Elizabeth Allen/R10/USEPA/US
01/25/2012 01:55 PM

To Chad Schulze
cc Sheila Fleming, williams.erin, Richard Kauffman, jae.p.douglas, keo1, karen.bishop, Scott Downey
bcc
Subject Re: Fw: Regarding Environmental Samples

Thanks Chad,

it wouldn't be the first time Day misunderstood our intent. If my failing memory serves me correctly, I don't think we expected any substantial residue would be present on broadleaf plants save visual evidence of damage. And I think in the longer term, if the results of air sampling do not appear to correlate well with urine data (meaning the urine results indicate exposure that can't be explained by our environmental data), then we may revise our conceptual model. But again, it seems unlikely that drift on to local crops would be noticeable just by visual monitoring. And if actual drift is occurring, it should influence air concentrations.

Richard, do want to remain the "response champ" and the consistent voice of the group? If so, we can prepare something for you to distribute. Otherwise, we'll have a trusted voice here send out a response after proper vetting.

Elizabeth

Chad Schulze | Ahhh ... I don't recall stating exactly those words... | 01/25/2012 01:34:31 PM

From: Chad Schulze/R10/USEPA/US
To: Elizabeth Allen/R10/USEPA/US@EPA
Cc: Sheila Fleming/R10/USEPA/US@EPA, williams.erin@epa.gov, Richard Kauffman/R10/USEPA/US@EPA, jae.p.douglas@state.or.us, keo1@cdc.gov, karen.bishop@state.or.us
Date: 01/25/2012 01:34 PM
Subject: Re: Fw: Regarding Environmental Samples

Ahhh ... I don't recall stating exactly those words (Erin?) ... I think what happened was that Day kept asking us to sample leaves from fruit trees, ornamentals, etc that were present during the last application. I stated that we were there to collect edible vegetation (at least that's what I thought our goal was) .... We gladly would have collected edible plants growing during the last spraying if they would have been available (kale for example) but they were not. I explained to Day that we were collecting samples of edible plants to support the exposure investigation and determine if there was a baseline concentration of the pesticides of concern. We had a discussion on the possibility of pesticide residues moving onto his property via delayed volatilization but not that we were trying to prove pesticides were moving in that way.

Regarding the honey ... they had harvested honey from the hives at some point in the year and were storing it in their home. They wanted us to sample it but I requested samples of honey still in the hive for chain of custody reasons. I again explained that this sampling effort was really just building an exposure baseline and that it was important to maintain a good chain of custody for any samples collected.

I did agreed that it would be important for any report to capture exactly how, what and where foliage was collected. I think we can respond by reiterating that the the exposure investigation was focused on edible vegetation and if there were any background concentration of pesticides in their food. I would also state that maintaining good chain of custody is crucial in such investigations and that any report describing the sampling event should describe how, what and where the samples were collected.

Let me know what you all think and I can respond to Day.

Thanks

****

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EPA DIVE TEAM:
http://yosemite.epa.gov/r10/oea.nsf/webpage/dive+team

Elizabeth Allen	I'm assuming Day has misinterpreted what happ...	01/25/2012 12:08:16 PM

From: Elizabeth Allen/R10/USEPA/US
To: Chad Schulze/R10/USEPA/US@EPA
Cc: Sheila Fleming/R10/USEPA/US@EPA
Date: 01/25/2012 12:08 PM
Subject: Fw: Regarding Environmental Samples

I'm assuming Day has misinterpreted what happened. The following excerpt from our QAPP indicates our intent to intentionally sample vegetation that exhibited signs of damage from drift. Obviously, anything planted subsequent to any spray would not exhibit such symptoms. And in the absence of any visible signs of drift damage, vegetation was to be collected in a random manner.

Vegetation Samples

The sampling team will collect materials (edible foliage, fruits, and other plant parts) from vegetation and place them in clean stainless steel bowl for visual examination. In cases where symptoms indicate herbicide exposed plant tissues, the damaged plant tissue will be isolated and transferred into a sample container (paper bag contained within a plastic Ziploc bag) and submitted to the lab for analysis. The sampling team will collect approximately one pound of each vegetation sample. If a herbicide drift pattern is apparent, the sampling team will collect samples in a gradient pattern sequentially from the area with least anticipated residue concentration to the greatest anticipated concentration. The most common signs of herbicide damage are leaves that are curling or cupped, discolored, or with dead spots. In cases where there is no apparent pattern, the sampling team will attempt to collect vegetation samples in a grid pattern.

