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EXECUTIVE SECRETARIAT

July 13, 2007

Hon. Stephen L. Johnson, Administrator
U.S. Environmental Protection Agency
Ariel Rios Building
1200 Pennsylvania Avenue, N.W.
Washington, D.C. 20460

Re: **Petition for Proposal and Promulgation of Revised Water Quality Standards for Alabama**

Dear Mr. Johnson:

Enclosed is a Petition for Proposal and Promulgation of Revised Water Quality Standards for Alabama submitted on behalf of the Alabama Rivers Alliance, Inc. and Conservation Alabama, Inc.

If you require any further information to respond to this petition, please do not hesitate to contact me.

Sincerely,

David A. Ludder
Attorney for Petitioners

cc: Jimmy I. Palmer, Regional Administrator
U.S. Environmental Protection Agency - Region 4

9150 McDougal Court ◆ Tallahassee ◆ Florida 32312-4208 ◆ Telephone 850-386-5671
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such reference (RfD) dose values which the Administrator determines to be in accordance with the Clean Water Act.

Respectfully submitted,

DAVID A. LUDDER
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Water Act that water quality standards "protect the public health or welfare, enhance the quality of water and serve the purposes of [the Clean Water Act]."

32. Promulgation of a federal rule establishing a reference dose (RfD) value of 0.3 mg/(kg-day) for Phenol and 0.0005 mg/(kg-day) for Acrolein to be used in lieu of the reference dose (RfD) values in Ala. Admin. Code Chap. 335-6-10, Appendix A (or, in the alternative, water quality criteria for Phenol and Acrolein based on such reference dose (RfD) values), is necessary to "protect the public health or welfare, enhance the quality of water and serve the purposes of [the Clean Water Act]."

Request for Relief

Petitioners request that the Administrator grant the following relief:

A. promptly prepare and publish a proposed federal regulation setting forth a reference dose (RfD) value of 0.3 mg/(kg-day) for Phenol and 0.0005 mg/(kg-day) for Acrolein to be used in lieu of the reference dose (RfD) values in Ala. Admin. Code Chap. 335-6-10, Appendix A (or, in the alternative, water quality criteria for Phenol and Acrolein based on such reference dose (RfD) values).

B. promulgate a federal regulation setting forth a reference dose (RfD) value of 0.3 mg/(kg-day) for Phenol and 0.0005 mg/(kg-day) for Acrolein to be used in lieu of the reference dose (RfD) values in Ala. Admin. Code Chap. 335-6-10, Appendix A (or, in the alternative, water quality criteria for Phenol and Acrolein based on such reference dose (RfD) values) not later than ninety (90) days after publication of the proposed federal regulation, unless prior to such promulgation, the Alabama Department of Environmental Management has adopted revised or new reference dose (RfD) values for Phenol and Acrolein and water quality criteria for Phenol and Acrolein based on

28. The conclusion of the Environmental Management Commission of the Alabama Department of Environmental Management that substantive, credible and relevant evidence, data and information supporting the proposed revision of the reference dose (RfD) values for Phenol and Acrolein was not presented is contradicted by the new scientific evidence of the toxicity of Phenol and Acrolein published in *IRIS Data Summary for Phenol* (Exhibit B), *Toxicological Review of Phenol*, EPA/635/R-02/006 (Sept. 2002) (Exhibit C), *IRIS Data Summary for Acrolein* (Exhibit E), and *Toxicological Review of Acrolein*, EPA/635/R-03/003 (May 2003) (Exhibit F), presented to the Commission on April 20, 2007.

29. The refusal of the Environmental Management Commission of the Alabama Department of Environmental Management to revise the reference dose (RfD) values for Phenol and Acrolein in Ala. Admin. Code Chap. 335-6-10, Appendix A contradicts the national policy expressed in the Clean Water Act that the discharge of toxic pollutants in toxic amounts be prohibited.

30. Promulgation of a federal rule establishing a reference dose (RfD) value of 0.3 mg/(kg-day) for Phenol and 0.0005 mg/(kg-day) for Acrolein to be used in lieu of the reference dose (RfD) values in Ala. Admin. Code Chap. 335-6-10, Appendix A (or, in the alternative, water quality criteria for Phenol and Acrolein based on such reference dose (RfD) values), is necessary to fulfill the national policy expressed in the Clean Water Act that the discharge of toxic pollutants in toxic amounts be prohibited.

31. The refusal of the Environmental Management Commission of the Alabama Department of Environmental Management to revise the reference dose (RfD) values for Phenol and Acrolein in Ala. Admin. Code Chap. 335-6-10, Appendix A contradicts the mandate of the Clean

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Admin. Code R. 335-6-10-.07(1)(g).

IRIS Data Summary for Acrolein (Exhibit E), and *Toxicological Review of Acrolein*, EPA/635/R-03/003 (May 2003) (Exhibit F), the Commission concluded that "[t]he petition is not supported by such substantive, credible and relevant evidence, data and information as would reasonable support the proposed rule . . .." This conclusion was likely reached because the U.S. Environmental Protection Agency has not updated its National Recommended Water Quality Criteria to reflect the new reference dose (RfD) values for Phenol and Acrolein in the Integrated Risk Information System (IRIS). *Transcript of June 29, 2007 Environmental Management Commission Meeting* at 118-121, 126-128 (Exhibit M).[3]

Argument

27. The refusal of the Environmental Management Commission of the Alabama Department of Environmental Management to revise the reference dose (RfD) values for Phenol and Acrolein in Ala. Admin. Code Chap. 335-6-10, Appendix A contradicts the EPA-approved requirement of Ala. Admin. Code R. 335-6-10-.07(1)(d)1.(iii) that reference dose values shall be values available through the U.S. Environmental Protection Agency's Integrated Risk Information System (IRIS).[4]

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[3] The Commission also dismissed the petition to amend Ala. Admin. Code Chap 335-6-10, Appendix A because the adoption of revised criteria "would impact the overall regulatory scheme of the Department." As explained by the Director of the Department, the "impact" would have been the discharge permit modifications made necessary by the adoption of the more protective reference dose (RfD) values. *Transcript of June 29, 2007 Environmental Management Commission Meeting* (Exhibit M) at 117-118 (referring to discussion at 41-44).

[4] Neither the Director of the Alabama Department of Environmental Management nor the Environmental Management Commission of the Alabama Department of Environmental Management has ever asserted or demonstrated that the reference dose (RfD) values for Phenol and Acrolein in Ala. Admin. Code Chap. 335-6-10, Appendix A were "determined by the Department in consultation with the Department of Public Health after review of information available from other sources other than the U.S. Environmental Protection Agency's Integrated Risk Information System (IRIS)." Ala.

for Acrolein published in the Integrated Risk Information System (IRIS) was explained by an official of the U.S. Environmental Protection Agency as follows:

> Criteria in the [National Recommended Water Quality Criteria] table are updated by the Office of Water on a periodic basis. The table was updated in 2002 and most recently in 2003 . . .. * * * Due to process time lines for updating criteria (i.e. publication in public dockets, etc.), the most recent updates to the table do not include the current RfDs for phenol and acrolein. This in no way reflects a rejection of the IRIS published RfDs. The Office of Water fully accepts IRIS values as the Agency's most recent quantitative risk assessment values which have been reviewed and agreed upon by an interdisciplinary group of scientists representing various Program Offices within the Agency and represents an Agency wide consensus.

Correspondence from Dr. Heidi Bethel, Health and Ecological Criteria Division, Office of Science and Technology, Office of Water, U.S. Environmental Protection Agency to David A. Ludder (Dec. 15, 2006) (Exhibit K).

25. On April 20, 2007, nineteen organizations petitioned the Environmental Management Commission of the Alabama Department of Environmental Management to amend Ala. Admin. Code Chap 335-6-10, Appendix A to revise the reference dose (RfD) values for Phenol and Acrolein to be consistent with the reference dose (RfD) values published in the Integrated Risk Information System (IRIS) (Exhibit L).

26. On June 29, 2007, the Environmental Management Commission of the Alabama Department of Environmental Management denied the petition to amend Ala. Admin. Code Chap 335-6-10, Appendix A to revise the reference dose (RfD) values for Phenol and Acrolein to be consistent with the reference dose values published in the Integrated Risk Information System (IRIS). *Transcript of June 29, 2007 Environmental Management Commission Meeting* (Exhibit M) at 115-132. Despite the fact that the petitioners submitted to the Commission the *IRIS Data Summary for Phenol* (Exhibit B), *Toxicological Review of Phenol*, EPA/635/R-02/006 (Sept. 2002) (Exhibit C),

22. On December 3, 2004, the Alabama Department of Environmental Management adopted several amendments to Ala. Admin. Code Chap. 335-6-10, Appendix A. The *Summary of Reasons* for the amendments states that it "will make the Department's toxic pollutant criteria consistent with EPA's recommended criteria reflected in its guidance document entitled 'National Recommended Water Quality Criteria: 2002' . . .." (Exhibit H). See also *Integrated Water Quality Assessment and Monitoring Report 2006, Chap. 1 Water Quality Standards* ("The adopted revisions make the Department's toxic pollutant criteria consistent with EPA's recommended criteria as reflected in its guidance document entitled 'National Recommended Water Quality Criteria: 2002,' as well as the fifteen (15) recommended water quality criteria EPA revised and published in the Federal Register on December 27, 2002 . . .") (Exhibit I). No revisions of the reference dose (RfD) values for Phenol or Acrolein were proposed or adopted to make such values consistent with values published by the U.S. Environmental Protection Agency in the Integrated Risk Information System (IRIS) in 2002 and 2003. Ala. Admin. Code Chap. 335-6-10, Appendix A (2005).

23. Ala. Admin. Code Chap. 335-6-10, Appendix A (2005) was approved on August 17, 2005 by James D. Giattina, Director, Water Management Division, U.S. Environmental Protection Agency, Region 4, pursuant to 33 U.S.C. § 1313.

24. In 2006, the U.S. Environmental Protection Agency published *National Recommended Water Quality Criteria* (2006) (Exhibit J). The recommended criteria for Acrolein and Phenol were not revised to incorporate the revised reference dose (RfD) values for Phenol (0.3 mg/(kg-day)) and new reference dose value for Acrolein (0.0005 mg/(kg-day)) published in the Integrated Risk Information System (IRIS). The reason the *National Recommended Water Quality Criteria* (2006) did not include the revised reference dose (RfD) value for Phenol and new reference dose (RfD) value

Management Division, U.S. Environmental Protection Agency, Region 4, pursuant to 33 U.S.C. § 1313.

18. On September 30, 2002, the U.S. Environmental Protection Agency published a revised reference dose (RfD) value for Phenol in the Integrated Risk Information System (IRIS). The revised reference dose (RfD) value for Phenol is 0.3 mg/(kg-day). *IRIS Data Summary for Phenol* (Exhibit B). The derivation of the revised reference dose (RfD) value for Phenol is more fully explained in *Toxicological Review of Phenol*, EPA/635/R-02/006 (Sept. 2002) (Exhibit C).

19. In November 2002, the U.S. Environmental Protection Agency published *National Recommended Water Quality Criteria: 2002* (EPA-822-R-02-047) (Exhibit D). The recommended criteria for Phenol incorporated a reference dose (RfD) value of 0.6 mg/(kg-day). The recommended criteria for Acrolein incorporated an Acceptable Daily Intake value of 0.0156 mg/(kg-day). See *National Recommended Water Quality Criteria: 2002 - Human Health Criteria Calculation Matrix* (EPA-822-R-02-012, Nov. 2002) (Exhibit A).

20. On June 3, 2003, the U.S. Environmental Protection Agency published a new reference dose (RfD) value for Acrolein in the Integrated Risk Information System (IRIS). The new reference dose (RfD) value for Acrolein is 0.0005 mg/(kg-day). *IRIS Data Summary for Acrolein* (Exhibit E). The derivation of the new reference dose (RfD) value for Acrolein is more fully explained in *Toxicological Review of Acrolein*, EPA/635/R-03/003 (May 2003) (Exhibit F).

21. On December 31, 2003, the U.S. Environmental Protection Agency published a table of updated national recommended water quality criteria for fifteen (15) toxic pollutants which did not include Phenol and Acrolein. 68 Fed. Reg. 75507 (2003) (Exhibit G).

$$conc.\ (mg/l) = (HBW \times RfD)/(FCR \times BCF) \qquad \textbf{Eq. 17}$$

where:

HBW = human body weight, set at 70 kg
RfD = reference dose, in mg/(kg-day)
FCR = fish consumption rate, set at 0.030 kg/day
BCF = bioconcentration factor, in l/kg
WCR = water consumption rate, set at 2 l/day

15. Ala. Admin. Code R. 335-6-10-.07(1)(d)1.(iii) (1994) provided:

> The values used for the reference dose (RfD) shall be values available through the U.S. Environmental Protection Agency's Integrated Risk Information System (IRIS), and values used for the bioconcentration factor (BCF) shall be values contained in ambient water quality criteria documents published by the U.S. Environmental Protection Agency, except where other values are established pursuant to subparagraph (1)(g). The RfD and BCF values for specific pollutants are provided in Appendix A.[2]

16. Ala. Admin. Code Chap. 335-6-10, Appendix A (1994) retained a reference dose (RfD) value for Phenol of 0.6 mg/(kg-day) and for Acrolein of 0.0156 mg/(kg-day).

17. Ala. Admin. Code R. 335-6-10-.07 (1994) and Ala. Admin. Code Chap. 335-6-10, Appendix A (1994) were approved on August 2, 1995 by Robert F. McGhee, Acting Director, Water

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[2] Also adopted on July 20, 1994 were revisions to Ala. Admin. Code R. 335-6-10-.07(1)(g) (1994) which, as revised, provided:

> Numeric criteria may be computed by the Department from equations 16, 17, 18, and 19 using values for the reference dose (RfD), cancer potency factor (CPF), and bioconcentration factor (BCF) determined by the Department in consultation with the Department of Public Health after review of information available from other sources other than the U.S. Environmental Protection Agency's Integrated Risk Information System (IRIS) or ambient water quality criteria documents. Such criteria, or the RfD, CPF, and BCF values used to compute criteria, shall not be effective until adopted following established rulemaking procedures.

11. Ala. Admin. Code Chap. 335-6-10, Appendix A (1991) included a reference dose (RfD) value for Phenol of 0.6 mg/(kg-day) and for Acrolein of 0.0156 mg/(kg-day).

12. Ala. Admin. Code R. 335-6-10-.07 (1991) and Ala. Admin. Code Chap. 335-6-10, Appendix A (1991) were approved on July 18, 1991 by Greer C. Tidwell, Regional Administrator, U.S. Environmental Protection Agency, Region 4, pursuant to 33 U.S.C. § 1313.

13. On July 20, 1994, the Alabama Department of Environmental Management adopted revisions to Ala. Admin. Code Chap. 335-6-10 addressing water quality criteria for toxic pollutants. These revisions included Ala. Admin. Code R. 335-6-10-.07 (1994) and Ala. Admin. Code Chap. 335-6-10, Appendix A (1994).

14. Water quality criteria for toxic pollutants were expressed as equations in Ala. Admin. Code R. 335-6-10-.07 (1994). For waters classified Public Water Supply (PWS), where consumption of contaminated water and fish may occur, the equation for non-carcinogenic toxic pollutants was as follows:

$$conc.\ (mg/l) = (HBW \times RfD)/((FCR \times BCF) + WCR) \qquad \textbf{Eq. 16}$$

For all other waters, where consumption of contaminated fish may occur, the equation for non-carcinogenic toxic pollutants was as follows:

Numeric criteria different than those specified in subparagraph (1)(d) hereof may be computed by the Department from equations 16, 17, 18, and 19 using values for the reference dose (RfD), cancer potency factor (CPF), and bioconcentration factor (BCF) determined by the Department in consultation with the Department of Public Health after review of information available from other sources other than the U.S. Environmental Protection Agency's Integrated Risk Information System (IRIS) or ambient water quality criteria documents. Such criteria shall not be effective until adopted following established rulemaking procedures, after which time the numeric criteria shall be listed in Table 1.

8. On February 20, 1991, the Alabama Department of Environmental Management adopted revisions to Ala. Admin. Code Chap. 335-6-10 addressing water quality criteria for toxic pollutants. These revisions included Ala. Admin. Code R. 335-6-10-.07 (1991) and Ala. Admin. Code Chap. 335-6-10, Appendix A (1991).

9. Water quality criteria for toxic pollutants were expressed as equations in Ala. Admin. Code R. 335-6-10-.07 (1991). For waters classified Public Water Supply (PWS), where consumption of contaminated water and fish may occur, the equation for non-carcinogenic toxic pollutants in Ala. Admin. Code R. 335-6-10-.07 (1991) was as follows:

$$conc.\ (mg/l) = (HBW \times RfD)/((FCR \times BCF) + WCR) \qquad \textbf{Eq. 16}$$

For all other waters, where consumption of contaminated fish may occur, the equation for non-carcinogenic toxic pollutants was as follows:

$$conc.\ (mg/l) = (HBW \times RfD)/(FCR \times BCF) \qquad \textbf{Eq. 17}$$

where:

HBW = human body weight, set at 70 kg
RfD = reference dose, in mg/(kg-day)
FCR = fish consumption rate, set at 0.0065 kg/day
BCF = bioconcentration factor, in l/kg
WCR = water consumption rate, set at 2 l/day

10. Ala. Admin. Code R. 335-6-10-.07(1)(d)1.(iii) (1991) provided:

> The values used for the reference dose (RfD) shall be values available through the U.S. Environmental Protection Agency's Integrated Risk Information System (IRIS), and values used for the bioconcentration factor (BCF) shall be values contained in ambient water quality criteria documents published by the U.S. Environmental Protection Agency. These values are provided in Appendix A.[1]

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[1] Also adopted on February 20, 1991 was Ala. Admin. Code R. 335-6-10-.07(1)(g) (1991) which provided:

> The Administrator shall promulgate any revised or new standard under this paragraph not later than ninety days after he publishes such proposed standards, unless prior to such promulgation, such State has adopted a revised or new water quality standard which the Administrator determines to be in accordance with this chapter.

5. 5 U.S.C. § 553(e) provides that "[e]ach agency shall give an interested person the right to petition for the issuance, amendment, or repeal of a rule."

Petitioners' Interests

6. The Petitioners are membership organizations dedicated to the protection of human health and the environment. Members of Petitioners use and enjoy the surface waters of the State of Alabama for fishing and drinking. Ala. Admin. Code Chap. 335-6-10 authorizes toxic pollutants to be present in surface waters. The U.S. Environmental Protection Agency has determined that Phenol and Acrolein are more toxic than indicated in Ala. Admin. Code Chap. 335-6-10, Appendix A. Exposure to Phenol and Acrolein in surface waters at levels authorized by Ala. Admin. Code Chap. 335-6-10 presents an excessive risk of adverse health effects to the members of Petitioners.

Facts

7. Immediately prior to February 20, 1991, the reference dose (RfD) value for Phenol published in the U.S. Environmental Protection Agency's Integrated Risk Information System (IRIS) was 0.6 mg/(kg-day). At that time, the Integrated Risk Information System (IRIS) did not include a reference dose (RfD) value for Acrolein. See *National Recommended Water Quality Criteria: 2002 - Human Health Criteria Calculation Matrix* (EPA-822-R-02-012, Nov. 2002) (Exhibit A). However, *Ambient Water Quality Criteria for Acrolein* (EPA 440/5-80-016) recommended a Acceptable Daily Intake value for Acrolein of 0.0156 mg/(kg-day).

BEFORE THE ADMINISTRATOR OF THE
UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

Alabama Rivers Alliance, Inc. and
Conservation Alabama, Inc.,

Petitioners.
_____/

PETITION FOR PROPOSAL AND PROMULGATION
OF REVISED WATER QUALITY STANDARDS FOR ALABAMA

Nature of Petition

1. This is a petition seeking to have the U.S. Environmental Protection Agency publish a proposed federal regulation and promulgate a final regulation establishing water quality criteria for Phenol and Acrolein applicable in the State of Alabama.

Authority

2. 33 U.S.C. § 1251(a)(3) provides that "it is the national policy that the discharge of toxic pollutants in toxic amounts be prohibited."

3. 33 U.S.C. § 1313(c)(2)A) requires that states adopt water quality standards that "protect the public health or welfare, enhance the quality of water and serve the purposes of this chapter."

4. 33 U.S.C. § 1313(c)(4) provides:

> The Administrator shall promptly prepare and publish proposed regulations setting forth a revised or new water quality standard for the navigable waters involved—
>
> (A) if a revised or new water quality standard submitted by such State under paragraph (3) of this subsection for such waters is determined by the Administrator not to be consistent with the applicable requirements of this chapter, or
>
> (B) in any case where the Administrator determines that a revised or new standard is necessary to meet the requirements of this chapter.

Exhibit A

United States
Environmental Protection
Agency

Office of Water
(4304T)

EPA-822-R-02-012
November 2002

National Recommended Water Quality Criteria: 2002

Human Health Criteria Calculation Matrix

Notices

This document contains information regarding the calculation of the human health criteria contained in the document entitled, *National Recommended Water Quality Criteria: 2002*. This document provides: cancer potency factors (q1*s); reference doses (RfDs); relative source contributions (RSCs); fish intake values; and equations used to derive the human health criteria in the aforementioned compilation.

This document is not a regulation and cannot substitute for the Clean Water Act or Environmental Protection Agency (EPA) regulations. Thus, the criteria in the calculation matrix cannot impose legally binding requirements on EPA, states, authorized tribes or the regulated community.

NRWQC: HUMAN HEALTH CRITERIA CALCULATION MATRIX (November 2002)

Priority Pollutants

	Chemical (CAS number)	Basis for 1998 nrwqc: q1* or RfD/ADI and BCF	1998 nrwqc (ug/l) w +o/ o only	Reference Cite[3]	Basis for 2002 nrwqc: q1*/ RfD/ADI, RSC and BCF[1,7,8]	Methodology: 2000 or 1980[2]	2002 nrwqc (ug/l) For Consumption of : Water + organism/ Organism Only	Reference Cite[3]
1	Antimony 77440 36 0	RfD = 4E-4 BCF = 1 FI = 6.5	14/4300	IRIS 02/01/91	RfD = 4E-4 RSC= 40% [a] BCF = 1 FI = 17.5	2000	5.6/640	IRIS 02/01/91
2	Arsenic 7440 38 2	q1* = 1.75 [9] BCF = 44 (for oysters) FI = 6.5	0.018/.14	57FR60848	q1* used = 1.75 [9] BCF = 44 FI = 6.5	1980	0.018/0.14	57FR60848
3	Berylium 7440 41 7	q1* = 4.3 BCF = 19	—	IRIS 09/01/92	—	NA	—	—
4	Cadmium 7440 43 9	RfD = 0.001(food) RfD= 0.0005(water)	—	IRIS 02/01/94	RfD = 1E-3 (food) RfD= 0.0005(water) RSC = 25%	NA	—	—
5	Chromium (III) 16065 83 1	RfD withdrawn BCF = 16	—	IRIS 03/01/88	1.5E+0	NA	—	—
5a	Chromium (VI) 18540 29 9	RfD = 5E-3 BCF = 16	—	IRIS 12/01/96	3E-3	NA	—	—
6	Copper 7440508	WQC based on DW Action level BCF = 36	1300/ --	AWQC ADDENDUM 1989 DRAFT final lead/copper rule 57 FR 26460	AWQC ADDENDUM 1989 DRAFT final lead/copper rule 57 FR 26460	Drinking water regulation	1,300/ --	WQC based on DW Action level

	Chemical (CAS number)	Basis for 1998 nrwqc: q1* or RfD/ADI and BCF	1998 nrwqc (ug/l) w +o/ o only	Reference Cite[3]	Basis for 2002 nrwqc: q1*/ RfD/ADI, RSC and BCF[1,7,8]	Methodology: 2000 or 1980[2]	2002 nrwqc (ug/l) For Consumption of : Water + organism/ Organism Only	Reference Cite[3]
7	Lead 7439921	WQC based on old Drinking water MCL	——	Lead/Copper Rule 57 FR 26460	——	NA	——	——
8	Mercury 7439976	RfD = 1E-4 BCF = 3760-9000 (PBCF = 7342.6) FI = 18.7	0.050/0.051	62 FR42160	Methylmercury CAS No. 22967926 RfD = 1E-4 ng/kg BW-day RSC = 2.7E-5 mg/kg BW-day (subtracted from RfD to account for marine fish consumption) FI = 17.5	2000	0.3 mg/kg	EPA 823-R-01-001
9	Nickel	RfD = 2E-2 BCF = 47 FI = 6.5	610/4600	IRIS 12/01/96 for Nickel, soluble salts used	RfD = 2E-2 BCF = 47 FI = 6.5	1980 (Undergoing Major reassessment, no revision)	610/4,600	IRIS12/01/96
10	Selenium 7782492	RfD = 5E-3 BCF =4.8 (1988 Addendum used) FI = 6.5	170/11,000[12]	IRIS 09/01/91	RfD = 5E-3 BCF =4.8 (1988 Addendum used) FI = 17.5	2000	170/4,200	IRIS 09/01/91
11	Silver 7440224	RfD = 5E-3 BCF = 0.5	——	IRIS 12/01/96	RfD = 5E-3 BCF = 0.5	NA	——	——
12	Thallium[5] 7440280	RfD = 6.8E-5[6] (RFD LISTED IS FOR THALLIUM SULFATE) BCF = 116 FI = 6.5	1.7/6.3	IRIS 09/01/90	RfD = 6.8E-5 [6,10,b] (RFD LISTED IS FOR THALLIUM (I) SULFATE 7446-18-6) BCF = 116 RSC = 20% (not used) FI = 6.5	1980	1.7/6.3	IRIS 09/01/90
13	Zinc 7440666	RfD = 3E-1 BCF = 47 FI = 6.5	9100/69,000[12]	IRIS 10/01/92	RfD = 3E-1 BCF = 47 FI = 17.5	2000	7,400/26,000	IRIS 10/01/92

	Chemical (CAS number)	Basis for 1998 nrwqc: q1* or RfD/ADI and BCF	1998 nrwqc (ug/l) w +o/ o only	Reference Cite[3]	Basis for 2002 nrwqc: q1*/ RfD/ADI, RSC and BCF[1,7,8]	Methodology: 2000 or 1980[2]	2002 nrwqc (ug/l) For Consumption of : Water + organism/ Organism Only	Reference Cite[3]
14	Cyanide 57125	RfD = 2E-2 BCF = 1 FI = 6.5	700/220,000	IRIS 02/01/93	RfD = 2E-2[10] BCF = 1 RSC = 20% [c] (not used) FI = 6.5	1980	700/220,000	IRIS 02/01/93
15	Asbestos 1332214	Based on drinking water MCL	7 million Fibers/L	DW MCL 56 FR 3526 1/30/91	Based on drinking water MCL		7 million Fibers/L	DW MCL 56 FR 3526 01/30/91
16	2,3,7,8-TCDD(Dioxin) 1746016	q1* = 1.56E+5 BCF = 5000 (q1* no longer listed on IRIS) FI = 6.5	1.3E-8/ 1.4E-8	1984 AWQC Document EPA 440/5-84-007	q1* = 1.56E+5 BCF = 5000 (q1* no longer listed on IRIS) FI = 17.5	2000	5.0E-9/5.1E-9	1984 AWQC Document EPA 440/5-84-007 + 2000 Methodology
17	Acrolein 107028	ADI = 15.6 ug/kg/day BCF = 215 FI = 6.5	320/780	1980 AWQC document EPA 440/5-80-016	ADI = 15.6 ug/kg/day (or 0.0156 mg/kg/day) BCF = 215 FI = 17.5	2000	190/290	1980 AWQC document EPA 440/5-80-016
18	Acrylonitrile 107131	q1* = 5.4E-1 BCF = 30 FI = 6.5	0.059/0.66	IRIS 01/01/91	q1* = 5.4E-1 BCF = 30 FI = 17.5	2000	0.051/0.25	IRIS 01/01/91
19	Benzene 71432	q1* = 2.9E-2 BCF = 5.2 FI = 6.5	1.2/71	IRIS 02/01/94	q1* = 1.5E-2 to 5.5E-2 BCF = 5.2 FI = 17.5	2000	0.61 - 2.2/14 - 51	IRIS 01/19/00
20	Bromoform 75252	q1* = 7.9E-3 BCF = 3.75 based on chloroform FI = 6.5	4.3/360	IRIS 01/01/91	q1* = 7.9E-3 BCF = 3.75 (based on chloroform) FI = 17.5	2000	4.3/140	IRIS 01/01/91
21	Carbon Tetrachloride 56235	q1* = 1.3E-1 BCF = 18.75 FI = 6.5	0.25/4.4	IRIS 06/01/91	q1* = 1.3E-1 BCF = 18.75 FI = 17.5	2000	0.23/1.6	IRIS 06/01/91

	Chemical (CAS number)	Basis for 1998 nrwqc: q1* or RfD/ADI and BCF	1998 nrwqc (ug/l) w +o/ o only	Reference Cite[3]	Basis for 2002 nrwqc: q1*/ RfD/ADI, RSC and BCF[1,7,8]	Methodology: 2000 or 1980[2]	2002 nrwqc (ug/l) For Consumption of : Water + organism/ Organism Only	Reference Cite[3]
22	Chlorobenzene 108907	RfD = 2E-2 BCF = 10.3 FI = 6.5	680/21,000	IRIS 07/01/93	RfD = 2E-2[10] BCF = 10.3 RSC = 20 % [d] (not used) FI =6.5	1980	680/21,000	IRIS 07/01/93
23	Chlorodi-bromomethane 124481	q1* = 8.4E-2 BCF = 3.75 based on chloroform FI = 6.5	0.41/34	IRIS 01/01/92	q1* = 8.4E-2[11] RfD = 2E-2 (not used) BCF = 3.75 (based on chloroform) RSC = 80% [k] (not used) FI = 17.5	2000	0.40/13	IRIS 01/01/92
24	Chloroethane 75003	——	——	——	——	NA-	——	——
25	2-Chloroethyl-vinyl Ether 110758	——	——	——	——	NA	——	——
26	Chloroform 67663	q1* = 6.1E-3 BCF = 3.75 FI = 6.5	5.7/470	IRIS 03/01/91	q1* = 6.1E-3 BCF = 3.75 FI = 6.5 (RfD = 1E-2; IRIS 10/19/01-not used)[4]	1980 (Undergoing Major reassessment, no revision)	5.7/470	IRIS 03/01/91
27	Dichloro-bromomethane 75274	q1* = 6.2E-2 BCF = 3.75 FI = 6.5	0.56/46	IRIS 03/01/93	q1* = 6.2E-2 BCF = 3.75 FI = 17.5	2000	0.55/17	IRIS 03/01/93
28	1,1-Dichloro-ethane 75343	q1* = 9.1	——	——	——	NA	——	——
29	1,2-Dichloro-ethane 107062	q1* = 9.1E-2 BCF = 1.2 FI = 6.5	0.38/99	IRIS 01/01/91	q1* = 9.1E-2 BCF = 1.2 FI = 17.5	2000	0.38/37	IRIS 01/01/91

	Chemical (CAS number)	Basis for 1998 nrwqc: q1* or RfD/ADI and BCF	1998 nrwqc (ug/l) w +o/ o only	Reference Cite[3]	Basis for 2002 nrwqc: q1*/ RfD/ADI, RSC and BCF[1,7,8]	Methodology: 2000 or 1980[2]	2002 nrwqc (ug/l) For Consumption of : Water + organism/ Organism Only	Reference Cite[3]
30	1,1-Dichloro-ethylene 75354	q1* = 6E-1 RfD = 9E-3 BCF = 5.6 FI = 6.5	0.057/3.2	IRIS 02/01/98	q1* = 6E-1[10] FI = 6.5 BCF = 5.6 (RfD = 5E-2 IRIS 08/13/02-not used) RSC = 20 % [e] (not used)	2000	0.057/3.2	IRIS 02/01/98
31	1,2-Dichlor-propane 78875	q1* = 6.7E-2 BCF = 4.1 (q1* not on IRIS, but verified at 6.7E-2) FI = 6.5	0.52/39	Draft IRIS Coversheet; DW reg. 56 FR 3526 1/30/91	q1* = 6.7E-2 BCF = 4.1 (q1* not on IRIS, but verified at 6.7E-2) FI = 17.5	2000	0.50/15	Draft IRIS Coversheet; DW reg. 56 FR 3526 1/30/91
32	1,3-Dichloro-propylene 542756	RfD = 3E-4 (low) BCF = 1.9 FI = 6.5	10 /1700	IRIS 10/01/90	RfD = 3E-4 [10] BCF = 1.9 FI = 6.5 (q1* = 0.1/ RfD = 3E-2 IRIS 05/25/00-not used)	1980	10/1,700	IRIS 10/01/90
33	Ethyl-benzene 100414	RfD = 1E-1 (low) BCF = 37.5 FI = 6.5	3100/29,000	IRIS 06/01/91	RfD = 1E-1 [10] BCF = 37.5 RSC = 20% [f] (not used) FI =6.5	1980	3,100/29,000	IRIS 06/01/91
34	Methyl-Bromide 74839	RfD = 1.4E-3 BCF = 3.75 Chloroform BCF used FI = 6.5	48/4000	IRIS 07/01/91	RfD = 1.4E-3 BCF = 3.75 (Chloroform BCF used) FI = 17.5	2000	47/1,500	IRIS 07/01/91
35	Methyl-Chloride 74873	q1* = 6.1E-3 (chloroform q1* and BCF used) BCF = 3.75 FI = 6.5	—	IRIS 03/01/91	—	—	—	—

	Chemical (CAS number)	Basis for 1998 nrwqc: q1* or RfD/ADI and BCF	1998 nrwqc (ug/l) w +o/ o only	Reference Cite[3]	Basis for 2002 nrwqc: q1*/ RfD/ADI, RSC and BCF[1,7,8]	Methodology: 2000 or 1980[2]	2002 nrwqc (ug/l) For Consumption of : Water + organism/ Organism Only	Reference Cite[3]
36	Methylene-Chloride 75092	q1* = 7.5E-3 BCF = 0.9 FI = 6.5	4.7/1600	IRIS 02/01/95	q1* = 7.5E-3 BCF = 0.9 FI = 17.5	2000	4.6/590	IRIS 02/01/95
37	1,1,2,2-Tetrachloro-ethane 79345	q1* = 2E-1 BCF = 5 FI = 6.5	0.17/11	IRIS 02/01/94	q1* = 2E-1 BCF = 5 FI = 17.5	2000	0.17/4.0	IRIS 02/01/94
38	Tetrachloro-ethylene 127184	q1* = 3.98E-2 BCF = 30.6 FI = 6.5	0.8/8.85	1980 AWQC DOCUMENT EPA 440/5-80-073	q1* = 3.98E-2 BCF = 30.6 FI = 17.5	2000	0.69/3.3	1980 AWQC DOCUMENT EPA 440/5-80-073 & 2000 FI
39	Toluene 108883	RfD = 2E-1 BCF = 10.7 FI = 6.5	6800/200,000	IRIS 04/01/94	RfD = 2E-1[10] BCF = 10.7 RSC = 20% [g] (not used) FI = 6.5	1980	6,800/200,000	IRIS 04/01/94
40	1,2-Trans-Dichloro-ethylene 156605	RfD = 2E-2 BCF = 1.58 FI = 6.5	700/140,000	IRIS 01/01/89	RfD = 2E-2[10] BCF = 1.58 RSC = 20% [h] (not used) FI = 6.5	1980	700/140,000	IRIS 01/01/89
41	1,1,1-Tri-chloroethane 71556	RfD = 9E-2 BCF = 5.6 RfD withdrawn 08/01/91	——	IRIS 09/01/90	——	NA	——	——
42	1,1,2-Trichloro-ethane 79005	q1* = 0.057 RfD = 0.004 BCF = 4.5 FI = 6.5	0.60/42	IRIS 07/01/94	q1* = 0.057[11] RfD = 4E-3 BCF = 4.5 RSC = 20% [i] FI = 17.5	2000 (based on q1* even though RSC and RfD are are available)	0.59/16	IRIS 02/01/1994
43	Trichloro-ethylene 79016	q1* = 1.26E-2 BCF = 10.6 RfD under rev q1* withdrawn FI = 6.5	2.7/81	1980 AWQC DOCUMENT EPA 440/5-80-077	q1* = 1.26E-2 BCF = 10.6 FI = 17.5	1980 AWQC DOCUMENT EPA 440/5-80-077 & 2000 FI	2.5/30	1980 AWQC DOCUMENT EPA 440/5-80-077 & 2000 FI

	Chemical (CAS number)	Basis for 1998 nrwqc: q1* or RfD/ADI and BCF	1998 nrwqc (ug/l) w +o/ o only	Reference Cite[3]	Basis for 2002 nrwqc: q1*/ RfD/ADI, RSC and BCF[1,7,8]	Methodology: 2000 or 1980[2]	2002 nrwqc (ug/l) For Consumption of : Water + organism/ Organism Only	Reference Cite[3]
44	Vinyl Chloride 75014	q1* = 1.74E-2 BCF = 1.17 FI = 6.5	2.0/525	1980 AWQC DOCUMENT EPA 440/5-80-078	q1* = 1.74E-2[10] (q1* = 1.4 LMS exposure from birth/RfD = 3E-3 IRIS 08/07/00- not used) BCF = 1.17 FI = 6.5	1980	2.0/530	1980 AWQC DOCUMENT EPA 440/5-80-078
45	2-Chlorophenol 95578	RfD = 5E-3 BCF = 134 FI = 6.5	120/400	IRIS 07/01/93	RfD = 5E-3 BCF = 134 FI = 17.5	2000	81/150	IRIS 07/01/93
46	2,4-Dichloro-phenol 120832	RfD = 3E-3 BCF = 40.7 FI = 6.5	93/790	IRIS 06/30/88	RfD = 3E-3 BCF = 40.7 FI = 17.5	2000	77/290	IRIS 06/30/88
47	2,4-Dimethyl-phenol 105679	RfD = 2E-2 BCF = 93.8 FI = 6.5	540/2300	IRIS 11/01/90	RfD = 2E-2 BCF = 93.8 FI = 17.5	2000	380/850	IRIS 11/01/90
48	2-Methyl-4,6-Dinitro-phenol (cresol) 534521	(0.039 mg/kg/day)/100 BCF = 5.5 FI = 6.5	13.4/765	1980 AWQC DOCUMENT EPA 440/5-80-063	(0.039 mg/kg/day)/100 BCF = 5.5 FI = 17.5	2000	13/280	1980 AWQC DOCUMENT EPA 440/5-80-063
49	2,4-Dinitro-phenol 51285	RfD = 2E-3 BCF = 1.5 FI = 6.5	70/14,000	IRIS 07/01/91	RfD = 2E-3 BCF = 1.5 FI = 17.5	2000	69/5,300	IRIS 07/01/91
50	2-Nitrophenol 88755	BCF = 2.33	———	———	———	NA	———	———
51	4-Nitrophenol 100027	BCF = 3.31	———	———	———	NA	———	———
52	3-Methyl-4-Chlorophenol 59507	RfD = 3E-2 (medium) q1* = 1.2E-1	———	IRIS MAY 1995	RfD = 3E-2 (medium) q1* = 1.2E-1	NA	———	———

	Chemical (CAS number)	Basis for 1998 nrwqc: q1* or RfD/ADI and BCF	1998 nrwqc (ug/l) w +o/ o only	Reference Cite[3]	Basis for 2002 nrwqc: q1*/ RfD/ADI, RSC and BCF[1,7,8]	Methodology: 2000 or 1980[2]	2002 nrwqc (ug/l) For Consumption of : Water + organism/ Organism Only	Reference Cite[3]
53	Pentachloro-phenol 87865	q1* = 0.12 BCF = 11 FI = 6.5	0.28/8.2	IRIS 07/01/93	q1* = 0.12 RfD = 3E-2 (not used) BCF = 11 FI = 17.5	2000	0.27/3.0	IRIS 07/01/93
54	Phenol 108952	RfD = 6E-1 BCF = 1.4 FI = 6.5	21,000/ 4,600,000	IRIS 02/01/90	RfD = 6E-1 BCF = 1.4 FI = 17.5	2000	21,000/1,700,000	IRIS 02/01/90
55	2,4,6-Trichlor-phenol 88062	q1* = 0.011 BCF = 150 FI = 6.5	2.1/6.5	IRIS 02/01/94	q1* = 0.011 BCF = 150 FI = 17.5	2000	1.4/2.4	IRIS 02/01/94
56	Acenaphthene 83329	RfD = 6E-2 BCF = 242 FI = 6.5	1200/2700	IRIS 04/01/94	RfD = 6E-2 BCF = 242 FI = 17.52	2000	670/990	IRIS 04/01/94
57	Acenaphthylene 208968	benzo (a) pyrene (BaP) q1* and BCF used	———	1980 AWQC document EPA/5-80-069	———	NA	———	———
58	Anthracene 120127	RfD = 3E-1 BCF = 30 (BaP BCF used) FI = 6.5	9600/110,000	IRIS 07/01/93	RfD = 3E-1 BCF = 30 (BaP BCF used) FI = 17.5	2000	8,300/40,000	IRIS 07/01/93
59	Benzidine 92875	q1* = 230 BCF = 87.5 FI = 6.5	0.00012/ 0.00054	IRIS 02/01/95	q1* = 230 RfD = 3E-3 (not used) BCF = 87.5 FI = 17.5	2000	0.000086/0.00020	IRIS 02/01/95
60	Benzo(a)-Anthracene 56553	q1* = 7.3E+0 (BaP q1* used) BCF =30 FI = 6.5	0.0044/0.049	IRIS 11/01/94 for CAS # 205992 was used	q1* = 7.3E+0 (BaP q1* used) BCF =30 FI = 17.5	2000	0.0038/0.018	IRIS 11/01/94 for CAS # 205992 was used
61	Benzo(a)-Pyrene 50328	q1* = 7.3E+0 BCF = 30 FI = 6.5	0.0044/0.049	IRIS 11/01/94	q1* = 7.3E+0 BCF = 30 FI = 17.5	2000	0.0038/0.018	IRIS 11/01/94

	Chemical (CAS number)	Basis for 1998 nrwqc: q1* or RfD/ADI and BCF	1998 nrwqc (ug/l) w +o/ o only	Reference Cite[3]	Basis for 2002 nrwqc: q1*/ RfD/ADI, RSC and BCF[1,7,8]	Methodology: 2000 or 1980[2]	2002 nrwqc (ug/l) For Consumption of : Water + organism/ Organism Only	Reference Cite[3]
62	Benzo(b)-Fluoranthene 205992	q1* = 7.3E+0 (BaP q1* used) BCF =30 FI = 6.5	0.0044/0.049	IRIS 11/01/94 for CAS # 205992 was used	q1* = 7.3E+0 (BaP q1* used) BCF =30 FI = 17.5	2000	0.0038/0.018	IRIS 11/01/94 for CAS # 205992 was used
63	Benzo(ghi)-Perylene 191242	BCF = 30 (BaP =7.3E+0, BCF)	———	———	——	NA	——	——
64	Benzo(k)-Fluoranthene 207089	q1* = 7.3E+0 (BaP q1* used) BCF =30 FI = 6.5	0.0044/0.049	IRIS 11/01/94 for CAS # 205992 was used	q1* = 7.3E+0 (BaP q1* used) BCF =30 FI = 17.5	2000	0.0038/0.018	IRIS 11/01/94 for CAS # 205992 was used
65	Bis(2-Chloro-ethoxy)-Methane 111911	BCF = 0.64	———	IRIS 3/1/91	——	NA	——	IRIS 3/1/91
66	Bis(2-Chloro-ethyl) Ether 111444	q1* = 1.1 BCF = 6.9 FI = 6.5	0.031/1.4	IRIS 02/01/94	q1* = 1.1 BCF = 6.9 FI = 17.5	2000	0.030/0.53	IRIS 02/01/94
67	Bis(2-Chloro-isopropyl)-Ether 108-60-1 (changed from 3963829)	RfD = 4E-2 BCF = 2.47 FI = 6.5	1400/170,000	IRIS 08/01/90	RfD = 4E-2 BCF = 2.47 FI = 17.5	2000	1,400/65,000	IRIS 08/01/90
68	Bis(2-Ethylhexyl)-Phthalate 117817	q1* = 0.014 BCF = 130 FI = 6.5	1.8/5.9	IRIS 02/01/93	q1* = 0.014 BCF = 130 FI = 17.5	2000	1.2/2.2	IRIS 02/01/93
69	4-Bromo-phenyl Phenyl-Ether 101553	BCF = 1640	——		——	NA	——	——
70	Butylbenzyl phthalate 85687	RfD = 2E-1 BCF = 414 FI = 6.5	3000/5200	IRIS 02/01/93	RfD = 2E-1 BCF = 414 FI = 17.5		1,500/1,900	IRIS 02/01/93

	Chemical (CAS number)	Basis for 1998 nrwqc: q1* or RfD/ADI and BCF	1998 nrwqc (ug/l) w +o/ o only	Reference Cite[3]	Basis for 2002 nrwqc: q1*/ RfD/ADI, RSC and BCF[1,7,8]	Methodology: 2000 or 1980[2]	2002 nrwqc (ug/l) For Consumption of : Water + organism/ Organism Only	Reference Cite[3]
71	2-Chloro-naphthalene 91587	RFD = 8E-2 BCF = 202 FI = 6.5	1700/4300	IRIS 11/01/90	RFD = 8E-2 BCF = 202 FI = 17.5	2000	1,000/1,600	IRIS 11/01/90
72	4-Chloro-Phenyl Phenyl Ether 7005723	BCF = 1200	———	———	———	NA	———	———
73	Chrysene 218019	q1* = 7.3E+0 (BaP q1* used) BCF =30 FI = 6.5	0.0044/0.049	IRIS 11/01/94 for CAS # 205992 was used	q1* = 7.3E+0 (BaP q1* used) BCF =30 FI = 17.5	2000	0.0038/0.018	IRIS 11/01/94 for CAS # 205992 was used
74	Dibenzo(a,h) Anthracene 53703	q1* = 7.3E+0 (BaP q1* used) BCF =30 FI = 6.5	0.0044/0.049	IRIS 11/01/94 for CAS # 205992 was used	q1* = 7.3E+0 (BaP q1* used) BCF =30 FI = 17.5	2000	0.0038/0.018	IRIS 11/01/94 for CAS # 205992 was used
75	1,2-Dichloro-benzene 95501	RfD = 9E-2 BCF = 55.6 FI = 6.5	2700/17,000	IRIS 03/01/91	RfD = 9E-2[10] BCF = 55.6 RSC = 20% [j] (not used) FI = 6.5	1980	2,700/17,000	IRIS 03/01/91
76	1,3-Dichloro-benzene 541731	ADI = 1.34E-2 (ADI for 1,2-DCB used) BCF = 55.6 FI = 6.5	400/2600	1980 AWQC DOC EPA 440/5-80-039	ADI = 1.34E-2 (ADI for 1,2-DCB used) BCF = 55.6 FI = 17.5	2000	320/960	1980 AWQC DOC EPA 440/5-80-039
77	1,4-Dichloro-benzene 106467	ADI = 1.34E-2 (ADI for 1,2-DCB used) BCF = 55.6 FI = 6.5	400/2600	1980 AWQC DOCUMENT EPA 440/5-80-039	ADI = 1.34E-2[10] (ADI for 1,2-DCB used) BCF = 55.6 RSC = 20% [j] (not used) FI = 6.5	1980	400/2,600	1980 AWQC DOCUMENT EPA 440/5-80-039
78	3,3'-Dichloro-benzidine 91941	q1* = 0.45 BCF = 312 FI = 6.5	0.04/0.077	IRIS 07/01/93	q1* = 0.45 BCF = 312 FI = 17.5	2000	0.021/0.028	IRIS 07/01/93

	Chemical (CAS number)	Basis for 1998 nrwqc: q1* or RfD/ADI and BCF	1998 nrwqc (ug/l) w +o/ o only	Reference Cite[3]	Basis for 2002 nrwqc: q1*/ RfD/ADI, RSC and BCF[1,7,8]	Methodology: 2000 or 1980[2]	2002 nrwqc (ug/l) For Consumption of : Water + organism/ Organism Only	Reference Cite[3]
79	Diethyl Phthalate 84662	RfD = 8E-1 BCF = 73 FI = 6.5	23,000/ 120,000	IRIS 02/01/93	RfD =8E-1 BCF = 73 FI = 17.5	2000	17,000/44,000	IRIS 02/01/93
80	Dimethyl Phthalate 131113	ADI = 10 BCF = 36 FI = 6.5	313,000/ 2,900,000	1980 AWQC document EPA 440/5-80-067	ADI = 10 BCF = 36 FI = 17.5	2000	270,000/1,100,000	1980 AWQC document EPA 440/5-80-067 & 2000 FI
81	Di-n-Butyl Phthalate 84742	RfD = 1E-1 BCF = 89 FI = 6.5	2700/12,000	IRIS 08/01/90	RfD = 1E-1 BCF = 89 FI = 17.5	2000	2,000/4,500	IRIS 08/01/90
82	2,4-Dinitro-toluene 121142	q1* = 3.11E-1 BCF = 3.8 FI = 6.5	0.11/9.1	1980 AWQC document EPA 440/5-80-045	q1* = 3.11E-1[11] RfD = 2E-3 (not used) BCF = 3.8 FI = 17.5	2000	0.11/3.4	1980 AWQC document EPA 440/5-80-045 & 2000 FI
83	2,6-Dinitro-toluene 606202	———	———	———	———	NA	———	———
84	Di-n-Octyl Phthalate 117840	———	———	———	———	NA	———	———
85	1,2-Diphenyl-hydrazine 122667	q1* = 0.8 BCF = 24.9 FI = 6.5	0.040/0.54	IRIS 01/01/91	q1* = 0.8 BCF = 24.9 FI = 17.5	2000	0.036/0.20	IRIS 01/01/91
86	Fluoranthene 206440	RfD = 4E-2 BCF = 1150 FI = 6.5	300/370	IRIS 07/01/93	RfD = 4E-2 BCF = 1150 FI = 17.5	2000	130/140	IRIS 07/01/93
87	Fluorene 86737	RfD = 4E-2 BCF = 30 BaP BCF used FI = 6.5	1300/14,000	IRIS 11/01/90	RfD = 4E-2 BCF = 30 (BaP BCF used) FI = 17.5	2000	1,100/5,300	IRIS 11/01/90

	Chemical (CAS number)	Basis for 1998 nrwqc: q1* or RfD/ADI and BCF	1998 nrwqc (ug/l) w +o/ o only	Reference Cite[3]	Basis for 2002 nrwqc: q1*/ RfD/ADI, RSC and BCF[1,7,8]	Methodology: 2000 or 1980[2]	2002 nrwqc (ug/l) For Consumption of : Water + organism/ Organism Only	Reference Cite[3]
88	Hexachloro-benzene 118741	q1* = 1.6 BCF = 8690 FI = 6.5	0.00075/ 0.00077	IRIS 11/01/96	q1* = 1.6[11] RfD = 8E-4 (not used) BCF = 8690 FI = 17.5	2000	0.00028/0.00029	IRIS 11/01/96
89	Hexachloro-butadiene 87683	q1* = 0.078 BCF = 2.78 FI = 6.5	0.44/50	IRIS 04/01/91	q1* = 0.078 BCF = 2.78 FI = 17.5	2000	0.44/18	IRIS 04/01/91
90	Hexachloro-cyclo-pentadiene 77474	RfD = 7E-3 BCF = 4.34 FI = 6.5	240/17,000	IRIS 09/01/90	RfD = 7E-3[10] BCF = 4.34 RSC = 20% [a] (not used) FI = 6.5 (RfD = 6E-3 IRIS 07/05/01-not used)	1980	240/17,000	IRIS 09/01/90
91	Hexachloro-ethane 67721	q1* = 0.014 BCF = 86.9 FI = 6.5	1.9/8.9	IRIS 02/01/94	q1* = 0.014 RfD = 1E-3 (not used) BCF = 86.9 FI = 17.5	2000	1.4/3.3	IRIS 02/01/94
92	Indeno(1,2,3-cd) Pyrene 193395	q1* = 7.3E+0 (BaP q1* used) BCF = 30 FI = 6.5	0.0044/0.049	IRIS 11/01/94 for CAS # 205992 was used	q1* = 7.3E+0 (BaP q1* used) BCF = 30 FI = 17.5	2000	0.0038/0.018	IRIS 11/01/94 for CAS # 205992 was used
93	Isophorone 78591	q1* = 9.5E-4 BCF = 4.38 FI = 6.5	36/2600	IRIS 11/01/92	q1* = 9.5E-4[11] RfD = 2E-1 (not used) BCF = 4.38 FI = 17.5	2000	35/960	IRIS 11/01/92
94	Naphthalene 91203	BCF = 10.5	———	———	———	NA	———	———
95	Nitrobenzene 98953	RfD = 5E-4 BCF = 2.89 FI = 6.5	17/1900	IRIS 01/01/91	RfD = 5E-4 BCF = 2.89 FI = 17.5	2000	17/690	IRIS 01/01/91

	Chemical (CAS number)	Basis for 1998 nrwqc: q1* or RfD/ADI and BCF	1998 nrwqc (ug/l) w +o/ o only	Reference Cite[3]	Basis for 2002 nrwqc: q1*/ RfD/ADI, RSC and BCF[1,7,8]	Methodology: 2000 or 1980[2]	2002 nrwqc (ug/l) For Consumption of : Water + organism/ Organism Only	Reference Cite[3]
96	N-Nitrosodi-methylamine 62759	q1* = 51 BCF = 0.026 FI = 6.5	0.00069/8.1	IRIS 07/01/93	q1* = 51 BCF = 0.026 FI = 17.5	2000	0.00069/3.0	IRIS 07/01/93
97	N-Nitrosodi-n-Propylamine 621647	q1* = 7.0 BCF = 1.13 FI = 6.5	0.005/1.4	IRIS 07/01/93	q1* = 7.0 BCF = 1.13 FI = 17.5	2000	0.0050/0.51	IRIS 07/01/93
98	N-Nitrosodi-phenylamine 86306	q1* = 0.0049 BCF = 136 FI = 6.5	5.0/16	IRIS 07/01/93	q1* = 0.0049 BCF = 136 FI = 17.5	2000	3.3/6.0	IRIS 07/01/93
99	Phenanthrene 85018	No RfD listed No q1* listed	———	———	———	NA	———	———
100	Pyrene 129000	RfD = 3E-2 BCF = 30 (BaP BCF used) FI = 6.5	960/11,000	IRIS 07/01/93	RfD = 3E-2 BCF = 30 (BaP BCF used) FI = 17.5	2000	830/4,000	IRIS 07/01/93
101	1,2,4-Trichloro-benzene 120821	RfD = 1E-2 BCF = 114 FI = 6.5	260/940[12]	IRIS 11/01/96	RfD = 1E-2[10] BCF = 114 RSC = 20% [a] (not used) FI = 6.5	1980	260/940	IRIS 11/01/96
102	Aldrin 309002	q1* = 17 BCF = 4670 FI = 6.5	0.00013/ 0.00014	IRIS 07/01/93	q1* = 17[11] RfD = 3E-5 (not used) BCF = 4670 FI = 17.5	2000	0.000049/0.000050	IRIS 07/01/93
103	alpha-BHC 319846	q1* = 6.3 BCF = 130 FI = 6.5	0.0039/0.013	IRIS 07/01/93	q1* = 6.3 BCF = 130 FI = 17.5	2000	0.0026/0.0049	IRIS 07/01/93
104	beta-BHC 319857	q1* = 1.8 BCf = 130 FI = 6.5	0.014/0.046	IRIS 01/01/91	q1* = 1.8 BCf = 130 FI = 17.5	2000	0.0091/0.017	IRIS 07/01/1993

	Chemical (CAS number)	Basis for 1998 nrwqc: q1* or RfD/ADI and BCF	1998 nrwqc (ug/l) w +o/ o only	Reference Cite[3]	Basis for 2002 nrwqc: q1*/ RfD/ADI, RSC and BCF[1,7,8]	Methodology: 2000 or 1980[2]	2002 nrwqc (ug/l) For Consumption of : Water + organism/ Organism Only	Reference Cite[3]
105	gamma-BHC 58899 lindane	q1* = 1.3 (Not listed on IRIS) BCF = 130 FI = 6.5	0.019/0.063	1980 AWQC DOCUMENT EPA 440/5-80-054	q1* = 1.3 (Not listed on IRIS)[10] BCF = 130 FI = 6.5 (RfD = 3E-4 IRIS 03/01/88- not used) RSC = 20% [1] (not used)	1980	0.019/0.063	1980 AWQC DOCUMENT EPA 440/5-80-054
106	delta-BHC 319868	BCF = 130	———	———	———	NA	———	———
107	Chlordane 57749	q1* = 3.5E-1 BCF = 14100 FI = 6.5	0.0021/0.0022	IRIS 02/07/98	q1* = 3.5E-1[11] RfD = 5E-4 (not used) BCF = 14100 FI = 17.5	2000	0.00080/0.00081	IRIS 02/07/98
108	4,4'-DDT 50293	q1* = 0.34 BCF = 53600 (one BCF applies for DDT, DDE and DDE) FI = 6.5	0.00059/ 0.00059	IRIS 05/01/91	q1* = 0.34[11] RfD = 5E-4 (not used) BCF = 53600 (one BCF applies for DDT, DDE and DDE) FI = 17.5	2000	0.00022/0.00022	IRIS 05/01/91
109	4,4'-DDE 72559	q1* = 0.34 BCF = 53600 FI = 6.5	0.00059/ 0.00059	IRIS 08/22/88	q1* = 0.34 BCF = 53600 (one BCF applies for DDT, DDE and DDE) FI = 17.5	2000	0.00022/0.00022	IRIS 08/22/88
110	4,4'-DDD 72548	q1* = 0.24 BCF = 53600 FI = 6.5	0.00083/ 0.00084	IRIS 08/22/88	q1* = 0.24 BCF = 53600 FI = 17.5	2000	0.00031/0.00031	IRIS 08/22/88
111	Dieldrin 60571	q1* = 16 BCF = 4670 FI = 6.5	0.00014/ 0.00014	IRIS 01/01/91	q1* = 16[11] RfD = 5E-5 (not used) BCF = 4670 FI = 17.5	2000	0.000052/0.000054	IRIS 07/01/1993

	Chemical (CAS number)	Basis for 1998 nrwqc: q1* or RfD/ADI and BCF	1998 nrwqc (ug/l) w +o/ o only	Reference Cite[3]	Basis for 2002 nrwqc: q1*/ RfD/ADI, RSC and BCF[1,7,8]	Methodology: 2000 or 1980[2]	2002 nrwqc (ug/l) For Consumption of : Water + organism/ Organism Only	Reference Cite[3]
112	alpha-Endosulfan 959988	BCF = 270 RfD = 6E-3 RfD for endosulfan used FI = 6.5	110/240	IRIS 10/1/94 ENDOSULFAN #115297 WAS USED	RfD = 6E-3 (RfD for endosulfan used) BCF = 270 FI = 17.5	2000	62/89	IRIS 10/1/94 ENDOSULFAN #115297 WAS USED
113	beta-Endosulfan 33213659	RfD = 6E-3 (RfD for endosulfan used) BCF = 270 FI = 6.5	110/240	IRIS 10/01/94 ENDOSULFAN #115297 WAS USED	RfD = 6E-3 (RfD for endosulfan used) BCF = 270 FI = 17.5	2000	62/89	IRIS 10/01/94 ENDOSULFAN #115297 WAS USED
114	Endosulfan Sulfate 1031078	RfD = 6E-3 (RfD for endosulfan used) BCF = 270 FI = 6.5	110/240	IRIS 10/1/94 ENDOSULFAN #115297 WAS USED	RfD = 6E-3 (RfD for endosulfan used) BCF = 270 FI = 17.5	2000	62/89	IRIS 10/01/94 ENDOSULFAN #115297 WAS USED
115	Endrin 72208	RfD = 3E-4 BCF = 3970 FI = 6.5	0.76/0.81	IRIS 09/07/88	RfD = 3E-4 [10] BCF = 3970 RSC = 20% [b] (not used) FI = 6.5	1980	0.76/0.81	IRIS 04/01/1991
116	Endrin Aldehyde 7421934	RfD = 3E-4 (RfD for Endrin used) BCF = 3970 FI = 6.5	0.76/0.81	IRIS 04/01/91 for CAS # 72208 was used	RfD = 3E-4 (RfD for Endrin used) BCF = 3970 FI = 17.5	2000	0.29/0.30	IRIS 04/01/91 for CAS # 72208 was used
117	Heptachlor 76448	q1* = 4.5 BCF = 11200 FI = 6.5	0.00021/ 0.00021	IRIS 07/01/93	q1* = 4.5[11] RfD = 5E-4 (not used) BCF = 11200 FI = 17.5	2000	0.000079/0.000079	IRIS 07/01/93
118	Heptachlor Epoxide 1024573	q1* = 9.1 BCF = 11200 (heptachor BCF used) FI = 6.5	0.00010/ 0.00011	IRIS 07/01/93	q1* = 9.1[11] RfD = 1.3E-5 (not used) BCF = 11200 (heptachor BCF used) FI = 17.5	2000	0.000039/0.000039	IRIS 07/01/93

	Chemical (CAS number)	Basis for 1998 nrwqc: q1* or RfD/ADI and BCF	1998 nrwqc (ug/l) w +o/ o only	Reference Cite[3]	Basis for 2002 nrwqc: q1*/ RfD/ADI, RSC and BCF[1,7,8]	Methodology: 2000 or 1980[2]	2002 nrwqc (ug/l) For Consumption of : Water + organism/ Organism Only	Reference Cite[3]
119	PCBs	q1* =2 (total PCBs/congener/ isomer) BCF = 31200 FI = 6.5	0.00017/ 0.00017	IRIS 06/01/97 for CASRN 1336-36-3	q1* = 2 (total PCBs/ congener/isomer) BCF = 31200 FI = 17.5	2000	0.000064/0.000064	IRIS 06/01/97 for CASRN 1336-36-3
120	Toxaphane 8001352	q1* = 1.1 BCF = 13100 FI = 6.5	0.00073/ 0.00075	IRIS 01/01/91	q1* = 1.1 BCF = 13100 FI = 17.5	2000	0.00028/0.00028	IRIS 01/01/91

FOOTNOTES :

1. IRIS values as of May 17, 2002 are presented. IRIS information is presented in some cases even though it may not be used to calculate criteria. All units are as follows: q1* = per mg/kg-day; RfD = mg/kg-d; BCF = L/kg; and FI = 17.5 g/day (unless otherwise noted).

2. Calculations are based on the following methodologies: *Guidelines and Methodologies Used in the Preparation of Health Effects Assessment Chapters of the Consent Decree Water Criteria Documents* (45FR79347) and *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health* (2000), EPA-822-B-00-004, October 2000).

3. Reference cite refers to an IRIS coversheet, AWQC criterion document or other document which serves as the basis for the criterion.

4. Although a new RfD is available in IRIS, the surface water criteria will not be revised until the National Primary Drinking Water Regulations: Stage 2 Disinfectants and Disinfection Byproducts Rule (Stage 2 DBPR) is completed, since public comment on the relative source contribution (RSC) for chloroform is anticipated.

5. In IRIS, EPA has listed thallium sulfate, not thallium alone. The thallium RfD included in the matrix is based on: NOAL = 0.25 mg TL_2SO_4/kg-d; UF = 3000; fraction TL in TL_2SO_4 = 0.81.

6. Original 1980 ADIs were expressed as mg/day. These have been converted to mg/kg-day to make them comparable to present day RfDs. All q1*s are in kg-day/mg or per mg/kg-day.

7. The fish tissue bioconcentration factor (BCF) from the 1980 criteria documents was retained unless otherwise noted. No BAFs were derived for this effort.

8. Criteria based on carcinogenicity (q1*), reflect 10^{-6} risk.

9. The q1* of 1.75 mg/kg-d was derived from the risk per unit concentration in drinking water from IRIS: $5x10^{-5}$ risk per ug/L in drinking water.

10. This criterion was not revised as part of this effort. EPA has published revisions for this criterion in the **Federal Register** and is soliciting scientific views on the revised value.

11. Although a q1* and RfD are available in IRIS, the q1*was used to derive the criterion because it resulted in the more stringent criterion.

12. These criteria were not published or promulgated in the NTR or CTR. These criteria were calculated based on the RfD presented in IRIS.

References for Relative Source Contribution (RSC)

a. FR 57 (138), July 17, 1992
b. FR 55 (143), July 25, 1990
c. Cyanide Health Advisory, March 31, 1987.
d. FR 56 (20), January 30, 1991
e. 1,1-dichloroethylene Health Advisory, March 31, 1987
f. Ethylbenzene Health Advisory, March 31, 1987
g. Toluene (Draft) Health Advisory, October 1993
h. FR 54 (97), May 22, 1989
i. 1,1,2-trichloroethane Health Advisory, September 1, 1989
j. Ortho-, meta- and para-dichlorobenzenes Health Advisory, March 31, 1987
k. FR 59 (145) July 29, 1994
l. Lindane Health Advisory, March 31, 1987.

1980 Methodology Calculations

Using cancer potency, q1*:

For consumption of water and organisms:

$$\text{AWQC } [\mu g/L] = \frac{10^{-6} \bullet 70 \text{ kg} \bullet 1000 \ \mu g/mg}{q1^* \ [kg\text{-}d/mg] \ (2 \text{ L/d} + (0.0065 \text{ kg/d} \bullet \text{BCF } [L/kg]))}$$

For consumption of organisms only:

$$\text{AWQC } [\mu g/L] = \frac{10^{-6} \bullet 70 \text{ kg} \bullet 1000 \ \mu g/mg}{q1^* \ [kg\text{-}d/mg] \bullet 0.0065 \text{ kg/d} \bullet \text{BCF } [L/kg]}$$

U sing Reference Dose:

For consumption of water and organisms:

$$\text{AWQC } [\mu g/L] = \frac{Rf\ D \ [mg/kg\text{-}d] \bullet 70 \text{ kg} \bullet 1000 \ \mu g/mg}{2 \text{ L/d} + (0.0065 \text{ kg/d} \bullet \text{BCF } [L/kg])}$$

F or consumption of organisms only:

$$\text{AWQC } [\mu g/L] = \frac{RfD \ [mg/kg\text{-}d] \bullet 70 \text{ kg} \bullet 1000 \ \mu g/mg}{0.0065 \text{ kg/d} \bullet \text{BCF } [L/kg]}$$

AWQC = Ambient water quality criteria = national recommended water quality criteria
q1* = Cancer potency factor kg-d/mg or per mg/kg-day
RfD = Reference dose mg/kg-d
DI = Drinking water intake 2 L/day
BW = Human body weight 70 kg
FI = Fish intake 0.0065 kg/day
BCF = Bioconcentration factor L/kg

2000 Methodology Calculations

Linear Approach

Using cancer potency, q1*:

For consumption of water and organisms:

$$\text{AWQC } [\mu g/L] = \frac{(10^{-6}/q1^*) \bullet 70 \text{ kg} \bullet 1000\ \mu g/mg}{(2 \text{ L/d} + (0.0175 \text{ kg/d} \bullet \text{BCF } [L/kg]))}$$

For consumption of organisms only:

$$\text{AWQC } [\mu g/L] = \frac{(10^{-6}/q1^*) \bullet 70 \text{ kg} \bullet 1000\ \mu g/mg}{0.0175 \text{ kg/d} \bullet \text{BCF } [L/kg]}$$

Using Reference Dose:

For consumption of water and organisms:

$$\text{AWQC } [\mu g/L] = [\text{ RfD } [mg/kg\text{-}d] \bullet \text{RSC} * (70 \text{ kg}/\ 2 \text{ L/d} + (0.0175 \text{ kg/d} \bullet \text{BCF } [L/kg])] \bullet 1000\ \mu g/mg$$
or (- RSC)

For consumption of organisms only:

$$\text{AWQC } [\mu g/L] = [\text{RfD } [mg/kg\text{-}d] \bullet \text{RSC} \bullet (70 \text{ kg}/ (0.0175 \text{ kg/d} \bullet \text{BCF } [L/kg]))] \bullet 1000\ \mu g/mg$$
(- RSC)

Nonlinear Approach (Presented for information only- no criteria in matrix based on nonlinear approach currently)
For consumption of water and organisms:

$$\text{AWQC } [ug/L] = \text{POD/UF} \bullet \text{RSC} \bullet (\text{BW/DI} + (\text{FI} \bullet \text{BCF})$$

For consumption of organisms only:

$$\text{AWQC } [ug/L] = \text{POD/UF} \bullet \text{RSC} \bullet * (\text{BW}/(\text{FI} \bullet \text{BCF})$$

AWQC = Ambient water quality criteria = national recommended water quality criteria
q1* = Cancer potency factor kg-d/mg or per mg/kg-day
RSD = Risk specific dose 10^{-6}/q1* mg/kg-day
RfD = Reference dose mg/kg-d
DI = Drinking water intake 2 L/day
BW = Human body weight 70 kg
FI = Fish intake 0.0175 kg/day
BCF = Bioconcentration factor L/kg
UF = Uncertainty factor (unitless)
RSC = Relative source contribution (percentage of subtraction)
POD = Point of departure mg/kg-day

NRWQC: HUMAN HEALTH CRITERIA CALCULATION MATRIX (November 2002)

NON PRIORITY POLLUTANTS

	Chemical (CAS number)	Gold Book or Criteria Document Value (ug/L) w + o/ o only	Basis (q1* or RfD/ADI) and BCF	1998 nrwqc (ug/L) w + o/o	Reference Cite	Basis (q1* or RfD/ADI) and BCF[1,2]	Methodology 2000 or 1980	2002 nrwqc (ug/L) w + o/ o only	Reference Cite[3]
6	Barium	1,000/—	RfD = 7E-2 (08/01/90) not used	1,000/—	Gold Book	RfD = 7E-2 (01/20/1999) not used	pre-1980	1,000/—	Gold Book
10	Chlorophenoxy Herbicide (2,4,5,-TP) 93721	10,—	RfD = 8E-3 (09/07/88) not used	10/—	Gold Book	RfD = 8E-3 (09/07/88) not used RSC = 20% [a]	pre-1980	10/—	Gold Book
11	Chlorophenoxy herbicide (2,4-D) 94757	100/—	RfD = 1E-2 (05/05/88)	100/—	Gold Book	RfD = 1E-2 (05/05/88) RSC = 20% [b]	pre-1980	100/—	Gold Book
15	Ether, Bis (Chloromethyl) 542881	0.00000376/ 0.00184 (based on q1* = 9299.8)	q1* = 2.2E+2 BCF = 63 FI = 6.5	0.00013/ 0.00078	IRIS 01/01/91	q1* = 2.2E+2 BCF = 63 FI = 17.5	2000	0.00010/ 0.00029	IRIS 01/01/91
20	Iron 7439896	300/—	—	300/—	Gold Book	—	pre-1980	300/—	Gold Book
22	Manganese 7439965	50/100	RfD = 1.4E-1 (01/01/93) not used	50/100	Gold Book	RfD = 1.4E-1 (05/01/1996) not used	pre-1980	50/100	Gold Book
23	Methoxychlor 72435	100/—	RfD = 5E-3 (08/01/91) not used	100/—	Gold Book	RfD = 5E-3 (08/01/91) RSC = 20 % [c,d] (not used)	pre-1980	100	Gold Book
25	Nitrates 14797558	10,000/—	1.6E+0 (10/01/91) not used	10,000/—	Gold Book	RfD = 1.6E+0 (10/01/91) not used	pre-1980	10,000/—	Gold Book
26	Nitrosamines	0.0008/1.24	B_H = 43.46 BCF = 0.20 (q1* = 1.5E+2 07/01/93 not used)	0.0008/1.24	EPA 440/5-80-064	B_H = 43.46 BCF = 0.20 (q1* = 1.5E+2 07/01/93 not used)	pre-1980	0.0008/1.24	EPA 440/5-80-064

	Chemical (CAS number)	Gold Book or Criteria Document Value (ug/L) w + o/ o only	Basis (q1* or RfD/ADI) and BCF	1998 nrwqc (ug/L) w + o/o	Reference Cite	Basis (q1* or RfD/ADI) and BCF[1,2]	Methodology 2000 or 1980	2002 nrwqc (ug/L) w + o/ o only	Reference Cite[3]
27	Dinitrophenols 25550587	70/14,300	BCF = 1.51 RfD = 0.002 FI = 6.5	70/14,300	EPA 440/5-80-063	BCF = 1.51 RfD = 2E-3 FI = 17.5	2000	69/5,300	EPA 440/5-80-063 & 2000 FI
28	Nitrosodibutylamine,N 924163	0.0064/0.587	q1* = 5.43 BCF = 3.38 FI = 6.5	0.0064/0.587	EPA 440/5-80-064	q1* = 5.43 BCF = 3.38 FI = 17.5	2000	0.0063/0.22	EPA 440/5-80-064 & 2000 FI
29	Nitrosodiethylamine,N 55185	0.0008/1.24	B_H = 43.46 BCF = 0.20 (q1* = 1.5E+2 07/01/93 not used)	0.0008/1.24	EPA 440/5-80-064	B_H = 43.46 BCF = 0.20 (q1* = 1.5E+2 07/01/93 not used)	pre-1980	0.0008/1.24	EPA 440/5-80-064
30	Nitrosopyrrolidine,N 930552	0.016/91.9	q1* = 2.13 BCF = 0.055 FI = 6.5	0.016/91.9	EPA 440/5-80-064	q1* = 2.13 BCF = 0.055 FI = 17.5	2000	0.016/34	EPA 440/5-80-064 & 2000 FI
34	Pentachlorobenzene 608935	74/85 (based on ADI = 1.17)	RfD = 8E-4 BCF = 2,125 FI = 6.5	3.5/4.1	IRIS 03/01/88	RfD = 8E-4 BCF = 2,125 FI = 17.5	2000	1.4/1.5	IRIS 03/01/88
38	Solids Dissolved and Salinity	250,000/—	—	250,000/—	Gold Book	—	pre-1980	250,000/—	Gold Book
43	Tetrachlorobenzene, 1,2,4,5- 95943	38/48 (based on ADI = 0.35)	RfD = 3E-4 BCF = 1,125 FI = 6.5	2.3/2.9	IRIS 03/01/91	RfD = 3E-4 BCF = 1,125 FI = 17.5	2000	0.97/1.1	IRIS 03/01/91
45	Trichlophenol,2,4,5 95954	2,600/9800 (based on ADI = 7)	RfD = 1E-1 BCF = 110 FI = 6.5	2,600/9800	IRIS 03/01/88	RfD = 1E-1 BCF = 110 FI = 17.5	2000	1,800/3,600	IRIS 03/01/88

FOOTNOTES:

1. Criteria based on carcinogenicity (q1*), reflect 10^{-6} risk.
2. The fish tissue bioconcentration factor (BCF) from the 1980 criteria documents was retained unless otherwise noted. No BAFs were derived for this effort.
3. Reference cite refers to an IRIS coversheet, AWQC criterion document or other document which serves as the basis for the criterion.

