oct 3, 2018, at 8:23 AM, Woods, Clint <woods.clint@epa.gov> wrote:

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

On Oct 3, 2018, at 7:36 AM, Konkus, John <konkus.john@epa.gov> wrote:

Sent from my iPhone

Begin forwarded message:

From: "Knickmeyer, Ellen" <EKnickmeyer@ap.org>
Date: October 3, 2018 at 7:23:13 AM EDT
To: Press <Press@epa.gov>, "Konkus, John" <konkus.john@epa.gov>
Subject: Questions on dose responses and the EPA proposal on scientific studies

Hi, John.
I'm sure we will be writing more on the EPA's proposal on scientific studies and wanted to nail down a couple points from the proposal. Please bear with me, because this is technical, and we have to be able to phrase it for readers in the simplest way possible.

One question – the EPA proposal says there is "growing empirical evidence of non-linearity in the concentration response function for specific pollutants and health effects."

Can you please send along and specify what studies, pollutants, and health effects are being cited there?

Second question – the EPA proposal below says in considering risks of low doses the EPA should look not just at linear models, but threshold, U-shaped, and bell-shaped models, and consider "model uncertainty" as a default.

The physics academics I talk to, and Dr. Calabrese in his writing, say U-shaped and J-

shaped curves apply only to models showing beneficial effects from lower doses of substances, or the "hormesis" that Calabrese writes about for radiation and toxins. Can you confirm that, or if not what does that refer to, specifically?

EPA Proposal:
In addition, this proposed regulation is designed to increase transparency of the assumptions underlying dose response models. As a case in point, there is growing empirical evidence of non-linearity in the concentrationresponse function for specific pollutants and health effects. The use of default models, without consideration of alternatives or model uncertainty, can obscure the scientific justification for EPA actions. To be even more transparent about these complex relationships, EPA should give appropriate consideration to high quality studies that explore: A broad class of parametric concentration-response models with a robust set of potential confounding variables; nonparametric models that incorporate fewer assumptions; various threshold models across the exposure range; and spatial heterogeneity. EPA should also incorporate the concept of model uncertainty when needed as a default to optimize low dose risk estimation based on major competing models, including linear, threshold, and U-shaped, Jshaped, and bell-shaped models.

Calabrese on "hormesis" in radiation and chemicals:
Enter an alternative model, which claims that the fundamental shape of the dose–response curve is neither linear nor threshold, but rather U-shaped. This so-called hormesis model—after the Greek

word 'to excite'—was first applied to describe dose–response relationships by Southam & Ehrlich (1943) more than 60 years ago. A typical hormetic curve is either Ushaped or has an inverted U-shaped dose–response, depending on the endpoint measured. If the endpoint is growth or longevity, the dose–response would be that of an inverted U-shape; if the endpoint is disease incidence, then the dose–response would be described as U- or J-shaped (Fig 1).

Thank you,
Ellen

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Message

From: Abboud, Michael [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=B6F5AF791A1842F1ADCC088CBF9ED3CE-ABBOUD, MIC]
Sent: 10/2/2018 8:16:04 PM
To: Woods, Clint [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=bc65010f5c2e48f4bc2aa050db50d198-Woods, Clin]
CC: Konkus, John [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=555471b2baa6419e8e141696f4577062-Konkus, Joh]
Subject: Re: APNewsBreak: Trump's EPA moving to loosen radiation limits

That was while we were on the phone. We'll give them more time.

Sent from my iPhone

On Oct 2, 2018, at 4:14 PM, Woods, Clint <woods.clint@epa.gov> wrote:

> Updated 18 mins ago but title/"radiation regulation" and other problems unaddressed
> https://apnews.com/

APNewsBreak: Trump's EPA moving to loosen radiation limits

WASHINGTON (AP) — The Trump administration is quietly moving to weaken U.S. radiation regulations, turning to scientific outliers who argue that a bit of radiation damage is actually good for you — like a little bit of sunlight.

The government's current, decades-old guidance says that any exposure to harmful radiation is a cancer risk. And critics say the proposed change could lead to higher levels of exposure for workers at nuclear installations and oil and gas drilling sites, medical workers doing X-rays and CT scans, people living next to Superfund sites and any members of the public who one day might find themselves exposed to a radiation release.

The Trump administration already has targeted a range of other regulations on toxins and pollutants, including coal power plant emissions and car exhaust, that it sees as costly and burdensome for businesses. Supporters of the EPA's new radiation guidance argue the government's current no-tolerance rule for radiation damage forces unnecessary spending for handling exposure in accidents, at nuclear plants, in medical centers and at other sites.

"This would have a positive effect on human health as well as save billions and billions and billions of dollars," Edward Calabrese, a toxicologist at the University of Massachusetts, said in 2016. He was to be the lead witness at a

congressional hearing Wednesday on EPA's push for what it calls transparency in science.

The proposed rule would require regulators to consider "various threshold models across the exposure range" when it comes to dangerous substances.

While it does not specify that it's addressing radiation and chemicals, an EPA news release on the rule quotes Calabrese as saying it would: "The proposal represents a major scientific step forward by recognizing the widespread occurrence of non-linear dose responses in toxicology and epidemiology for chemicals and radiation and the need to incorporate such data in the risk assessment process."

The comment period for the science regulation has ended and the agency is currently reviewing the comments. There's no specific date for final action by the administration. The EPA declined to make an official with its radiation-protection program available.

Radiation is everywhere, from potassium in bananas to the microwaves popping our popcorn. Most of it is benign. But what's of concern is the higher-energy, shorter-wave radiation, like X-rays, that can penetrate and disrupt living cells, sometimes causing cancer.

As recently as this March, the EPA's online guidelines for radiation effects advised: "Current science suggests there is some cancer risk from any exposure to radiation."

"Even exposures below 100 millisieverts" — an amount roughly equivalent to 25 chest X-rays or about 14 CT chest scans — "slightly increase the risk of getting cancer in the future," the agency's guidance said.

But that online guidance — separate from the rule-change proposal — was edited in July to add a section emphasizing the low individual odds of cancer: "According to radiation safety experts, radiation exposures of ...100 millisieverts usually result in no harmful health effects, because radiation below these levels is a minor contributor to our overall cancer risk," the revised policy says.

Calabrese and his supporters argue that smaller exposures of cell-damaging radiation and other carcinogens can serve as stressors that activate the body's

repair mechanisms and can make people healthier. They compare it to physical exercise or sunlight.

Mainstream scientific consensus on radiation is based on deceptive science, says Calabrese, who argued in a 2014 essay for "righting the past deceptions and correcting the ongoing errors in environmental regulation."

EPA spokesman John Konkus said in an email that the proposed rule change is about "increasing transparency on assumptions" about how the body responds to different doses of dangerous substances and that the agency "acknowledges uncertainty regarding health effects at low doses" and supports more research on that.

The radiation regulation is supported by Steven Milloy, a Trump transition team member for the EPA who is known for challenging widely accepted ideas about manmade climate change and the health risks of tobacco. He has been promoting Calabrese's theory of healthy radiation on his blog.

But Jan Beyea, a physicist whose work includes research with the National Academies of Science on the 2011 Fukushima nuclear power plant accident, said the EPA science proposal represents voices "generally dismissed by the great bulk of scientists."

The EPA proposal would lead to "increases in chemical and radiation exposures in the workplace, home and outdoor environment, including the vicinity of Superfund sites," Beyea wrote.

At the level the EPA website talks about, any one person's risk of cancer from radiation exposure is perhaps 1 percent, Beyea said.

"The individual risk will likely be low, but not the cumulative social risk," Beyea said.

"If they even look at that — no, no, no," said Terrie Barrie, a resident of Craig, Colorado, and an advocate for her husband and other workers at the now-closed Rocky Flats nuclear-weapons plant, where the U.S. government is compensating certain cancer victims regardless of their history of exposure.

"There's no reason not to protect people as much as possible," said Barrie.

U.S. agencies for decades have followed a policy that there is no threshold of radiation exposure that is risk-free.

The National Council on Radiation Protection and Measurements reaffirmed that principle this year after a review of 29 public health studies on cancer rates among people exposed to low-dose radiation, via the U.S. atomic bombing of Japan in World War II, leak-prone Soviet nuclear installations, medical treatments and other sources.

Twenty of the 29 studies directly support the principle that even low-dose exposures cause a significant increase in cancer rates, said Roy Shore, chief of research at the Radiation Effects Research Foundation, a joint project of the United States and Japan. Scientists found most of the other studies were inconclusive and decided one was flawed.

None supported the theory there is some safe threshold for radiation, said Shore, who chaired the review.

If there were a threshold that it's safe to go below, "those who profess that would have to come up with some data," Shore said in an interview.

"Certainly the evidence did not point that way," he said.