----- Forwarded by Elizabeth Allen/R10/USEPA/US on 01/25/2012 12:03 PM -----

From: esseneinfo@aol.com
To: Richard Kauffman/R10/USEPA/US@EPA, (b) (6) [redacted], Chad Schulze/R10/USEPA/US@EPA, (b) (6) [redacted]
Cc: (b) (6) [redacted], (b) (6) [redacted], Elizabeth Allen/R10/USEPA/US@EPA, jae.p.douglas@state.or.us, keo1@cdc.gov, karen.bishop@state.or.us
Date: 01/25/2012 11:55 AM
Subject: Regarding Environmental Samples

Richard and all, this is Day with a request.
In regard to the upcoming report on environmental samples and any related communications from you to government, citizens, or media, I request that the following important point be clearly articulated.
When Chad and Erin from Seattle EPA came to my property to take environmental samples, they explained something that you need to likewise make clear in your report. They explained that the reason that they were NOT taking samples from plants that had been growing on the land at the time of the spring sprays; rather, they were intentionally taking samples of plant foilage that was more recent, for the following reason. Chad said that the purpose was not to find evidence that the past sprays had drifted to our property **-- that if that were the goal they would indeed be sampling different foilage --** but that instead the purpose was to test the theory that, if as some persons have feared might be the case, our region was so saturated with pesticide residue that, even in the absence of a recent spray, it continues to volatize and/or otherwise move onto our properties. Chad and Erin supplied that information when I inquired why they were taking samples of plants **THAT HAD NOT EVEN BEEN ALIVE DURRING THE SPRAY SEASON.** Also, though we had honey that was from the spray season, honey was selected that was from more recent hives that would not relate to the spring spray season. At that time, I explained to Chad and he agreed, that, at the time of the written report on the environmental samples, **it would be very important that the above information be clearly communicated, since otherwise negative results -- not finding pesticide on the samples -- will be wrongly interpreted by everyone not privy to the above to mean that the foilage that was alive durring the spray season was not found to have pesticide residue on it when tested in the fall.**
My request, then, is that the above be made very very clear in the report and every other communication to the community, government -- including the report to the Governor -- and media. Thanks, Day Owen

-----Original Message-----
From: Richard Kauffman <Kauffman.Richard@epamail.epa.gov>
To: danandmaya (b) (6) [redacted]
Cc: spiralmom (b) (6) [redacted]; esseneinfo <esseneinfo@aol.com>; gary (b) (6) [redacted]; Elizabeth Allen <Allen.Elizabeth@epamail.epa.gov>; jae.p.douglas <jae.p.douglas@state.or.us>; keo1 <keo1@cdc.gov>; karen.bishop <karen.bishop@state.or.us>
Sent: Tue, Jan 24, 2012 9:06 am
Subject: Highway 36 Comments and Questions from D/M Gee

Marijana and Dan,

I am sending this response on behalf of the exposure investigation team.
I apologize for the delay in responding which was due to tracking down how to obtain answers to your questions about NHANES.

Thank you for the article on Dr. Watson's research below. It was great to have the opportunity to hear from her this morning.

Regards,

Richard

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1. A & B
The National Center for Health Statistics which houses the NHANES data does not generally release this information. NHANES participants provide written consent for testing. Part of the consent form ensures that individual and community specific information, including the town, individual addresses, or the date of sampling will not be released. Therefore, to ensure compliance with federal human subjects' protection guidelines, this information is not available to us unless we develop and submit a proposal to the CDC's Research Data Center requesting access to the data. The proposal requires that a valid research question be developed and accepted. Since the data is strictly protected, access to the data by the researcher is allowed only under tightly controlled conditions once the proposal is accepted. Once a report on the data is developed, significant review and clearance is required before the results can be published. More information can be found at: http://www.cdc.gov/rdc. The resources necessary for the development of such a proposal is extensive, and a successful project may take 6-9 months to complete. Proposals can be submitted by anyone, including citizens. Since we are struggling to find the necessary resources to carry out the current exposure investigation, we are unable develop a proposal to answer questions related to the geography and season of the NHANES 2,4-D data.

1. C
The reference article in the following link describes the basis for the health interpretation of the test results. It was co-authored by Dr. Dana Barr.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2831914/

The only pesticide we detected in urine samples was 2,4-D. Without data that document exposure to other pesticides, we cannot speculate on possible interactive effects.