References for Relative Source Contribution (RSC)

a. Silvex (2,4,5-TP) Health Advisory 1988
b. 2,4-dichlorophenoxyacetic acid (2,4-D) Health Advisory, March 31, 1987
c. FR 56 (20), January 30, 1991
d. Methoxychlor Health Advisory, March 31, 1987

Exhibit B

U.S. Environmental Protection Agency

Integrated Risk Information System

Phenol (CASRN 108-95-2)

Note: A TOXICOLOGICAL REVIEW is available for this chemical in Adobe* PDF format (213 Pages, 2.5 Mbytes). Similar documents can be found in the List of Available IRIS Toxicological Reviews.

Links to specific pages in the toxicological review are available throughout this summary. To utilize this feature, your Web browser and Adobe program must be configured properly so the PDF displays within the browser window. If your browser and Adobe program need configuration, please go to the IRIS Help page for instructions.

SUBSTANCE SUMMARY INDEX

Chronic Health Hazards for Non-Carcinogenic Effects

Reference Dose for Chronic Oral Exposure (RfD)

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- Principal and Supporting Studies
- Uncertainty and Modifying Factors
- Additional Studies/ Comments
- Confidence in the Oral RfD
- EPA Documentation and Review

Reference Concentration for Chronic Inhalation Exposure (RfC)

- Inhalation RfC Summary
- Principal and Supporting Studies
- Uncertainty and Modifying Factors
- Additional Studies/ Comments
- Confidence in the Inhalation RfC
- EPA Documentation and Review

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Phenol; CASRN 108-95-2; 09/30/2002

Health assessment information on a chemical substance is included in IRIS only after a comprehensive review of chronic toxicity data by U.S. EPA health scientists from several Program Offices and the Office of Research and Development. The summaries presented in Sections I and II represent a consensus reached in the review process. Background information and explanations of the methods used to derive the values given in IRIS are provided in the Background Documents.

STATUS OF DATA FOR Phenol

File First On-Line 01/31/1987

Category (section)	Status	Last Revised
Oral RfD Assessment (I.A.)	on-line	09/30/2002
Inhalation RfC Assessment (I.B.)	Not avail.; message	09/30/2002
Carcinogenicity Assessment (II.)	on-line	09/30/2002

_I. Chronic Health Hazard Assessments for Noncarcinogenic Effects

_I.A. Reference Dose for Chronic Oral Exposure (RfD)

Phenol

CASRN -- 108-95-2
Last Revised -- 09/30/2002

The oral RfD is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis. It is expressed in units of mg/kg-day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. Please refer to the Background Document for an elaboration of these concepts. RfDs can also be derived for the noncarcinogenic health effects of substances that are also carcinogens. Therefore, it is essential to refer to other sources of information concerning the carcinogenicity of this substance. If the U.S. EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation will be contained in Section II of this file.

Carcinogenicity Assessment for Lifetime Exposure
Evidence for Human Carcinogenicity
- Weight-of-Evidence Characterization
- Human Carcinogenicity Data
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Revision History
Synonyms

__I.A.1. Oral RfD Summary

This RfD replaces the previous RfD of 0.6 mg/kg-day entered on IRIS 6/1/89, which was based on a developmental toxicity study in rats (NTP, 1983a), with a NOAEL of 60 mg/kg-day. New studies published since the previous RfD include a new two-generation study (Ryan et al., 2001; available in unpublished form as IIT Research Institute, 1999), a new developmental toxicity study using divided gavage dosing (Argus Research Laboratories, 1997), and a 13-week drinking water neurotoxicity study (ClinTrials BioResearch, 1998). Although these new studies result in a stronger database, another new study (Hsieh et al., 1992) raises questions as to whether the critical effect has been appropriately identified, or whether immunotoxicity is the critical effect. A database uncertainty factor of 3 was added to account for this uncertainty. The new developmental toxicity study (Argus Research Laboratories, 1997) is the new principal study, with a NOAEL of 60 mg/kg-day and a BMDL of 93 mg/kg-day. The RfD is based on the BMDL because, unlike the NOAEL, the BMDL is not limited to one of the experimental doses. The NTP (1983a) study was not considered appropriate as a co-principal study due to the equivocal nature of the identified LOAEL and because the effect observed was not supported in the more recent study in rats using a more environmentally relevant dosing protocol (divided gavage dosing rather than a single bolus dose).

Critical Effect	Experimental Doses*	UF	MF	RfD
Decreased maternal weight gain	BMDL: 93 mg/kg-day	300	1	3E-1 mg/kg-day
Rat developmental study	BMD: 157 mg/kg-day			
Argus Research Laboratories, 1997				

*Conversion Factors and Assumptions -- This RfD is applied to ingested phenol only and is in addition to phenol formed endogenously in the gut by bacterial metabolism of protein.
BMDL = 95% lower confidence limit on the maximum likelihood estimate of the dose corresponding to a one standard deviation change in the mean
BMD = Maximum likelihood estimate of the dose corresponding to a one standard deviation change in the mean

__I.A.2. Principal and Supporting Studies (Oral RfD)

Argus Research Laboratories. (1997) Oral (gavage) developmental toxicity study of phenol in rats. Horsham, PA. Protocol number: 916-011.

In an unpublished developmental toxicity study conducted according to GLP guidelines (Argus Research Laboratories, 1997), pregnant Crl:CDRBR VAF/Plus Sprague-Dawley rats (25/group) received phenol by oral gavage on gestation days (GDs) 6 through 15. Dosing was three times daily with 0, 20, 40, or 120 mg phenol/kg/dosage, using a dosing volume of 10 mL/kg. The corresponding daily doses were 0, 60,

120, and 360 mg/kg-day. The exposed dams were observed twice a day for viability and daily for clinical signs, abortions, and premature deliveries. In addition, the maternal body weights were recorded every day, and food consumption was also recorded periodically. The rats were sacrificed on GD 20 and gross necropsy was performed and the number of corpora lutea in each ovary was recorded. The uterus of each rat was excised and examined for number and distribution of implantations, live and dead fetuses, and early and late resorptions. Each fetus was weighed, sexed, and examined for gross external alterations. One half of the fetuses were examined for soft tissue alterations and the rest were examined for skeletal alterations.

One high-dose dam died on GD 11. The study authors attributed this death to phenol treatment, because it occurred only at the high dose, although there were no adverse clinical observations and no abnormal necropsy findings in this animal. Other high-dose animals exhibited excess salivation and tachypnea (rapid breathing). There were no other treatment-related clinical observations and no treatment-related necropsy findings. Dose-dependent decreases in body weight of the exposed animals as compared with the controls were observed. Statistically significant decreases in both maternal body weight (8%) and body weight gain (38% for GDs 6–16) were observed at the high dose; although a statistically significant decrease in body weight gain (11%) was observed at the mid dose, the decrease at the mid dose (relative to controls) in absolute maternal weight at the end of dosing (3%) was not statistically significant. Dose-dependent decreases in food consumption were also observed during the dosing period.

Fetal body weights in the high-dose group were significantly lower than those of controls—by 5–7%. The high-dose group had a statistically significant decrease in ossification sites on the hindlimb metatarsals, but it is unlikely that this small change is biologically significant. The incidence of litters with incompletely ossified or unossified sternal centra was 0/23, 0/25, 3/23, and 3/24; this increase was not statistically significant. There were small, dose-related increases in the number of litters with fetuses with "any alteration" and with "any variation" at 120 mg/kg/day and higher. However, neither of these changes was statistically significant, and the response was not clearly dose-related. In addition, an increase in total variations is of questionable significance in the absence of any increase in individual variations. No other treatment-related effects were observed in uterine contents, malformations, or variations.

The maternal NOAEL was 60 mg/kg-day, based on small decreases in maternal body weight gain at 120 mg/kg-day, and the developmental NOAEL was 120 mg/kg-day, based on decreased fetal body weight and delayed ossification at 360 mg/kg-day. Benchmark dose (BMD) modeling was also conducted for the decreased maternal weight. Defining the benchmark response as a one-standard-deviation decrease in maternal body weight gain, the 95% lower confidence limit on the BMD (i.e., the BMDL) was 93 mg/kg-day. This BMDL was calculated using the polynomial model, which gave slightly better fit than the power and Hill models, using BMDS Version 1.3.

No human studies that addressed the developmental toxicity of phenol were identified. In a well-designed developmental toxicity study (NTP, 1983a), timed-mated CD rats were administered phenol by gavage at 0, 30, 60, or 120 mg/kg-day in 5 mL/kg distilled water on GD 6 to 15 and sacrificed on GD 20. Females were weighed on GDs 0, 6 through 15 (prior to daily dosing), and 20 (immediately following sacrifice), and they were also observed during treatment for clinical signs of toxicity. A total of 20–22 females per group were confirmed to be pregnant at sacrifice on GD 20. The dams were evaluated at sacrifice for body weight, liver weight, gravid uterine weight, and status of uterine implantation sites. Live fetuses were weighed, sexed, and examined for gross morphological abnormalities and malformations in the viscera and skeleton. Results of this study did not show any dose-related signs of maternal toxicity or any clinical symptoms of toxicity related to phenol treatment. The number of implantation sites was slightly higher in the dosed groups, but this change could not be treatment-related, because implantations in this strain take place prior to GD 6 (prior to dosing).

Significant increases in the litters with nonlive (dead plus resorbed) were observed in the low- and mid-dose groups but not in the high-dose group, but this effect was not considered treatment related, because this response was not dose dependent, and the response in the high-dose group was comparable with that of the control. In addition, there was no effect on the more appropriate measure of nonlive per litter. There was also no effect on live fetuses, sex ratio, malformations, or variations.

However, a clear dose-related downward trend in fetal body weight was observed, although the changes at the two lower doses were small and the effect was statistically significant only at the high dose. Fetal body weights in the high-dose group were 93% of the average in the control group; fetal body weights were not reported separately for males and females. Historical control data from the supplier report the average fetal body weight in this strain as being well below the weight in the high-dose group (Charles River Laboratories, 1988). (Concurrent control weight was 4.14 g, high-dose weight was 3.84 g, and historical control weight was 3.39 g.)

The litter size in the high-dose group was also somewhat higher (but not statistically significant) than in the controls, possibly contributing to the smaller fetal weight at the high dose. The total pup burden (total fetal weight) and the gravid uterine weight were highest in the low-dose group, and then in the high-dose group; both of these values were higher than those in the control group. In addition, the treatment-period maternal weight gain was very similar in the control and high-dose groups (but higher in the low-dose group), but the absolute maternal weight gain (i.e., adjusted for the gravid uterine weight) was much lower in the high-dose group than in the controls. The results from the low-dose group suggest that the dams could have borne a somewhat higher burden of the total in utero package. However, the results also suggest that the dams were near the limit of what they could carry, based on the lower absolute weight gain but unaffected treatment-period weight gain in the high-dose group. No dose-related signs of maternal toxicity and no clinical symptoms of toxicity related to phenol treatment were observed in this study. On the basis of these considerations and the potential for the decreased fetal weight to reflect primarily the larger litter size, the decreased fetal weight in this study could be considered an equivocal LOAEL. Thus, on the basis of decreased fetal body weight, the mid dose in this study of 60 mg/kg-day was a NOAEL for developmental toxicity and the high dose of 120 mg/kg-day was an equivocal LOAEL. The high dose (120 mg/kg-day) was a maternal NOAEL. BMD modeling could not be done for the decreased fetal weight, because NTP did not have information on the fetal weight by sex, either in the report or in its archives. Data on fetal weight by sex is needed for meaningful modeling, because the average weight of males and females is different and the number of males per group varied.

Although the same NOAEL of 60 mg/kg-day was identified for this study as in the principal study (Argus Research Laboratories, 1997), this study was not considered adequate to be a co-principal study in light of the equivocal nature of the LOAEL and the absence of an effect on fetal weight in another gavage developmental study in rats (Argus Research Laboratories, 1997) at a maternally toxic dose in that study of 120 mg/kg-day.

In a standard mouse developmental toxicity study (NTP, 1983b), phenol was administered by gavage in water at 0, 70, 140, or 280 mg/kg-day on GDs 6 to 15 to groups of 31–36 plug-positive female CD-1 mice. The pregnancy rate in the controls was only 83%; the pregnancy rate in dosed animals ranged from approximately 83% in the low- and mid-dose groups to 71% at the high dose. In addition, 4/36 high-dose mice died; no deaths occurred in any other groups. The average maternal body weight gain during treatment was statistically significantly reduced at the high dose, as was the maternal body weight at terminal sacrifice on GD 17 (by 10%, compared with the control group). In addition, tremors were observed at the high dose throughout the dosing period. As in the rat study, a highly statistically significant decrease in fetal body weight per litter (18%) was observed at the high dose. An increased incidence of cleft palate was also reported at the highest dose level, although the incidence was not significantly different from that of the other groups, and there was no statistically significant increase in the incidence of litters with malformations. There was no other evidence of altered prenatal viability or structural development.

Thus, the high dose of 280 mg/kg-day was a maternal frank effect level based on the observed deaths; tremors and decreased body weight also occurred at this dose. The high dose was also a developmental LOAEL based on decreased fetal body weight (accompanied by a possible increase in the incidence of cleft palate) in the fetuses, an effect that was likely secondary to the severe toxicity in the dams. The study NOAEL for maternal and developmental toxicity was 140 mg/kg-day.

Hsieh et al. (1992) investigated the effects of phenol exposure on hematological, immune, and neurochemical endpoints in a study of 6-week-old male CD-1 mice (5 per dose) administered actual concentrations of 0, 4.7, 19.5, or 95.2 ppm in drinking water for 28 days. On the basis of measured

concentrations and water intake, the authors reported that the corresponding daily doses were 0, 1.8, 6.3, and 33.6 mg/kg-day. After 28 days, the mice were sacrificed by decapitation, gross pathological examinations were performed, and the liver, spleen, thymus, and kidney were weighed. Blood was taken at sacrifice for analysis. Splenocytes were prepared for analysis of antibody production response, mitogen-stimulated lymphocyte proliferation, mixed lymphocyte response, and cell-mediated cytolysis response.

During the 28-day exposure, no mortality and no overt clinical signs occurred in exposed mice. Phenol treatment had no effects on food or water consumption or on body weight gain. Exposed mice had no gross lesions in the liver, kidney, spleen, thymus, lung, heart, and brain, and no effect on organ weights for the liver, kidney, spleen, and thymus was seen. A dose-related decrease in erythrocyte counts was statistically significant at all doses. The hematocrit was decreased only at the high dose. A decreased erythrocyte count in the absence of an effect on hematocrit may have been due to macrocytosis (enlarged erythrocytes), but insufficient data were provided to evaluate this possibility. The erythrocyte counts in all dosed groups were markedly lower than the historical control values provided by the animal distributor (Charles River Laboratories, 1986), although the hematocrit concentration in all groups was above the historical control mean. There was no effect on total or differential leukocyte counts.

A decreased antibody response to sheep red blood cells was observed, as indicated by both the plaque-forming cell (PFC) assay (expressed as PFC/million spleen cells and PFC/spleen) and the antibody titer using an enzyme-linked immunosorbent assay (ELISA). Two of these measures were statistically significantly decreased at the mid dose, and PFC/spleen was significantly decreased only at the high dose. These decreases reached 40% (a value often used by immunotoxicologists as a rule of thumb for clinically relevant decreases) at the high dose. Decreases in the absolute splenocyte lymphoproliferative responses to mitogens and the mixed lymphocyte response (the proliferative ability of splenic lymphocytes in response to alloantigens) were also observed at the high dose; there was no effect on the cytolytic response to tumor cells at any dose.

Although these assays were conducted according to the methods of the day, the latter two do not conform to modern protocols, and there is little biological significance to the results of the mitogen response assay. Identification of a NOAEL in this study is somewhat problematic, because immunotoxicity risk assessment guidelines have not been developed. The determination of what degree of decrease is adverse is also problematic, because the clinical relevance of a decrement in immune function will depend on the magnitude and type of immune challenge, with a sufficiently large challenge resulting in illness even for unimpaired individuals. In a report on the use of immunotoxicity data for risk assessment, Selgrade (1999) recommended that any statistically significant and consistent change be considered a risk for the purposes of hazard identification, but the degree of change considered adverse for the purposes of dose-response assessment was not addressed.

On the basis of the magnitude of the decreases in antibody response observed in three related assays, supported by decreased hematocrit and red blood cells, the high dose (33.6 mg/kg-day) can be considered the study LOAEL, and the mid dose (6.2 mg/kg-day) can be considered the study NOAEL. There is, however, considerable uncertainty regarding the reliability of these values due to issues of study interpretation and because the study used only 5 animals per group as compared with the recommended 8 per group (U.S. EPA, 1998).

__I.A.3. Uncertainty and Modifying Factors (Oral RfD)

UF = 300

A factor of 10 is used to protect sensitive human subpopulations (intraspecies variability). The data on the within-human variability in the toxicokinetics and toxicodynamics of ingested phenol are insufficient to adjust the default uncertainty factor for intraspecies variability. In a sample of liver fractions from 10 people, Seaton et al. (1995) found that the kinetics of phenol sulfation and hydroquinone conjugation varied by up to approximately threefold. Much larger variability is observed in CYP2E1 (the cytochrome P450 enzyme that oxidizes phenol to potentially toxic metabolites), particularly between neonates and adults (Vieira et al., 1996). These data on inter-individual variability in enzymatic metabolism are not

adequate to move from the default UF_H of 10 because they do not reflect potential variability in portal-of-entry metabolism of phenol or uncertainty regarding the identity of the toxic moiety.

A factor of 10 is used to extrapolate from animals to humans (UF_A). The absorption, distribution, and metabolism of ingested phenol in rats and humans appear to be generally qualitatively similar, although the data are insufficient for a quantitative comparison. Comparison of laboratory animal and human phenol toxicokinetics is also limited by the lack of knowledge regarding the identity of the toxic moiety. It is not possible to quantitatively use the toxicokinetic data to adjust the default 10-fold factor for interspecies variability, and the default UFA of 10 is judged to be appropriate. It may be possible to reduce this default value of 10 following review and evaluation of data comparing the toxicokinetics of phenol and its metabolites in rats and humans (perhaps supplemented by a physiologically based pharmacokinetic model), if such data become available.

The BMDL was based on an effect of minimal severity (decreased maternal weight *gain*), and a higher BMDL and NOAEL were obtained for the related endpoint of effects on maternal weight. The BMDL is also within 50% of the NOAEL identified for the decreased maternal weight endpoint. Therefore, no uncertainty factor is required for extrapolation from a NOAEL to a LOAEL. No uncertainty factor for extrapolation across duration is needed, because this developmental study is supported by chronic bioassays in two species in which toxicity was observed only at higher doses. An additional uncertainty factor for sensitive populations such as infants and children is not needed for phenol because sufficient studies of reproductive and developmental toxicity have been performed, with the observation of decreased fetal body weight (in the absence of other indications of fetal toxicity or teratogenicity) only at doses equal to or higher than the LOAEL for the endpoint used for developing the oral RfD.

The toxicity database for phenol by the oral route can be considered complete. It includes 2-year drinking water studies conducted in rats and mice (NCI, 1980), a two-generation drinking water study conducted in rats (Ryan et al., 2001; available in unpublished form as IIT Research Institute, 1999), and gavage developmental toxicity studies in rats (Argus Research Laboratories, 1997; NTP, 1983a; Narotsky and Kavlock, 1995) and mice (NTP, 1983b). However, the range of endpoints evaluated in the chronic toxicity studies was limited and did not include hematological or serum biochemistry evaluations. Immunological and hematological effects in mice were observed at low doses by Hsieh et al. (1992) in a 28-day drinking water study. These endpoints were evaluated, and no significant hematological or serum biochemistry effects were observed at doses of up to >300 mg/kg-day in the two-generation rat study (IIT Research Institute, 1999; Ryan et al., 2001). The difference in these results suggest species differences between mice and rats, but confirmation of the immunological and hematological effects in an assay done according to modern test methods would be useful.

The results of a study of the effects of phenol on bone marrow cellularity in mice dosed intraperitoneally at up to 300 mg/kg-day (Eastmond et al., 1987) and an in vitro study with mouse bone marrow cells (Corti and Snyder, 1998) also do not indicate that mouse blood cells are highly susceptible to effects of phenol. However, these studies did not evaluate the same parameter measured by Hsieh et al. (1992), and significant interspecies differences in immunotoxicity are not unusual. It is of interest that the endpoints affected in the Hsieh et al. (1992) study (two measures of effects on antibody production, the PFC and ELISA) are the immune endpoints most highly predictive of effects on host resistance (Luster et al., 1992, 1993). Therefore, to account for the uncertainties regarding the immunological and hematological effects in mice, a database uncertainty factor of 3 is used. The database factor could be reconsidered with results of an immunotoxicity study in mice that is compliant with EPA immunotoxicity test guidelines (U.S. EPA, 1998).

An additional degree of public health protection may also be provided by the use of a gavage study rather than the more environmentally relevant route of drinking water. This is because gavage administration results in a higher peak blood level–presumably even using a divided dosing protocol–than does ingestion of the same daily dose in drinking water, and at least some effects of phenol are related to peak blood levels. Thus, a composite uncertainty factor of 300 was used, based on default factors of 10 each for interspecies extrapolation and intraspecies variability and a database factor of 3 to account for uncertainties regarding the immunotoxic potential of phenol.

MF = 1

No MF is applied because the existing uncertainties have been addressed with the standard uncertainty factors.

__I.A.4. Additional Studies/Comments (Oral RfD)

Phenol is produced endogenously by bacteria in the gut at a rate estimated at 1 to 10 mg/day, corresponding to approximately 0.014–0.14 mg/kg-day (Bone et al., 1976; Lawrie and Renwick, 1987; Renwick et al., 1988), based on total phenol (free plus conjugated) levels in urine. Because endogenous phenol is formed in the gut, the toxicokinetics would be similar to that of ingested phenol. Both humans and laboratory animals efficiently conjugate and excrete phenol at low doses, resulting in only a small degree of systemic exposure to free phenol (or any of its oxidative metabolites) at these low levels. The phenol conjugation capacity of the liver is an important determinant of the ingested dose that would result in toxicity, but there is no information on the degree of phenol conjugation by humans at doses in the range of the RfD.

Human variability exists in both the levels of endogenous phenol production and in the conjugative capacity of the liver. In the absence of more detailed information, it is reasonable to assume that humans have adapted by having adequate conjugation capacity for the range of endogenous phenol production. Therefore, the default total uncertainty factor of 10 for human variability in toxicokinetics and toxicodynamics described above is considered adequate. Determining whether oxidative metabolites are formed in people with high endogenous levels of phenol formation would enhance the confidence in the determination of the intraspecies uncertainty factor. The RfD is at least twice the endogenous rate of phenol formation in humans, meaning that endogenous production is approximately 5–50% of the RfD.

An extensive database for the effects of orally administered phenol in laboratory animals is available. Two-year drinking water studies have been conducted in groups of F344 rats and B6C3F1 mice (50 animals/sex/dose/species). The rats were exposed to 0, 2500, or 5000 ppm, corresponding to 0, 260, and 585 mg/kg-day for male rats and 0, 280, and 630 mg/kg-day for female rats. The mice were exposed to 0, 2500, or 5000 ppm in drinking water, corresponding to estimated doses of 0, 450, and 660 mg/kg-day for both sexes. These studies identified NOAELs of 260 mg/kg-day and 480 mg/kg-day for rats and mice, respectively, based on decreased body weight gain and decreased water consumption (NCI, 1980). A complete histopathology evaluation was included, but no increases in noncancer lesions were found. Hematology and serum biochemical evaluations were not included in those chronic studies, but they were included in a recent two-generation drinking water study conducted in Sprague-Dawley rats (Ryan et al., 2001; available in unpublished form as IIT Research Institute, 1999), as described below.

Toxicity in gavage studies with phenol is typically much higher than that in drinking water studies. NOAELs for systemic effects were 5- to10-fold lower in gavage studies (Berman et al., 1995; Moser et al., 1995; Dow Chemical Co., 1945) than those seen in drinking water studies. Many (but not all) of the effects in drinking water studies appeared to be due to decreased water consumption resulting from poor palatability. Effects observed in gavage studies included tremor and liver and kidney histopathology; effects in drinking water studies were less severe. As described in greater detail in the Toxicological Review, this difference between gavage and drinking water exposure is consistent with toxicokinetic data that suggest that toxicity is correlated with peak blood concentrations rather than being a measure of total dose, such as the area under the phenol blood concentration curve (AUC). Due to this marked difference in toxicity between gavage and drinking water, the RfD was not based on gavage studies of systemic effects, even though those effects occurred at lower doses.

Although the principal study for the development of the RfD (Argus Research Laboratories, 1997) used gavage dosing, it is not clear whether this difference in toxicity also applies to the endpoint of decreased maternal weight gain. In addition, Argus Research Laboratories (1997) used a divided dosing protocol, a significant enhancement that made the gavage dosing more closely resemble an environmentally relevant route of exposure.

In an unpublished 13-week neurotoxicity study conducted according to good laboratory practice (GLP) guidelines (ClinTrials BioResearch Ltd., 1998), groups of 15 male and 15 female Sprague-Dawley rats received phenol via drinking water at concentrations of 0, 200, 1000, or 5000 ppm for 13 weeks followed by a 4-week recovery period. The study authors calculated that the average doses were 0, 18.1, 83.1, and 308.2 mg/kg-day for males and 0, 24.6, 107.0, and 359.8 mg/kg-day for females. During the exposure period, clinical signs and water intake were recorded daily and body weight and food consumption were recorded weekly. In addition, a functional observational battery and a motor activity test were conducted pre-study and once each during weeks 4, 8, 13, and 17. At the end of the exposure and at the end of the recovery period, five rats/sex in the control and 5000 ppm groups underwent neuropathological evaluations (including a thorough evaluation of the brain and several nerves). The rest of the rats were sacrificed at the end of the 4-week recovery and were subjected to gross necropsy.

The primary clinical sign was dehydration, which was associated with marked decreases in water consumption at the high dose and smaller decreases at the mid-dose. Decreases in water consumption were more pronounced in females than in males and were most evident during the first week of dosing. Water consumption was decreased to approximately 90% of the control level in mid-dose males and females, to approximately 60% of control levels in high-dose males, and to approximately 55% (40% during the first week) of control levels in high-dose females. Water consumption rebounded to levels higher than those of controls during the recovery period. The decreased water consumption was likely due to the poor palatability of phenol at high concentrations rather than being a manifestation of an overt toxicological effect. In addition, the high-dose group had decreased body weights as compared with the controls (8% for males and 12% for females) and decreased food intake (approximately 10% for males and 10–20% for females). The only toxicologically significant neurological effect was decreased motor activity in females. A statistically significant reduction in total group mean motor activity counts was observed at week 4 in the 5000 ppm group. The authors reported that the rate of linear change of motor activity with time was also significantly decreased at weeks 8 and 13 in the 1000 ppm and 5000 ppm groups. The authors attributed the decreased activity to dehydration, noting that the control group mean total activity increased by >20% at week 4 as compared with prestudy levels, whereas activity of the dehydrated females in the 5000 ppm group at week 4 was decreased by 17% and activity of the females in this group that were not dehydrated increased by 2%. However, a detailed analysis of the individual animal data, as discussed in the Toxicological Review, did not support the hypothesis that all of the decreased motor activity could be attributed to dehydration; phenol at least contributed to the decreased motor activity. On the basis of decreased motor activity, the study NOAEL in females was 1000 ppm phenol (107 mg/kg-day) and the LOAEL was 5000 ppm (360 mg/kg-day). No LOAEL was identified in males; the high dose of 308 mg/kg-day was a NOAEL. A BMDL of 219 mg/kg-day was calculated for decreased motor activity in females in week 4 in this study

In a two-generation reproductive toxicity study following modern GLP guidelines (Ryan et al., 2001; full unpublished study available as IIT Research Institute, 1999), 30 Sprague-Dawley rats/sex/group were exposed to 0, 200, 1000, or 5000 ppm phenol in drinking water. The authors calculated that the average daily phenol intake during week 10 was 0, 14.7, 70.9, and 301.0 mg/kg-day for P1 males and 0, 20.0, 93.0, and 320.5 mg/kg-day for P1 females. For the F1 generation, the average phenol intake during week 10 was 0, 13.5, 69.8, and 319.1 mg/kg-day for males and 0, 20.9, 93.8, and 379.5 mg/kg-day for females. Most of the treatment-related changes in P1 rats were observed in the high-dose groups.

The only significant observed clinical sign was redness around the nose fur, which occurred in the high-dose males and females of the F1 generation before mating and in P1 dams during lactation. This redness likely reflected a nonspecific stress response. A significant decrease in water consumption was observed throughout the study in both P1 and F1 animals of both sexes, which was attributed to poor palatability. The low water consumption at the high dose was accompanied by decreased body weights as compared with the controls.

Decreased absolute organ weights and increased relative organ weights were observed for a number of organs at the high dose in both the P1 and F1 generations. Most of these changes likely reflected the lower body weight and overall dehydration in these groups. F1 females had a statistically significant, dose-related decrease in absolute uterine weights at all doses, but P1 females were not affected. The decreased uterine weight was not considered adverse because there was no evidence of a dose-response relationship for relative uterine weight, no effect on reproductive function, and no

histopathological changes in the uterus and the individual animal data showed that the uterine weight was below the control range for only a few rats in each dose group. No other organ weight changes in either the P1 or the F1 generation were considered adverse. The histopathological examinations showed no treatment-related lesions in the kidneys, spleen, liver, thymus, or reproductive organs.

An immunotoxicity screen in this study found no significant effects on spleen weight, cellularity, or antibody-forming cells for any test group as compared with the control group. Complete hematological evaluations and serum biochemical evaluations were conducted on P1 males prior to sacrifice, and no biologically significant changes were observed. No effect on fecundity or fertility in either generation was observed. In addition, there was no effect on other indicators of reproductive toxicity, including the frequency of estrus, testicular sperm count, sperm motility and sperm morphology.

The survival of the high-dose F1 pups was significantly decreased on prenatal day 4 (pre-culling), although there was no effect on overall F1 pup survival. In the F2 generation, high-dose pup survival was significantly decreased throughout the lactation period. This decreased survival of both generations of pups was likely secondary to the decreased maternal water intake and associated decreases in milk production. In the F1 generation, delayed vaginal patency and delayed preputial separation were observed at the high dose. The delay was considered secondary to decreased fetal growth at the high dose and as resulting from decreased water consumption due to poor palatability and associated decreased food consumption.

Thus, all of the adverse systemic and reproductive effects of phenol in the Ryan et al. (2001) study occurred at the high dose, and they appear to be secondary to decreased water consumption due to poor palatability rather than a toxic effect of phenol. On the basis of decreased parental and pup body weight (compared with the controls) and decreased pup survival, the high dose is a LOAEL. The study NOAEL is 70.9 mg/kg-day (based on the NOAEL corresponding to the lowest LOAEL in this study, in P1 males). BMD modeling was not conducted for this study because the observed effects appeared to be secondary to decreased water consumption and not reflective of phenol toxicity.

Phenol is readily absorbed by the inhalation, oral, and dermal routes (Piotrowski, 1971; Capel et al., 1972; Dow Chemical Co., 1994). Portal-of-entry metabolism for the inhalation and oral routes appears to be extensive and involves sulfate and glucuronide conjugation and, to a lesser extent, oxidation, primarily by CYP2E1. The primary oxidative metabolites include hydroquinone and catechol, which are also substrates for conjugation. Secondary products of hydroquinone or catechol metabolism, including benzoquinone and trihydroxybenzene, can also be formed (Capel et al., 1972; Dow Chemical Co., 1994; Kenyon et al., 1995). Once absorbed, phenol is widely distributed in the body, although the levels in the lung, liver, and kidney are often reported as being higher than those in other tissues (on a per-gram-tissue basis) (Tanaka et al., 1998; Liao and Oehme, 1981; Dow Chemical Co., 1994). Elimination from the body is rapid, primarily as sulfate and glucuronide conjugates in the urine, regardless of route of administration; phenol does not appear to accumulate significantly in the body (Ohtsuji and Ikeda, 1972; Deichmann and Witherup, 1944; Dow Chemical Co., 1994).

For more detail on Susceptible Populations, exit to the toxicological review, Section 4.7 (PDF).

__I.A.5. Confidence in the Oral RfD

Study -- Medium
Database -- Medium to high
RfD -- Medium to high

The principal study (Argus Research Laboratories, 1997) used an adequate number of animals and evaluated an appropriate array of endpoints for a developmental toxicity study. Although gavage dosing was used, the divided-dosing protocol provided a significant enhancement that made the gavage dosing more closely resemble an environmentally relevant route of exposure. Although the use of gavage dosing lowers the confidence in the study, the dosing frequency in the divided-dose gavage study may be fairly similar to that in drinking water studies, in which rodents typically consume water in a few larger doses, often in association with food consumption.

Confidence in the supporting database is medium to high. Although the oral toxicity database meets the minimal criteria for a high-confidence database (chronic studies in two species, developmental toxicity studies in two species, and a multigeneration reproduction study), the chronic studies did not evaluate a sufficient array of endpoints. In particular, the chronic mouse study (NCI, 1980) did not evaluate hematological and immunological effects, making interpretation of the results of the Hsieh et al. (1992) study difficult. Considering the above issues results in medium to high confidence in the RfD.

For more detail on Characterization of Hazard and Dose Response, exit to the toxicological review, Section 6 (PDF).

__I.A.6. EPA Documentation and Review of the Oral RfD

Source Document -- U.S. EPA, 2002

This assessment was peer reviewed by external scientists. Their comments have been evaluated carefully and incorporated in the finalization of this IRIS summary. A record of these comments is included as an appendix to the Toxicological Review. ***To review this appendix, exit to the toxicological review, Appendix A, Summary of and Response to External Peer Review Comments (PDF)***.

Other EPA Documentation -- Summary Review of the Health Effects Associated with Phenol: Health Issue Assessment. Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH. U.S. EPA. 1986.

Agency Consensus Date -- 08/28/2002

Screening-Level Literature Review Findings -- A screening-level review conducted by an EPA contractor of the more recent toxicology literature pertinent to the RfD for phenol conducted in August 2003 did not identify any critical new studies. IRIS users who know of important new studies may provide that information to the IRIS Hotline at hotline.iris@epa.gov or 202-566-1676.

__I.A.7. EPA Contacts (Oral RfD)

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS in general at (202) 566-1676 (phone), (202)566-1749 (FAX), or hotline.iris@epa.gov (email address).

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_I.B. Reference Concentration for Chronic Inhalation Exposure (RfC)

Phenol
CASRN –108-95-2
Last Revised -- 09/30/2002

The inhalation RfC is analogous to the oral RfD and is likewise based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis. The inhalation RfC considers toxic effects for the respiratory system (portal of entry) and effects peripheral to the respiratory system (extrarespiratory effects). It is generally expressed in units of mg/m^3. In general, the RfC is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily inhalation exposure of the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202) 566-1676 (phone), (202)566-1749 (FAX) or hotline.iris@epa.gov (internet address).

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_VI. Bibliography

Phenol
CASRN -- 108-95-2
Last Revised -- 09/30/2002

_VI.A. Oral RfD References

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Exhibit C

EPA/635/R-02/006

EPA

TOXICOLOGICAL REVIEW

OF

Phenol

(CAS No. 108-95-2)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

September 2002

U.S. Environmental Protection Agency
Washington D.C.

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FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard identification and dose-response assessment in IRIS pertaining to chronic exposure to phenol. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of phenol.

In Section 6, EPA has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose-response. Matters considered in this characterization include knowledge gaps, uncertainties, quality of data, and scientific controversies. This characterization is presented in an effort to make apparent the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's IRIS Hotline at 301-345-2870.

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This document and summary information on IRIS have received peer review by both EPA scientists and independent scientists external to EPA. Subsequent to external review and incorporation of comments, this assessment underwent an Agency-wide review process whereby the IRIS Program Manager achieved a consensus approval among the Office of Research and Development; Office of Air and Radiation; Office of Prevention, Pesticides, and Toxic Substances; Office of Solid Waste and Emergency Response (OSWER) of Water; Office of Policy, Economics, and Innovation; Office of Children's Health Protection; Office of Environmental Information; and the Regional Offices.

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Summaries of the external peer reviewers' comments and the disposition of their recommendations are presented in Appendix A.

1. INTRODUCTION

This document presents background and justification for the hazard and dose-response assessment summaries in U.S. Environmental Protection Agency's (EPA's) Integrated Risk Information System (IRIS). IRIS summaries may include an oral reference dose (RfD), an inhalation reference concentration (RfC), and a carcinogenicity assessment.

The RfD and RfC provide quantitative information for noncancer dose-response assessments. The RfD is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis but may not exist for other toxic effects such as some carcinogenic responses. It is expressed in units of milligrams per kilogram per day (mg/kg-day). In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer effects during a lifetime. The inhalation RfC is analogous to the oral RfD, but it provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for the respiratory system (portal of entry) and effects peripheral to the respiratory system (extrarespiratory or systemic effects). It is generally expressed in units of milligrams per cubic meter (mg/m^3).

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral exposure and inhalation exposure. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates are presented in three ways. The *slope factor* is the result of application of a low-dose extrapolation procedure and is presented as the risk per mg/kg/day. The *unit risk* is the quantitative estimate in terms of either risk per μg/L drinking water or risk per $\mu g/m^3$ air breathed. Another form in which risk is presented is a drinking water or air concentration that provide cancer risks of 1 in 10,000 1 in 100,000 or 1 in 1,000,000.

Development of these hazard identification and dose-response assessments for phenol has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidelines that were used in the development of this assessment may include the following: *Guidelines for Carcinogen Risk Assessment* (U.S. EPA,1986a), *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986b), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986c), *Guidelines for Developmental Toxicity Risk Assessment*

(U.S. EPA, 1991), *Proposed Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1996a), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996b), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a); *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988); (proposed) *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a); *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b); *Peer Review and Peer Involvement at the U.S. Environmental Protection Agency* (U.S. EPA, 1994c); *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995); *Draft Revised Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1999); *Science Policy Council Handbook: Peer Review* (U.S. EPA, 1998b, 2000a); *Science Policy Council Handbook: Risk Characterization* (U.S. EPA, 2000b).

The literature search strategy employed for this compound was based on the CASRN and at least one common name. At a minimum, the following databases were searched: RTECS, HSDB, TSCATS, CCRIS, GENETOX, EMIC, EMICBACK, DART, ETICBACK, TOXLINE, CANCERLINE, MEDLINE, and MEDLINE backfiles. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. The literature search was conducted in June 1999; selected key articles published after that date are also included.

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

Phenol is a monosubstituted aromatic hydrocarbon. In its pure state, it exists as a colorless or white solid. This pure compound is mixed with water and commercially sold as a liquid product. Phenol gives off a sweet, acrid smell detectable to most people at 40 ppb in air and at about 1–8 ppm in water (ATSDR, 1998). It evaporates more slowly than water and is moderately soluble in water. Phenol is also combustible.

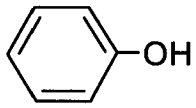
Phenol is produced through both natural and anthropogenic processes. It is naturally occurring in some foods, in human and animal wastes, and in decomposing organic material, and it is produced endogenously in the gut from the metabolism of aromatic amino acids. Phenol has been isolated from coal tar, but it is now synthetically manufactured. Currently, the largest use of phenol is as an intermediate in the production of phenolic resins, which are used in the plywood, adhesive,

construction, automotive, and appliance industries. Phenol is also used in the production of synthetic fibers such as nylon and for epoxy resin precursors such as bisphenol-A. Phenol is toxic to bacteria and fungi, and it is used as a slimicide and disinfectant. Because of its anesthetic effects, phenol is used in medicines such as ointments, ear and nose drops, cold sore lotions, throat lozenges and sprays (such as those sold under the Cepastat® and Chloraseptic® labels), and antiseptic lotions.

The greatest potential source of exposure to phenol is in the occupational setting, where phenol is used in manufacturing processes. People are also exposed via consumer products, such as medicines and lotions, and some foods and tobacco smoke. Phenol has been found in drinking water.

The physical and chemical properties of phenol are shown in Table 1.

Table 1. Physical Properties and Chemical Identity of Phenol

CAS Registry Number	108-95-2	Lide, 1993
Synonym(s)	Benzenol, hydroxybenzene, monophenol, oxybenzene, phenyl alcohol, phenyl hydrate, phenyl hydroxide	ATSDR, 1998
Registered trade name(s)	Carbolic acid, phenic acid, phenic alcohol	ATSDR, 1998
Melting point, °C	43	Lide, 1993
Boiling point, °C	181.8	Lide, 1993
Vapor pressure, at 25 °C	0.3513	HDSB, 1996
Density, at 20 °C relative to the density of H_2O at 4 °C	1.0576	Lide, 1993
Flashpoint (open cup)	85 °C	ATSDR, 1998
Water solubility, g/L at 25 °C	87	Lide, 1993
Log K_{OW}	1.46	HDSB, 1996
Odor threshold	0.047 ppm (0.18 mg/m^3) - 100% response 0.006 ppm (0.02 mg/m^3) - sensitive	U.S. EPA, 1986d
Molecular weight	94.12	Calculated
Conversion factors	1 ppm (v/v) = mg/m^3 x 0.260 1 mg/m^3 = ppm (v/v) x 3.85	Calculated
Empirical formula	C_6H_6O	Lide, 1993
Chemical structure		

3. TOXICOKINETICS RELEVANT TO ASSESSMENTS

Phenol is readily absorbed by the inhalation, oral, and dermal routes. The portal-of-entry metabolism for the inhalation and oral routes appears to be extensive and involves sulfate and glucuronide conjugation and, to a lesser extent, oxidation. The primary oxidative metabolites include hydroquinone and catechol, which are also substrates for conjugation. Secondary products of hydroquinone or catechol metabolism, including benzoquinone and trihydroxybenzene, can also be formed. Once absorbed, phenol is widely distributed in the body, although the levels in the lung, liver, and kidney are often reported as being higher than in other tissues (on a per-gram-tissue basis). Elimination from the body is rapid, primarily as sulfate and glucuronide conjugates in the urine, regardless of the route of administration. Phenol does not appear to accumulate significantly in the body.

3.1. ABSORPTION

Extensive absorption following inhalation exposure has been demonstrated in both human and laboratory animal studies. Piotrowski (1971) studied lung and skin absorption of phenol in volunteers (seven male and one female) exposed to air concentrations of 6 to 20 mg/m^3 for 8 hours. The study subjects were staff of the research institute in Poland, and all had undergone a previous medical examination. In the lung absorption studies, the subjects inhaled phenol through a face mask, eliminating the potential for most dermal absorption. These subjects retained 60–88% of the inhaled phenol, and the percent retained did not vary with exposure concentration. The absorption rate leveled off after approximately 3 hours of exposure, indicating that absorption had reached steady state. In the skin absorption studies, subjects wore underwear and denim coveralls or were unclothed for different trials of the experiment; in each case they were supplied with fresh air from outside the chamber for breathing. The absorption coefficient did not appear to vary greatly with exposure for 6 hours to concentrations in air ranging from about 5 to 25 mg/m^3, and clothing did not appear alter the absorption rate. The mean absorption coefficient was 0.35 m^3/hr, indicating that the amount of phenol present in 0.35 m^3 of air was absorbed through the skin per hour. These data show that dermal absorption can contribute significantly to the systemic dose of phenol following exposure to phenol in air. However, the quantitative data from the dermal exposure study are limited for the development of an RfC because of the short duration of the exposure and the absence of a direct determination of whether the absorption rate had reached steady state.

Other studies of workers exposed to phenol provide evidence for significant absorption via the inhalation route; however, the contribution of dermal absorption from direct contact with liquid phenol or from phenol in air was not assessed in these studies. Ohtsuji and Ikeda (1972) studied the urinary free and conjugated phenol levels in Bakelite® factory workers. The total and conjugated phenol levels tended to increase with increasing air concentration, but free phenol levels were not affected. This suggests that at the concentrations studied (up to 12.5 mg/m^3), phenol conjugation was not rate limiting. The investigators did not specifically evaluate the levels of oxidative phenol metabolites, so no conclusion can be made regarding whether Phase I metabolism was rate limiting. On the basis of mass balance analysis, the authors concluded that phenol is efficiently absorbed from the lung, because the phenol dose (air concentration * air volume/hr) was similar to the total mass excreted in the urine.

Other occupational studies provide qualitative evidence for lung absorption, reporting increasing urinary excretion of phenol metabolites with increasing workplace air concentrations. In many cases, the data are not adequate to estimate the rate and degree of absorption through this route, and potential contributions of dermal absorption are often inadequately described (Ogata et al., 1986; ACGIH, 1991).

Absorption through the lung has also been evaluated in laboratory animals report following inhalation exposure or intratracheal administration. In an unpublished study, Dow Chemical Co. (1994) studied the kinetics of ^{14}C-phenol in Fischer 344 (F344) rats following inhalation exposure to 25 ppm (96 mg/m^3) for 6 hours (nose only).[1] Radioactivity in the blood was at steady-state levels at the first measured time point (120 minutes after beginning the 6-hour exposure), indicating rapid absorption kinetics. Hughes and Hall (1995) evaluated the disposition of phenol following intratracheal and intravenous (i.v.) administration of 63.5 nmol of ^{14}C-phenol to female Fisher 344 rats. The recovery of radioactivity in tissues and excreta for both routes was approximately 90% of the administered dose within 72 hours. Because the amount of radioactivity recovered was nearly equal for the intratracheal and the i.v. dose routes (and fecal excretion is minimal), the authors concluded that absorption was near 100%.

Hogg et al. (1981) administered ^{14}C-phenol intratracheally in isolated perfused rat lungs from MRC hooded rats. At the end of the experiment (perfusions were approximately 85 minutes),

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[1]This study has not been peer-reviewed, but it was well-conducted according to EPA guidelines for a pharmacokinetics study (with minor deviations).

approximately 92% of the administered radioactivity was in the perfusate, 6% was recovered in the lung lavage, and approximately 3% was associated with lung tissue. The high recovery in the perfusate indicated that phenol is nearly completely absorbed across the airways.

Human evidence for oral absorption indicates rapid and complete absorption. In a study of three human volunteers, Capel et al. (1972) found that 85–98% of a 0.01 mg/kg oral dose of ^{14}C-phenol was excreted in the urine—primarily as phenylsulfate and phenylglucuronide—within 24 hours. In addition, case reports of oral poisoning provide qualitative evidence for gastrointestinal absorption of phenol, but the ingested and absorbed dose were not estimated in these reports, and in some cases both oral and dermal exposure were involved (Tanaka et al., 1998).

Numerous laboratory animal studies have found that orally administered phenol is readily absorbed. In most cases, absorption rates were not calculated, but the rapid recovery of high percentages of administered doses in the urine—with only minimal recovery in feces—demonstrated nearly complete absorption. In various studies in the rat (Kao et al., 1979; Edwards et al., 1986; Kenyon et al., 1995), the percentage of the administered dose recovered in urine ranged from 65 to 96.5% over a wide range of doses. Varying degrees of absorption have been observed in a variety of other species. In a study of 18 animal species orally administered single doses ranging from 20 to 50 mg/kg ^{14}C-phenol, Capel et al. (1972) found that the percentage of radiolabel recovered in the urine within 24 hours varied from 31% of the administered dose in squirrel monkeys to 95% in Wistar rats. It is not clear, however, if these differences represent differences in the degree of absorption or in the rate of elimination.

Hughes and Hall (1995) found that in female F344 rats administered 63.5 nmol of ^{14}C-phenol by oral gavage, total recovery of radioactivity (in tissues and excreta) was approximately 90% of the administered dose within 72 hours. Because fecal excretion is approximately 1–3%, and the recovered dose was nearly equal for the oral and the i.v. dose routes, the authors concluded that the absorption was near 100%. The difference between the 90% recovery and 100% total absorption was attributed to losses that were consistent across doses.

Rapid absorption of orally administered phenol has been observed in a number of studies. Dow Chemical Co. (1994) studied the kinetics of ^{14}C-phenol in F344 rats following oral dosing by gavage or in drinking water. Total recovery of the administered radioactivity in the urine, feces, tissues and carcass, and exhaled air was approximately 95%, regardless of the dosing protocol. In the high-dose gavage animals (150 mg/kg), peak levels of radioactivity in blood were detected 15

minutes post-administration, indicating rapid uptake kinetics. Humphrey et al. (1980) found that free phenol levels in the plasma of rats given an oral dose of 300 mg/kg radiolabeled phenol reached a maximum of 26 μg/mL at the first measured time, about 10 minutes after dosing, and declined rapidly to background by 60 minutes. They observed similar results in beagle dogs given a 40 mg/kg dose, with rapid peak levels of 7.8 μg/mL and complete removal of free phenol by 1 hour.

More quantitative kinetics data are available from in situ perfusion studies. Humphrey et al. (1980) administered ^{14}C- phenol (1 mg/mL) to the gut lumen of rats by means of a duodenal cannula. The remaining radioactivity was measured at 3-minute intervals over 30 minutes in perfusate collected by an ileal cannula. The results from the intestinal perfusion studies indicated that removal of ^{14}C-phenol obeys first-order kinetics, with a lumenal $T_{1/2}$ of 5.5 minutes and a rate constant for absorption of 0.127 min^{-1}. These authors also measured the plasma concentrations of phenol in the portal vein and posterior vena cava of dogs following intraduodenal dosing with either 40 or 160 mg/kg phenol. At either dose, the concentration was already maximal in the portal vein plasma within 3 minutes after dosing (the first measurement taken) and had decreased to nondetectable levels within 1 hour at the low dose and to 33% at the high dose. These data show that in both species phenol is rapidly absorbed from the gut.

Powell et al. (1974) added ^{14}C-phenol to the mucosal medium of isolated rat gut preparations and measured the level of radioactivity in the mucosal and serosal medium over 2 hours. They found that 78% of the administered radiolabel had been transferred to the serosal medium over this period. Kao et al. (1979) administered ^{14}C-phenol (12.5 or 25 mg/kg) to rats intraduodenally. Recovery of the radioactivity was rapid, with more than 70% recovered in the urine within 2 hours.

The dermal route of exposure is an important one. Both absorption of phenol liquid directly in contact with skin and dermal absorption from exposure to phenol vapor are of concern. Significant dermal absorption can result from phenol in air, so that phenol in air results in both dermal and inhalation exposure (Piotrowski, 1971). On the basis of an analysis of the Piotrowski (1971) data, ATSDR (1998) concluded that in air concentrations ranging from 5 to 25 mg/m^3, the amount of phenol absorbed through the skin will be about half of that absorbed through the lungs. The conclusion was reached by estimating the amount of phenol absorbed through the lung as the product of the human ventilation rate of 0.8 m^3/hour and the steady-state lung retention fraction of 0.7 reported by Piotrowski (1971). The resulting lung absorption coefficient of 0.6 m^3/hr is nearly twice the skin absorption coefficient of 0.35 m^3/hr. This analysis is limited, however, because it is

not clear that the exposure duration was long enough for steady state to be reached in the dermal absorption studies. Absorption via the dermal route may be lower at steady state due to the potential for a back-pressure from phenol levels in blood.

A number of case reports and in vitro studies have led to quantitative estimates of phenol absorption through the skin. Baranowska-Dutkiewicza (1981) applied a reservoir of 2.5, 5, or 10 g/L phenol solution on a small area of the forearm of 12 male volunteers. The absorption rate was dependent on the concentration and ranged from 0.08 $mg/cm^2/hr$ at the low concentration to 0.301 $mg/cm^2/hr$ at the high concentration. At the low concentration, the total amount of phenol absorbed—but not the absorption rate—increased with increased time; approximately 13% of the applied dose was absorbed over a 30-minute period. In an in vitro study, 20% of applied doses ranging from 1.3 to 2.7 $\mu g/cm^2$ were absorbed from unoccluded human skin patches within 72 hours; addition of a Teflon cap resulted in 47% absorption over this same period (Hotchkiss et al., 1992).

Bentur et al. (1998) reported an accidental dermal poisoning case in which a solution of 90% phenol was spilled on the left foot (3% of body surface). The exposure site remained occluded, and no attempt at decontamination was made until the onset of symptoms, which began within 4.5 hours. Following admission to the hospital shortly afterwards, peak serum phenol levels of 21.6 μg/mL were measured. This study is presented here for completeness, but quantitative exposure data from studies at lower phenol concentrations are more relevant to environmental exposures.

The ability of phenol to be absorbed through the skin has also been evaluated in laboratory animals. Hughes and Hall (1995) administered 63.5 nmol of labeled phenol to an occluded dermal patch (2.5 cm^2) of female F344 rats. Maximal recovery of the radioactivity was approximately 70%. The site of dermal application was washed 72 hours post-treatment and yielded 14% of the recovered dose; 1.6% of the recovered dose was present in the skin at this site. Thus, approximately 15% of the dose was not absorbed within 72 hours. In an in vitro study, Hotchkiss et al. (1992) found that phenol absorption by rat skin is similar to that of human skin: approximately 20–50% in 72 hours, depending on the conditions.

Taken together, the human and laboratory animal data demonstrate that phenol is readily absorbed following exposure by all dose routes. The recovery of greater than 90% of the administered phenol dose as urinary metabolites provides direct evidence that the administered dose was nearly completely absorbed. The route of administration appears to play a limited role, with skin absorption reported as less extensive than absorption from the lung or gut. In most studies,

absorption rate constants have not been calculated; however, the measurement of peak blood phenol concentrations within minutes of dosing indicates that absorption is rapid

3.2. DISTRIBUTION

Studies in humans and laboratory animals indicate that phenol is widely distributed throughout the body regardless of exposure route. Because phenol is rapidly excreted, studies on tissue distribution typically evaluate only a small fraction of the absorbed dose.

Several fatal poisoning case studies evaluated phenol concentrations in multiple tissues (Tanaka et al., 1998). Generally, phenol is widely distributed. Higher tissue concentrations relative to blood have been reported for some organs, particularly for the liver and kidneys, although this finding has not been reported consistently across all studies.

Morrison et al. (1991) reported on the kinetics of phenol injected intramuscularly in a motor point block procedure in pediatric patients. Administered doses ranged from 6.7 to 70 mg/kg, and the blood phenol concentration was measured at 5, 15, 30, 60, and 120 minutes after the last injection. Phenol reached peak levels 5 to 15 minutes after administration and rapidly declined to 3 to 34% of peak levels within 120 minutes. Peak phenol concentration (μg/mL) in blood as a function of administered dose (x, in mg/kg) was determined ($y = 0.483x - 3.244$; $r = 0.873$). Pretreatment levels of blood phenol ranged from 0.3 to 0.8 μg/mL and post-treatment levels ranged from 2.5 to 36 μg/mL.

The laboratory animal data provide additional evidence for elevated tissue concentrations in the lung, liver, and kidney, although the magnitude of the tissue differences varies from study to study. Liao and Oehme (1981) evaluated the tissue distribution of 207 mg/kg ^{14}C-phenol orally administered to male Sprague-Dawley rats. Total radioactivity in tissues declined rapidly from a maximum of 28.4% of the administered dose at 0.5 hours to 16.6% at 1 hour and 0.3% at 16 hours. Tissue concentrations of radioactivity measured at time points between 0.5 and 16 hours were significantly greater than in plasma for the liver, spleen, kidney, and adrenal gland; tissue concentrations in lungs and thyroid were also marginally elevated. The liver had the greatest amount of radioactivity, accounting for 29–56% of the total radioactivity recovered from tissues at the various time points. The study authors attributed the high levels in the liver to both an elevated tissue concentration and the large relative organ size. Because the study measured total radioactivity without further identification of the radiolabeled compounds, it is not known whether the observed radioactivity represented phenol or its metabolites.

Dow Chemical Co. (1994) conducted a study of ^{14}C-phenol administered to F344 rats by oral gavage at 1.5, 15, or 150 mg/kg in drinking water at 5000 ppm or via nose-only inhalation at 25 ppm for 6 hours. Tissue levels of radioactivity were measured in the kidneys, liver, lung, muscle, skin, spleen, testes, ovaries, and carcass 24 hours after exposure by the various routes. The only sites with a statistically significant increase in radioactivity levels were the kidney and liver (levels 5- to 10-fold higher than in other tissues). This finding was consistent across dosing regimens.

Hughes and Hall (1995) evaluated the disposition of radiolabeled phenol administered dermally, by oral gavage, intravenously, or intratracheally to female F344 rats. When the rats were sacrificed 72 hours after administration by any of these four routes, tissue concentrations represented only 1–5% of the recovered dose. No tissue appeared to have higher concentrations of radiolabel following oral dosing, but the lung concentrations were markedly higher following intratracheal administration. There was no substantive difference across tissues following dermal dosing, although untreated skin had a slightly higher level. Marginal elevations in the liver and kidneys were observed following i.v. dosing. The authors concluded that phenol is distributed widely in tissues, with some accumulation in the large organs (lung, liver, and kidney, based on within-route comparisons to the levels in blood).

Powell et al. (1974) treated juvenile rats (50 g) with less than 1 mg/kg ^{14}C-phenol orally or intraperitoneally. Whole-body radiograms indicated that the liver was not a site for accumulation of the phenol; rather, it was widely distributed. It is not clear whether the difference between the findings of this study and others is due to the differences in the sensitivity of the analysis or to differences in dose levels. Thus, the data from animals studies at doses ranging from 1.5 to 207 mg/kg, which included several doses higher and lower than the chronic No Observed Adverse Effect Level (NOAEL) of 60 mg/kg (NTP 1983a; Argus Research Laboratories, 1997) (see Chapter 5), showed that phenol is rapidly distributed to a wide range of tissues.

No direct studies of the placental transfer of phenol were identified. However, Ghantous and Danielsson (1986) evaluated the placental transfer of benzene, of which phenol is a primary metabolite. B6 mice were exposed for 10 minutes to benzene (at a target concentration of 2000 ppm) in air on gestation day (GD) 11, 14, or 17. The investigators conducted whole-body radiography analysis and determined of tissue concentrations. Radioactivity was distributed to the fetuses, but it was not specifically identified as phenol. The concentration of volatile and nonvolatile radioactivity in the fetuses was, however, lower than that in maternal tissues.

The human and laboratory animal data indicate that phenol is widely distributed in the body. Although the human data are inconclusive, the laboratory animal data consistently indicate that highly perfused organs such as the liver, kidney, and lung have higher tissue concentrations in comparison to the blood concentration.

3.3. METABOLISM

Metabolic pathways for phenol are shown in Figure 1. Phenol is directly conjugated with sulfate or glucuronic acid. Phenol that is not directly conjugated can also be a substrate for oxidation reactions. The cytochrome P450 2E1 isozyme (CYP2E1) catalyzes the addition of one oxygen atom to a variety of low-molecular-weight substrates such as benzene and chloroform, and it is thought to be the primary P450 isozyme for phenol oxidation, although a minor role by other cytochrome P450 enzymes cannot be discounted. The oxidation products of phenol generated by CYP2E1 activity appear to be primarily hydroquinone and catechols, which can themselves undergo further oxidation by CYP2E1 to trihydroxybenzene or by peroxidation to benzoquinone. Alternatively, the hydroquinone or catechol metabolites can undergo conjugation reactions. In addition to P450-mediated oxidation, some studies have suggested that peroxidative metabolism of phenol can also take place, producing biphenols and diphenoquinones.

Direct sulfate and glucuronic acid conjugations are detoxifying mechanisms that represent the bulk of phenol metabolism, as evidenced by the metabolic profiles observed in both humans

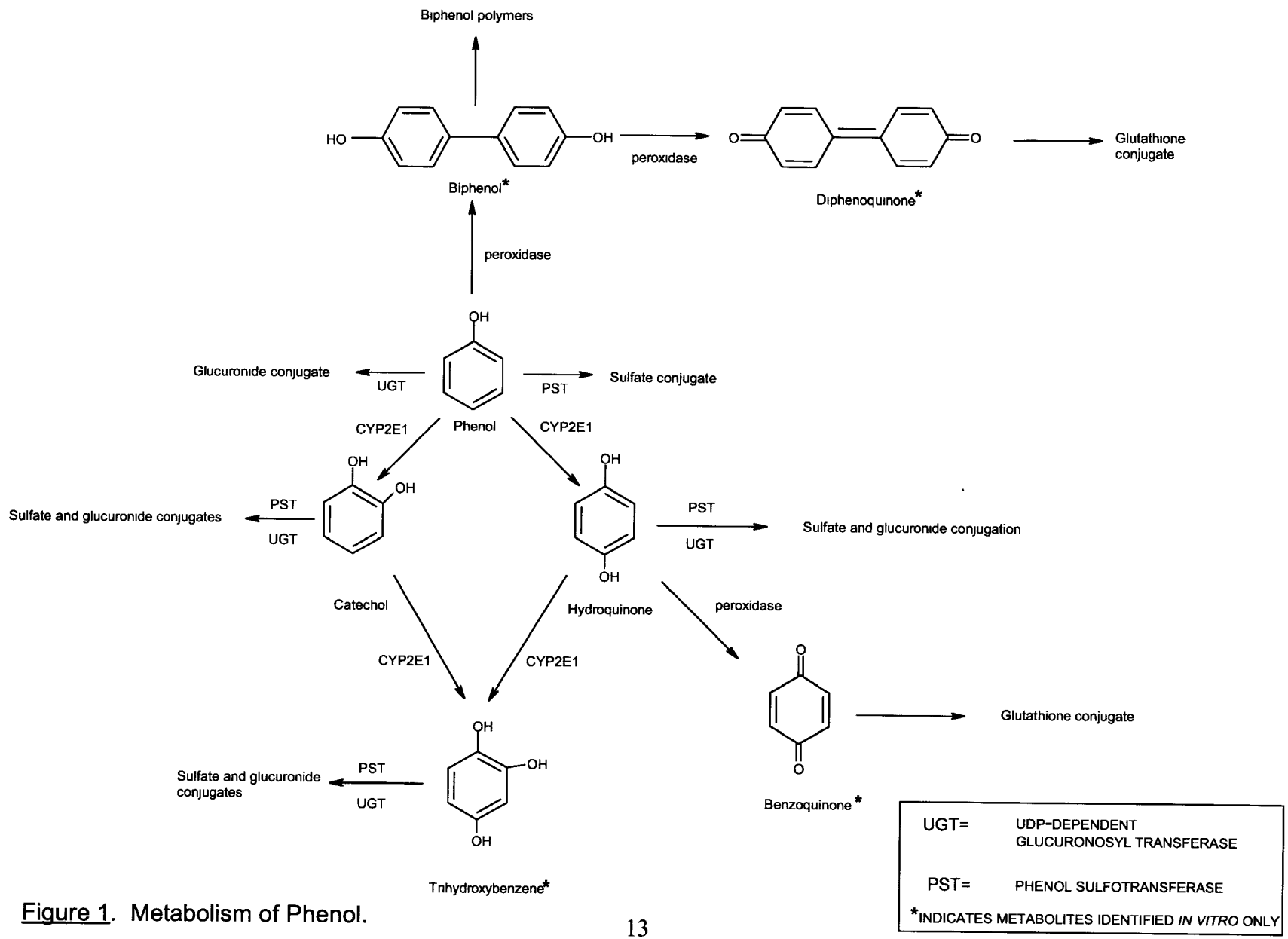

Figure 1. Metabolism of Phenol.

and laboratory animals. In humans and in most other species tested, sulfation predominates at the lower doses. Capel et al. (1972) studied the urinary metabolites following oral administration of 0.01 mg/kg ^{14}C-phenol to three male volunteers. In these men, 85–98% of the dose was excreted in 24 hours—69–90% as phenyl sulfate, 4–23% as phenyl glucuronide, and trace amounts as hydroquinone conjugates. This high degree of conjugation indicates that, at low doses, ingested phenol is nearly completely conjugated.

Laboratory animal studies have clearly shown that as the dose increases, the role of glucuronidation increases, until at sufficiently high doses it becomes the predominant reaction. The formation of oxidative metabolites (primarily by CYP2E1) also increases with increasing dose. These dose-dependent changes have been best characterized in rat studies, which show that at low doses sulfation predominates, with the glucuronidation beginning to predominate at approximately 133 μmol/kg (12.5 mg/kg) (Kao et al., 1979; Powell et al., 1974; Hogg et al., 1981; Koster et al., 1981; Edwards et al., 1986; Meerman et al., 1987; Dow Chemical Co., 1994), a dose that is below the chronic NOAEL of 60 mg/kg for phenol toxicity (NTP 1983a; Argus Research Laboratories, 1997), as discussed later in detail. There is considerable interspecies variation, however, in the contribution of the sulfation and glucuronidation pathways (Capel et al., 1972; Mehta et al., 1978).

The formation of oxidative metabolites increases at high doses. Dow Chemical Co. (1994) reported that hydroquinone conjugates represented 3, 7.8, and 17.0% of the eluted radioactivity in an analysis of urinary metabolites following single oral doses of 1.5, 15, and 150 mg/kg ^{14}C-phenol, respectively. Legathe et al. (1994) administered an intraperitoneal (i.p.) dose of 75 mg/kg to B6 mice and reported urinary metabolites as 34.5% phenyl sulfate, 28.5% phenyl glucuronide, and 32.4% hydroquinone glucuronide, indicating substantial contribution of oxidative metabolism at this high dose.

The formation of oxidative metabolites is thought to result primarily from reactions catalyzed by CYP2E1. Koop et al. (1989), using hepatic microsomes prepared from male New Zealand white rabbits, showed that CYP2E1 was the most active of six P450 isoforms tested. Treatment of the lysates with an antibody to CYP2E1 inhibited hydroquinone formation by 68% and 89% in acetone-induced and uninduced microsomes, respectively. Snyder et al. (1993) studied phenol metabolism in vitro in rat hepatic microsomal preparations. Addition of phenol to the CYP2E1 microsome preparation yielded hydroquinone and, to a lesser degree, catechol metabolites. Incubation of ^{14}C-phenol and ^{3}H-glutathione in the CYP2E1 microsome preparation yielded an additional metabolite that cochromatographed with the compound formed from the reaction of

benzoquinone with glutathione. The formation of the glutathione adduct was not dependent on addition of glutathione-S-transferase. Lunte and Kissinger (1983) also reported the formation of glutathione conjugates in microsomal (prepared from liver of male Swiss mice) metabolism of phenol to hydroquinone. In addition to benzoquinone, hydroquinone and catechol can also be oxidized to trihydroxybenzene (Sawahata and Neal, 1983).

Other in vitro studies using hepatic microsomes from rats treated with various P450 inducers or inhibitors have also provided evidence for the importance of CYP2E1 in phenol metabolism (Sawahata and Neal, 1983; Gilmour et al., 1986; Chapman et al., 1994; Kenyon et al., 1998). CYP2E1 appears to predominate phenol oxidation. Contributions by other P450 enzymes cannot be excluded, however, because only 68% of the induced hydroquinone formation was blocked by anti-CYP2E1 antibody, and several inducers of other P450 enzymes (such as phenobarbital and arochlor) enhanced phenol metabolism in these studies.

An alternative oxidative pathway involving peroxidation has been described for phenol. Several investigators have used in vitro cell preparations with high peroxidase activity, such as peritoneal macrophages or neutrophil preparations (Eastmond et al., 1986; Post et al., 1986; Eastmond et al., 1987; Kalf et al., 1990), purified peroxidase enzymes (Smart and Zannoni, 1984; Subrahmanyam and O'Brien, 1985), or cell lines that have high myeloperoxidase activity (Kolachana et al., 1993), to show that phenol can be metabolized in these reactions. Metabolites resulting from these reactions include 4,4'-biphenol and diphenoquinone. Although the peroxidation of phenol has been demonstrated in vitro, no direct in vivo evidence for these peroxidative reactions was identified.

The shift from sulfation to glucuronidation at increasing doses has been postulated to result from depletion of sulfate pools (Kim et al., 1995). Alternatively, it has been suggested that the difference between the K_m values for sulfate and glucuronide conjugation drives the conjugation shift (Weitering et al., 1979). The effects of differing metabolizing enzyme activity across the zones of the liver has also been suggested as an explanation for the metabolic profiles of phenol (Medinsky et al., 1995). The functional units of the liver include lobules with blood supply provided from the perimeter (periportal region) of the lobule though the portal vein and the hepatic artery. The blood flows from the periphery of the lobule toward the terminal hepatic vein (also called the central vein) at the center of the lobule through a series of differing metabolic regions or zones. Both sulfotransferases and glucuronosyltransferases are present in periportal zone 1, with the sulfotransferases predominating. Glucuronosyltransferases are present in zone 2, while both

glucuronosyltransferases and monooxygenases such as CYP2E1 are present in pericentral zone 3.

According to the model, phenol entering the hepatic circulation would be metabolized first in the periportal region, where sulfation predominates. Because the blood flows from the periportal region to the pericentral region and then to the central vein and general circulation, little unconjugated phenol is available for glucuronide conjugation or oxidation when it reaches the pericentral regions of the liver. This model is consistent with the shift from sulfation to glucuronidation at increasing doses. As the dose increases, more of the phenol reaches the pericentral region unconjugated, and thus is available for glucuronidation. The model also explains the increase in oxidative metabolites at high doses that exceed the conjugating capacity of zones 1 and 2 (Kenyon et al., 1995).

The model is also consistent with the observation that oral dosage with benzene results in greater production of hydroquinone than that seen after oral dosage with phenol, even though benzene is metabolized to hydroquinone via phenol. Benzene enters the liver in the periportal region, is oxidized to phenol and other metabolites in the pericentral region, and then leaves the liver via the hepatic vein. Because benzene must be oxidized before it is conjugated, more unconjugated phenol would be released into the blood following benzene exposure than following phenol exposure (Medinsky et al., 1995).

Direct evidence for this model was presented by Ballinger et al. (1995), who studied phenol and metabolite transport kinetics in isolated perfused liver from rats, and by Hoffmann et al. (1999), who conducted similar experiments in mice. The effects of enzyme distribution in the zones of the liver were studied by contrasting phenol metabolite profiles resulting from antero- and retrograde perfusions. It is noteworthy that the importance of the location of enzyme activities within the liver would only be significant at oral phenol doses that were not conjugated at the portal of entry, and thus were available for transport to the liver via the hepatic portal vein.

There is some evidence that the capacity for phenol conjugation varies with the portal of entry. Cassidy and Houston (1984) conducted an elegant series of experiments in which they injected rats intra-arterially, intravenously, or intraduodenally with phenol and then measured the systemic availability of phenol. This allowed them to evaluate the first-pass metabolism by different organ systems at doses ranging from 0.4 to 15 mg/kg. They were able to use this approach to determine metabolism by the liver and gut. However, results on metabolism by the respiratory tract from this study should be treated with caution, because environmental exposure results in exposure of the epithelial respiratory tract (i.e., the portion exposed to the outside), whereas this study

involved exposure of the endothelial respiratory tract (i.e., the portion exposed to the inside of the body). Thus, any differences between the metabolic capacity of the endothelial and epithelial cells would not be taken into account by the study design.

In this series of experiments, phenol that was systemically available had not been conjugated or metabolized. The doses at which phenol became systemically available thus reflected the doses at which the relevant metabolic enzyme systems became saturated. Metabolism became nearly saturated between 4.5 and 15 mg/kg for the endothelial lung, and between 0.4 and 1.5 mg/kg in the liver, and it was not saturated at the high dose for the gut. The affinity of metabolic pathways also varied among the organ systems. The liver and gut, which removed 88% and 86% (respectively) of phenol at the 0.4 mg/kg dose, demonstrated high affinity in comparism with the endothelial lung, which removed 58% of the phenol at this same dose. Taken together, these data indicate that the gut is a high-affinity and high-capacity site of metabolism, and the liver has high affinity but its capacity is readily exceeded.

The data also suggest that the lung provides substantial metabolizing capacity but has lower affinity than the gut and liver. Clear conclusions regarding the metabolic capacity of the lung following exposure by the inhalation route are not possible because of the potential for differences between the metabolic capacity of the epithelial and endothelial cells of the lung. However, one would expect the potential for metabolism of inhaled phenol to be similar to that seen in this study, because systemically absorbed phenol must pass through the endothelial cell layer.

The area under the blood concentration curve (AUC) for ^{14}C-phenol was route- and dose-dependent, reflecting the effects of portal-of-entry metabolism. In contrast, the AUC for phenol metabolites did not differ by dosing route, indicating that phenol is extensively metabolized and the effect of portal-of-entry metabolism is to reduce the amount of parent compound available for metabolism by other organ systems.

Studies using isolated perfused rat liver were also conducted and correlated well with the in vivo data. The percent of phenol removal from blood by first-pass metabolism declined from 73% at a blood concentration of 2.8 μg/mL to 26% at 26 μg/mL, indicating extensive saturation at the higher dose level.

Dow Chemical Co. (1994) studied the differential metabolism kinetics of phenol by differing exposure routes. This study evaluated the kinetics of ^{14}C-phenol in F344 rats following dosing regimens that included single or eight daily oral gavage doses of 1.5, 15, or 150 mg/kg 5000 ppm in

drinking water for 1 or 8 days or 25 ppm via inhalation for 6 hours (nose-only) for 1 or 8 days. The authors estimated the doses resulting from the drinking water and inhalation exposures. For drinking water administration (males only), doses were estimated by measurement of daily water consumption. The administered dose was 291 mg/kg for the single-day protocol and 405 mg/kg for the last day of the 8-day treatment, based on the water intake and the weight of each animal; thus, the drinking water doses were higher than the oral gavage doses.

The doses used in the drinking water study and the high dose in the gavage study were all higher than the chronic NOAEL of 60 mg/kg (NTP 1983a; Argus Research Laboratories, 1997), as discussed in detail in Section 5. In contrast, the inhalation doses were estimated as 11.5 and 17.8 mg/kg for males and females, respectively, following a single exposure period, and the dose was 21.4 mg/kg (males only) on the last day of the 8-day exposure protocol. Thus, the inhalation route more closely resembled the middle gavage dose level, and the absorbed dose via inhalation was lower than the chronic NOAEL (NTP 1983a; Argus Research Laboratories, 1997).