The U.S. Food and Drug Administration, which regulates electronic devices that emit radiation, advises, broadly, that a single CT scan with a dose of 10 millisieverts may increase risks of a fatal cancer by about 1 chance in 2,000.

Supporters of the proposal say it's time to rethink radiation regulation.

"Right now we spend an enormous effort trying to minimize low doses" at nuclear power plants, for example, said Brant Ulsh, a physicist with the California-based consulting firm M.H. Chew and Associates. "Instead, let's spend the resources on minimizing the effect of a really big event."

Message

From: Konkus, John [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=555471B2BAA6419E8E141696F4577062-KONKUS, JOH]
Sent: 10/1/2018 5:49:40 PM
To: Woods, Clint [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=bc65010f5c2e48f4bc2aa050db50d198-Woods, Clin]
Subject: RE: AP requesting interview with EPA radiation-protection officials regarding LNT standards vs. threshold, hormetic standards

Deliberative Process / Ex. 5

From: Woods, Clint
Sent: Monday, October 1, 2018 1:49 PM
To: Konkus, John <konkus.john@epa.gov>
Subject: Re: AP requesting interview with EPA radiation-protection officials regarding LNT standards vs. threshold, hormetic standards

Deliberative Process / Ex. 5

On Oct 1, 2018, at 12:48 PM, Konkus, John <konkus.john@epa.gov> wrote:

Deliberative Process / Ex. 5

From: Woods, Clint
Sent: Monday, October 1, 2018 1:44 PM
To: Konkus, John <konkus.john@epa.gov>
Subject: Re: AP requesting interview with EPA radiation-protection officials regarding LNT standards vs. threshold, hormetic standards

Deliberative Process / Ex. 5

On Oct 1, 2018, at 12:37 PM, Konkus, John <konkus.john@epa.gov> wrote:

Deliberative Process / Ex. 5

From: Knickmeyer, Ellen [mailto:EKnickmeyer@ap.org]
Sent: Monday, October 1, 2018 1:33 PM
To: Konkus, John <konkus.john@epa.gov>
Cc: Jones, Enesta <Jones.Enesta@epa.gov>; Press <Press@epa.gov>; Edwards, Jonathan <Edwards.Jonathan@epa.gov>; Veal, Lee <Veal.Lee@epa.gov>; Griggs, John <Griggs.John@epa.gov>
Subject: RE: AP requesting interview with EPA radiation-protection officials regarding LNT standards vs. threshold, hormetic standards

Hi, John,
On the part of the Transparency rule that deals with dose-response for radiation and chemicals, and says regulators should consider alternate theories in addition to LNT, did Edward Calabrese and or Steven Milloy play a role in crafting it?
Best,
Ellen

From: Konkus, John <konkus.john@epa.gov>
Sent: Friday, September 14, 2018 10:53 AM
To: Knickmeyer, Ellen <EKnickmeyer@ap.org>
Cc: Jones, Enesta <Jones.Enesta@epa.gov>; Press <Press@epa.gov>; Edwards, Jonathan <Edwards.Jonathan@epa.gov>; Veal, Lee <Veal.Lee@epa.gov>; Griggs, John <Griggs.John@epa.gov>
Subject: RE: AP requesting interview with EPA radiation-protection officials regarding LNT standards vs. threshold, hormetic standards

The Radiation Health Effects site reflects recent information and was last updated in July 2018.

EPA's proposed rule on Strengthening Transparency in Regulatory Science did not discuss radiation. The Agency did seek comment on increasing transparency of the assumptions underlying dose response models. As noted previously, the timeline for any future action on this proposal has not yet been determined and EPA is reviewing more than 500,000 comments.

EPA continues to use the linear-no-threshold model for population-level radiation protection purposes. The EPA's Radiation Protection Program acknowledges uncertainty regarding health effects at low doses and is supportive of continued research in this area. More information in support of this approach can be found in the International Commission on Radiological Protection Report 103 and the 2018 National Council on Radiation Protection and Measurements Commentary 27.

From: Knickmeyer, Ellen [mailto:EKnickmeyer@ap.org]
Sent: Friday, September 14, 2018 8:34 AM
To: Konkus, John <konkus.john@epa.gov>
Cc: Jones, Enesta <Jones.Enesta@epa.gov>; Press <Press@epa.gov>; Edwards, Jonathan <Edwards.Jonathan@epa.gov>; Veal, Lee <Veal.Lee@epa.gov>; Griggs, John <Griggs.John@epa.gov>
Subject: Re: AP requesting interview with EPA radiation-protection officials regarding LNT standards vs. threshold, hormetic standards

Thank you.

Sent from my iPhone

On Sep 14, 2018, at 8:30 AM, Konkus, John <konkus.john@epa.gov> wrote:

> Ellen: We're working on something more robust for you. Should have it this morning.
>
> John
>
> Sent from my iPhone
>
> On Sep 14, 2018, at 5:52 AM, Knickmeyer, Ellen <EKnickmeyer@ap.org> wrote:
>
> > Hi, Enesta,

That EPA website entry you sent me a link to seems to be a contradictory statements saying exposures below 10 rem usually have no harmful health effects but the EPA still follows the guideline that no exposure is safe.

When does that entry date from? When was it revised and what part was revised?

Your other statement provides no information at all, except to indicate the EPA may decide to reverse its longstanding policy on radiation exposure without any advance notice to the public it is considering doing that.

Can you answer my questions and provide an EPA expert to speak to? These are non-answers and public silence on important public health questions for Americans.

Best,
Ellen

Sent from my iPhone

On Sep 13, 2018, at 6:01 PM, Jones, Enesta <Jones.Enesta@epa.gov> wrote:

> Ellen,
> I inadvertently cut off the end of our response. Here it is:
>
> Find more information here: https://www.epa.gov/radiation/radiation-health-effects
>
> Ellen,
>
> "EPA is currently reviewing the more than 590,000 comments on the Strengthening Transparency in Regulatory Sciences proposal. The EPA will determine a timeline for a decision after it has more fully assessed the comments." — **EPA spokesperson**
>
> On Sep 13, 2018, at 10:32 AM, Knickmeyer, Ellen <EKnickmeyer@ap.org> wrote:
>
> Hello – wanted to also ask, is this still EPA policy?

https://www.epa.gov/sites/production/files/2015-05/documents/low-dose-284-291.pdf
"Radiation protection, like the regulation of other carcinogenic agents, is—in the absence of compelling evidence to the contrary—predicated on the linear, no-threshold (LNT) hypothesis, which assumes that the risk of cancer due to a low dose exposure is proportional to dose, with no threshold."

"Given the current state of the science, the consensus positions of key scientific and governmental bodies, as well as the conservatism and calculational convenience of the LNT assumption, it is unlikely that EPA will modify this approach in the near future."

Best,
Ellen

From: Knickmeyer, Ellen
Sent: Thursday, September 13, 2018 10:06 AM
To: 'press@epa.gov' <press@epa.gov>; 'Konkus, John' <konkus.john@epa.gov>
Cc: 'edwards.jonathan@epa.gov' <edwards.jonathan@epa.gov>; 'veal.lee@epa.gov' <veal.lee@epa.gov>; 'griggs.john@epa.gov' <griggs.john@epa.gov>
Subject: AP requesting interview with EPA radiation-protection officials regarding LNT standards vs. threshold, hormetic standards

Hi, all,
I'm doing a story on the current EPA expressing openness to moving away from the linear non-threshold standard for radiation protection and toward standards that maintain lower doses of radiation and other carcinogens can be of acceptably low risk or beneficial. That's as with Dr. Calabrese's

comments in April on Mr. Pruitt's proposed science "transparency" rule, which the EPA cited in announcing the proposal:

Dr. Edward J. Calabrese, Professor, Environmental Health Sciences, University of Massachusetts: "The proposal represents a major scientific step forward by recognizing the widespread occurrence of non-linear dose responses in toxicology and epidemiology for chemicals and radiation and the need to incorporate such data in the risk assessment process

And as with last year's EPA guidance last year that emergency responders can safely tolerate "low doses" of radiation

https://www.epa.gov/sites/production/files/2017-07/documents/pags_comm_tool_p9.pdf

I need to finish the story by Tuesday afternoon. Can you please make an EPA radiation-protection or analytics senior official available to talk to me by Monday on the topic? Where has the EPA moved since April on the LNT vs. threshold vs. hormesis argument, have there been any more moves within EPA away from LNT, and what are EPA radiation officials' thoughts on the topic?