1. D
In reviewing the ATSDR urine sampling protocol, we were unable to find the information you reference above. Are you perhaps referring to the EPA QAPP for environmental samples which is appendix B (pages 37-47) of the protocol? All urine samples were frozen immediately upon collection and stored on dry ice to ensure stability until analyzed at the NCEH laboratory.

2. A

We welcome all credible research and scientific information that anyone would like to submit to us. We rely upon our published reports (health consultations, public health assessments, toxicological profiles, and associated materials) to communicate our findings, including a discussion of relevant research studies.

From: "Marijana(pronounced maari-yanna) and Dan Gee"
(b) (6) [redacted]
To: Eron king (b) (6) [redacted]
Cc: Richard Kauffman/R10/USEPA/US@EPA, Clare Howard
(b) (6) [redacted], Elizabeth Allen/R10/USEPA/US@EPA,
"esseneinfo@aol.com Owen" <esseneinfo@aol.com>,
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Orloff" <keo1@cdc.gov>
Date: 12/15/2011 07:01 PM
Subject: Re: Note from Clare Howard, NOW COMMENTS from D/M Gee

Greetings to all,

1. In winter of 2011 over 30 of our community members' urine ,including ours tested positive for Atrazine and 2,4-D . In Spring of 2011 urine of our family showed up positive for 2,4-D and Atrazine, AGAIN ! (through Dr. Dana Barr testing). In late summer of August 2011 our urine tested positive for 2,4-D AGAIN! (through OHA testing). THIS IS INDICATING A CHRONIC LOW LEVEL EXPOSURE ! NOTE: THIS IS ONLY FOR 2011, AND ONLY TWO MAIN PESTICIDES ARE TESTED!

It is logical that due to the obviously known source of our exposure to presume that this is a year after year recurrence. Our questions for this column are:

A. WAS THE CONTROL GROUP (NHANES) TESTED IN SUMMER, SPRING, FALL OR WINTER ?

B. OR WHERE THEY TESTED IN ALL SEASONS?

C. In your report ,Mr.Kenneth Orloff you wrote that our levels "are below the concentrations that would be expected to cause adverse health effects". CAN YOU PLEASE POINT US TO THE STUDY THAT PROVES THE FINDINGS THAT YOU STATED TO ALL OF THE PARTICIPANTS?

ALSO, CAN YOU PLEASE POINT US TO THE STUDY THAT CAN SUPPORT YOUR TEST REPORT STATEMENT IN THE LIGHT OF THE TRUTH THAT THIS CHEMICAL IS NOT THE ONLY CHEMICAL IN THE MIX OF PESTICIDES USED THAT HAS ENCROACHED INTO OUR BODIES ? SO, PLEASE POINT US TO THE STUDY THAT PROVES THE SAFETY OF 2,4-D IN THE CONSTANT LOW LEVEL SYNERGISTIC EFFECT WITH OTHER PESTICIDES SPRAYED ON THE HILLS AROUND OUR COMMUNITIES IN OR?

D. It has also come to our attention from the scientists that are reviewing your protocol that the temperature the urine samples were

stored at was way less than optimal: "Disturbing item in the QAPP which was that samples and extractions appear to be recommended to be stored at 4° C [pp. 19-21]. This temperature is inappropriate for vouchers since they will quickly degrade at this temperature if unprotected. They should be stored at -70° C maximum - even -20° C storage in an 'ordinary' freezer still allows enzymatic chemical conversions to occur at measurable rates. Notice also that these are "Data Quality Objectives" and not "Data Quality Guarantees".

QUESTION: WHY WERE THE TESTS STORED AT 4° C,RATHER THAN -70° C MAXIMUM - EVEN -20° C ???