Metabolic profiles revealed ratios of 0.61 for glucuronide/sulfate conjugates in urine at the two lower gavage doses and were similar following inhalation (0.24–0.39). The ratio at the high gavage dose was 1.16, and it was similar following drinking water exposure (1.43 and 1.87 for the single and 8 day exposures). The observed formation of oxidative products, as shown by urine levels of hydroquinone glucuronide, was also dependent on total dose. The formation of oxidative metabolites following inhalation paralleled the low-dose gavage data, whereas the drinking water levels paralleled the high-dose gavage levels. The pattern of phenol metabolism correlated with the magnitude of the absorbed dose and did not appear to be dependent on the route of administration.

Metabolism of phenol appears extensive in the lung, liver, and gastrointestinal tract; however limited data are available for other organs. Metabolism appears to be extensive in the kidney (Tremaine et al., 1984). No data were identified that addressed portal-of-entry metabolism for the skin.

One consequence of the portal-of-entry metabolism of phenol is that phenol serum levels are not necessarily linear with dose or exposure levels. At low doses, almost all of the absorbed phenol is conjugated and excreted, without entering the bloodstream. At higher doses, free phenol and its metabolites appear in the blood and increase with dose. This nonlinearity of blood phenol levels with dose is illustrated by the data of Dow Chemical Co. (1994). Peak phenol blood concentrations in rats following an oral bolus dose of 150 mg/kg were 2320-fold higher than the peak blood concentrations following an oral bolus dose of 1.5 mg/kg.

The role of peak levels may be significant for induction of at least some aspects of systemic toxicity. Dow Chemical Co. (1994) sheds some light on the relationship between metabolism and toxicity. The high-dose gavage group in this study developed a cluster of behaviors that the authors termed "phenol twitching behavior (PTW)" that included tremors, sudden jerks, hyper-reactivity to stimuli, and excessive blinking. PTW began almost immediately after dosing and disappeared by 37 minutes post-dosing. Blood phenol levels also peaked almost immediately after dosing, and PTW was not apparent at blood phenol concentrations below approximately 3 μg/mL. PTW was not observed at the lower gavage doses or following inhalation exposure; peak blood phenol levels in these groups were well below 1 μg/mL.

Interestingly, PTW was also not observed in the drinking water exposure groups, even though the total dose in these groups was higher than the high gavage dose, and the drinking water doses had a similar metabolic profile to the high gavage dose. Unfortunately, blood phenol levels were not sampled in the drinking water groups, so the peak blood phenol level is not available. However, given the rapid clearance of phenol from the blood, it is likely that the peak blood level was much lower in the drinking water group than in the high-dose gavage group. This suggests that PTW is more closely related to peak phenol blood levels than to a measure of total dose, such as AUC. Because phenol metabolite levels paralleled those of phenol, these data cannot be used to distinguish between phenol and its metabolites being the toxic agent. These data do not identify the appropriate dose metric (e.g., peak concentration vs AUC) for other toxic endpoints.

One indication that the oxidative metabolites are important determinates of toxicity is based on experiments by Chapman et al. (1994). They studied the dysmorphogenic and embryotoxic effects of benzene and its metabolites to the whole rat conceptus in vitro. Phenol at 1.6 mM elicited only minor effects, but inclusion of S9 microsomal fractions greatly increased the potency of phenol, with significant effects observed at doses as low as 0.01 mM. Metabolite analysis indicated that hydroquinone and catechol were the primary metabolites. When evaluated singly, hydroquinone, catechol, and benzoquinone induced similar embryotoxicity, producing 100% lethality at 0.1 mM. The addition of phenol and hydroquinone together induced a more-than-additive embryotoxicity, which the authors suggested as evidence for a peroxidative mechanism for phenol bioactivation, based on the potential for electron cycling between phenol and hydroquinone.

Intraspecies variability has also been studied. Campbell et al. (1987) isolated human liver sulfotransferases, the enzymes responsible for the conjugation of phenol with sulfate, and analyzed their apparent activities toward *p*-nitrophenol (as a model compound for simple phenols).

The average phenol sulfotransferase (PST) activity measured in liver samples of 20 patients (13 male, 7 female) was 35.8±10.6 standard error of the mean (SEM) units/mg protein. No correlation between enzyme activity and patient age or gender was found, although the power to detect any such correlation was not noted. Seaton et al. (1995) studied the kinetics of phenol sulfation and hydroquinone conjugation, both of which varied over a range of approximately three-fold in a sample of liver fractions from 10 humans. Using lysates from a single human liver, saturation of phenol sulfation was apparent above 800 μM; the observed kinetics were consistent with two contributing enzymes, PST1 and PST2. The expression of two distinct PST enzymes has also been demonstrated in human nasal epithelium (Beckmann et al., 1995).

Kawamoto et al. (1996) studied the effect of various lifestyle factors and of genetic polymorphisms in five metabolizing enzymes, including aldehyde dehydrogenase (ALDH2), N-acetyl transferase (NAT2), cytochrome P450 1A1 (CYP1A1), CYP2E1, and glutathione-*S*-transferase mu (GSTM1) on urinary levels of phenol in a cohort of men who were not occupationally exposured. Step-wise multiple regression analysis was performed to identify important determinates of urinary phenol levels. On the basis of this analysis, there was no relationship between polymorphisms (including for CYP2E1) and background urinary phenol levels. In the total sample (n = 351), the geometric mean urinary phenol level was 7.64 mg/L and the geometric standard deviation was 2.9. No data are available, however, on how genetic polymorphisms affect the levels of metabolites produced from exogenously dosed phenol.

The changes in enzyme activity or expression of genes that encode enzymes important for phenol metabolism with age have been studied. The status of CYP2E1 in fetuses remains unclear, with conflicting results reported. Most of the existing studies indicate that this enzyme is expressed in human adults but not in human fetuses, even when measured using sensitive assays (reviewed in Hakkola et al., 1998). However, at least two studies (Carpenter et al., 1996; Vieira et al., 1996) indicate that CYP2E1 is expressed at least to some degree in fetal liver. Vieira et al. found that CYP2E1 protein could not be detected immunochemically in fetal human liver, and there was only minimal evidence of CYP2E1 mRNA or CYP2E1 activity in fetal liver microsomes. (The difference in assay results may be due to differences in sensitivity or to cross-reaction of CYP1A1 activity.) However, the authors found, that CYP2E1 protein levels rise rapidly in the first few hours after birth, with a slow increase in protein levels and in CYP2E1 mRNA levels during childhood.

Results of animal studies of developmental CYP2E1 regulation are consistent with the human data in providing uniform evidence of the rapid induction of this gene soon after birth (Song

et al., 1986; Umeno et al., 1988; Schenkman et al., 1989; Ueno and Gonzalez, 1990). Thus, overall, the data show that if CYP2E1 activity exists in human fetuses, levels are much lower than those in adults. Regardless of fetal CYP2E1 expression, the enzyme is rapidly induced upon birth. For this reason, children would be expected to be capable of phenol metabolism, although the amount of CYP2E1 may be less than that present in adults.

Age-dependent changes in phase II conjugation have also been evaluated. In an evaluation of how PST activity varies with age in rats, Iwasaki et al. (1993) studied ß-naphthol metabolism by PST in fetal rat liver, in the liver of 2-, 9-, 17-, and 25-day-old neonates, and in adult rats. Activity was analyzed in the livers of both sexes. The fetal liver had little conjugating ability, but this activity developed rapidly after birth. However, activity was substantially lower in neonates of all ages evaluated when compared with adult levels. Heaton and Renwick (1991) administered i.p. doses of 25 mg/kg ^{14}C-phenol to rats varying in age from 3 to 16 weeks and measured metabolites in urine collected in 24 hours. The percentage of the administered dose recovered in the urine in 24 hours ranged from 61–90% in males and 63–99% in females, with increasing recovery with age.

Importantly, the formation of hydroquinone conjugates was greater in the younger animals. In males, 38% of the administered dose was recovered as hydroquinone conjugates in the 3-week-old animals; 8.2% of the urinary metabolites was recovered in 16-week-old rats. In females, 17.8% of the administered dose was recovered as hydroquinone conjugates in 4-week-old rats and 10.5% was recovered in 15-week-old rats. Taken together, the evidence indicates that both sulfate conjugation and P450 metabolism are lower early in life and increase as adulthood is reached. However, even in the face of limited P450 activity, significant formation of oxidation products can occur because of limited sulfation capacity. The oxidative products become substrates for glucuronidation, and this does not appear to be limited in the young.

Phenol metabolism may also be gender dependent, although the data are less substantial than those for differences due to age. Iwasaki et al. (1986) reported that PST activity was similar in both sexes up to 3 weeks of age and was higher in males than in females in 7-week-old rats. Activity in 2-year-old rats of both sexes was similar and fell between the levels for males and females at 7 weeks to 1 year. Kenyon et al. (1995) administered ^{14}C-phenol to B6 mice of both sexes and observed that, males excreted a greater proportion of HQ-glucuronide than did females at all doses; the difference was roughly twofold at a dose of 40 μmol/kg. These results are consistent with the greater degree of hydroquinone conjugates excreted in the urine of male versus female rats reported by Heaton and Renwick (1991). Sex-based differences in metabolism have also been reported in

rats (Meerman et al., 1987), with slightly lower total recovered radioactivity in the urine of females versus males (i.e., more rapid metabolism in males). However, the magnitude of this difference (91.2% vs 87.3%) was limited.

Interspecies differences in phenol metabolism have also been evaluated. Seaton et al. (1995) found that the rates of both phenol sulfation and hydroquinone conjugation in mouse and rat liver were comparable to those of human liver preparations. Schlosser et al. (1993) reported that mouse liver microsomes metabolized approximately twice as much phenol as did rat liver microsomes, although the relative proportions of metabolites were roughly similar.

Phenol is formed endogenously in the gut by bacterial metabolism of aromatic amino acids in protein. The amount formed is related to the amount of protein ingested, but the amount in humans typically varies from 1 to 10 mg/day, corresponding to approximately 0.014 to 0.14 mg/kg-day (Bone et al., 1976; Lawrie and Renwick, 1987; Renwick et al., 1988).

A physiologically based pharmacokinetic (PBPK) model for the distribution of benzene and metabolites was developed by Bois et al. (1991). The model was developed to predict phenol and metabolite distributions to fat, well-perfused tissue, poorly-perfused tissue, bone marrow, liver, lung, and gut using Monte Carlo simulations of 64 parameters. The model was not validated using empirical data. The Bois et al. model consistently predicted that phenol administration would produce higher levels of phenol and hydroquinone in the blood than seen following benzene administration.

The first phase in the development of a model of the in vitro kinetics of phenol and benzene biotransformation by liver microsomes was described by Schlosser et al. (1993) and enhanced by Medinsky et al. (1995). The model described the following reaction sequences: benzene > phenol > catechol > trihydroxybenzene and phenol > hydroquinone > trihydroxybenzene. All reaction steps were assumed to be catalyzed by cytochrome P450 2E1, and benzene, phenol, catechol, and hydroquinone were all assumed to compete through reversible binding for the same reaction site on cytochrome P450. Parameters were identified that were successful at predicting the concentration with time of all five chemicals in incubations with rat or mouse liver microsomes (Schlosser et al., 1993). The observation of a lag time in the production of hydroquinone from benzene—in comparison to the rate of production of hydroquinone from phenol—supported the assumption that all of the substrates compete for the same enzyme reaction site.

Medinsky et al. (1995) extended the data into a conceptual model of the differences between phenol and benzene metabolism. The goals of the conceptual model included explaining the

observed differences between the carcinogenicity and genotoxicity of phenol and benzene and explaining why urinary hydroquinone levels are higher after benzene dosing than after phenol dosing. The latter observation would appear to be inconsistent with the prediction of the Bois et al. (1991) model that blood hydroquinone levels are higher following phenol dosing than following benzene dosing. As described earlier in this section, differences between benzene and phenol toxicity were attributed to zonal differences in the distribution of hepatic metabolic enzymes.

In summary, phenol is an endogenous metabolite that undergoes further metabolism efficiently. At low doses the bulk of the phenol appears to be conjugated with sulfate or glucuronide at the portal of entry. As the dose increases, the sulfation pathway becomes saturated, and the relative contribution of glucuronidation and oxidation reactions increases. Saturation of first-pass metabolism may be important for producing peak levels of phenol that correlate with acute systemic toxicity. In addition, saturation of conjugation, which leads to increases in oxidative metabolism, may also be an important determinate of toxicity. The data on intraspecies variability are limited, but they do not indicate great variation in metabolic capacity in humans. In rodents, males and younger animals appear to rely more heavily than females and adult animals on oxidative metabolism, respectively, but the differences are no more than twofold. The metabolism of phenol in humans and rodents appears to be similar, although some evidence suggests that mice metabolize phenol more rapidly than do humans or rats.

3.4. EXCRETION

The existing human and laboratory animal studies consistently report that phenol is rapidly excreted, with little tendency for accumulation. Elimination is primarily in the urine in both humans and laboratory animals, with only a minor contribution of elimination in the bile. Ohtsuji and Ikeda (1972) studied the urinary free and conjugated phenol levels in Bakelite® factory workers. Workers were exposed to phenol vapor by inhalation on a daily basis. The workers were also possibly exposed by the dermal route, but the contribution of this route to the total exposure was not directly measured. Analysis of urinary phenol levels at different times during the work shift and across work shifts indicated that in workers exposed to 7.8 to 9.6 mg/m^3, the urinary levels increased significantly from the beginning of the work shift to the end of the work shift, but they did not tend to accumulate across the work shifts. A slight increase in the morning sample on the sixth consecutive work day was observed, but after two days off, pre-shift samples were no longer elevated.

Rapid clearance from the blood in humans has also been observed. Bentur et al. (1998) presented a case report from a dermal poisoning in which a solution of 90% phenol was spilled on the left foot (3% of body surface). Even at this high exposure level, clearance from the blood was rapid, with blood levels decreasing from 21.6 to 2.8 μg/mL in the first 12 hours. The authors estimated that the half-life elimination was 13.86 hours, but they did not include the initial rapid decline in serum concentration that was apparent over the first 12 hours post-admission. Both absorption and clearance would be expected to be more rapid at lower exposure levels, as high exposure levels can lead to protein denaturation and saturation kinetics.

Laboratory animal studies have consistently found that phenol is rapidly excreted. Clearance of phenol from the blood is rapid. Rats given an oral dose of 300 mg/kg, a level comparable to the rodent median lethal dose (LD_{50})of 340 mg/kg (Deichmann and Witherup, 1944), had maximum blood concentrations of 26 μg/mL at the first measured time point (about 10 minutes), and blood levels declined rapidly to background by 60 minutes (Humphrey et al., 1980). Similar results were observed by the same authors in dogs given a 40 mg/kg dose, with rapid peak levels (7.8 μg/mL) and complete removal of free phenol by 1 hour. A half-life of 12 minutes in blood was reported for rats administered 150 mg/kg by gavage (Dow Chemical Co., 1994). Legathe et al. (1994) reported biphasic elimination kinetics from the blood, with a terminal half-life of 22 minutes. Similarly, Cassidy and Houston (1984) reported biphasic kinetics with a half-life of approximately 5 minutes following intra-arterial administration.

The elimination kinetics in multiple tissues was studied by Liao and Oehme (1981). Total radioactivity in tissues was maximal within 30 minutes of dosing, representing 28.4% of the administered dose. Tissue levels accounted for 16.6% of the administered dose at 2 hours and 0.3% at 16 hours. Although maximum levels varied considerably across tissues, the rate of elimination did not appear to differ with tissue type. Numerous laboratory animal studies indicate that urinary elimination of sulfate and glucuronide conjugates accounts for most of the excretion, ranging from 70 to 90% of the administered dose within 24 hours, whereas excretion in feces represents only a small fraction of the administered dose, approximately 1–3% (Edwards et al., 1986; Meerman et al., 1987; Dow Chemical Co., 1994; Hughes and Hall, 1995).

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS—EPIDEMIOLOGY, CASE REPORTS, CLINICAL CONTROLS

The epidemiology data on phenol are limited. Epidemiology studies have typically included confounding exposures and have not adequately adjust for smoking. Kauppinen et al. (1986) reported a significant increase in respiratory cancer in phenol-exposed workers, but this observation appears to be due to confounding exposures, because there was no dose-response, and the effect decreased after accounting for latency. No effect on cancer mortality was observed in workers exposed to phenol in the rubber industry (Wilcosky et al., 1984) or in workers exposed to formaldehyde and phenol (Dosemeci et al., 1991). An occupational study (Shamy et al., 1994) and case studies (e.g., Merliss, 1972) have reported liver effects following exposure to phenol. Immune effects were also been reported in an occupational study of workers exposed to phenol as part of a mixture of solvents (Baj et al., 1994). Studies of populations whose drinking water was contaminated with phenol found elevated incidences of diarrhea, nausea, mouth sores, and dark urine (Jarvis et al., 1985; Baker et al., 1978).

4.1.1. Oral

Estimated lethal oral doses of phenol in adults vary widely, from 1 g (14 mg/kg, assuming an adult body weight of 70 kg) to as much as 65 g (930 mg/kg) (Deichmann and Klepinger, 1981). In another report (Bruce et al., 1987), the minimum lethal oral dose in adults was estimated as 140 mg/kg.

Jarvis et al. (1985) reported on illness associated with consuming water contaminated with phenol from a spill into the river that served as the drinking water source. A retrospective mail survey was sent to a total of 594 English households: 250 that were highly exposed, 94 that were exposed at low concentrations (from a reservoir that diluted the contaminated river water), and 250 that were unexposed (selected from a telephone book). On the basis of data from the water authority, the estimated phenol concentrations in drinking water in the low-exposure area (0.05 mol/L, equivalent to 4.7 μg/L) was roughly half that in the high-exposure area (0.11 μmol/L, equivalent to 10 μg/L) for the first 24 hours. The next day, the phenol concentration for both groups was 0.05 μmol/L, and the concentration was < 0.01 μmol/L (< 0.9 μg/L) by the third day after the contamination incident.

Chlorination of the water resulted in production of chlorophenols. The chlorophenol concentration followed a similar pattern, but the chlorophenol concentrations, which ranged from

0.43 to 0.2 μmol/L at the first measurement (84.7–39.4 μg/L, assuming all chlorophenols were in the form of trichlorophenols), were higher than those for phenol. There were no data on phenol concentrations in the unexposed area, but an unspecified local press report implicated a possibility of phenol contamination. Due to the similarity of the two exposed areas in the measured concentrations of phenol and chlorophenol, these two areas were combined in the data analysis.

The percentage of responding households was similar in all of the groups and ranged from 69 to 77%, resulting in 172 households (448 people) in the unexposed area and 254 households (754 people) in the exposed area being evaluated. The two groups had similar distributions in sex, age, and usual water usage. Compared with the unexposed individuals, those in the exposed area had significantly higher incidences of gastrointestinal illnesses, such as diarrhea, nausea, vomiting, and abdominal pain. Other symptoms, such as headache, rash, and malaise, were also observed at a significantly elevated incidence in the exposed group. The day of onset of symptoms corresponded with the period of elevated phenol concentrations in the contaminated drinking water. The associations were stronger among those who reported that they drank the water than among those in the exposed area who reported not drinking the water. (Others may have consumed the water in cooking.) In another analysis, gastrointestinal symptoms did not significantly correlate with whether the water tasted bad. However, because of the confounding exposure of chlorophenol in the water, the usefulness of the result for risk assessment is limited.

Baker et al. (1978) reported on phenol poisoning in humans due to an accidental contamination of their drinking water on July 16, 1974. A train derailment resulted in a spillage of 37,900 L of pure phenol onto the ground, and the spillage caused contamination of drinking water in wells of nearby houses. Two wells near the spill were tested initially on July 23 and were found to have phenol concentrations of 0.21 and 3.2 mg/L. Further testing in late July and August of the six wells nearest the spill found peak concentrations between 15 and 126 mg/L. Within approximately 2 months after the spill, "most families" began to obtain water from other sources (from neighbors or bottled water). Phenol concentrations in well water as high as 1130 mg/L were reported over the next 6 months, with the higher levels observed after flushing of the spill site (unspecified number of wells tested). The authors investigated the health effects in three groups of people. Group 1 (n = 39) consisted of all families living 120–310 m from the spill site and having at least one water test greater than 0.1 mg phenol/L (at least once between July and February). Group 2 (neighborhood control) (n = 61) consisted of all families adjacent to Group 1 (210–670 meters from the spill) whose wells had phenol concentrations of between 0.1 and 0.0001 mg/L. Group 3 (distant control)

(n = 58) lived at least 1.9 km from the spill and had no phenol in its wells.

Group 1 reported significantly more diarrhea, mouth sores, burning mouth, and dark urine than the combined control groups. About 44% of the individuals in Group 1 reported at least two of these responses in the 7 months following the accident and were considered "affected individuals"; only 8% and 3% of Group 2 or 3 subjects, respectively, had the same responses. Responses in Group 1 were primarily restricted to the first 2 months of exposure, before the use of bottled water began. Responses in the other two groups tended to occur throughout the 8-month period (July through February). Other than the four reported symptoms, no abnormal observations in physical examinations and serum biochemical evaluations were evident in Group 1 as compared with controls when monitoring was done in February.

On the basis of water testing data and water preference histories, the authors estimated that the daily oral dose of phenol for the 17 affected individuals in Group 1 was between 10 and 240 mg. However, this range may overestimate the amount of phenol ingested because phenol's unpleasant odor might have discouraged ingestion of water with concentrations above 0.1 mg/L. In contrast, this range does not include phenol that may have been absorbed during skin contact with contaminated water. It was also not clear whether the subjects continued to shower with the contaminated water after switching their drinking water source. Based on a default adult body weight of 70 kg, this daily oral dose corresponds to 0.14 to 3.4 mg phenol/kg-day. Thus, there is a considerable range in the estimated phenol dose associated with symptoms. In addition, because "most" (but apparently not all) families switched to other water sources within the first 2 months of exposure, the exposure duration for the affected individuals is not known. Therefore, it is difficult to use these data for quantitative analysis, although they might be useful for placing bounding estimates on the risk values for systemic effects estimated from laboratory animal studies.

4.1.2. Inhalation

Kauppinen et al. (1986) reported a case-control study on respiratory cancers and chemical exposures in the wood industry. A cohort of 3805 Finnish men who worked in the particle board, plywood, sawmill, or formaldehyde glue industries for at least 1 year between 1944 and 1965 was followed until 1981. From the cohort, 60 cases of respiratory malignant tumors were identified. The tissue locations of these tumors included tongue (1), pharynx (1), larynx or epiglottis (4), and lung or trachea (54). No cases with tumor in the mouth, nose, or sinuses were identified. Among the 60 cases, 2 were rejected due to a false preliminary diagnosis of cancer and 1 was rejected as

chronic lymphocytic leukemia. The final size of the group of cases was thus 57. The control group contained three subjects for each case, selected from the cohort and matched by birth year, for a total size of 171.

The job exposure was estimated from the industrial hygiene data of the plant, general hygiene data on exposures, and information on ventilation, work procedures, and other relevant factors at the plants. However, the authors, gave no information on direct phenol measurements; thus, the quality of the estimated exposure levels could not be evaluated. The work histories of the subjects were assessed primarily from plant registers and supplemented with personal interviews. Individual phenol exposures were determined qualitatively as "yes" or "no" and as a function of exposure time.

Smoking histories were determined by a mail survey that resulted in smoking information on 39 of 57 cases (68%) and on 130 of 171 controls (76%). Because there were few nonsmokers and information on the amount smoked was not as complete as on years of smoking, the subjects were compared only as light or heavy smokers, based on whether their years of smoking exceeded 35.

Phenol exposure resulted in a statistically significant odds ratio (OR) of 3.98 or 4.94 for respiratory tumors with or without the adjustment for smoking years, respectively. When the duration of phenol exposure was considered, both exposures ≤ 5 years and > 5 years resulted in a statistically significant OR $\leq$ of 5.86 or 4.03, respectively (i.e., no duration response). When a provision for a 10-year latency was introduced (excluding exposure during the 10 years immediately preceding the diagnosis of cases), phenol exposure resulted in a nonsignificant OR of 2.86 adjusted for smoking years but a significant or of 3.98 without smoking adjustment. Of the 39 cases for which smoking information was available, 12 had been exposed to phenol (9 to phenol in wood dust), and 7 had been exposed to phenol with a 10-year latency (4 to phenol in wood dust). Because the OR did not increase with duration of phenol exposure and the provision for the 10-year latency period resulted in lower values of ORs, a confounding factor may have been responsible for the observed statistically significant ORs.

One of the confounding factors could have been concurrent exposure to multiple pesticides, which was in the same study shown to increase the OR for respiratory tumors. An exclusion of workers exposed to both phenol and pesticides resulted in a change of the OR from a significant 4.9 to a nonsignificant 2.6. Thus, a confounding effect due to exposures to pesticides was very possible. Considering the location of the tumors, formaldehyde exposure was also a likely confounder.

Generally similar results were observed in this study for workers exposed to phenol in wood

dust. Exposure to phenol in wood dust resulted in a statistically significant OR with or without adjustment for smoking. For the workers exposed to phenol but not wood dust, provision for a latency period eliminated the observed statistically significant OR. Among the workers exposed to phenol in wood dust, however, the OR did increase with exposure duration and was statistically significant in those exposed > 5 years (OR of 4.77) but not in those exposed for ≤ 5 years (OR of 3.84). On the basis of these results, the phenol-exposed workers had an elevated risk of respiratory cancer, but phenol itself did not appear to be the causative agent; rather, it appears that there was a confounding exposure.

Wilcosky et al. (1984) reported a case-control study of cancer mortality and solvent exposures in the rubber industry. From a cohort of 6678 active and retired male rubber workers of a large plant in Ohio, 183 decedents from stomach cancer, prostate cancer, lymphosarcoma and reticulum cell sarcoma, lymphatic leukemia, and respiratory cancer were selected as cases. As a control, 20% of an age-stratified random sample of the cohort (calculated as 1336 subjects) was selected. Including phenol, a total of 25 solvents were authorized to be used in the plant. The exposure to any particular solvent was determined from the records of annual authorization for use of these solvents in each work area. Only workers who had cumulative exposures of more than 1 year were considered exposed.

On the basis of the analysis of the age-adjusted exposure ORs, no association was seen between phenol exposure and mortality from stomach cancer, prostate cancer, lymphosarcoma and reticulum cell sarcoma, lymphatic leukemia, or respiratory cancer. However, this study had several major limitations. One limitation was that the estimation of whether workers were exposed to a solvent was based solely on authorization and not on actual usage, which would tend to lead to an overestimation of exposure. In addition, the analysis was based solely on a qualitative evaluation of whether a given solvent was used; no estimates of exposure were made, and so no exposure-response assessment was conducted. Although smoking can confound evaluation of cancer risk, this factor was not investigated. Finally, it was common for workers to be simultaneously exposed to multiple solvents; therefore, solvents other than phenol may have affected the study outcome. In this study, phenol exposure was not associated with a risk of several cancers, but this lack of an association cannot be considered definitive because of the study limitations mentioned above.

In an occupational epidemiology study, Dosemeci et al. (1991) evaluated mortality among 14,861 white male workers in five companies that used formaldehyde and phenol. Unfortunately, the phenol exposure was confounded by co-exposure to other compounds, such as formaldehyde,

asbestos, urea, melamine, hexamethylenediamine, wood dust, plasticizers, carbon black, ammonia, and antioxidants. On the basis of phenol concentrations obtained from historical monitoring and industrial hygiene surveys, the investigators assigned each job/department/year combination to groups with no, low, medium, or high phenol exposure and then calculated cumulative exposure.

Compared with the entire U.S. population, the entire cohort, had no significant increases in standardized mortality ratios (SMRs) for all causes of death or any diseases. The phenol-exposed workers as a group had slightly elevated SMRs for cancers of the esophagus (1.6), rectum (1.4), kidney (1.3), and Hodgkin's disease (1.7); however, none of these increases were statistically significant when compared with those in general population. In addition, an analysis of mortality by level of cumulative exposure showed that none of these increases had dose-response relationships with exposure to phenol. The only significant observations were decreases of SMRs for infective and parasitic diseases and for accidents in the entire cohort and exposed workers. These observations were attributed to the healthy worker effect. This study provided no evidence of phenol-induced morbidity, mortality, or carcinogenicity.

Baj et al. (1994) reported an epidemiology study of 22 Polish office workers (18 females and 4 males) exposed to Ksylamit® vapor for 6 months and 27 age- and sex-matched healthy volunteers from the same town. The exact composition of the Ksylamit® vapor was not reported. The study authors stated that Ksylamit® consists of "a mixture of chlorinated benzenes, pentachlorophenol, -chloronaphthalene, chloroparaffin and kerosene." The only exposure information reported was that at the end of 6-month exposure period the concentrations of formaldehyde and phenol in the workplace atmosphere were 0.8 mg/m^3 and 1.3 mg/m^3, respectively. The study authors did not address how exposure to formaldehyde or phenol resulted from the reported product constituents. In addition, it cannot be determined from the presented information whether the analytical methods used would differentiate between phenol and pentachlorophenol (ATSDR, 1998).

The exposed workers reported chronic symptoms such as headache, cough and sore throats, burning eyes, and fatigue, but morbidity during the 6-month exposure period was comparable to that of the controls. Although all evaluated hematological parameters were normal in the exposed workers as a group, some statistically significant changes were observed in a subset of eight workers who had elevated urinary phenol levels 3 days after the last day of exposure (mean of 18.2 mg/L, compared with 12.1 mg/L in the exposed workers and 7.9 mg/L for the general population). Compared with the matched controls, there was a small, but statistically significant decrease among

in erythrocyte counts and a statistically significant increase in eosinophil and monocyte counts. Levels of CD3, CD4, and CD8 lymphocytes were also decreased in the exposed group, but there was no effect on the CD4/CD8 ratio, and the effect was not stronger in the apparently more highlyexposed subset. Decreases in lymphocyte proliferation induced by phytohemagglutinin and alloantigens were also observed in exposed workers, whereas reactivity to concanavalin A (Con A) was unchanged.

These results suggest that exposure to Ksylamit® could affect the immune and hematological systems. However, the poor characterization of the chemical exposure, including uncertainties regarding the source of the phenol as well as the marginal dose-response for phenol in urine, mean that conclusions regarding the contribution of phenol to the observed effects are limited.

Shamy et al. (1994) reported a cross-sectional investigation of phenol-induced biochemical changes in workers at an oil refining plant in Egypt. The study included 20 workers who were exposed to a time=weighted average concentration of 5.4 ppm phenol and 30 office workers who had no exposure to organic solvents. The phenol-exposed workers worked in the aromatic extraction of distillates; other potential exposures were not described. The mean concentration of phenol in spot urine samples was 68.6 and 11.5 mg/g creatinine in the exposed and control groups, respectively. The average duration of exposure was 13.15 years. At the end of the shift of the last working day of the week, blood samples were collected for hematological and serum biochemistry evaluations.

Small but statistically significant increases (approximately 55% and 80%, respectively) were observed in serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT). There were also small but statistically significant increases in hemoglobin, hematocrit, mean corpuscular hemoglobin, and mean corpuscular volume, although there was no effect on red blood cell count. This finding is in contrast with those of laboratory animal studies, in which decreases in erythrocytes and hematocrit have been reported. Other small, but statistically significant changes included increased basophils and neutrophils, decreased monocytes, and increased clotting time. A nonsignificant increase in prothrombin time and decrease in platelets was also observed.

Overall, these data suggest subclinical effects on the liver and hematopoietic system, based on the small changes in SGOT and SGPT; the observed increases in the hematology endpoints are not adverse. Although the authors described the workers as exposed to phenol alone and compared them with other workers exposed to mixed solvents, it appears that the phenol-exposed workers may

have also been exposed to other organic compounds that can cause hepatic or hematologic effects, and the observed effects cannot be clearly attributed to phenol exposure.

Merliss (1972) reported a case of phenol marasmus. A laboratory technician had been frequently exposed to phenol through vapor or skin contact for 13.5 years. He developed lessened appetite, weight loss, muscle pain in his legs and arms, and dark color in his urine. Serum biochemical evaluations indicated liver damage, with SGOT and SGPT at much higher than normal levels. The patient's symptoms improved after the exposure ended.

4.2. PRE-CHRONIC, CHRONIC STUDIES AND CANCER BIOASSAYS IN LABORATORY ANIMALS

4.2.1. Oral

Acute lethality of oral phenol has been evaluated in numerous animal studies. Oral LD_{50} values in rodents range from 300 mg/kg to 650 mg/kg (Deichmann and Witherup, 1944; Von Oettingen and Sharpless, 1946; Flickinger, 1976; Berman et al., 1995). The acute toxicity of phenol when administered by gavage appears to be at least partly dependent on phenol concentration or total administered volume (Deichmann and Witherup, 1944; NTP, 1983a), and it may be more severe in young animals (Deichmann and Witherup, 1944). In addition to lethality, acute oral dosing has been reported to induce adverse renal (tubular necrosis, protein casts, papillary hemorrhage), hematological (reduction in poly/normochromatic erythrocyte ratio), respiratory (dyspnea and rales), neurological (muscle twitching, decreased motor activity, ataxia, tremors, convulsions, coma), and reproductive and developmental effects (Berman et al., 1995; Narotsky and Kavlock, 1995; Liao and Oehme, 1981; Moser et. al. 1995; NTP, 1983a,b).

As shown in Table 2, there is an extensive database of oral studies relevant to the RfD. Chronic drinking water studies have been conducted in rats and mice, but the only noncancer endpoints evaluated were body weight and histopathology (NCI, 1980). Hematology and serum biochemical evaluations were included in a recent two-generation drinking water study conducted in rats (Ryan et al., 2001; available in unpublished form as IIT Research Institute, 1999). A specialized subchronic neurotoxicity study was conducted with rats exposed to phenol in drinking water (ClinTrials BioResearch, 1998).

These drinking water studies consistently found effects only at exposure levels where water consumption was also decreased, sometimes by as much as 80%. The decreased water consumption was presumably due to poor palatability of the drinking water. Effects seen in these studies included

tremors, decreased final body weight as compared with the controls (possibly as a result of dehydration), decreased motor activity, and kidney inflammation. Decreased pup survival was also observed in the two-generation study. The only drinking water study in which effects were seen in the absence of decreased water consumption was a 28-day study with mice by Hsieh et al. (1992). In that study, decreased hematocrit and decreased immune endpoints were observed at doses at least an order of magnitude lower than the NOAELs in the other drinking water studies. Although the Hsieh et al. study is useful for hazard identification, its findings need to be confirmed before they can be used in dose-response assessment. The differing findings of this study and the above-mentioned two-generation study in rats in which no immunological effects were observed suggest marked interspecies differences between rats and mice for this endpoint.

Toxicity in gavage studies with phenol is typically much higher than that in the drinking water studies. NOAELs for systemic effects were 5–10 - fold lower in gavage studies (Berman et al., 1995; Moser et al., 1995; Dow Chemical Co., 1945) than those seen in the drinking water studies. Effects observed included tremor and liver and kidney histopathology. As described in greater detail in Section 4.5, this difference between gavage and drinking water exposure is

Table 2. Summary of Oral Toxicity Studies

Strain, Species, Sex	Reference	Dose	Route/ Volume	Duration	Response	NOAEL mg/kg-day	LOAEL mg/kg-day	Comments
Systemic Toxicity								
F344 rat, 50/sex/ group	NCI, 1980	0, 2500, 5000 ppm; 0, 260, 585 (M), 0, 280, 630 (F) mg/kg-day	Drinking water	103 weeks	Kidney inflammation and decreased body weight (compared to controls) in both sexes, decreased water consumption at high dose	260	585	Study authors stated there were no noncancer effects, but independent evaluation for this assessment found significant increase in kidney inflammation.
B6C3F1 mouse, 50/sex/ group	NCI, 1980	0, 2500, 5000 ppm; 0, 450, or 660 mg/kg-day	Drinking water	103 weeks	Decreased body weight, decreased water consumption	450	660	Effect apparently secondary to decreased water consumption.
F344 rat, 10/sex/ group	NCI, 1980	0, 100, 300, 1000, 3000, 10,000 ppm; 0, 16, 48, 160, 480, 800 (M), 0, 17, 51, 170, 510, 1140 (F) mg/kg-day	Drinking water	13 weeks	Decreased body weight, decreased water consumption.	480	800	Range-finding for bioassay; effect apparently secondary to decreased water consumption.
B6C3F1 mouse, 10/group	NCI, 1980	0, 100, 300, 1000, 3000, 10,000 ppm; 0, 25, 75, 250, 450, 500 (M), 0, 26, 78, 260, 468, 520 (F) mg/kg-day	Drinking water	13 weeks	Decreased body weight, decreased water consumption.	450	500	Range-finding for bioassay; effect apparently secondary to decreased water consumption.

Strain, Species, Sex	Reference	Dose	Route/ Volume	Duration	Response	NOAEL mg/kg-day	LOAEL mg/kg-day	Comments
Sprague-Dawley rat, 15/sex/ group	ClinTrials BioResearch, 1998	0, 200, 1000, 5000 ppm; 0, 18, 83.1, 308 (M), 0, 24.6, 107, 360 (F) mg/kg-day	Drinking water	13 weeks	Decreased motor activity in females, decreased body weight in males and females, decreased water consumption	107	360	Specialized neurotoxicity study; decreased body weight apparently secondary to decreased water consumption; unpublished GLP study BMDL = 219 mg/kg-day
CD-1 mouse, 5 M/group	Hsieh et al., 1992	0, 4.7, 19.5, 95.2 ppm; 0, 1.8, 6.2 or 33.6 mg/kg-day	Drinking water	28 days	Decreased immune endpoints (particularly in plaque-forming cell assay and ELISA), decreased red blood cells and hematocrit	6.2	33.6	Study limited by small sample size; confirmation in a study conducted according to modern methods would be useful.
Rat 10/group, strain & sex NS	Dow Chemical Co., 1945	0, 50, 100 duration adjusted: 0, 35.7, 71.4 mg/kg-day	Gavage/ volume NS	6 months, 5 days/wk	Liver and kidney histopathology; mortality in 4/10 at low and high doses	None	35.7	Unpublished study, small group size, incomplete reporting; authors raised questions about the mortality, although it is unclear what the issue was.
F344 rat, 8 F/group	Berman et al., 1995; Moser et al., 1995	0, 4, 12, 40, 120 mg/kg-day	Gavage/ 1 mL/kg	14 days	Tubular degeneration, tremor, increased rearing post-exposure	12	40	Small group size, small dosage volume
Reproductive and Developmental Toxicity								
Sprague-Dawley rat 30/sex/ group P1; 20/sex/ group F1	Ryan et al., 2001; IIT Research Institute, 1999	0, 200, 1000, 5000 ppm; 0, 14.7, 70.9, 301 (P1 M), 0, 20, 93, 320.5 (P1 F) mg/kg-day	Drinking water	2 genera-tions	Decreased parental and pup body weight, decreased pup survival, decreased water consumption	70.9	301	Study also included evaluation of hematology, serum biochemistry, and developmental landmarks. Effects may be secondary to decreased water consumption. Decreased uterine weight at all doses, but not considered adverse

Strain, Species, Sex	Reference	Dose	Route/ Volume	Duration	Response	NOAEL mg/kg-day	LOAEL mg/kg-day	Comments
Sprague-Dawley Rat 25 F/group	Argus Research Laboratories, 1997	0, 60,120 or 360 mg/kg-day	Gavage 10 mL/kg	GD 6-15	Decreased maternal weight gain; Decreased fetal body weight and delayed ossification	60 (maternal) 120 (dev)	120 (maternal) 360 (dev)	Doses were divided into 3 administrations/day One dam died at 360 mg/kg-day Unpublished GLP study BMDL = 93 mg/kg-day
CD Rats 20-22 F/group	NTP, 1983a	0, 30, 60 or 120 mg/kg-day	Gavage 5 mL/kg	GD 6-15	Decreased fetal body weight	120 (maternal) 60 (dev)	None (maternal) 120 (dev)	None Developmental LOAEL is equivocal BMDL not calculated, because data on fetal weight by sex was not available
CD Rat 5-10 F/group	NTP, 1983a	0-250 mg/dg-day	Gavage 1-7.5 mL/kg	GD 6-15	Toxicity (tremors, liver and lung pathology, death) markedly higher in smaller dosing volume.	N/A	N/A	Range-finding studies.
CD-1 Mouse, 31-36 F/group	NTP, 1983b	0, 70, 140 or 280 mg/kg-day	Gavage 10 mL/kg	GD 6-15	4/36 dams died, tremors, reduced maternal body weight (10%); reduced fetal body weight	140 (maternal) 140 (dev)	280 (maternal FEL) 280 (dev)	None
F344 Rat 15-20 F/group	Narotsky and Kavlock, 1995	0, 40, 53.3 mg/kg-day	Gavage 1 mL/kg	GD 6-19	Maternal rales and dyspnea, marginal decreases in maternal body weight	N/A	N/A	Screening study

NS = Not Stated; dev = developmental; ELISA = enzyme-linked immunosorbent assay

consistent with toxicokinetic data suggesting that toxicity is correlated with peak blood concentrations rather than with total daily intake.

Several developmental toxicity studies are available (Argus Research Laboratories, 1997; NTP, 1983a; NTP, Narotsky and Kavlock, 1995). All of these studies were conducted via the gavage route, although the Argus Research Laboratories study used large dosing volumes and a divided-dosing protocol, apparently to reduce the effect of peak blood levels. The developmental toxicity studies found that the primary fetal effect is decreased body weight, which occurs at doses similar to those that cause decreased maternal body weight gain. The National Toxicology Program (NTP, 1983a) also found that toxicity increased markedly if the same phenol dose was administered in a lower dosing volume. Because the observed signs of toxicity included tremors and liver and kidney pathology and were not targeted to the portal of entry, the effect of dosing volume is not related to the concentration of a direct-contact toxicant.

The National Cancer Institute (NCI, 1980) conducted a carcinogenicity bioassay in which F344 rats (50/sex/group) received analytical-grade phenol (approximately 98.5% pure) in drinking water at concentrations of 0, 2500, or 5000 ppm for 103 weeks and were sacrificed 1–2 weeks later. Using the reference water intake of 0.13 and 0.14 L/kg-day for chronic exposure of male and female F344 rats, respectively (U.S. EPA, 1988), the doses can be estimated as 0, 260, and 585 mg/kg-day for male rats and 0, 280, and 630 mg/kg-day for female rats. The doses shown here were adjusted to account for the reported water consumption of 80% and 90% of control at the low and high doses, respectively. The animals were observed daily for clinical signs and examined weekly for palpable masses. Body weights and food consumption were recorded every 2 weeks for the first 12 weeks and then monthly thereafter; water consumption was recorded weekly.

At the end of study, the animals were sacrificed, and complete gross and histopathological examinations were performed. Organs and tissues examined included the bone marrow, spleen, cervical and mesenteric lymph nodes, heart, liver, kidney, thyroid, reproductive organs, brain, and other major tissues. No evaluation of other noncancer endpoints, such as hematological effects or serum biochemistry, was conducted.

The survival rate at study termination was comparable among all three groups of males (approximately 50%) and females (approximately 75%). Dose-related decreases in body weight compared with the controls were observed in male and female rats, with a decrease of approximately 15% in high-dose males and approximately 10% in high-dose females. Water consumption was reduced by approximately 10% at the high dose. The authors stated that the non-neoplastic lesions were similar to those occurring naturally in aged F344 rats. However, an analysis conducted for this assessment found statistically significant increases (using a chi square test) in chronic kidney

inflammation in high-dose males and females; there were no significant changes at the low dose. No other differences in the incidence of non-neoplastic lesions between the controls and exposed rats were observed.

On the basis of increased kidney inflammation and decreased body weight as compared with controls at the high dose of 5000 ppm (585 mg/kg-day for males and 630 mg/kg-day for females), the NOAEL in this study can be considered to be the low dose, 260 mg/kg-day in males and 280 mg/kg-day for females, resulting in an overall study NOAEL of 260 mg/kg-day. These effects also indicate that the maximum tolerated dose (MTD) was reached.

In the NCI (1980) rat study, there were no dose-related trends in cancer incidence in male or female rats, but the authors reported several tumors for which statistically significant increases were seen in low-dose males only, based on pairwise comparisons. These increases were seen in the incidences of pheochromocytomas of the adrenal medulla (13/50, 22/50, and 9/50 in the control, low-, and high-dose groups, respectively) and "leukemias or lymphomas" (18/50, 31/50, and 25/50). The historical control incidences of pheochromocytomas in the bioassay program was 9% (data for the test laboratory were not reported), and the historical control incidence of leukemias or lymphomas in the test laboratory was 26%. The study authors stated that the leukemias were "of the type usually seen in untreated F344 rats." There were no significant increases in tumor incidence in any tissue in female rats. Because there was no clear dose-response in males and the tumors were not observed in female rats, an association between the tumors and phenol exposure cannot be established. NCI concluded that phenol was "not carcinogenic in male or female F344 rats." However, the report noted uncertainties regarding the possible increase in leukemia in male rats, and the NCI reviewers recommended that phenol be considered for a retest.

In a parallel study, NCI (1980) administered phenol at 0, 2500, or 5000 ppm in drinking water to B6C3F1 mice (50/sex/group) for 103 weeks and sacrificed the mice 1–2 weeks later. For B6C3F1 mice, the reference water intake was 0.24 L/kg-day for both sexes. The study found that water consumption was decreased to 75% and 50–60% of the control levels at the low and high doses, respectively. The resulting doses (adjusting for decreased water intake) were 0, 450, and 660 mg/kg-day for both sexes. Dose-related decreases in body weight as compared with the controls were attributed to the decrease in water consumption. No other clinical signs of toxicity were observed, and mortality rates (approximately 10% in males and 20% in females) were comparable between experimental and control groups.

Histopathological examination and statistical analyses revealed no phenol-related signs of toxicity or carcinogenicity; lesions in all systems observed in the dosed groups were comparable to those in the controls. NCI concluded that, under the conditions of the assay, phenol was not

carcinogenic in male or female B6C3F1 mice. On the basis of the decreased body weight as compared with controls observed at 5000 ppm, the low dose of 2500 ppm (450 mg/kg-day) can be considered the study NOAEL. The observed effect, however, is likely secondary to the decreased water consumption due to poor palatability.

In light of the marked decrease in water consumption, higher doses of phenol in drinking water probably could not have been tested. If the authors had attempted to overcome the palatability issue by administering the high dose by gavage rather than in drinking water, high toxicity would have been expected in light of the higher toxicity of phenol administered by gavage (see Section 4.5 and Table 2). These considerations suggest that an MTD was also reached in mice, although the conclusion is less clear than for rats.

In the range-finding test for the carcinogenicity bioassay (NCI, 1980), F344 rats and B6C3F1 mice (10/sex/group) were given drinking water containing 0, 100, 300, 1000, 3000, or 10,000 ppm phenol (at a purity of 98.47%) for 13 weeks. Using the reference water intake of 0.16 and 0.17 L/kg-day for subchronic exposure of male and female F344 rats, respectively (U.S. EPA, 1988), the doses can be estimated as 0, 16, 48, 160, 480, and 800 mg/kg-day for male rats and 0, 17, 51, 170, 510, and 1140 mg/kg-day for female rats. The high doses shown here were adjusted to account for the decreased water consumption described below. For B6C3F1 mice, the reference water intake was 0.25 L/kg-day for males and 0.26 L/kg-day for females. The corresponding doses (adjusting for decreased water intake at the high dose) were 0, 25, 75, 250, 450, and 500 mg/kg-day for males and 0, 26, 78, 260, 468, and 520 mg/kg-day for female mice.

Body weights, appearance, behavior, and food and water consumption were recorded weekly. After 13 weeks, all animals were sacrificed and tissues were subjected to histopathological examinations. All of the rats and mice survived the phenol treatment. The only significant observation was the decreased final body weights (compared to controls) in rats of both sexes (11–14%) and in male mice (12%) that received 10,000 ppm. Because drinking water consumption in these groups was decreased to 50–70% (rats) and 20–60% (mice) of the control value, the decreased body weight was likely due to the low water consumption. No histopathological changes attributable to phenol treatment were observed.

This study suggests that the second-highest dose (480 mg/kg-day for male rats, 510 mg/kg-day for female rats, 450 mg/kg-day for male mice, and 470 mg/kg-day for female mice) was a NOAEL, based on the decreased final body weight (compared to controls) at 10,000 ppm, which was secondary to decreased water consumption due to poor palatability at the high dose.

In an unpublished 13-week neurotoxicity study conducted according to good laboratory practices (GLP) guidelines (ClinTrials BioResearch, 1998), groups of 15 male and 15 female Sprague-

Dawley rats received phenol via drinking water at concentrations of 0, 200, 1000, or 5000 ppm (at a purity of 100%) for 13 weeks, followed by a 4-week recovery period.[2] The authors calculated that the average doses were 0, 18.1, 83.1, and 308.2 mg/kg-day for males and 0, 24.6, 107.0, and 359.8 mg/kg-day for females. These concentrations were chosen on the basis of preliminary palatability studies conducted at a different laboratory (IITRI project No. L08657).[3]

During the exposure period, clinical signs and water intake were recorded daily, and body weight and food consumption were recorded weekly. In addition, a functional observational battery (FOB) and a motor activity test were conducted pre-study and once each during weeks 4, 8, 13, and 17. At the end of the exposure and the end of the recovery period, five rats/sex in the control and 5000 ppm groups underwent neuropathological evaluations (including a thorough evaluation of the brain and several nerves). The rest of the rats in the ClinTrials BioResearch (1988) study were sacrificed at the end of the 4-week recovery and were subjected to gross necropsy.

One high-dose female was euthanized on day 14 due to poor condition. Clinical signs observed in this female prior to sacrifice included dehydration, hunched posture, tremors, reduced activity, and cold to touch. Among the rest of the high-dose animals, the primary clinical sign was dehydration, which was accompanied by reduced activity and tremors in one female and by a thin appearance in additional animals. Dehydration was also observed in mid-dose rats (2/15 in each sex). Dehydration was assessed qualitatively and independently of drinking water consumption by grabbing the scruff on the back of the animal's neck; a delay in returning to the normal position was considered dehydration.

Dehydration was associated with marked decreases in water consumption at the high dose and smaller decreases at the mid dose. Decreases in water consumption were more pronounced in females than in males and were most evident during the first week of dosing. Water consumption was decreased to approximately 90% of the control level in mid-dose males and females, to approximately 60% of control levels in high-dose males, and to approximately 55% (40% during the first week) of control levels in high-dose females. Water consumption rebounded to levels higher than those of

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[2]This study has not been peer-reviewed, but it was conducted (with minor deviations) according to EPA guidelines for a neurotoxicity screening battery, it is well documented, and it contributes useful information to the hazard identification and dose-response portions of the assessment. The study was designed to comply with the U.S. EPA Enforceable Consent Agreement for Phenol (Docket No. OPPTS-42150).

[3]Results of the palatability study were not provided in the IITRI study report (IIT Research Institute, 1999; Ryan et al., 2001), which reports the results of the two-generation reproduction study. The reproduction study was conducted during the same time period as the neurotoxicity study and reported similar problems of markedly decreased drinking water consumption at the high dose of 5000 ppm (see Section 4.3).

controls during the recovery period. The decreased water consumption was likely due to the poor palatability of phenol at high concentrations rather than being a manifestation of an overt toxicological effect. In addition, the high-dose group had decreased body weights when compared to controls (8% for males and 12% for females) and decreased food intake (approximately 10% for males and 10–20% for females).

The only toxicologically significant neurological effect was decreased motor activity in females. There was a statistically significant reduction in total group mean motor activity counts at week 4 in the 5000 ppm group. The authors reported that the rate of linear change of motor activity with time was also significantly decreased at weeks 8 and 13 in the 1000 ppm and 5000 ppm groups, although supporting data were not provided. Motor activity in females at week 4 exhibited a dose dependency at the first five (of six) analysis intervals, although the total counts for the low- and mid-dose groups were not significantly different from control. High-dose females also had markedly lower total activity counts than did controls and lower counts in the first four intervals, at week 4, although there was no statistically significant difference in mean total counts (Table 3). By contrast, the high-dose males had markedly lower group mean activity counts pre-study but activity comparable to or higher than the controls at weeks 4, 8, and 13.

The authors attributed the decreased activity to dehydration, noting that the control group mean total activity increased by >20% at week 4 as compared with pre-study levels, whereas activity of dehydrated females in the 5000 ppm group at week 4 was decreased by 17%, and activity of females in this group that were not dehydrated increased by 2%. To address whether the decreased activity could be attributed to dehydration, this assessment evaluated the data in greater detail. Table 4 presents the individual animal data for week 4 total motor activity counts and compares them with the individual animal dehydration data. If the individual clinical data reported an animal as dehydrated, the days of that notation are shown. With the exception of animal 4502 (which died) and animal 4507 (which had severe dehydration) dehydration was noted as slight or moderate. For clarity of presentation, the individual animal data are shown for the control and high-dose groups, but only the average data are shown for the low- and mid-dose groups.

Table 3. Total Activity Counts in Rats Provided Phenol in Drinking Water (ClinTrials BioResearch, 1998)

Dose Group	Prestudy (Mean ±SD)	Week 4 (Mean ±SD)	Week 8 (Mean ±SD)	Week 13 (Mean ±SD)
Females				

Control	384±116	468±118	436±75	309±77
200 ppm	386±89	451±149	440±99	338±66
1000 ppm	384±103	394±78	436±104¶	343±124¶
5000 ppm	372±142	337±127**	363±111¶¶	366±145¶¶
Males				
Control	354±109	339±89	320±90	260±68
200 ppm	340±107	346±132	323±88	256±78
1000 ppm	335±126	356±137	359±105	274±103
5000 ppm	277±59	321±95	352±91	275±116

**Significantly different from control, $p<0.01$ (T-test)

¶Linear constructed variable significantly different from control, $p<0.05$ (T-test)

¶¶Linear constructed variable significantly different from control, $p<0.01$ (T-test)

Table 4. Individual Data on Dehydration and Week 4 Motor Activity in Rats Provided Phenol in Drinking Water (ClinTrials BioResearch, 1998)

Group 1 (Control)			Group 2 (200 ppm)			Group 3 (1000 ppm)			Group 4 (5000 ppm)		
Animal #	Total Counts	Days Dehydrated	Animal #	Total Counts	Days Dehydrated	Animal #	Total Counts	Days Dehydrated	Animal #	Total Counts	Days Dehydrated
1501	383	No	2501	397	No	3501	322	No	4601	501	14, 21
1502	321	No	2502	529	No	3502	402	18, 21	4502	No data - sacrificed day 14 due to poor condition	
1502	621	No	2503	427	No	3603	270	No	4503	227	7, 14, 21, 28
1504	437	No	2504	558	No	3504	370	No	4504	258	14, 21, 28, 35, 42, 70
1505	630	No	2505	245	No	3505	572	21, 28, 35, 42, 49, 56, 70	4505	396	No
1506	365	No	2506	537	No	3506	452	No	4506	277	70
1507	591	No	2507	470	No	3507	461	No	4507	399	7-9, 11, 12, 13-15, 17, 20, 21, 70, 77
1508	318	No	2508	284	No	3508	342	No	4508	271	No

Group 1 (Control)			Group 2 (200 ppm)			Group 3 (1000 ppm)			Group 4 (5000 ppm)		
Animal #	Total Counts	Days Dehydrated	Animal #	Total Counts	Days Dehydrated	Animal #	Total Counts	Days Dehydrated	Animal #	Total Counts	Days Dehydrated
1509	479	No	2509	527	No	3509	462	No	4509	387	No
1510	469	No	2510	823	No	3510	452	No	4510	450	No
1511	309	No	2511	561	No	3511	390	No	4511	439	7
1512	574	No	2512	424	No	3512	383	No	4512	130	No
1513	566	No	2513	302	No	3513	320	No	4513	242	49, 56
1514	381	No	2514	289	No	3514	403	No	4514	180	7, 14, 21, 28
1515	578	No	2515	386	No	3515	311	No	4515	556	No
Overall Average	468	--		451	--		394	--		337	--
Average - Dehydrated animals	N/A	--		N/A	--		487 (n=2)	--		315	--
Average - non-dehydrated animals	468	--		451	--		380	--		365	--

N/A = Not applicable

Only two animals in the mid-dose group were reported as dehydrated on any day, and neither of these animals had decreased motor activity. As shown, the average activity was lower in the dehydrated high-dose females than in those not reported as dehydrated, but an association of decreased activity with dehydration was not consistently supported on an individual-animal basis. (For the purpose of calculating averages, animals were considered dehydrated if they dehydrated at any point in the study. This is a limitation to the analysis, because some were reported as dehydrated only prior to week 4 and others were reported as dehydrated only after week 4. In addition, basing the analysis on the clinical sign of dehydration may not appropriately reflect whether the animals were dehydrated, because no objective measure of dehydration was used and because decreased water consumption in this group occurred throughout the study.)

As shown in Table 4, animal 4601 was reported as dehydrated on days 14 and 21, but it had one of the highest total activity counts. Conversely, animal 4512 had the lowest activity count, but it was never reported as being dehydrated. Furthermore, the mean activity of the dehydrated high-dose females was 67% of concurrent controls, compared to 78% of concurrent controls for the nondehydrated high-dose females. These data indicate that the difference between the control and high-dose animals was greater than the difference between the dehydrated and nondehydrated animals at the high dose.

Overall, the data indicate that there was not a tight linkage between dehydration and decreased motor activity in the high-dose females. The data for high-dose males also did not indicate a clear correlation between low activity and dehydration. The clinical signs for one high-dose male (4003) for week 2 included severe dehydration and decreased activity, but no effect (i.e., no dehydration or decreased activity) was seen when the animal underwent the objective activity analysis in week 4. The finding of dehydration in males without the accompanying decrease in activity further supports the conclusions that only severe (not mild or moderate) dehydration results in decreased motor activity levels and that the decrease observed in females was phenol related. Conversely, the absence of other findings in the FOB and the presence of a statistically significant effect on motor activity only at 4 weeks and not at later time points argue against a neurotoxic effect of phenol.

As an additional investigation of whether decreased motor activity was related to dehydration, the very limited literature on water deprivation and motor activity was reviewed. Campbell and Cicala (1962) evaluated the effects of terminal water and food deprivation (i.e., deprivation until death from dehydration or starvation) on motor activity of male and female Wistar rats. Motor activity was measured using a stabilimeter, which is similar to the figure-8 mazes used in

the ClinTrials BioResearch (1998) study in that ambulation (as opposed to simply movement) is measured. The study found that water deprivation alone did not result in decreased motor activity until approximately days 5–7 (depending on age), at which time activity continuously declined until death. By contrast, food deprivation resulted in an initial increase in activity followed by decreasing activity until death.

Only the pooled data for males and females were reported. These results are not directly comparable to the results of the ClinTrials study because the latter involved long-term, lower-level dehydration; however, they do support the conclusion that the decreased motor activity in high-dose females was due at least partially to phenol exposure. The most appropriate way to address this issue would be to conduct the neurotoxicity study with a water-restricted control group. Overall, based on the decreased motor activity, the study NOAEL in females was 1000 ppm phenol (107 mg/kg-day) and the Lowest Observed Adverse Effect Level(LOAEL) was 5000 ppm (360 mg/kg-day). No LOAEL was identified in males; the high dose of 308 mg/kg-day was a NOAEL. A 95% lower confidence limit on the benchmark dose (BMD) of 219 mg/kg-day was calculated for decreased motor activity in week 4 in this study (see Appendix B).

Hsieh et al. (1992) investigated the effects of phenol exposure on hematological, immune, and neurochemical endpoints in a study of 6-week-old male CD-1 mice administered actual concentrations of 0, 4.7, 19.5, or 95.2 ppm in drinking water for 28 days. On the basis of measured concentrations and water intake, the authors reported that the corresponding daily doses were 0, 1.8, 6.2, and 33.6 mg/kg-day.

The mice were housed in groups of five per cage. Drinking water was prepared and changed every 3 days. Drinking water was provided in glass water bottles with stainless sipper tubes containing ball bearings to minimize evaporation; the bottles were shaken frequently during treatment. Food and water consumption were monitored continuously, and the animals were weighed weekly. After 28 days, the mice were sacrificed by decapitation, gross pathological examinations were performed, and the liver, spleen, thymus, and kidney were weighed. Blood was taken at sacrifice for analysis. Splenocytes were prepared for analysis of mitogen-stimulated lymphocyte proliferation, mixed lymphocyte response, and cell-mediated cytolytic response.

Data were reported for five animals per group for each assay. During the 28-day exposure, no mortality and no overt clinical signs occurred in exposed mice. Phenol treatment had no effect on food or water consumption or on body weight gain. Exposed mice had no gross lesions in the liver, kidney, spleen, thymus, lung, heart, or brain, and there were no effects on organ weights for the liver, kidney, spleen, and thymus. As shown in Table 5, a decreased antibody response to sheep red

blood cells was observed, as indicated by both the plaque-forming cell (PFC) assay (expressed as PFC/million spleen cells and PFC/spleen) and the antibody titer using an enzyme-linked

Table 5. Effects of Phenol Exposure on Spleen Cellularity and Selected Blood Parameters in Mice and Rats

Concentration (mg/L)	Dose (mg/kg-day)	PFC/10^6 splenic cells	PFC/total spleen	Antibody titer [b]
Hsieh et al. (1992) - 4-week study in CD-1 mice				
0	0	1,123 ± 99[a]	265,947 ± 53,099	0.446 ± 0.039
4.7	1.8	896 ± 60	214,678 ± 17,500	0.392 ± 0.068
19.5	6.3	795 ± 49[c]	207,659 ± 18,886	0.325 ± 0.019[c]
95.2	33.6	616 ± 83[c]	130,185 ± 18,202[c]	0.263 ± 0.037[c]
IIT Research Institute (1999); Ryan et al. (2001) - 2-generation study in Sprague-Dawley rats, effects in P1 generation				
0	0	1343±890[d]	$5.54 \times 10^5 \pm 3.70 \times 10^5$	Not assayed
200	15	1668±788	$6.42 \times 10^5 \pm 3.40 \times 10^5$	Not assayed
1000	71	1781±1151	$9.01 \times 10^5 \pm 7.16 \times 10^5$	Not assayed
5000	301	1880±865	$9.81 \times 10^5 \pm 5.02 \times 10^5$	Not assayed
Positive control (n=5)	cyclo-phosphamide	0±0	0±0	Not assayed

[a] Values are given as mean ± S.E. (n=5). [b]Arbitrary as change in absorbance at 490 nm, using 1:2000 diluted serum. [c] Significant ($P<0.05$) difference from the control value. [d] Values are given as mean ±SD (n=8-9)

immunosorbent assay (ELISA). Two of these measures were statistically significantly decreased at the mid dose, and PFC/spleen was significantly decreased only at the high dose. Decreases in the absolute splenocyte lymphoproliferative responses to mitogens and the mixed lymphocyte response (the proliferative ability of splenic lymphocytes in response to alloantigens) were also observed at the high dose; there was no effect on the cytolytic response to tumor cells at any dose.

Although these assays were conducted according to the methods of the day, the latter two assays do not conform to modern protocols, and there is little biological significance in the results of the mitogen response assay. In particular, the approach used cannot distinguish between an effect on the lymphocyte's ability to initiate a proliferative response and confounding due to contamination by nucleated red blood cell precursors.

A statistically significant, dose-related decrease in erythrocyte counts was observed at all doses (Table 6). The hematocrit was decreased only at the high dose. A decreased erythrocyte count in the absence of an effect on hematocrit may have been due to macrocytosis (enlarged erythrocytes), but insufficient data were provided to evaluate this possibility. In the absence of such data, the decreased erythrocyte counts are insufficient to form the basis for identification of a LOAEL. The erythrocyte counts in all dosed groups were markedly lower than the historical control values provided by the animal distributor (Charles River Laboratories, 1986), although the hematocrit concentration in all groups was above the historical control mean. There was no effect on total or differential leukocyte counts. Interestingly, total white blood cells for all groups, including the controls, were below the historical control data provided by the distributor.

Hsieh et al. (1992) also observed dose-related decreases in the concentration of several neurotransmitters and their metabolites in the brain, including levels of norepinephrine, indoleamine serotonin, and dopamine and their metabolites. In the absence of a clear correlation with clinical effects, the toxicological significance of these neurobiochemical findings is unclear.

Thus, this study found dose-related, statistically significant decreases in red blood cells at all doses, but the significance of this finding is uncertain, because decreased hematocrit was observed only at the high dose. Statistically significant decreases in antibody response were observed at the mid dose, and these decreases reached 40% (a value often used by immunotoxicologists as a rule of thumb for clinically relevant decreases) at the high dose. Identification of a NOAEL in this study is somewhat problematic, because immunotoxicity risk assessment guidelines have not been developed.

The determination of what degree of decrease is adverse is also problematic, because the clinical relevance of a decrement in immune function will depend on the magnitude and type of

Table 6. Effects of Phenol Exposure on Spleen Cellularity and Selected Blood Parameters in Mice and Rats

Concentration (mg/L)	Dose (mg/kg-day)	Spleen cellularity (x 10^{-7})	WBC[a] (x 10^{-3})	RBC[a] (x 10^{-6})	Hematocrit %	Differential counts as % of WBCs		
						Lymphocyte	Neutrophil	Monocyte
Hsieh et al. (1992) - 4-week study in CD-1 mice								
0	0	8.59 ± 0.34[b]	6.06 ± 0.17	7.17 ± 0.56	48.00 ± 0.52	74.20 ± 1.83	17.00 ± 1.00	4.60 ± 0.51
4.7	1.8	7.94 ± 0.20	5.82 ± 0.60	4.90 ± 0.54[c]	49.10 ± 0.68	71.80 ± 2.06	19.40 ± 0.75	4.80 ± 1.02
19.5	6.3	7.31 ± 0.40	5.05 ± 0.53	4.64 ± 0.76[c]	48.20 ± 1.24	69.20 ± 3.25	21.80 ± 2.40	4.60 ± 0.81
95.2	33.6	7.26 ± 0.55	5.68 ± 0.69	3.23 ± 0.68[c]	44.10 ± 0.81[c]	73.60 ± 2.32	17.00 ± 1.55	6.20 ± 1.16
Historical control value[d]		Not available	9.0 (8.9-9.1)	7.6 (7.2-8.0)	42 (36-48)	70 (52-86)	25 (10-42)	4 (0-8)
IIT Research Institute (1999); Ryan et al. (2001) - 2-generation study in Sprague-Dawley rats, effects in P1 generation								
0	0	43.2±13.5[e]	13.1±2.01	9.22±0.37	46.5±1.44	N/A	N/A	N/A
200	15	38.3±8.78[f]	13.8±1.98	9.08±0.62	46.2±3.65	N/A	N/A	N/A
1000	71	48.1±11.0	14.5±2.42	9.03±0.34	46.4±1.56	N/A	N/A	N/A
5000	301	52.7±13.4	14.9±2.93	8.81±0.44	45.1±1.75	N/A	N/A	N/A

[a]Cells/mm^3. [b]Values are given as mean ± S.E. (n=5); N/A = not assayed. [c]Significant ($P < 0.05$) difference from the control value. [d]Mean (Range ±2S.D) for mice 6-8 weeks of age, based on 20 studies, from Charles River Laboratories (1986). [e]Mean ±SD (n=9-10 for hematology endpoints and n=8-9 for spleen cellularity). [f]Standard deviation reported in Ryan et al. (2001) has a typographical error; correct standard deviation obtained from IIT Research Institute (1999)

immune challenge, with a sufficiently large challenge resulting in illness even for unimpaired individuals. In a paper on the use of immunotoxicity data for risk assessment, Selgrade (1999) recommended that any statistically significant and consistent change be considered a risk for the purposes of hazard identification, but the degree of change considered adverse for the purposes of dose-response assessment was not addressed.

On the basis of magnitude of the decreases in antibody response observed in three related—assays, supported by decreased hematocrit and red blood cells—the high dose (33.6 mg/kg-day) can be considered the study LOAEL, and the mid dose (6.2 mg/kg-day) can be considered the study NOAEL. There is, however, considerable uncertainty regarding the reliability of these values because of issues of study interpretation and because the study used only five animals per group as opposed to the recommended eight per group (U.S. EPA, 1998c).

The results of BMD modeling conducted for this study are presented in Appendix B for completeness. However, it is unclear what the appropriate benchmark response (BMR) would be for an in vivo/in vitro immunotoxicity study, and so the modeling results are considered very preliminary. In particular, it is unclear whether the default of one standard deviation is appropriate as the BMR for this study design in light of the small sample size (n = 5) but the relatively tight data.

In contrast to the minimal effects observed in these drinking water studies, gavage dosing with phenol produces severe toxicity, including liver and kidney pathology, and death at doses that cause only minimal effects when delivered in drinking water.

Dow Chemical Co. (1945) administered 0, 50, or 100 mg/kg phenol by gavage 5 days/wk to 10 rats per group (sex and strain not reported) for 6 months (0, 35.7, or 71.4 mg/kg-day after adjusting for intermittent dosing). The dosing volume was not reported. Mortality occurred in 1/10, 4/10, and 4/10 rats in the control, low-, and high-dose groups. The authors raised questions about whether the mortality was treatment-related, but it is not clear whether they questioned whether the deaths were due to phenol or to gavage accidents. Other observed effects were slight cloudy swelling of the liver and of the tubular epithelium at the high dose and slight tubular degeneration at the low dose. This unpublished study[4] is limited by the incomplete reporting of methods and results, but the low dose of 35.7 mg/kg-day appears to be a LOAEL.

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[4]Although this unpublished study is not a primary reference for this assessment, it is presented here because it contributes some useful information to the overall hazard identification phase of the phenol assessment.

In a series of toxicological screening tests, the systemic, neurological, and developmental effects of phenol in F344 rats following acute and short-term oral exposure were examined (Narotsky and Kavlock, 1995; Berman et al., 1995; and Moser et al., 1995; MacPhail et al., 1995). In these tests, systemic and neurological effects were examined on the same animals following exposure by gavage to a single dose of phenol or to 14 consecutive daily doses. Developmental toxicity was also examined in pregnant rats that received phenol by gavage on GDs 6–19. The dosing volume was 1 mL/kg (Moser et al., 1995; Narotsky and Kavlock, 1995).

In the acute toxicity study of this series, groups of eight female rats were given a single gavage dose (1 mL/kg volume) of phenol at 0, 12, 40, 120, or 224 mg/kg in water (Berman et al., 1995; Moser et al., 1995). An FOB evaluating autonomic and neuromuscular functions, activity, excitability, and sensorimotor and physiological measures was conducted prior to the exposure and at approximately 4 and 24 hours after exposure. Immediately after the 24-hour FOB, the animals were sacrificed, blood samples were collected for serum chemistry analyses, and the liver, kidneys, spleen, thymus, and adrenals were weighed and subjected to histopathological examinations. Two rats (25%) died within 4 hours of exposure to 224 mg/kg, and one rat died 24 hours after exposure to 120 mg/kg phenol. The only treatment-related effects observed were confined to these two dose groups, and they included tremor, decreased motor activity, and kidney pathology (necrosis, protein casts, and papillary hemorrhage). Hepatocyte necrosis was also observed at 40 and 120 mg/kg but not at 224 mg/kg. No other effects were reported at the lower doses, although the primary data were not provided.

In the short-term study, groups of eight female rats were given daily gavage doses of phenol in water at 0, 4, 12, 40, or 120 mg/kg-day for 14 consecutive days (Berman et al., 1995; Moser et al., 1995). As in the acute study, the FOB was conducted prior to exposure as well as on days 4 and 9 (before the daily dose) and approximately 24 hours after the last dose. After the last FOB, blood samples were collected for serum chemistry analyses, and internal organs were removed, weighed, and subjected to histopathological examinations. All rats administered the high dose died during the study, but deaths occurred over the entire dosing period. Tremor was also seen in the high-dose (120 mg/kg) group immediately after the first administration but not after subsequent treatment. Vacuolar degeneration of the liver, kidney necrosis and protein casts, and "necrosis or atrophy of spleen or thymus" were reported at 40 mg/kg-day. The increased incidences were not large enough to be statistically significant; the statistical power of the study was also low, with only 8 rats per group.

Additional information on this study is available from a preliminary abstract (Schlicht et al., 1992) and from a recent WHO (1994) review. According to these sources, the renal pathology

consisted of 3/8 rats with renal vascular stasis, 2/8 rats with tubular degeneration in the papillar region, and 1/8 rats with protein casts in the tubules. WHO (1994) states that, according to a personal communication from one of the study authors, the pathology report attributed the renal findings to decreased vascular perfusion.

The study also found slight but not statistically significant decreases in motor activity at 40 mg/kg-day. The only statistically significant effect in this group was increased rearing in the post-exposure measurement. The only effect at 12 mg/kg-day was "necrosis or atrophy in the spleen or thymus" in 1/8 rats. On the basis of the liver, kidney, and thymus/spleen pathology findings, which are rarely observed in control animals in 2-week studies, and the decreased motor activity, the second dose (40 mg/kg-day) was the study LOAEL, and the mid dose of 12 mg/kg-day was the study NOAEL.

4.2.2. Inhalation

The laboratory animal inhalation data for phenol are very limited, with only one 2-week toxicity study being conducted using modern methodology and documentation (Hoffman et al., 2001; additional details available in the unpublished version, Huntingdon, 1998). Although a subchronic study conducted with multiple laboratory animal species is available (Sandage, 1961), this latter unpublished study tested only one concentration and was insufficiently documented for definitive risk assessment purposes. Other short-term (Dalin and Kristoffersson, 1974) or subchronic (Deichmann et al., 1944) inhalation toxicity studies are limited by short duration, inadequate documentation, or lack of a modern exposure protocol. Nonetheless, the data are consistent that the respiratory tract, kidney, and nervous system are targets of inhalation exposures.

In conducting dosimetric conversions from animal studies to human exposure scenarios, U.S. EPA (1994b) classifies gases according to their water solubility and reactivity. Category 1 gases are highly water-soluble and/or rapidly reactive and do not penetrate the blood. Category 3 gases are water insoluble, and uptake from the lungs is limited by perfusion. Category 2 gases are intermediate between these two groups. They are moderately water-soluble and rapidly-reversibly reactive or moderately-to-slowly irreversibly metabolized in the respiratory tissue. On the basis of phenol's chemical/physical properties (see Table 1) of moderate water solubility and moderate reactivity (based on the evidence of irritation and corrosivity seen following direct contact), it can be considered a Category 2 gas. This conclusion is supported by the finding of both respiratory effects (from direct contact) and systemic (extrarespiratory) effects (from absorbed phenol) following inhalation exposure, as described below.