All best,
Ellen Knickmeyer

Ex. 6

eknickmeyer@ap.org
ellen.knickmeyer@gmail.com

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Message

From: Jackson, Ryan [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=38BC8E18791A47D88A279DB2FEC8BD60-JACKSON, RY]
Sent: 10/1/2018 5:28:21 PM
To: Woods, Clint [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=bc65010f5c2e48f4bc2aa050db50d198-Woods, Clin]
CC: Bolen, Brittany [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=31e872a691114372b5a6a88482a66e48-Bolen, Brit]; Lyons, Troy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=15e4881c95044ab49c6c35a0f5eef67e-Lyons, Troy]; Konkus, John [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=555471b2baa6419e8e141696f4577062-Konkus, Joh]
Subject: Re: EPW Hearing

Deliberative Process / Ex. 5

Ryan Jackson
Chief of Staff
U.S. EPA

Ex. 6

On Oct 1, 2018, at 1:26 PM, Woods, Clint <woods.clint@epa.gov> wrote:

Deliberative Process / Ex. 5

On Oct 1, 2018, at 12:13 PM, Bolen, Brittany <bolen.brittany@epa.gov> wrote:

Deliberative Process / Ex. 5

Sent from my iPhone

On Oct 1, 2018, at 1:00 PM, Lyons, Troy <lyons.troy@epa.gov> wrote:

Please note that EPW is holding a hearing this week on science transparency

Oversight of the Environmental Protection Agency's Implementation of Sound and

Transparent Science in Regulation

October 3, 2018 02:15 PM

The Senate Committee on Environment and Public Works Subcommittee on Superfund, Waste Management, and Regulatory Oversight will hold a subcommittee hearing entitled, *"Oversight of the Environmental Protection Agency's Implementation of Sound and Transparent Science in Regulation."*

WEDNESDAY, October 3, 2018

2:15PM

Room 406 of the Dirksen Senate Office Building

Troy M. Lyons
Associate Administrator
Office of Congressional & Intergovernmental Relations
U.S. Environmental Protection Agency
Ex. 6 (cell)

Message

From: Woods, Clint [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=BC65010F5C2E48F4BC2AA050DB50D198-WOODS, CLIN]
Sent: 10/4/2018 1:51:57 PM
To: Konkus, John [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=555471b2baa6419e8e141696f4577062-Konkus, Joh]
CC: Abboud, Michael [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=b6f5af791a1842f1adcc088cbf9ed3ce-Abboud, Mic]
Subject: Re: Questions on risk models and EPA's proposal on scientific studies-on deadline

Deliberative Process / Ex. 5

On Oct 4, 2018, at 9:01 AM, Konkus, John <konkus.john@epa.gov> wrote:

Deliberative Process / Ex. 5

Sent from my iPhone

Begin forwarded message:

Resent-From: <Press@epa.gov>
From: "Knickmeyer, Ellen" <EKnickmeyer@ap.org>
Date: October 4, 2018 at 7:37:53 AM EDT
To: Press <Press@epa.gov>, "Konkus, John" <konkus.john@epa.gov>
Cc: "Feldman, Carole" <CFeldman@ap.org>
Subject: RE: Questions on risk models and EPA's proposal on scientific studies-on deadline

Hi, John,
Can you please explain what the reference to J and U shaped models means in the proposed regulation. It's very specific language, it's in the proposal for a regulation, what does it mean?

EPA proposal:
'The use of default models, without consideration of alternatives or model uncertainty, can obscure the scientific justification for EPA actions. To be even more transparent about these complex relationships, EPA should give appropriate consideration to high quality studies that explore: A broad class of parametric concentration-response models with a robust set of potential confounding variables; nonparametric models that incorporate fewer assumptions; various threshold models across the exposure range; and spatial heterogeneity. EPA should also incorporate the concept of model uncertainty when needed as a default to optimize low dose risk estimation based on major competing models, including linear, threshold, and U-shaped, J-shaped, and bell-shaped models.'"

Below are four explanations from experts of all ranges of opinion on the EPA proposal, all saying the U and J shaped model is Calabrese's hormesis model, meaning the model for the theory that exposure to a hazardous substance is beneficial at lower doses.

If that isn't what it means, what does it mean? All you're saying to these expert assessments is "Nope."

EPA'S EXPERT:
Edward Calabrese:
"Enter an alternative model, which claims that the fundamental shape of the dose–response curve is neither linear nor threshold, but rather U-shaped. This so-called hormesis model—after the Greek word 'to excite'—was first applied to describe dose–response relationships by Southam & Ehrlich (1943) more than 60 years ago. A typical hormetic curve is either Ushaped or has an inverted U-shaped dose–response, depending on the endpoint measured. If the endpoint is growth or longevity, the dose–response would be that of an inverted U-shape; if the endpoint is disease incidence, then the dose–response would be described as U- or J-shaped (Fig 1). "
Calabrese says this rule as it reads would apply to radiation and chemicals.

2nd expert:
Marco Kaltofen, PhD, PE (Civil, MA), C. NSE Affiliate Research Engineer
Nuclear Science and Engineering Program, Dept. of Physics, Worcester Polytechnic Institute
"EPA has used a straight line model. That means more poison equals more harm. 500 hundred years of scientific data supports this model.Now
EPA wants to use a squiggly model. That means that a little poison might be good for you, so let's try it.
EPA calls it a J-model because harm goes down first, then goes up, like a J shape.

3rd expert:
Jennifer Sass, Ph.D.
Senior Scientist, Natural Resources Defense Council
Part-time faculty, George Washington University
Linear no-threshold: "The idea is that any amount of exposure is associated with some amount of risk. The higher the exposure, the higher the risk. The only time you're going to have zero risk is when you have zero exposure."
"What this (EPA proposal) is trying to pretend is that EPA's models are uncertain as if they had a problem and somehow these other models are better....J or U shape."
Sasse says this rule as it reads would apply to radiation and chemicals.

4th expert:
John Peterson Myers
Environmental Health Sciences
609 East High St
Charlottesville VA 22902
Calabrese bases his arguments on widespread occurrence of 'hormetic' responses in the literature. A 'hormetic' response is a special case of a larger set of dose-response curves

called 'non-monotonic dose response curves' (NMDRCs), named thus because the slope of the curve changes sign somewhere along the curve (a term out of mathematics). Hence a J-shaped or U-shaped or inverted-U shaped curve are all non-monotonic.

From: Abboud, Michael <abboud.michael@epa.gov>
Sent: Wednesday, October 03, 2018 5:13 PM
To: Knickmeyer, Ellen <EKnickmeyer@ap.org>; Press <Press@epa.gov>; Konkus, John <konkus.john@epa.gov>
Subject: RE: Questions on risk models and EPA's proposal on scientific studies-on deadline

Please see below.

"The proposed rule does not refer to cancer-causing agents or hormesis. It stated: 'The use of default models, without consideration of alternatives or model uncertainty, can obscure the scientific justification for EPA actions. To be even more transparent about these complex relationships, EPA should give appropriate consideration to high quality studies that explore: A broad class of parametric concentration-response models with a robust set of potential confounding variables; nonparametric models that incorporate fewer assumptions; various threshold models across the exposure range; and spatial heterogeneity. EPA should also incorporate the concept of model uncertainty when needed as a default to optimize low dose risk estimation based on major competing models, including linear, threshold, and U-shaped, J-shaped, and bell-shaped models.'"
– EPA spokesman

From: Knickmeyer, Ellen [mailto:EKnickmeyer@ap.org]
Sent: Wednesday, October 3, 2018 4:25 PM
To: Press <Press@epa.gov>; Konkus, John <konkus.john@epa.gov>
Subject: RE: Questions on risk models and EPA's proposal on scientific studies-on deadline

Hi, John,
Dr. Calabrese just now and the independent physicists I talked to said that the J and U shaped models that the EPA proposal cites refers only to hormesis, which is the idea that cancer-causing agents are actually beneficial at lower doses.

Can you comment on that, specifically? Would the proposal require regulators to consider that hazardous substances at low doses are healthy, and if not, what does the reference to U and J shaped models mean?
Thanks,
Ellen

From: Knickmeyer, Ellen
Sent: Wednesday, October 03, 2018 8:25 AM
To: Press <Press@epa.gov>; Konkus, John <konkus.john@epa.gov>
Subject: Questions on risk models and EPA's proposal on scientific studies

(Sent a few minutes ago but it looks like it may have hung up in my email – repeating again just in case.)

Hi, John.
I'm sure we will be writing more on the EPA's proposal on scientific studies and wanted to nail down a couple points from the proposal. Please bear with me, because this is technical, and we have to be able to phrase it for readers in the simplest way possible.

One question – the EPA proposal says there is "growing empirical evidence of non-linearity in the concentration response function for specific pollutants and health effects."

Can you please send along and specify what studies, pollutants, and health effects are being cited there?

Second question – the EPA proposal below says in considering risks of low doses the EPA should look not just at linear models, but threshold, U-shaped, and bell-shaped models, and consider "model uncertainty" as a default.

The physics academics I talk to, and Dr. Calabrese in his writing, say U-shaped and J-shaped curves apply only to models showing beneficial effects from lower doses of harmful substances, or the "hormesis" that Calabrese writes about for radiation and toxins. Can you confirm that, or if not what does that refer to, specifically?