E. It has come to our attention that OHA's David Farrer and Dr. Matthew Dubrow have been asked by community members "where do they think the exposure is occurring" and are pointing to the sources of everything BUT THE VERY HILLS SOAKED IN THIS AND OTHER PESTICIDES!? WHY???? This is very disrespectful to the intelligence of people that have been personally effected by these sprays and ARE VERY AWARE and do know the source!
It is also disrespectful to the ones that are now starting to find a correlation to their cancers, aches and pains, diabetes, Parkinson's, miscarriages, etc, etc. and were previously clueless to that correlation. We want you to know that even several community members that were using these pesticides, and thought that they were safe ,after discovering that they now have cancer are starting to do their own research, and are finding that many respected scientists, especially the ones that are not financially tied to the Chemical Industry have already shown the studies of the correlation.
THE POINT HERE IS THAT THERE IS NO RUNNING AWAY FROM TRUTH, AND EVERYONE THAT IS NOW GIVEN A CHANCE TO STAND UP FOR THAT TRUTH SHOULD TAKE THAT OPPORTUNITY!
FOR THE DECEPTION AND DESTRUCTIVE QUALITIES OF THE POLITICS INVOLVED IN THIS POLLUTION AND HEALTH DEGRADATIONS MUST CONSUME IT'S SELF!
I am not speaking to anyone here personally, but to the force behind this corrupt system, and to the ones that choose to feed it by allowing any wrong activities to continue!

2.
WE WOULD APPRECIATE YOUR OPENNESS TO THE INFORMATION AND RESEARCH DONE BY SCIENTISTS THAT HAVE STEPPED OUT OF THE "SYSTEM" RUN BY THE CORPORATE AGENDAS !
When your agencies are getting pressured by the ignorance of the very polluters ,we ask you to ask yourselves:" how are my actions effecting lives of others?" " how will my actions effect me in the future ? "how am I responsible?" " how do I stand up for the truth" There needs to be a letter to all the participants that OHA fact sheets were not the same as the independent study fact sheets, and we request that you allow us to submit to you the data for your review to be sent to ALL PARTICIPANTS. THESE ARE ALL CREDIBLE STUDIES, AND SHOW DIFFERENT FACTS THAN THOSE THAT HAVE BEEN GIVEN/ PRESENTED AT THE OHA OPEN HOUSE.

A.
WILL YOU ACCEPT CREDIBLE RESEARCH DATA OTHER THAN CORPORATE,AND DISTRIBUTE IT AS WELL, NOW THAT YOU HAVE ALREADY DISTRIBUTED THE CORPORATE 'FACT" SHEETS THAT DO NOT SEAM TO SEE ANY HEALTH PROBLEMS RELATED TO THEIR POISONS? WILL YOU SEND THE COUNTERING CREDIBLE DATA WE PROVIDE from our research TO THE PUBLIC?

3.

I am professionally trained in the ancient health diagnoses through the Chinese Medicine of nail, tongue, skin and eyes. This system of diagnosing is over 5,000 years old and has been proven more advanced than modern technology in the light of it's ability to diagnose early stages of the disease . In over 40 years of experience and research ,my mentor ,Dr .Chi has been able to pre-diagnose modern diseases many years in advance ,at their developmental stages. I was certified and trained in this ancient art and have observed the necessary markers in many families of our community. We have found that many families ,including children have shown to exhibit the markers of an early progress of disease ,long before the clinical tests will be able to show and diagnose. For an instance ,there are certain physical markers of bad estrogen, or xenoetrogens. But before I go ahead and explain some of those physical markers ,let me briefly explain what xenoestrogens are.

Xenoestrogens,also called environmental hormones or endocrine disrupting chemicals , and are substances that mimic the effects of estrogen. They attach to the receptors and disrupt endocrine functions! Common xenoestrogens are PESTICIDES!

CONSTANT LOW DOSAGE EXPOSURE to XENOESTROGENS can cause damage to the reproductive system and other organs and lead to cancer! In man it can reduce the sperm count, has feminizing qualities, increases the risk of testicular cancer (which by the way has been shown elevated in our community!),and are predominant precursors of prostate cancer. In woman it causes the early puberty, increases the risk of breast cancer. It also causes hormonal imbalance, gaining of weight and difficulty managing weight, uterine fibroids, ovarian cyst, fybrocystic breasts. High Bad Estrogen and Low Testosterone are also related to diabetes and insulin resistance. There are many studies already linking elevated mimicking estrogen from the environmental source (pesticides) and cardiovascular risk in both men and women. Those who have elevated levels have increased risk for blood cloths, arteriosclerosis, heart attack! No wonder why one of our community members who used to spray RoundUp comments that he had multiple symptoms of heart attacks. His wife , after his decades of spraying now has cancer!

Recent studies indicate that a high level of bad estrogen has pro -inflammatory effects and thus can increase damage to the blood vessels and increase the chance for stroke! (Gary, this is what Jan ,your wife, our dear friend was a victim of this year!,and what many others are dealing with due to the negligence and bureaucracy that needs to be eliminated forever!)