Because the equations for the regional gas dosimetry ratio (RGDR) for Category 2 gases are currently undergoing EPA reevaluation (eqs. 4-29 through 4-44, pages 4-52 through 4-57 of U.S. EPA, 1994b), dosimetric adjustments for extrarespiratory effects were made using the Category 3 equations (eq. 4-48, page 4-60 of U.S. EPA, 1994b), in which the RGDR is based on the blood:air partition coefficient for the chemical in the experimental animal species and in humans. No data on the blood:air partition coefficient for phenol in laboratory animals or in humans were located. Therefore, the default value of 1 for the ratio of the laboratory animal-to-human partition coefficient was used, and the human equivalent concentration (HEC) for systemic effects was the same as the duration-adjusted concentration.

HECs for respiratory tract effects were calculated using the equations of U.S. EPA (1994b) for a Category 1 gas (eqs. 4-17 through 4-28, pages 4-47 through 4-51). When the EPA reanalysis is complete, revised dosimetric conversions may be calculated. The inhalation toxicity data for phenol are summarized in Table 7.

The acute toxicity studies support the findings of the short-term and subchronic studies that the respiratory tract and nervous system are targets of inhaled phenol. For example, tremors were seen in rats and guinea pigs exposed to 187 or 540 ppm (720 or 2080 mg/m^3) phenol for 30 minutes in a whole-body inhalation chamber (UBTL, 1991). By contrast, no tremors were observed in rats exposed via nose-only inhalation at 25 ppm (96 mg/m^3) for 6 hours (Dow Chemical Co., 1994). Phenol also caused sensory irritation in mice, as evidenced by decreased respiratory rate (De Ceaurriz et al., 1981). The concentration associated with a 50% decrease in rate (RD_{50}) was estimated to be 166 ppm (639 mg/m^3). No acute lethality studies were identified for phenol following exposure by the inhalation route.

In a 2-week inhalation study conducted according to GLP guidelines (Hoffman et al., 2001; full study report available as Huntingdon, 1998), groups of 20 F344 rats per sex were exposed nose-only to actual concentrations of 0, 0.52, 4.9, or 25 ppm phenol (0, 2.0, 18.9, or 96.2 mg/m^3) 6 hrs/day, 5 days/wk for 2 weeks. The duration-adjusted concentrations were 0, 0.36, 3.4, and 17 mg/m^3, respectively. The animals were observed twice daily for mortality and abnormal clinical signs. Animal body weights and food consumption were recorded twice pre-test, weekly thereafter, and just prior to sacrifice. At the end of 2 weeks of exposure, 10 rats of each sex in each group were sacrificed. The rest of the rats were sacrificed after 2 weeks of recovery. Blood samples were collected just prior to sacrifice for hematological (including differential leukocyte count) and

Table 7. Summary of Inhalation Toxicity Studies

Strain Species, Sex	Reference	Exposure mg/m³	Duration	Duration-Adjusted mg/m³	Response	NOAEL/ LOAEL mg/m³	NOAEL/ LOAEL (HEC) mg/m³	Comments
F344 Rat (20/sex)	Huntingdon 1998	0, 2.0, 18.9, 96.2 Nose-only	6 hr/d 5 d/week 2 weeks	0, 0.36, 3.4, 17	Red nasal discharge, but no histopathology lesions.	17/None	2.5 or 17 /None	Well-conducted study, but authors did not note the clinical signs
Rat, 7 exposed, 11-12 controls (Strain & sex NS)	Dalin and Kristoffersson 1974	0, 100	15 days continuous	0, 100	Nervous system effects, increased serum liver enzymes	None/100	None/100	Exposure measurement not done according to modern methods, no histopathology exam
Guinea pig, 12 (Strain & sex NS)	Deichmann et al. 1944	100-200	7 hr/day 5 d/week 6 weeks	31 (based on midpoint of range)	FEL – 5/12 dead	None	None/ 31 is FEL	Minimal documentation, outdated exposure methods, no controls
Rabbit 6 exposed (Strain and sex NS)	Deichmann et al. 1944	100-200	7 hr/day 5 d/week 13 weeks	31 (based on midpoint of range)	Pneumonia, heart inflammation, liver necrosis, kidney tubular degeneration	None	None/ 31	Minimal documentation, outdated exposure methods, no controls
Rat, 12 (Strain and sex NS)	Deichmann et al. 1944	100-200	7 hr/day 5 d/week 74 days	31 (based on midpoint of range)	No effect, no evidence of histopathology	31/None	31/None	Minimal documentation, sensitivity of assay unclear, outdated exposure methods, no controls
Rhesus monkey 10 M/ group	Sandage 1961	0, 18.2	90 days, continuous	0, 18.2	Liver and kidney pathology (Not further described)	None/18.2	None/18.2	Pathology reported to be minimal, but limited by minimal description Unpublished study

Strain Species, Sex	Reference	Exposure mg/m³	Duration	Duration-Adjusted mg/m³	Response	NOAEL/ LOAEL mg/m³	NOAEL/ LOAEL (HEC) mg/m³	Comments
Sprague-Dawley rat 50 M/ group	Sandage 1961	0, 18.2	90 days, continuous	0, 18.2	Liver and kidney pathology (Not further described)	None/18.2	None/18.2	Pathology reported to be minimal, but limited by minimal description Unpublished study
Albino mouse 100 M/group	Sandage 1961	0, 18.2	90 days, continuous	0, 18.2	Lung pathology (Not further described)	None/18.2	Not determinable*	Pathology reported to be minimal, but limited by minimal description Unpublished study

NS = Not stated

* HEC cannot be determined because the region of the respiratory tract affected is not clear.

biochemical examinations. Gross pathological evaluations were conducted on all of the animals, and organ weights were determined. Histopathological examinations were conducted on the liver, kidney, and respiratory tract tissues (including three sections of the lungs with mainstem bronchi, the pharynx, and three sections of the nasal turbinates) of the control and high-exposure groups; the spleen of mid-concentration females was also analyzed.

During the exposure, one male rat in the low-concentration group accidentally died from trauma caused by turning itself within the nose-only restraint tube. All of the other rats survived until sacrifice at the end of the 2-week exposure or 2-week recovery periods. During exposure and recovery, there were no treatment-related changes in weekly physical examinations, body weight, weight gain, or food consumption. The authors reported no effect on clinical signs. However, there was a concentration- and duration-related increase in the incidence of a red nasal discharge in the males. The incidence was 0/20, 0/20, 3/20, and 4/20 at 0, 0.52, 4.9, and 25 ppm, respectively, in the first week and 0/20, 0/20, 7/20, and 10/20 in the second week of exposure. This detailed information on nasal discharge was presented only in the unpublished report (Huntingdon, 1998); the published version reported scattered observations of chromodacryorrhea and nasal discharge.

In an analysis done for this assessment, the incidence at the mid and high concentrations was statistically significant, using the Fisher exact test. In females, nasal discharge was seen in 1/20 at the low concentration and 3/20 at the mid concentration in the second week, but no discharge was reported in high-concentration females. Prior to exposure, a nasal discharge was observed in a single control male and a single high-concentration female. Considering the exposure chamber design, it does not appear that the discharge was an artifact of the rats' noses being in contact with phenol condensate on the chamber walls. Instead, in the absence of nasal histopathology, it is likely that the discharge reflected a nonspecific response to stress in the rats. A tear-like nasal discharge in rats can be a generalized response to stress from a variety of causes. Porphyrins in the discharge lead to a red color. In light of the dose-related response in males, it appears that the stress in this study was related to an effect of phenol, either as an irritant or a toxicant.

Hematological and biochemical examinations showed slight but statistically significant increases in prothrombin time at the low concentration only and in albumin concentration in high-exposure females; these changes were not considered to be biologically significant. No other significant changes in hematology or biochemistry were observed. The only statistically significant changes in organ weights were decreases in liver-to-body, spleen-to-body and spleen-to-brain weight ratios in mid-concentration (18.9 mg/m^3) females. Because the changes in organ weights did not occur at the highest phenol exposure concentration (96.2 mg/m^3), and the same responses occurred in

female rats but not in male rats, these responses were considered by the authors to be not toxicologically significant.

Gross pathological and microscopic examinations of these organs did not exhibit any differences from those of the controls. Although there were a number of histopathology findings in the respiratory tract (e.g., inflammatory cells in the nasolacrimal ducts, alveolar macrophages, and eosinophilic and basophilic material), these findings occurred at similar incidences in the control and exposed groups. The lesions reported were also those typically seen in control animals. The only lesion of concern was minimal to slight lung hemorrhage, which was reported in 4/10 control males and 6/10 high-exposure males at the terminal sacrifice. However, there was no clear concentration-related increase in incidence or severity, this lesion was not found in the females, and this lesion was not seen in exposed animals post-recovery or in control animals.

Thus, it appears that the only effect of concern in this study was the red nasal discharge, which was observed in males but not in females, and this effect was probably due to a nonspecific response to stress. In addition, no supporting histopathology was observed in a thorough examination. On the basis of these considerations, the highest concentration (96.2 mg/m^3) in this study was NOAEL. The HEC cannot be definitively determined in the absence of an affected endpoint. However, assuming that the respiratory tract would be affected first, as shown in other studies, a conservative NOAEL (HEC) based on a nasal effect would be 2.5 mg/m^3. Assuming that the nasal discharge reflects a nonadverse systemic stress response, the NOAEL(HEC) would be 17 mg/m^3. No LOAEL was identified.

Deichmann et al. (1944) conducted subchronic inhalation studies of phenol toxicity in rabbits, rats, and guinea pigs. Twelve guinea pigs, 6 rabbits, and 12 rats (strain and sex not reported) were exposed (whole body) in a single exposure chamber to phenol vapor at "a concentration ranging from 0.1 to 0.2 mg/L (100-200 mg/m^3)" for 7 hrs/day, 5 days/wk for 6 weeks, 13 weeks, or approximately 11 weeks, respectively. The actual exposure concentration apparently could not be controlled more precisely. Using the midpoint of 150 mg/m^3 as the exposure concentration, the duration-adjusted concentration was 31 mg/m^3.

Among the three tested species, the guinea pig was the most sensitive and the rat was the least sensitive to phenol exposure. Deaths were observed in 5/12 guinea pigs during the 6-week exposure period. Other signs of toxicity in the guinea pigs included decreased activity during the first week and respiratory difficulties and paralysis of hind quarters after 4 weeks of exposure. Histopathological evaluations revealed lesions of the lungs (pneumonia and bronchitis), heart (inflammation, fibrosis, and necrosis), liver (fatty changes and necrosis), and kidneys (tubular

degeneration and edema). At the end of exposure, the surviving guinea pigs had a concentration of 1.0 mg free phenol/100 mL blood and 0.4 mg conjugated phenol/100 mL blood. (Details on the analytical procedures used to measure phenol in blood were not reported.).

No deaths or clinical signs of toxicity were observed in the rabbits exposed for 13 weeks, but lobular pneumonia and fibrosis was observed in these animals. Histopathology lesions in the heart, kidney, and liver were similar to, but less severe than, those reported in the guinea pigs. After 37 days on study, the rabbits had a concentration of 0.5 mg free phenol/100 mL blood and 0.7 mg conjugated phenol/100 mL blood; similar concentrations were observed at the end of the exposure period.

The rats did not show any clinical signs of toxicity during the 74-day exposure period (approximately 11 weeks), and there was no histopathological evidence of any effect. Blood phenol levels were not reported for the rats, but an analysis of carcass homogenate found 0.2 mg free phenol and 0.35 mg conjugated phenol per 100 g carcass homogenate. These levels were reported to be within the normal range in unexposed rats. This study is limited by the use of only one exposure concentration, the absence of controls, the inadequate control of exposure levels, and the absence of reporting of the primary data. However, the results do appear to show that rats are much less sensitive than rabbits or guinea pigs to the inhalation effects of phenol. However, a comparison of blood levels suggests that the interspecies differences are due to both toxicokinetic and toxicodynamic differences.

In an unpublished 90-day study (Sandage, 1961), groups of 10 male rhesus monkeys, 50 male Sprague-Dawley rats, and 100 male albino mice were exposed to average phenol concentrations of 0 or 4.72 ppm (18.2 mg/m^3) continuously for 90 days.[5] Exposure was interrupted for 14 hours on day 39 and for 36 hours on days 68–69. The frequency of monitoring of the test atmosphere was not reported, but the phenol concentration was reported to remain in the desired range of 4.5–5.5 ppm "after the first three days." No further information on the concentrations during the first 3 days was reported.

During the exposure, no deaths were observed in the test animals. Body weight gain in mice was comparable to that in controls but was slightly higher in exposed rats and monkeys. A complete hematological examination showed no significant changes in the three test species following phenol

[5]Although this unpublished study is not a primary reference for this assessment, it is presented here because it contributes some useful information to the overall hazard identification phase of the phenol assessment.

exposure. Blood biochemistry (alkaline phosphatase, cholinesterase, amylase, lipase, and glutamic oxalacetic transaminase) was evaluated in monkeys only. Urinalysis was apparently conducted in all species, but kidney function tests (urine volume and specific gravity) were conducted only in monkeys and rats. The study authors reported that there were no effects on any of these endpoints but did not provide any supporting data.

At the end of the exposure period, "approximately half" of the animals underwent a stress test in which the animals swam in a smooth-walled tank until exhausted. These animals were sacrificed immediately after the test, and the other animals were held for a 2-week recovery period prior to sacrifice. Histopathological evaluations were conducted on only 5–8 organs (including the liver, kidney, and lung). It appears that all of the monkeys and about half of the rats and mice were evaluated, although it is not clear whether some of the rodents were evaluated after the recovery period.

The authors considered the histopathology findings "essentially negative" and did not provide any description of the observed lesions or the number of animals examined histopathologically. Liver and kidney pathology was observed in 30% and 20%, respectively, of the monkeys (compared with 0% of the controls). However, the authors did not consider these changes to be significant, and they noted that 6/7 reports of pathology in monkeys were considered "minimal or doubtful." Liver and kidney pathology was also reported in 20% of phenol-exposed rats (compared with 0% of the controls) and lung pathology was reported in 20% of the phenol-exposed mice (compared with 6% of the controls). The incidences of liver and kidney pathology in the rat and lung pathology in the mouse were statistically significant in a Fisher's exact test done for this assessment. Although the incidence of lung pathology was not affected in monkeys and rats, a relatively high incidence of lung pathology in the control animals (30% and 65%, respectively) decreased the sensitivity of the evaluation. No other significant pathological changes were reported in the test animals.

Although the authors concluded that there was no evidence that phenol exposure resulted in significant damage, there is some indication of liver, kidney, and lung pathology in this study, but the inadequate reporting precludes the determination of whether there was a treatment-related effect. For the purposes of this assessment, the single exposure level tested, 18.2 mg/m^3, should be considered a free-standing LOAEL, although it might be considered a minimal LOAEL if additional histopathology data were available. The LOAEL (HEC) for the kidney and liver lesions is also 18.2 mg/m^3. In the absence of additional information on the nature of the lung lesions, the LOAEL (HEC) for the lung cannot be determined. The study is also limited by the poor control of exposure levels and limited reporting of effects.

In a study published by Dalin and Kristoffersson (1974), seven white rats of an in-house strain were exposed to phenol at a concentration of 100 mg/m^3 continuously for 15 days. There is some uncertainty about this exposure measure, however, because the exposure chamber was not set up according to modern designs, and it does not appear that continuous monitoring of exposure levels was conducted. Unexposed rats (n = 11–12) were used as controls. Nervous system effects were observed from the first day after the start of exposure. These effects progressed from increased activity to imbalance, twitches, and disordered walking rhythm on days 3–4. These signs disappeared by day 5 and were replaced by sluggish behavior until the end of the exposure. A tilting-plane test was conducted before and after exposure in both groups, and a significant effect was observed on the exposed rats. There were no significant changes in food intake or water consumption during the exposure period. Although there was no significant difference in body weight of the exposed group compared with that of the controls, the average body weight of the exposed group decreased during exposure, whereas the controls gained weight.

The serum biochemical evaluations showed large, statistically significant increases in SGOT, SGPT, lactic dehydroganese (LDH), and glutamate dehydrogenase activities, indicating liver damage. Plasma potassium and magnesium levels were also increased. Although the significance of these changes is unknown, the authors suggested that the increased magnesium levels may have caused some of the nervous system effects. Hemoglobin and hematocrit were unaffected. No histopathology examination was conducted. On the basis of the observed nervous system effects as well as the serum enzyme changes indicating liver damage, the only exposure concentration was a free-standing LOAEL. The LOAEL (HEC) is 100 mg/m^3, but the actual exposure measurement is of low quality.

4.2.3. Dermal

Phenol is quite irritating, and dermal exposure to liquid phenol or to concentrated phenol vapor can result in inflammation and necrosis of the skin (Conning and Hayes, 1970; Patrick et al., 1985; Pullin et al., 1978). As discussed in Section 3.1, phenol is readily absorbed from dermal contact with phenol liquid or phenol vapor, so systemic effects can also result from dermal exposure. Several acute lethality assays have been reported. Conning and Hayes (1970) reported a dermal LD_{50} of 669.4 mg/kg for undiluted phenol applied for 24 hours to the skin of female Alderly Park rats. Acute dermal toxicity appears to be dependent on the concentration of phenol, with increased lethality observed with decreased concentration when the same total dose is applied (Deichmann and Witherup, 1944; Conning and Hayes, 1970). In addition to lethality, renal effects (severe

hemoglobinuria and hematin casts in the tubules), cardiovascular effects (cardiac arrhythmias and ventricular tachycardia), and neurological effects (severe muscle tremors, marked twitching, generalized convulsions, loss of consciousness, and prostration) were observed at 107.1 mg/kg in female Alderly Park rats following dermal exposure to undiluted phenol for 24 hours (Conning and Hayes, 1970). A similar array of effects have been reported in humans following accidental dermal exposures to large volumes of phenol (ATSDR 1998).

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES

No studies of the reproductive or developmental toxicity of phenol following inhalation exposure of laboratory animals were located. Several developmental toxicity studies in rats and mice conducted via the gavage route are available (Argus Research Laboratories, 1997; NTP, 1983a,b; Narotsky and Kavlock, 1995); the only developmental effect reported in these studies was decreased fetal body weight. In a two-generation drinking water study in rats (Ryan et al., 2001; available in unpublished form as IIT Research Institute, 1999), decreased pup weight and decreased survival of pups pre-culling were observed, but these effects appeared to be secondary to decreased water consumption.

In the Ryan et al study, 30 Sprague-Dawley rats/sex/group were exposed to 0, 200, 1000 or 5000 ppm phenol in drinking water. Parental (P1) rats were given phenol for 10 weeks prior to mating, during a 2-week mating period, throughout gestation, lactation, and until sacrifice. The males were sacrificed after successful mating. All of the P1 females were allowed natural parturition and were sacrificed at F1 weaning. The authors calculated that the average daily phenol intake during week 10 was 0, 14.7, 70.9, and 301.0 mg/kg-day for P1 males and 0, 20.0, 93.0, and 320.5 mg/kg-day for P1 females.

For the F1 generation, the average phenol intake during week 10 was 0, 13.5, 69.8, and 319.1 mg/kg-day for males and 0, 20.9, 93.8, and 379.5 mg/kg-day for females. The F1 generation (20 rats/sex/group) was treated following a protocol similar to that used for the P1 generation, and F2 pups were sacrificed after weaning, on postnatal day (PND) 22. During treatment, rats were monitored for mortality, clinical signs, body weight, and food and water consumption. At sacrifice, the animals were necropsied, and reproductive organs from 20 animals per sex in the control and high-dose groups from the P1 and F1 generations were examined microscopically. In addition, the spleen, thymus, liver, and kidneys from 10 randomly selected P1 and F1 animals of each sex in the control and high-dose groups were examined.

Most of the treatment-related changes in P1 rats were observed in the high-dose groups. No treatment-related mortality was observed in P1 rats of either sex. Three high-dose F1 female pups died shortly after weaning. The deaths appeared to be associated with decreased water consumption associated with poor palatability, as these pups refused to drink the water containing the phenol. No other treatment-related mortality was reported in the F1 generation. The only significant observed clinical sign was redness around the nose fur, which occurred in the high-dose males and females of the F1 generation before mating and in P1 dams during lactation. As noted in the context of the Hoffman et al. (2001) inhalation study, this redness likely reflects a nonspecific stress response. A significant decrease in water consumption was observed throughout the study in P1 animals of both sexes (up to 23% for males and up to 39% for females) and was attributed to poor palatability. Decreased water consumption in the F1 rats at the high dose was of a similar or larger magnitude. The low water consumption at the high dose was accompanied by decreased body weights as compared with the controls (9% in P1 males and 16% in P1 females at sacrifice).

At birth, the high-dose F1 and F2 pups had decreased body weights as compared with the controls, and the differences were larger by PND 21. The authors noted that pups began drinking the water prior to weaning and that the decreased water consumption was also observed in the high-dose pups. Decreased absolute organ weights and increased relative organ weights were observed for a number of organs at the high dose in both the P1 and F1 generations. Most of these changes likely reflected the lower body weight and overall dehydration in these groups.

F1 females had a statistically significant, dose-related decrease in uterine weights at all doses, but P1 females were not affected. The authors suggested that some of this decrease may have been related to a lower incidence of uterine dilatation at the high dose. Because the stage in the estrus cycle can affect uterine weight, estrus cycle staging was also considered as an explanation, but the authors did not consider the small decreases in the number of rats in estrus (16/24, 15/24, 13/25, 9/22 in the control, low-, mid-, and high-dose groups) sufficient to account for the decreased uterine weight. Nonetheless, the decreased uterine weight was not considered adverse for several reasons. There was no evidence of a dose-response relationship for relative uterine weight across the three dose groups (Table 8). There was no effect on reproductive function and no histopathological changes in the uterus. Finally, the uterine weight was below the control range for only a few rats in each dose group, and the control group appeared to have greater variability (particularly at the high end) than each of the experimental groups.

Table 8. Selected Results of Two-Generation Drinking Water Study (Ryan et al., 2001; IIT Research Institute, 1999)

Endpoint	Control	200 ppm (14-21 mg/kg-day)	1000 ppm (70-94 mg/kg-day)	5000 ppm (301-380 mg/kg-day)
P1 body weight - males week 10 (g)	414±39.8	434±34.4	423±40.3	382±40.8*
P1 body weight - females week 10 (g)	259±24.5	260±21.8	258±19.3	234±21.2*
P1 water consumption - males week 10 (g/day)	30±4.6	32±4.6	30±3.3	23±3.1*
P1 water consumption - females week 10 (g/day)	24±3.7	26±5.5	24±4.1	15±2.1*
F1Absolute uterine weight (g) Uterine weight relative to body weight	0.81±0.33 0.27±0.11	0.62±0.13* 0.21±0.044*	0.60±0.17* 0.20±0.058*	0.53±0.17* 0.20±0.068*
F1 Absolute prostate weight (g) Prostate weight relative to body weight	0.90±0.20 0.18±0.039	0.77±0.17* 0.15±0.031	0.74±0.17* 0.15±0.027*	0.76±0.16* 0.18±0.036
F1 pnd0 litter weight (g)	6.73±0.42	6.85±0.51	6.63±0.51	6.38±0.27*
F2 pnd0 litter weight (g)	6.67±0.38	6.80±0.44	6.58±0.42	6.20±0.48*
Preputial separation (age, days) Body weight at onset (g)	44.0±2.16 229±23.0	45.4±2.05 236±23.4	44.4±2.02[2] 227±19.7	47.8±3.13* 195±23.6*
Vaginal patency (age, days) Body weight at onset (g)	34.6±1.85 126±13.7	34.5±1.83 127±13.5	34.1±2.25 121±12.6	38.3±2.21* 112±7.8*

[1]Mean ±Standard deviation

[2]Standard deviation reported in Ryan et al. (2001) has a typographical error; correct standard deviation obtained from IIT Research Institute (1999)

*Statistically significant, p 0.05

Absolute prostate weights were significantly reduced—by approximately 15%—in all dose groups of the F1 generation but not the P1 generation. Relative prostate weight was decreased in the low-dose (but not statistically significantly) and mid-dose F1 groups but not at the high dose. In the absence of a dose-response, the changes in prostate weight were not considered adverse. Dose-related statistically significant decreases in absolute adrenal and spleen weights were also observed in the mid- and high-dose F1 males. However, there was no effect on relative weights of these organs in these groups, there was no effect on these organ weights at any dose in the P1 males, and P1 females were only affected at the high dose (where decreased body weight would have played a role).

The pathological examinations showed no treatment-related lesions in the kidneys, spleen, liver, thymus, or reproductive organs. An immunotoxicity screen conducted with 10 male P1 rats per group found no significant effects on spleen weight, cellularity, or antibody-forming cells for any test group when compared with the control group; the expected results were found with a positive control group. The strength of this finding is reduced, however, by the relatively large standard variability in response (based on the standard error as a percent of the mean).

Complete hematological evaluations (including hematocrit, erythrocyte count, and differential white cell count) and serum biochemical evaluations were conducted on 8–10 P1 males/group prior to sacrifice. The only significant change in these evaluations was increased blood urea nitrogen (BUN) in the high-dose group. Because this change was not accompanied by increased creatinine and there was no associated kidney pathology, the BUN increase was not considered to be biologically significant. The authors also noted that all but one of the high-dose BUN values were within the control range.

There was no effect on fecundity or fertility in either generation. In addition, there was no effect on other indicators of reproductive toxicity, including the frequency of estrus, testicular sperm count, sperm motility, and sperm morphology. The survival of the high-dose F1 pups was significantly decreased on PND 4 (pre-culling), although there was no effect on overall F1 pup survival. In the F2 generation, high-dose pup survival was significantly decreased throughout the lactation period. This decreased survival of both generations of pups was likely secondary to the decreased maternal water intake and associated decreases in milk production. In the F1 generation, delayed vaginal patency and delayed preputial separation were observed at the high dose. The delay was considered secondary to decreased fetal growth at the high dose resulting from decreased water consumption due to poor palatability and associated decreased food consumption.

Thus, all of the adverse systemic and reproductive effects of phenol in this study occurred at the high dose, and they appear to be secondary to decreased water consumption due to poor

palatability rather than to a toxic effect of phenol. On the basis of the decreased parental and pup body weight (compared to the controls) and decreased pup survival, the high dose is a LOAEL. The study NOAEL is 70.9 mg/kg-day (based on the NOAEL corresponding to the lowest LOAEL in this study, in P1 males). BMD modeling was not conducted for this study because the observed effects appeared to be secondary to decreased water consumption and not reflective of phenol toxicity.

In an unpublished developmental toxicity study conducted according to GLP guidelines (Argus Research Laboratories, 1997), pregnant Crl:CDRBR VAF/Plus Sprague-Dawley rats (25 per group) received phenol by oral gavage on GDs 6 through 15.[6] Dosing was three times daily with 0, 20, 40, or 120 mg phenol/kg/dosage using a dosing volume of 10 mL/kg. The corresponding daily doses were 0, 60, 120, and 360 mg/kg-day. The authors noted that the test material was 90% phenol United States Pharmacopeia (USP); the authors adjusted the dosage calculations for test material purity.

The exposed dams were observed twice a day for viability and daily for clinical signs, abortions, and premature deliveries. In addition, the maternal body weights were recorded every day, and food consumption was also recorded periodically (every 1–2 days). The rats were sacrificed on GD 20, and gross necropsy of the thoracic, abdominal, and pelvic viscera was performed. The number of corpora lutea in each ovary was recorded. The uterus of each rat was excised and examined for number and distribution of implantations, live and dead fetuses, and early and late resorptions. Each fetus was weighed, sexed, and examined for gross external alterations. One-half of the fetuses were examined for soft tissue alterations, and the rest were examined for skeletal alterations.

One high-dose dam died on GD 11. The study authors attributed this death to phenol treatment because it occurred only at the high dose, although there were no adverse clinical observations and no abnormal necropsy findings in this animal. Other high-dose animals exhibited excess salivation and tachypnea (rapid breathing). There were no other treatment-related clinical observations and no treatment-related necropsy findings. Dose-dependent decreases in body weight of the exposed animals as compared with the controls were observed. Statistically significant decreases in both maternal body weight (8%) and body weight gain (38% for GD 6–16) were observed at the high dose; although a statistically significant decrease in body weight gain (11%) was

[6]This study has not been peer-reviewed, but it was conducted (with minor deviations) according to EPA guidelines for developmental toxicity studies, it is well documented, and it contributes useful information to the hazard identification and dose-response portions of the assessment. The study was designed to meet the U.S. EPA Pesticide Assessment Guidelines, Subdivision F, 83-3.

observed at the mid dose, the decrease at the mid dose (relative to controls) in absolute maternal weight at the end of dosing (3%) was not statistically significant. Dose-dependent decreases in food consumption were also observed during the dosing period (see Table 9).

Fetal body weights in the high-dose group were significantly lower than those of the controls, by 5–7%. The high-dose group had a statistically significant decrease in ossification sites on the hindlimb metatarsals, but it is unlikely that this small change is biologically significant. The incidence of litters with incompletely ossified or unossified sternal centra was 0/23, 0/25, 3/23, and 3/24; this increase was not statistically significant (Table 9). There were small, dose-related increases in the number of litters with fetuses with "any alteration" and with "any variation" at 120 mg/kg/day and higher. However, neither of these changes was statistically significant, and the response was not clearly dose-related. In addition, an increase in total variations is of questionable significance in the absence of any increase in individual variations. There were no other treatment-related effects on uterine contents, malformations, or variations.

The maternal NOAEL was 60 mg/kg-day, based on small decreases in maternal body weight gain at 120 mg/kg-day, and the developmental NOAEL was 120 mg/kg-day, based on decreased fetal body weight and delayed ossification at 360 mg/kg-day. Benchmark dose (BMD) modeling was also conducted for the decreased maternal weight. Defining the BMR as a one standard deviation decrease in maternal body weight gain, the BMDL was 93 mg/kg-day. Details on the BMD modeling are provided in Appendix B.

In a well-designed developmental toxicity study (NTP, 1983a), timed-mated CD rats were administered phenol by gavage at 0, 30, 60, or 120 mg/kg-day in 5 mL/kg distilled water on GDs 6 to 15 and sacrificed on GD 20. Females were weighed on GDs 0, 6–15 (prior to daily dosing), and 20 (immediately following sacrifice), and they were also observed during treatment for clinical signs of toxicity. A total of 20–22 females per group were confirmed to be pregnant at sacrifice on GD 20. The dams were evaluated at sacrifice for body weight, liver weight, gravid uterine weight, and status of uterine implantation sites. Live fetuses were weighed, sexed, and examined for gross morphological abnormalities and malformations in the viscera and skeleton.

The results of this study did not show any dose-related signs of maternal toxicity or any clinical symptoms of toxicity related to phenol treatment. The number of implantation sites was slightly higher in the dosed groups, but this change could not be treatment related because implantations in this strain

Table 9. Key Results in Argus Research Laboratories, (1997) Rat Developmental Toxicity Study

	Control	60 mg/kg-day	120 mg/kg/day	**360 mg/kg-day**
Maternal body weight GD 16 (g)	363.9±22.2	359.0±18.7	354.3±17.5	**334.2±20.1****
Maternal body weight gain (GD 6-16) (g)	64.0±10.7	58.0±9.4	56.8±10.8*	**39.8±9.5****
Maternal food consumption (GD 6-16) (g/day)	26.7±2.7	26.0±2.6	24.8±1.9**	**21.9±2.1****
Fetal body weight - all fetuses (g)	3.62±0.30	3.63±0.24	3.60±0.30	**3.41±0.35***
Fetal body weight - males (g)	3.71±0.30	3.73±0.25	3.71±0.31	**3.53±0.34**
Fetal body weight - females (g)	3.53±0.30	3.53±0.25	3.49±0.28	**3.29±0.35****
% Fetuses with any alteration/litter	1.0±2.6	2.4±4.6	3.7±4.4	**4.1±7.8**
% Fetuses with any variation/litter	1.0±2.6	2.4±4.6	3.4±4.4	**3.8±7.3**
Incompletely ossified or unossified sternal centra litter incidence	0	0	37337	**37338**
Ossification sites per fetus per litter, hindlimb metatarsals	4.00±0.00	4.00±0.00	4.00±0.00	**3.98±0.07***

[1]Mean ±Standard deviation

*Statistically significant, p 0.05

**Statistically significant, p 0.01

take place prior to GD 6 (prior to dosing). Significant increases in the litters with nonlive (dead plus resorbed) pups were observed in the low- and mid-dose groups but not in the high-dose group (Table 10). Because this response was not dose dependent, and the response in the high-dose group was comparable to that in the control group, this observation is not considered treatment-related. In addition, there was no effect on the more appropriate measure of nonlive pups per litter.

There was also no effect on live fetuses, sex ratio, malformations, or variations, but there was a clear dose-related downward trend in fetal body weight, although the changes at the two lower doses were small and the effect was statistically significant only at the high dose (Table 10). Fetal body weights in the high-dose group were 93% of the average of those in the control group; fetal body weights were not reported separately for males and females. Historical control data from the supplier report the average fetal body weight in this strain as being well below the weight in the high-dose group (Charles River Laboratories, 1988). (Concurrent control weight was 4.14 g, high-dose weight was 3.84 g, and historical control weight was 3.39 g.) The litter size in the high-dose group was also somewhat higher (but not statistically significantly) than in the controls, possibly contributing to the smaller fetal weight at the high dose.

As shown in Table 10, the total pup burden (total fetal weight) and the gravid uterine weight were highest in the low-dose group and then in the high-dose group; both values higher than those in the control group. In addition, the treatment-period maternal weight gain was very similar in the control and high-dose groups (but higher in the low-dose group), but the absolute maternal weight gain (i.e., adjusted for the gravid uterine weight) was much lower in the high-dose group than in the controls. The results from the low-dose group suggest that the dams could have borne a somewhat higher burden of the total in utero package. However, the results also suggest that the dams were near the limit of what they could carry, considering the lower absolute weight gain but unaffected treatment-period weight gain in the high-dose group. No dose-related signs of maternal toxicity and no clinical symptoms of toxicity related to phenol treatment were observed in this study.

From these considerations and the potential for the decreased fetal weight to reflect primarily the larger litter size, the decreased fetal weight in this study could be considered an equivocal LOAEL. Thus, on the basis of decreased fetal body weight, the mid dose in this study of 60 mg/kg-day was a NOAEL for developmental toxicity and the high dose of 120 mg/kg-day was an equivocal LOAEL. The high dose was a maternal NOAEL. BMD modeling could not be done for the decreased fetal weight because NTP did not have information on the fetal weight by sex,

Table 10. Key Results from Developmental Toxicity Study in Rats Administered Phenol by Gavage (NTP, 1983a)

	Control	30 mg/kg-day	60 mg/kg-day	120 mg/kg-day
Live fetuses/litter[1]	12.23±0.51	13.32±0.51	12.14±0.56	13.75±0.62
No. litters with nonlive (%)	0 (0%)	5 (22.7%)	7 (31.8%)	3 (15%)
Nonlive/liver	0	0.41±0.18	0.32±0.10	0.25±0.14
Historical control data for this strain on nonlive/litter (Implants/pregnant female minus live fetuses/pregnant female) (Charles River Laboratories, 2001): 0.6-0.8, based on 62 studies.				
Males/litter	6.23±0.38	7.36±0.52	5.14±0.56	7.35±0.57
Average fetal body weight per litter (g)	4.14±0.07	4.10±0.05	4.03±0.07	3.84±0.05**
Historical control fetal weight (from Charles River Laboratories, 1988)	Mean 3.39 g Range 3.04-3.52 g			
Total ave. fetal weight/dam (g) (calculated)	50.6	54.6	48.9	52.8
Gravid uterine weight (g)	76.9±3.0	82.9±3.4	75.4±3.2	81.7±3.7
Treatment period maternal weight gain (g)	41.0±1.1	47.2±1.7**	40.2±1.6	41.2±2.9
Absolute maternal weight gain (adjusted for gravid uterine weight) (g)	58.0±3.1	58.4±2.2	52.7±2.4	51.8±3.2

[1]Mean ±Standard error of the mean.

**Statistically significant, $p<0.01$

either in the report or in its archives.[7] Data on fetal weight by sex is needed for meaningful modeling because the average weight of males and females is different and the number of males per group varied.

The preliminary rat developmental toxicity studies (NTP, 1983a) found that phenol toxicity is increased by the use of small dosing volumes. For example, when phenol was administered by gavage on GDs 6–15 to pregnant CD rats at doses of 0, 125, 160, 200, or 250 mg/kg-day in a volume of 1 mL/kg, the mortality was 0% (0/9), 70% (7/10), 78% (7/9), 100% (9/9), and 100% (9/9), respectively. The deaths were preceded by dose-related signs of toxicity, including tremors, convulsion, and respiratory distress; mottled liver and congested lungs were found on necropsy. In contrast, when the same doses were administered in a volume of 5–7.5 mL/kg, the respective mortality was only 0% (0/24), 0% (0/5), 17% (1/6), 17% (1/6), and 71% (5/7), respectively. On the basis of these results, a volume of 5 mL/kg was used in the main developmental toxicity study.

In preliminary toxicity studies conducted with doses of 60–250 mg/kg-day in a volume of 5–7.5 mL/kg, decreased maternal body weight gain (or body weight loss) during dosing was observed at 160 mg/kg-day and up, doses at which mortality was also observed. In addition, tremors were observed sporadically in the phenol-dosed groups, without any clear dose-response. There were no treatment-related changes in prenatal viability, fetal sex ratio, or fetal structural development.

The study authors stated that when results of all of the preliminary studies were pooled, a statistically significant trend of decreasing fetal weight was observed, but there were no statistically significant differences from controls in pairwise analyses. The power of the pairwise tests was limited because only 4–6 litters were produced in the dose range 100–200 mg/kg-day.

In a standard mouse developmental toxicity study (NTP, 1983b), phenol was administered by gavage in water at 0, 70, 140, or 280 mg/kg-day on GDs 6 to 15 in a volume of 10 mL/kg. Groups of 31–36 plug-positive female CD-1 mice were used in each treatment group. The pregnancy rate in the controls was only 83%; the pregnancy rate in dosed animals ranged from approximately 83% in the low- and mid-dose groups to 71% at the high dose. In addition, 4/36 high-dose mice died; no deaths occurred in any other groups. The average maternal body weight gain during treatment was statistically significantly reduced at the high dose, as was the maternal body weight at terminal sacrifice on GD 17 (by 10%, compared with the control group). In addition, tremors were observed at the high dose throughout the dosing period. As in the rat study, there was a highly statistically significant decrease in fetal body weight per litter (18%) at the high dose. An increased incidence of cleft palate was also reported at the highest dose level, although the incidence was not significantly

[7]Michael Shelby, NTP, personal communication to Lynne Haber (TERA), March 13, 2002.

different from that of the other groups and there was no statistically significant increase in the incidence of litters with malformations. There was no other evidence of altered prenatal viability or structural development.

Thus, the high dose of 280 mg/kg-day was a maternal frank effect level based on the observed deaths; tremors and decreased body weight also occurred at this dose. The high dose was also a developmental LOAEL based on decreased fetal body weight (accompanied by a possible increase in the incidence of cleft palate) in the fetuses, an effect that was likely secondary to the severe toxicity in the dams. The study NOAEL for maternal and developmental toxicity was 140 mg/kg-day.

The series of oral screening studies mentioned above (Narotsky and Kavlock, 1995; Berman et al., 1995; Moser et al., 1995; MacPhail et al., 1995) also included a developmental toxicity screening study in which groups of pregnant F344 rats (15–20 animals/group) were given phenol at doses of 0, 40, or 53.3 mg/kg by gavage once daily on GDs 6–19 and then sacrificed on postnatal day (PND) 6 (Narotsky and Kavlock, 1995). The dosing volume was 1 mL/kg. Pups in each litter were examined and counted on PND 1, 3, and 6 and were weighed on PND 1 and 6. Uterine implantation sites were counted after the dams were sacrificed. Only minimal quantitative data were presented. No maternal deaths were observed. The authors reported that phenol treatment altered respiration (rales and dyspnea) at both dose levels, but no quantitative data were presented. Decreased (but not statistically significant) maternal body weight (compared with the controls) and decreased (statistically significant) maternal body weight gain were also reported at both doses, but there was no clear dose-response.

No statistically significant evidence of developmental toxicity due to phenol exposure was observed. The only evidence of developmental toxicity came from dams that exhibited severe respiratory signs. These signs of developmental toxicity included a dose-dependent increase in full-litter resorptions (one at the low dose and two at the high dose) and an excessive incidence of perinatal mortality and reduced pup weights on PND 1 in one litter at the high dose. However, these changes as a group were not significantly different from those in the controls. Nonetheless, the respiratory effects from oral dosing reported in this study are of interest, particularly as they were not reported in the related study of systemic toxicity (Moser et al., 1995). This difference may reflect differences in the completeness of the study reporting. Alternatively, it may suggest that pregnant females may be more sensitive than nonpregnant females to the toxic effects of phenol.

4.4. OTHER STUDIES

4.4.1 Initiation/PromotionStudies, Other Short-Term Tumorigenicity Assays, and Cancer Mechanism Studies

Several studies have tested the promotion potential of dermally administered phenol. These studies found that phenol promotes tumors initiated with dimethyl=benzanthracene (DMBA), but the phenol doses tested caused ulceration (Salaman and Glendenning, 1957; Boutwell and Bosch, 1959) and thus were well above the MTD. One study (Wynder and Hoffman, 1961) found promotion of DMBA-initiated tumors by dermally administered phenol at a concentration that caused "no toxic reactions."

Salaman and Glendenning (1957) conducted an initiation/promotion study in which groups of 20 male "S" strain mice were initiated with a single dermal treatment with DMBA and promoted with dermal treatment with 0.5 mg/mouse phenol in acetone using two different treatment concentrations for the same applied dose. The phenol was applied beginning 3 weeks after the DMBA application for either 24 weeks in a volume of 0.025 mL as a 20% solution or for 32 weeks in a volume of 0.1 mL as a 5% solution (rotating the weekly applications among four application sites for both concentrations). The study did not report whether the application site(s) were covered or whether the animals were restrained from licking the site.

The high concentration produced local ulceration that healed just in time for the next treatment 4 weeks later, whereas the low concentration produced only transient light crusting that tended to decrease as the experiment progressed. It is unclear how severe the skin effects would have been if the low concentration had been repeatedly applied to the same site rather than being rotated among four sites. Tumors were observed in both treatment groups, with a shorter time to first tumor and a higher tumor burden in the group treated with the higher phenol concentration. A few histologically confirmed malignant tumors (primarily squamous epitheliomas) were observed in both groups. In mice that underwent the same phenol treatment but were not pretreated with DMBA, seven papillomas were observed at the high concentration. No tumors were observed at the low concentration, even though the weekly dose was the same and the total dose per mouse was higher (because the duration was longer).

This study had no control group on DMBA-only group, but the absence of tumors at the low concentration indicates that the observed tumors were phenol related. The authors noted that the observed tumors might have been related to the significant skin injury produced by phenol. This suggestion is supported by the strong effect of the concentration of applied phenol at the same total dose.

Boutwell and Bosch (1959) conducted a series of initiation/promotion studies with different strains of mice. The mice were pretreated with a single application of 75 μg DMBA in benzene followed by 5% or 10% phenol (1.25 or 2.5 mg per application) in benzene or with either dose of phenol alone twice weekly for 52 to 72 weeks. An additional group received DMBA alone, apparently followed by benzene vehicle, although there is some inconsistency between the text and the summary tables regarding whether the control group received the benzene vehicle. At the high phenol dose, dermal treatment with phenol resulted in decreased body weight (compared with the controls) and decreased survival. Skin wounds, hair loss, and reparative hyperplasia were also seen at the high dose, with the wounds predominantly seen in the first 6 weeks of treatment. By contrast, the authors stated that there was no evidence of ill effects of 5% phenol except for its promoting activity. This statement was based on external observation; no histopathology was conducted.

A dose-related increase in papillomas and in carcinomas was observed in the groups initiated with DMBA and promoted with phenol. Increased papillomas were also observed in one strain treated with the high dose of phenol alone. There was evidence of decreased activity when phenol was further purified, indicating that the activity was not due to a contaminant. Because the benzene vehicle is a defatting agent, it is unclear whether it would have contributed to the effect of phenol.

Wynder and Hoffmann (1961) also found that dermally applied phenol is a promoter. Female Swiss mice (28–30/group) were initiated with a single application of DMBA followed by treatment with 5% phenol three times weekly or 10% phenol two or three times weekly. The dilution vehicle was not reported. "No toxic reactions" were reported at 5% phenol, although the higher concentration was reported to be "rather toxic." Treatment was for 12 months, and the mice were observed for an additional 3 months; the percentage of animals with papillomas and with cancers was recorded monthly.

At 10 months, papillomas were seen in 33% of the low-dose group and > 80% of the high-dose group; cancer was seen in 3% of the low-dose animals and 30–60% of the high-dose groups. By contrast, there were no papillomas or cancers in female Swiss mice treated with phenol alone and only 10% papillomas (no cancer until week 12, and only 7% of the animals had cancer at study termination) in the mice treated with DMBA alone. Survival decreased markedly after week 10 in the high-dose groups but not the other groups. In another experiment, the onset of tumor formation was much earlier in mice treated with 0.005% benzo[a]pyrene three times weekly plus 5% phenol twice weekly than in mice treated with benzo[a]pyrene alone. Papillomas were observed by the second week in the groups receiving benzo[a]pyrene and phenol and were present in at least 33% of the animals by week 5, compared with 3% of the mice at week 5 in the benzo[a]pyrene-only group.

In a short-term assay, Stenius et al. (1989) found that phenol did not increase the production of gamma-glutamyl transpeptidase (GGT)-positive foci. Groups of 7–9 male Sprague-Dawley rats were partially hepatectomized and treated with diethylnitrosamine and then with 100 mg/kg phenol by gavage for 5 days (gavage volume not reported). Phenobarbital, the positive control, produced a marked increase in GGT-positive foci. This assay was based on the assumption that GGT-positive cells in enzyme-altered foci represent initiated cells and the observation that these cells are often resistant to toxic effects.

A decrease in tumor formation was seen in a co-carcinogenesis study of phenol and benzo[a]pyrene (Van Duuren and Goldschmidt, 1976; Van Duuren et al., 1971, 1973). Phenol was applied at a dose of 3 mg/application in acetone to the clipped skin of female ICR/Ha Swiss mice (50/group) three times a week for 1 year simultaneously with 5 μg of benzo[a]pyrene. The resulting number of tumors (both papillomas and squamous cell carcinomas) was markedly lower than in the animals receiving the benzo[a]pyrene alone. Phenol alone did not cause skin tumors. Neither the application volume nor the application surface area were reported, and no information was provided on any skin effects other than tumors.

In a test of a (TG •AC) transgenic mouse line carrying a v-Ha-ras gene fused to a ζ globin promoter, Spalding et al. (1993) found that phenol did not produce papillomas. This strain has genetically initiated skin and has been shown to be sensitive to the known promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA). Five male mice were dermally treated twice per week for 20 weeks with 3 mg phenol. Only one papilloma was observed; in contrast, strongly promoting agents produced five or more papillomas per mouse. This dose did, however, cause chronic irritation and hair loss.

A number of studies have investigated the reason why benzene is carcinojenic but orally administered phenol is not, in light of the fact that when phenol and many of its metabolites are significant products of benzene metabolism. Medinsky et al. (1995) noted that, on the basis of the urinary metabolite profile, higher levels of hydroquinone are produced after benzene exposure than after exposure to comparable doses of phenol. The potential production of other toxic metabolites, such as muconaldehyde, following benzene exposure but not phenol exposure was also noted. The authors explained the different metabolite profiles of phenol and benzene using the zonal distribution of metabolizing enzymes in the liver. As described in Section 3.3, phenol is conjugated in the gut and in zone 1 of the liver. This reduces the amount of phenol that reaches zone 3 of the liver, where oxidative activity is highest, and so decreases hydroquinone production. By contrast, conjugation of benzene in the gut and zone 1 is low, because benzene must be oxidized prior to conjugation. This

results in more free phenol reaching zone 3 of the liver following benzene exposure than after phenol exposure, and hence more production of hydroquinone.

Equivocal or negative results were obtained with phenol in a well-conducted and well-controlled interlaboratory study evaluating the usefulness of the Chinese hamster V79 cell metabolic cooperation assay for detecting tumor promoters (Bohrman et al., 1988). The study authors noted, however, that the assay was conducted in the absence of exogenous metabolic activation, and V79 cells have low intrinsic metabolic capacity.

Miyagawa et al. (1995) conducted a validation test in male B6C3F1 mice of the in vivo-in vitro replicative DNA synthesis test. The test was based on the hypothesis that nongenotoxic carcinogens are likely to increase cell proliferation. Phenol was negative in this assay, which was conducted at 0, 300, and 600 mg/kg administered via oral gavage.

4.4.2. Genotoxicity

The genotoxic potential of phenol appears to depend on the competing processes of activation to a genotoxic form and metabolic inactivation (e.g., via conjugation). Phenol tended to be negative in bacterial gene mutation assays (Pool and Lin, 1982; Rapson et al., 1980; Haworth et al., 1983) but was positive or equivocal in mammalian cell gene mutation assays (McGregor et al., 1988a,b; Paschin and Bahitova, 1982; Tsutsui et al., 1997) (Table 11). Phenol tended to induce micronuclei in mice when administered intraperitoneally (Marrazzini et al., 1994; Chen and Eastmond,1995a; Ciranni et al., 1988b) but was negative (or positive only at very high doses) when administered orally (Ciranni et al., 1988b; Gocke et al., 1981). This difference is likely due to the first-pass conjugation and inactivation of orally administered phenol. Phenol was also positive in in vitro micronucleus tests with human lymphocytes (Yager et al., 1990) and Chinese hamster ovary (CHO) cells (Miller et al., 1995) and caused chromosome aberrations in the presence of S9 activation in CHO cells (Ivett et al., 1989). Results from DNA damage assays are inconsistent, but they tend to show that phenol can cause sister chromatid exchanges (Erexson et al., 1985; Ivett et al., 1989) or cell transformation (Tsutsui et al., 1997) if it is not metabolically inactivated.

Phenol was negative in a well-conducted assay *Salmonella typhimurium* reverse mutation assay performed with up to cytotoxic doses in the presence and absence of varying concentrations of S9 activation with strains TA1535, TA1537, TA1538, TA98, and TA100 (Pool and Lin, 1982). Phenol was tested in two independent laboratories as part of a large-scale test by NTP in salmonella strains TA1535, TA1537, TA98, and TA100 in the presence and absence of S9 activation (Haworth et al., 1983). Both laboratories found that phenol was negative. Rapson et al. (1980) also reported

that phenol was negative in a test in strain TA100, although no primary data were presented and it was unclear whether sufficiently high doses were tested. A weak positive response was reported with phenol in strain TA98 in the presence of S9 but not in the absence of S9 (Gocke et al., 1981). Other strains were also tested in that assay, but the results were unclear.

Positive or equivocal results have been reported in mammalian cell gene mutation assays. McGregor et al. (1988a, b) reported on a well-conducted mouse lymphoma L5178Y tk+/tk- assay of phenol performed as part of a test of 72 coded chemicals. In the absence of S9, the results were considered questionable or inconclusive in two independent assays because of the absence of a dose-related trend and increases occurring only in the presence of high cytotoxicity. In the presence of S9, the first test was questionable (no dose-related trend but statistically significant results at several doses), but a clear positive result was obtained in the confirmatory test. Overall, the study authors concluded that no definitive conclusion was possible.

Table 11. Summary of Genotoxicity Studies

Endpoint	Assay system	Results (wo/w Activation)	Comments	Reference
In Vitro Studies				
Gene mutation-bacteria	*Salmonella typhimurium* TA1535, TA1537, TA1538, TA98, TA100	-/-	Tested to cytotoxic doses, varying S9 concentrations	Pool and Lin, 1982
	Salmonella typhimurium TA1535, TA1537, TA98, TA100	-/-	Part of NTP testing; tested in 2 laboratories	Haworth et al. 1983
	Salmonella typhimurium TA100	-1	Unclear if sufficiently high doses tested	Rapson et al. 1980
	Salmonella typhimurium TA98	-/w [2]	Other strains also tested, but results with them unclear	Gocke et al. 1981
Gene mutation - mammalian cell	Mouse lymphoma L5178Y cells	?/?[2]	Two independent assays conducted +/- S9	McGregor et al. 1988a, 1988b
	Chinese hamster V79 cells	NT[2]/+	S9 from phenobarbital-induced mice	Paschin and Bahitova 1982
	Syrian hamster embryo (SHE) cells	NT/+	None	Tsutsui et al. 1997
Clastogenicity	Micronuclei in human lymphocytes	+/NT	None	Yager et al. 1990
	Micronuclei in CHO cells	+/+	S9 from phenobarbital/beta-naphthoflavone induced rats	Miller et al. 1995
Chromosome aberration	CHO cells	-/+	Part of NTP testing	Ivett et al. 1989

Endpoint	Assay system	Results (wo/w Activation)	Comments	Reference
DNA damage	Sister chromatid exchange, human lymphocytes	-/NT	Unclear if sufficiently high doses tested	Jansson et al. 1986
	Sister chromatid exchange, human lymphocytes	+/NT	Small increases	Morimoto et al. 1983; Morimoto and Wolff 1980
	Sister chromatid exchange, human lymphocytes	+/NT	None	Erexson et al. 1985
	Sister chromatid exchange, CHO cells	+/w	Part of NTP testing	Ivett et al. 1989
	Unscheduled DNA synthesis, SHE cells	+/NT	None	Tsutsui et al. 1997
	Single strand breaks mouse lymphoma cells	-	None	Pellack-Walker and Blumer, 1986
	Single strand breaks CHO cells	-	None	Sze et al. 1996
Cell transformation	Syrian hamster embryo (SHE) cells	+/NT	None	Tsutsui et al. 1997
In Vivo Studies				
Gene mutation	*Drosophila* sex-linked recessive lethal	-	None	Gocke et al. 1981
	Drosophila sex-linked recessive lethal	-	None	Sturtevant 1952
	Drosophila sex-linked recessive lethal	-	None	Woodruff et al. 1985
Clastogenicity	Mouse micronucleus i.p.	+	None	Marazzini et al. 1994

Endpoint	Assay system	Results (wo/w Activation)	Comments	Reference
	Mouse micronucleus i.p.	+	Weak response	Chen and Eastmond 1995a
	Mouse micronucleus i.p.	-	No positive control, unclear if sufficiently high doses tested	Barale et al. 1990
	Mouse micronucleus i.p.	-	Small sample size	Gocke et al. 1981
	Mouse micronucleus oral	w	None	Ciranni et al. 1988b
	Mouse micronucleus i.p.	+	Clear effect at same dose as oral study	Ciranni et al. 1988b
	Mouse micronucleus oral	-	Number tested not reported	Gad el-Karim et al. 1985
	Mouse micronucleus oral	+	Pregnant females on GD13	Ciranni et al. 1988a
Chromosome aberration	Mouse, spermatogonia and spermatocytes	+	Inconsistencies in reporting	Bulsiewicz 1977
DNA damage	Single strand breaks, testicular cells, i.p.	-	None	Skare and Schrotel 1984

[1]Apparently in the absence of S9 - the presence of absence of S9 was not addressed.
[2]w = weak positive response; ? = questionable or inconclusive; NT = not tested

Other authors have also reported positive results in mammalian gene mutation assays. Paschin and Bahitova (1982) found that phenol was mutagenic in an in vitro assay for mutagenicity at the HGPRT locus of V79 Chinese hamster cells in the presence of S9 from the livers of phenobarbital-induced mice. Tsutsui et al. (1997) also reported that phenol induced a dose-related increase in mutation frequency in Syrian hamster embryo cells.

In contrast with these positive results in mammalian cells, a number of authors (Gocke et al., 1981; Sturtevant, 1952; Woodruff et al., 1985) found that phenol was negative in sex-linked recessive lethal assays conducted in drosophila using the feeding and injection routes.

The potential for phenol to induce micronuclei (a measure of clastogenicity) appears to be related to the route of dosing, with generally positive results when phenol is administered intraperitoneally but negative or equivocal results when it is administered orally. This route-related difference is likely due to the potential for first-pass detoxification of phenol when it is administered via the oral route but not when administered intraperitoneally. Several authors have suggested that the chromosomal effects of phenol result from phenol interactions with the spindle apparatus (Bulsiewicz, 1977; Yager et al., 1990). No in vitro studies of phenol clastogenesis were located.

Phenol was positive in the micronucleus test in male mice at an i.p. dose of 120 mg/kg (Marrazzini et al., 1994). Similarly, Chen and Eastmond (1995a) reported a weak increase in bone marrow micronuclei of male CD-1 mice treated with up to 160 mg/kg-day phenol intraperitoneally for 3 days. By contrast, Barale et al. (1990) found that phenol was negative when administered at i.p. doses of up to 160 mg/kg to male CD-1 mice. However, it is unclear whether sufficiently high doses were tested in that study, since no cytotoxicity and no clinical signs of toxicity were reported; also, the study did not include a positive control. Gocke et al. (1981) also found that phenol was negative in male and female NMRI mice treated with i.p. phenol at two daily doses of up to 188 mg/kg per dose, although the sample size of tested animals was small.

Ciranni et al. (1988b) found that an oral dose of 265 mg/kg phenol caused a slight increase in micronuclei and some myelotoxicity (decreased ratio of polychromatic to normochromatic erythrocytes, PCE/NCE ratio), but i.p. administration of the same dose caused clear increases in micronuclei and stronger myelotoxicity. Gad-el-karim et al. (1985) reported that a single oral dose of phenol (250 mg/kg) was negative for micronucleus formation in male CD mice, but they did not report the number of animals tested. This difference between the effects of i.p. and oral administration of phenol is also consistent with the metabolic effects of first-pass metabolism mentioned in Section 3.3.

Ciranni et al. (1988a) found that a single gavage dose of 265 mg/kg phenol caused a small but statistically significant increase in bone marrow micronuclei—accompanied by cytotoxicity—in pregnant female CD-1 mice treated on GD 13. There was no effect on fetal liver. Although no positive control was included, benzene did cause micronuclei in fetal liver, confirming the sensitivity of the assay.

Phenol was positive in an in vitro assay for the development of micronuclei in human lymphocytes in the absence of exogenous metabolic activation, although the dose-response was weak (Yager et al., 1990). Miller et al. (1995) also found that phenol was positive in an in vitro micronucleus test in CHO cells in the presence or absence of S9 from livers of phenobarbital/beta-naphthoflavone induced rats, although a stronger response was observed in the presence of S9.

Phenol was evaluated in the chromosome aberration assay in CHO cells as part of a series of tests by NTP to evaluate genotoxicity assays (Ivett et al., 1989). No significant increase was observed in the absence of S9 activation. In the presence of S9, significant increases in both simple and complex aberrations were observed. A delayed harvest time was used due to cell cycle delay.

In a five-generation study of chromosome aberrations in spermatogonia and spermatocytes in Porton strain inbred mice, Bulsiewicz (1977) observed dose-related increases in aberrations that tended to increase with successive generations. Polyploidy was also observed. Three dose groups and a control were treated by oral gavage. The dosing volume was reported as "2 mL of a solution of phenol" (sic) for the low-dose group; volumes were not reported for the other groups. Phenol was reported as being administered in a concentration of 0, 0.08, or 0.8 mg/L per day, or "8 g per liter" (sic).

A number of studies reported synergistic effects between phenol and hydroquinone in the micronucleus assay in mice (Marrazzini et al., 1994; Barale et al., 1990; Chen and Eastmond, 1995a). At least part of this interaction is likely due to phenol enhancing the peroxidase-dependent metabolic activation of hydroquinone.

A number of studies have evaluated the potential for phenol to cause DNA damage. These studies tend to show that phenol can produce effects when it is metabolized to an active form, but that inactivation is likely to predominate over activation following oral dosing.

Jansson et al. (1986) found no effect on sister chromatid exchanges (SCEs) in an in vitro assay with human lymphocytes, although it was unclear whether sufficiently high doses were tested. Small but statistically significant increases in SCEs in cultured human lymphocytes were reported by Morimoto et al. (1983) and Morimoto and Wolff (1980). By contrast, Erexson et al. (1985) found a

dose-related increase in SCEs in human lymphocytes exposed to phenol in vitro. They attributed the difference between their results and negative results in other studies to differences in the procedure used. In particular, Erexson and colleagues used mitogenic stimulation of the lymphocytes 24 hours prior to the phenol exposure. This mean that the cells were blast-transformed and exposed in the G1-S phase (and so there was less opportunity for repair prior to DNA replication), and cytochrome P450 activity was elevated as a result of the blast transformation. Negative controls showed that the increases were not due to the mitogenic stimulation alone. As part of a series of tests by NTP to evaluate genotoxicity assays, phenol was tested for the induction of SCEs in CHO cells (Ivett et al., 1989). Phenol was positive in the absence of S9 and weakly positive in the presence of S9. Cell cycle delay was observed at all positive doses.

In an assay with Syrian hamster embryo cells, Tsutsui et al. (1997) reported that phenol induced a slight dose-related increase in transformed colonies as well as a dose-related increase in unscheduled DNA synthesis in the same cell line.

In an evaluation of effects on germ cells in vivo, Skare and Schrotel (1984) found no effect on single strand-breaks in testicular cells of Sprague-Dawley rats receiving a single i.p. injection of up to 79 mg/kg or five daily i.p. injections of up to 39.5 mg/kg-day. Phenol also did not induce single strand breaks in mouse lymphoma L5178YS cells (Pellack-Walker and Blumer, 1986) or in CHO cells in a test up to cytotoxic concentrations (Sze et al., 1996).

Reddy et al. (1990) reported that DNA adducts were produced in cultured rat Zymbal glands orally dosed with 750 μg/mL of either phenol or hydroquinone. The adducts were not chemically characterized and their intensities were not quantified, but no spots were observed autoradiographically in the untreated controls. By contrast, many different adducts were seen in the analyzed tissues (bone marrow, Zymbal gland, liver, spleen) from both untreated female Sprague-Dawley rats and from rats treated for 4 days by oral gavage with a dose of 75 mg/kg-day phenol or 150 mg/kg-day of a 1:1 mixture of phenol and hydroquinone. The adduct patterns and levels of adducts did not differ significantly between control and treated animals.

The authors noted that endogenous adducts would interfere with the determination of treatment-induced adducts that chromatograph similarly. To address this possibility, they compared the chromatograms resulting from in vitro and in vivo treatments. The absence of the major in vitro adducts of hydroquinone or benzoquinone in the in vivo samples suggested that these adducts were not formed in the whole animal. Conversely, the primary adduct of phenol formed in vitro was also observed in vivo, although the levels relative to controls were much higher under in vitro conditions.

The authors suggested that the higher level of adducts following in vitro treatment versus in vivo treatment could be attributed to detoxification of orally administered phenol, but they did not further address the possibility that there may be a significant basal load of adducts formed by endogenously produced phenol.

Using a fluorescence *in situ* hybridization approach, Chen and Eastmond (1995a) found that treatment with phenol alone resulted in micronuclei and breaks in euchromatin, whereas hydroquinone affected chromosome loss and chromosomal breakage, particularly in centromeric heterochromatin. They suggested that the different pattern of effects with phenol and hydroquinone indicates that the synergism between phenol and hydroquinone is not due solely to phenol-induced increases in hydroquinone metabolism. Instead, they suggested, phenol or its metabolites may also be inhibiting DNA repair. In a follow-up study, Chen and Eastmond (1995b) found that phenol alone did not affect the DNA repair enzymes topoisomerase I or topoisomerase II in vitro. However, mixing phenol with horseradish peroxidase to mimic the peroxidase metabolism of the bone marrow resulted in complete inhibition of topoisomerase II; no effect was seen when glutathione was added to the peroxidase mixture.

In an assay with NCTC 929 mouse fibroblast cells, Yang and Duerksen-Hughes (1998) found that phenol caused a dose-related increase in levels of the p53 protein. The authors noted that cells increased p53 levels in response to DNA damage.

4.4.3. Neurological Effects

As described above, tremors have been observed following relatively high exposures to phenol via the oral (Dow Chemical Co., 1994; Moser et al., 1995) or inhalation (Dalin and Kristoffersson, 1974) routes. Decreased motor activity and a statistically significant increase in rearing post-exposure were also reported in a screening study with rats (Moser et al., 1995), and altered balance was reported in rats exposed continuously via inhalation for 15 days (Dalin and Kristoffersson, 1974). However, in a 13-week drinking water neurotoxicity study that included extensive neurohistological analyses (ClinTrials BioResearch, 1998), the only observed effects were decreased motor activity and decreased body weight (compared with the controls), which were probably secondary to decreased water consumption as a result of poor palatability. On the basis of the results of a short-term screening study, neurotoxic effects do not occur at lower exposures than other systemic effects of phenol (Berman et al., 1995; Moser et al., 1995).

4.4.4. Immunotoxicity

As described in Section 4.2, Hsieh et al. (1992) reported immune effects in CD-1 mice administered phenol in drinking water for 28 days. The reported effects included decreased antibody response (based on the PFC assay and direct antibody quantification using ELISA), with some evidence of decreased lymphoproliferative response and decreased mixed lymphocyte response. The clearest response was seen in the PFC and ELISA, which are highly predictive of effects on host resistance (Luster et al., 1992, 1993). Confidence in the study results is decreased by the somewhat small sample size (five rather than eight per group). Berman et al. (1995) reported atrophy of the spleen or thymus of rats gavaged with phenol under conditions that greatly enhanced toxicity in comparison with drinking water exposure.

Although no effects on spleen weight, cellularity, or antibody-forming cells in the spleen were observed in a two-generation study of Sprague-Dawley rats exposed to phenol in drinking water at concentrations of up to 5000 ppm (approximately 300 mg/kg-day) (Ryan et al., 2001; reported in unpublished form as IIT Research Institute, 1999), qualitative and quantitative differences between rats and mice in the effects of chemicals on the immune response are not unusual (e.g., U.S. EPA, 2000c). There is also no consistent pattern between mice and rats regarding which species is more predictive of immunotoxic effects in humans. In light of Hsieh et al. (1992) who reported immunotoxic effects of phenol at unusually low doses, it would be useful to confirm the results of that study in mice using a protocol compliant with EPA immunotoxicity test guidelines (U.S. EPA, 1998c).

The National Institute of Environmental Health Sciences (NIEHS) and the NTP have elected to conduct a comprehensive series of tests to evaluate the potential of phenol to adversely affect the immune system (verbal communication from Dori Germolec, NIEHS to Monica Barron, EPA, 2002). Using test protocols designed to be consistent with EPA testing guidelines and GLP standards, the first assay considered the same dosing regimen used in the Hsieh et al. (1992) study. That is, inbred female B6C3F1 mice–rather than outbred CD-1 male mice–were exposed to drinking water concentrations of 0, 5, 20, and 100 mg/L phenol (approximately 0, 1.7, 6.7, and 33 mg/kg-day). The study also included a positive control. Preliminary results demonstrated immunosuppression (reduced antibody response) at all levels of exposure, confirming the Hsieh study outcome.

In order to characterize a wider range of response levels, a second assay was initiated using drinking water concentrations of 0, 1.25, 2.5, 5.0, 20, and 40 mg/L (approximately 0, 0.4, 0.8, 1.7, 6.7, and 13.3 mg/kg-day), overlapping the previous study's range of exposures. In addition, NIEHS/NTP has initiated host resistence studies. Depending on the final outcome of this series of

tests, including NIEHS/NTP peer review, EPA may reconsider and, if appropriate, reopen this assessment.

4.4.5. Other Studies

Eastmond et al. (1987) investigated the role of phenol in benzene-induced myelotoxicity. No suppression of bone marrow cellularity was observed in male B6C3F1 mice treated intraperitoneally with doses as high as 150 mg/kg twice daily for 12 days (daily doses up to 300 mg/kg). Only minimal suppression was observed in mice dosed with hydroquinone at up to 100 mg/kg twice per day. By contrast, marked statistically significant, dose-related suppression was seen in mice treated with 75 mg/kg phenol and 75 mg/kg hydroquinone under the same conditions. In further in vitro studies, the authors showed that phenol stimulates the horseradish peroxidase-mediated metabolism of hydroquinone, and they hypothesized that similar stimulation of local peroxidases occurs in the bone marrow. The observation of myelotoxicity following benzene treatment–but only minimally or not at all following phenol or hydroquinone treatment– was therefore explained by a more-than-additive interaction between phenol and hydroquinone.

Corti and Snyder (1998) evaluated gender- and age-specific differences in cytotoxicity of benzene metabolites in vitro. Bone marrow cells were harvested from adult unexposed male and female Swiss Webster mice as well as from pregnant females and from fetal males and females. Cultures of CFU-e (colony forming units-erythroid, an erythroid precursor cell particularly susceptible to benzene toxicity) were prepared and then exposed to different concentrations of the metabolites. Although most of the benzene metabolites caused marked cytotoxicity, only minimal toxicity (0–20% cytolethality) of phenol was observed up to the highest concentration tested (40 μM), compared with nearly 100% cytolethality at the same concentration of catechol or hydroquinone. The effects were strongest in cells isolated from fetal females or from virgin adult females, but the dose-response was inconsistent, and it appears that no statistical comparisons with the untreated control of the same life stage were done.

Zamponi et al. (1994) studied the mechanism of phenol-induced cardiac arrhythmia, including ventricular tachycardia. In an abbreviated report, the authors suggested that phenol caused cardiac arrhythmia by blocking batrachotoxin-activated cardiac sodium channels. Testing conditions, including doses tested, were not provided in the abbreviated report.

Bishop et al. (1997) investigated the effect of phenol exposure on total reproductive capacity in mice. Groups of 26 female hybrid (SEC x C57BL6) F1 mice were given a single i.p. injection of 0 (buffer solution) or 350 mg/kg of phenol, and the females were caged individually with an untreated male hybrid (C3H/R1 x C57BL10) F1 mouse following the day of injection for 347 days. The

animals were observed daily for producing newborn mice; the young mice were counted and discarded immediately after birth. Female reproductive performance was evaluated on the basis of the total number of offspring per female and the average number of litters per female. The numbers of offspring per female and litters per female in phenol-treated mice were comparable to those in the controls. Thus, phenol had no measurable detrimental effect on the parameters used for evaluating long-term reproductive effects in this study.

4.5. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS AND MODE OF ACTION

Studies investigating the effects of orally administered phenol are summarized in Table 2. When phenol is administered in drinking water, the most common effect is decreased water consumption, presumably due to poor palatability. Effects seen concurrently with decreased water consumption, and probably secondary to dehydration, include body weights lower than those of the controls, decreased maternal body weight compared with that of developmental toxicity studies, and decreased pup survival prior to culling. Decreased motor activity was also seen in a drinking water neurotoxicity study, but it does not appear to be secondary to the decreased water consumption. The data also indicate that phenol causes immune effects in mice, but not in rats.

Oral exposure to phenol also affects the kidney and liver. Kidney inflammation was observed in the chronic drinking water study in rats (NCI, 1980). Liver and kidney pathology (tubular degeneration, kidney necrosis, and vacuolar degeneration) in rats also has been observed in short-term and subchronic toxicity studies using gavage dosing (Berman et al., 1995; Moser et al., 1995; Dow Chemical Co., 1945). These results from experimental animal studies support the observations in case studies and epidemiology studies (Shamy et al., 1994; Merliss, 1972) that the liver is a target of phenol in humans.

A number of nervous system effects have been observed following phenol dosing. Tremors were observed in one animal that later died (apparently of dehydration) following dosing in drinking water (ClinTrials BioResearch, 1998). Tremors have also been observed in several gavage studies in rats and mice (NTP, 1983a; Dow Chemical Co., 1994; Moser et al., 1995). However, in a specialized 13-week neurotoxicity study in male and female rats that included an FOB and a detailed neurohistopathology evaluation (ClinTrials BioResearch, 1998), the only observed nervous system effects were tremors in one animal and decreased motor activity in females. A short-term gavage screening study (Moser et al., 1995) found that the only effect in an FOB was a marginal decrease in motor activity and increased rearing post-exposure.

Headaches and weakness were reported in workers exposed to a mixture of phenol and a number of other aromatic compounds (Baj et al., 1994). Due to the mixed nature of the exposure, the effects cannot be clearly attributed to phenol. Muscle pain in a laboratory technician who developed phenol marasmus after being frequently exposed to phenol vapor (Merliss, 1972) may have reflected neurological damage.

The data regarding the hematotoxic potential of phenol are conflicting. No hematological effects were observed in rats in a well-conducted two-generation study in Sprague-Dawley rats (Ryan et al., 2001; available in unpublished form as IIT Research Institute, 1999). By contrast, decreased hematocrit and erythrocyte counts were seen at much lower doses in a 28-day drinking water study in mice (Hsieh et al., 1992), although this study is limited by the use of only five males per dose. Data from these two studies are contrasted in Table 6. The differences between the two studies cannot be resolved by considering the results of the chronic drinking water studies conducted by NCI (1980) in mice and rats, because no hematological evaluation was conducted in those studies.

The negative finding in rats following oral exposure are supported by the absence of hematological effects in rhesus monkeys, male Sprague-Dawley rats, and male albino mice (strain not further identified) exposed to an average phenol concentration of 18.2 mg/m^3 continuously for 90 days (Sandage, 1961). Hemoglobin and hematocrit were also unaffected in a small study of rats exposed to phenol in air at 100 mg/m^3 continuously for 15 days (Dalin and Kristoffersson, 1974). Neither of these inhalation studies used modern exposure protocols, and both were limited by inadequate exposure monitoring. However, Dalin and Kristoffersson (1974) did both report systemic effects (nervous system and liver effects) in rats at exposure levels that did not cause hematological effects. Sandage (1961) found an indication of liver and kidney histopathology in the monkeys and rats, although not in the mice. Assuming that all of the inhaled phenol was absorbed (see Section 3.2), the systemic dose to mice in the Sandage (1961) study can be estimated at approximately 30 mg/kg-day, based on a body weight of 0.03 kg and an inhalation rate of 0.052 mg/m^3/day. This dose is comparable to the high dose in the Hsieh et al. (1992) study.

The negative results in the inhalation studies raise further questions about the reliability of the hematotoxicity effects seen by Hsieh et al. (1992) in mice–particularly in the light of the small sample size–as well as the relevance of those results to humans. Because portal-of-entry conjugation is more efficient following ingestion rather than following inhalation of phenol (see Section 3.3), it is not surprising that the systemic toxicity (i.e., liver and kidney effects) of a given absorbed dose may be higher for inhaled phenol. Human data on hematotoxic effects of phenol are limited. Baj et al. (1994) reported a small but statistically significant decrease in erythrocytes in workers exposed to a mixture

containing phenol, chlorinated benzenes, and other compounds. Due to the mixed nature of the exposure, the effect, if any, cannot be clearly attributed to phenol.