EPA Proposal:
In addition, this proposed regulation is designed to increase transparency of the assumptions underlying dose response models. As a case in point, there is growing empirical evidence of non-linearity in the concentrationresponse function for specific pollutants and health effects. The use of default models, without consideration of alternatives or model uncertainty, can obscure the scientific justification for EPA actions. To be even more transparent about these complex relationships, EPA should give appropriate consideration to high quality studies that explore: A broad class of parametric concentration-response models with a robust set of potential confounding variables; nonparametric models that incorporate fewer assumptions; various threshold models across the exposure range; and spatial heterogeneity. EPA should also incorporate the concept of model uncertainty when needed as a default to optimize low dose risk estimation based on major

competing models, including linear, threshold, and U-shaped, Jshaped, and bell-shaped models.

Calabrese on "hormesis" in radiation and chemicals:
Enter an alternative model, which claims that the fundamental shape of the dose–response curve is neither linear nor threshold, but rather U-shaped. This so-called hormesis model—after the Greek word 'to excite'—was first applied to describe dose–response relationships by Southam & Ehrlich (1943) more than 60 years ago. A typical hormetic curve is either Ushaped or has an inverted U-shaped dose–response, depending on the endpoint measured. If the endpoint is growth or longevity, the dose–response would be that of an inverted U-shape; if the endpoint is disease incidence, then the dose–response would be described as U- or J-shaped (Fig 1).

Thank you,
Ellen

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From: Woods, Clint [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=BC65010F5C2E48F4BC2AA050DB50D198-WOODS, CLIN]
Sent: 10/4/2018 2:30:39 PM
To: Konkus, John [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=555471b2baa6419e8e141696f4577062-Konkus, Joh]
CC: Abboud, Michael [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=b6f5af791a1842f1adcc088cbf9ed3ce-Abboud, Mic]
Subject: Re: Questions on risk models and EPA's proposal on scientific studies-on deadline

Deliberative Process / Ex. 5

On Oct 4, 2018, at 10:29 AM, Konkus, John <konkus.john@epa.gov> wrote:

See below...

From: Knickmeyer, Ellen [mailto:EKnickmeyer@ap.org]
Sent: Thursday, October 4, 2018 10:25 AM
To: Konkus, John <konkus.john@epa.gov>; Press <Press@epa.gov>
Cc: Feldman, Carole <CFeldman@ap.org>
Subject: RE: Questions on risk models and EPA's proposal on scientific studies-on deadline

Hi, John. The published literature on risk assessment and the specialists I talked to in risk assessment say the U and J curve in risk-assessment refer only to hormesis, or Dr. Calabrese's theory that hazardous substances are good for you at lower doses.
Michael told me yesterday when I asked about the J and U curves that hormesis is not in the proposal.
What do the J and U curves in the proposed regulation refer to, then?
Thanks.
Ellen

From: Konkus, John <konkus.john@epa.gov>
Sent: Thursday, October 04, 2018 10:20 AM
To: Knickmeyer, Ellen <EKnickmeyer@ap.org>; Press <Press@epa.gov>
Cc: Feldman, Carole <CFeldman@ap.org>
Subject: RE: Questions on risk models and EPA's proposal on scientific studies-on deadline

"The list of shapes in the proposal is a description of virtually every potential shape of a curve in a dose-response relationship." – EPA Spokesperson

From: Knickmeyer, Ellen [mailto:EKnickmeyer@ap.org]
Sent: Thursday, October 4, 2018 10:02 AM
To: Konkus, John <konkus.john@epa.gov>; Press <Press@epa.gov>
Cc: Feldman, Carole <CFeldman@ap.org>
Subject: RE: Questions on risk models and EPA's proposal on scientific studies-on deadline

What does the U and J shaped model mean in the proposal?

From: Konkus, John <konkus.john@epa.gov>
Sent: Thursday, October 04, 2018 9:59 AM
To: Knickmeyer, Ellen <EKnickmeyer@ap.org>; Press <Press@epa.gov>

Cc: Feldman, Carole <CFeldman@ap.org>
Subject: RE: Questions on risk models and EPA's proposal on scientific studies-on deadline

Ellen:

"The proposed regulation takes comment on ensuring transparency and consideration of high quality studies evaluating models and model assumptions, including high quality studies that examine different potential dose-response relationships – the list below is literally a description of every potential shape of a curve. You need look no further than the 590,000 public comments provided to EPA on this proposal to see literally thousands of different viewpoints on particular studies, and the EPA is currently reviewing all of those comments to ensure the best proposal is put forward." – EPA Spokesperson

Also, you mistakenly list Dr. Calabrese as an "EPA Expert." He is not affiliated with EPA. The hearing yesterday was a Congressional hearing not an EPA hearing. EPA did not present at the hearing.

Thank you,

John Konkus

From: Knickmeyer, Ellen [mailto:EKnickmeyer@ap.org]
Sent: Thursday, October 4, 2018 7:38 AM
To: Press <Press@epa.gov>; Konkus, John <konkus.john@epa.gov>
Cc: Feldman, Carole <CFeldman@ap.org>
Subject: RE: Questions on risk models and EPA's proposal on scientific studies-on deadline

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EPA proposal:
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Below are four explanations from experts of all ranges of opinion on the EPA proposal, all saying the U and J shaped model is Calabrese's hormesis model, meaning the model for the theory that exposure to a hazardous substance is beneficial at lower doses.

If that isn't what it means, what does it mean? All you're saying to these expert assessments is "Nope."

EPA'S EXPERT:
Edward Calabrese:
"Enter an alternative model, which claims that the fundamental shape of the dose–response curve is neither linear nor threshold, but rather U-shaped. This so-called hormesis model—

after the Greek word 'to excite'—was first applied to describe dose–response relationships by Southam & Ehrlich (1943) more than 60 years ago. A typical hormetic curve is either Ushaped or has an inverted U-shaped dose–response, depending on the endpoint measured. If the endpoint is growth or longevity, the dose–response would be that of an inverted U-shape; if the endpoint is disease incidence, then the dose–response would be described as U- or J-shaped (Fig 1). "
Calabrese says this rule as it reads would apply to radiation and chemicals.

2nd expert:
Marco Kaltofen, PhD, PE (Civil, MA), C. NSE Affiliate Research Engineer
Nuclear Science and Engineering Program, Dept. of Physics, Worcester Polytechnic Institute
"EPA has used a straight line model. That means more poison equals more harm. 500 hundred years of scientific data supports this model.Now
EPA wants to use a squiggly model. That means that a little poison might be good for you, so let's try it.
EPA calls it a J-model because harm goes down first, then goes up, like a J shape.

3rd expert:
Jennifer Sass, Ph.D.
Senior Scientist, Natural Resources Defense Council
Part-time faculty, George Washington University
Linear no-threshold: "The idea is that any amount of exposure is associated with some amount of risk. The higher the exposure, the higher the risk. The only time you're going to have zero risk is when you have zero exposure."
"What this (EPA proposal) is trying to pretend is that EPA's models are uncertain as if they had a problem and somehow these other models are better....J or U shape."
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John Peterson Myers
Environmental Health Sciences
609 East High St
Charlottesville VA 22902
Calabrese bases his arguments on widespread occurrence of 'hormetic' responses in the literature. A 'hormetic' response is a special case of a larger set of dose-response curves called 'non-monotonic dose response curves' (NMDRCs), named thus because the slope of the curve changes sign somewhere along the curve (a term out of mathematics). Hence a J-shaped or U-shaped or inverted-U shaped curve are all non-monotonic.

From: Abboud, Michael <abboud.michael@epa.gov>
Sent: Wednesday, October 03, 2018 5:13 PM
To: Knickmeyer, Ellen <EKnickmeyer@ap.org>; Press <Press@epa.gov>; Konkus, John <konkus.john@epa.gov>
Subject: RE: Questions on risk models and EPA's proposal on scientific studies-on deadline

Please see below.

"The proposed rule does not refer to cancer-causing agents or hormesis. It stated: 'The use of default models, without consideration of alternatives or model uncertainty, can obscure the scientific

justification for EPA actions. To be even more transparent about these complex relationships, EPA should give appropriate consideration to high quality studies that explore: A broad class of parametric concentration-response models with a robust set of potential confounding variables; nonparametric models that incorporate fewer assumptions; various threshold models across the exposure range; and spatial heterogeneity. EPA should also incorporate the concept of model uncertainty when needed as a default to optimize low dose risk estimation based on major competing models, including linear, threshold, and U-shaped, J-shaped, and bell-shaped models.'" **– EPA spokesman**

From: Knickmeyer, Ellen [mailto:EKnickmeyer@ap.org]
Sent: Wednesday, October 3, 2018 4:25 PM
To: Press <Press@epa.gov>; Konkus, John <konkus.john@epa.gov>
Subject: RE: Questions on risk models and EPA's proposal on scientific studies-on deadline

Hi, John,
Dr. Calabrese just now and the independent physicists I talked to said that the J and U shaped models that the EPA proposal cites refers only to hormesis, which is the idea that cancer-causing agents are actually beneficial at lower doses.