Going back to some of the physical markers that according to the Nail ,Tongue and Eye Chinese Medicine Diagnoses can show the early progress of the disease and the exposure to the xenoestrogens:
1. Red dots on the tongue
2. Cherry angiomas (little red dots) on the abdomen ,face and other parts of the body (chest,etc).
3. White spots on the nails
4. Facial hyperpigmentation
5. Exposed blood vessels in the eyes
These are just some common markers that often appear early in the progress of disease and long before clinical tests will show estrogen dominance. Again we have found these markers on MANY in our community members
observed ,including children! We are also aware of many still born, deformed children and recently have found out about several stunted

growth children in our community, as well ADHD and behavioral problems, etc.
There has been many miscarriages in our community as well early puberty, brain tumors ,cancers ,etc. We are not going to go into details of our own health experiences here, but are documenting everything as much as all the other community groups are doing now so we heard! It is very obvious that this community and others are not standing for this ignorance and that denial of the correlation to our chronic exposure to these pesticides must cease !
Here is the link to the article on the behavioral effects of the Xenoestrogens. by Prof Giancarlo Panzica University of Torino :
http://www.scitopics.com/Behavioral_effects_of_Xenoestrogens.html

When one understands the magnitude of suffering these substances cause one must face the responsibility to eliminate self destructive substances from their use, and as Eron said in the below e mail everyone needs to stand up sooner or later, for lies can only live so long! Humans are the only species that self destruct ,yet call themselves intelligent? Humans that are knowingly or ignorantly feeding the destructiveness in any way must realize that the actions they are taking through their own will can only come back to the it's source! Looking at the information test report conclusions, fact sheets given to the public ,so far this study has not served the public truthfully and it has not encompassed independent ,non corporate scientific facts! THIS CAN STILL CHANGE, BUT IF IT DOES NOT IT WILL NOT BE ACCEPTED BY WHAT OUR RESEARCH ,EXPERIENCE HAS SHOWN TO US TO BE TRUE!

THIS BELOW IS VERY IMPORTANT INFORMATION TO CONSIDER IN REGARDS TO LOW LEVEL ,CHRONIC EXPOSURE TO THE XENOESTROGENS IN THE ENVIRONMENT:

Invasion of the Endocrine Disruptors Are tiny amounts of man made chemicals having a big effect on human beings? UTMB professor Cheryl Watson thinks she's found how they might be.

By Jim Kelly

The first time UTMB professor Cheryl Watson heard of the endocrine disruptor hypothesis, she says, “I thought, now there’s a scary idea.” This was in the early 1980s, and the scary idea was that chemicals in the environment might interfere with the vital hormonal signaling networks that govern animal and human reproduction, development, and behavior.

A small but growing group of wildlife biologists, environmental toxicologists, cancer researchers, and specialists in developmental disorders suggested such interference could be causing a host of problems that recently seemed to have emerged in both animals and humans. Endocrine disruption caused by pollution, they said, was the best explanation for why certain populations of birds near the Great Lakes had mysteriously become unable to produce viable eggs, or in some cases had become completely uninterested in courtship and mating. It might also explain the sudden appearance in England of fish possessing both male and female sex characteristics, and male alligators in a Florida lake with sex organs one-third to one-half normal size. In humans, some asserted, exposure to endocrine disruptors before or just after birth could account for an apparent decline in the average quantity and quality of sperm, as well as an increase in the rates of breast, prostate, and testicular cancer.

We knew that animals were being affected," Watson says. "You had these classic cases of DDT causing thin-shelled bird eggs and alligators in Lake Apopka in Florida having very small reproductive organs, things like that." There was no reason, she remembers thinking, to assume that humans were immune to these effects.

And yet, many scientists seemed to be doing just that. Their strongest arguments centered on the lack of an explanation for how the very low doses of endocrine-disrupting chemicals to which most people were exposed could produce any effect at all. Take the largest group of endocrine disruptors, the so-called "estrogen mimics" or "xenoestrogens," which included such notoriously toxic substances as DDT, dioxins, and PCBs. Certainly they seemed to be able to imitate the body's own dominant estrogen, estradiol, in some ways—they could make breast cancer cells grow. But at realistic exposure levels they barely triggered any response at all in experiments designed to measure traditional mechanisms of hormone action. They were, at worst, thought to be "weakly estrogenic."