The results of Hsieh et al. (1992) also indicate that phenol can be immunotoxic to mice. The investigators observed a clear dose-related decrease in two related measures of antibody formation (the PFC assay and a direct measure of antibody titer using ELISA), along with some evidence of a decreased cell-mediated response at the high dose. Confirmation of their results in a repeat assay conducted according to EPA test guidelines would be useful in light of the small number of animals used and the limited number of risk assessments that have been based on in vivo/in vitro immunotoxicity assays.

No effect on spleen weight, cellularity, or antibody-forming cells (in the PFC assay) were observed in a two-generation study of Sprague-Dawley rats exposed to phenol in drinking water at much higher doses (Ryan et al., 2001; reported in unpublished form as IIT Research Institute, 1999), but qualitative and quantitative differences in effects of chemicals on the immune response of rats and mice are not unusual (e.g., see dioxin, as described in U.S. EPA, 2000c). "Necrosis or atrophy in the spleen or thymus" (not further described) was observed in a 14-day screening study of rats gavaged with phenol (Berman et al., 1995), supporting the immune organs as targets of phenol.

Baj et al. (1994) reported in an epidemiology study of Polish workers that exposure to Ksylamit® vapor resulted in immune effects, but it is unclear whether phenol is the causative agent because Ksylamit® contains a number of different aromatic compounds. Overall, the data indicate that phenol by itself may cause immunotoxicity in humans, but more data are needed to address this possibility. Interaction between phenol and benzene metabolites may also cause immune effects, as described below.

Benzene (which is metabolized to phenol) among other compounds, causes immunological effects, including lymphopenia and leukopenia (reviewed in ATSDR, 1998). However, although benzene is a leukemogen in humans, it has not been shown to induce leukemias in experimental animals. For example, in the NTP gavage studies of benzene (NTP, 1986), it was carcinogenic to both male and female F344 rats and $B6C3F_1$ mice, inducing tumors at multiple sites. There was a statistically significant increase in lymphomas in male and female mice but not in rats of either sex. No significant increase in leukemias was noted in either species.

One proposed mechanism by which this immunotoxicity is induced involves the interaction between phenol and hydroquinone, in which phenol stimulates the metabolism of hydroquinone. Eastmond et al. (1987) observed decreased bone marrow cellularity in male mice dosed intraperitoneally with phenol and hydroquinone but not with phenol alone at doses of up to 300 mg/kg-day for 12 days and only minimally with hydroquinone. These results appear to contradict those seen

at much lower doses (Hsieh et al., 1992), although the target tissue examined by Eastmond et al. (1987) was bone marrow, whereas Hsieh et al. examined the spleen. The former study was also conducted via the i.p. rather than oral route, but toxicity might be expected to be higher via the i.p. route because first-pass metabolism would be lower.

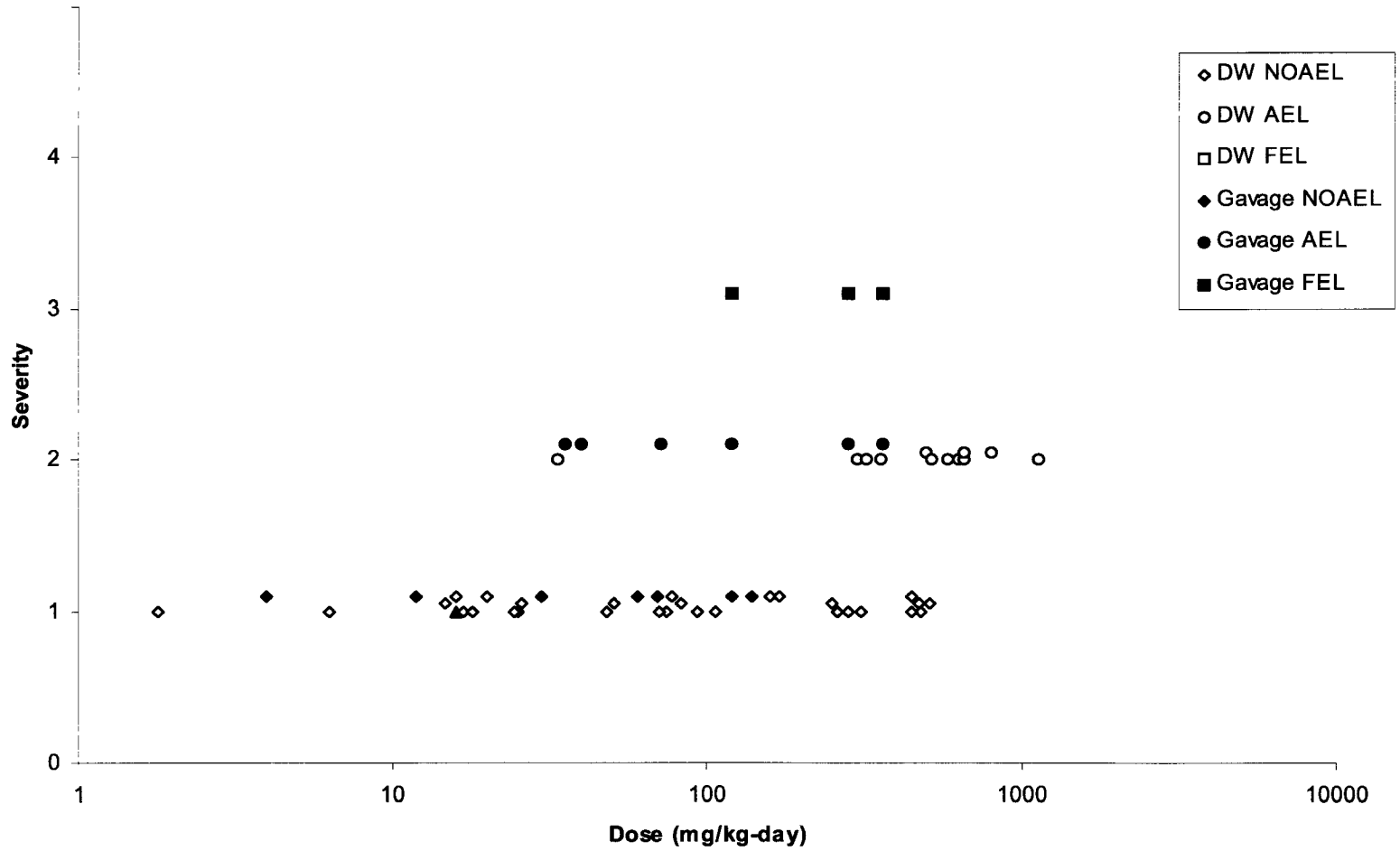
It is not known with certainty whether the toxic effects of phenol are due to the parent compound or to its metabolites. Distinguishing between effects of each is complicated by the lack of adequate data on concurrent blood levels of parent compound and metabolites. Phenol and metabolite levels in blood would be expected to rise in parallel as portal-of-entry metabolism becomes saturated. However, several lines of reasoning suggest that many of the toxic effects are due to the oxidative metabolites of phenol. In an in vitro study of the dysmorphogenic and embryotoxic effects of benzene and phenol and their metabolites on whole rat conceptuses, Chapman et al. (1994) found that phenol toxicity was much higher in the presence of S9.

The target tissues of phenol toxicity (kidney, liver, lung, and possibly bone marrow) are also those in which phenol can be oxidatively metabolized. In a 2-week inhalation study, Dalin and Kristoffersson (1974) observed altered balance and twitches in the absence of increased amounts of phenol in the blood, suggesting that a phenol metabolite rather than phenol itself is the toxic agent. Alternatively, the analytical method used may not have been sensitive enough or specific enough to detect any changes in blood levels of phenol. Phenol could produce portal-of-entry and systemic toxicity as a result of its ability to react with and to denature protein.

A key point with regard to the evaluation of the toxicity of orally administered phenol is whether gavage studies accurately represent the toxicity under environmental exposure conditions. Gavage studies are typically done using a single bolus dose per day, whereas environmental exposure is more likely to involve exposure distributed over the course of the day. Although laboratory animals consume drinking water in a few larger doses primarily during the active period rather than in continuous small sips, the toxicokinetics of environmental exposure are more closely modeled by a drinking water study or a gavage study using divided dosing, than by a study using a single gavage dose per day.

Figure 2 compares the doses and observed severity of effects in drinking water and gavage

Figure 2. Plot of severity with dose for drinking water (DW) (open symbols) or gavage (filled-in symbols). Values of 1, 2, 3 correspond with NOAEL, AEL, or FEL, respectively.



studies. Of particular interest is the number of gavage studies in which death, a frank effect, was observed, whereas drinking water studies at comparable or higher daily doses produced only tremors, kidney inflammation, and effects secondary to decreased water intake. The gavage NOAELs that occur at the same doses as gavage adverse effect levels are for maternal and developmental toxicity. The sole exception to the large difference between gavage and drinking water studies in doses that cause effects is the 28-day drinking water study by Hsieh et al. (1992), which reported hematological and immune effects at doses comparable to NOAELs in drinking water studies. As noted above, it would be useful to obtain independent confirmation in mice of the results reported by Hsieh et al. (1992).

Toxicokinetic data support this difference between gavage and drinking water studies and indicate that toxicity is correlated with peak blood concentrations rather than the area under the curve. Dow Chemical Co. (1994) observed "phenol twitching behavior" (including tremors and eye blinking) in rats gavaged with 150 mg/kg phenol; the behavior disappeared in less than an hour post-dosing, as phenol blood levels declined below peak values. By contrast, no twitching behavior was observed following a similar daily dose of phenol in drinking water. Unfortunately, blood levels of phenol or its metabolites were not determined in the drinking water phase of the study, but they are likely much lower than in the gavage phase, in light of the rapid blood clearance.

The higher systemic toxicity of gavaged phenol when it is administered in smaller volumes (NTP, 1983a) also supports the idea that toxicity is related to peak blood concentrations, because smaller dosing volumes would be expected to enhance the absorption rate. An unpublished GLP range-finding study for maternal toxicity (International Research and Development Corp., 1993) also found higher systemic toxicity for phenol when it was administered in smaller dosing volumes.[8] Data on the relationship between dosing volume and peak phenol blood concentrations are not available. Data on the relationship between peak blood concentrations and effects also are not available for the endpoints relevant to the critical effect.

The inhalation data on the effects of phenol are very limited (Table 7). Only one study conducted according to modern toxicological methods was located (Hoffman et al., 2001; available in unpublished form as Huntingdon, 1998), and the exposures in this study were for only 2 weeks.

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[8]Although this unpublished study is not a primary reference for this assessment, it is presented here because it contributes some useful information to the overall hazard identification phase of the phenol assessment.

Other studies ranged from 2 weeks (Dalin and Kristoffersson, 1974) to 90 days (Deichmann et al., 1944; Sandage, 1961), but they included incomplete documentation of the study results, and they did not use modern methods for controlling exposure levels. In addition, the authors of some of the studies (e.g., Sandage, 1961) appear to have been looking for marked effects and thus dismissed statistically significant incidences of organ pathology of lesser severity. Nonetheless, the studies are fairly consistent with regard to the target organs and the effects observed. Exposure to high concentrations produced nervous system effects, and liver, kidney, and lung pathology occurred at lower concentrations. Rats were reported to be much less sensitive than rabbits or guinea pigs (Deichmann et al., 1944). The systemic targets observed following inhalation exposure to phenol are supported by data from the oral exposure route.

Information on the mode of action of inhaled phenol toxicity is also quite limited, but some extrapolation from other routes is possible. On the basis of the irritative and corrosive effects seen following dermal exposure to phenol, respiratory tract effects are likely due to direct contact of phenol with the respiratory tract tissue. As noted in Section 3, phenol is extensively absorbed following inhalation exposure. The lung can metabolize phenol prior to absorption, but the efficiency of metabolism in the lung is lower than that for the gut or liver (Cassidy and Houston, 1984). After the inhaled phenol (and its metabolites) reaches the blood stream, the same points described above for the oral route are relevant. In brief, it is not known whether the systemic toxic effects of inhaled phenol are due to phenol itself or to its metabolite(s), but at least some of the toxic effects appear to be attributable to phenol metabolite(s) (Chapman et al., 1994). Systemic toxicity appears to be related to peak concentrations in blood rather than to total daily intake.

4.6. WEIGHT OF EVIDENCE EVALUATION AND CANCER CHARACTERIZATION–SYNTHESIS OF HUMAN, LABORATORY ANIMAL, AND OTHER SUPPORTING EVIDENCE, CONCLUSIONS ABOUT HUMAN CARCINOGENICITY, AND LIKELY MODE OF ACTION

Chronic drinking water bioassays of phenol have been conducted in rats and mice (NCI, 1980). In these studies, NCI concluded that phenol was "not carcinogenic" in male or female F344 rats or B6C3F1 mice. However, the report also noted that leukemia and lymphoma were statistically significantly increased in low-dose male rats, although there was no significant increase at the high dose. The increases in leukemia are of particular interest in light of the leukemogenic effects of benzene (for which phenol is a metabolite) in humans. (In experimental animals, benzene has not

been shown to induce leukemia, although increases in lymphoma have been observed [e.g., NTP, 1986].)

The MTD was clearly reached in the rat study (NCI, 1980), on the basis of decreased body weight compared to controls and on kidney histopathology. Although the only sign of toxicity in the mouse study was decreased body weight (compared to the controls) secondary to decreased water consumption, higher doses probably could not have been tested, because of the decreased water consumption. Higher toxicity probably could have been achieved in a gavage study in mice at lower doses. These considerations suggest that an MTD was also reached in mice, although a definitive conclusion is difficult.

No other long-term oral carcinogenicity studies of phenol are available. No inhalation studies of phenol were of a sufficient duration to assess phenol carcinogenicity. The only long-term study that has assessed the carcinogenicity of phenol applied dermally without initiation was that of Boutwell and Bosch (1959), in which increased papillomas were seen at a dose that also caused ulceration.

In contrast with these negative results for oral carcinogenicity, dermally administered phenol has been consistently observed to be a promoter. Several authors (Salaman and Glendenning, 1957; Boutwell and Bosch, 1959; Wynder and Hoffmann, 1961) observed that dermally applied phenol promoted DMBA-initiated skin tumors. These studies have generally reported significant skin ulceration at all phenol doses tested. The exception is Wynder and Hoffman (1961), who reported that 5% phenol promoted DMBA-initiated tumors in mice in the absence of any toxic reactions. When the same phenol dose was administered in different volumes, higher promotion activity was exhibited by the more concentrated solution, which also produced severe skin ulceration, suggesting that some of the promotion activity may have been related to the rapid cell division in the repairation of skin damage (Salaman and Glendenning, 1957). The observed response was dose-related (Boutwell and Bosch, 1959), but marked systemic toxicity was also observed at these doses. Co-carcinogenesis with dermally administered benzo[a]pyrene has also been observed (Wynder and Hoffmann, 1961). Because the benzo[a]pyrene was co-administered with the phenol, this assay cannot be classified as a true initiation/promotion assay. Production of papillomas by dermally administered phenol (in the absence of an initiator) was observed only at a concentration that caused ulceration, and hence was above the MTD.

Genotoxicity studies have found that phenol tends not to be mutagenic in bacteria (Pool and Lin, 1982; Rapson et al., 1980; Haworth et al., 1983), but positive or equivocal results have been

obtained in gene mutation assays in mammalian cells (McGregor et al., 1988a, 1988; Paschin and Bahitova, 1982; Tsutsui et al., 1997). Increases were larger in the presence of S9 activation. Phenol tended to induce micronuclei in mice when administered intraperitoneally (Marrazzini et al., 1994; Chen and Eastmond,1995a; Ciranni et al., 1988b), but it produced negative (or positive only at very high doses) results when administered orally (Ciranni et al., 1988b; Gocke et al., 1981). This difference is likely due to the first-pass conjugation and inactivation of orally administered phenol. Phenol was also positive in in vitro micronucleus tests with human lymphocytes (Yager et al., 1990) and CHO cells (Miller et al., 1995), and it caused chromosome aberrations in the presence of S9 activation in CHO cells (Ivett et al., 1989). Phenol has been observed to act synergistically with hydroquinone in the production of genotoxic effects (Marrazzini et al., 1994; Barale et al., 1990; Chen and Eastmond, 1995a).

Epidemiology data do not shed further light on the carcinogenic potential of phenol. Some studies (Kauppinen et al., 1986; Dosemeci et al., 1991) have reported elevated risks in phenol-exposed workers, whereas others have observed no effect (Wilcosky et al., 1984). However, the usefulness of each of these studies for risk assessment is limited by (depending on the study) an absence of an effect when latency was considered, a lack of a dose-response, and potential for confounding.

Although phenol was negative in oral bioassays conducted in rats and mice (NCI, 1980), questions remain regarding its carcinogenic potential in light of the positive results in initiation/promotion assays (albeit at exposures typically above the MTD), the increases in leukemia in low-dose male rats in the oral bioassay, and the observation of gene mutations in mammalian cells in vivo and micronuclei in vivo following i.p. dosing. No inhalation studies of sufficient duration to assess phenol carcinogenicity have been conducted. Dermal carcinogenicity or initiation/promotion studies with phenol at exposures below the MTD have not been conducted. The carcinogenic potential of phenol via inhalation exposure has not been evaluated at all. Under the draft revised *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1999), the data regarding the carcinogenicity of phenol via the oral, inhalation, and dermal exposure routes *are inadequate for an assessment of human carcinogenic potential.* Under the current guidelines (U.S. EPA, 1986a), phenol falls in Category D: not classifiable as to human carcinogenicity.

Similar conclusions have been reached by other groups in recent assessments of the carcinogenicity of phenol. IARC (1999) concluded that there is *inadequate evidence* in humans and in experimental animals for the carcinogenicity of phenol. Overall, IARC concluded that phenol

is *not classifiable as to its carcinogenicity to humans (Group 3).* Phenol is not included in the *9th Report on Carcinogens* (NTP, 2000a), and it is not listed as being considered for inclusion in the *10th Report on Carcinogens* (NTP, 2000b). This report contains only chemicals and substances that have been reviewed and classified as known human carcinogens or as reasonably anticipated to be carcinogens.

4.7. SUSCEPTIBLE POPULATIONS

People with decreased ability to conjugate and eliminate phenol, such as those with low activities of PST or glucuronyltransferase, may be more susceptible to phenol toxicity. If the toxicity of phenol is due to oxidative metabolites such as hydroquinone or catechol, individuals with increased oxidative activity would also be more sensitive to phenol toxicity. The activity of CYP2E1, the enzyme responsible for the oxidation of phenol, can be increased by exposure to a variety of agents, including ethanol and chlorinated solvents, so people with high exposures to these agents may be more sensitive to phenol.

4.7.1. Possible Childhood Susceptibility

As discussed in Section 4.3, a gavage study in rats (NTP, 1983a) reported decreased fetal body weight at a dose below that at which maternal toxicity occurred. In contrast, maternal toxicity occurred at a lower dose than did decreased fetal body weight in a gavage developmental toxicity study that used a divided-dosing protocol (Argus Research Laboratories, 1997). The observation of a fetal effect at a dose as low as 120 mg/kg-day (NTP, 1983a) suggests that the developing fetus is a possible susceptible population. The strength of this conclusion is weakened, however, by the small magnitude of the fetal weight decrease together with the increased litter size, which led to the high dose being identified as an *equivocal* LOAEL (see Section 4.3). The strength of the conclusion is also weakened by the observation in another gavage rat study (Argus Research Laboratories, 1997) that decreased fetal weight occurs only at doses above those that cause decreased maternal weight gain, and the observation of decreased fetal weight in a drinking water study (Ryan et al., 2001) only at concentrations that also resulted in reduced water consumption.

Only one study was located that specifically addressed age-related differences in the systemic toxicity of phenol. Deichmann and Witherup (1944) compared the lethality of an oral dose of 600 mg/kg phenol (administered as a 5% aqueous solution) in 10-day-old, 5-week-old, and adult rats. Mortality was 90%, 30%, and 60% in the neonates, young rats, and adult rats, respectively. Although the young and adult rats died within 1.5 hours of dosing, the neonates died 12–24 hours

after dosing. The data from this study suggest that neonates are more sensitive than adults and young rats may be less sensitive than adults. Alternatively, the age-related differences observed in this study could reflect inter-individual variability that was not a consequence of age. This study has not been replicated; therefore, definitive conclusions are not possible.

Data from humans and rats are consistent in showing very little fetal expression of CYP2E1, which is rapidly induced shortly after birth and rises though childhood (reviewed in Hakkola et al., 1998). The age after parturition at which CYP2E1 levels peak has been studied in laboratory animals, with inconsistent results. Some studies suggest that peak levels are reached during childhood, with a subsequent decrease to adult levels (Schenkman et al., 1989), whereas others have shown a rapid rise in CYP2E1 levels during childhood to a maximum level that is sustained during adulthood (Song et al., 1986). If the toxic moiety is a metabolite, decreased P450 metabolism could be expected to result in decreased toxicity. However, sulfate conjugation is also lower early in life (Iwasaki et al., 1993), so more of the dose is available for oxidative metabolism. Indeed, Heaton and Renwick (1991) found higher production of oxidative metabolites in young rats. This difference was higher in males, with the percentage of the administered dose recovered as hydroquinone conjugates decreasing from 38% of the administered dose in 3-week-old males to 8.2% in 16-week-old rats. Smaller decreases with age (from 17.8% of the administered dose in 4-week-old rats to 10.5% in 15-week-old rats) were observed in females. These data suggest the potential for children to be more sensitive than adults to the systemic effects of phenol.

4.7.2. Possible Gender Differences

Kenyon et al. (1995) (in mice) and Heaton and Renwick (1991) (in rats) reported higher excretion of hydroquinone conjugates in males than in females, suggesting higher levels of hydroquinone production in males. By contrast, Meerman et al. (1987) reported only slightly faster metabolism in male rats. These data would tend to suggest that if hydroquinone is the toxic moiety, phenol would be more toxic in males. However, few differences in phenol toxicity between males and females were identified; differences in NOAELs reflect differences in water consumption per unit weight, resulting in differences in estimated intake. Acute oral lethality data do suggest that phenol is more toxic to males (Thompson and Gibson, 1984).

5. DOSE RESPONSE ASSESSMENTS

5.1 Oral Reference Dose (RfD)

5.1.1. Choice of Principal Study and Critical Effect

An extensive database for the effects of orally administered phenol is available. The studies relevant to the development of the RfD are summarized in Table 2. Two-year drinking water studies conducted in rats and mice are available (NCI, 1980). Hematology and serum biochemical evaluations were not included in those chronic studies, but they were included in a recent two-generation drinking water study conducted in rats (Ryan et al., 2001; available in unpublished form as IIT Research Institute, 1999). The only study evaluating hematological effects in mice is a 28-day drinking water study (Hsieh et al., 1992). A specialized subchronic neurotoxicity study was conducted with rats exposed to phenol in drinking water (ClinTrials BioResearch, 1998). A number of developmental toxicity studies have been conducted in rats and mice, all using the gavage route (Argus Research Laboratories, 1997; NTP, 1983a; NTP, 1983b, Narotsky and Kavlock, 1995).

As shown in Table 2, the study with the lowest NOAEL/LOAEL boundary is the 28-day drinking water study in mice by Hsieh et al. (1992). In this study, the NOAEL was 6.3 mg/kg-day, and the LOAEL was 33.6 mg/kg-day, based on decreased antibody response, supported by decreases in hematocrit and red blood cells. Statistically significant decreases in erythrocyte counts were observed at the low and mid doses, but these results were considered biologically questionable in the absence of effects on hematocrit, in the absence of data addressing whether the apparent inconsistency was due to macrocytosis, and in light of the lack of support from other studies.

As noted in Section 4.5, this study is useful for hazard identification. However, confirmation of the study results in an independent assay in mice would be useful before using the data for dose-response assessment, considering the small number of animals tested and the limited number of risk assessments that have been based on in vivo/in vitro immunotoxicity assays. In addition, although qualitative differences between rats and mice in immune assays are not unusual (e.g., dioxin, see U.S. EPA, 2000c), it is of interest that Hsieh et al. (1992) observed immune effects in mice at very low doses, but the only other study evaluating similar immune parameters (Ryan et al., 2001; IIT Research Institute, 1999) did not observe effects in rats at 10-fold higher doses. No other studies in mice have directly evaluated effects on antibody forming cells.

In another study that evaluated immune effects of phenol on mice (Eastmond et al., 1987), no effect on bone marrow cellularity was observed at phenol doses of up to 300 mg/kg-day in mice dosed intraperitoneally for 12 days. Similarly, Corti and Snyder (1998) evaluated the effects of benzene metabolites on CFU-e cells (an erythroid progenitor cell sensitive to benzene) harvested from

mice and found that the cytotoxicity of phenol was much lower than that of other benzene metabolites. The effects in mice and rats were not compared in that study.

In light of these issues, and in the absence of other data supporting the observed effects at such low doses, the results of Hsieh et al. (1992) are too preliminary to be used as the basis for the phenol RfD. However, this study does raise concerns regarding the potential of phenol to cause hematological and immune effects, and it would be worthwhile to address these questions in a mouse immunotoxicity study conducted according to modern methods. The uncertainties regarding these endpoints and the use of a database uncertainty factor to address these uncertainties are further addressed in Section 5.1.3.

The next lowest NOAEL/LOAEL combination was observed in a 14-day gavage study in rats conducted as part of a large-scale screening study of a number of chemicals (Berman et al., 1995; Moser et al., 1995). Tremor, kidney tubular degeneration, and increased rearing in an FOB were observed at the high dose of 40 mg/kg-day but not at the next lower dose of 12 mg/kg-day. Although the incidence of kidney histopathology was not statistically significant, the high dose can be considered a LOAEL in light of the low statistical power of the study (only eight female rats per group) and the rarity of these lesions in short-term studies. The corresponding NOAEL is 12 mg/kg-day.

The relevance of this NOAEL to environmental exposures is questionable, however, due to the markedly higher toxicity observed in gavage studies than in drinking water studies, as discussed in Section 4.5, and the absence of supporting toxicity in drinking water studies of much longer duration. In particular, drinking water studies found no kidney histopathology in rats exposed to 260 mg/kg-day for 2 years (NCI, 1980) (although kidney inflammation was observed at higher doses), in mice exposed to doses up to 660 mg/kg-day for 2 years (NCI, 1980), or in parental rats in a two-generation reproduction study (Ryan et al., 2001; IIT Research Institute, 1999).

The only other study reporting kidney histopathology at low doses was a poorly documented and unpublished 6-month gavage study in rats (Dow Chemical Co., 1945). The very small dosing volume used by Berman et al. (1995) also may have contributed to the high toxicity, considering the findings of NTP (1983a).

The principal study for development of the RfD is Argus Research Laboratories (1997), in which decreased maternal weight gain was observed in rats gavaged on GD 6–15 with 120 mg/kg-day phenol; the maternal NOAEL was 60 mg/kg-day, based on decreased body weight gain, and the developmental NOAEL was 120 mg/kg-day. The BMDL was 93 mg/kg-day. No effect on body

weight was reported at 120 mg/kg-day in systemic toxicity studies using drinking water as the exposure route (NCI, 1980; ClinTrials BioResearch, 1998), but it is not surprising that maternal toxicity following 9 days of dosing occurs at a lower dose than does chronic systemic toxicity, considering the different physiological status during pregnancy. Although the principal study was conducted via the gavage route, measures were taken to decrease the impact of bolus dosing by dividing the daily dose into three administrations per day.

It is of interest that rats consume drinking water not in many little sips, but in a few larger doses primarily associated with food consumption during the active period of the day. Therefore, the toxicokinetic profile of the divided-dose gavage study may actually be fairly similar to the toxicokinetic profile that would be observed with drinking water exposure. In addition, a more precise measurement of administered dose is possible in gavage studies, because spillage can occur in drinking water studies.

The NOAEL identified in the Argus Research Laboratories (1997) study is supported by a developmental toxicity study (NTP, 1983a) in which decreased fetal weight was observed in CD rats gavaged on GDs 6–15 with 120 mg/kg-day; the NOAEL was 60 mg/kg-day. The high dose of 120 mg/kg-day was considered an *equivocal* LOAEL for developmental effects, in light of the small magnitude of the weight decrease, the increased litter size (which can result in decreased fetal weight), and the absence of an effect on fetal weight at a maternally toxic dose in another gavage developmental study in rats (Argus Research Laboratories, 1997). In the NTP (1983a) study, the maternal toxicity NOAEL was the high dose, 120 mg/kg-day.

Because of the uncertainties regarding identification of the critical effect level for the NTP (1983a) study, it was not considered to be an appropriate co-principal study. BMD modeling could not be conducted on the fetal weight endpoint, because fetal weights were reported only as an average across both sexes for all litters; no individual animal data were available. Because fetal weights of male fetuses tend to be heavier than those of females, and because the number of fetuses per litter affects the fetus weight, it was not appropriate to model the pooled data.

Although the decreased maternal weight gain (Argus Research Laboratories, 1997) was a mild effect and was possibly confounded by the gavage dosing, these results are supported by a drinking water study. Decreased motor activity was seen in female rats consuming the high concentration of phenol (5000 ppm, corresponding to 360 mg/kg-day) in the 13-week neurotoxicity study (ClinTrials BioResearch, 1998). The NOAEL in females was 107 mg/kg-day; no adequate fit could be obtained using BMD modeling. As discussed in Section 4.2, the authors considered the

decreased motor activity to be secondary to dehydration, but an analysis of the individual animal data and comparison with the literature could not confirm this assumption.

Ryan et al. (2001) conducted a two-generation drinking water study (also available in unpublished form as IIT Research Institute, 1999) in rats in which decreased parental and pup weight occurred at a LOAEL of 301 mg/kg-day, with a NOAEL of 71 mg/kg-day. However, these lower body weights, compared with control, are likely to be secondary to decreased water consumption and not an indication of phenol toxicity.

5.1.2. Method of Analysis: Benchmark Dose

The RfD was derived by the BMD approach using BMDS Version 1.3, which downloaded from the National Center for Environmental Assessment's web site. The BMR was defined as the default of a change of one standard deviation (U.S. EPA, 2000d). A BMDL of 93 mg/kg-day was derived for decreased maternal weight gain (Argus Research Laboratories, 1997) using the polynomial model. Similar BMDL values of 125 and 129 mg/kg-day were calculated using the power and Hill models, respectively, although the fit (based on the Akaike Information Criterion [AIC]) was slightly better using the polynomial model, and a more conservative BMDL was obtained using this model.

An alternative BMDL for this endpoint could be calculated using the geometric mean of the BMDLs from all three models, 114 mg/kg-day, on the rationale that the small difference in AICs was not meaningful. Other measures of fit (based on the goodness-of-fit p value and on visual fit) also indicated that all three models are comparable. However, in this case the slightly more conservative approach was used, in part as an added degree of protection because of the uncertainties regarding immunotoxicity. Details of the model results are presented in Appendix B.

5.1.3. RfD Derivation

The data on the within-human variability in the toxicokinetics and toxicodynamics of ingested phenol are insufficient to adjust the default uncertainty factor for intraspecies variability (UF_A). In a sample of liver fractions from 10 people, Seaton et al. (1995) found that the kinetics of phenol sulfation and hydroquinone conjugation varied by up to approximately threefold. Much larger variability in CYP2E1 has been found, particularly between neonates and adults (Vieira et al., 1996). These data on inter-individual variability in enzymatic metabolism are not adequate to move

from the default UF_H of 10, because they do not reflect potential variability in portal-of-entry metabolism of phenol or uncertainty regarding the identity of the toxic moiety. Furthermore, variability in CYP2E1 does not necessarily translate directly into variability in tissue dose of because metabolism by CYP2E1 may be limited by blood flow to the liver.

The absorption, distribution, and metabolism of ingested phenol in rats and humans appear to be generally qualitatively similar, although the data are insufficient for a quantitative comparison. Comparison of laboratory animal and human phenol toxicokinetics is also limited by incomplete information regarding the identity of the toxic moiety. As discussed in Section 4.5, the data suggest that most of the toxic effects of phenol can be attributed to its oxidative metabolites, but the data are insufficient to rule out the possibility that some effects may be attributable to phenol itself. In the absence of adequate data on which to based a toxicokinetic or toxicodynamic comparison of rodents and humans, the default UF_A of 10 is used for interspecies extrapolation. However, it may be possible to reduce this default value of 10 after review and evaluation of data (perhaps supplemented by a PBPK model) that compare the toxicokinetics of phenol and its metabolites in the placenta and fetus of rats and humans, if such data become available.

The BMDL was based on an effect of minimal severity (decreased maternal weight *gain*), and a higher BMDL and NOAEL were obtained for effects on maternal weight. The BMDL is also within 50% of the NOAEL identified for the decreased maternal weight endpoint. Therefore, no uncertainty factor (UF) is required for extrapolation from a NOAEL to a LOAEL. No UF for extrapolation across duration is needed because this developmental study is supported by chronic bioassays in two species in which toxicity was observed only at higher doses.

The database for phenol by the oral route can be considered complete. It includes 2-year drinking water studies conducted in rats and mice (NCI, 1980), a two-generation drinking water study conducted in rats (Ryan et al., 2001; available in unpublished form as IIT Research Institute, 1999), and gavage developmental toxicity studies in rats (Argus Research Laboratories, 1997; NTP, 1983a; Narotsky and Kavlock, 1995) and mice (NTP, 1983b). However, the range of endpoints evaluated in the chronic toxicity studies was limited and did not include hematological or serum biochemistry evaluations. Immunological and hematological effects in mice were observed by Hsieh et al. (1992) in a 28-day drinking water at low doses. These endpoints were evaluated, and no significant hematological or serum biochemistry effects were observed at doses of up to >300 mg/kg-day in the two-generation rat study (IIT Research Institute, 1999; Ryan et al., 2001). The difference in these results suggest species differences between mice and rats, but confirmation of the

immunological and hematological effects in an assay done according to modern test methods would be useful.

An i.p. study of the effects of phenol on bone marrow cellularity in mice at doses of up to 300 mg/kg-day (Eastmond et al., 1987) and an in vitro study with mouse bone marrow cells (Corti and Snyder, 1998) also do not indicate that mouse blood cells are highly susceptible to effects of phenol. However, these studies did not evaluate the same parameter measured by Hsieh et al. (1992), and significant interspecies differences in immunotoxicity are not unusual. It is of interest that the endpoints affected in the Hsieh et al. (1992) study (two measures of effects on antibody-forming cells, PFC and ELISA) are the immune endpoints most highly predictive of effects on host resistance (Luster et al., 1992; Luster et al., 1993). Therefore, to account for the uncertainties regarding the immunological and hematological effects in mice, a database uncertainty factor of 3 is used.

An additional degree of public health protection may also be provided by the use of a gavage study rather than the more environmentally relevant route of drinking water. This is because gavage administration results in a higher peak blood level–presumably even using a divided-dosing protocol–than does ingestion of the same daily dose in drinking water. Because at least some toxic effects of phenol are related to peak blood levels rather than to total intake, toxicity would be expected to be higher following gavage exposure than drinking water exposure.

A composite UF of 300 results. No modifying factor is applied because the existing uncertainties have been addressed with the standard uncertainty factors.

RfD = 93 mg/kg-day /300 = 0.3 mg/kg-day, or 3E-1 mg/kg-day.

Note that this RfD is applied to ingested phenol *in addition to* the normal daily endogenous production of phenol, as discussed in further detail in Section 6.1.2.

An additional uncertainty factor for sensitive populations such as infants and children is not needed for phenol because sufficient studies of reproductive and developmental toxicity have been performed.

5.2. Inhalation Reference Concentration (RfC)

5.2.1. Choice of Principal Study and Critical Effect

The minimal database needed for the development of an RfC is a well-conducted subchronic inhalation study that has adequately evaluated a comprehensive array of endpoints, including the respiratory tract, and established a NOAEL and a LOAEL (U.S. EPA, 1994b). This criterion was not met for phenol. Neither of the two available subchronic studies (Deichmann et al., 1944; Sandage, 1961) are adequate for exposure-response assessment, because neither included adequate documentation of the histopathology results, and neither used modern methods for generating or monitoring exposure levels. These studies can, however, be used for hazard identification, and they identify the respiratory tract, liver, and kidney as targets of inhalation exposure to phenol.

The phenol database also includes a well-conducted 2-week inhalation study with rats that used modern exposure methods, evaluated a wide array of endpoints, and included a thorough histopathology evaluation of the respiratory tract (Hoffman et al., 2001; the full unpublished study report is available as Huntingdon, 1998). The only treatment-related effect observed was a red nasal discharge in male rats, which was observed with a statistically significant duration-related and concentration-related incidence in the mid- and high-concentration groups. However, because the red nasal discharge was likely due to a nonspecific response to stress, this response is not considered adverse.

In the absence of an inhalation study of sufficient duration, no RfC for phenol can be derived. A route-to-route extrapolation is not appropriate, because phenol can be a direct-contact irritant, and so portal of entry effects are a potential concern.

5.2.2. RfC Derivation

No RfC could be derived, due to insufficiencies of the database.

5.3. CANCER ASSESSMENT

As discussed in Section 4.6, the data regarding the carcinogenicity of phenol are *inadequate for assessment of human carcinogenic potential.* Phenol was negative in oral carcinogenicity studies in rats and mice, but questions remain regarding increased leukemia in male rats in the bioassay as well as the positive gene mutation data and the positive results in dermal initiation/promotion studies at doses at or above the MTD. No inhalation studies of an appropriate duration exist. Therefore, no quantitative assessment of carcinogenic potential via any route is possible.

6. MAJOR CONCLUSIONS IN CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

6.1.1. Oral Noncancer

In most studies of phenol administered in drinking water, water consumption was markedly decreased at the highest dose, presumably due to poor palatability. A number of toxic effects secondary to the decreased water consumption have been observed, including decreased body weight compared to controls, decreased pup weight, and decreased pup survival pre-culling. Other effects that may not have been secondary to decreased water consumption were kidney inflammation (NCI, 1980) and decreased motor activity (ClinTrials BioResearch, 1998). Gavage studies found more severe effects and reported these effects at lower doses. Observed effects included lung, liver, and kidney pathology; tremors and other nervous system effects; and, at sufficiently high doses, death.

These data suggest that the toxicity of phenol is higher via gavage dosing than via administration in drinking water. The suggestion is supported by the finding that a series of behaviors termed "phenol twitching behavior" correlate with peak blood levels rather than area under the curve (Dow Chemical Co., 1994). For a given daily dose, peak blood levels would be much higher following gavage dosing than following continuous administration in water. A direct comparison of the toxicity of phenol when administered via these two routes could determine definitively whether route-specific differences exist. Nonetheless, the data supporting the higher toxicity of phenol administered by gavage were considered sufficiently strong to consider it inappropriate to use the Berman et al. (1995) study (which also used small dosing volumes) as the principal study.

Developmental toxicity studies have been conducted only via the gavage route. In the principal study (Argus Research Laboratories, 1997), decreased maternal weight gain was observed in rats administered 120 mg/kg-day in a divided-dosing protocol. The BMDL for this study was 93 mg/kg-day and the NOAEL was 60 mg/kg-day. Although exposure in this study was for only 9 days, comparison with the entire database for phenol via the oral route indicates that this study appropriately identifies the critical effect. Because a maternal effect (decreased maternal body weight gain during gestation) is considered the critical effect for phenol (i.e., the first adverse effect or its known precursor that occurs to the most sensitive species as the dose rate of an agent increases),

protection from this effect would also be protective from systemic effects of chronic exposure, which occur at higher doses. In light of the different physiological status during pregnancy, it is not unreasonable for maternal toxicity following 9 days of dosing to occur at a lower dose than does chronic systemic toxicity.

Although a NOAEL of 60 mg/kg-day was identified for decreased fetal weight in the NTP (1983a) study, this study was not considered adequate to be a co-principal study. The high dose of 120 mg/kg-day was considered an *equivocal* LOAEL for developmental effects, in light of the small magnitude of the weight decrease and the absence of an effect on fetal weight at a maternally toxic dose in another gavage developmental study in rats (Argus Research Laboratories, 1997). In addition, although the observed decrease in fetal weight was small (but statistically significant), increased litter size was also seen at this dose. It is possible, therefore, that the dams were near the limit of what they could carry in terms of pup burden (total fetal weight).

The NOAEL was supported quantitatively by the NOAEL of 107 mg/kg-day for decreased motor activity in a 90-day drinking water neurotoxicity study (ClinTrials BioResarch, 1998). A NOAEL of 71 mg/kg-day for decreased parental and pup body weights was also identified in a drinking water two-generation reproduction study (Ryan et al., 2001; available in unpublished form as IIT Research Institute, 1999), although these effects are likely secondary to decreased water consumption. There is, however, some uncertainty in the identification of the NOAEL for this latter study. As described in Section 4.3, a statistically significant decrease in uterine weight was observed at all three doses in this study. The decrease was not considered adverse for a number of reasons, including the absence of a dose-response and the small number of animals outside the control range. This consideration, however, is rather subjective and is based on considerable professional judgement.

A key uncertainty in the development of the RfD is the interpretation of the study by Hsieh et al. (1992). Immunotoxicity (decreased response of antibody-forming cells) and hematotoxicity (decreased red blood cells and hematocrit) were observed in this 28-day drinking water study in mice at doses much lower than the doses than produced toxicity in other studies. No immunological or hematotoxic effects were seen at much higher doses in a two-generation drinking water study in rats (Ryan et al., 2001; IIT Research Institute, 1999). These differing results suggest species differences between mice and rats, but confirmation of the immunological and hematological effects in an assay done in mice according to modern test methods would be needed before using the data for dose-response assessment, considering the small number of animals tested and the limited number of risk

assessments that have been based on in vivo/in vitro immunotoxicity assays.

Similarly, in the absence of hematotoxicity in monkeys, rats, and mice following inhalation exposure to phenol levels resulting in comparable or higher systemic doses of phenol (Sandage, 1961; Dalin and Kristoffersson, 1974), confirmation of the reported hematological effects would also be useful. A database uncertainty factor of 3 was used to account for the uncertainty regarding immunotoxicity. This factor could be removed if an immunotoxicity study conducted according to U.S. EPA (1998c) guidelines became available.

Although it does not directly affect the determination of the RfD, uncertainty also exists regarding whether the decreased motor activity in females reported by ClinTrials BioResearch, (1998) was due to dehydration only or whether phenol exposure also contributed to the effect. The NOAEL from this study was used as supporting data for the principal study. The study authors attributed the decreased motor activity to dehydration, because of the marked decrease in water intake and the absence of supporting changes in the FOB. By contrast, this assessment concluded that phenol at least contributed to the effect, because there was no clear correlation between individual animals with dehydration and those with decreased activity and because the limited literature on the topic reports no effect on motor activity of water deprivation for several days. A neurotoxicity study in which the controls were allowed only limited access to drinking water would also address this issue.

6.1.2. Inhalation Noncancer

The database for inhalation toxicity of phenol is very limited. A well-conducted 2-week study is available (Hoffman et al., 2001; available in unpublished form as Huntingdon, 1998), but the duration is less than that appropriate for serving as the basis for the RfC. Longer-term studies have been conducted (Deichmann et al., 1944; Sandage, 1961), but they are limited by inadequate control of exposure levels, unclear sensitivity of the evaluation, and limited reporting.

However, the inhalation toxicity studies are sufficient however, to identify the respiratory tract, liver, kidney, and nervous system as targets of inhaled phenol toxicity. A significant uncertainty exists regarding which species is the most appropriate for extrapolation to humans. Deichmann et al. (1944) reported marked systemic toxicity in rabbits and deaths in guinea pigs at exposure concentrations that caused no histopathology in rats. No inhalation studies in guinea pigs or rabbits have been conducted to confirm these findings. In addition, it is unclear which of these species is most like humans.

The primary data need for developing an RfC is a 90-day inhalation study that includes a thorough examination of the respiratory tract. Pharmacokinetic studies of inhaled phenol would also aid in the extrapolation from experimental animals to humans.

6.1.3. Cancer

Several epidemiology studies have evaluated the carcinogenesis of phenol, but they have not found a consistent dose-related association. Because all of the subjects were also exposed to other chemicals and there was no correction for smoking, these studies are not adequate to reach conclusions on the carcinogenic potential of phenol.

Phenol was negative in drinking water bioassays with rats and mice (NCI, 1980), although an increased incidence of leukemias was observed in low-dose male rats. No inhalation studies of sufficient duration to assess carcinogenicity were found. In short-term dermal assays, tumorigenicity (production of papillomas in the absence of treatment by an initiating agent) was observed only at a dose/concentration combination that produced ulceration and thus was well above the MTD (Salaman and Glendenning, 1957). Similarly, although phenol was a promoter when tested in initiation/promotion studies, the doses tested typically caused ulceration (Salaman and Glendenning, 1957; Boutwell and Bosch, 1959) and death (Boutwell and Bosch, 1959). There were two exceptions. First, the low concentration tested by Salaman and Glendenning) caused promotion as well as "transient light crusting." Because the site of the weekly treatment was rotated across four sites on the body, it is unclear whether more severe effects would have been observed if the same site had been treated for the entire study. The second exception was that the low concentration tested by Wynder and Hoffmann (1961) was reported as causing no toxicity, although the sensitivity of the evaluation is unclear. On the basis of the high observed toxicity, it is not clear whether the promoting activity observed for phenol in several studies was secondary to the repeated injury and healing of the skin. From these considerations, the data regarding the carcinogenic potential of phenol are *inadequate for an assessment of human carcinogenic potential.*

6.2. DOSE-RESPONSE

No human data that are adequate for the derivation of a phenol RfD were located. Therefore, laboratory animal data were used.

The RfD of 0.3 mg/kg-day was based on a BMDL of 93 mg/kg-day for decreased maternal body weight gain in a gavage rat developmental toxicity study that used a divided-dosing protocol

(Argus Research Laboratories, 1997). There was a corresponding NOAEL of 60 mg/kg-day and a LOAEL for maternal toxicity of 120 mg/kg-day. A composite UF of 300 was used. This factor is based on a default factor of 10 for extrapolation from laboratory animals to humans, a default factor of 10 to account for intrahuman variability, and a factor of 3 to account for database insufficiencies.

Although the database for phenol can be considered "complete," there are uncertainties regarding the immunotoxicity potential of phenol in light of the immunotoxicity (decreased antibody forming cells) reported by Hsieh et al. (1992). The database factor may be reconsidered with results from an immunotoxicity study in mice that is compliant with EPA immunotoxicity test guidelines (U.S. EPA, 1998c). This RfD is at least twice the endogenous rate of phenol formation in humans, estimated as 0.014–0.14 mg/kg-day (Bone et al., 1976; Lawrie and Renwick, 1987; Renwick et al., 1988), based on total phenol (free plus conjugated) levels in urine. This means that endogenous production is approximately 5–50% of the RfD.

Note also that the RfD is meant to apply to ingested phenol *in addition to* the endogenous formation of phenol. Endogenous phenol is produced by bacteria in the gut, so endogenous phenol and ingested phenol would have similar toxicokinetics. Both humans and laboratory animals efficiently conjugate and excrete phenol at low doses, resulting in only a small degree of systemic exposure to free phenol (or any of its oxidative metabolites) at these low levels. The primary difference between endogenous and exogenous phenol would result, because endogenous phenol is formed in the intestines and some phenol may reach the colon and rectum, where some will escape the hepatic portal circulation and be absorbed directly without conjugation. By contrast, a smaller amount of ingested phenol would be expected to reach the colon and rectum.

The data are insufficient to determine the degree of conjugation of endogenously formed phenol in humans because the available data are based on analysis of daily urinary excretion of total phenol (i.e., phenol conjugates plus any trace amounts of free phenol) (Lawrie and Renwick, 1987; Renwick et al., 1988); oxidative metabolites of phenol were not measured. The phenol conjugation capacity of the liver is an important determinant of the ingested dose that would result in toxicity, but there is no information on the degree of phenol conjugation by humans at doses in the range of the RfD. Human variability exists in both the levels of endogenous phenol production and in the conjugative capacity of the liver.

In the absence of more detailed information, it is reasonable to assume that humans have adapted by having adequate conjugation capacity for the range of endogenous phenol production. Therefore, the default total uncertainty factor of 10 for human variability in toxicokinetics and

toxicodynamics is considered adequate. Determining whether oxidative metabolites are formed in individuals who have high endogenous levels of phenol formation would enhance the confidence in determining the intraspecies uncertainty factor.

The principal study (Argus Research Laboratories, 1997) used an adequate number of animals and evaluated an appropriate array of endpoints for a developmental toxicity study. Although gavage dosing was used, the divided-dosing protocol provided a significant enhancement that made the gavage dosing more closely resemble an environmentally relevant route of exposure. The principal study is judged to have medium confidence. Although the use of gavage dosing lowers the confidence in the study, the dosing frequency may be fairly similar to that in drinking water studies, in which rodents typically consumed water in a few larger doses and often in association with food consumption.

Confidence in the supporting database is medium to high. Although the oral toxicity database meets the minimal criteria for a high-confidence database (chronic studies in two species, developmental toxicity studies in two species, and a multigeneration reproduction study), the chronic studies did not evaluate a sufficient array of endpoints. In particular, the chronic mouse study (NCI, 1980) did not evaluate hematological and immunological effects, making interpretation of the results of the Hsieh et al. (1992) study difficult. Consideration of the above issues results in medium to high confidence in the RfD.

The RfD developed in this document can be compared with other limits on phenol exposure, partially as a test of the reasonableness of the RfD. Phenol is used in a number of industrial products, as well as in over-the-counter medicines such as cough drops, throat sprays, and mouthwashes (e.g., Cepastat® and Chloraseptic® brands). Use of these consumer products can result in short-term, high-level phenol exposures, but prolonged exposure (more than a week) at these levels is not recommended. The short duration of exposures to the cough medicines suggests that safe exposure levels would be higher than those for lifetime exposure. On the other hand, at least some aspects of phenol toxicity appear to be related to peak concentrations in blood, and higher peak blood concentrations could result from the consumer product exposure.

ATSDR (1998) estimated that intake of the maximum recommended dosage of 300 mg phenol/day would result in an approximate dose of 4–8 mg/kg-day. No documentation of this maximum recommended dosage could be located. Use of these products on a daily basis over the course of a lifetime would result in a dose approximately 10-fold higher than the RfD derived in this document; however, these products are not intended for use over a prolonged period. Evaluation of

potential health effects in individuals who do consume these products in large amounts or over long periods of time could provide additional information about human health effects of phenol and safe exposure levels.

The use of a higher dose of phenol than the RfD in over-the-counter medicines suggests that this RfD provides an adequate degree of public health protection. However, the maximum recommended dosage may not be protective of pregnant women and fetuses, as consumers are advised "as with any drug, if you are pregnant or nursing a baby, seek the advice of a medical professional before using this drug."

Although a substantial amount of data on phenol toxicokinetics are available, they are not sufficient to move away from the default UFs for interspecies extrapolation and intraspecies variability (IPCS, 2001). Data on how blood levels of phenol and its metabolites relate to doses in rats and humans would be useful in addressing the interspecies UF, as would data on the potential for phenol to cross the placenta. Similarly, data on how differences in enzyme activities relate to phenol and metabolite blood levels would be useful in addressing intrahuman variability. Finally, a drinking water study compliant with EPA test guidelines (U.S. EPA, 1998c) that evaluated hematological effects and immunological effects in mice could address the uncertainties associated with the Hsieh et al. (1992) study and lead to reconsideration for the need for a database UF.

The available data are inadequate to derive an RfC. As noted above, a 90-day inhalation study that evaluated the respiratory tract would be necessary for development of an RfC.

Because the data were considered inadequate to assess the carcinogenicity of phenol, no quantitative assessment was conducted.

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APPENDIX A. SUMMARY OF EXTERNAL PEER REVIEW COMMENTS AND DISPOSITION

The support document and IRIS summary for phenol have undergone both internal peer review by scientists within EPA and a more formal external peer review by scientists outside EPA in accordance with EPA guidance on peer review (U.S. EPA, 1998b, 2000a). Comments made by the internal reviewers were addressed prior to submitting the documents for external peer review and are not part of this appendix. The three external peer reviewers were tasked with providing written answers to general questions on the overall assessment and on chemical-specific questions in areas of scientific controversy or uncertainty. A summary of significant comments made by the external reviewers and EPA's response to these comments follows.

(1) General Comments

A. Data Presentation

All three reviewers agreed that the document is well organized. Two reviewers recommended specific changes to increase the clarity of certain sections.

Response to comments: The specific changes requested by the reviewers to clarify the text were made.

B. Are there additional data/studies that should be included?

One reviewer was not aware of any other data/studies that should be included. Another reviewer recommended a developmental toxicity study but noted that it uses an unconventional study design. A third reviewer criticized EPA for superficially exploring human health literature and relying heavily on animal experiments. This third reviewer also suggested that EPA expand its discussion of studies looking at phenol usage in over-the-counter drugs.

Response to comments: We reviewed the developmental toxicity study (Minor and Becker, 1971) recommended by one reviewer and concluded that it should not be included in the document because it would not contribute any significant information to the database. The specific reasons for the exclusion are: (1) this study used intraperitoneal (i.p.) dosing, a route of exposure of limited relevance for human environmental exposure; because several well-conducted oral developmental toxicity studies of phenol are available, it is not useful or necessary to supplement the database with an i.p. study; (2) phenol was administered on gestation days 9–11 or days 12–14; such studies of short windows of exposure can be useful for mechanistic purposes, but they are inadequate to fully assess developmental toxicity; (3) this study is presented only as an abstract published 30 years ago, and the full study was never published, raising questions about the reliability of the results, and in addition, the abstract provides insufficient information for evaluation of the study.

In response to the request for additional human data, additional reviews of literature databases and secondary review articles were conducted, but no additional data were identified. Trade associations were also contacted, with the same results. However, information relating the effects observed in animals to effects observed in the available human studies was added to Section 4.5. Due to limitations such as confounding exposures and uncertain exposure estimation, the available human

data are useful for hazard identification but not for dose-response assessment.

Manufacturers of over-the-counter drugs containing phenol, the relevant trade associations were contacted, and reference books were consulted, in order to obtain information on the basis for the recommended maximal dosage, but the information was not available.

C. For the RfD, has the most appropriate critical effect (decreased fetal body weight in the NTP (1983a) study and decreased maternal weight gain in the Argus Research Laboratories (1997) study) been chosen?

One reviewer agreed with the selection of decreased fetal body weight as the critical effect, with a NOAEL of 60 mg/kg-day. This reviewer noted that although this is a relatively nonspecific toxic effect, it could be appropriately considered adverse. In contrast, a second reviewer stated that the reduction in body weight was a weak basis for the RfD because it was nonspecific and because of other changes affecting body weight (e.g., increased litter size). On the basis of these considerations, the reviewer did not consider the observed reduction in body weights to be an adverse effect and suggested that the corresponding dose might be considered a NOAEL. Neither of these reviewers commented on the appropriateness of the co-critical effect of decreased maternal weight gain endpoint, also with a NOAEL of 60 mg/kg-day (and a BMDL of 93 mg/kg-day).

A third reviewer asserted that the critical effect occurs at a lower dose and that the RfD should be based on a NOAEL lower than 60 mg/kg-day. The reviewer stated that the data from the NTP (1983a) study are consistent with a LOAEL of 30 mg/kg-day, based on statistically significant increases in the number of litters with nonlive fetuses in the low- and mid-dose groups and a trend comparison of the fetal weight data. The reviewer also stated that data from the Argus Research Laboratories (1997) study support the conclusion that 60 mg/kg-day is a LOAEL, based on an increase in the percent litters with fetuses with alterations. The reviewer also recommended that the LOAEL in the IIT Research Institute (1999) study should be 20 mg/kg-day (with no NOAEL identified), based on decreased uterine weight and decreased prostate weight, rather than the NOAEL of 70.0 mg/kg-day identified in the Toxicological Review. The reviewer asked for additional discussion of the interpretation of these results but did not recommend a specific critical effect. This reviewer also recognized the uncertainties in the Hsieh et al. (1992) study but suggested that macrocytosis could explain the inconsistency in that study between erythrocyte count and hematocrit.

Response to comments: The relationship between litter size and fetal weight was noted, and the analysis of the NTP (1983a) study notes that 120 mg/kg-day is an *equivocal* LOAEL, with a NOAEL of 60 mg/kg-day. Due to the uncertainties in the identification of the NOAEL/LOAEL boundary for this study, the NTP (1983a) study was then judged not appropriate as a co-principal study. If the NOAEL in the NTP (1983a) study were changed to 120 mg/kg-day, there would not be a significant effect on the RfD because the Argus Research Laboratories (1997) study identified a NOAEL of 60 mg/kg-day and a LOAEL of 120 mg/kg-day, with a BMDL of 93 mg/kg-day. In addition, the NOAEL identified in the principal study (Argus Research Laboratories, 1997) is supported by a NOAEL of 107 mg/kg-day in the ClinTrials BioResearch (1998) study, a value that is very close to the BMDL used to derive the RfD.

Additional information about the litters with nonlive fetuses, decreased uterine weight, and other findings mentioned by the third reviewer were added to the document (Section 4.3). The former endpoint was not considered treatment-related due to the absence of a dose-response. The data on percent litters with fetuses with alterations were independently analyzed using the chi-square test, and no significant effect was observed. Text was also added to Section 6.1.1 regarding uncertainties in the interpretation of the uterine weight data.

D. Has the noncancer assessment been based on the most appropriate studies?

Two of the reviewers agreed that the noncancer assessment is based on the most appropriate studies, although, as indicated in the previous question, one of the reviewers raised some concerns about the interpretation of those studies. Two of the reviewers also agreed with EPA's assessment of the deficiencies of the study by Hsieh et al. (1992). One reviewer agreed with the selection of significant endpoints in the phenol document; however, this reviewer disagreed with the use of gavage studies for the derivation of the RfD. This reviewer asserted that EPA should have used the two-generation drinking water study by IIT Research Institute (1999) to derive the RfD.

Response to comments: As described in the Toxicological Review, the effects at the LOAEL (the high dose) of the IIT Research Institute (1999) study appear to be secondary to decreased water consumption due to poor palatability, and so do not appropriately reflect phenol toxicity. Text was added to the Toxicological Review noting that rats consume water only intermittently during the day, and so a divided-dose gavage protocol is fairly similar to drinking water consumption.

E. For the noncancer (RfD) assessment, are there other data that should be considered in developing uncertainty factors or the modifying factor? Do the data support the use of different values from those proposed?

All three reviewers agreed with the uncertainty factors presented in the phenol document and EPA's rationale for selecting these factors.

Response to comments: None.

F. Do the confidence and weight-of-evidence statements present a clear rationale and accurately reflect the utility of the studies chosen, the relevancy of the effects (cancer and noncancer) to humans, and the comprehensiveness of the database? Do these statements make sufficiently apparent all the underlying assumptions and limitations of these assessments?

The reviewers agreed that the confidence statements are carefully reasoned and clearly stated. Two of the reviewers agreed that the weight-of-evidence statements are appropriate, and a third reviewer noted that no weight-of-evidence statement is used for noncarcinogenicity.

Response to comments: None needed.

(2) Chemical-Specific Comments

A. When endogenously produced phenol is taken into account, can the RfD be supported? Note that the RfD is applied to ingested phenol in addition to the normal daily endogenously

produced phenol. Are there differences in endogenous phenol production between rats and humans that should be taken into account in the development of the RfD?

The reviewers agreed that the RfD applied to ingested phenol in addition to the normal daily endogenously produced phenol is appropriate. One reviewer noted that endogenous production is a relatively small fraction (~2–20%) of the RfD. (This fraction became 5–50% after addition of the database uncertainty factor during consensus review.) Another reviewer noted that both the experimental animals and humans would have similar baseline levels of endogenous phenol production.

Response to comments: Additional supporting information provided by the reviewers was incorporated into the text. Toxicokinetic considerations regarding quantitatively accounting for endogenous production were also incorporated into the text.

B. Do you agree/disagree with the recommendation that there are not sufficient data to generate a scientifically defensible RfC and cancer slope factor?

Two reviewers agreed that data are insufficient to generate an RfC and cancer slope factor. One of these reviewers also commented on statements made by EPA related to red nasal discharge identified in study animals. This reviewer stated that the secretion/discharge is not directly suggestive of, or a precursor to, a nasal or ocular lesion but is simply the animal's response to stress. A third reviewer did not comment on this question because it was beyond this person's area of expertise.

Response to comments: The information on red nasal discharge provided by the reviewer was incorporated into the text.

C. Was the interpretation of the decreased fetal body weight in rats in the National Toxicology Program (NTP) study (NTP, 1983a) appropriate?

One reviewer raised some issues regarding the adversity of the effect but generally agreed with EPA's decision to designate the decreased fetal body weight finding at 120 mg/kg-day as an equivocal LOAEL, resulting in a NOAEL of 60 mg/kg-day. A second reviewer disagreed that decreased fetal body weight is an adverse effect and asserted that 120 mg/kg-day should be classified as a NOAEL instead. A third reviewer supported interpreting the data using trend analysis rather than pairwise comparisons, resulting in a LOAEL of 30 mg/kg-day for fetal body weight reduction.

Response to comments: On the basis of the weight of evidence, the weight of the reviewers' comments, and the supporting data from the Argus Research Laboratories (1997) study, the high dose in the NTP (1983a) study was retained as an equivocal LOAEL, but the study was removed from being a co-principal study for the derivation of the RfD. Although there was a dose-response trend at the low and mid doses, the decreases in the fetal body weight at these doses were marginal (1%–2%) and were not considered biologically significant. Only the response (7%) at the high dose was significantly different from the control. A meaningful benchmark dose could not be calculated for these data, because fetal body weight by sex were not in the published study and not available in

NTP's archives.

D. Please comment on the choice of gavage developmental toxicity studies as the co-critical studies in light of the differences between phenol toxicity when administered in drinking water and by gavage.

One reviewer agreed with EPA's willingness to use gavage studies but suggested that the IIT Research Institute (1999) drinking water study represents a more relevant exposure scenario and supports a lower LOAEL, based on decreased absolute and relative uterine weight. A second reviewer asserted that the drinking water study is more appropriate because a divided gavage dose is not equivalent to more extended intake during *ad libitum* water consumption. A third reviewer supported the use of the gavage study and asserted that the divided dose administered in the Argus Research Laboratories study (1997) is not unrealistic because animals in drinking water studies tend to drink when they eat, not continuously throughout the day. This reviewer believed that the NOAEL and the LOAEL are not overly conservative.

Response to comments: Additional information about the interpretation of the decreased uterine weight in the IIT Research Institute (1999) drinking water study and associated uncertainties was added Section 4.3. In particular, even though the decrease in relative uterine weight was statistically significant at all dose levels, there was no dose-response. Information was also added to the text noting that rats drink water in a small number of periods during the day rather than continuously through the day, so a divided-dose gavage study is fairly similar to drinking water exposure.

E. Was the interpretation of decreased motor activity in the 13-week oral neurotoxicity study appropriate?

One reviewer agreed with EPA's interpretation of decreased motor activity in the 13-week oral neurotoxicity study. A second reviewer found the interpretation difficult to comment on, given the confounded results of the study, and a third reviewer did not respond to this question because it was beyond this person's area of expertise.

Response to comments: The issues potentially confounding the neurotoxicity were noted in the document.

OVERALL RECOMMENDATION

All three reviewers stated that the document is acceptable with revisions.

New Reference:

Minor, JL; Becker, BA. (1971). A comparison of the teratogenic properties of sodium salicylate, sodium benzoate, and phenol. Toxicol Appl Pharmacol 19:373.

Appendix B. Benchmark Dose Modeling Results

Benchmark dose (BMD) modeling was performed to identify potential critical effect levels for derivation of the RfD for phenol. The modeling was conducted according to draft EPA guidelines (U.S. EPA, 2000d) using Benchmark Dose Software Version 1.3 (BMDS), which is available from EPA (U.S. EPA, 2001). The BMD modeling results are summarized in Table B-1, and the output is attached as Appendix C. A brief discussion of the modeling results for each endpoint is presented below.

Because all the following endpoints are continuous variables, the continuous models available with BMDS (power, polynomial, and Hill models) were used. The hybrid model was not used, because the hybrid model software in BMDS is still undergoing Beta-testing and was not considered sufficiently validated to provide a BMDL as the basis for the quantitative dose-response assessment. (The hybrid modeling approach defines the benchmark response [BMR] directly in terms of risk, whereas the standard approach, defines the BMR in terms of a change in the mean.) For all of the modeling conducted, the BMR was defined as a 1.0 SD change in the mean because this is the default measure recommended by the EPA (U.S. EPA, 2000d) in the absence of a clear biological rationale for selecting an alternative response level.

Argus Research Laboratories, 1997

Two endpoints were modeled from this study: decreased maternal body weight gain and the related endpoint of decreased maternal body weight. The decrease in weight gain was the more sensitive endpoint, with a NOAEL of 60 mg/kg/day and a LOAEL of 120 mg/kg/day.

As summarized in Table B-1, the BMD and BMDL estimates for the endpoint of decreased maternal body weight gain was similar for all three models. The model fit was also generally similar. A visual analysis of the data fit in the regions of the BMDLs indicated that the data fitting from the three model was adequate and comparable across all models. The goodness-of-fit *p*-values calculated for the power and polynomial models were very similar, but no *p*-value could be computed for the Hill model because there were no degrees of freedom for the calculation. (This was because the number of parameters included in the model was equal to the number of data points.) The Akaike Information Criterion (AIC), a measure of goodness of fit that takes into account the number of degrees of freedom, was very similar for all three models but was marginally better (i.e., lower) for the polynomial model. The polynomial model was chosen as the basis for the BMDL for this endpoint, based on the slightly better fit and as a slightly more health-protective value. An alternative BMDL for this endpoint could be calculated using the geometric mean of the BMDLs from all three models, 114 mg/kg-day, based on the rationale that the small difference in AIC observed was not meaningful.

For the endpoint of decreased maternal body weight, all three continuous models gave a similar BMD estimate and provided adequate data fits, with goodness-of-fit *p*-values larger than 0.1. However, the best data fit was obtained with the polynomial model, which had a *p*-value of 0.92, compared with a *p*-value of 0.30 obtained with the power model. The *p*-value for the Hill model was not computed due to insufficient degrees of freedom. A visual analysis of the data fit in the region of

the BMDLs also indicated that good fit was obtained with all three models, with the best fit obtained using the polynomial model. In addition, the AIC analysis also indicated that the best data fit was obtained with the polynomial model. Comparable BMDLs of 143 and 147 mg/kg/day were obtained using the polynomial and Hill models, respectively. Thus, the BMDL chosen for this endpoint was 143 mg/kg-day, obtained with the polynomial model, with a corresponding BMD of 345 mg/kg. This BMDL is higher than the BMDL for maternal body weight gain, the other endpoint modeled for this study.

ClinTrials BioResearch (1998)

Only one endpoint from this study was modeled: decreased total activity counts in a motor activity assay in females at week 4. Acceptable fits (p=0.35 and p=0.17) were obtained with the polynomial and power models, respectively. The visual fit for both models in the region of the BMDL was acceptable but not as good as would be desired. Much better fit was obtained with the Hill model, based on visual fit, but no BMDL could be calculated for this model. The same BMDL of 219 mg/kg-day was calculated with both the power and polynomical models and was chosen as the study BMDL.

Hsieh et al. (1992)

Three related endpoints were modeled for this study: plaque-forming cells, plaque-forming cells/10^6 spleen cells, and antibody titer. The study NOAEL was 6.2 mg/kg-day, with a LOAEL of 33.6 mg/kg-day.

As summarized in Table B-1, unacceptable fits were obtained with the power and polynomial models for the endpoint of plaque-forming cells/10^6 spleen cells. No p-value could be obtained for the Hill model due to insufficient degrees of freedom, but a visual analysis of the results indicated that the Hill model provided an acceptable fit. Based on the Hill model, the BMD and BMDL for decreased plaque-forming cells/10^6 spleen cells were 1.26 mg/kg-day and 0.38 mg/kg-day, respectively.

Similar results were obtained for plaque forming cells/spleen. Inadequate fits were obtained with the power and polynomial models, and the Hill model provided no p-value at all due to insufficient degrees of freedom. A visual analysis of results indicated that the Hill model provided an overall adequate fit, but not a very good fit in the range of the BMD. In addition, this model failed to estimate BMD or BMDL. Therefore, no BMD and BMDL can be identified for this particular endpoint.

For the endpoint of decreased antibody titer, the power and polynomial models both had marginal fit, based on the goodness-of-fit p-values; visual inspection of the data indicated that these models had inadequate fit. No p-value could be calculated for the Hill model, due to insufficient degrees of freedom, but a visual analysis of the model results indicated an adequate fit. Therefore, based on the Hill model, the BMD and BMDL for decreased antibody titer were 3.51 mg/kg-day and 0.73 mg/kg-day, respectively.

The lowest BMDL for this study was 0.38 mg/kg-day, calculated for plaque-forming cells/10^6

spleen cells. However, this BMDL is not used for risk assessment due to uncertainties in the appropriate BMR for this in vivo/in vitro study design.

Table B-1. Benchmark Dose Modeling Results for Phenol

Model	*p*-value	AIC	BMD[a]	BMDL[a]
Argus Research Laboratories (1997)				
Maternal Body Weight Gain				
Power	0.3165	545	152	125
Polynomial	0.3191	543	157	93
Hill	N/A	545	151	129
Maternal Body Weight				
Power	0.3013	731	354	244
Polynomial	0.9188	729	345	**143**
Hill	N/A[b]	733	345	147
ClinTrials BioResearch Ltd. (1998)				
Motor Activity - Total Activity Counts in Females in Week 4				
Power	0.1701	629	337	219
Polynomial	0.3477	625	336	**219**
Hill	N/A	630	246	---
Hsieh et al., 1992				
Plaque-Forming Cells				
Power	0.008	236	15.7	10.5
Polynomial	0.003	227	14.3	9.7
Hill	NA	229	1.26	0.38
Plaque-Forming Cells/Total Spleen				
Power	0.054	469	23	15.3
Polynomial	<0.0001	509	23.4	5.87
Hill	N/A	467	6.14	--- [c]

Antibody Titer				
Power	0.102	-66.2	21.4	13.1
Polynomial	0.102	-70.2	21.4	13.1
Hill	N/A	-66.9	3.51	0.73
a. BMD and BMDL are based on benchmark response of 1.0 SD. Results are presented in units of mg/kg/day.				
b. NA: the information is not available because there are insufficient degrees of freedom for the test				
c. ---: failed to estimated this value.				