Can you comment on that, specifically? Would the proposal require regulators to consider that hazardous substances at low doses are healthy, and if not, what does the reference to U and J shaped models mean?
Thanks,
Ellen

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Sent: Wednesday, October 03, 2018 8:25 AM
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(Sent a few minutes ago but it looks like it may have hung up in my email – repeating again just in case.)

Hi, John.
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One question – the EPA proposal says there is "growing empirical evidence of non-linearity in the concentration response function for specific pollutants and health effects."

Can you please send along and specify what studies, pollutants, and health effects are being cited there?

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The physics academics I talk to, and Dr. Calabrese in his writing, say U-shaped and J-shaped curves apply only to models showing beneficial effects from lower doses of harmful substances, or the "hormesis" that Calabrese writes about for radiation and toxins. Can you confirm that, or if not what does that refer to, specifically?

EPA Proposal:
In addition, this proposed regulation is designed to increase transparency of the assumptions underlying dose response models. As a case in point, there is growing empirical evidence of non-linearity in the concentrationresponse function for specific pollutants and health effects. The use of default models, without consideration of alternatives or model uncertainty, can obscure the scientific justification for EPA actions. To be even more transparent about these complex relationships, EPA should give appropriate consideration to high quality studies that explore: A broad class of parametric concentration-response models with a robust set of potential confounding variables; nonparametric models that incorporate fewer assumptions; various threshold models across the exposure range; and spatial heterogeneity. EPA should also incorporate the concept of model uncertainty when needed as a default to optimize low dose risk estimation based on major competing models, including linear, threshold, and U-shaped, Jshaped, and bell-shaped models.

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Enter an alternative model, which claims that the fundamental shape of the dose–response curve is neither linear nor threshold, but rather U-shaped. This so-called hormesis model—after the Greek word 'to excite'—was first applied to describe dose–response relationships by Southam & Ehrlich (1943) more than 60 years ago. A typical hormetic curve is either Ushaped or has an inverted U-shaped dose–response, depending on the endpoint measured. If the endpoint is growth or longevity, the dose–response would be that of an inverted U-shape; if the endpoint is disease incidence, then the dose–response would be described as U- or J-shaped (Fig 1).

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Sent: 10/4/2018 2:14:51 PM
To: Konkus, John [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=555471b2baa6419e8e141696f4577062-Konkus, Joh]
CC: Abboud, Michael [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=b6f5af791a1842f1adcc088cbf9ed3ce-Abboud, Mic]
Subject: Re: Questions on risk models and EPA's proposal on scientific studies-on deadline

Deliberative Process / Ex. 5

On Oct 4, 2018, at 10:06 AM, Konkus, John <konkus.john@epa.gov> wrote:

Deliberative Process / Ex. 5

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Message

From: Woods, Clint [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=BC65010F5C2E48F4BC2AA050DB50D198-WOODS, CLIN]
Sent: 9/14/2018 1:56:11 PM
To: Konkus, John [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=555471b2baa6419e8e141696f4577062-Konkus, Joh]
Subject: RE: AP requesting interview with EPA radiation-protection officials regarding LNT standards vs. threshold, hormetic standards

Working with ORIA on a couple updates, and will get something back to you by noon.

From: Konkus, John
Sent: Friday, September 14, 2018 9:27 AM
To: Woods, Clint <woods.clint@epa.gov>
Subject: FW: AP requesting interview with EPA radiation-protection officials regarding LNT standards vs. threshold, hormetic standards

See below…

From: Jones, Enesta
Sent: Friday, September 14, 2018 8:45 AM
To: Konkus, John <konkus.john@epa.gov>
Cc: Press <Press@epa.gov>
Subject: Re: AP requesting interview with EPA radiation-protection officials regarding LNT standards vs. threshold, hormetic standards

Deliberative Process / Ex. 5

On Sep 14, 2018, at 8:30 AM, Konkus, John <konkus.john@epa.gov> wrote:

Ellen: We're working on something more robust for you. Should have it this morning.

John

Sent from my iPhone

On Sep 14, 2018, at 5:52 AM, Knickmeyer, Ellen <EKnickmeyer@ap.org> wrote:

Hi, Enesta,

That EPA website entry you sent me a link to seems to be a contradictory statements saying exposures below 10 rem usually have no harmful health effects but the EPA still follows the guideline that no exposure is safe.

When does that entry date from? When was it revised and what part was revised?

Your other statement provides no information at all, except to indicate the EPA may decide to reverse its longstanding policy on radlation exposure without any advance notice to the public it is considering doing that.

Can you answer my questions and provide an EPA expert to speak to? These are non-answers and public silence on important public health questions for Americans.

Best,
Ellen

Sent from my iPhone

On Sep 13, 2018, at 6:01 PM, Jones, Enesta <Jones.Enesta@epa.gov> wrote:

Ellen,
I inadvertently cut off the end of our response. Here it is:

Find more information here: https://www.epa.gov/radiation/radiation-health-effects

Ellen,

"EPA is currently reviewing the more than 590,000 comments on the Strengthening Transparency in Regulatory Sciences proposal. The EPA will determine a timeline for a decision after it has more fully assessed the comments." — **EPA spokesperson**

On Sep 13, 2018, at 10:32 AM, Knickmeyer, Ellen <EKnickmeyer@ap.org> wrote:

Hello – wanted to also ask, is this still EPA policy?
https://www.epa.gov/sites/production/files/2015-05/documents/low-dose-284-291.pdf
"Radiation protection, like the regulation of other carcinogenic agents, is—in the absence of compelling evidence to the contrary—predicated on the linear, no-threshold (LNT) hypothesis, which assumes that the risk of cancer due to a low dose exposure is proportional to dose, with no threshold."

"Given the current state of the science, the consensus positions of key scientific and governmental bodies, as well as the conservatism and calculational convenience of the LNT assumption, it is unlikely that EPA will modify this approach in the near future."

Best,

Ellen

From: Knickmeyer, Ellen
Sent: Thursday, September 13, 2018 10:06 AM
To: 'press@epa.gov' <press@epa.gov>; 'Konkus, John' <konkus.john@epa.gov>
Cc: 'edwards.jonathan@epa.gov' <edwards.jonathan@epa.gov>; 'veal.lee@epa.gov' <veal.lee@epa.gov>; 'griggs.john@epa.gov' <griggs.john@epa.gov>
Subject: AP requesting interview with EPA radiation-protection officials regarding LNT standards vs. threshold, hormetic standards

Hi, all,
I'm doing a story on the current EPA expressing openness to moving away from the linear non-threshold standard for radiation protection and toward standards that maintain lower doses of radiation and other carcinogens can be of acceptably low risk or beneficial. That's as with Dr. Calabrese's comments in April on Mr. Pruitt's proposed science "transparency" rule, which the EPA cited in announcing the proposal:

Dr. Edward J. Calabrese, Professor, Environmental Health Sciences, University of Massachusetts: "The proposal represents a major scientific step forward by recognizing the widespread occurrence of non-linear dose responses in toxicology and epidemiology for chemicals and radiation and the need to incorporate such data in the risk assessment process

And as with last year's EPA guidance last year that emergency responders can safely tolerate "low doses" of radiation

https://www.epa.gov/sites/production/files/2017-07/documents/pags_comm_tool_p9.pdf

I need to finish the story by Tuesday afternoon. Can you please make an EPA radiation-protection or analytics senior official available to talk to me by Monday on the topic? Where has the EPA moved since April on the LNT vs. threshold vs. hormesis argument, have there been any more moves within EPA away from LNT, and what are EPA radiation officials' thoughts on the topic?

All best,
Ellen Knickmeyer
Ex. 6
eknickmeyer@ap.org
ellen.knickmeyer@gmail.com

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Sent: 4/20/2018 6:20:52 PM
To: Bowman, Liz [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=c3d4d94d3e4b4b1f80904056703ebc80-Bowman, Eli]; Bolen, Brittany [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=31e872a691114372b5a6a88482a66e48-Bolen, Brit]; Gordon, Stephen [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=7c8fb4d82bff4eec98f5c5d00a47f554-Gordon, Ste]; Konkus, John [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=555471b2baa6419e8e141696f4577062-Konkus, Joh]; Letendre, Daisy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=b691cccca6264ae09df7054c7f1019cb-Letendre, D]; Schwab, Justin [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=eed0f609c0944cc2bbdb05df3a10aadb-Schwab, Jus]
Subject: Transparency/Data Access Deliberative Process / Ex. 5

Happy to work on some talking points for a release to accompany Tuesday's announcement.