It was a messy question, and not just scientifically. The issue had become politically controversial as well, as environmentalists and breast-cancer activists called for tighter regulation of dozens of economically important manmade chemicals known to have estrogenic activity—everything from pesticides and herbicides to widely used detergents and plastics.

Some researchers might have just shrugged their shoulders and walked away. But Watson had a different reaction. "I always had a kind of curiosity about how we could see these effects in animals, and yet when we tested mechanistically in the laboratory, we didn't see any effects," she says. That curiosity combined with something else—years spent on the leading edge of estrogen signaling research — to produce an idea. Experiments conducted with her frequent collaborator Bahiru Gametchu had convinced her that steroids could act on cells through a "non-traditional pathway," one that at the time was not yet accepted by the majority of steroid signaling specialists and virtually was unknown outside the field. If a natural estrogen could trigger this mechanism, she reasoned, then it was possible that xenoestrogens could do the same thing. And if she could measure that effect, it might be possible to begin to determine the true threat these chemicals posed.

Now—after years of fine-tuning experiments, struggling against entrenched ideas about how hormones work and submitting and re-submitting papers to journal after journal—she believes she's succeeded.

To Watson, the results she and her team have produced seem both exciting and deeply disturbing. Watson's data, published in the National Institute of Environmental Health Sciences-sponsored journal Environmental Health Perspectives, suggest that xenoestrogens can act at concentrations almost too small to imagine, plugging themselves into cellular circuits that biologists are only beginning to understand. "We see xenoestrogens causing the same type of responses that physiological estrogens can, triggering responses at the same low doses for the most part," Watson says. "They're doing them in a different timing pattern, but they're still just as potent. The literature says they're weak estrogens, and they don't do anything unless you use high concentrations of them, and we're saying that's not true."

The literature says they're weak estrogens, and they don't do anything unless you use high concentrations of them, and we're saying that's not true."

Watson's journey to uncover a new signaling mechanism for xenoestrogens began with a colleague's mistake—the kind of serendipitous stumble so often recounted in tales of scientific discovery. That colleague was Gametchu, an immunologist, who in the late 1980s was pushing the limits of the then-new technology of fluorescent antibody labeling. These antibody "fluorescent tags" were designed to attach to only one kind of molecule. Once they got into a cell, examination with a microscope would show exactly where those molecules—in this case glucocorticoid receptors, important players in leukemia therapy—were located. Antibodies are not small enough to slip through a cell's membrane on their own, so Gametchu had to use a detergent to "punch holes" in his cells to get the fluorescent antibodies in. One day, Watson remembers, Gametchu forgot to use the detergent. By the time he realized his mistake, he was so close to the end of the experiment that he decided to go ahead and take a look through the microscope anyway.

What he saw surprised him—and it surprised Watson, too, when he invited her to come down the hall and look a few minutes later. "It was really weird," she remembers. "He was trying to determine if the receptor was in the cell nucleus or in the cytoplasm. You would expect to see either a cell with the nucleus all lit up or a cell with an empty nucleus and the cytoplasm all lit up. What I saw was a cell with glowing polka dots all over its surface."

Both the glucocorticoid and the estrogen signaling molecules that Gametchu and Watson studied were classified as steroid hormones. According to current theory, that meant they slid straight through the plasma membrane surrounding a cell to dock with glucocorticoid and estrogen receptor molecules inside the cell—the only place, according to the conventional wisdom of the time, that such receptors existed. And yet, now Gametchu seemed to have found glucocorticoid receptors on the cell's outside.

Talking it over with Gametchu a few days later, Watson remembered hearing about a Stanford researcher named Clara Szego who had published data in the 1970s that seemed to show that steroid hormone receptors—estrogen receptors, in fact—existed on the outer membranes of cells. Szego's claims had been widely disputed, but now it looked as if she might have been right.

Immediately, Watson began thinking about how she might do antibody experiments to look for estrogen receptors on the cell membrane. Estrogen signaling was known to play a critical part in the genesis of breast cancer, and estrogen receptors on the cell membrane might act differently from those inside the cell, activating previously unsuspected biochemical circuits. If they could be found, membrane estrogen receptors could help supply a major piece of the breast-cancer puzzle.