Appendix C. Benchmark Dose Modeling Output

maternal body weight gain

```
==============================================================
    Power Model. $Revision: 2.1 $ $Date: 2000/10/11 20:57:36 $
    Input Data File: F:\BMDS\MA.(d)
    Gnuplot Plotting File:  F:\BMDS\MA.plt
                                          Fri May 10 11:56:51 2002
                    ==========================================
```

BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~

The form of the response function is:

Y[dose] = control + slope * dose^power

Dependent variable = MEAN
Independent variable = dose
rho is set to 0
The power is restricted to be greater than or equal to 1
A constant variance model is fit

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

alpha =	101.864	
rho =	0	Specified
control =	64	
slope =	-43.1882	
power =	-0.0984037	

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	rho	control	slope	power
alpha	1	-1	0.01	-0.049	-0.053
rho	-1	1	-0.011	0.049	0.054
control	0.01	-0.011	1	-0.77	-0.74
slope	-0.049	0.049	-0.77	1	1
power	-0.053	0.054	-0.74	1	1

Parameter Estimates

Variable	Estimate	Std. Err
alpha	98.6453	328.851
~~~~~~~~~~~~~~~~~~~~~~~~~

rho	0	0.834088
control	63.4328	2.12406
slope	-0.0653203	0.11847
power	1	0.30005

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	23	64	10.7	63.4	9.93	0.0571
60	25	58	9.4	59.5	9.93	-0.152
120	23	56.8	10.8	55.6	9.93	0.121
360	25	39.8	9.5	39.9	9.93	-0.0118

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2 Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model R: Yi = Mu + e(i
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-267.891852	5	545.783705
A2	-267.502264	8	551.004528
fitted	-268.393473	4	544.786945
R	-297.755244	2	599.510487

Test 1: Does response and/or variances differ among dose levels (A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	df	p-value
Test 1	60.506	6	<.00001
Test 2	0.779177	3	0.8544
Test 3	1.00324	1	0.3165

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels It seems appropriate to model the data

The p-value for Test 2 is greater than .05 A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .05. The model chosen appears to adequately describe the data

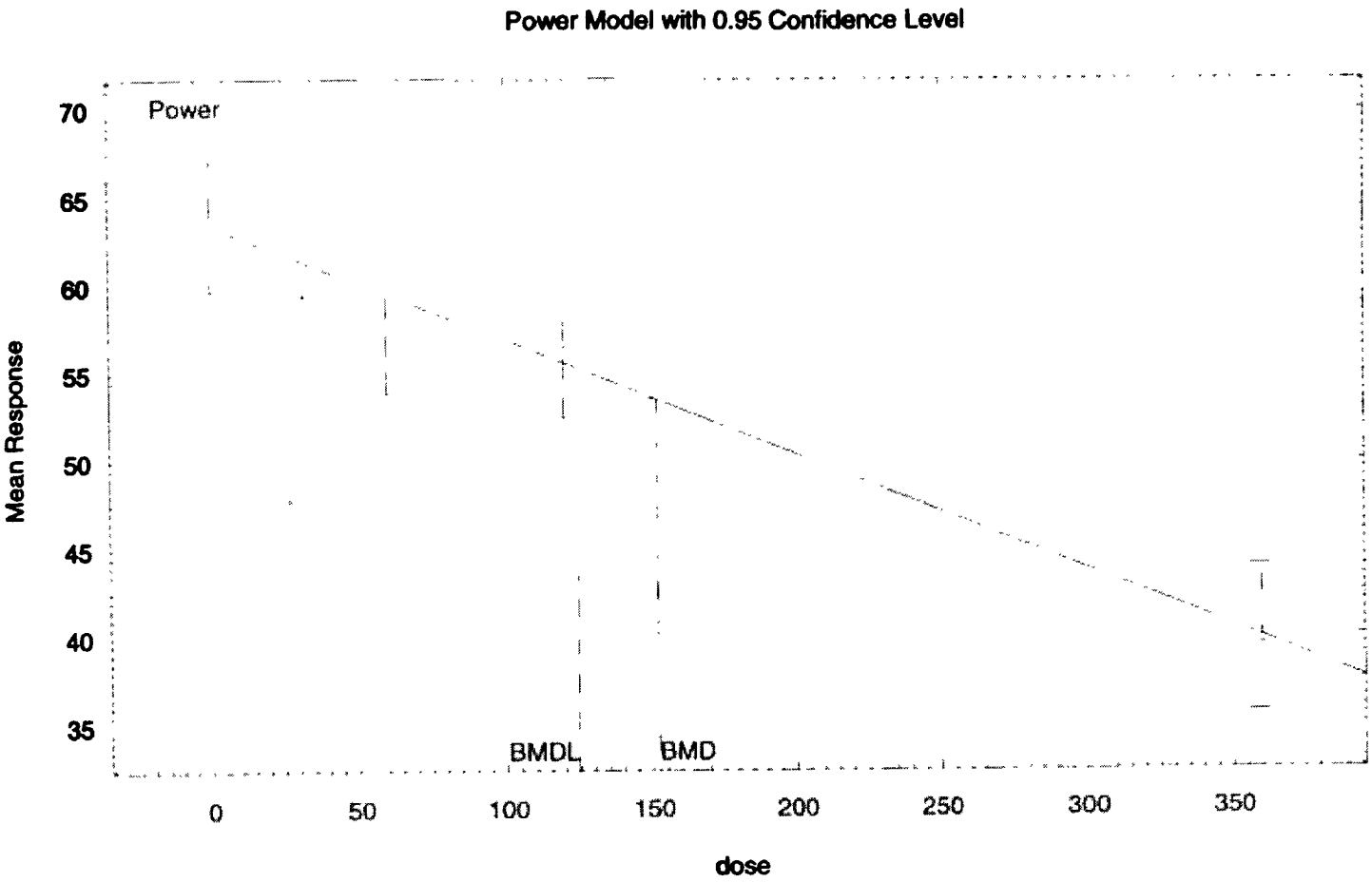
Benchmark Dose Computation
Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 152.051

BMDL = 124.591



11:56 05/10 2002

maternal body weight gain

```
==================================================================
         Polynomial Model. $Revision: 2.1 $ $Date: 2000/10/11 17:51:39 $
         Input Data File: F:\BMDS\MA.(d)
         Gnuplot Plotting File:  F:\BMDS\MA.plt
                                                    Fri May 10 11:55:32 2002
      ==============================================================
```

BMDS MODEL RUN
~~~~~~~~~~~~~~~

The form of the response function is:

Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2

Dependent variable = MEAN
Independent variable = dose
rho is set to 0
Signs of the polynomial coefficients are not restricted
A constant variance model is fit

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

alpha =	101.864	
rho =	0	Specified
beta_0 =	63.3326	
beta_1 =	-0.0615798	
beta_2 =	-1.00086e-005	

Parameter Estimates

Variable	Estimate	Std. Err
alpha	98.6343	14.2366
beta_0	63.2966	1.94612
beta_1	-0.0618515	0.0343059
beta_2	-8.99169e-006	8.68663e-005

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	beta_0	beta_1	beta_2
alpha	1	-1.2e-006	1.9e-006	-2e-006
beta_0	-1.2e-006	1	-0.77	0.68
beta_1	1.9e-006	-0.77	1	-0.98
beta_2	-2e-006	0.68	-0.98	1

Table of Data and Estimated Values of Interest
~~~~~~~~~~~~~~~

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	23	64	10.7	63.3	9.93	1.63
60	25	58	9.4	59.6	9.93	-3.91
120	23	56.8	10.8	55.7	9.93	2.44
360	25	39.8	9.5	39.9	9.93	-0.163

Model Descriptions for likelihoods calculated

Model A1 Yij = Mu(i) + e(ij
Var(e(ij)) = Sigma^2

Model A2 Yij = Mu(i) + e(ij)
Var(e(ij)) = Sigma(i)^2

Model R Yi = Mu + e(i)
Var(e(i)) = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-267.891852	5	545.783705
A2	-267.502264	8	551.004528
fitted	-268.388117	3	542.776234
R	-297.755244	2	599.510487

Test 1 Does response and/or variances differ among dose levels
(A2 vs. R)
Test 2 Are Variances Homogeneous (A1 vs A2)
Test 3 Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	60.506	6	<.0001
Test 2	0.779177	3	0.8544
Test 3	0.992529	1	0.3191

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels.
It seems appropriate to model the data

The p-value for Test 2 is greater than 05. A homogeneous variance
model appears to be appropriate here

The p-value for Test 3 is greater than 05 The model

chosen appears
to adequately describe the data

Benchmark Dose Computation
Specified effect = 1

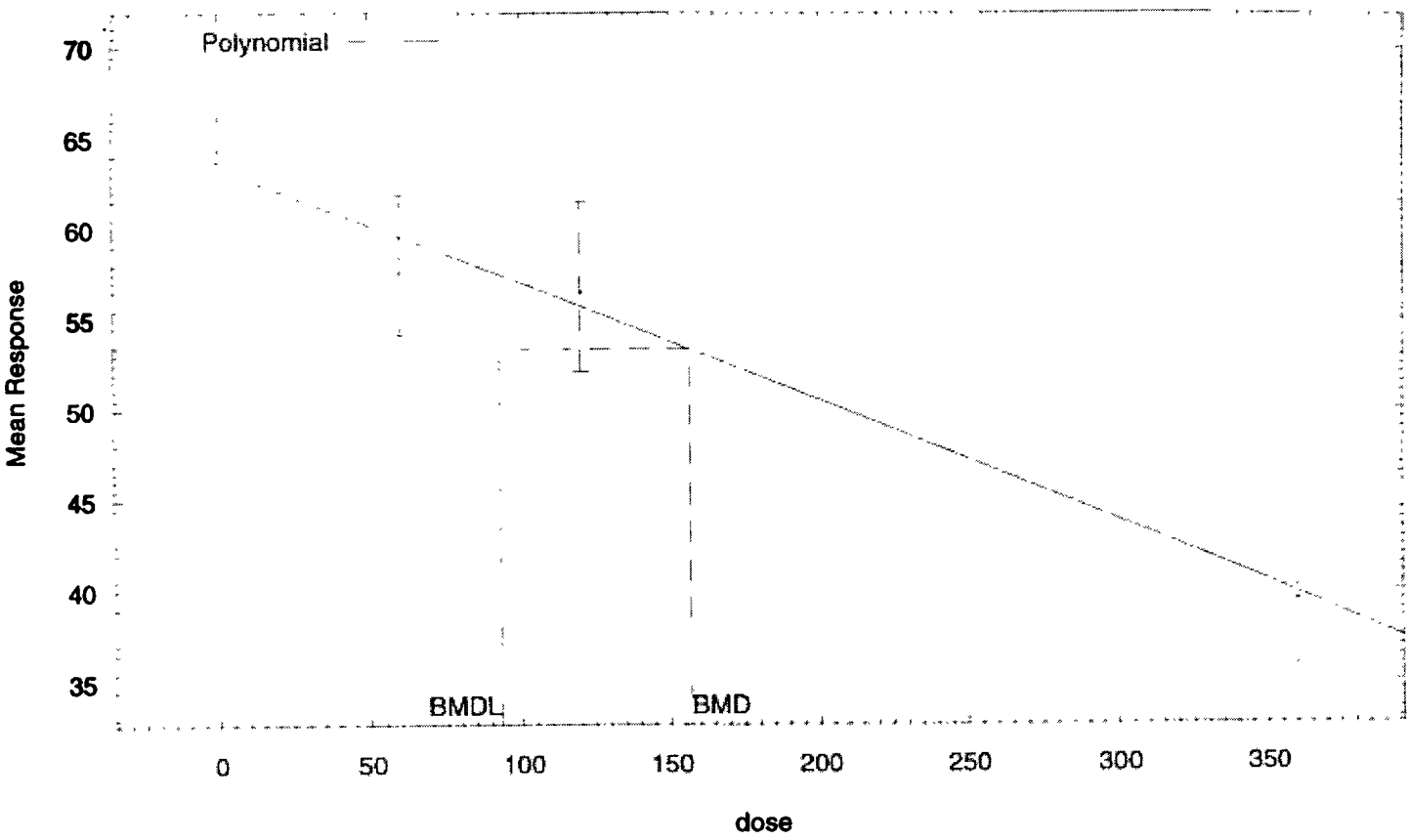
Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 156.987

BMDL = 92.9272

Polynomial Model with 0.95 Confidence Level



11:55 05/10 2002

maternal body weight gain

```
==========================================================
 Hill Model. $Revision: 2.1 $ $Date: 2000/10/11 21:21:23 $
 Input Data File: F:\BMDS\MA.(d)
 Gnuplot Plotting File:  F:\BMDS\MA.plt
                                          Fri May 10 11:57:36 2002
==================================================================

BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~

  The form of the response function is

  Y[dose] = intercept + v*dose^n/(k^n + dose^n)


  Dependent variable = MEAN
  Independent variable = dose
  rho is set to 0
  Power parameter restricted to be greater than
  A constant variance model is fit

  Total number of dose groups = 4
  Total number of records with missing values = 0
  Maximum number of iterations = 250
  Relative Function Convergence has been set to: 1e-008
  Parameter Convergence has been set to: 1e-008


                 Default Initial Parameter Values
                         alpha =      101.585
                           rho =            0   Specified
                     intercept =           64
                             v =        -24.2
                             n =      1.42687
                             k =      189.176


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -n
                have been estimated at a boundary point, or have been specified
by the user,
                and do not appear in the correlation matrix )
```

	alpha	rho	intercept	v	k
alpha	1	0	0	0	0
rho	0	1	0	0	0
intercept	0	0	1	0	0
v	0	0	0	1	0
k	0	0	0	0	1

Parameter Estimates

Variable	Estimate	Std Err
alpha	98.6445	1
rho	0	1
intercept	63.4595	1
v	-2388.7	1
n	1	NA
k	36185.3	

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	23	64	10.7	63.5	9.93	0.0544
60	25	58	9.4	59.5	9.93	-0.152
120	23	56.8	10.8	55.6	9.93	0.124
360	25	39.8	9.5	39.9	9.93	-0.013

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Degrees of freedom for Test A1 vs fitted <= 0

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-267.891852	5	545.783705
A2	-267.502264	8	551.004528
fitted	-268.395812	4	544.791623
R	-297.755244	2	599.510487

Test 1: Does response and/or variances differ among dose levels (A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	60.506	6	<.0001
Test 2	0.779177	3	0.8544
Test 3	1.00792	0	NA

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels It seems appropriate to model the data

The p-value for Test 2 is greater than .05. A homogeneous variance model appears to be appropriate here

NA - Degrees of freedom for Test 3 are less than or equal to 0 The Chi-Square test for fit is not valid

Benchmark Dose Computation
Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

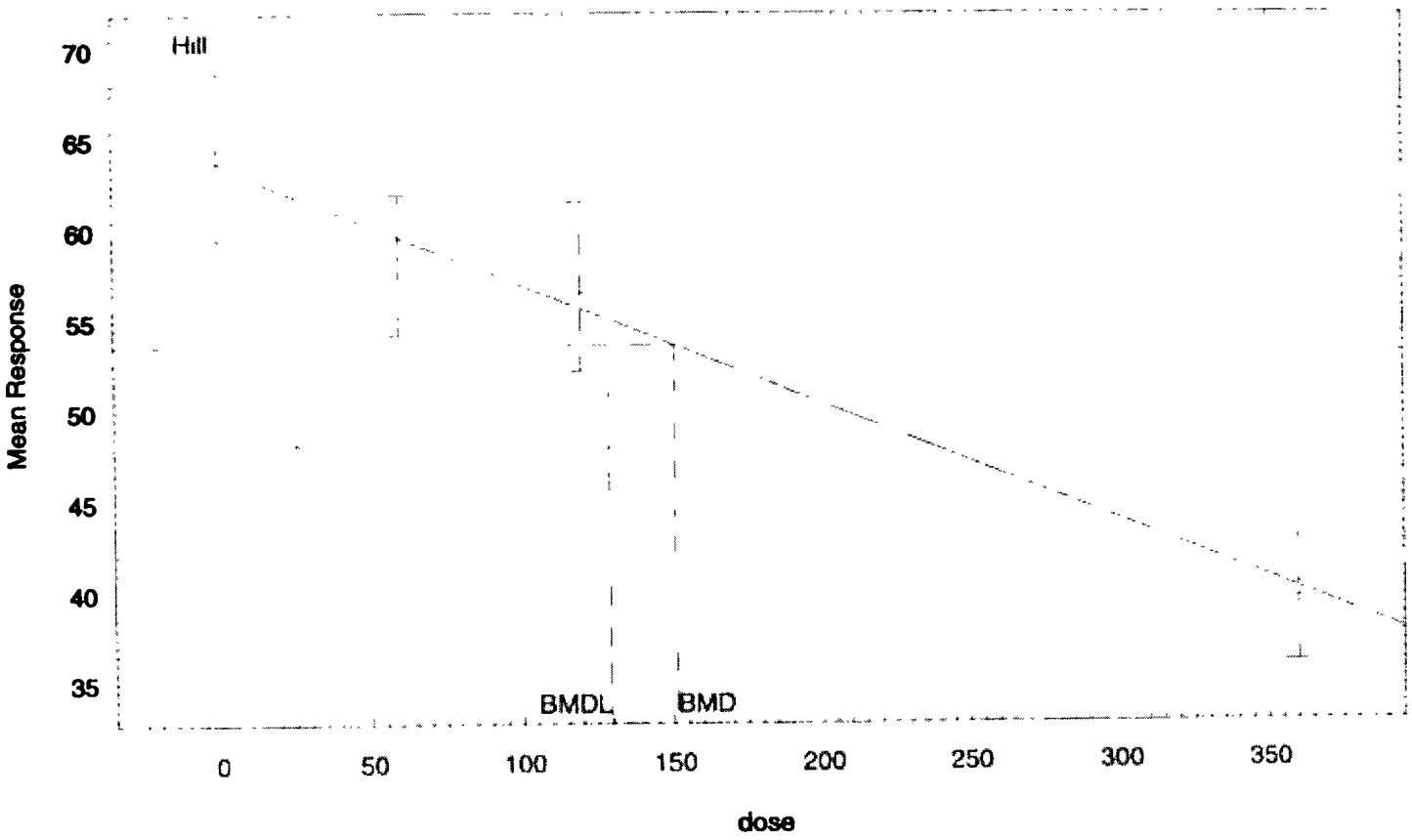
BMD = 151.083

BMDL = 129.177

Warning optimum may not have been found Bad completion code in Optimization r outine.

BMDL computation failed for one or more point on the BMDL curve
The BMDL curve will not be plotted

Hill Model with 0.95 Confidence Level



11:57 05/10 2002

BMR=1.0 SD

```
==========================================================================
          Power Model. $Revision: 2.1 $ $Date: 2000/10/11 20:57:36 $
          Input Data File: C:\DOCUMENTS AND SETTINGS\JZHAO\MY
DOCUMENTS\PHENOL\DAMWEIGHT.(d)
          Gnuplot Plotting File:  C:\DOCUMENTS AND SETTINGS\JZHAO\MY
DOCUMENTS\PHENOL\DAMWEIGHT.plt
                                                    Thu Jan 10 16:36:20 2002
==========================================================================

BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = control + slope * dose^power


   Dependent variable = MEAN
   Independent variable = dose
   rho is set to 0
   The power is restricted to be greater than or equal to 1
   A constant variance model is fit

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                 Default Initial Parameter Values
                       alpha =      779.391
                         rho =            0   Specified
                     control =        440.4
                       slope =     -357.319
                       power =    -0.432011
```

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	rho	control	slope	power
alpha	1	-1	-0.095	0.16	0.16
rho	-1	1	0.094	-0.16	-0.16
control	-0.095	0.094	1	-0.78	-0.76
slope	0.16	-0.16	-0.78	1	1
power	0.16	-0.16	-0.76	1	1

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	747.101	25868.4
rho	0	5.71247
control	439.654	6.06278

slope	-0.0770251	0.338099
power	1	0.724264

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	23	440	29	440	27.3	0.0273
60	25	435	28.2	435	27.3	0.00247
120	23	429	25.1	430	27.3	-0.0443
360	24	412	29.1	412	27.3	0.0137

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Warning: Likelihood for model A1 larger than the Likelihood for model A2.

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-361.236054	5	732.472108
A2	-361.411149	8	738.822299
fitted	-361.769541	4	731.539082
R	-368.360689	2	740.721377

Test 1: Does response and/or variances differ among dose levels (A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	df	p-value
Test 1	13.8991	6	0.003046
Test 2	-0.350191	3	<.00001
Test 3	1.06697	1	0.3016

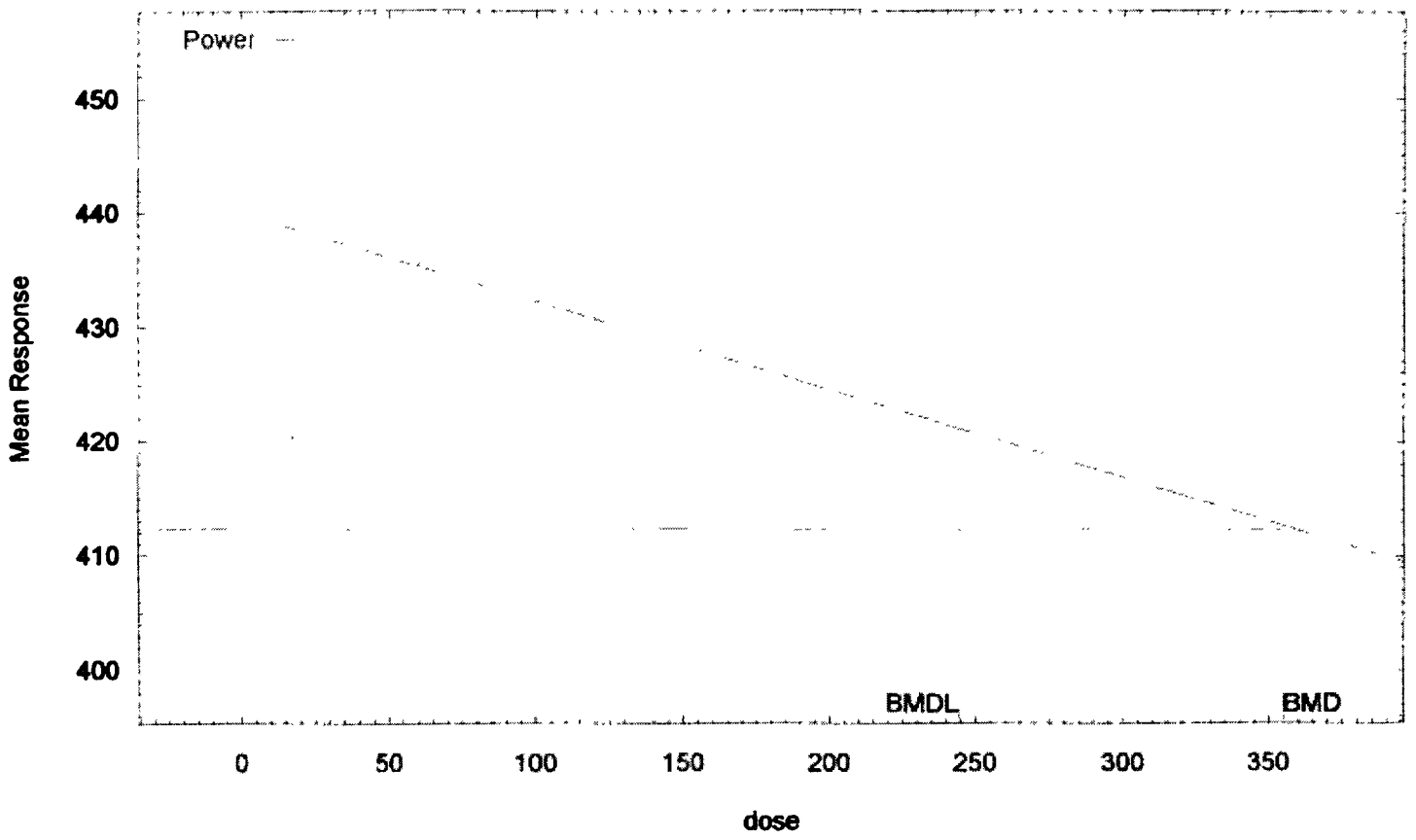
The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels. It seems appropriate to model the data

The p-value for Test 2 is less than .05. Consider running a non-homogeneous variance model

The p-value for Test 3 is greater than .05. The model chosen appears to adequately describe the data

```
 Benchmark Dose Computation
Specified effect =              1

Risk Type        =     Estimated standard deviations from the control mean

Confidence level =           0.95

             BMD =        354.861

            BMDL =        244.116
```

Power Model with 0.95 Confidence Level



16:37 01/10 2002

Maternal body weight

```
==================================================================
        Polynomial Model. $Revision: 2.1 $ $Date: 2000/10/11 17:51:39 $
        Input Data File: F:\BMDS\Argus maternal bw.(d)
        Gnuplot Plotting File:  F:\BMDS\UNSAVED1.plt
                                                    Fri May 10 10:18:09 2002
==================================================================
```

BMDS MODEL RUN

The form of the response function is

Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 +

Dependent variable = MEAN
Independent variable = dose
rho is set to 0
Signs of the polynomial coefficients are not restricted
A constant variance model is fit

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

alpha =	779.391	
rho =	0	Specified
beta_0 =	440.188	
beta_1 =	-0.0971452	
beta_2 =	5.21988e-005	

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	746.657	108.336
beta_0	440.199	5.35447
beta_1	-0.0970673	0.0943987
beta_2	5.19026e-005	0.000239328

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	beta_0	beta_1	beta_2
alpha	1	-5.5e-008	7.6e-008	-7.5e-008
beta_0	-5.5e-008	1	-0.77	0.68
beta_1	7.6e-008	-0.77	1	-0.98
beta_2	-7.5e-008	0.68	-0.98	1

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	23	440	29	440	27.3	-0.167
60	25	435	28.2	435	27.3	0.401
120	23	429	25.1	429	27.3	-0.251
360	24	412	29.1	412	27.3	0.0167

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model R: Yi = Mu + e(i
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-361.736054	5	733.472108
A2	-361.411149	8	738.822299
fitted	-361.741255	3	729.482510
R	-368.365774	2	740.731548

Test 1 Does respqnse and/or variances differ among dose levels
(A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	13.9092	6	0.003031
Test 2	0.649809	3	0.8849
Test 3	0.010402	1	0.9188

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels.
It seems appropriate to model the data

The p-value for Test 2 is greater than .05. A homogeneous variance
model appears to be appropriate here

The p-value for Test 3 is greater than 05 The model

chosen appears
to adequately describe the data

Benchmark Dose Computation
Specified effect = 1

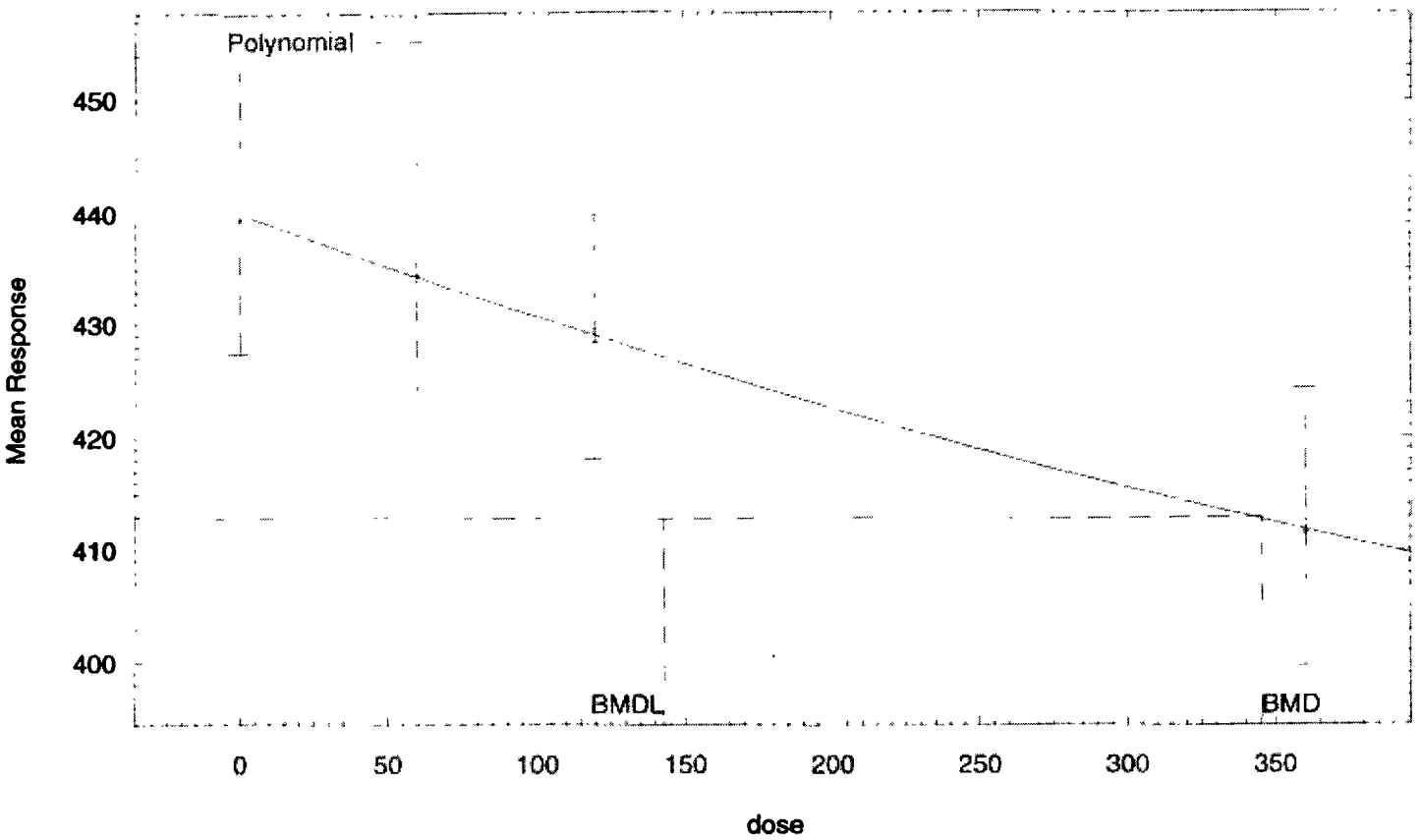
Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 345.237

BMDL = 142.794

Polynomial Model with 0.95 Confidence Level



10:18 05/10 2002

```
==========================================================================
          Hill Model. $Revision: 2.1 $ $Date: 2000/10/11 21:21:23 $
          Input Data File: C:\DOCUMENTS AND SETTINGS\JZHAO\MY
DOCUMENTS\PHENOL\DAMWEIGHT.(d)
          Gnuplot Plotting File:  C:\DOCUMENTS AND SETTINGS\JZHAO\MY
DOCUMENTS\PHENOL\DAMWEIGHT.plt
                                                    Thu Jan 10 16:38:54 2002
==========================================================================

BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

  The form of the response function is:

  Y[dose] = intercept + v*dose^n/(k^n + dose^n)


  Dependent variable = MEAN
  Independent variable = dose
  rho is set to 0
  Power parameter restricted to be greater than 1
  A constant variance model is fit

  Total number of dose groups = 4
  Total number of records with missing values = 0
  Maximum number of iterations = 250
  Relative Function Convergence has been set to: 1e-008
  Parameter Convergence has been set to: 1e-008


                 Default Initial Parameter Values
                       alpha =      772.871
                         rho =            0   Specified
                   intercept =        440.4
                           v =        -28.1
                           n =      1.44931
                           k =      160.473

          Asymptotic Correlation Matrix of Parameter Estimates

                 alpha        rho    intercept          v          n
 k
     alpha         1          0          0          0          0
 0
       rho         0          1          0          0          0
 0
intercept          0          0          1          0          0
 0
         v         0          0          0          1          0
 0
         n         0          0          0          0          1
 0
         k         0          0          0          0          0
```

Parameter Estimates

Variable	Estimate	Std Err.
alpha	746.575	1
rho	0	1
intercept	440.4	1
v	-57.271	1
n	1.25328	1
k	370.906	1

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res
0	23	440	29	440	27.3	5.28e-008
60	25	435	28.2	435	27.3	3.13e-007
120	23	429	25.1	429	27.3	-3.6e-007
360	24	412	29.1	412	27.3	9.88e-007

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Warning: Likelihood for fitted model larger than the Likelihood for model A1

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-361.736054	5	733.472108
A2	-361.411149	8	738.822299
fitted	-361.736054	5	733.472107
R	-368.860689	2	741.721377

Test 1: Does response and/or variances differ among dose levels (A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	14.8991	6	0.001905
Test 2	0.649809	3	0.8849
Test 3	1.01313e-007	0	NA

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels. It seems appropriate to model the data

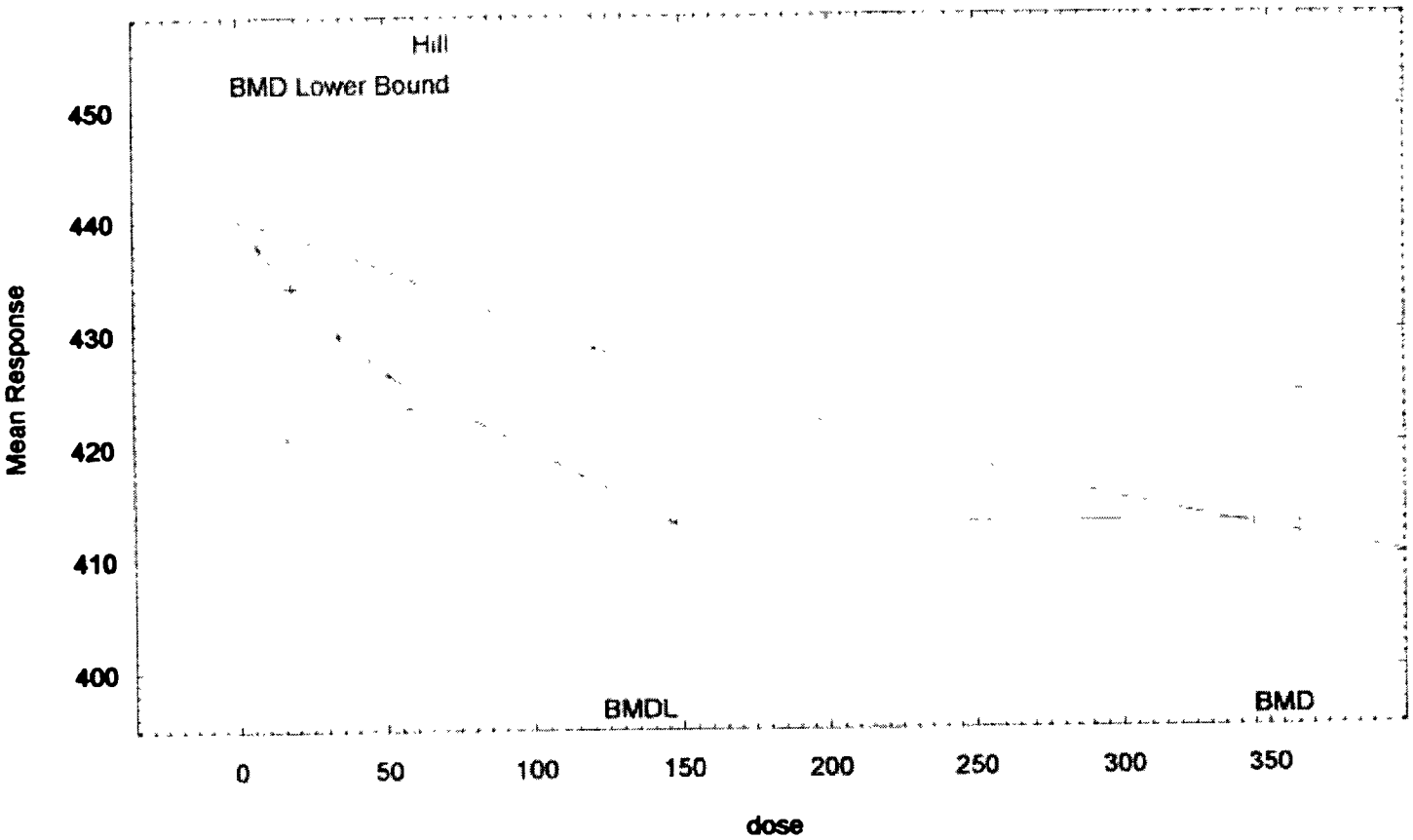
The p-value for Test 2 is greater than .05. A homogeneous variance model appears to be appropriate here

NA - Degrees of freedom for Test 3 are less than or equal to 0. The Chi-Square test for fit is not valid

Benchmark Dose Computation

Specified effect	=	1
Risk Type	=	Estimated standard deviations from the control mean
Confidence level	=	0.95
BMD	=	344.737
BMDL	=	147.428

Hill Model with 0.95 Confidence Level



16:38 01/10 2002

```
Clintrials motor activity.  BMR=1 SD
 ==================================================================
          Power Model. $Revision: 2.1 $ $Date: 2000/10/11 20:57:36 $
          Input Data File: F:\BMDS\UNSAVED1.(d)
          Gnuplot Plotting File:  F:\BMDS\UNSAVED1.plt
                                                   Fri May 17 13:46:34 2002
                                  ==================================

 BMDS MODEL RUN
 ----------------------------------

   The form of the response function is:

   Y[dose] = control + slope * dose^power


   Dependent variable = MEAN
   Independent variable = COLUMN1
   rho is set to 0
   The power is restricted to be greater than or equal to 1
   A constant variance model is fit

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                          alpha =      14556.4
                            rho =            0   Specified
                        control =          468
                          slope =     -2101.71
                          power =    -0.471502

          Asymptotic Correlation Matrix of Parameter Estimates

                 alpha          rho      control        slope        power

    alpha            1           -1       NA             NA           NA

      rho           -1            1       NA             NA           NA

  control       NA             NA             NA             NA             NA

    slope       NA             NA             NA             NA             NA

    power       NA             NA             NA             NA             NA


NA - This parameter's variance has been estimated at zero
```

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	13773.9	81444
rho	0	0.995906
control	455.234	58.2246
slope	-0.348708	5.10257
power	1	2.40548

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	15	468	118	455	117	0.109
24.6	15	451	149	447	117	0.037
107	15	394	78	418	117	-0.204
360	14	337	127	330	117	0.0622

Model Descriptions for likelihoods calculated

```
Model A1:        Yij = Mu(i) + e(ij)
            Var(e(ij)) = Sigma^2

Model A2:        Yij = Mu(i) + e(ij)
            Var(e(ij)) = Sigma(i)^2

Model  R          Yi = Mu + e(i)
            Var(e(i)) = Sigma^2
```

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-309.709699	5	629.419399
A2	-307.217620	8	630.435240
fitted	-310.650690	4	629.301380
R	-315.381614	2	634.763229

Test 1: Does response and/or variances differ among dose levels (A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	df	p-value
Test 1	16.328	6	0.0009712
Test 2	4.98416	3	0.173
Test 3	1.88198	1	0.1701

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels It seems appropriate to model the data

The p-value for Test 2 is greater than 05. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .05. The model chosen appears to adequately describe the data

Benchmark Dose Computation
Specified effect = 1

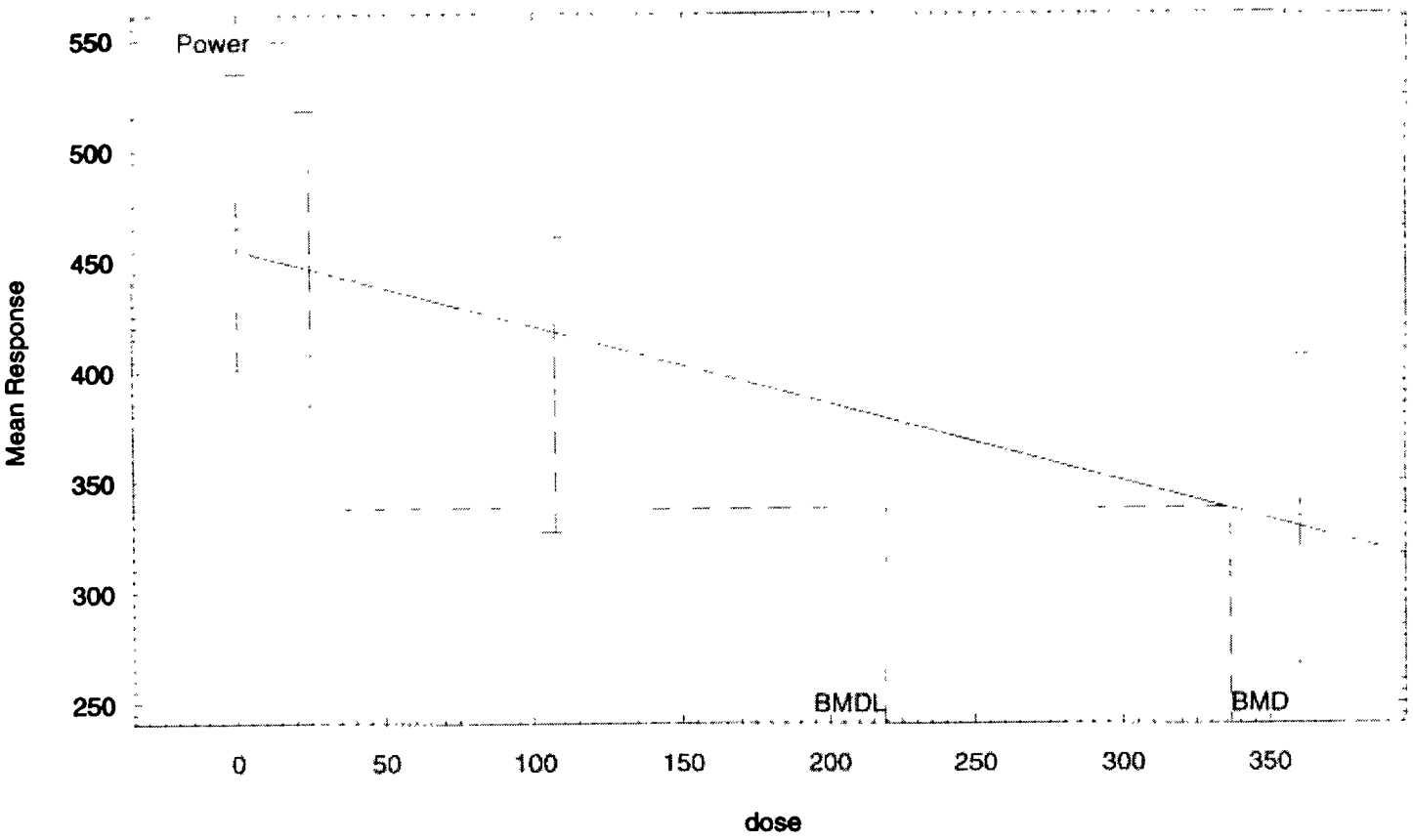
Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

336.564

219.138

Power Model with 0.95 Confidence Level



13:46 05/17 2002

Clintrials motor activity in females at 4 weeks, BMR = 1 SD

Polynomial Model. $Revision: 2.1 $ $Date: 2000/10/11 17:51:39 $
Input Data File: F:\BMDS\UNSAVED1.(d)
Gnuplot Plotting File: F:\BMDS\UNSAVED1.plt
Fri May 17 13:53:20 2002

BMDS MODEL RUN

The form of the response function is:

Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 +

Dependent variable = MEAN
Independent variable = COLUMN1
rho is set to 0
The polynomial coefficients are restricted to be negative
A constant variance model is fit

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

alpha =	1	
rho =	0	Specified
beta_0 =	469.177	
beta_1 =	-0.83776	
beta_2 =	0	

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	13763.4	2532.12
beta_0	455.234	20.0191

beta_1	-0.348708	0.108862
beta_2	0	NA

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	beta_0	beta_1
alpha	1	7.1e-010	-2.3e-01
beta_0	7.1e-010	1	-0.65
beta_1	-2.3e-011	-0.65	1

The following parameter(s) have been estimated at a boundary
point or have been specified. Correlations are not computed:

beta_2

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	15	468	118	455	117	1.63
24.6	15	451	149	447	117	0.555
107	15	394	78	418	117	-3.06
360	14	337	127	330	117	0.871

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-310.209699	5	630.419399
A2	-307.217620	8	630.435240
fitted	-310.650699	2	625.301398
R	-315.381614	2	634.763229

Test 1: Does response and/or variances differ among dose levels
(A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	16.328	6	0.0009712
Test 2	5.98416	3	0.1124
Test 3	0.881999	1	0.3477

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels.
It seems appropriate to model the data

The p-value for Test 2 is greater than .05. A homogeneous variance
model appears to be appropriate here

The p-value for Test 3 is greater than .05. The model chosen appears
to adequately describe the data

Benchmark Dose Computation
Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

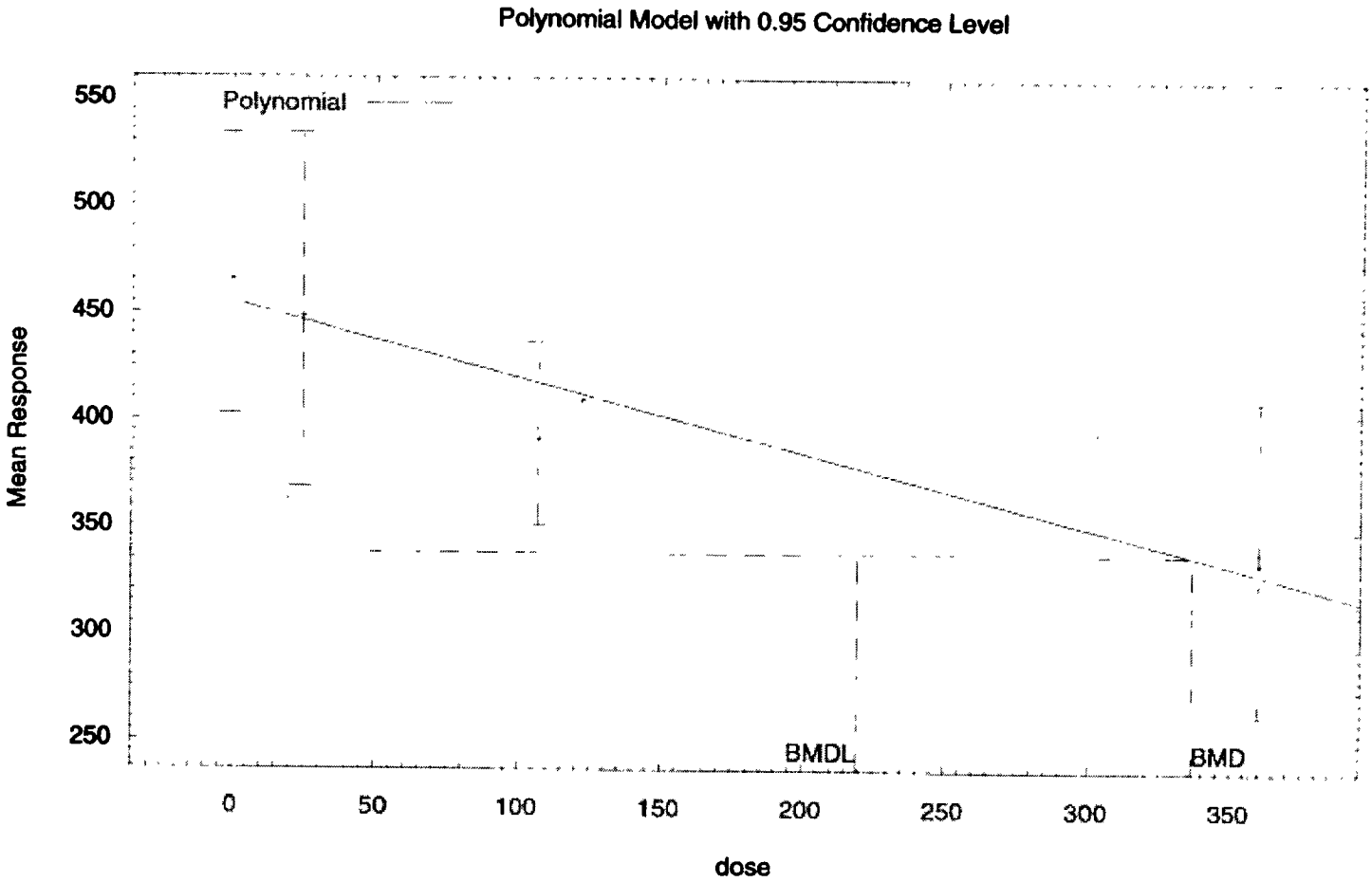
Confidence level = 0.95

BMD = 336.435

BMDL = 219.137

BMDL computation failed for one or more point on the BMDL curve.

The BMDL curve will not be plotted



13:53 05/17 2002

Clintrials motor activity BMR=1 SD

```
==============================================================
  Hill Model. $Revision: 2.1 $ $Date: 2000/10/11 21:21:23 $
  Input Data File: F:\BMDS\UNSAVED1.(d)
  Gnuplot Plotting File:  F:\BMDS\UNSAVED1.plt
                                          Fri May 17 13:48:20 2002
==================================================================

BMDS MODEL RUN
------------------------------------------------------------------

  The form of the response function is:

  Y[dose] = intercept + v*dose^n/(k^n + dose^n)


  Dependent variable = MEAN
  Independent variable = COLUMN1
  rho is set to 0
  Power parameter restricted to be greater than 1
  A constant variance model is fit

  Total number of dose groups = 4
  Total number of records with missing values = 0
  Maximum number of iterations = 250
  Relative Function Convergence has been set to: 1e-008
  Parameter Convergence has been set to: 1e-008


                Default Initial Parameter Values
                       alpha =      13197.2
                         rho =            0   Specified
                   intercept =          468
                           v =         -131
                           n =      1.77566
                           k =      94.7123


          Asymptotic Correlation Matrix of Parameter Estimates

                 alpha          rho    intercept            v            n
     k

     alpha           1            0            0            0            0
     0

       rho           0            1            0            0            0
     0

 intercept           0            0            1            0            0
     0

         v           0            0            0            1            0
     0

         n           0            0            0            0            1
     0
```

k	0	0	0	0	0
1					

Parameter Estimates

Variable	Estimate	Std Err
alpha	13569.6	
rho		
intercept	468	1
v	-161.338	1
n	1.34226	1
k	121.061	1

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	15	468	118	468	116	1.17e-007
24.6	15	451	149	451	116	-3.09e-007
107	15	394	78	394	116	-2.44e-007
360	14	337	127	337	116	7.32e-007

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2 Yij = Mu(i) + e(ij
Var{e(ij)} = Sigma(i)^2

Model R Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Degrees of freedom for Test A1 vs fitted <= 0

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-310.209699	5	630.419399
A2	-307.217620	8	630.435240
fitted	-310.209699	5	630.419399
R	-315.881614	2	635.763229

Test 1: Does response and/or variances differ among dose levels (A2 vs. R)
Test 2 Are Variances Homogeneous (A1 vs A2)
Test 3 Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	17.328	6	0.000605
Test 2	5.98416	3	0.1124
Test 3	2.93312e-011	0	NA

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels It seems appropriate to model the data

The p-value for Test 2 is greater than 05 A homogeneous variance model appears to be appropriate here

NA - Degrees of freedom for Test 3 are less than or equal to 0. The Chi-Square test for fit is not valid

Benchmark Dose Computation
Specified effect = 1

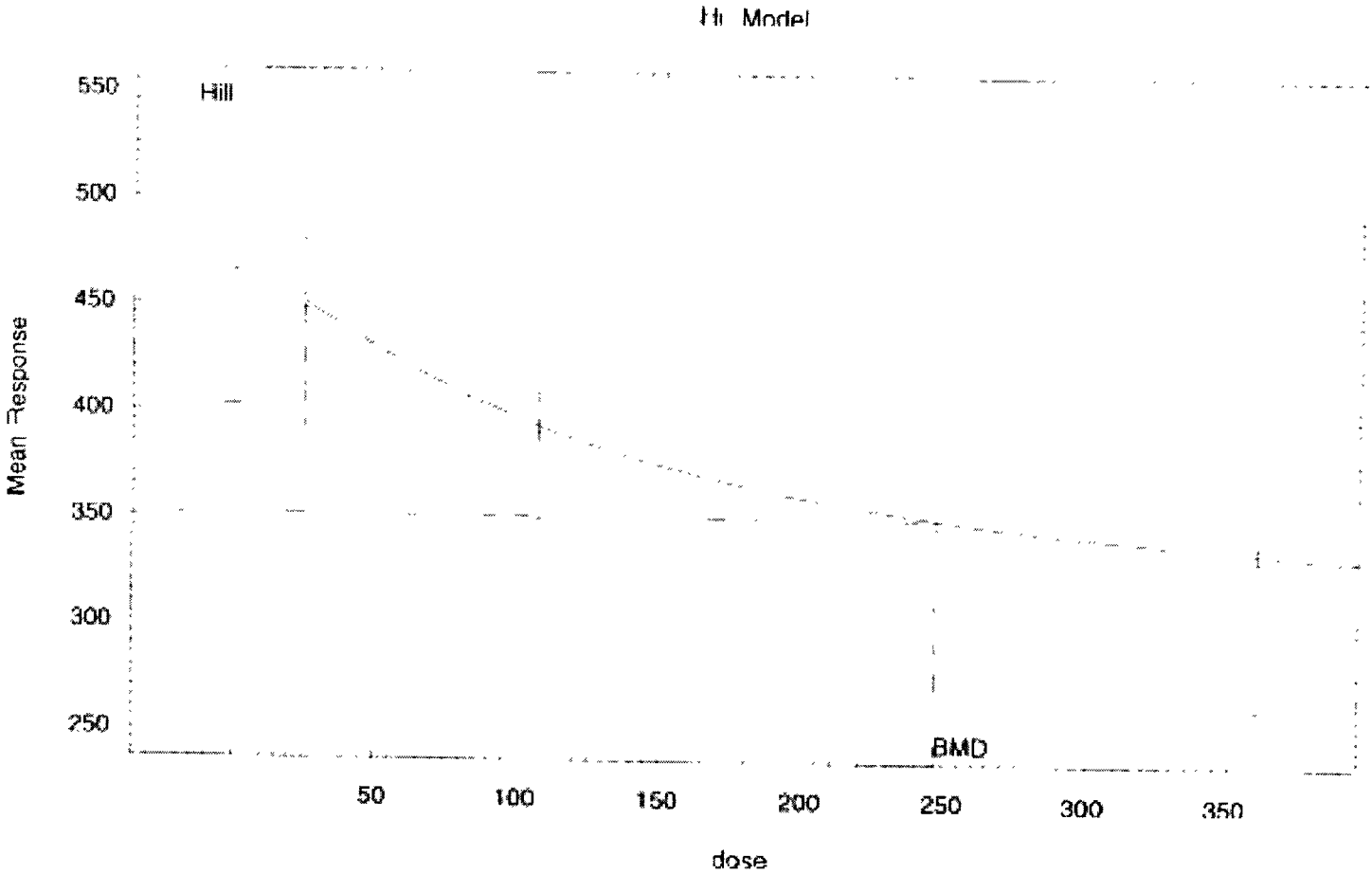
Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 246.508

Warning optimum may not have been found Bad completion code in Optimization r outine.

BMDL computation failed


Hill Model
Hill
BMD
Mean Response
550
500
450
400
350
300
250
50
100
150
200
250
300
350
dose

3:48 05/1 2002

PFC response, BMR=1.0 SD

```
==========================================================================
        Power Model. $Revision: 2.1 $ $Date: 2000/10/11 20:57:36 $
        Input Data File: C:\BMDS\DATA\CONT.(d)
        Gnuplot Plotting File:  C:\BMDS\DATA\CONT.plt
                                                    Tue May 14 14:01:03 2002
==========================================================================

BMDS MODEL RUN
--------------------------------------------------------------------------

  The form of the response function is:

  Y[dose] = control + slope * dose^power


  Dependent variable = MEAN
  Independent variable = Dose
  rho is set to 0
  The power is restricted to be greater than or equal to 1
  A constant variance model is fit

  Total number of dose groups = 4
  Total number of records with missing values = 0
  Maximum number of iterations = 250
  Relative Function Convergence has been set to: 1e-008
  Parameter Convergence has been set to: 1e-008


                 Default Initial Parameter Values
                         alpha =      28373.3
                           rho =            0   Specified
                       control =         1123
                         slope =     -1807.87
                         power =    -0.361754


          Asymptotic Correlation Matrix of Parameter Estimates

                 alpha          rho      control        slope        power

   alpha           1           -1        0.065       NA           NA

     rho          -1            1       -0.056       NA           NA

 control       0.065       -0.056            1       NA           NA

   slope      NA           NA           NA           NA           NA

   power      NA           NA           NA           NA           NA


NA - This parameter's variance has been estimated at zero.


                         Parameter Estimates

     Variable            Estimate            Std. Err
```

alpha	32160.5	346106
rho	0	1.59091
control	976.333	37.4625
slope	-11.4263	18.1954
power	1	0.423031

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res
0	5	1.12e+003	221	976	179	0.818
1.8	5	896	134	956	179	-0.333
6.2	5	795	110	905	179	-0.616
33.6	5	616	186	592	179	0.132

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-110.300585	5	230.601171
A2	-108.879712	8	233.759424
fitted	-113.784950	4	235.569899
R	-119.888342	2	243.776683

Test 1: Does response and/or variances differ among dose levels (A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	df	p-value
Test 1	22.0173	6	6.469e-005
Test 2	2.84175	3	0.4167
Test 3	6.96873	1	0.008295

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels. It seems appropriate to model the data

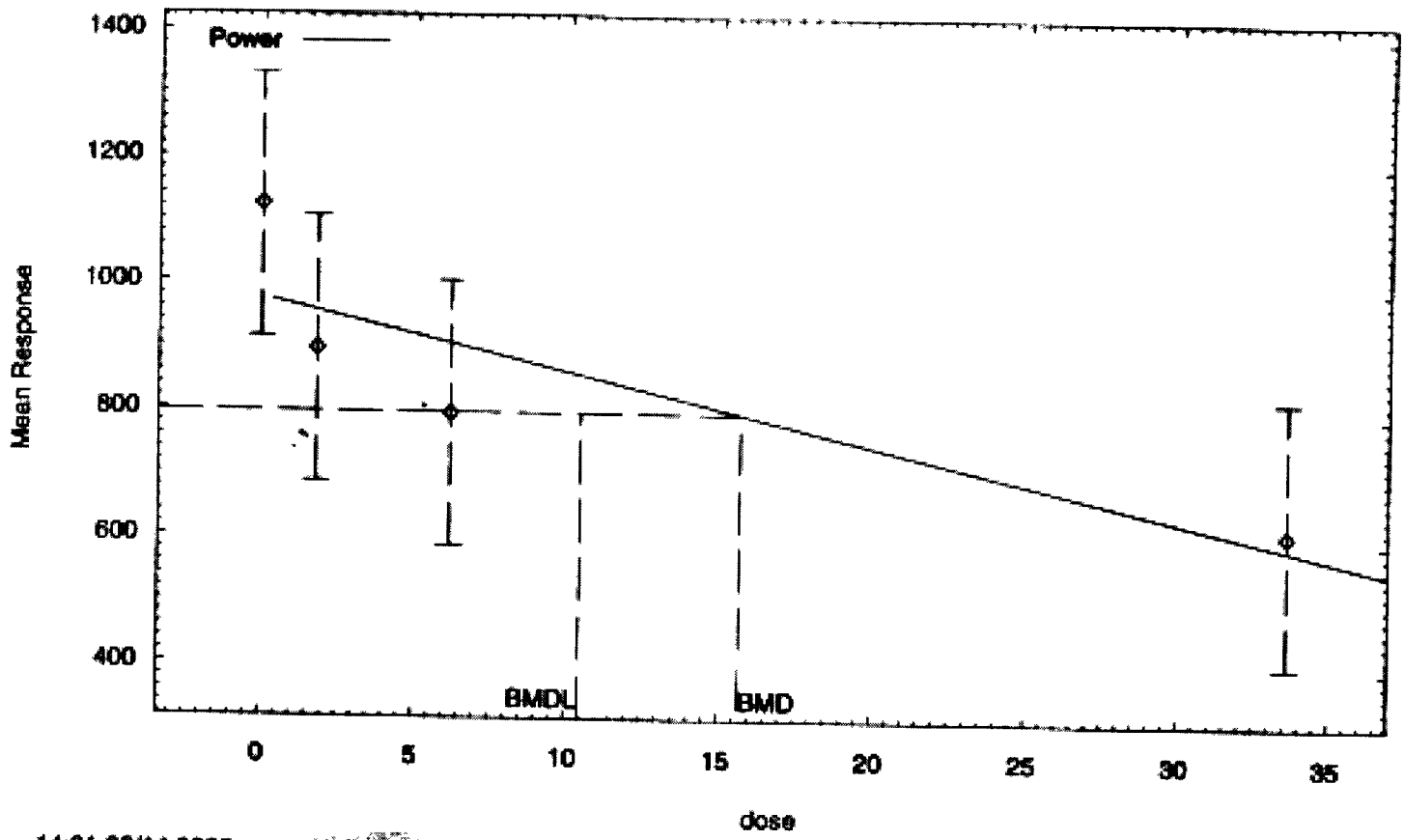
The p-value for Test 2 is greater than .05. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is less than .05. You may want to try a

different model

```
 Benchmark Dose Computation
Specified effect =             1

Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =       15.6948

            BMDL =       10.4544
```

Power Model with 0.95 Confidence Level



14:01 05/14 2002

PFC response BMR = 1SD

```
=================================================================
        Polynomial Model. $Revision: 2.1 $ $Date: 2000/10/11 17:51:39 $
        Input Data File: F:\BMDS\MA.(d)
        Gnuplot Plotting File:  F:\BMDS\MA.plt
                                                  Tue May 14 14:20:26 2002
                                          ============================

BMDS MODEL RUN
~~~~~~~~~~~~~~~~

  The form of the response function is:

  Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2


  Dependent variable = MEAN
  Independent variable = dose
  rho is set to 0
  The polynomial coefficients are restricted to be negative
  A constant variance model is fit

  Total number of dose groups = 4
  Total number of records with missing values = 0
  Maximum number of iterations = 250
  Relative Function Convergence has been set to: 1e-008
  Parameter Convergence has been set to: 1e-008


                 Default Initial Parameter Values
                         alpha =            1
                           rho =            0   Specified
                        beta_0 =      1071.13
                        beta_1 =     -56.7007
                        beta_2 =            0


                          Parameter Estimates

      Variable            Estimate            Std. Err
         alpha              26722.6             8451.07
        beta_0              976.316             46.0371
        beta_1             -11.4246             2.69108
        beta_2                    0                NA

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.


          Asymptotic Correlation Matrix of Parameter Estimates

                   alpha       beta_0       beta_1
     alpha           1     -0.00012      0.00019
    beta_0     -0.00012            1        -0.61
    beta_1      0.00019        -0.61            1
```

The following parameter(s) have been estimated at a boundary
point or have been specified. Correlations are not computed:

beta_2

Table of Data and Estimated Values of Interest

Dose Res.	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2
0	5	1.12e+003	221	976	163	4.49
1.8	5	896	134	956	163	-1.83
6.2	5	795	110	905	163	-3.38
33.6	5	616	86	592	163	0.72

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var(e(ij)) = Sigma^2

Model A2 Yij = Mu(i) + e(ij)
Var(e(ij)) = Sigma(i)^2

Model R: Yi = Mu + e(i
Var(e(i)) = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-107.560658	5	225.121317
A2	-105.022715	8	226.045431
fitted	-111.931872	2	227.863744
R	-118.871191	2	241.742383

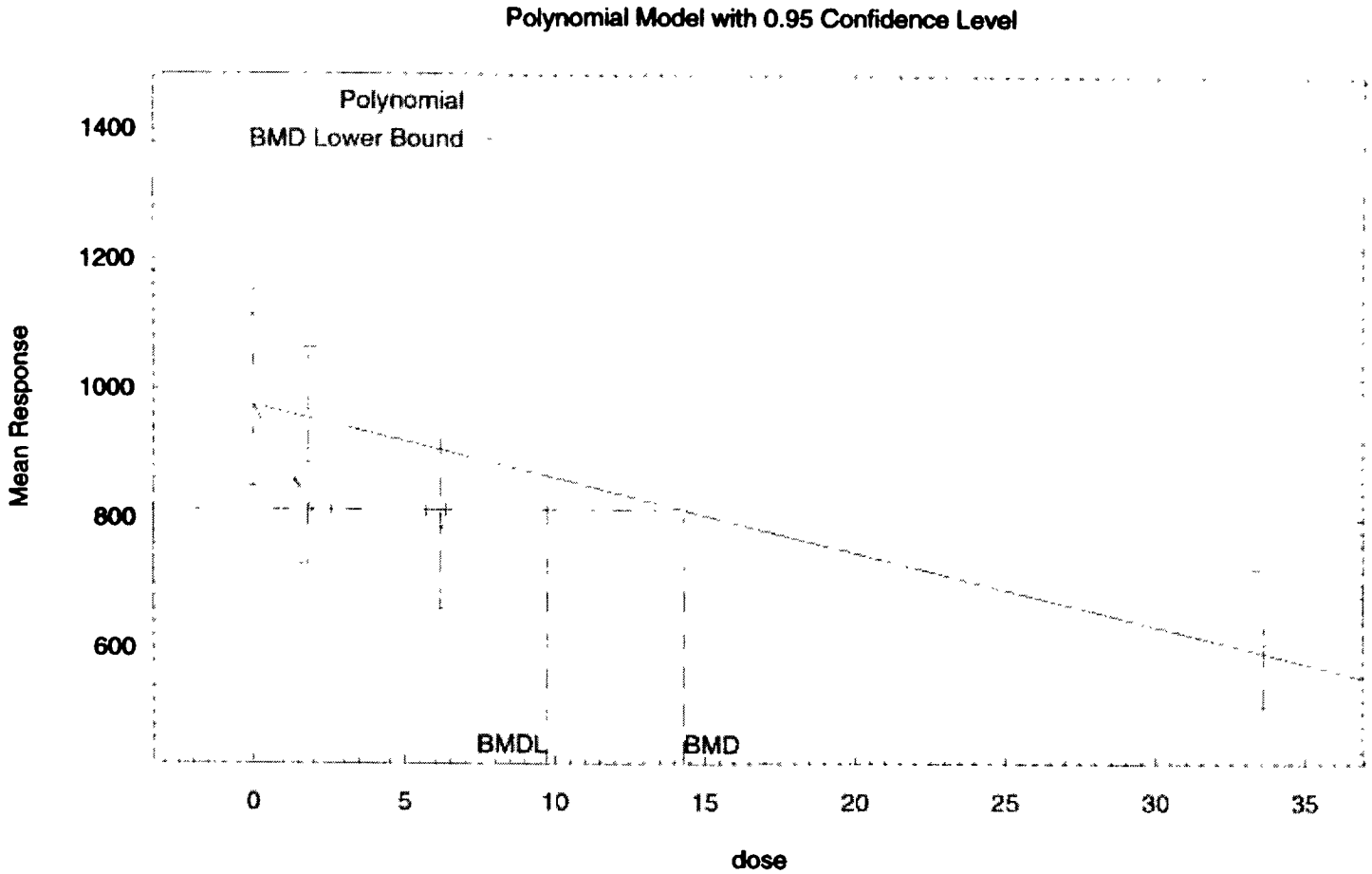
Test 1: Does response and/or variances differ among dose levels
(A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	27.697	6	<.0001
Test 2	5.07589	3	0.1663
Test 3	8.74243	1	0.003109

The p-value for Test 1 is less than 05. There appears to be a

```
difference between response and/or variances among the
dose levels.
It seems appropriate to model the data

The p-value for Test 2 is greater than  05   A
homogeneous variance
model appears to be appropriate here


The p-value for Test 3 is less than .05.  You may want
to try a
different model


 Benchmark Dose Computation
Specified effect =             1

Risk Type        =     Estimated standard deviations from the control mean


Confidence level =          0.95

             BMD =       14.3086


            BMDL =       9.73181
```



14:20 05/14 2002

PFC response, BMR=1.0 SD

```
Hill Model. $Revision: 2.1 $ $Date: 2000/10/11 21:21:23 $
Input Data File: C:\BMDS\DATA\CONT.(d)
Gnuplot Plotting File:  C:\BMDS\DATA\CONT.plt
                                                Tue May 14 14:02:22 2002
```

BMDS MODEL RUN

The form of the response function is:

Y[dose] = intercept + v*dose^n/(k^n + dose^n)

Dependent variable = MEAN
Independent variable = Dose
rho is set to 0
Power parameter restricted to be greater than 1
A constant variance model is fit

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

alpha =	24615.1	
rho =	0	Specified
intercept =	1123	
v =	-507	
n =	0.62026	
k =	2.95446	

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -n
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	alpha	rho	intercept	v	k
alpha	1	0	0	0	0
rho	0	1	0	0	0
intercept	0	0	1	0	0
v	0	0	0	1	0
k	0	0	0	0	1

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	23185.3	1
rho	0	1
intercept	1115.7	1
v	-533.126	1
n	1	NA
k	3.17243	1

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	5	1.12e+003	221	1.12e+003	152	0.048
1.8	5	896	134	923	152	-0.175
6.2	5	795	110	763	152	0.21
33.6	5	616	186	629	152	-0.0825

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Degrees of freedom for Test A1 vs fitted <= 0

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-110.300585	5	230.601171
A2	-108.879712	8	233.759424
fitted	-110.512739	4	229.025477
R	-119.888342	2	243.776683

Test 1: Does response and/or variances differ among dose levels (A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	22.0173	6	<.0001
Test 2	2.84175	3	0.4167
Test 3	0.424307	0	NA

The p-value for Test 1 is less than .05. There appears to be a

difference between response and/or variances among the dose levels.
It seems appropriate to model the data

The p-value for Test 2 is greater than .05. A homogeneous variance model appears to be appropriate here

NA - Degrees of freedom for Test 3 are less than or equal to 0. The Chi-Square test for fit is not valid

Benchmark Dose Computation
Specified effect = 1

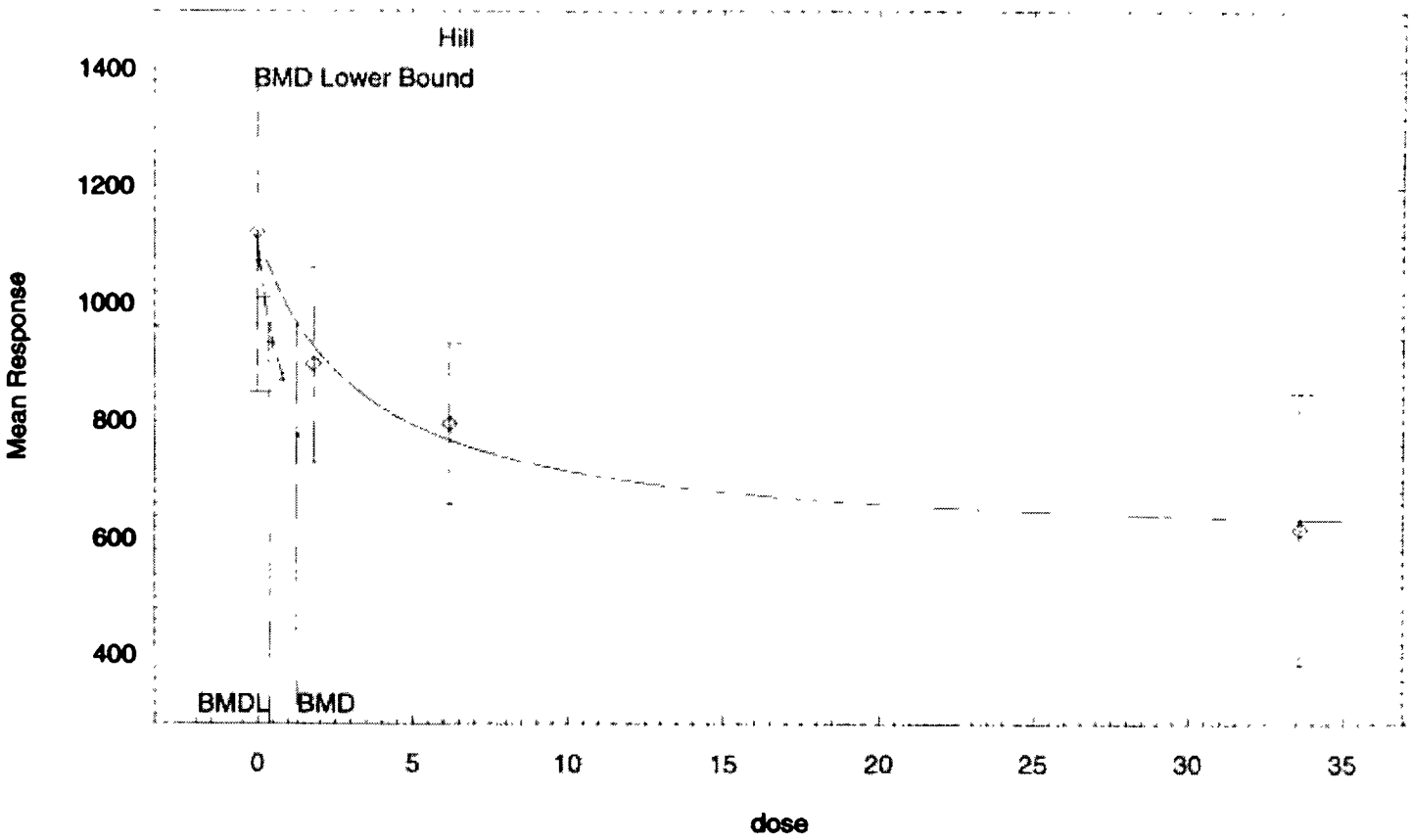
Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 1.26834

BMDL = 0.383495

Hill Model with 0.95 Confidence Level



14:02 05/14 2002

PFC/total spleen

```
Power Model. $Revision: 2.1 $ $Date: 2000/10/11 20:57:36 $
Input Data File: C:\BMDS\DATA\CONT.(d)
Gnuplot Plotting File:  C:\BMDS\DATA\CONT.plt
                                        Tue May 14 15:50:01 2002
```

BMDS MODEL RUN

The form of the response function is:

Y[dose] = control + slope * dose^power

Dependent variable = MEAN
Independent variable = Dose
The power is restricted to be greater than or equal to 1
The variance is to be modeled as Var(i) = alpha*mean(i)^rho

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

alpha =	4.76625e+009
rho =	0
control =	265947
slope =	-173704
power =	-0.0701236

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	rho	control	slope	power
alpha	1	-1	0.39	NA	NA
rho	-1	1	-0.4	NA	NA
control	0.39	-0.4	1	NA	NA
slope	NA	NA	NA	NA	NA
power	NA	NA	NA	NA	NA

NA - This parameter's variance has been estimated at zero.