Deliberative Process / Ex. 5

Clint Woods
Deputy Assistant Administrator
Office of Air and Radiation, U.S. EPA
202.564.6562

Message

From: Woods, Clint [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=BC65010F5C2E48F4BC2AA050DB50D198-WOODS, CLIN]
Sent: 9/14/2018 11:53:19 AM
To: Konkus, John [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=555471b2baa6419e8e141696f4577062-Konkus, Joh]
CC: Yamada, Richard (Yujiro) [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=4c34a1e0345e4d26b361b5031430639d-Yamada, Yuj]
Subject: Re: AP requesting interview with EPA radiation-protection officials regarding LNT standards vs. threshold, hormetic standards

Deliberative Process / Ex. 5

On Sep 14, 2018, at 6:32 AM, Konkus, John <konkus.john@epa.gov> wrote:

Deliberative Process / Ex. 5

Sent from my iPhone

Begin forwarded message:

Resent-From: <Press@epa.gov>
From: "Knickmeyer, Ellen" <EKnickmeyer@ap.org>
Date: September 14, 2018 at 5:52:06 AM EDT
To: "Jones, Enesta" <Jones.Enesta@epa.gov>
Cc: Press <Press@epa.gov>, "Konkus, John" <konkus.john@epa.gov>, "Edwards, Jonathan" <Edwards.Jonathan@epa.gov>, "Veal, Lee" <Veal.Lee@epa.gov>, "Griggs, John" <Griggs.John@epa.gov>
Subject: Re: AP requesting interview with EPA radiation-protection officials regarding LNT standards vs. threshold, hormetic standards

Hi, Enesta,

That EPA website entry you sent me a link to seems to be a contradictory statements saying exposures below 10 rem usually have no harmful health effects but the EPA still follows the guideline that no exposure is safe.

When does that entry date from? When was it revised and what part was revised?

Your other statement provides no information at all, except to indicate the EPA may decide to reverse its longstanding policy on radlation exposure without any advance notice to the public it is considering doing that.

Can you answer my questions and provide an EPA expert to speak to? These are non-answers and public silence on important public health questions for Americans.

Best,
Ellen

Sent from my iPhone

On Sep 13, 2018, at 6:01 PM, Jones, Enesta <Jones.Enesta@epa.gov> wrote:

Ellen,
I inadvertently cut off the end of our response. Here it is:

Find more information here: https://www.epa.gov/radiation/radiation-health-effects

Ellen,

"EPA is currently reviewing the more than 590,000 comments on the Strengthening Transparency in Regulatory Sciences proposal. The EPA will determine a timeline for a decision after it has more fully assessed the comments." — **EPA spokesperson**

On Sep 13, 2018, at 10:32 AM, Knickmeyer, Ellen <EKnickmeyer@ap.org> wrote:

Hello – wanted to also ask, is this still EPA policy?
https://www.epa.gov/sites/production/files/2015-05/documents/low-dose-284-291.pdf
"Radiation protection, like the regulation of other carcinogenic agents, is—in the absence of compelling evidence to the contrary—predicated on the linear, no-threshold (LNT) hypothesis, which assumes that the risk of cancer due to a low dose exposure is proportional to dose, with no threshold."

"Given the current state of the science, the consensus positions of key scientific and governmental bodies, as well as the conservatism and calculational convenience of the LNT assumption, it is unlikely that EPA will modify this approach in the near future."

Best,
Ellen

From: Knickmeyer, Ellen
Sent: Thursday, September 13, 2018 10:06 AM
To: 'press@epa.gov' <press@epa.gov>; 'Konkus, John' <konkus.john@epa.gov>
Cc: 'edwards.jonathan@epa.gov' <edwards.jonathan@epa.gov>; 'veal.lee@epa.gov' <veal.lee@epa.gov>; 'griggs.john@epa.gov' <griggs.john@epa.gov>
Subject: AP requesting interview with EPA radiation-protection officials regarding LNT standards vs. threshold, hormetic standards

Hi, all,
I'm doing a story on the current EPA expressing openness to moving away from the linear non-threshold standard for radiation protection and toward standards that maintain lower doses of radiation and other carcinogens can be of acceptably low risk or beneficial. That's as with Dr. Calabrese's comments in April on Mr. Pruitt's proposed science "transparency" rule, which the EPA cited in announcing the proposal:

Dr. Edward J. Calabrese, Professor, Environmental Health Sciences, University of Massachusetts: "The proposal represents a major scientific step forward by recognizing the widespread occurrence of non-linear dose responses in toxicology and epidemiology for chemicals and radiation and the need to incorporate such data in the risk assessment process

And as with last year's EPA guidance last year that emergency responders can safely tolerate "low doses" of radiation

https://www.epa.gov/sites/production/files/2017-07/documents/pags_comm_tool_p9.pdf

I need to finish the story by Tuesday afternoon. Can you please make an EPA radiation-protection or analytics senior official available to talk to me by Monday on the topic? Where has the EPA moved since April on the LNT vs. threshold vs. hormesis argument, have there been any more moves within EPA away from LNT, and what are EPA radiation officials' thoughts on the topic?

All best,
Ellen Knickmeyer

Ex. 6

eknickmeyer@ap.org
ellen.knickmeyer@gmail.com

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Message

From: Woods, Clint [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=BC65010F5C2E48F4BC2AA050DB50D198-WOODS, CLIN]
Sent: 10/3/2018 3:33:40 PM
To: Konkus, John [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=555471b2baa6419e8e141696f4577062-Konkus, Joh]
Subject: RE: Media Request: Question About Science Transparency Rule & Radiation Guidelines

Deliberative Process / Ex. 5

From: Konkus, John
Sent: Wednesday, October 3, 2018 11:11 AM
To: Woods, Clint <woods.clint@epa.gov>
Subject: FW: Media Request: Question About Science Transparency Rule & Radiation Guidelines

Clint: See question below...

From: Zahra Hirji [mailto:zahra.hirji@buzzfeed.com]
Sent: Wednesday, October 3, 2018 11:06 AM
To: Konkus, John <konkus.john@epa.gov>
Cc: Press <Press@epa.gov>
Subject: Re: Media Request: Question About Science Transparency Rule & Radiation Guidelines

Hi John,

Thanks so much for this comment. I have two follow ups. Can I attribute this quote to you -- it seems the AP article does, and I'm curious why it was provided to me on background.

Second, you say "EPA's policy is to continue to use the linear-no-threshold model for population-level radiation protection purposes" -- but what about using the model for individual-level radiation protection purposes? My understanding is there are times when the EPA sets a protection radiation or contamination standard based on the individual with the highest risk, say for a community near a Superfund site. Are you saying the rule change leaves the door open for changing individual-level radiation protection purpose -- or not?

Thanks,
Zahra

On Wed, Oct 3, 2018 at 8:17 AM Konkus, John <konkus.john@epa.gov> wrote:

> "The proposed regulation doesn't talk about radiation or any particular chemicals. EPA's policy is to continue to use the linear-no-threshold model for population-level radiation protection purposes which would not – under the proposed regulation that has not been finalized – trigger any change in that policy." - - EPA Spokesperson
>
> The extended comment period has closed. We received over 590,000 comments.
>
> On Oct 3, 2018, at 8:04 AM, Zahra Hirji <zahra.hirji@buzzfeed.com> wrote:
>
> Dear John Konkus,

I'm a reporter for BuzzFeed News covering EPA's science transparency rule, and I have a few questions about the impacts. Yesterday, the AP published a story suggesting the rule proposal could impact EPA's radiation guidelines. While it's true the rule proposal does not mention radiation or the guidelines specifically, it does mention opening the discussion on various models. (Here's a sentence from the docket page for the rule: "EPA shall evaluate the appropriateness of using default assumptions, including assumptions of a linear, no-threshold dose response, on a case-by-case basis.") Some scientists I've spoken to say it is possible reconsidering the linear non-threshold model and the radiation guidelines could be impacted by the larger rule proposal, even though it's not spelled out so clearly in the proposal -- and it appears some people have provided comments to the proposal on this subject.

Can you confirm the public comment period on the rule proposal asked for input on the types of models that dictate the EPA's radiation guidelines and could open the door to changing these guidelines down the road? And can you comment on why University of Massachusetts scientist Edward Calabrese is quoted in EPA's press release for the rule saying essentially just that?