For the next five years, with the help of Gametchu and graduate student Todd Pappas, Watson worked to develop a technique that would enable her to use antibodies to see membrane estrogen receptors. The system she settled on employed a line of rat pituitary gland tumor cells known to respond quickly to estrogen, and a custom-made antibody to the estrogen receptor. (Estrogen receptor antibodies were just beginning to be

commercially available, but were prohibitively expensive because they were being marketed for breast-cancer clinical diagnosis, not research.) At the end of a long, difficult process of trial and error, she succeeded: “Using our antibody to the estrogen receptor, we saw essentially the same thing that we had seen with the membrane glucocorticoid receptors.”

Seeing estrogen receptors on the membrane was a great achievement, but getting other people to see them, too, turned out to be much harder than Watson had anticipated. The orthodox view that steroid hormone receptors were found only inside cells was strongly held by the researchers who reviewed articles on this subject for scientific journals. “We spent a lot of energy in the first few years of this effort submitting papers over and over again, re-writing and re-submitting—papers typically would take four and five different submissions to different journals before we could get them accepted,” Watson says.

But in 1995, they managed to break through with a paper in FASEB Journal , a widely respected and read scientific publication. Around the same time, Watson published a paper titled “The Other Estrogen Receptor in the Plasma Membrane: Implications for the Actions of Environmental Estrogens” in Environmental Health Perspectives. (“Environmental estrogens” is a term used for xenoestrogens or estrogen mimics that are environmental contaminants.) In that paper, Watson described her group’s finding that estradiol could interact with membrane estrogen receptors and within minutes trigger inflows of calcium ions that led to the release of large quantities of prolactin, a powerful reproductive hormone whose wide range of effects included the promotion of lactation and maternal behavior.

The speed of this response meant that it had to be what researchers called “non-genomic” or “non-nuclear”—that is, it had to be happening through a different mechanism than the relatively slow process by which scientists had traditionally thought hormones worked. That pathway required the involvement of DNA (the genome, thus—“genomic”) and RNA in the cell’s nucleus to produce new proteins like prolactin, and it took hours or even days, not minutes. The implication was that if membrane estrogen receptors could respond to estradiol in such a rapid, unexpected fashion, they might be doing something similar in response to xenoestrogens—and doing it so much faster that scientists weren’t catching it.

“People were so wed to the idea of a genomic response, and when you’re looking at genomic responses you usually don’t check until twenty-four or forty-eight hours, when you’re sure you can get a good signal,” Watson says. “Well, you could be completely missing a whole mechanism because you chose time points that didn’t let you see it.”

If researchers were “missing a whole mechanism,” Watson recognized, it could shed light on why they’d had so much trouble explaining the apparent xenoestrogen effects observed in wildlife—and, increasingly, in laboratory animals—with test-tube experiments on cells. Those experiments had been designed to detect genomic responses, and they all seemed to show that xenoestrogens only produced such effects at very high concentrations, a thousand or even ten thousand times as high as estradiol. “People look at that and say, that’s a lot,” Watson says. “What are we worried about? Even if a chemical company spills a lot of this stuff it gets quickly diluted below that level. Very rarely would you ever have that kind of quantity around.” But if, like estradiol,

xenoestrogens could also work through a membrane receptor, who knew how little might be needed to produce a significant response?

Watson had a hunch the answer would be interesting, and so in 2001 she and postdoctoral researcher Nataliya Bulayeva began developing a series of experiments to look at the responses of rat pituitary tumor cells to a number of known xenoestrogens.

The chemicals studied included pesticides (endosulfan and dieldrin) and their breakdown products (DDE, for example, produced when DDT is metabolized); coumestrol, a xenoestrogen produced by plants and found in alfalfa sprouts and sunflower seeds; and two compounds widely used to make plastics, nonylphenol and bisphenol A. They also tested DES, a synthetic estrogen that had been given to pregnant women from 1940 to the early 1970s in the mistaken belief that it prevented miscarriages. In fact, researchers later found that the daughters of women who had taken DES during pregnancy faced a far greater risk of a rare type of vaginal cancer and malformations of the reproductive tract.

“Our method is more convenient—it gives you more data faster, and that’s the point, the power of numbers,”

Primarily carried out by Bulayeva and research assistant Ann Wozniak, the lab work focused on detecting whether exposure to the different xenoestrogens caused rapid responses in calcium inflow and prolactin release; it also compared the level and time course of any responses to those induced by estradiol. It aimed as well to measure changes in extracellular-regulated kinases (ERKs), enzymes inside the cell that were known to participate in many different signaling processes—some of which led to quick non-genomic responses and others of which influenced complicated processes like cell division.