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	0.00015149	0.0025216

rho	2.52122	1.36567
control	237600	17339.2
slope	-3207.96	6498.98
power	1	0.554928

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	5	2.66e+005	1.19e+005	2.38e+005	7.36e+004	0.385
1.8	5	2.15e+005	3.91e+004	2.32e+005	7.14e+004	-0.24
6.2	5	2.08e+005	4.22e+004	2.18e+005	6.59e+004	-0.152
33.6	5	1.3e+005	4.07e+004	1.3e+005	3.44e+004	0.0108

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = alpha*(Mu(i))^rho

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-230.616816	5	471.233632
A2	-225.384337	8	466.768674
A3	-227.759631	6	467.519262
fitted	-229.618859	5	469.237718
R	-235.936079	2	475.872158

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)

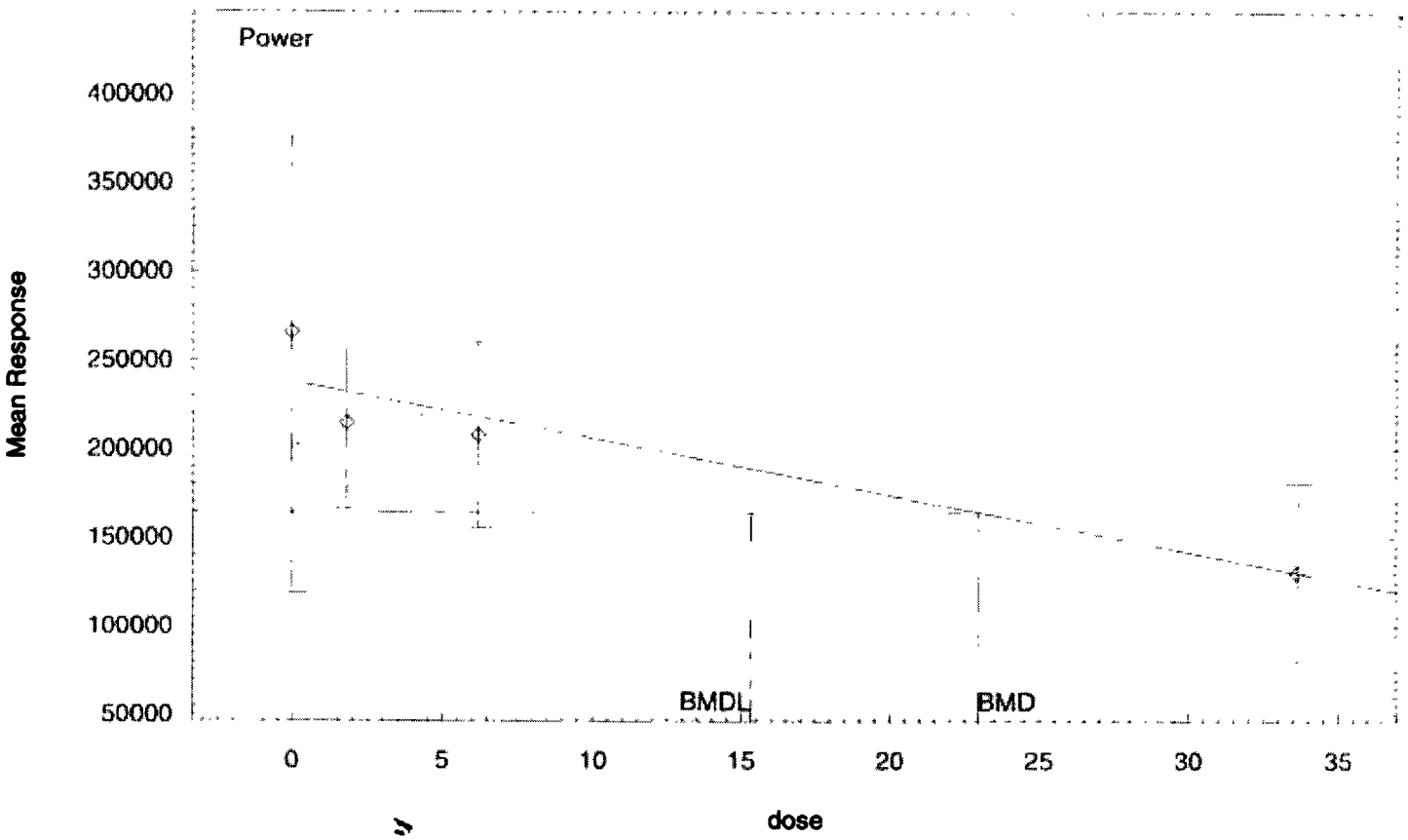
Tests of Interest

Test	-2*log(Likelihood Ratio)	d.f	p-value
Test 1	21.1035	6	0.001758
Test 2	10.465	3	0.015
Test 3	4.75059	2	0.09299
Test 4	3.71846	1	0.05381

The p-value for Test 1 is less than .05. There appears to be a

```
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .05.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .05.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .05.  The model chosen seems
to adequately describe the data


 Benchmark Dose Computation
Specified effect =             1

Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

                          22.9511


                          15.3302
```

Power Model with 0.95 Confidence Level



15:50 05/14 2002

PFC/total spleen

```
=============================================== ==============
    Polynomial Model. $Revision: 2.1 $ $Date  2000/10/11 17:51:39 $
    Input Data File: F:\BMDS\MA.(d)
    Gnuplot Plotting File:  F:\BMDS\MA.plt
                                                  Tue May 14 16:19:41 2002
          ==================================================
```

BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~

The form of the response function is

Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2

Dependent variable = MEAN
Independent variable = dose
The polynomial coefficients are restricted to be negative
The variance is to be modeled as Var(i) = alpha*mean(i)^rho

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

alpha =	1
rho =	0
beta_0 =	251869
beta_1 =	-9322.72
beta_2 =	0

Parameter Estimates

Variable	Estimate	Std. Err
alpha	216454	344531
rho	0.982068	0.138912
beta_0	188775	63404
beta_1	-7724.65	20131.8
beta_2	-1.63675	555.248

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	rho	beta_0	beta_1	beta_2
alpha	1	-1	0.14	-0.027	0.017
rho	-1	1	-0.053	0.021	0.0082
beta_0	0.14	-0.053	1	-0.79	0.71
beta_1	-0.027	0.021	-0.79	1	-1
beta_2	0.017	0.0082	0.71	-1	1
~~~~~~~~~~~~~~~~~~~~

Table of Data and Estimated Values of Interest

Dose Res.	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2
0	5	2.66e+005	1.19e+005	1.[illegible]9e+005	1.81e+005	2.13
1.8	5	2.15e+005	3.91e+004	1.[illegible]5e+005	1.75e+005	1.14
6.2	5	2.08e+005	4.22e+004	1.[illegible]1e+005	1.57e+005	2.13
33.6	5	1.3e+005	4.07e+004	-7.[illegible]6e+004	1.13e+005	8.94

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2 Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = alpha*(Mu(i))^rho

Model R: Yi = Mu + e(i
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-230.616816	5	471.233632
A2	-225.384337	8	466.768674
A3	-227.759622	6	467.519244
fitted	-249.295307	5	508.590613
R	-235.936079	2	475.872158

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	21.1035	6	0.001758
Test 2	10.465	3	0.015
Test 3	4.75057	2	0.09299
Test 4	43.0714	1	<.0001

The p-value for Test 1 is less than 05 There appears to be a

```
difference between response and/or variances among the
dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .05   A
non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than  05.  The
modeled variance appears
 to be appropriate here

The p-value for Test 4 is less than  05   You may want
to try a different
model


 Benchmark Dose Computation
Specified effect =             1

Risk Type        =     Estimated standard deviations from the control mean


Confidence level =          0.95

             BMD =       23.3523


            BMDL =       5.86859
```

PFC/total spleen

```
Hill Model. $Revision: 2.1 $ $Date: 2000/10/11 21:21:23 $
Input Data File: C:\BMDS\DATA\CONT.(d)
Gnuplot Plotting File:  C:\BMDS\DATA\CONT.plt
                                        Tue May 14 15:50:56 2002
```

BMDS MODEL RUN

The form of the response function is:

Y[dose] = intercept + v*dose^n/(k^n + dose^n)

Dependent variable = MEAN
Independent variable = Dose
Power parameter restricted to be greater than 1
The variance is to be modeled as Var(i) = alpha * mean(i) ^ rho

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

alpha =	1
rho =	2.30623
intercept =	265947
v =	-135762
n =	0.475261
k =	9.59273

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -n
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	alpha	rho	intercept	v	k
alpha	1	-1	NA	-0.99	
rho	-1	1	NA	0.99	
intercept	NA	NA	NA	NA	NA
v	-0.99	0.99	NA	1	
k	0.21	-0.22	NA	-0.62	1

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	9.6535e-006	1.06893e-008
rho	2.73759	8.76021e-005
intercept	265131	1.22396
v	-136181	2.5363
n	1	NA
k	4.00666	8.61636e-005

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	5	2.66e+005	1.19e+005	2.65e+005	8.24e+004	0.0099
1.8	5	2.15e+005	3.91e+004	2.23e+005	6.5e+004	-0.127
6.2	5	2.08e+005	4.22e+004	1.82e+005	4.94e+004	0.511
33.6	5	1.3e+005	4.07e+004	1.43e+005	3.56e+004	-0.373

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = alpha*(Mu(i))^rho

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Degrees of freedom for Test A3 vs fitted <= 0

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-230.616816	5	471.233632
A2	-225.384337	8	466.768674
A3	-227.759620	6	467.519240
fitted	-228.438245	5	466.876489
R	-235.936079	2	475.872158

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	21.1035	6	0.001758
Test 2	10.465	3	0.015
Test 3	4.75057	2	0.09299
Test 4	1.35725	0	NA

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels It seems appropriate to model the data

The p-value for Test 2 is less than .05. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is greater than .05. The modeled variance appears to be appropriate here

NA - Degrees of freedom for Test 4 are less than or equal to 0. The Chi-Square test for fit is not valid

Benchmark Dose Computation
Specified effect = 1

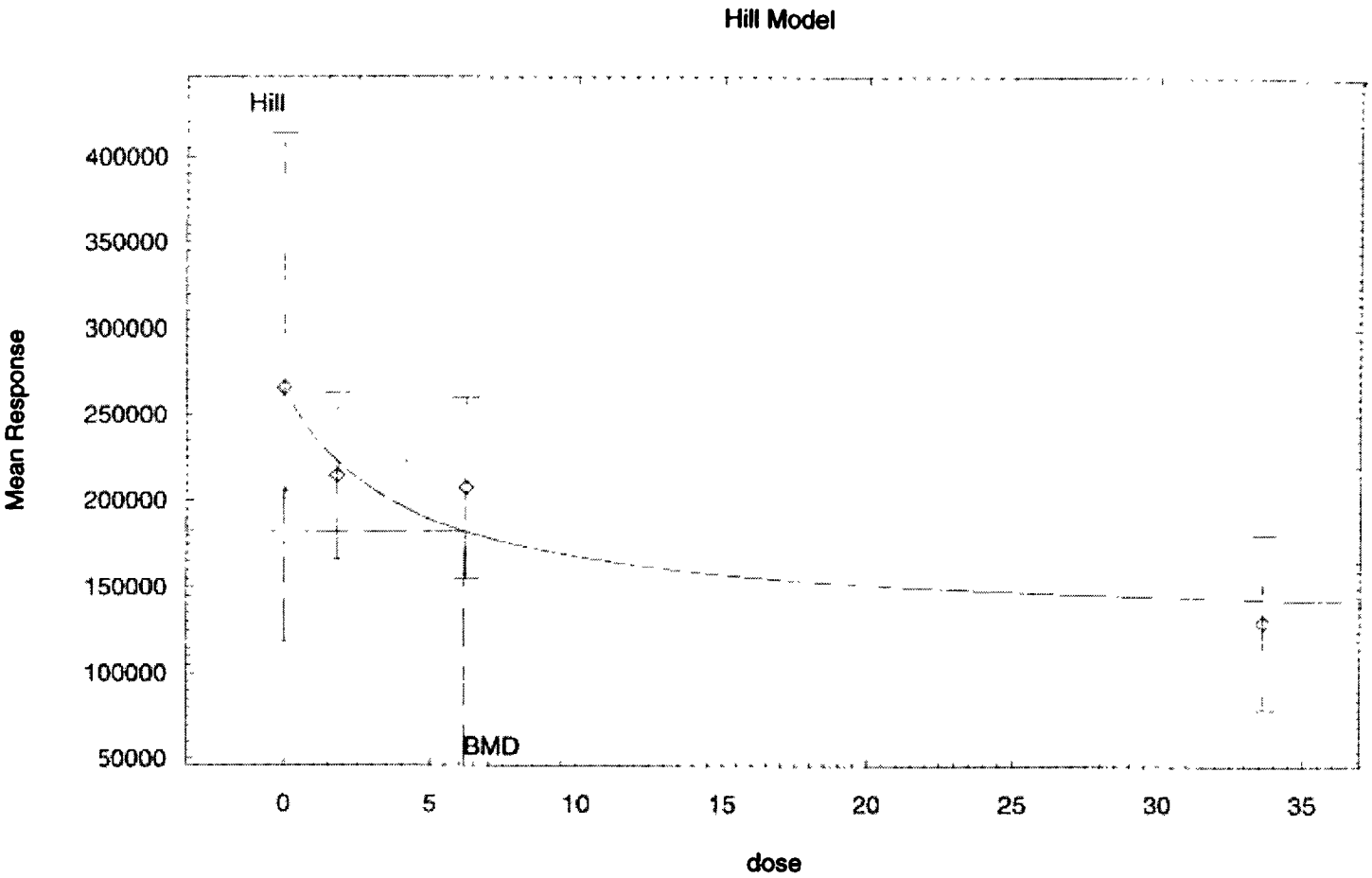
Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 6.13974

Warning: optimum may not have been found. Bad completion code in Optimization routine.

BMDL computation failed.



15:50 05/14 2002

Antibody titer

```
Power Model. $Revision: 2.1 $ $Date: 2000/10/11 20:57:36 $
Input Data File: C:\BMDS\DATA\CONT.(d)
Gnuplot Plotting File:  C:\BMDS\DATA\CONT.plt
                                        Tue May 14 15:52:46 2002
```

BMDS MODEL RUN

The form of the response function is:

Y[dose] = control + slope * dose^power

Dependent variable = MEAN
Independent variable = Dose
rho is set to 0
The power is restricted to be greater than or equal to 1
A constant variance model is fit

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

alpha =	0.0098315	
rho =	0	Specified
control =	0.446	
slope =	-1.46784	
power =	-0.592416	

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	rho	control	slope	power
alpha	1	0.99	0.091	NA	NA
rho	0.99	1	0.041	NA	NA
control	0.091	0.041	1	NA	NA
slope	NA	NA	NA	NA	NA
power	NA	NA	NA	NA	NA

NA - This parameter's variance has been estimated at zero

Parameter Estimates

Variable	Estimate	Std. Err

alpha	0.00898754	0.018154
rho	0	1.9925
control	0.402624	0.0174706
slope	-0.00443496	0.0109976
power	1	0.658352

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	5	0.446	0.087	0.403	0.0948	0.458
1.8	5	0.392	0.152	0.395	0.0948	-0.0279
6.2	5	0.325	0.042	0.375	0.0948	-0.529
33.6	5	0.263	0.083	0.254	0.0948	0.0991

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model R: Yij = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	38.453073	5	-66.906146
A2	42.155047	8	-68.310094
fitted	37.119156	4	-66.238312
R	33.214605	2	-62.429209

Test 1: Does response and/or variances differ among dose levels (A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	df	p-value
Test 1	17.8809	6	0.0004654
Test 2	7.40395	3	0.06008
Test 3	2.66783	1	0.1024

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels. It seems appropriate to model the data

The p-value for Test 2 is greater than .05. A homogeneous variance model appears to be appropriate here

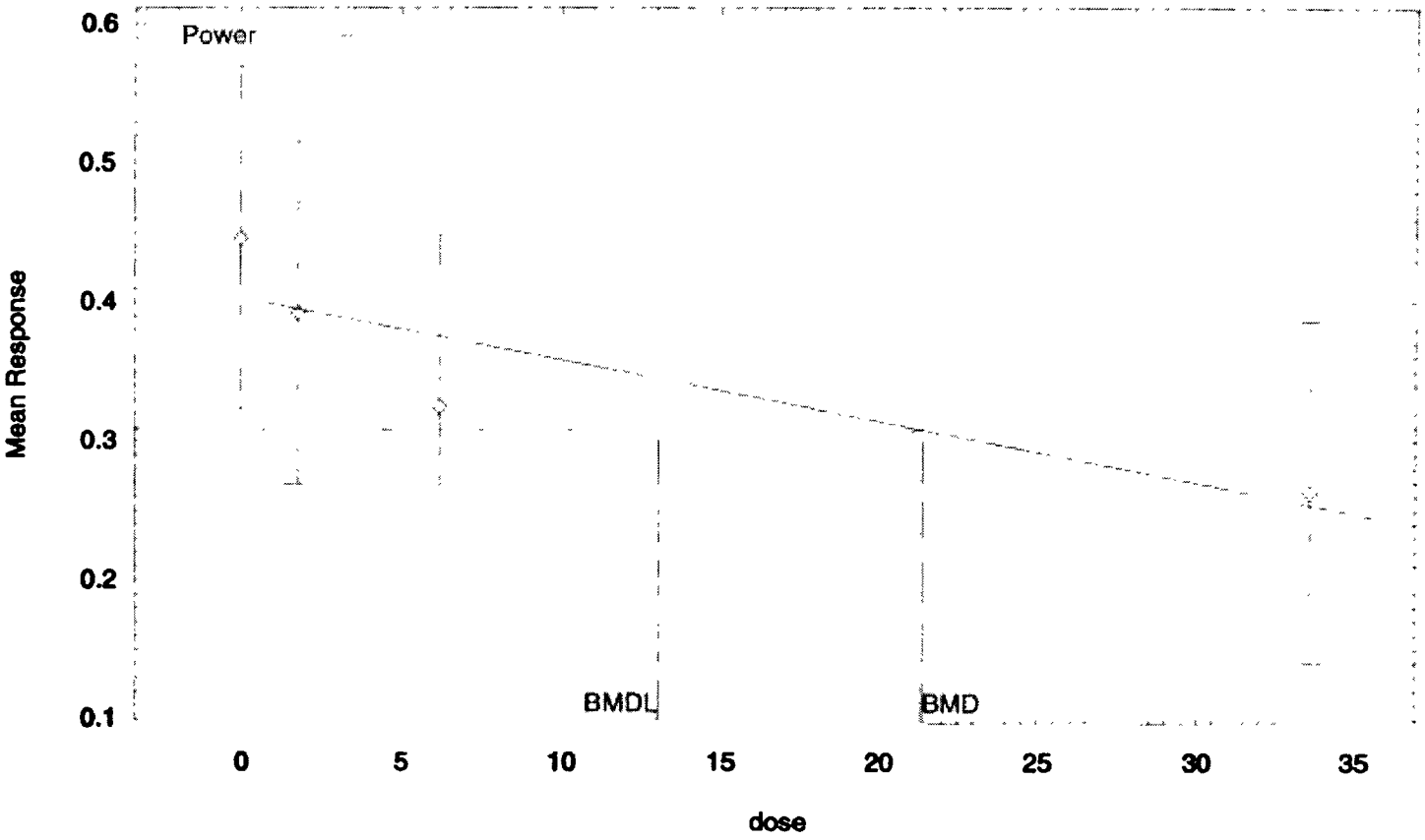
The p-value for Test 3 is greater than .05. The model chosen appears

to adequately describe the data

Benchmark Dose Computation

Specified effect	=	1
Risk Type	=	Estimated standard deviations from the control mean
Confidence level	=	0.95
BMD	=	21.3762
BMDL	=	13.0626

Power Model with 0.95 Confidence Level



15:52 05/14 2002

antibody titer

```
=============================================================
Polynomial Model. $Revision: 2.1 $ $Date: 2000/10/11 17:51:39 $
Input Data File: F:\BMDS\MA.(d)
Gnuplot Plotting File:  F:\BMDS\MA.plt
                                                Tue May 14 16:22:39 2002
=================================================
```

BMDS MODEL RUN
~~~~~~~~~~~~~~~~~

The form of the response function is:

Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 +

Dependent variable = MEAN
Independent variable = dose
rho is set to 0
The polynomial coefficients are restricted to be negative
A constant variance model is fit

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

alpha =	0.0098315	
rho =	0	Specified
beta_0 =	0.439645	
beta_1 =	-0.0221168	
beta_2 =	0	

Parameter Estimates

Variable	Estimate	Std. Err
alpha	0.00898754	0.00284211
beta_0	0.402624	0.0266986
beta_1	-0.00443496	0.00156066
beta_2	0	NA

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	beta_0	beta_1
alpha	1	-2e-008	5.4e-009
beta_0	-2e-008	1	-0.61
beta_1	5.4e-009	-0.61	1
~~~~~~~~~~~~~~~~~

The following parameter(s) have been estimated at a boundary
point or have been specified. Correlations are not computed:

beta

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	5	0.446	0.087	0.403	0.0948	2.29
1.8	5	0.392	0.152	0.395	0.0948	-0.139
6.2	5	0.325	0.042	0.375	0.0948	-2.64
33.6	5	0.263	0.083	0.254	0.0948	0.495

Model Descriptions for likelihoods calculated

```
Model A1          Yij = Mu(i) + e(ij)
            Var{e(ij)} = Sigma^2

Model A2          Yij = Mu(i) + e(ij)
            Var{e(ij)} = Sigma(i)^2

Model  R:          Yi = Mu + e(i
             Var{e(i)} = Sigma^2
```

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	38.453073	5	-66.906146
A2	42.155047	8	-68.310094
fitted	37.119156	2	-70.238312
R	33.214605	2	-62.429209

Test 1 Does response and/or variances differ among dose levels
(A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	17.8809	6	0.0004654
Test 2	7.40395	3	0.06008
Test 3	2.66783	1	0.1024

The p-value for Test 1 is less than .05. There appears to be a

difference between response and/or variances among the
dose levels.
It seems appropriate to model the data

The p-value for Test 2 is greater than 05. A
homogeneous variance
model appears to be appropriate here

The p-value for Test 3 is greater than .05 The model
chosen appears
to adequately describe the data

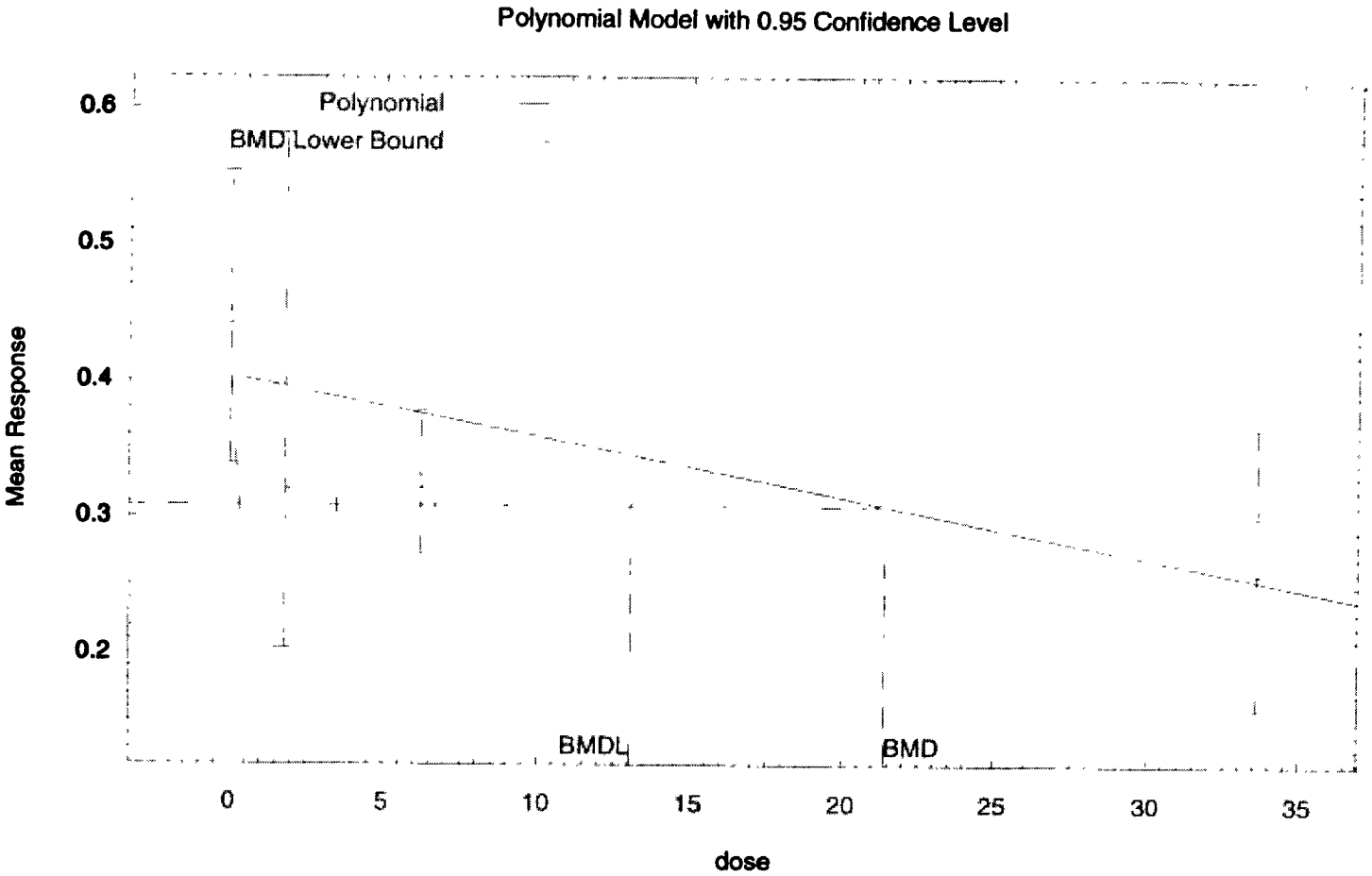
Benchmark Dose Computation
Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 21.3762

BMDL = 13.0626



16:22 05/14 2002

Antibody titer

```
==========================================================================
        Hill Model. $Revision: 2.1 $ $Date: 2000/10/11 21:21:23 $
        Input Data File: C:\BMDS\DATA\CONT.(d)
        Gnuplot Plotting File:  C:\BMDS\DATA\CONT.plt
                                                    Tue May 14 15:53:39 2002
==========================================================================

BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

  The form of the response function is:

  Y[dose] = intercept + v*dose^n/(k^n + dose^n)


  Dependent variable = MEAN
  Independent variable = Dose
  rho is set to 0
  Power parameter restricted to be greater than 1
  A constant variance model is fit

  Total number of dose groups = 4
  Total number of records with missing values = 0
  Maximum number of iterations = 250
  Relative Function Convergence has been set to: 1e-008
  Parameter Convergence has been set to: 1e-008


                 Default Initial Parameter Values
                         alpha =   0.00678961
                           rho =          0   Specified
                     intercept =      0.446
                             v =     -0.183
                             n =    1.39743
                             k =    4.26269