I am on deadline, and am looking to publish a story ahead of today's Senate EPW hearing on the transparency rule (where Calabrese is one of three invited speakers) and available on my cell at Ex. 6 Ex. 6

Best,
Zahra Hirji

--
Zahra Hirji
Reporter
BuzzFeed News
Office: Ex. 6
Cell: Ex. 6
Zahra.Hirji@buzzfeed.com
@zhirji28

--
Zahra Hirji
Reporter
BuzzFeed News
Office: Ex. 6
Cell: Ex. 6
Zahra.Hirji@buzzfeed.com
@zhirji28

Message

From: Woods, Clint [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=BC65010F5C2E48F4BC2AA050DB50D198-WOODS, CLIN]
Sent: 10/2/2018 9:36:06 PM
To: Konkus, John [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=555471b2baa6419e8e141696f4577062-Konkus, Joh]
CC: Abboud, Michael [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=b6f5af791a1842f1adcc088cbf9ed3ce-Abboud, Mic]; Block, Molly [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=60d0c681a16441a0b4fa16aa2dd4b9c5-Block, Moll]
Subject: Re: For the record

Deliberative Process / Ex. 5

On Oct 2, 2018, at 5:24 PM, Konkus, John <konkus.john@epa.gov> wrote:

Deliberative Process / Ex. 5

From: Feldman, Carole [mailto:CFeldman@ap.org]
Sent: Tuesday, October 2, 2018 5:22 PM
To: Konkus, John <konkus.john@epa.gov>
Subject: RE: For the record
Importance: High

John,
One more question.
Can you say why EPA included Calabrese's comments in its news release if it did not agree with his interpretation re the impact of the regulation on radiation and chemicals.
Thanks,
Carole

From: Konkus, John <konkus.john@epa.gov>
Sent: Tuesday, October 02, 2018 4:35 PM
To: Feldman, Carole <CFeldman@ap.org>
Subject: RE: For the record

Yes please.

From: Feldman, Carole [mailto:CFeldman@ap.org]
Sent: Tuesday, October 2, 2018 4:34 PM
To: Konkus, John <konkus.john@epa.gov>
Subject: RE: For the record

Quote you?

From: Konkus, John <konkus.john@epa.gov>
Sent: Tuesday, October 02, 2018 4:32 PM

To: Feldman, Carole <CFeldman@ap.org>
Cc: Press <Press@epa.gov>
Subject: For the record

"The proposed regulation doesn't talk about radiation or any particular chemicals. And as we indicated in our response, EPA's policy is to continue to use the linear-no-threshold model for population-level radiation protection purposes which would not – under the proposed regulation that has not been finalized – trigger any change in that policy."

John Konkus
Environmental Protection Agency
Deputy Associate Administrator for Public Affairs
Mobile: (Ex. 6

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Message

From: Woods, Clint [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=BC65010F5C2E48F4BC2AA050DB50D198-WOODS, CLIN]
Sent: 10/2/2018 8:28:31 PM
To: Konkus, John [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=555471b2baa6419e8e141696f4577062-Konkus, Joh]; Abboud, Michael [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=b6f5af791a1842f1adcc088cbf9ed3ce-Abboud, Mic]
Subject: Fwd: AP requesting interview with EPA radiation-protection officials regarding LNT standards vs. threshold, hormetic standards

Deliberative Process / Ex. 5

Begin forwarded message:

From: "Woods, Clint" <woods.clint@epa.gov>
Date: September 14, 2018 at 10:31:57 AM EDT
To: "Konkus, John" <konkus.john@epa.gov>
Subject: RE: AP requesting interview with EPA radiation-protection officials regarding LNT standards vs. threshold, hormetic standards

Deliberative Process / Ex. 5

From: Konkus, John
Sent: Friday, September 14, 2018 9:27 AM
To: Woods, Clint <woods.clint@epa.gov>
Subject: FW: AP requesting interview with EPA radiation-protection officials regarding LNT standards vs. threshold, hormetic standards

See below...

From: Jones, Enesta
Sent: Friday, September 14, 2018 8:45 AM
To: Konkus, John <konkus.john@epa.gov>
Cc: Press <Press@epa.gov>
Subject: Re: AP requesting interview with EPA radiation-protection officials regarding LNT standards vs. threshold, hormetic standards

Deliberative Process / Ex. 5

On Sep 14, 2018, at 8:30 AM, Konkus, John <konkus.john@epa.gov> wrote:

Ellen: We're working on something more robust for you. Should have it this morning.

John

Sent from my iPhone

On Sep 14, 2018, at 5:52 AM, Knickmeyer, Ellen <EKnickmeyer@ap.org> wrote:

> Hi, Enesta,
>
> That EPA website entry you sent me a link to seems to be a contradictory statements saying exposures below 10 rem usually have no harmful health effects but the EPA still follows the guideline that no exposure is safe.
>
> When does that entry date from? When was it revised and what part was revised?
>
> Your other statement provides no information at all, except to indicate the EPA may decide to reverse its longstanding policy on radiation exposure without any advance notice to the public it is considering doing that.
>
> Can you answer my questions and provide an EPA expert to speak to? These are non-answers and public silence on important public health questions for Americans.
>
> Best,
> Ellen

Sent from my iPhone

On Sep 13, 2018, at 6:01 PM, Jones, Enesta <Jones.Enesta@epa.gov> wrote:

Ellen,
I inadvertently cut off the end of our response. Here it is:

Find more information here: https://www.epa.gov/radiation/radiation-health-effects

Ellen,

"EPA is currently reviewing the more than 590,000 comments on the Strengthening Transparency in Regulatory Sciences proposal. The EPA will determine a timeline for a decision after it has more fully assessed the comments." — **EPA spokesperson**

On Sep 13, 2018, at 10:32 AM, Knickmeyer, Ellen <EKnickmeyer@ap.org> wrote:

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https://www.epa.gov/sites/production/files/2015-05/documents/low-dose-284-291.pdf
"Radiation protection, like the regulation of other carcinogenic agents, is—in the absence of compelling evidence to the contrary—predicated on the linear, no-threshold (LNT) hypothesis, which assumes that the risk of cancer due to a low dose exposure is proportional to dose, with no threshold."

"Given the current state of the science, the consensus positions of key scientific and governmental bodies, as well as the conservatism and calculational convenience of the LNT assumption, it is unlikely that EPA will modify this approach in the near future."

Best,
Ellen

From: Knickmeyer, Ellen
Sent: Thursday, September 13, 2018 10:06 AM
To: 'press@epa.gov' <press@epa.gov>; 'Konkus, John' <konkus.john@epa.gov>
Cc: 'edwards.jonathan@epa.gov' <edwards.jonathan@epa.gov>; 'veal.lee@epa.gov' <veal.lee@epa.gov>; 'griggs.john@epa.gov' <griggs.john@epa.gov>
Subject: AP requesting interview with EPA radiation-protection officials regarding LNT standards vs. threshold, hormetic standards

Hi, all,
I'm doing a story on the current EPA expressing openness to moving away from the linear non-threshold standard for radiation protection and toward standards that maintain lower doses of radiation and other

carcinogens can be of acceptably low risk or beneficial. That's as with Dr. Calabrese's comments in April on Mr. Pruitt's proposed science "transparency" rule, which the EPA cited in announcing the proposal:

Dr. Edward J. Calabrese, Professor, Environmental Health Sciences, University of Massachusetts: "The proposal represents a major scientific step forward by recognizing the widespread occurrence of non-linear dose responses in toxicology and epidemiology for chemicals and radiation and the need to incorporate such data in the risk assessment process

And as with last year's EPA guidance last year that emergency responders can safely tolerate "low doses" of radiation

https://www.epa.gov/sites/production/files/2017-07/documents/pags_comm_tool_p9.pdf

I need to finish the story by Tuesday afternoon. Can you please make an EPA radiation-protection or analytics senior official available to talk to me by Monday on the topic? Where has the EPA moved since April on the LNT vs. threshold vs. hormesis argument, have there been any more moves within EPA away from LNT, and what are EPA radiation officials' thoughts on the topic?

All best,
Ellen Knickmeyer

Ex. 6

eknickmeyer@ap.org
ellen.knickmeyer@gmail.com

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Message

From: Woods, Clint [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=BC65010F5C2E48F4BC2AA050DB50D198-WOODS, CLIN]
Sent: 4/23/2018 2:00:42 PM
To: Bowman, Liz [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=c3d4d94d3e4b4b1f80904056703ebc80-Bowman, Eli]; Gordon, Stephen [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=7c8fb4d82bff4eec98f5c5d00a47f554-Gordon, Ste]; Ford, Hayley [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=4748a9029cf74453a20ee8ac9527830c-Ford, Hayle]
CC: Letendre, Daisy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=b691cccca6264ae09df7054c7f1019cb-Letendre, D]; Bolen, Brittany [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=31e872a691114372b5a6a88482a66e48-Bolen, Brit]; Jackson, Ryan [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=38bc8e18791a47d88a279db2fec8bd60-Jackson, Ry]
Subject: RE: Science Transparency Event--Run of Show

I can do 1:30 **Deliberative Process / Ex. 5**

Deliberative Process / Ex. 5

From: Bowman, Liz
Sent: Monday, April 23, 2018 9:58 AM
To: Gordon, Stephen <gordon.stephen@epa.gov>; Ford, Hayley <ford.hayley@epa.gov>
Cc: Letendre, Daisy <letendre.daisy@epa.gov>; Bolen, Brittany <bolen.brittany@epa.gov>; Woods, Clint <woods.clint@epa.gov>; Jackson, Ryan <jackson.ryan@epa.gov>
Subject: RE: Science Transparency Event--Run of Show

Sounds good. Could we all meet at 1/1:30 p.m., prior to senior staff?