ERK measurements had been done before, but Bulayeva had adapted a more efficient and precise mechanized process that enabled her to rapidly and accurately scan large numbers of cell preparations. “Our method is more convenient—it gives you more data faster, and that’s the point, the power of numbers,” Bulayeva says. Still, “faster” is a relative term when one is testing the effects of varying concentrations of multiple chemicals at exposure times ranging from three to thirty minutes. Once the ERK analysis system was ready, it took more than six months for Bulayeva to gather the needed data.

The results of the ERK studies and the calcium and prolactin experiments, though, looked like they were worth the wait. “We looked at these data and said these things are just as potent as physiological estrogens like estradiol if you look at these mechanisms,” Watson says.

Take bisphenol A, for example. The chemical is a near-ubiquitous component of the polycarbonate plastics used to make beverage bottles and other food packaging, and it easily leaches into its surroundings, making exposure virtually impossible to avoid; a 2005 Centers for Disease Control study examining American urine samples found that 95 percent contained measurable levels of bisphenol A. The Watson lab’s results showed that cells exposed to less than one part per trillion concentrations of bisphenol A doubled their prolactin output.

Watson’s work was cited in two recent papers by bisphenol A expert Frederick vom Saal of the University of Missouri. One, published in the Proceedings of the National Academy of Sciences, described prostate

gland abnormalities found in male mouse fetuses whose mothers had been fed doses of bisphenol A lower than those normally consumed by pregnant women. The other, a commentary published in Environmental Health Perspectives, reviewed the latest test-tube, animal, and epidemiological studies of the low-dose effects of bisphenol A, noted significant differences in results produced by industry- and government-sponsored research, and called on the EPA to re-assess the risks posed by the chemical.

Other data from the Watson group were almost as disturbing. They showed prolactin output activity equal to that prompted by the human hormone estradiol at levels below one part per billion of the pesticide endosulfan and the DDT breakdown product DDE. And all the xenoestrogens tested—except bisphenol A—appeared to be causing strong ERK activation within a matter of minutes at very low concentrations.

"People thought they could ignore such low levels, that in real life unless you bathe in this stuff you don't have to worry about it," Watson says. "There are hundreds of articles over the last ten years or so, all saying that you need micromolar quantities of these compounds to get a reaction through the nuclear estrogen receptor gene expression route, and now we come along and say we've got nanomolar responses—responses at concentrations a thousand times lower. Well, at the last meeting when I presented this it got people's attention. They went yikes!"

The Watson group's results had implications that went beyond simple shock value. One of the most profound was the way their unusually detailed data showed the paradoxical responses of living things to different levels of xenoestrogens at different points in time. When it comes to endocrine signaling effects, the toxicological truism that "the dose makes the poison"—the greater the level of a toxic substance, the greater the response—has recently come to be seen as too simplistic. Reactions to low doses of hormones or hormone mimics can be much greater than reactions to high doses, and such reactions do not simply increase or decrease in a simple fashion over time.

"Here's the estradiol ERK response," Watson says, sketching a curve in purple ink on a piece of scrap paper. "Here's the response in this case to a phytoestrogen, a plant estrogen. Now they both do the trick, but look at the complete difference in phasing." The two curves don't match up—one peaks sooner than the other, and then both show second, non-matching peaks. Watson uses the sketch to pose a straightforward question: Do the two add together, cancel each other out, or interact in some other fashion? This opens the door to an incredibly complex problem.

Watson's results are based on work within a single cell line, testingone xenoestrogen at a time. Even so they seem to show an incredible range of effects. What happens at the tissue level, or at the level of a whole organism that might be exposed at any stage of development? For that matter, what happens to a human—a developing fetus, a newborn baby, a child, an adolescent, an adult—exposed, as we all to some degree have been, to multiple xenoestrogens at the same time?

"That's a really important question," Watson says. "We're at the point of depicting these mechanisms that each compound elicits in a dose and time-dependent way. But when you put them all together, which is undoubtedly how we see them—we know these things are in combinations in the environment—God only knows what these things do together. I think

that needs to be studied. We’re really only at the beginning of this.”

Thanks for your time ,and please address our questions,

D/M