From: Gordon, Stephen
Sent: Monday, April 23, 2018 9:57 AM
To: Ford, Hayley <ford.hayley@epa.gov>
Cc: Letendre, Daisy <letendre.daisy@epa.gov>; Bolen, Brittany <bolen.brittany@epa.gov>; Woods, Clint <woods.clint@epa.gov>; Bowman, Liz <Bowman.Liz@epa.gov>; Jackson, Ryan <jackson.ryan@epa.gov>
Subject: Re: Science Transparency Event--Run of Show

I am going to have a call with Clint this morning to discuss run of show, and I will let you know.

On Apr 23, 2018, at 9:49 AM, Ford, Hayley <ford.hayley@epa.gov> wrote:

> Is there a run of show for the science transparency event tomorrow? I have 2-4PM blocked but not sure what is included in that. Is he doing any press after? Can someone please send to me?
>
> Thanks!

Hayley Ford
Deputy White House Liaison and Personal Aide to the Administrator
Environmental Protection Agency
ford.hayley@epa.gov
Phone: 202-564-2022
Cell: Ex. 6

Message

From: Woods, Clint [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=BC65010F5C2E48F4BC2AA050DB50D198-WOODS, CLIN]
Sent: 9/13/2018 9:43:00 PM
To: Konkus, John [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=555471b2baa6419e8e141696f4577062-Konkus, Joh]; Gunasekara, Mandy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=53d1a3caa8bb4ebab8a2d28ca59b6f45-Gunasekara,]
CC: Yamada, Richard (Yujiro) [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=4c34a1e0345e4d26b361b5031430639d-Yamada, Yuj]
Subject: RE: Another OAR draft response

Yep – sorry for delay

From: Konkus, John
Sent: Thursday, September 13, 2018 4:58 PM
To: Woods, Clint <woods.clint@epa.gov>; Gunasekara, Mandy <Gunasekara.Mandy@epa.gov>
Cc: Yamada, Richard (Yujiro) <yamada.richard@epa.gov>
Subject: RE: Another OAR draft response

Deliberative Process / Ex. 5

From: Woods, Clint
Sent: Thursday, September 13, 2018 4:55 PM
To: Konkus, John <konkus.john@epa.gov>; Gunasekara, Mandy <Gunasekara.Mandy@epa.gov>
Cc: Yamada, Richard (Yujiro) <yamada.richard@epa.gov>
Subject: RE: Another OAR draft response

+ Yamada

Deliberative Process / Ex. 5

From: Konkus, John
Sent: Thursday, September 13, 2018 4:27 PM
To: Gunasekara, Mandy <Gunasekara.Mandy@epa.gov>; Woods, Clint <woods.clint@epa.gov>
Subject: Another OAR draft response

Deliberative Process / Ex. 5

On Sep 13, 2018, at 4:18 PM, Konkus, John <konkus.john@epa.gov> wrote:

Deliberative Process / Ex. 5

From: Jones, Enesta
Sent: Thursday, September 13, 2018 4:02 PM
To: Konkus, John <konkus.john@epa.gov>; Block, Molly <block.molly@epa.gov>; Abboud, Michael <abboud.michael@epa.gov>; Hewitt, James <hewitt.james@epa.gov>
Cc: Jones, Enesta <Jones.Enesta@epa.gov>; Lynn, Tricia <lynn.tricia@epa.gov>; Grantham, Nancy <Grantham.Nancy@epa.gov>
Subject: Open Inquiries, 9/13/18

Six Awaiting Approval:

From: "Lynn, Tricia" <lynn.tricia@epa.gov>
Date: September 13, 2018 at 3:36:17 PM EDT
To: Press <Press@epa.gov>
Subject: FOR REVIEW: AP (Ellen Knickmeyer) RE: LNT standards vs. threshold, hermetic standards (9/17)

Deliberative Process / Ex. 5

From: Knickmeyer, Ellen [mailto:EKnickmeyer@ap.org]
Sent: Thursday, September 13, 2018 10:33 AM
To: Press <Press@epa.gov>; Konkus, John <konkus.john@epa.gov>
Cc: Edwards, Jonathan <Edwards.Jonathan@epa.gov>; Veal, Lee <Veal.Lee@epa.gov>; Griggs, John <Griggs.John@epa.gov>
Subject: RE: AP requesting interview with EPA radiation-protection officials regarding LNT standards vs. threshold, hormetic standards

Hello – wanted to also ask, is this still EPA policy?
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"Given the current state of the science, the consensus positions of key scientific and governmental bodies, as well as the conservatism and calculational convenience of the LNT assumption, it is unlikely that EPA will modify this approach in the near future."

Best,
Ellen

From: Knickmeyer, Ellen
Sent: Thursday, September 13, 2018 10:06 AM
To: 'press@epa.gov' <press@epa.gov>; 'Konkus, John' <konkus.john@epa.gov>
Cc: 'edwards.jonathan@epa.gov' <edwards.jonathan@epa.gov>; 'veal.lee@epa.gov' <veal.lee@epa.gov>; 'griggs.john@epa.gov' <griggs.john@epa.gov>
Subject: AP requesting interview with EPA radiation-protection officials regarding LNT standards vs. threshold, hormetic standards

Hi, all,
I'm doing a story on the current EPA expressing openness to moving away from the linear non-threshold standard for radiation protection and toward standards that maintain lower doses of radiation and other carcinogens can be of acceptably low risk or beneficial. That's as with Dr. Calabrese's comments in April on Mr. Pruitt's proposed science "transparency" rule, which the EPA cited in announcing the proposal:

Dr. Edward J. Calabrese, Professor, Environmental Health Sciences, University of Massachusetts: "The proposal represents a major scientific step forward by recognizing the widespread occurrence of non-linear dose responses in toxicology and epidemiology for chemicals and radiation and the need to incorporate such data in the risk assessment process

And as with last year's EPA guidance last year that emergency responders can safely tolerate "low doses" of radiation

https://www.epa.gov/sites/production/files/2017-07/documents/pags_comm_tool_p9.pdf

I need to finish the story by Tuesday afternoon. Can you please make an EPA radiation-protection or analytics senior official available to talk to me by Monday on the topic? Where has the EPA moved since April on the LNT vs. threshold vs. hormesis argument, have there been any more moves within EPA away from LNT, and what are EPA radiation officials' thoughts on the topic?

All best,
Ellen Knickmeyer

Message

From: Woods, Clint [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=BC65010F5C2E48F4BC2AA050DB50D198-WOODS, CLIN]
Sent: 9/25/2018 1:20:27 PM
To: Jackson, Ryan [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=38bc8e18791a47d88a279db2fec8bd60-Jackson, Ry]
Subject: Fwd: New IQG Case: RFC 18003 - NATA/Ethylene Oxide
Attachments: RFC 18003 Request.pdf; ATT00001.htm

Deliberative Process / Ex. 5

Begin forwarded message:

From: "Shoaff, John" <Shoaff.John@epa.gov>
To: "Wehrum, Bill" <Wehrum.Bill@epa.gov>, "Woods, Clint" <woods.clint@epa.gov>, "Tsirigotis, Peter" <Tsirigotis.Peter@epa.gov>, "Koerber, Mike" <Koerber.Mike@epa.gov>
Cc: "Lewis, Josh" <Lewis.Josh@epa.gov>
Subject: FW: New IQG Case: RFC 18003 - NATA/Ethylene Oxide

Bill et al,

Deliberative Process / Ex. 5

John

JOHN SHOAFF | DIRECTOR
OFFICE OF AIR POLICY & PROGRAM SUPPORT (OAPPS)
OFFICE OF AIR & RADIATION | U.S. EPA | WJC NORTH 5442-B
1200 PENNSYLVANIA AVE. NW | MC 6103A | WASHINGTON, D.C. | 20460 | USA
Shoaff.john@epa.gov | 1-202-564-0531 DIRECT | Ex. 6 MOBILE

From: Kirby, Kevin
Sent: Monday, September 24, 2018 10:05 AM
To: Eagles, Tom <Eagles.Tom@epa.gov>
Subject: New IQG Case: RFC 18003 - NATA/Ethylene Oxide

Deliberative Process / Ex. 5

Thanks,
Kevin

Kevin J. Kirby
Enterprise Data Architect
US Environmental Protection Agency

OEI/OEIP/EQMD
202 566-1656 desk