          Asymptotic Correlation Matrix of Parameter Estimates

                   alpha        rho    intercept          v          n
 k

     alpha           1          0            0          0          0
 0

       rho           0          1            0          0          0
 0

 intercept           0          0            1          0          0
 0

         v           0          0            0          1          0
 0

         n           0          0            0          0          1
 0

         k           0          0            0          0          0
 1
```

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	0.0078652	1
rho	0	1
intercept	0.446	1
v	-0.200218	1
n	1.14792	1
k	4.2868	1

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	5	0.446	0.087	0.446	0.0887	-1.73e-008
1.8	5	0.392	0.152	0.392	0.0887	3.45e-007
6.2	5	0.325	0.042	0.325	0.0887	1.51e-007
33.6	5	0.263	0.083	0.263	0.0887	-1.34e-007

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Degrees of freedom for Test A1 vs fitted <= 0

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	38.453073	5	-66.906146
A2	42.155047	8	-68.310094
fitted	38.453073	5	-66.906146
R	33.214605	2	-62.429209

Test 1: Does response and/or variances differ among dose levels (A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	17.8809	6	0.0004654
Test 2	7.40395	3	0.06008
Test 3	5.46761e-007	0	NA

The p-value for Test 1 is less than .05. There appears to be a

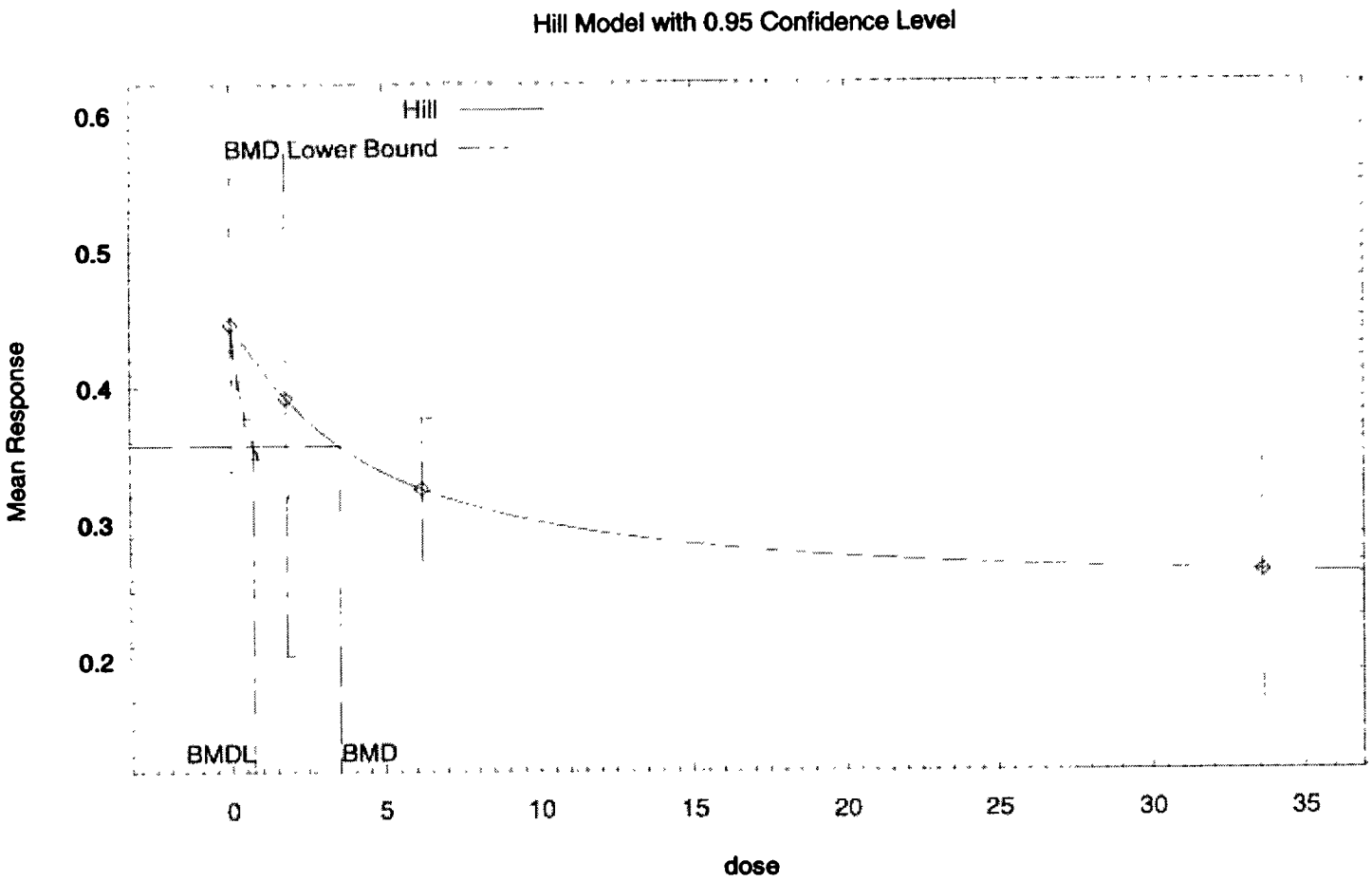
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .05. A homogeneous variance model appears to be appropriate here

NA - Degrees of freedom for Test 3 are less than or equal to 0. The Chi-Square test for fit is not valid

Benchmark Dose Computation

Specified effect	=	1
Risk Type	=	Estimated standard deviations from the control mean
Confidence level	=	0.95
BMD	=	3.51089
BMDL	=	0.726794



15:53 05/14 2002

Exhibit D

United States
Environmental Protection
Agency

Office of Water
Office of Science and Technology
(4304T)

EPA-822-R-02-047
November 2002

National Recommended Water Quality Criteria: 2002

NOTICE

This document has been reviewed by the Health and Ecological Criteria Division, Office of Science and Technology, U.S. Environmental Protection Agency, and approved for publication.

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NOTICE i

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I Introduction

The U.S. Environmental Protection Agency (EPA) is publishing an updated compilation of its national recommended water quality criteria for 158 pollutants, developed pursuant to section 304(a) of the Clean Water Act (CWA or the Act). Section 304(a)(1) of the Act requires EPA to develop and publish, and from time to time revise, criteria for water quality accurately reflecting the latest scientific knowledge. Water quality criteria developed under section 304(a) are based solely on data and scientific judgments on the relationship between pollutant concentrations and environmental and human health effects. Section 304(a) criteria do not reflect consideration of economic impacts or the technological feasibility of meeting the chemical concentrations in ambient water.

The recommended water quality criteria contained in this document provide guidance for states and tribes authorized to establish water quality standards under the CWA to protect human health and aquatic life. Under the CWA, states and authorized tribes are to establish water quality standards to protect designated uses. Such standards are used in implementing a number of environmental programs, including setting discharge limits in National Pollutant Discharge Elimination System (NPDES) permits. While this document constitutes the EPA's guidance regarding ambient concentrations for various pollutants, this document does not substitute for the CWA or EPA's regulations; nor is it a regulation itself. Thus, it cannot impose legally binding requirements on the EPA, states, authorized tribes or the regulated community, and might not apply to a particular situation based upon the circumstances. State and tribal decision-makers retain the discretion to adopt approaches on a case-by-case basis that differ from this guidance when appropriate. The EPA may change this guidance in the future.

II What is in this Updated Compilation?

EPA developed an updated compilation of its national recommended water quality criteria (NRWQC) for pollutants. The criteria in this document supercede any Clean Water Act (CWA) section 304(a) water quality criteria published in EPA's previous criteria compilations including: the "*Blue Book*," "*Red Book*," "*Gold Book*" and EPA's last compilation of national recommended water quality criteria published on December 10, 1998 (63 FR 68354 or *National Recommended Water Quality Criteria– Correction*, April 1999, EPA 822-Z-99-001). Many of the human health criteria in this compilation have been revised based on EPA's new methodology for deriving human health criteria *(See: Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health* (2000), EPA-822-B-00-004, October 2000). Water quality criteria contained in this document may be superceded by the publication of section 304(a) water quality criteria subsequent to the publication of this document.

This compilation is presented as a summary table containing EPA's water quality criteria for 158 pollutants. For each set of criteria, EPA lists a **Federal Register** citation, EPA document number or Integrated Risk Information System (IRIS) entry (www.epa.gov/iris/index.html). Information pertinent to the derivation of individual criteria may be found in the cited references or footnotes. Still, these references may not be a single complete source of information for the

criteria derivation. You may need to refer to more than one document for complete information. A calculation matrix that contains all of the components (e.g., cancer potency factors (q1*s), reference doses (RfDs) and bioconcentration factors (BCFs)) and formulas used to derive the human health criteria in the compilation is available at http://www.epa.gov/waterscience/criteria/. EPA does not have national recommended water quality criteria for pollutants with no criteria listed.

The national recommended water quality criteria include: previously published criteria that are unchanged, criteria that have been recalculated from earlier criteria (63 FR68354, 12/10/1998) and newly calculated criteria based on peer-reviewed assessments and data.

The Agency intends to revise this compilation of national recommended water quality criteria from time to time to keep states and authorized tribes informed as to the most current recommended section 304(a) water quality criteria.

III What is the Relationship Between These Criteria and Your State or Tribal Water Quality Standards?

As part of the water quality standards triennial review process defined in Section 303(c)(1) of the CWA, the states and authorized tribes are responsible for maintaining and revising water quality standards. Water quality standards consist of designated uses, water quality criteria to protect those uses, a policy for antidegradation, and general policies for application and implementation. Section 303(c)(1) requires States and Tribes to review, and modify if appropriate, their water quality standards at least once every three years.

States and authorized tribes must adopt water quality criteria that protect designated uses. Protective criteria are based on a sound scientific rationale and contain sufficient parameters or constituents to protect the designated uses. Criteria may be expressed in either narrative or numeric form. States and authorized tribes have four options when adopting water quality criteria for which EPA has published section 304(a) criteria. They can: (1) establish numerical values based on recommended section 304(a) criteria; (2) adopt section 304(a) criteria modified to reflect site-specific conditions; (3) adopt criteria derived using other scientifically defensible methods; or (4) establish narrative criteria where numeric criteria cannot be determined (40 CFR 131.11).

Consistent with 40 CFR131.21 (See: *EPA Review and Approval of State and Tribal Water Quality Standards* (65 FR 24641, April 27, 2000)), water quality criteria adopted by law or regulation by States and Tribes prior to May 30, 2000, are in effect for CWA purposes unless superseded by federal regulations (see, for example, the *National Toxics Rule*, 40 CFR 131.36; *Water Quality Standards for Idaho*, 40 CFR 131.33). New or revised water quality criteria adopted into law or regulation by States and Tribes on or after May 30, 2000 are in effect for CWA purposes only after EPA approval.

IV What is the Status of Existing Recommended Criteria While They Are Under Revision?

Water quality criteria published by EPA remain the Agency's recommended water quality criteria until EPA revises or withdraws the criteria. For example, while undertaking recent reassessments of certain chemicals, EPA has consistently supported the use of the existing section 304(a) criteria for these chemicals and considers them to be scientifically sound until the Agency reevaluates the 304(a) criteria, subjects the criteria to appropriate peer review, and subsequently publishes revised 304(a) criteria.

V What's New in the Compilation?

Human Health Criteria

Revised Human Health Criteria

EPA revised the methodology it uses to develop water quality criteria for protection of human health. The revised methodology entitled, *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health* (2000) (EPA-822-B-00-004, October 2000) and a **Federal Register** notice (65 FR 66443, 11/3/2000) both describe the Agency's current approach for deriving national recommended water quality criteria to protect human health.

EPA has revised many of the human health criteria in the compilation based on this new methodology. By and large, these represent partial updates of the 304(a) criteria as described in both the draft Methodology revisions and the **Federal Register** notice that accompanied the final Methodology (65 FR 66443). EPA received much support for revising criteria based on partially updated components of the criteria equations as a way of increasing the frequency of scientific improvements to the nationally recommended criteria that currently available information would allow. For a water quality criterion revision based on a partial update to be considered acceptable to EPA, a component of the criterion (e.g., the toxicological risk assessment) would need to be comprehensive (e.g., a new or revised RfD or cancer dose-response assessment, as opposed to simply a new scaling factor), should stand alone and be based on new national or local data. These recalculations of water quality criteria integrate an updated national default freshwater/estuarine fish consumption rate (17.5 g/day) and, in some cases, previously determined relative source contribution (RSC) values obtained from primary drinking water standards as well as new cancer potency information from the Agency's Integrated Risk Information System (IRIS).

EPA has not revised the human health criteria in today's compilation to include bioaccumulation factors (BAFs) based on the 2000 Methodology. The BAF component of criteria development is comparatively time- and resource-intensive. As previously indicated, EPA received support for partial updates rather than waiting a substantially longer period of time for BAFs to be developed. EPA also received support for the idea of not automatically developing BAFs for all of the currently published criteria, but rather revising criteria for

pollutants of high priority and national importance. EPA intends to focus its limited resources on developing BAFs for pollutants that the Agency considers highest priority in terms of toxicological concern, frequency of occurrence and bioaccumulation potential. EPA is in the process of determining candidate pollutants for BAF derivations. The human health criteria were developed with BCFs or field-measured BAFs previously developed using the 1980 Methodology. The BCFs used in deriving these criteria are consistent with BCFs used in promulgating human health criteria for priority toxic pollutants in rules such as the 1992 *National Toxics Rule* and the 2000 *California Toxics Rule.*

Not all of EPA's national recommended water quality criteria for protection of human health were revised in this compilation. Criteria currently undergoing major reassessments, such as arsenic, chloroform and nickel, were not revised at this time. Water quality criteria that predate EPA's 1980 human health methodology were not revised for this effort. EPA believes revisions for these criteria, which include barium, 2,4-D and 2,4,5-TP, iron, manganese, methoxychlor, nitrates and solids (dissolved) and salinity may warrant major reassessments. Updated IRIS information and/or RSC values are available for vinyl chloride, 1,1-dichloroethylene, 1,3-dichloropropene, thallium, chlorobenzene, cyanide, ethylbenzene, toluene, 1,2-trans-dichloroethylene, 1,2-dichlorobenzene, 1,4-dichlorobenzene, hexachlorocyclopentadiene, 1,2,4-trichlorobenzene, Lindane and Endrin. Yet, EPA did not update the criteria for the preceeding 15 chemicals in this document. EPA intends to publish revisions for the aformentioned chemicals in a separate **Federal Register** notice, soliciting scientific views regarding these revisions.

New IRIS information was available and incorporated for benzene. EPA revised the criteria for benzene based on the new cancer slope factors, which are presented in IRIS as a range. The set of risk estimates falling within this interval reflects both the inherent uncertainties in the risk assessment of benzene and the limitations of the epidemiological studies in determining dose-response and exposure data. EPA modeled the unit risk values for the carcinogenicity of benzene from epidemiological data on occupationally exposed humans. The unit risk is expressed as a range rather than a single value because in the case of this particular pollutant there is no scientific basis for choosing a single result from various model estimates. (See IRIS Benzene CASRN 71-43-2 (01/19/2000) at: www.epa.gov/iris/subst/0276.htm). The resulting ranges of criteria for protection of human health from consumption of water and organisms and consumption of organisms only are 0.61- 2.2 ug/L and 14- 51 ug/L, respectively. The Agency calculated the criteria as ranges, but is recommending the use of the upper limits in the criteria table. EPA considers any criterion in each range scientifically defensible, yet only the upper limit of each range is presented to establish the upper bound of the average ambient concentration that should not be exceeded.

For copper and asbestos, the Agency chose to base the section 304(a) water quality criteria on the Agency's drinking water maximum contaminant levels (MCLs) or action levels which are established on drinking water regulation methodologies.

A notice of intent to revise or develop human health criteria for arsenic, methylmercury and carbofuran was published in the **Federal Register** on October 12, 2000 (65 FR 60664). This

notice informed the public that EPA was in the process of revising or developing criteria for these pollutants and solicited any significant scientific information the public may be aware of that was not identified in EPA's literature search. A human health criterion for methylmercury is now available. Work is ongoing for arsenic and carbofuran.

Methylmercury

On January 8, 2001, EPA announced the availability of a recommended water quality criterion for methylmercury (66 FR 1344). In the January 8, 2001 notice, EPA withdrew its previous ambient human health water quality criteria for mercury (see 63 FR 68354, December 10, 1998; correction in 64 FR 19781, April 22, 1999) as the recommended section 304(a) water quality criteria. This updated compilation contains the new methylmercury criterion. This new water quality criterion describes the concentration of methylmercury in freshwater and estuarine fish and shellfish tissue that should not be exceeded to protect consumers of fish and shellfish among the general population. EPA expects the criterion recommendation to be used as guidance by states, tribes, and EPA in establishing or updating water quality standards for waters of the United States and in issuing fish and shellfish consumption advisories. This is the first time EPA has issued a water quality criterion expressed as a fish and shellfish tissue value rather than as a water column value. This approach is a direct consequence of the scientific consensus that consumption of contaminated fish and shellfish is the primary human route of exposure to methylmercury. EPA recognizes that this approach differs from traditional water column criteria, and will pose implementation challenges. In the January 8, 2001 notice EPA provided suggested approaches for relating the fish and shellfish tissue criterion to concentrations of methylmercury in the water column. EPA also plans to develop more detailed guidance to assist states and authorized tribes with implementation of the methylmercury criterion in water quality standards and related programs.

Dioxin

The section 304(a) water quality criteria for dioxin contained in this compilation is expressed in terms of 2,3,7,8-Tetrachloro-dibenzo-p-dioxin (2,3,7,8-TCDD) and should be used in conjunction with the national/international convention of toxicity equivalence factors (TEF/TEQs) to account for the additive effects of other dioxin-like compounds (dioxins). EPA supports the use of either the 1989 interim procedures or the 1998 World Health Organization (WHO) TEF scheme, but prefers the 1998 WHO TEF scheme because it is based on more recent data and is internationally accepted. (See: *Update to the Interim Procedures for Estimating Risks Associated with Exposures to Mixtures of Chlorinated Dibenzo-p-dioxins and -dibenzofurans*, EPA/625/3-89/016, March 1989 and Van den Berg M., 1998). By applying the TEF/TEQ approach, the other highly toxic dioxins will be properly taken into account.

Aquatic Life Criteria

Revised Definition of Total PCBs for Aquatic Life Criteria

The aquatic life criteria for Polychlorinated Biphenyls (PCBs) in this compilation apply to total PCBs. The definition of total PCBs is the sum of all homolog, all isomer, all congener, or all Aroclor analyses. The aquatic life criteria contained in the previous publication of the NRWQC (63 FR 68354, 12/10/1998) were based on total PCB concentrations, but the definition of total PCBs only applied to the sum of seven particular Aroclors (1242, 1254, 1221, 1232, 1248, 1260 and 1016, CAS numbers 53469219, 11097691, 11104282, 11141165, 12672296, 11096825, and 12674112, respectively). This revision of the aquatic life criteria harmonizes the total PCB definition with that used for EPA's human health criteria for PCBs.

Saltwater Dissolved Oxygen (Cape Cod to Cape Hatteras)

This new compilation of NRWQC contains saltwater criteria for dissolved oxygen (D.O.). EPA's new water quality criteria are the result of a 10-year research effort to produce sufficient information to support their development. The water quality criteria presented today represent EPA's best estimates, based on the data available, of D.O. concentrations necessary to protect aquatic life and uses associated with aquatic life.

EPA published saltwater criteria for dissolved oxygen because hypoxia (low dissolved oxygen) is a significant problem for certain coastal waters that receive runoff containing nutrients (e.g., nitrogen, phosphorous) and other oxygen demanding biological wastes. Excessive amounts of nutrients in aquatic systems stimulate algal growth which can deplete available dissolved oxygen required to maintain healthy fish and shellfish populations. EPA's Environmental Monitoring and Assessment Program (EMAP) for the estuaries in the Virginian Province (defined as Cape Cod to Cape Hatteras) has shown that 25% of the area of the Virginian Province exhibits dissolved oxygen concentrations of less than 5 mg/L. For many fish and shellfish, extended periods of D.O. below 5 mg/L can cause adverse effects to larval life stages. EMAP also has generated field observations that correlate many of the biologically degraded benthic areas with low dissolved oxygen in the lower water column. These two reports emphasize that hypoxia is a major concern within the Virginian Province, among other coastal locations of the United States.

The geographic scope of the saltwater dissolved oxygen water quality criteria are limited to coastal saltwaters of the Virginian Province of the Atlantic coast of the United States (i.e., southern Cape Cod, MA, to Cape Hatteras, NC). However, the saltwater dissolved oxygen criteria can be applied elsewhere if the species and data used to derive the criteria for the Virginia Province are applicable to location-specific biological, physical, and water quality conditions. EPA believes that the overall approach for deriving marine water quality criteria for D.O. contained in *Ambient Water Quality Criteria for Dissolved Oxygen (Saltwater): Cape Cod to Cape Hatteras* is applicable to regions outside the Virginian Province.

For additional information see: *Ambient Water Quality Criteria for Dissolved Oxygen (Saltwater): Cape Cod to Cape Hatteras* (EPA-822R-00-012).

Freshwater Aquatic Life Criteria for Ammonia

This compilation includes EPA's latest revision (*1999*) of the freshwater aquatic life criteria for ammonia. The new water quality criteria reflect research and data since 1984, including the pH and temperature relationship of the acute and chronic criteria and the averaging period of the chronic criterion. The revised acute criterion for ammonia is now dependent on pH and fish species, and the chronic criterion is dependent on pH and temperature. At lower temperatures, the chronic criterion incorporates the presence or absence of early life stages of fish. The temperature dependency results in a gradual increase in the criterion as temperature decreases. At temperatures below 15 °C the criterion is more stringent, when early life stages of fish are expected to be present. EPA's recommendations in the 1999 criteria represent a change from both the 1984 chronic ammonia criterion, which was dependent mainly on pH, and from the 1998 ammonia criteria, in which the chronic criterion was dependent on pH and the presence of early life stages of fish.

For additional information see: *1999 Update of Ambient Water Quality Criteria for Ammonia* (EPA-822-R-99-014).

Cadmium

EPA revised its aquatic life criteria for cadmium. A notice was published in the **Federal Register** (65 FR 50201, 8/17/2000) announcing the availability of the peer review draft and soliciting any significant scientific input from the public. EPA has addressed the peer review comments and significant issues raised by the public. A notice of availability for the completed document, entitled *2001 Update of Ambient Water Quality Criteria for Cadmium* (EPA-822-R-01-001) was published in the **Federal Register** on April 12, 2001 (66 FR18935).

Guidance on the Calculation of Hardness-Dependent Metals Criteria

Freshwater aquatic life criteria for certain metals are expressed as a function of hardness because hardness can affect the toxicities of these metals. Hardness is used as a surrogate for a number of water quality characteristics which affect the toxicity of metals. Increasing hardness has the effect of decreasing the toxicity of metals. Water quality criteria to protect aquatic life may be calculated at different concentrations of hardness measured in milligrams per liter (mg/L) as calcium carbonate ($CaCO_3$).

Appendix B of this document presents the hardness-dependent equations for freshwater metals criteria. The specific values in the table are calculated at a hardness of 100 mg/L ($CaCO_3$) for illustrative purposes only. The hardness equations included in this compilation were developed based on results from laboratory toxicity tests that were conducted in fresh waters encompassing a range of hardness values. Although the amount of data and the strength of the

relationship vary for different metals, almost all data for hardness and toxicity are in the 20 to 400 mg/L hardness range.

In the past, EPA recommended that when the hardness of fresh surface water is less than 25 mg/L, 304(a) criteria concentrations be calculated as if the hardness is 25 mg/L. Available toxicity data in this range for copper, zinc and cadmium (EPA 440/5-84-031, EPA 440/5-87-003,and EPA-822-R-01-001) are somewhat limited, and are quite limited for silver, lead, chromium III and nickel (EPA 440/5-80-071, EPA 440/5-84-027,EPA 440/5-84-029 and EPA 440/5-86-004). Even fewer data are available below 20 mg/L hardness for copper, zinc and cadmium and none are available for silver, lead, chromium III and nickel. EPA evaluated these limited data, available in the current metals' criteria documents, and determined that they are inconclusive. Capping hardness at 25 mg/L without additional data or justification may result in criteria that provide less protection than that intended by EPA's *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (EPA 822/R-85-100) or "the Guidelines." Therefore, EPA now recommends that hardness not be capped at 25 mg/L, or any other hardness on the low end. If there is a state or tribal regulatory requirement that hardness be capped at 25 mg/L, or if there are any situation-specific questions about the applicability of the hardness-toxicity relationship, a Water Effect Ratio (WER) procedure should be used to provide the level of protection intended by the Guidelines. When an ambient hardness of less than 25 mg/L is used to establish criteria for lead or cadmium, the hardness dependent Conversion Factor (CF) should not exceed one.

For hardness over 400 mg/L, EPA recommends two options: (1) calculate the criterion using a default WER of 1.0 and using a hardness of 400 mg/L in the hardness equation; or (2) calculate the criterion using a WER and the actual ambient hardness of the surface water in the equation. The second option is expected to result in the level of protection intended in the Guidelines whereas the first option is thought to result in an even more protective aquatic life criterion. At high hardness there is an indication that hardness and related inorganic water quality characteristics do not have as much of an effect on toxicity of metals as they do at lower hardnesses. Related water quality characteristics do not correlate as well at higher hardnesses as they do at lower hardnesses. There is also increased uncertainty in this range because very limited data are available to clearly quantify the relationship between hardness and toxicity. Therefore, if hardness is over 400 mg/L as $CaCO_3$, EPA continues to recommend that a hardness of 400 mg/L be used with a default WER of 1.0; alternatively, the WER and actual hardness of the surface water may be used.

Where applicable water quality standards require the use of a default hardness (e.g., 25 mg/L) to calculate a criterion, states and authorized tribes should use the WER procedure to adjust that criterion so that it provides the level of protection intended by the Guidelines. As the WER is inherently a site-specific procedure, any WER developed for a given site would be applicable only for that site unless its applicability at other sites is demonstrated. In any case, states and authorized tribes electing to use the WER Guidance should ensure that their water quality standards provide for them. Consistent with the "Performance-Based Approach" discussed in detail in EPA's recent modification of its implementing regulations at 40 CFR

131.21 (See 65 FR 24641, April 27, 2000), EPA encourages states and authorized tribes to identify all opportunities for adoption, and EPA approval of, such site-specific criteria. A performance-based approach relies on the adoption of a standard method or process (e.g., WER procedures) into state or tribal water quality standards, rather than adoption of a specific outcome (e.g., a site-specific criterion). When such an approach is sufficiently detailed and has suitable safeguards to ensure predictable, repeatable outcomes, EPA approval of the approach can serve as approval of the outcomes as well.

National Guidance on the Applicability of Freshwater and Saltwater Criteria

EPA recommends that the aquatic life criteria in this compilation apply as follows:

(1) For water in which the salinity is equal to or less than 1 part per thousand 95% or more of the time, the applicable criteria are the freshwater criteria.

(2) For water in which the salinity is equal to or greater than 10 parts per thousand 95% or more of the time, the applicable criteria are the saltwater criteria in Column C; and

(3) For water in which the salinity is between 1 and 10 parts per thousand the applicable criteria are the more stringent of the freshwater or saltwater criteria, as described in items (1) and (2) of this section. However, an alternative freshwater or saltwater criteria may be used if scientifically defensible information and data demonstrate that on a site-specific basis the biology of the water body is dominated by freshwater aquatic life and that freshwater criteria are more appropriate; or conversely, the biology of the water body is dominated by saltwater aquatic life and that saltwater criteria are more appropriate.

Nutrient Criteria

EPA recently developed section 304(a) water quality criteria for nutrients because excessive levels of nutrients are a major cause of the nonattainment of designated uses and more scientific information is needed to evaluate and address these conditions. Availability of these nutrient criteria recommendations was announced in the **Federal Register** on January 9, 2001 (66 FR1671). EPA's nutrient criteria will cover four major types of waterbodies: lakes and reservoirs, rivers and streams, estuarine and coastal areas, and wetlands across 14 major ecoregions of the United States. EPA's section 304(a) criteria are intended to provide for the protection and propagation of aquatic life and recreation. To support the development of the nutrient criteria, EPA published Technical Guidance Manuals that describe a process for assessing nutrient conditions in the four waterbody types. (For example See: *Nutrient Criteria Technical Guidance Manual: Lakes and Reservoirs*, April 2000; *Nutrient Criteria Technical Guidance Manual; Rivers and Streams*, July 2000; *Nutrient Criteria Technical Guidance Manual: Estuarine and Coastal Marine Waters*, October 2001). This information is intended to serve as a starting point for the states, authorized tribes and others to develop more refined

nutrient criteria, as appropriate, using EPA waterbody-specific technical guidance manuals and other scientifically defensible approaches.

In cases where no new ecoregional nutrient criteria are available, the phosphate phosphorus information presented in the Gold Book (*Quality Criteria for Water:1986*, EPA 440/5-86-001) may still be applicable. The phosphate phosphorus information in the Gold Book address eutrophication in freshwater systems on a national basis without any consideration of regional differences that may occur. The new ecoregional nutrient criteria recommendations reflect regional differences in eutrophication for different water body types and ecological conditions. The criteria in the compilation for elemental phosphorus is based on the toxic effects of phosphorus to aquatic organisms and is not intended to prevent eutrophication.

VI Comments on the December 10, 1998 National Recommended Water Quality Criteria Compilation

When the national recommended water quality criteria compilation was published in 1998, the Agency requested comment and observations on the compilation format and on the revised criteria development process. No comments were received on the format or process. Comments were, however, received on specific criteria and errors in formulas. The comments stated that certain criteria did not reflect the latest scientific knowledge and also that some criteria were derived based on data where an improper form of chemical was tested because of bioavailability issues. The errors in the formulas have been corrected. EPA does not agree that an improper form of the specified chemicals was tested.

VII Where Can I Find More Information About Water Quality Criteria and Water Quality Standards?

For more information about water quality criteria and Water Quality Standards refer to the following: *Water Quality Standards Handbook* (EPA 823-B94-005a); *Advanced Notice of Proposed Rule Making (ANPRM)*, (63FR36742); *Draft Strategy for Water Quality Standards and Criteria: Strengthening the Foundation of Programs to Protect and Restore the Nation's Water (EPA-823-R-02-001, May 2002); Water Quality Criteria and Standards Plan -- Priorities for the Future* (EPA 822-R-98-003); *Guidelines and Methodologies Used in the Preparation of Health Effects Assessment Chapters of the Consent Decree Water Criteria Documents* (45FR79347); *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health* (2000), EPA-822-B-00-004, October 2000); *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (EPA 822/R-85-100); *National Strategy for the Development of Regional Nutrient Criteria* (EPA 822-R-98-002); and *EPA Review and Approval of State and Tribal Water Quality Standards* (65FR24641).

These publications may also be accessed through EPA's National Service Center for Environmental Publications (NSCEP, previously NCEPI) or on the Office of Science and Technology's Homepage (www.epa.gov/OST).

VIII What Are the National Recommended Water Quality Criteria?

The following compilation and its associated footnotes and notes presents the EPA's latest national recommended water quality criteria.

NATIONAL RECOMMENDED WATER QUALITY CRITERIA FOR PRIORITY TOXIC POLLUTANTS

	Priority Pollutant	CAS Number	Freshwater CMC (µg/L)	Freshwater CCC (µg/L)	Saltwater CMC (µg/L)	Saltwater CCC (µg/L)	Human Health For Consumption of: Water + Organism (µg/L)	Human Health For Consumption of: Organism Only (µg/L)	FR Cite/ Source
1	Antimony	7440360					5.6 B	640 B	65FR66443
2	Arsenic	7440382	340 A,D,K	150 A,D,K	69 A,D,bb	36 A,D,bb	0.018 C,M,S	0.14 C,M,S	65FR31682 57FR60848
3	Beryllium	7440417					Z		65FR31682
4	Cadmium	7440439	2.0 D,E,K,bb	0.25 D,E,K,bb	40 D,bb	8.8 D,bb	Z		EPA-822-R-01-001 65FR31682
5a	Chromium (III)	16065831	570 D,E,K	74 D,E,K			Z Total		EPA820/B-96-001 65FR31682
5b	Chromium (VI)	18540299	16 D,K	11 D,K	1,100 D,bb	50 D,bb	Z Total		65FR31682
6	Copper	7440508	13 D,E,K,cc	9.0 D,E,K,cc	4.8 D,cc,ff	3.1 D,cc,ff	1,300 U		65FR31682
7	Lead	7439921	65 D,E,bb,gg	2.5 D,E,bb,gg	210 D,bb	8.1 D,bb			65FR31682
8a	Mercury	7439976	1.4 D,K,hh	0.77 D,K,hh	1.8 D,ee,hh	0.94 D,ee,hh			62FR42160
8b	Methylmercury	22967926						0.3 mg/kg J	EPA823-R-01-001
9	Nickel	7440020	470 D,E,K	52 D,E,K	74 D,bb	8.2 D,bb	610 B	4,600 B	65FR31682
10	Selenium	7782492	L,R,T	5.0 T	290 D,bb,dd	71 D,bb,dd	170 Z	4200	62FR42160 65FR31682 65FR66443
11	Silver	7440224	3.2 D,E,G		1.9 D,G				65FR31682
12	Thallium	7440280					1.7 B	6.3 B	65FR31682

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13	Zinc	7440666	120 D,E,K	120 D,E,K	90 D,bb	81 D,bb	7,400 U	26,000 U	65FR31682 65FR66443
14	Cyanide	57125	22 K,Q	5.2 K,Q	1 Q,bb	1 Q,bb	700 B	220,000 B,H	EPA820/B-96-001 57FR60848
15	Asbestos	1332214					7 million fibers/L I		57FR60848
16	2,3,7,8-TCDD (Dioxin)	1746016					5.0E-9 C	5.1E-9 C	65FR66443
17	Acrolein	107028					190	290	65FR66443
18	Acrylonitrile	107131					0.051 B,C	0.25 B,C	65FR66443
19	Benzene	71432					2.2 B,C	51 B,C	IRIS 01/19/00 &65FR66443
20	Bromoform	75252					4.3 B,C	140 B,C	65FR66443
21	Carbon Tetrachloride	56235					0.23 B,C	1.6 B,C	65FR66443
22	Chlorobenzene	108907					680 B,Z,U,	21,000 B,H,U	65FR31682
23	Chlorodibromomethane	124481					0.40 B,C	13 B,C	65FR66443
24	Chloroethane	75003							
25	2-Chloroethylvinyl Ether	110758							
26	Chloroform	67663					5.7 C,P	470 C,P	62FR42160
27	Dichlorobromomethane	75274					0.55 B,C	17 B,C	65FR66443

	Priority Pollutant	CAS Number	Freshwater CMC (µg/L)	Freshwater CCC (µg/L)	Saltwater CMC (µg/L)	Saltwater CCC (µg/L)	Human Health For Consumption of: Water + Organism (µg/L)	Human Health For Consumption of: Organism Only (µg/L)	FR Cite/ Source
28	1,1-Dichloroethane	75343							
29	1,2-Dichloroethane	107062					0.38 B,C	37 B,C	65FR66443
30	1,1-Dichloroethylene	75354					0.057 C	3.2 C	65FR66443
31	1,2-Dichloropropane	78875					0.50 B,C	15 B,C	65FR66443
32	1,3-Dichloropropene	542756					10	1,700	57FR60848
33	Ethylbenzene	100414					3,100 B	29,000 B	65FR31682
34	Methyl Bromide	74839					47 B	1,500 B	65FR66443
35	Methyl Chloride	74873							65FR31682
36	Methylene Chloride	75092					4.6 B,C	590 B,C	65FR66443
37	1,1,2,2-Tetrachloroethane	79345					0.17 B,C	4.0 B,C	65FR66443
38	Tetrachloroethylene	127184					0.69 C	3.3 C	65FR66443
39	Toluene	108883					6,800 B,Z	200,000 B	65FR31682
40	1,2-Trans-Dichloroethylene	156605					700 B,Z	140,000 B	65FR31682
41	1,1,1-Trichloroethane	71556					Z		65FR31682
42	1,1,2-Trichloroethane	79005					0.59 B,C	16 B,C	65FR66443
43	Trichloroethylene	79016					2.5 C	30 C	65FR66443
44	Vinyl Chloride	75014					2.0 C	530 C	57FR60848

	Priority Pollutant	CAS Number	Freshwater CMC (µg/L)	Freshwater CCC (µg/L)	Saltwater CMC (µg/L)	Saltwater CCC (µg/L)	Human Health For Consumption of: Water + Organism (µg/L)	Human Health For Consumption of: Organism Only (µg/L)	FR Cite/ Source
45	2-Chlorophenol	95578					81 B,U	150 B,U	65FR66443
46	2,4-Dichlorophenol	120832					77 B,U	290 B,U	65FR66443
47	2,4-Dimethylphenol	105679					380 B	850 B,U	65FR66443
48	2-Methyl-4,6-Dinitrophenol	534521					13	280	65FR66443
49	2,4-Dinitrophenol	51285					69 B	5,300 B	65FR66443
50	2-Nitrophenol	88755							
51	4-Nitrophenol	100027							
52	3-Methyl-4-Chlorophenol	59507					U	U	
53	Pentachlorophenol	87865	19 F,K	15 F,K	13 bb	7.9 bb			65FR31682
							0.27 B,C	3.0 B,C,H	65FR66443
54	Phenol	108952					21,000 B,U	1,700,000 B,U	65FR66443
55	2,4,6-Trichlorophenol	88062					1.4 B,C	2.4 B,C,U	65FR66443
56	Acenaphthene	83329					670 B,U	990 B,U	65FR66443
57	Acenaphthylene	208968							
58	Anthracene	120127					8,300 B	40,000 B	65FR66443
59	Benzidine	92875					0.000086 B,C	0.00020 B,C	65FR66443
60	Benzo(a)Anthracene	56553					0.0038 B,C	0.018 B,C	65FR66443
61	Benzo(a)Pyrene	50328					0.0038 B,C	0.018 B,C	65FR66443

	Priority Pollutant	CAS Number	Freshwater CMC (µg/L)	Freshwater CCC (µg/L)	Saltwater CMC (µg/L)	Saltwater CCC (µg/L)	Human Health For Consumption of: Water + Organism (µg/L)	Human Health For Consumption of: Organism Only (µg/L)	FR Cite/ Source
62	Benzo(b)Fluoranthene	205992					0.0038 B,C	0.018 B,C	65FR66443
63	Benzo(ghi)Perylene	191242							
64	Benzo(k)Fluoranthene	207089					0.0038 B,C	0.018 B,C	65FR66443
65	Bis(2-Chloroethoxy)Methane	111911							
66	Bis(2-Chloroethyl)Ether	111444					0.030 B,C	0.53 B,C	65FR66443
67	Bis(2-Chloroisopropyl)Ether	108601					1,400 B	65,000 B	65FR66443
68	Bis(2-Ethylhexyl)Phthalate[X]	117817					1.2 B,C	2.2 B,C	65FR66443
69	4-Bromophenyl Phenyl Ether	101553							
70	Butylbenzyl Phthalate[W]	85687					1,500 B	1,900 B	65FR66443
71	2-Chloronaphthalene	91587					1,000 B	1,600 B	65FR66443
72	4-Chlorophenyl Phenyl Ether	7005723							
73	Chrysene	218019					0.0038 B,C	0.018 B,C	65FR66443
74	Dibenzo(a,h)Anthracene	53703					0.0038 B,C	0.018 B,C	65FR66443
75	1,2-Dichlorobenzene	95501					2,700 B	17,000 B	65FR31682
76	1,3-Dichlorobenzene	541731					320	960	65FR66443
77	1,4-Dichlorobenzene	106467					400 Z	2,600	65FR31682
78	3,3'-Dichlorobenzidine	91941					0.021 B,C	0.028 B,C	65FR66443

	Priority Pollutant	CAS Number	Freshwater CMC (µg/L)	Freshwater CCC (µg/L)	Saltwater CMC (µg/L)	Saltwater CCC (µg/L)	Human Health For Consumption of: Water + Organism (µg/L)	Human Health For Consumption of: Organism Only (µg/L)	FR Cite/ Source
79	Diethyl Phthalate[W]	84662					17,000 B	44,000 B	65FR66443
80	Dimethyl Phthalate[W]	131113					270,000	1,100,000	65FR66443
81	Di-n-Butyl Phthalate[W]	84742					2,000 B	4,500 B	65FR66443
82	2,4-Dinitrotoluene	121142					0.11 C	3.4 C	65FR66443
83	2,6-Dinitrotoluene	606202							
84	Di-n-Octyl Phthalate	117840							
85	1,2-Diphenylhydrazine	122667					0.036 B,C	0.20 B,C	65FR66443
86	Fluoranthene	206440					130 B	140 B	65FR66443
87	Fluorene	86737					1,100 B	5,300 B	65FR66443
88	Hexachlorobenzene	118741					0.00028 B,C	0.00029 B,C	65FR66443
89	Hexachlorobutadiene	87683					0.44 B,C	18 B,C	65FR66443
90	Hexachlorocyclopentadiene	77474					240 U,Z	17,000 H,U	57FR60848
91	Hexachloroethane	67721					1.4 B,C	3.3 B,C	65FR66443
92	Ideno(1,2,3-cd)Pyrene	193395					0.0038 B,C	0.018 B,C	65FR66443
93	Isophorone	78591					35 B,C	960 B,C	65FR66443
94	Naphthalene	91203							
95	Nitrobenzene	98953					17 B	690 B,H,U	65FR66443

	Priority Pollutant	CAS Number	Freshwater CMC (µg/L)	Freshwater CCC (µg/L)	Saltwater CMC (µg/L)	Saltwater CCC (µg/L)	Human Health For Consumption of: Water + Organism (µg/L)	Human Health For Consumption of: Organism Only (µg/L)	FR Cite/ Source
96	N-Nitrosodimethylamine	62759					0.00069 B,C	3.0 B,C	65FR66443
97	N-Nitrosodi-n-Propylamine	621647					0.0050 B,C	0.51 B,C	65FR66443
98	N-Nitrosodiphenylamine	86306					3.3 B,C	6.0 B,C	65FR66443
99	Phenanthrene	85018							
100	Pyrene	129000					830 B	4,000 B	65FR66443
101	1,2,4-Trichlorobenzene	120821					260	940	IRIS11/01/96
102	Aldrin	309002	3.0 G		1.3 G		0.000049 B,C	0.000050 B,C	65FR31682 65FR66443
103	alpha-BHC	319846					0.0026 B,C	0.0049 B,C	65FR66443
104	beta-BHC	319857					0.0091 B,C	0.017 B,C	65FR66443
105	gamma-BHC (Lindane)	58899	0.95 K		0.16 G		0.019 C	0.063 C	65FR31682 65FR66443
106	delta-BHC	319868							
107	Chlordane	57749	2.4 G	0.0043 G,aa	0.09 G	0.004 G,aa	0.00080 B,C	0.00081 B,C	65FR31682 65FR66443
108	4,4'-DDT	50293	1.1 G,u	0.001 G,aa,ii	0.13 G,ii	0.001 G,aa,u	0.00022 B,C	0.00022 B,C	65FR31682 65FR66443
109	4,4'-DDE	72559					0.00022 B,C	0.00022 B,C	65FR66443
110	4,4'-DDD	72548					0.00031 B,C	0.00031 B,C	65FR66443

	Priority Pollutant	CAS Number	Freshwater CMC (µg/L)	Freshwater CCC (µg/L)	Saltwater CMC (µg/L)	Saltwater CCC (µg/L)	Human Health For Consumption of: Water + Organism (µg/L)	Human Health For Consumption of: Organism Only (µg/L)	FR Cite/ Source
111	Dieldrin	60571	0.24 K	0.056 K,O	0.71 G	0.0019 G,aa	0.000052 B,C	0.000054 B,C	65FR31682 65FR66443
112	alpha-Endosulfan	959988	0.22 G,Y	0.056 G,Y	0.034 G,Y	0.0087 G,Y	62 B	89 B	65FR31682 65FR66443
113	beta-Endosulfan	33213659	0.22 G,Y	0.056 G,Y	0.034 G,Y	0.0087 G,Y	62 B	89 B	65FR31682 65FR66443
114	Endosulfan Sulfate	1031078					62 B	89 B	65FR66443
115	Endrin	72208	0.086 K	0.036 K,O	0.037 G	0.0023 G,aa	0.76 B	0.81 B,H	65FR31682
116	Endrin Aldehyde	7421934					0.29 B	0.30 B,H	65FR66443
117	Heptachlor	76448	0.52 G	0.0038 G,aa	0.053 G	0.0036 G,aa	0.000079 B,C	0.000079 B,C	65FR31682 65FR66443
118	Heptachlor Epoxide	1024573	0.52 G,V	0.0038 G,V,aa	0.053 G,V	0.0036 G,V,aa	0.000039 B,C	0.000039 B,C	65FR31682 65FR66443
119	Polychlorinated Biphenyls PCBs:			0.014 N,aa		0.03 N,aa	0.000064 B,C,N	0.000064 B,C,N	65FR31682 65FR66443
120	Toxaphene	8001352	0.73	0.0002 aa	0.21	0.0002 aa	0.00028 B,C	0.00028 B,C	65FR31682 65FR66443

Footnotes:

A This recommended water quality criterion was derived from data for arsenic (III), but is applied here to total arsenic, which might imply that arsenic (III) and arsenic (V) are equally toxic to aquatic life and that their toxicities are additive. In the arsenic criteria document (EPA 440/5-84-033, January 1985), Species Mean Acute Values are given for both arsenic (III) and arsenic (V) for five species and the ratios of the SMAVs for each species range from 0.6 to 1.7.

Chronic values are available for both arsenic (III) and arsenic (V) for one species; for the fathead minnow, the chronic value for arsenic (V) is 0.29 times the chronic value for arsenic (III). No data are known to be available concerning whether the toxicities of the forms of arsenic to aquatic organisms are additive.

B This criterion has been revised to reflect The Environmental Protection Agency's q1* or RfD, as contained in the Integrated Risk Information System (IRIS) as of May 17, 2002. The fish tissue bioconcentration factor (BCF) from the 1980 Ambient Water Quality Criteria document was retained in each case.

C This criterion is based on carcinogenicity of 10^{-6} risk. Alternate risk levels may be obtained by moving the decimal point (e.g., for a risk level of 10^{-5}, move the decimal point in the recommended criterion one place to the right).

D Freshwater and saltwater criteria for metals are expressed in terms of the dissolved metal in the water column. The recommended water quality criteria value was calculated by using the previous 304(a) aquatic life criteria expressed in terms of total recoverable metal, and multiplying it by a conversion factor (CF). The term "Conversion Factor" (CF) represents the recommended conversion factor for converting a metal criterion expressed as the total recoverable fraction in the water column to a criterion expressed as the dissolved fraction in the water column. (Conversion Factors for saltwater CCCs are not currently available. Conversion factors derived for saltwater CMCs have been used for both saltwater CMCs and CCCs). See "Office of Water Policy and Technical Guidance on Interpretation and Implementation of Aquatic Life Metals Criteria," October 1, 1993, by Martha G. Prothro, Acting Assistant Administrator for Water, available from the Water Resource center, USEPA, 401 M St., SW, mail code RC4100, Washington, DC 20460; and 40CFR§131.36(b)(1). Conversion Factors applied in the table can be found in Appendix A to the Preamble- Conversion Factors for Dissolved Metals.

E The freshwater criterion for this metal is expressed as a function of hardness (mg/L) in the water column. The value given here corresponds to a hardness of 100 mg/L. Criteria values for other hardness may be calculated from the following: CMC (dissolved) = $\exp\{m_A [\ln(\text{hardness})] + b_A\}$ (CF), or CCC (dissolved) = $\exp\{m_C [\ln(\text{hardness})] + b_C\}$ (CF) and the parameters specified in Appendix B- Parameters for Calculating Freshwater Dissolved Metals Criteria That Are Hardness-Dependent.

F Freshwater aquatic life values for pentachlorophenol are expressed as a function of pH, and are calculated as follows: CMC = exp(1.005(pH)-4.869); CCC = exp(1.005(pH)-5.134). Values displayed in table correspond to a pH of 7.8.

G This Criterion is based on 304(a) aquatic life criterion issued in 1980, and was issued in one of the following documents: Aldrin/Dieldrin (EPA 440/5-80-019), Chlordane (EPA 440/5-80-027), DDT (EPA 440/5-80-038), Endosulfan (EPA 440/5-80-046), Endrin (EPA 440/5-80-047), Heptachlor (EPA 440/5-80-052), Hexachlorocyclohexane (EPA 440/5-80-054), Silver (EPA 440/5-80-071). The Minimum Data Requirements and derivation procedures were different in the 1980 Guidelines than in the 1985 Guidelines. For example, a "CMC" derived using the 1980 Guidelines was derived to be used as an instantaneous maximum. If assessment is to be done using an averaging period, the values given should be divided by 2 to obtain a value that is more comparable to a CMC derived using the 1985 Guidelines.

H No criterion for protection of human health from consumption of aquatic organisms excluding water was presented in the 1980 criteria document or in the *1986 Quality Criteria for Water*. Nevertheless, sufficient information was presented in the 1980 document to allow the calculation of a criterion, even though the results of such a calculation were not shown in the document.

I This criterion for asbestos is the Maximum Contaminant Level (MCL) developed under the Safe Drinking Water Act (SDWA).

J This fish tissue residue criterion for methylmercury is based on a total fish consumption rate of 0.0175 kg/day.

K This recommended criterion is based on a 304(a) aquatic life criterion that was issued in the *1995 Updates: Water Quality Criteria Documents for the Protection of Aquatic Life in Ambient Water*, (EPA-820-B-96-001, September 1996). This value was derived using the GLI Guidelines (60FR15393-15399, March 23, 1995; 40CFR132 Appendix A); the difference between the 1985 Guidelines and the GLI Guidelines are explained on page iv of the 1995 Updates. None of the decisions concerning the derivation of this criterion were affected by any considerations that are specific to the Great Lakes.

L The CMC = 1/[(f1/CMC1) + (f2/CMC2)] where f1 and f2 are the fractions of total selenium that are treated as selenite and selenate, respectively, and CMC1 and CMC2 are 185.9 µg/l and 12.82 µg/l, respectively.

M EPA is currently reassessing the criteria for arsenic.

N This criterion applies to total pcbs, (e.g., the sum of all congener or all isomer or homolog or Aroclor analyses.)

O The derivation of the CCC for this pollutant (Endrin) did not consider exposure through the diet, which is probably important for aquatic life occupying upper trophic levels.

P Although a new RfD is available in IRIS, the surface water criteria will not be revised until the National Primary Drinking Water Regulations: Stage 2 Disinfectants and Disinfection Byproducts Rule (Stage 2 DBPR) is completed, since public comment on the relative source contribution (RSC) for chloroform is anticipated.

Q This recommended water quality criterion is expressed as µg free cyanide (as CN)/L.

R This value for selenium was announced (61FR58444-58449, November 14, 1996) as a proposed GLI 303(c) aquatic life criterion. EPA is currently working on this criterion and so this value might change substantially in the near future.

S This recommended water quality criterion for arsenic refers to the inorganic form only.

T This recommended water quality criterion for selenium is expressed in terms of total recoverable metal in the water column. It is scientifically acceptable to use the conversion factor (0.996- CMC or 0.922- CCC) that was used in the GLI to convert this to a value that is expressed in terms of dissolved metal.

U The organoleptic effect criterion is more stringent than the value for priority toxic pollutants.

V This value was derived from data for heptachlor and the criteria document provides insufficient data to estimate the relative toxicities of heptachlor and heptachlor epoxide.

W Although EPA has not published a completed criteria document for butylbenzyl phthalate it is EPA's understanding that sufficient data exist to allow calculation of aquatic criteria. It is anticipated that industry intends to publish in the peer reviewed literature draft aquatic life criteria generated in accordance with EPA Guidelines. EPA will review such criteria for possible issuance as national WQC.

X There is a full set of aquatic life toxicity data that show that DEHP is not toxic to aquatic organisms at or below its solubility limit.

Y This value was derived from data for endosulfan and is most appropriately applied to the sum of alpha-endosulfan and beta-endosulfan.

Z A more stringent MCL has been issued by EPA. Refer to drinking water regulations (40 CFR 141) or Safe Drinking Water Hotline (1-800-426-4791) for values.

aa This criterion is based on a 304(a) aquatic life criterion issued in 1980 or 1986, and was issued in one of the following documents: Aldrin/Dieldrin (EPA 440/5-80-019), Chlordane (EPA 440/5-80-027), DDT (EPA 440/5-80-038), Endrin (EPA 440/5-80-047), Heptachlor (EPA 440/5-80-052), Polychlorinated biphenyls (EPA 440/5-80-068), Toxaphene (EPA 440/5-86-006). This CCC is currently based on the Final Residue Value (FRV) procedure. Since the publication of the Great Lakes Aquatic Life Criteria Guidelines in 1995 (60FR15393-15399, March 23, 1995), the Agency no longer uses the Final Residue Value procedure for deriving CCCs for new or revised 304(a) aquatic life criteria. Therefore, the Agency anticipates that future revisions of this CCC will not be based on the FRV procedure.

bb This water quality criterion is based on a 304(a) aquatic life criterion that was derived using the 1985 Guidelines (*Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses*, PB85-227049, January 1985) and was issued in one of the following criteria documents: Arsenic (EPA 440/5-84-033), Cadmium (EPA 882-R-01-001), Chromium (EPA 440/5-84-029), Copper (EPA 440/5-84-031), Cyanide (EPA 440/5- 84-028), Lead (EPA 440/5-84-027), Nickel (EPA 440/5-86-004), Pentachlorophenol (EPA 440/5-86-009), Toxaphene, (EPA 440/5-86-006), Zinc (EPA 440/5-87- 003).

cc When the concentration of dissolved organic carbon is elevated, copper is substantially less toxic and use of Water-Effect Ratios might be appropriate.

dd The selenium criteria document (EPA 440/5-87-006, September 1987) provides that if selenium is as toxic to saltwater fishes in the field as it is to freshwater fishes in the field, the status of the fish community should be monitored whenever the concentration of selenium exceeds 5.0 µg/L in salt water because the saltwater CCC does not take into account uptake via the food chain.

ee This recommended water quality criterion was derived on page 43 of the mercury criteria document (EPA 440/5-84-026, January 1985). The saltwater CCC of 0.025 ug/L given on page 23 of the criteria document is based on the Final Residue Value procedure in the 1985 Guidelines. Since the publication of the Great Lakes Aquatic Life Criteria Guidelines in 1995 (60FR15393-15399, March 23, 1995), the Agency no longer uses the Final Residue Value procedure for deriving CCCs for new or revised 304(a) aquatic life criteria.

ff This recommended water quality criterion was derived in *Ambient Water Quality Criteria Saltwater Copper Addendum* (Draft, April 14, 1995) and was promulgated in the Interim final National Toxics Rule (60FR22228-222237, May 4, 1995).

gg EPA is actively working on this criterion and so this recommended water quality criterion may change substantially in the near future.

hh This recommended water quality criterion was derived from data for inorganic mercury (II), but is applied here to total mercury. If a substantial portion of the mercury in the water column is methylmercury, this criterion will probably be under protective. In addition, even though inorganic mercury is converted to methylmercury and methylmercury bioaccumulates to a great extent, this criterion does not account for uptake via the food chain because sufficient data were not available when the criterion was derived.

ii This criterion applies to DDT and its metabolites (i.e., the total concentration of DDT and its metabolites should not exceed this value).

NATIONAL RECOMMENDED WATER QUALITY CRITERIA FOR NON PRIORITY POLLUTANTS

	Non Priority Pollutant	CAS Number	Freshwater CMC (µg/L)	Freshwater CCC (µg/L)	Saltwater CMC (µg/L)	Saltwater CCC (µg/L)	Human Health For Consumption of: Water + Organism (µg/L)	Human Health For Consumption of: Organism Only (µg/L)	FR Cite/Source
1	Alkalinity	--		20000 F					Gold Book
2	Aluminum pH 6.5 - 9.0	7429905	750 G,I	87 G,I,L					53FR33178
3	Ammonia	7664417	FRESHWATER CRITERIA ARE pH, Temperature and Life-stage DEPENDENT -- SEE DOCUMENT D						EPA822-R-99-014
			SALTWATER CRITERIA ARE pH AND TEMPERATURE DEPENDENT						EPA440/5-88-004
4	Aesthetic Qualities	--	NARRATIVE STATEMENT-- SEE DOCUMENT						Gold Book
5	Bacteria	--	FOR PRIMARY RECREATION AND SHELLFISH USES -- SEE DOCUMENT						Gold Book
6	Barium	7440393					1,000 A		Gold Book
7	Boron	--	NARRATIVE STATEMENT-- SEE DOCUMENT						Gold Book
8	Chloride	16887006	860,000 G	230,000 G					53FR19028
9	Chlorine	7782505	19	11	13	7.5	C		Gold Book
10	Chlorophenoxy Herbicide (2,4,5,-TP)	93721					10 A		Gold Book
11	Chlorophenoxy Herbicide (2,4-D)	94757					100 A,C		Gold Book
12	Chloropyrifos	2921882	0.083 G	0.041 G	0.011 G	0.0056 G			Gold Book
13	Color	--	NARRATIVE STATEMENT-- SEE DOCUMENT F						Gold Book
14	Demeton	8065483		0.1 F		0.1 F			Gold Book
15	Ether, Bis(Chloromethyl)	542881					0.00010 E, H	0.00029 E,H	65FR66443

	Non Priority Pollutant	CAS Number	Freshwater CMC (µg/L)	Freshwater CCC (µg/L)	Saltwater CMC (µg/L)	Saltwater CCC (µg/L)	Human Health For Consumption of: Water + Organism (µg/L)	Human Health For Consumption of: Organism Only (µg/L)	FR Cite/Source
16	Gases, Total Dissolved	--	NARRATIVE STATEMENT -- SEE DOCUMENT F						Gold Book
17	Guthion	86500		0.01 F		0.01 F			Gold Book
18	Hardness	--	NARRATIVE STATEMENT-- SEE DOCUMENT						Gold Book
19	Hexachlorocyclo-hexane-Technical	319868					0.0123	0.0414	Gold Book
20	Iron	7439896		1,000 F			300 A		Gold Book
21	Malathion	121755		0.1 F		0.1 F			Gold Book
22	Manganese	7439965					50 A,O	100 A	Gold Book
23	Methoxychlor	72435		0.03 F		0.03 F	100 A,C		Gold Book
24	Mirex	2385855		0.001 F		0.001 F			Gold Book
25	Nitrates	14797558					10,000 A		Gold Book
26	Nitrosamines	--					0.0008	1.24	Gold Book
27	Dinitrophenols	25550587					69	5300	65FR66443
28	Nitrosodibutylamine,N	924163					0.0063 A,H	0.22 A,H	65FR66443
29	Nitrosodiethylamine,N	55185					0.0008 A,H	1.24 A,H	Gold Book
30	Nitrosopyrrolidine,N	930552					0.016 H	34 H	65FR66443
31	Oil and Grease	--	NARRATIVE STATEMENT -- SEE DOCUMENT F						Gold Book

	Non Priority Pollutant	CAS Number	Freshwater CMC (µg/L)	Freshwater CCC (µg/L)	Saltwater CMC (µg/L)	Saltwater CCC (µg/L)	Human Health For Consumption of: Water + Organism (µg/L)	Human Health For Consumption of: Organism Only (µg/L)	FR Cite/Source
32	Oxygen, Dissolved Freshwater	7782447	WARMWATER AND COLDWATER MATRIX -- SEE DOCUMENT N						Gold Book
	Oxygen, Dissolved Saltwater		SALTWATER – SEE DOCUMENT						EPA-822R-00-012
33	Parathion	56382	0.065 J	0.013 J					Gold Book
34	Pentachlorobenzene	608935					1.4 E	1.5 E	65FR66443
35	pH	--		6.5 - 9 F		6.5 - 8.5 F,K	5 - 9		Gold Book
36	Phosphorus Elemental	7723140				0.1 F,K			Gold Book
37	Nutrients	--	See EPA's Ecoregional criteria for Total Phosphorus, Total Nitrogen, Chlorophyll *a* and Water Clarity (Secchi depth for lakes; turbidity for streams and rivers) (& Level III Ecoregional criteria)						P
38	Solids Dissolved and Salinity	--					250,000 A		Gold Book
39	Solids Suspended and Turbidity	--	NARRATIVE STATEMENT -- SEE DOCUMENT F						Gold Book
40	Sulfide-Hydrogen Sulfide	7783064		2.0 F		2.0 F			Gold Book
41	Tainting Substances	--	NARRATIVE STATEMENT-- SEE DOCUMENT						Gold Book
42	Temperature	--	SPECIES DEPENDENT CRITERIA -- SEE DOCUMENT M						Gold Book
43	Tetrachlorobenzene,1,2,4,5-	95943					0.97 E	1.1 E	65FR66443
44	Tributyltin (TBT)	--	0.46 Q	0.063 Q	0.37 Q	0.010 Q			EPA 822-F-00-008
45	Trichlorophenol,2,4,5-	95954					1,800 B,E	3,600 B,E	65FR66443

Footnotes:

A This human health criterion is the same as originally published in the Red Book which predates the 1980 methodology and did not utilize the fish ingestion BCF approach. This same criterion value is now published in the Gold Book.

B The organoleptic effect criterion is more stringent than the value presented in the non priority pollutants table.

C A more stringent Maximum Contaminant Level (MCL) has been issued by EPA under the Safe Drinking Water Act. Refer to drinking water regulations 40CFR141 or Safe Drinking Water Hotline (1-800-426-4791) for values.

D According to the procedures described in the *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses*, except possibly where a very sensitive species is important at a site, freshwater aquatic life should be protected if both conditions specified in Appendix C to the Preamble- Calculation of Freshwater Ammonia Criterion are satisfied.

E This criterion has been revised to reflect EPA's q1* or RfD, as contained in the Integrated Risk Information System (IRIS) as of May 17, 2002. The fish tissue bioconcentration factor (BCF) used to derive the original criterion was retained in each case.

F The derivation of this value is presented in the Red Book (EPA 440/9-76-023, July, 1976).

G This value is based on a 304(a) aquatic life criterion that was derived using the 1985 Guidelines (*Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses*, PB85-227049, January 1985) and was issued in one of the following criteria documents: Aluminum (EPA 440/5-86-008); Chloride (EPA 440/5-88-001); Chloropyrifos (EPA 440/5-86-005).

H This criterion is based on carcinogenicity of 10^{-6} risk. Alternate risk levels may be obtained by moving the decimal point (e.g., for a risk level of 10^{-5}, move the decimal point in the recommended criterion one place to the right).

I This value for aluminum is expressed in terms of total recoverable metal in the water column.

J This value is based on a 304(a) aquatic life criterion that was issued in the *1995 Updates: Water Quality Criteria Documents for the Protection of Aquatic Life in Ambient Water* (EPA-820-B-96-001). This value was derived using the GLI Guidelines (60FR15393-15399, March 23, 1995; 40CFR132 Appendix A); the differences between the 1985 Guidelines and the GLI Guidelines are explained on page iv of the 1995 Updates. No decision concerning this criterion was affected by any considerations that are specific to the Great Lakes.

K According to page 181 of the Red Book:

> For open ocean waters where the depth is substantially greater than the euphotic zone, the pH should not be changed more than 0.2 units from the naturally occurring variation or any case outside the range of 6.5 to 8.5. For shallow, highly productive coastal and estuarine areas where naturally occurring pH variations approach the lethal limits of some species, changes in pH should be avoided but in any case should not exceed the limits established for fresh water, i.e., 6.5-9.0.

L There are three major reasons why the use of Water-Effect Ratios might be appropriate. (1) The value of 87 µg/l is based on a toxicity test with the striped bass in water with pH= 6.5-6.6 and hardness <10 mg/L. Data in "Aluminum Water-Effect Ratio for the 3M Plant Effluent Discharge, Middleway, West Virginia" (May 1994) indicate that aluminum is substantially less toxic at higher pH and hardness, but the effects of pH and hardness are not well quantified at this time. (2) In tests with the brook trout at low pH and hardness, effects increased with increasing concentrations of total aluminum even though the concentration of dissolved aluminum was constant, indicating that total recoverable is a more appropriate measurement than dissolved, at least when particulate aluminum is primarily aluminum hydroxide particles. In surface waters, however, the total recoverable procedure might measure aluminum associated with clay particles, which might be less toxic than aluminum associated with aluminum hydroxide. (3) EPA is aware of field data indicating that many high quality waters in the U.S. contain more than 87 µg aluminum/L, when either total recoverable or dissolved is measured.

M U.S. EPA. 1973. Water Quality Criteria 1972. EPA-R3-73-033. National Technical Information Service, Springfield, VA.; U.S. EPA. 1977. Temperature Criteria for Freshwater Fish: Protocol and Procedures. EPA-600/3-77-061. National Technical Information Service, Springfield, VA.

N U.S. EPA. 1986. Ambient Water Quality Criteria for Dissolved Oxygen. EPA 440/5-86-003. National Technical Information Service, Springfield, VA.

O This criterion for manganese is not based on toxic effects, but rather is intended to minimize objectionable qualities such as laundry stains and objectionable tastes in beverages.

P Lakes and Reservoirs in Nutrient Ecoregion: II EPA 822-B-00-007, III EPA 822-B-01-008, IV EPA 822-B-01-009, V EPA 822-B-01-010, VI EPA 822-B-00-008 , VII EPA 822-B-00-009, VIII EPA 822-B-01-015, IX EPA 822-B-00-011, XI EPA 822-B-00-012, XII EPA 822-B-00-013, XIII EPA 822-B-00-014, XIV EPA 822-B-01-011; Rivers and Streams in Nutrient Ecoregion: I EPA 822-B-01-012, II EPA 822-B-00-015, III EPA 822-B-00-016, IV EPA 822-B-01-013, V EPA 822-B-01-014, VI EPA 822-B-00-017, VII EPA 822-B-00-018, VIII EPA 822-B-01-015, IX EPA 822-B-00-019, X EPA 822-B-01-016, XI EPA 822-B-00-020, XII EPA 822-B-00-021, XIV EPA 822-B-00-022; and Wetlands in Nutrient Ecoregion XIII EPA 822-B-00-023.

Q EPA announced the availability of a draft updated tributyltin (TBT) document on August 7, 1997 (62FR42554). The Agency has reevaluated this document and anticipates releasing an updated document for public comment in the near future.

NATIONAL RECOMMENDED WATER QUALITY CRITERIA FOR ORGANOLEPTIC EFFECTS

	Pollutant	CAS Number	Organoleptic Effect Criteria (µg/L)	FR Cite/Source
1	Acenaphthene	83329	20	Gold Book
2	Monochlorobenzene	108907	20	Gold Book
3	3-Chlorophenol	--	0.1	Gold Book
4	4-Chlorophenol	106489	0.1	Gold Book
5	2,3-Dichlorophenol	--	0.04	Gold Book
6	2,5-Dichlorophenol	--	0.5	Gold Book
7	2,6-Dichlorophenol	--	0.2	Gold Book
8	3,4-Dichlorophenol	--	0.3	Gold Book
9	2,4,5-Trichlorophenol	95954	1	Gold Book
10	2,4,6-Trichloropehnol	88062	2	Gold Book
11	2,3,4,6-Tetrachlorophenol	--	1	Gold Book
12	2-Methyl-4-Chlorophenol	--	1,800	Gold Book
13	3-Methyl-4-Chlorophenol	59507	3,000	Gold Book
14	3-Methyl-6-Chlorophenol	--	20	Gold Book
15	2-Chlorophenol	95578	0.1	Gold Book
16	Copper	7440508	1,000	Gold Book
17	2,4-Dichlorophenol	120832	0.3	Gold Book
18	2,4-Dimethylpehnol	105679	400	Gold Book
19	Hexachlorocyclopentadiene	77474	1	Gold Book
20	Nitrobenzene	98953	30	Gold Book

	Pollutant	CAS Number	Organoleptic Effect Criteria (µg/L)	FR Cite/Source
21	Pentachlorophenol	87865	30	Gold Book
22	Phenol	108952	300	Gold Book
23	Zinc	7440666	5,000	45 FR79341

General Notes:

1. These criteria are based on organoleptic (taste and odor) effects. Because of variations in chemical nomenclature systems, this listing of pollutants does not duplicate the listing in Appendix A of 40 CFR Part 423. Also listed are the Chemical Abstracts Service (CAS) registry numbers, which provide a unique identification for each chemical.

NATIONAL RECOMMENDED WATER QUALITY CRITERIA

Additional Notes:

1. Criteria Maximum Concentration and Criterion Continuous Concentration

The Criteria Maximum Concentration (CMC) is an estimate of the highest concentration of a material in surface water to which an aquatic community can be exposed briefly without resulting in an unacceptable effect. The Criterion Continuous Concentration (CCC) is an estimate of the highest concentration of a material in surface water to which an aquatic community can be exposed indefinitely without resulting in an unacceptable effect. The CMC and CCC are just two of the six parts of an aquatic life criterion; the other four parts are the acute averaging period, chronic averaging period, acute frequency of allowed exceedence, and chronic frequency of allowed exceedence. Because 304(a) aquatic life criteria are national guidance, they are intended to be protective of the vast majority of the aquatic communities in the United States.

2. Criteria Recommendations for Priority Pollutants, Non Priority Pollutants and Organoleptic Effects

This compilation lists all priority toxic pollutants and some non priority toxic pollutants, and both human health effect and organoleptic effect criteria issued pursuant to CWA §304(a). Blank spaces indicate that EPA has no CWA §304(a) criteria recommendations. For a number of non-priority toxic pollutants not listed, CWA §304(a) "water + organism" human health criteria are not available, but EPA has published MCLs under the SDWA that may be used in establishing water quality standards to protect water supply designated uses. Because of variations in chemical nomenclature systems, this listing of toxic pollutants does not duplicate the listing in Appendix A of 40 CFR Part 423. Also listed are the Chemical Abstracts Service CAS registry numbers, which provide a unique identification for each chemical.

3. Human Health Risk

The human health criteria for the priority and non priority pollutants are based on carcinogenicity of 10^{-6} risk. Alternate risk levels may be obtained by moving the decimal point (e.g., for a risk level of 10^{-5}, move the decimal point in the recommended criterion one place to the right).

4. Water Quality Criteria published pursuant to Section 304(a) or Section 303(c) of the CWA

Many of the values in the compilation were published in the California Toxics Rule. Although such values were published pursuant to Section 303(c) of the CWA, they represent the Agency's most recent calculation of water quality criteria and are thus the Agency's 304(a) criteria.

5. Calculation of Dissolved Metals Criteria

The 304(a) criteria for metals, shown as dissolved metals, are calculated in one of two ways. For freshwater metals criteria that are hardness-dependent, the dissolved metal criteria were calculated using a hardness of 100 mg/l as $CaCO_3$ for illustrative purposes only. Saltwater and freshwater metals' criteria that are not hardness-dependent are calculated by multiplying the total recoverable criteria before rounding by the appropriate conversion factors. The final dissolved metals' criteria in the table are rounded to two significant figures. Information regarding the calculation of hardness dependent conversion factors are included in the footnotes.

6. Maximum Contaminant Levels

The compilation includes footnotes for pollutants with Maximum Contaminant Levels (MCLs) more stringent than the recommended water quality criteria in the compilation. MCLs for these pollutants are not included in the compilation, but can be found in the appropriate drinking water regulations (40 CFR 141.11-16 and 141.60-63), or can be accessed through the Safe Drinking Water Hotline (800-426-4791) or the Internet (http://www.epa.gov/waterscience/drinking/standards/dwstandards.pdf).

7. Organoleptic Effects

The compilation contains 304(a) criteria for pollutants with toxicity-based criteria as well as non-toxicity based criteria. The basis for the non-toxicity based criteria are organoleptic effects (e.g., taste and odor) which would make water and edible aquatic life unpalatable but not toxic to humans. The table includes criteria for organoleptic effects for 23 pollutants. Pollutants with organoleptic effect criteria more stringent than the criteria based on toxicity (e.g., included in both the priority and non-priority pollutant tables) are footnoted as such.

8. Gold Book

The "Gold Book" is Quality Criteria for Water: 1986. EPA 440/5-86-001.

9. Correction of Chemical Abstract Services Number

The Chemical Abstract Services number (CAS) for Bis(2-Chlorisoprpyl) Ether, has been revised in IRIS and in the table. The correct CAS number for this chemical is 108-60-1. The previous CAS number for this pollutant was 39638-32-9.

10. Contaminants with Blanks

EPA has not calculated criteria for contaminants with blanks. However, permit authorities should address these contaminants in NPDES permit actions using the States' existing narrative criteria for toxics.

11. Specific Chemical Calculations

A. Selenium

Aquatic Life

This compilation contains aquatic life criteria for selenium that are the same as those published in the proposed CTR. In the CTR, EPA proposed an acute criterion for selenium based on the criterion proposed for selenium in the Water Quality Guidance for the Great Lakes System (61 FR 58444). The GLI and CTR proposals take into account data showing that selenium's two prevalent oxidation states in water, selenite and selenate, present differing potentials for aquatic toxicity, as well as new data indicating that various forms of selenium are additive. The new approach produces a different selenium acute criterion concentration, or CMC, depending upon the relative proportions of selenite, selenate, and other forms of selenium that are present.

EPA is currently undertaking a reassessment of selenium, and expects the 304(a) criteria for selenium will be revised based on the final reassessment (63FR26186). However, until such time as revised water quality criteria for selenium are published by the Agency, the recommended water quality criteria in this compilation are EPA's current 304(a) criteria.

Appendices:

Appendix A - Conversion Factors for Dissolved Metals

Metal	Conversion Factor freshwater CMC	Conversion Factor freshwater CCC	Conversion Factor saltwater CMC	Conversion Factor saltwater CCC[1]
Arsenic	1.000	1.000	1.000	1.000
Cadmium	1.136672-[(ln hardness)(0.041838)]	1.101672-[(ln hardness)(0.041838)]	0.994	0.994
Chromium III	0.316	0.860	--	--
Chromium VI	0.982	0.962	0.993	0.993
Copper	0.960	0.960	0.83	0.83
Lead	1.46203-[(ln hardness)(0.145712)]	1.46203-[(ln hardness)(0.145712)]	0.951	0.951
Mercury	0.85	0.85	0.85	0.85
Nickel	0.998	0.997	0.990	0.990
Selenium	--	--	0.998	0.998
Silver	0.85	--	0.85	--
Zinc	0.978	0.986	0.946	0.946

Appendix B - Parameters for Calculating Freshwater Dissolved Metals Criteria That Are Hardness-Dependent

Chemical	m_A	b_A	m_C	b_C	Freshwater Conversion Factors (CF)	
					CMC	CCC
Cadmium	1.0166	-3.924	0.7409	-4.719	1.136672-[(ln hardness)(0.041838)]	1.101672-[(ln hardness)(0.041838)]
Chromium III	0.8190	3.7256	0.8190	0.6848	0.316	0.860
Copper	0.9422	-1.700	0.8545	-1.702	0.960	0.960
Lead	1.273	-1.460	1.273	-4.705	1.46203-[(ln hardness)(0.145712)]	1.46203-[(ln hardness)(0.145712)]
Nickel	0.8460	2.255	0.8460	0.0584	0.998	0.997
Silver	1.72	-6.59	--	--	0.85	--
Zinc	0.8473	0.884	0.8473	0.884	0.978	0.986

Hardness-dependant metals' criteria may be calculated from the following:
CMC (dissolved) = $\exp\{m_A [\ln(\text{hardness})] + b_A\}$ (CF)
CCC (dissolved) = $\exp\{m_C [\ln(\text{hardness})] + b_C\}$ (CF)

Appendix C - Calculation of Freshwater Ammonia Criterion

1. The one-hour average concentration of total ammonia nitrogen (in mg N/L) does not exceed, more than once every three years on the average, the CMC (acute criterion) calculated using the following equations.

Where salmonid fish are present:

$$CMC = \frac{0.275}{1 + 10^{7.204-pH}} + \frac{39.0}{1 + 10^{pH-7.204}}$$

Or where salmonid fish are not present:

$$CMC = \frac{0.411}{1 + 10^{7.204-pH}} + \frac{58.4}{1 + 10^{pH-7.204}}$$

2A The thirty-day average concentration of total ammonia nitrogen (in mg N/L) does not exceed, more than once every three years on the average, the CCC (chronic criterion) calculated using the following equations.

When fish early life stages are present:

$$CCC = \left(\frac{0.0577}{1 + 10^{7.688-pH}} + \frac{2.487}{1 + 10^{pH-7.688}} \right) \bullet \quad MIN\ (2.85,\ 1.45 \cdot 10^{0.028 \cdot (25-T)})$$

When fish early life stages are absent:

$$CCC = \left(\frac{0.0577}{1 + 10^{7.688-pH}} + \frac{2.487}{1 + 10^{pH-7.688}} \right) \bullet \quad 1.45 \cdot 10^{0.028 \cdot (25-MAX\ (T,7))}$$

2B. In addition, the highest four-day average within the 30-day period should not exceed 2.5 times the CCC.

Exhibit E

U.S. Environmental Protection Agency
Integrated Risk Information System

Acrolein (CASRN 107-02-8)

MAIN CONTENTS

Reference Dose for Chronic Oral Exposure (RfD)

SUBSTANCE SUMMARY INDEX

Chronic Health Hazards for Non-Carcinogenic Effects

Reference Dose for Chronic Oral Exposure (RfD)

- Oral RfD Summary
- Principal and Supporting Studies
- Uncertainty and Modifying Factors
- Additional Studies/ Comments
- Confidence in the Oral RfD
- EPA Documentation and Review

Reference Concentration for Chronic Inhalation Exposure (RfC)

- Inhalation RfC Summary
- Principal and Supporting Studies
- Uncertainty and Modifying Factors
- Additional Studies/

Note: A TOXICOLOGICAL REVIEW is available for this chemical in Adobe* PDF format (106 Pages, 853 Kbytes). Similar documents can be found in the List of Available IRIS Toxicological Reviews.

Links to specific pages in the toxicological review are available throughout this summary. To utilize this feature, your Web browser and Adobe program must be configured properly so the PDF displays within the browser window. If your browser and Adobe program need configuration, please go to the IRIS Help page for instructions.

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Acrolein; CASRN 107-02-8

Health assessment information on a chemical substance is included in IRIS only after a comprehensive review of chronic toxicity data by U.S. EPA health scientists from several Program Offices and the Office of Research and Development. The summaries presented in Sections I and II represent a consensus reached in the review process. Background information and explanations of the methods used to derive the values given in IRIS are provided in the Background Documents.

STATUS OF DATA FOR Acrolein

File First On-Line 09/07/1988

Category (section)	Status	Last Revised
Oral RfD Assessment (I.A.)	on-line	06/03/2003
Inhalation RfC Assessment (I.B.)	on-line	06/03/2003
Carcinogenicity Assessment (II.)	on-line	06/03/2003

_I. Chronic Health Hazard Assessments for Noncarcinogenic Effects

_I.A. Reference Dose for Chronic Oral Exposure (RfD)

Substance Name -- Acrolein
CASRN — 107-28-8
Last Revised — 06/03/2003

The oral Reference Dose (RfD) is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis. It is expressed in units of mg/kg-day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. Please refer to the Background Document for an elaboration of these concepts. RfDs can also be derived for the noncarcinogenic health effects of substances that are also carcinogens. Therefore, it is essential to refer to other sources of information concerning the carcinogenicity of this substance. If the U.S. EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation will be contained in Section II of this file.

An RfD for acrolein was not previously available on IRIS.

__I.A.1. Oral RfD Summary

Critical Effect	Experimental Doses*	UF	MF	RfD
Decreased survival Chronic gavage rat study Parent et al., 1992a	NOAEL: 0.05 mg/kg-day FEL*: 0.5 mg/kg-day	100	1	5×10^{-4} mg/kg-day

* FEL – frank effect level (an objective, clinically evident effect)

__I.A.2. Principal and Supporting Studies (Oral RfD)

Parent et al. (1992a) administered acrolein in water daily via gavage to Sprague-Dawley rats, 70/sex/group, at dose levels of 0, 0.05, 0.5, and 2.5 mg/kg BW. Dosing volume was 10 ml/kg. Ten animals from each group were sacrificed after one year and the remainder after two years. An extensive array of tissues was examined

Comments
- Confidence in the Inhalation RfC
- EPA Documentation and Review

Carcinogenicity Assessment for Lifetime Exposure

Evidence for Human Carcinogenicity
- Weight-of-Evidence Characterization
- Human Carcinogenicity Data
- Animal Carcinogenicity Data
- Supporting Data for Carcinogenicity

Quantitative Estimate of Carcinogenic Risk from Oral Exposure
- Summary of Risk Estimates
- Dose-Response Data
- Additional Comments
- Discussion of Confidence

Quantitative Estimate of Carcinogenic Risk from Inhalation Exposure
- Summary of Risk Estimates
- Dose-Response Data
- Additional Comments
- Discussion of Confidence

EPA Documentation, Review and, Contacts

- Bibliography
- Revision History
- Synonyms

microscopically, including stomach tissue. Although it was not explicitly stated that both the glandular stomach and forestomach were examined, it is unlikely that both parts of the stomach were not examined. Daily observations were made and various clinical, hematological and urinary parameters were measured after 3, 6, 12, and 18 months of treatment and immediately prior to sacrifice. There were no statistically significant increased incidences of microscopic lesions in the treated rats, whether neoplastic or non-neoplastic. Food consumption and body weights were unaffected by treatment. With the exception of a statistically significant depression of creatinine phosphokinase (creatine kinase) at all dose levels and at most time intervals (except 12 months), clinical chemistry parameters, hematology and urinalysis measurements were unaffected by treatment.

The most definitive responses reported were treatment-related increases in early cumulative mortality. Data were provided in the form of survival curves. Among high-dose males, survival was significantly reduced after one year ($p<0.05$), and marginally reduced among mid-dose males (p value not reported). Among high-dose males, a trend test for survival during the first year indicated a highly statistically significant ($p=0.003$) decrease; however, the statistical differences are nullified when the survival data for two years are included in the analysis. Survival among females during the first year corresponded closely to those obtained for males. A statistically significant decrease in survival ($p<0.05$) was reported in the high-dose group, while a decrease in survival in the mid-dose group was marginally significant (p value not reported). Unlike responses in males, the significant associations between dosing and survival persisted in females through the end of the study. After two years, a statistically significant reduction in survival was noted based on four different statistical tests for the mid-dose group and in three of four statistical tests in the high-dose group (p values not reported). Although the differences in survival were statistically significant in females after two years, it should be noted that the differences were relatively small. No differences in survival compared to controls were seen in either the male or female low-dose groups (0.05 mg/kg/day). Thus, 0.5 mg/kg/day is considered a frank effect level (FEL) for the rat, and 0.05 mg/kg/day the no-observed-adverse-effect level (NOAEL). The FEL is defined as "a level of exposure or dose which produces irreversible, adverse effects at a statistically or biologically significant increase in frequency or severity between those exposed and those not exposed" (IRIS, 2003).

Other studies support the findings of reduced survival in laboratory animals exposed to acrolein as reported by Parent et al. (1992a). In a study designed to evaluate the potential carcinogenicity of acrolein (Parent et al., 1991), Swiss albino CD-1 mice (70-75/sex/group) were dosed via gavage (acrolein in distilled water and stabilized with hydroquinone) with 0, 0.5, 2.0 or 4.5 mg/kg/day for 18 months. The primary effect was increased mortality only in high-dose males of the 4.5 mg/kg/day group; mortality in the mid- and low-dose groups was less than control. There were no dose-related adverse histopathological or clinical findings.

In a 13-week daily gavage study of acrolein (in 0.5% methyl cellulose) in F344 rats and B6C3F1 mice conducted for the National Toxicology Program (NTP, 1995), 10 rats/sex/dose were administered 0, 0.75, 1.25, 2.5, 5.0, and 10 mg acrolein/kg; 10 mice/sex/dose received 0, 1.25, 2.5, 5.0, 10 and 20 mg/kg. Dose volume was 5 ml/

kg for rats and 10 ml/kg for mice. Treatment resulted in similar dose-related effects in both sexes of both species: hemorrhage and necrosis and other lesions of the forestomach and glandular stomach and secondary changes associated with acrolein-induced mortality in high-dose animals (NTP, 1995; Pathology Working Group Review, 1997). Abnormal breathing and nasal/eye discharge were among the clinical findings in high-dose rats. Nearly all high-dose animals died or were removed from study because of gastrointestinal toxicity. The NOAEL was 0.75 mg/kg for female rats and 1.25 mg/kg for males, based on forestomach squamous epithelial hyperplasia in the 1.25 mg/kg group and 2.5 mg/kg group, respectively. There were no clinical signs of toxicity in mice. The forestomach lesions in mice were similar to those in the rat. Glandular stomach lesions were only seen in the 10 and 20 mg/kg males and in the 20 mg/kg females. Statistically significant increases in absolute and relative liver weights were seen in male mice at 10 mg/kg without attendant hepatic histopathology. There was no NOAEL for the male mouse (i.e., one male had squamous epithelial hyperplasia at the lowest dose of 1.25 mg/kg). The NOAEL for female mice was 1.25 mg/kg. Reasons for no reported observations of stomach lesions in Sprague-Dawley female rats at the highest dose (2.5 mg/kg) of the Parent et al. (1992a) study compared with forestomach squamous epithelial hyperplasia observed in female F344 rats in the NTP study at 1.25 mg/kg/day are not readily apparent, but may relate to differences in strain sensitivity or vehicle. The vehicle dose volume was 5 ml/kg in the NTP (1995) study and 10 ml/kg in the Parent et al. (1992a) study for rats, and there may have been a reduced local gastric mucosal irritation and pathology by virtue of dilution. There were also differences in the vehicle solution and, possibly, the stability of the dosing solutions. Parent et al. (1992a) conducted stability studies on acrolein in water, and monitored the stability of their dosing solutions (reporting losses of less than 10% for 3 hours at room temperature). They used a stabilizing agent, 0.25% hydroquinone, in the stock solution, and prepared dosing solutions daily. The NTP study used a dose vehicle of 0.5% methylcellulose in deionized water, and no information was available on stability or stabilizing agents.

For the mouse results, there is a similar divergence between the absence of reported forestomach lesions in the CD-1 mice at 4.5 mg/kg in the Parent et al. (1991) study compared with effects observed in female B6C3F1 at 2.5 mg/kg in the NTP study. Species differences and dose volume again may have accounted for observed differences in response. Dose volume in the NTP study for mice was 10 ml/kg, and was unspecified in the Parent et al. (1991) study.

A benchmark dose approach was unsuitable for RfD development because the data in the Parent et al. (1992a) study were presented graphically, with statistical evaluation at one and two-year time points, but no numerical values. Moreover, the NOAEL derived from the Parent et al. study and used as the basis for the RfD is from a statistically significant increase in mortality, a frank effect. A benchmark dose analysis would not be appropriate when the dose-response is for early cumulative mortality.

__I.A.3. Uncertainty and Modifying Factors (Oral RfD)

UF = 100.

A default UF_A of 10 was applied to account for interspecies differences between laboratory animals and humans. No information was available to support a change from the default.

A default UF_H of 10 was applied for intraspecies uncertainty to account for human variability and sensitive subpopulations, i.e., to account for human variability in the severity or range of response from any given acrolein exposure amongst different individuals.

A UF_D was not applied because the database for acrolein was considered complete. The available oral database includes chronic toxicity studies in the rat and mouse, an oral reproductive toxicity study in Sprague-Dawley rats and an oral developmental toxicity study in New Zealand white rabbits. The findings from the oral reproductive and developmental toxicity studies are supported by an inhalation reproductive toxicity study of acrolein in Fisher 344 rats that revealed no reproductive or developmental effects. Acrolein's high reactivity at the point of contact and the evidence for minimal systemic distribution of acrolein obviates the need for additional repeat dose studies.

The RfD is based on a NOAEL from a chronic study, which obviates the need for an uncertainty factor for LOAEL to NOAEL extrapolation or for subchronic to chronic extrapolation.

MF = 1.

__I.A.4. Additional Studies/Comments (Oral RfD)

Administration of acrolein in water by oral gavage at 0.05, 0.5, and 5.0 mg/kg to male and female Sprague-Dawley rats (30/sex/group) daily, 5 days per week for 13 weeks did not produce any significant adverse effects on mortality, on clinical, hematological, or urinalysis parameters, or on histopathology (Bioassay Systems Corp., 1981). This study was a precursor to Parent et al. (1992a), identified as the principal study.

In a range-finding gavage study in artificially inseminated New Zealand white rabbits (3-4/group), acrolein (0, 0.5, 1.0, 2.0, 4.0, and 6.0 mg/kg/day) produced high incidences of maternal mortality, spontaneous abortion, resorption, clinical signs, gastric ulceration, and/or sloughing of the gastric mucosa. The dose-response curve for mortality was steep. A factor of 2 in dose (from 2 to 4 mg/kg) resulted in 25% mortality in the high-dose animals compared to 0% in lower-dose animals (Parent et al., 1993). Mortality was 100% at 6 mg/kg.

In a two-generation reproductive toxicity study, four groups of 30 male and 30 female Sprague-Dawley rats were gavaged with 70 daily doses of acrolein at levels of 0, 1, 3, or 6 mg/kg in a dosing volume of 5 ml/kg (Parent, 1992b). Rats within each dosing

group (F_0 generation) were then assigned to a 21-day period of cohabitation. Dosing continued for females through cohabitation, gestation, and lactation. A similar regime was carried out for F_1 generation offspring, resulting in F_2 generation pups. Mortality was significant (at 6 mg/kg) in both males and female of the F_0 and F_1 generations with the pattern continuing with F1 mid-dose animals, the latter showing signs of respiratory distress and histopathological lesions in the lungs and stomach. Reproductive parameters (i.e., mating performance and fertility indices) were unaffected. No treatment-related gross or microscopic effects were observed in the reproductive tissues of the F_0 or F_1 animals, and no gross abnormalities were observed in F_2 generation pups. The data provide evidence that acrolein is not a selective reproductive toxicant but does produce toxicological effects at doses as low as 3 mg/kg/day.

Arumugam et al. (1999) exposed male Wistar rats, 5 animals/group, daily to acrolein via intubation (2.5 mg/kg BW) for 45 days. The incidence of mortality, if any, was not reported in this study. This study clearly showed damage to mitochondria (through the loss of mitochondrial lamellae of the cristae), a decrease in the availability of reduced glutathione (a substrate for glutathione peroxidase), and a 30-56% decrease in activities of citric acid cycle enzymes, resulting in decreased energy production in liver cells. These results indicate that at least some uptake occurs from the oral route; however, the stomach was not examined by light microscopy.

Because of the highly reactive nature of acrolein, the concentration of a dose administered by gavage can affect the time course and degree of severity of toxicity at the point of entry and the relevance of the gavage bolus dose to human exposure. Rats have both a forestomach and a glandular stomach, while humans have only a glandular stomach. The glandular stomach is more resistant than the forestomach to pH changes and irritation. The residence time in the forestomach (of approximately 2 hours) is sufficiently long compared to the reaction time for toxicity with airway tissue observed in inhalation studies (i.e., microseconds) so that the dose to the glandular stomach may be much lower than that to the forestomach (TERA, 1998). The dog is a better model for glandular stomach toxicity than the rat, however, Parent et al. (1992c) administered acrolein (0.1% aqueous) in gelatin capsules to beagle dogs, so the dose concentration to the glandular tissue is not known. In lieu of studies that provide data on glandular stomach toxicity, the Parent et al. (1992a) study in the rat remains the most suitable choice for the principal study.

For more detail on Susceptible Populations, exit to the toxicological review, Section 4.8 (PDF).

__I.A.5. Confidence in the Oral RfD

Study -- Medium
Database -- High
RfD -- Medium to High

The overall confidence in this RfD assessment is medium to high. Confidence in the principal study is medium. Several supporting studies involving other species also indicated that mortality increases sharply with elevated dose. The research demonstrating acrolein's high reactivity, low systemic distribution, toxicity at the point of entry, pronounced decreases in serum creatinine phosphokinase (creatine kinase), citric acid cycle enzymes and liver GSH, and increased mitochondrial damage in the Wistar rat suggest interference with normal metabolic processes or possibly the absorption of essential nutrients sufficient to lead to early mortality. Further research is needed, however, to support a definitive mode of action. In the NTP (1995) study, glandular stomach and forestomach lesions were reported at higher doses and likely played a role in the observed mortality. Confidence in the database is judged high with chronic exposure studies in 2 species. Moreover, two studies (Parent et al., 1992b; Parent et al., 1993) provide evidence that reproductive and developmental effects are not critical endpoints although only one species was tested for reproductive effects (rat) and for developmental effects (rabbit). While the possibility of some transport of acrolein or a metabolite of acrolein to systemic sites remains, the critical target sites (discussed further in the Toxicological Review of Acrolein) are at the point of contact, e.g., the respiratory system, the gastrointestinal tract, mucous membranes, and skin. The high reactivity of acrolein and the lack of significant systemic distribution obviates the need to examine reproductive/ developmental effects in a second species. The overall confidence in this RfD assessment is medium-to-high; a variety of studies across different durations of exposure and in several different laboratory animal species has been consistent in demonstrating that in the absence of mortality there are no clear indications of adverse effects.

For more detail on Characterization of Hazard and Dose Response, exit to the toxicological review, Section 6 (PDF).

__I.A.6. EPA Documentation and Review of the Oral RfD

Source Document – U.S. EPA (2003)

This assessment was peer reviewed by external scientists. Their comments have been evaluated carefully and incorporated in finalization of the IRIS summary. A record of these comments is included as an appendix to the Toxicological Review of Acrolein (U.S. EPA, 2003). ***To review this appendix, exit to the toxicological review, Appendix A, Summary of External Peer Review Comments and Disposition (PDF)***.

Agency Consensus Date – 05/16/2003

__I.A.7. EPA Contacts (Oral RfD)

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202)566-1676 (phone), (202)566-1749 (FAX), or hotline.iris@epa.gov (email address).

in general, at (202)566-1676 (phone), (202)566-1749 (FAX) or hotline.iris@epa.gov (email address).

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_III. [reserved]
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_V. [reserved]

_VI. Bibliography

Substance Name -- Acrolein
CASRN -- 107-02-8
Last Revised -- 06/03/2003

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Exhibit F

EPA/635/R-03/003

TOXICOLOGICAL REVIEW

OF

ACROLEIN

(CAS No. 107-02-8)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

May 2003

US Environmental Protection Agency
Washington, DC

DISCLAIMER

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FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to chronic exposure to acrolein. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of acrolein.

In Section 6, EPA has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose response. Matters considered in this characterization include knowledge gaps, uncertainties, quality of data, and scientific controversies. This characterization is presented in an effort to make apparent the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's IRIS Hotline at 202-566-1676.

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Summaries of the external peer reviewers' comments and the disposition of their recommendations are in Appendix A.

1. INTRODUCTION

This document presents background and justification for the hazard and dose-response assessment summaries in EPA's Integrated Risk Information System (IRIS). IRIS Summaries may include an oral reference dose (RfD), inhalation reference concentration (RfC) and a carcinogenicity assessment.

The RfD and RfC provide quantitative information for noncancer dose-response assessments. The RfD is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis but may not exist for other toxic effects such as some carcinogenic responses. It is expressed in units of mg/kg-day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer effects during a lifetime. The inhalation RfC is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). It is generally expressed in units of mg/m^3.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral exposure and inhalation exposure. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates are presented in three ways. The *slope factor* is the result of application of a low-dose extrapolation procedure and is presented as the risk per mg/kg-day. The *unit risk* is the quantitative estimate in terms of either risk per μg/L drinking water or risk per $\mu g/m^3$ air breathed. Another form in which risk is presented in a drinking water or air concentration providing cancer risks of 1 in 10,000; 1 in 100,000; or 1 in 1,000,000.

Development of these hazard identification and dose-response assessments for acrolein has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidelines that were used in the development of this assessment may include the following: *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986a), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a), *Draft Revised Guidelines for Carcinogen Assessment* (U.S. EPA, 1999), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), (proposed) *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 1998b, 2000a), *Science Policy Council Handbook: Risk Characterization* (U.S. EPA, 2000b), *Benchmark Dose Technical Guidance Document* (U.S.

EPA, 2000c) and *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA 2000d).

The literature search strategy employed for this compound was based on the CASRN and at least one common name. At a minimum, the following data bases were searched: RTECS, HSDB, TSCATS, CCRIS, GENE-TOX, DART/ETIC, EMIC, TOXLINE, CANCERLIT, and MEDLINE. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document.

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

Acrolein is also known as acrylaldehyde, acrylic aldehyde, allyl aldehyde, ethylene aldehyde, 2-propenal, and prop-2-en-1-al (Izard and Libermann, 1978). Trade names include aqualin, aqualine, biocide, magnacide, magnacide B, and Slimicide (Ghilarducci and Tjeerdema, 1995). Some relevant physical and chemical properties are listed below (HSDB, 2003; unless otherwise referenced).

CASRN: 107-02-8
Empirical formula: C_3H_4O
Structure: C=C-C=O
Molecular weight: 56.06 g/mol
Vapor pressure: 274 mm Hg @ 25°C
Vapor density: 1.94 (Air = 1)
Specific gravity: 0.8389 @ 20°C
Boiling point: 52.5°C at 760 mm Hg
Melting point: -88°C
Water solubility: 208 g/L @ 20°C
Log K_{ow} (octanol / water partition coefficient): -0.01 (high water solubility)
Log K_{oc} (organic carbon / water partition coefficient): 0.5 (low adsorption to soil)
pH: 6.0 (max); a 10% solution in water @ 25°C
Eye irritation: beginning at 0.09 ppm for 5 minutes (Weber-Tschopp et al., 1977)
Odor threshold: 0.160 ppm (Amoore and Hautala, 1983)
Conversion factor: 1 ppm = 2.3 mg/m^3; 1 mg/m^3 = 0.44 ppm

At room temperature acrolein is a colorless to yellowish flammable liquid with a disagreeable, choking odor. It is extremely acrid and is irritating to mucous membranes (ACGIH, 1991). Reported values for the odor thresholds include 0.21 ppm (0.5 mg/m^3) (Leonardos et al., 1969) and 0.16 ppm (0.4 mg/m^3) (Amoore and Hautala, 1983).

The principal use of acrolein is as an intermediate in the synthesis of acrylic acid, which is used to make acrylates, and of DL-methionine, an essential amino acid used as an animal feed supplement. Other derivatives of acrolein are glutaraldehyde, pyridines,

tetrahydrobenzaldehyde, allyl alcohol and glycerol, 1,4-butanediol and 1,4-butenediol, 1,3-propanediol, DL-glyceraldehyde, flavors and fragrances, polyurethane and polyester resins. Acrolein is unstable and polymerizes (especially under light or in the presence of alkali or strong acid) to form diacryl, a plastic solid (Merck Index, 1966).

The most important direct use of acrolein is as a biocide. As an herbicide, acrolein is used to control algae, aquatic weeds and mollusks in recirculating process water systems. Acrolein also controls the growth of microorganisms in liquid fuel, the growth of algae in oil fields, the formation of slime in paper manufacture. It is used to promote cross-linking of protein collagen in leather tanning, and as a tissue fixative for histological samples (IARC, 1995).

Due to its high vapor pressure and water solubility, acrolein is expected to be highly mobile when released into the environment, although degradation processes are likely to limit its transport. Acrolein is released to the environment through manufacturing processes and its use as an intermediate for glycerine, methionine, glutaraldehyde and other organic chemicals. It is also released into the environment through exhaust gas from combustion processes, including tobacco smoke, emissions from forest fires, and auto exhaust. Acrolein has also been detected in sugar cane molasses, souring salted pork, the fish odor of cooked horse mackerel, the volatiles in white bread, the volatile components of chicken breast muscle, the aroma volatiles of ripe arctic bramble berries and the products from heating animal fats and vegetable oils (HSDB, 2003).

If released to air, a vapor pressure of 274 mm Hg at 25°C indicates acrolein will exist solely in the vapor-phase in the ambient atmosphere. Vapor-phase acrolein will be degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals, ozone, and nitrate radicals; the half-lives for these reactions in air are estimated to be 20 hours, 15 days, and 28 days, respectively. Acrolein in hexane solvent showed moderate absorption of UV light >290 nm, which indicated potential for photolytic transformation under environmental conditions (HSDB, 2003). Other reports for half-life are on the order of 4 to 20 hours with removal from the atmosphere primarily by reaction with hydroxyl radicals (Grosjean, 1990; Atkinson, 1985).

If released to soil, acrolein is expected to have very high mobility based upon an estimated Koc of 3 (log Koc = 0.5). Volatilization from moist soil surfaces is expected to be an important fate process based upon a Henry's Law constant of 1.22E-4 atm-m^3/mole. Acrolein may volatilize from dry soil surfaces based upon its vapor pressure (HSDB, 2003).

If released into water, acrolein is not expected to adsorb to suspended solids and sediment based upon the estimated Koc (HSDB, 2003). In deionized water at a concentration of 0.5 mg/ml, there was no decomposition of acrolein at 4 and 24 hours, but at 6 mg/ml, losses were reported of 0.5% by 4 hours and 3.9% by 24 hours (Parent et al., 1993). Lijinsky and Reuber (1987) measured loss of acrolein at 18% after 6 days at a temperature of 5°C, and 27% after 3 days at 22°C.

The half-life of acrolein in natural unsterilized water was 29 hours compared with 43 hours in sterilized (thymol-treated) water. Volatilization from water surfaces is expected to be

an important fate process based upon the compound's Henry's Law constant. Estimated volatilization half-lives for a model river and model lake are 4.4 hours and 4.6 days, respectively. An estimated bioconcentration factor (BCF) of 3 suggests the potential for bioconcentration in aquatic organisms is low (HSDB, 2003).

Occupational exposure to acrolein may occur through inhalation and dermal contact with this compound at workplaces where acrolein is produced or used. The half-life of acrolein in drinking water suggests some potential for water to be a source of exposure to humans. Howard et al. (1991) estimated groundwater half-lives of 11 days under aerobic conditions and 14-56 days under anaerobic conditions. However, limited studies indicate that it has rarely been detected in drinking or well water (Glaze et al., 1989; Staples et al., 1985), and the short half-lives of acrolein in surface waters make long range aquatic transport unlikely (CICAD, 2002).

Exposure of the general population occurs primarily through atmospheric contact (HSDB, 2003). EPA reported mean ambient acrolein concentrations of 14.3 $\mu g/m^3$ (6.2 ppb), ranging from 8.2 to 24.6 $\mu g/m^3$ (3.6 to 10.7 ppb), for two urban locations based upon data from 1961 to 1980 (U.S. EPA, 1993). Acrolein has been detected in exhaust gases from both gasoline engines (0.05-27.7 mg/m^3) and diesel engines (0.12-0.21 mg/m^3) (IARC, 1995).

Concentrations in indoor air may exceed outdoor levels 2- to 20-fold times (Environment Canada, 2000). Levels between 2.3 and 275 $\mu g/m^3$ have been reported in smoky indoor environments such as bars and restaurants (IARC, 1995). In residences where wood stoves were used, concentrations from 0.7-6.0 $\mu g/m^3$ have been reported (IARC, 1995). IARC (1995) noted that the acrolein concentrations in the smoke from various cigarettes ranged from 3-220 μg/cigarette. Levels as high as 463-684 μg/cigarette were reported in Japan (Kuwata et al., 1979). Jones et al. (1999) reported concentrations of acrolein in mainstream smoke (defined as smoke that is directly exhaled from the smoker) ranging from 10 – 140 μg per cigarette, and estimated concentrations in sidestream smoke (i.e., smoke emitted from the smoldering tobacco between puffs) in the range of 100 – 1700 μg per cigarette.

EPA's Toxic Release Inventory (TRI) lists the release of acrolein at on-site and off-site facilities for all industries in the US in calendar year 2000 as follows: Total Air Emissions - 208,108 lbs; Surface Water Discharges - 643 lbs; Underground Injection - 201,020 lbs; Releases to Land - 404 lbs; Total On-site Releases - 410,175 lbs; Total Off-site Releases - 410 lbs; Total On- and Off-site Releases - 410,585 lbs (TRI, 2003).

3. TOXICOKINETICS RELEVANT TO ASSESSMENTS

3.1. ABSORPTION AND DISTRIBUTION

Respiratory uptake studies with acrolein in dogs indicate that acrolein is retained at rates of 75-80% in the upper respiratory tract (URT) with a lesser rate of retention (65-70%) for the lower respiratory tract. At inhaled concentrations of 176-264 ppm (400-600 mg/m^3), 80-85% was retained in the total respiratory track at varying ventilation rates, suggesting little distribution elsewhere (Egle, 1972). Acrolein's strong reactivity with tissues is proposed to result in little systemic distribution (Beauchamp et al., 1985). This hypothesis is supported by the results from McNulty et al. (1984) who observed no reduction in liver glutathione (GSH) following inhalation of acrolein by rats, indicating that inhaled acrolein does not reach the liver to any great extent.

Deposition efficiency of inhaled acrolein (nominal concentrations of 0, 0.9, 4.5 and 9.1 ppm or 0, 2.1, 10.4, and 20.9 mg/m^3) in the upper respiratory tract of the anesthetized male F344 rat was examined by Morris (1996). During nose-only exposures of the surgically-isolated URT for 40 minutes, steady-state concentrations were not attained or maintained during the exposure, and uptake slowly decreased, suggesting limited uptake at these concentrations and durations.

Evidence for systemic absorption of acrolein from the gastrointestinal tract was reported by Draminski et al. (1983), who identified low levels of acrolein-derived conjugates in the urine of rats after ingestion of a single dose of 10 mg/kg body weight. This dose, however, resulted in 50% mortality and would be expected to cause severe gastrointestinal damage under these conditions. Damage to the stomach lining, especially endothelial cells (Patel and Block, 1993), may allow some absorption to occur. The likelihood of significant absorption from the gastrointestinal tract at lower concentrations is uncertain.

The distribution of [2,3-14C]acrolein administered to Sprague-Dawley rats (5/sex/group) after intravenous (iv) or oral gavage was evaluated by Parent et al. (1996a, 1998). Doses were 2.5 mg/kg (iv and oral), 2.5 mg/kg after 14 consecutive days of unlabeled acrolein (oral), and 15 mg/kg (oral). Radiolabel in expired air, urine, and feces was measured at 4, 12, and 16 and 24 hours post-dosing, then every 24 hours for the next 6 days. Data in the report demonstrate that the large majority of label (>96%) was recovered in excreta within the first 24 hours. Tissue concentrations (including blood) of radioactivity were minimal (<1.2% from the iv dosing and <0.7% from the oral dosing) and time course of excretion for all groups was similar except for delayed excretion in the high-dose group. Radiolabel measured in excreta and in tissues was associated with various acrolein metabolites and not attributed to parent compound. The radiolabel in feces was later determined to be associated with a homopolymer of acrolein, which was apparently formed in the gastrointestinal tract (Parent et al., 1998). These studies indicate little systemic distribution of acrolein.

The high reactivity of acrolein is due to the polarization of the double bond by the aldehyde group, and the resulting increased potential for nucleophilic addition. Because acrolein

readily reacts with sulfhydryl and amino groups on proteins, it is unlikely to distribute systemically, and thus its adverse effects are characterized in terms of cytotoxicity at the site of entry. Additional evidence of the reactivity of acrolein can be seen in conflicting data reported in the literature for the *in vitro* mutagenic potential of acrolein. In a series of Ames assays, Parent et al. (1996b) resolve many of the different outcomes by considering the presence or absence of non-DNA nucleophiles from the S9 activation mixture, in the test chemical solution, or in the plating solutions. If non-DNA nucleophiles were present, acrolein would rapidly and indiscriminately react with any available species and not reach the DNA target.

While the possibility of some transport of acrolein or a metabolite of acrolein to systemic sites remains, the critical target sites, as noted in the toxicology section, are those at the point of contact, the respiratory system, the gastrointestinal tract, mucous membranes, and skin.

3.2. METABOLISM AND EXCRETION

Absorbed acrolein reacts directly with protein and non-protein sulfhydryl groups, and with primary and secondary amines found in proteins and nucleic acids (Ghilarducci and Tjeerdema, 1995). In proteins, it preferentially attacks free SH groups of cysteine residues, ϵ-amino groups of lysine residues and histidine residues (Esterbauer et al., 1991). Uchida et al. (1998a,b) has shown that, *in vitro*, acrolein binds to serum albumin and low-density lipoproteins. Acrolein's role as a lipid peroxidation byproduct and possible mediator in various human diseases has been recently reviewed by Uchida (1999). It is well-documented that the conjugation of the β-carbon of acrolein with sulfhydryl groups is rapid and essentially irreversible (Esterbauer et al., 1976), and leads to thiazolidine derivatives and a decrease in glutathione (GSH) stores without an increase in oxidized GSH (GSSG). This pathway results in an acrolein-GSH adduct which is then further metabolized by both high- and low-affinity forms of mitochondrial and cytosolic aldehyde and alcohol dehydrogenase (Mitchell and Peterson, 1989); one resultant product has been identified as 3-hydroxypropylmercapturic acid (Clapp et al., 1969; Kaye and Young, 1970). This product has been isolated from urine of rats after subcutaneous injection of acrolein (Kaye, 1973) and after inhalation and intraperitoneal (ip) injection of Wistar rats (Linhart et al., 1996). The reduction of the acrolein-GSH adduct by alcohol dehydrogenase to 3-hydroxypropylmercapturic acid was postulated as a potentially important pathway (Mitchell and Peterson, 1989). There is increasing evidence that aldehydes such as acrolein are generated endogenously during the process of lipid peroxidation (Esterbauer et al., 1991); the rate constant for reaction of acrolein with cysteine at pH 7.4 was 220 M^{-1} sec^{-1} compared to 121 with GSH. Among all α, β-unsaturated aldehydes, acrolein is the strongest electrophile, which accounts for its high reactivity with nucleophiles (Witz, 1989). Thiol adducts of acrolein are considerably more stable than adducts formed by all other α, β-unsaturated aldehydes (Esterbauer et al., 1991).

When radiolabeled acrolein was administered by gavage (0.82 mg/kg) to one lactating goat, incorporation of radioactivity appeared to follow incorporation of metabolites into normal biosynthetic pathways (Sharp et al., 2001).

Elucidation of the major pathways of metabolism has been greatly enhanced by the

studies of Parent and colleagues. Parent et al. (1998) synthesized and characterized the potential metabolites of acrolein in the feces and urine of rats administered acrolein either orally or intravenously. The pathways of metabolism proposed by Parent et al. (1998) are illustrated in Figure 1. The main pathway appears to be an addition of GSH to the activated double bond, followed by processing to mercapturic acid derivatives, the three compounds at the bottom of the figure, which are then excreted in the urine after either oxidation or reduction of the aldehyde, with reduction predominating. Another pathway of metabolism is that of epoxidation of the double bond followed by attack of GSH on the epoxide. A third pathway involves addition of water to acrolein to form 3-hydroxypropionaldehyde, which is further oxidized to malonic acid and ultimately oxalic acid. Some of these compounds can be incorporated into normal metabolic pathways. For example, glycidaldehyde can be hydrated to glyceraldehyde (Patel et al., 1980).

None of the unconjugated metabolites resulting from the epoxidation of acrolein, such as those reported by Patel et al. (1980), were found in the excreta by Parent et al. (1998). A polar and a nonpolar fraction were extracted with a molecular weight range of 2,000-20,000 Da (Parent et al., 1998). They concluded that these compounds were either homopolymers of acrolein, or that the polyacrolein in this fraction was originally a copolymer with a natural polymer, either a protein or polysaccharide.

Marinello et al. (1984) incubated [^{14}C]acrolein with purified cytochrome P450 in the absence of NADPH and observed the binding of label. GSH inhibited the binding of label to hepatic microsomes by 90%. Binding to microsomes was substantially enhanced in the presence of NADPH. Addition of the P450 inhibitor, SKF-525A, in the presence of NADPH prevented binding of label.

Incubation of Wistar liver microsomes with 5 mM acrolein for 30 seconds resulted in a two-fold stimulation of GSH transferase and 0.1 mM for 30 minutes reduced GSH protection against lipid peroxidation (Haenen et al., 1988).

3.3. PHYSIOLOGICALLY-BASED TOXICOKINETIC MODELS

No physiologically-based toxicokinetic models are available for acrolein.

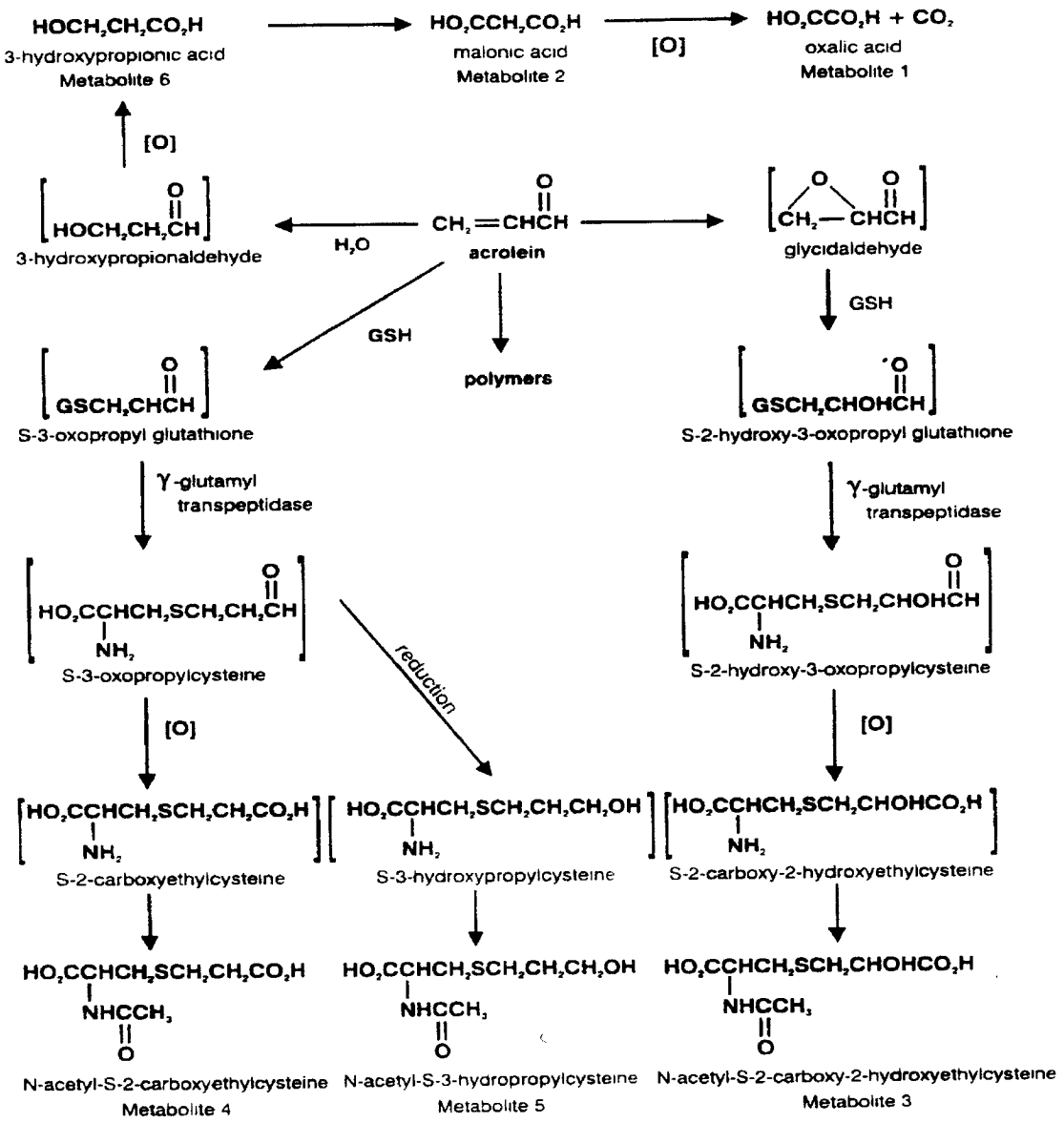

Figure 1. Proposed metabolism of acrolein in rats. The structures in brackets represent postulated intermediates.

Source: Reprinted from Toxicol Sci (1998) by Parent et al., with permission of the Society of Toxicology.

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS--EPIDEMIOLOGY, CASE REPORTS, CLINICAL CONTROLS

EPA has an interim policy on the use of third-party studies submitted by regulated entities (U.S. EPA, 2001). For these purposes, EPA is considering "third party studies" as studies that have not been conducted or funded by a federal agency pursuant to regulations that protect human subjects. Under the interim policy, the Agency will not consider or rely on any such human studies (third-party studies involving deliberate exposure of human subjects when used to identify or quantify toxic endpoints such as those submitted to establish a NOAEL or NOEL for systemic toxicity) in its regulatory decision making, whether previously or newly submitted. Some of the supporting studies discussed in this Toxicological Review are third-party studies; however, the scientific and technical strengths and weaknesses of these studies were described before this Agency policy was articulated. In addition, the studies cited provide data that suggest and inform a public health concern for acrolein, but were not designed or used as principal studies in the derivation of any quantitative value for acrolein based on NOAELs or LOAELs. The Agency is requesting that the National Academy of Sciences conduct an expeditious review of the complex scientific and ethical issues posed by EPA's possible use of third-party studies that intentionally dose human subjects with toxicants to identify or quantify their effects.

4.1.1. Acute Exposures (<24 hours)

A clinical study by Weber-Tschopp et al. (1977) provides the most comprehensive description of acute effects in humans. Three experiments were performed using male and female student volunteers: (1) a continuous exposure at constantly increasing acrolein concentrations, (2) short exposures to successively increasing concentrations, and (3) a 1-hour exposure to a constant concentration.

In experiment (1), 31 males and 22 females were exposed to acrolein for 40 minutes during which the acrolein concentration was gradually increased to 0.6 ppm (1.4 mg/m^3) during the first 35 minutes, then remained constant. The standard deviation in the acrolein concentration used was 0.023 ppm (3.8%). Groups of unexposed students were used as controls. The subjects had to fill out a questionnaire for the first 5 minutes. After that, the eye blinking frequency of two subjects was measured as well as the breathing frequency of a third subject during the entire exposure. The incidence (not stated) of complaints about eye irritation after 35 minutes of slowly increasing exposure from zero to a specified level and then held at that level for another 5 minutes was significantly higher ($p<0.01$) than controls beginning at 0.09 ppm (0.21 mg/m^3) and was increasing even at 0.6 ppm (1.4 mg/m^3). Nasal irritation was significantly higher ($p<0.01$) than controls beginning at 0.26 ppm (0.6 mg/m^3) and was increasing even at 0.6 ppm (1.4 mg/m^3). Throat irritation increased significantly through 0.43 ppm (1 mg/m^3). The eye blink frequency increased significantly beginning at 0.26 ppm (0.6 mg/m^3) ($p<0.01$).

In experiment (2), 17 males and 25 females were exposed, in groups of five, for 1 ½ minutes to successive concentrations of 0, 0.15, 0.3, 0.45, and 0.6 ppm (0, 0.3, 0.7, 1.0, and 1.4 mg/m^3). After one minute of exposure they were administered a questionnaire. Between each exposure they were allowed to recuperate in a clean room for 8 minutes. As in the first experiment, eye blink frequency and respiration rate were measured. The same controls as for the first experiment were used. Eye and nasal irritation was significantly higher ($p<0.05$) than controls beginning at 0.3 and 0.6 ppm (0.7 and 1.4 mg/m^3), respectively. Throat irritation was not evident.

In experiment (3), 21 males and 25 females were distributed into three groups and exposed for 60 minutes to a constant acrolein concentration of 0.3 ppm (0.7 mg/m^3). As in the other two experiments, eye blink frequency and respiration rate were measured. In controls, measurements of eye blink and breathing frequency, and subjective symptoms of irritation were assessed at the beginning of exposure. Each of the effects increased significantly ($p<0.01$) during the first 20-30 minutes of exposure compared to controls, after which the irritation effects reached a plateau. Eye blink frequency reached a steady rate after 10 minutes of exposure. During exposure there was a decrease in the average respiration rate (16 individuals) after 40 minutes ($p<0.01$). Each individual that demonstrated an increase in eye blink frequency also reported a sharp increase in eye irritation. Throat irritation, not a significant response in the previous two experiments, was increased compared to controls after 10 minutes of exposure.

It was concluded by the investigators that the average threshold of sensation lies in the range of 0.09 (eye irritation) to 0.30 ppm (respiration rate, throat irritation) with nasal irritation at 0.15 ppm (0.35 mg/m^3). No adaptation to these effects was observed.

According to the review by Esterbauer et al. (1991), a level of 5.5 ppm (12.6 mg/m^3) resulted in painful eye and nose irritation after 20 seconds, and 22 ppm (51 mg/m^3) was immediately intolerable. In one case report, exposure to 153 ppm (352 mg/m^3) for 10 minutes was fatal.

Sim and Pattle (1957) exposed volunteers (12 males/group) to 0.8 and 1.2 ppm (1.88 and 2.80 mg/m$^{3)}$ acrolein for 10 and 5 minutes, respectively. Acrolein concentration was determined via reaction with hydroxylamine hydrochloride, followed by back titration to pH 4.5. Volunteers were exposed simultaneously in 100 m^3 exposure chambers with no restrictions on movement or smoking within the chamber. The vapor was described by the volunteers as "extremely irritating" to all exposed mucosal surfaces, with lacrimation occurring within 20 and 5 seconds in the low and high exposures, respectively. Ten minutes of low-dose exposure was described as "only just tolerable," and high-dose exposure for more than 5 minutes "would have been extremely distressing." The comments by the volunteers were subjective, and it does not appear that any other endpoints were monitored. The effects of exposure to acrolein were considerably more apparent than exposure to much higher concentrations of several other aldehydes.

In one of two additional case reports, a 27-month-old boy was exposed to probable high levels of acrolein (and other chemicals) from burning vegetable oil for one hour (Mahut et al., 1993). No exposure measurements were reported. Initial acute respiratory failure regressed in a

few hours, but in the months following exposure diffuse bronchiectasis developed. In the second case report, a chemical worker was exposed to a sudden release of acrolein from a rupture in the workplace. The principal effect was chemical pneumonia and eye irritation, both of which resolved with treatment (Champeix et al., 1966).

In summary, based upon the available human data, levels as low as 0.09 ppm (0.21 mg/m^3) for 5 minutes may elicit subjective complaints of eye irritation with increasing concentrations leading to more extensive eye, nose and respiratory symptoms.

4.1.2. Exposures (> 24 Hours)

No chronic studies of humans exposed to acrolein are available.

The only study relating to cancer was a nested case control study by Ott et al. (1989), in which individuals were classified as having been exposed to one of a large number of chemicals in the work environment. The study investigators reported non-Hodgkin's lymphoma (52 cases), multiple myeloma (20 cases), nonlymphocytic leukemia (39 cases), and lymphocytic leukemia (18 cases) within a cohort of employed men from two chemical manufacturing facilities and a research and development center. Exposure odds ratios were examined in relation to 111 work areas, 21 specific chemicals, and 52 chemical activity groups. Odds ratios of 2.6 (2 cases) for non-Hodgkin's lymphoma, 1.7 (1 case) for multiple myeloma, and 2.6 (3 cases) for nonlymphocytic leukemia were reported for workers exposed to acrolein. None of the lower 95% confidence limits exceeded 1.0. Because of a lack of a statistically significant increase in the cancer endpoints and the likelihood of confounding by concomitant exposure to other chemicals in the workplace, the results must be considered equivocal.

4.2. ACUTE STUDIES IN ANIMALS—ORAL AND INHALATION

4.2.1. Lethality Studies

Ballantyne et al. (1989) examined the effects of 1- and 4-hour exposures to acrolein in male and female Sprague-Dawley rats (5/sex/exposure). Animals were exposed to 14, 22, 24, 31, and 81 ppm (32, 50, 55, 71, and 186 mg/m^3)[1] acrolein for 1 hour or 4.8, 7.0, 9.1, and 12.1 ppm (11, 16, 20.8, and 27.7 mg/m^3) for 4 hours. One- and 4-hour LC_{50} values of 65 and 25.8 mg/kg, respectively, were calculated for the combined sexes. Clinical signs of sensory irritation and toxicities were observed at all exposure concentrations. Lachrymation, perinasal and periocular wetness and encrustation, mouth and audible breathing, decreased breathing rate, and hypoactivity were observed during exposure in all animals. Signs of respiratory distress and hypoactivity were observed for post-exposure days 1-6. Body weights of survivors decreased during the first post-exposure week but the weight was regained during the second week.

A necropsy of animals that died during the post-exposure period revealed perinasal and

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[1]Conversion to mg/m^3: 1 ppm = 2.3 mg/m^3

perioral encrustation, mottled discoloration of the lungs and liver, clear fluid in the trachea and thoracic cavity, gas-filled stomach and intestine, and opaque or cloudy corneas. Histology of the lungs revealed congestion and intra-alveolar hemorrhage, fibrin disposition in the smaller airways, and necrosis and exfoliation of the bronchiolar epithelium. Death was attributed to lung injury. Histopathology was not performed on surviving animals.

In another study examining acute exposure effects of acrolein in rats, Crane et al. (1986) exposed Sprague-Dawley rats to acrolein at concentrations ranging from 580-41,550 ppm (1,330-95,268 mg/m^3) for exposure durations ranging from 2.8 to 36.5 minutes until animals were incapacitated. The time-to-incapacitation endpoint (i.e., when rats could no longer perform a coordinated act of walking in a rotating cage, and exhibiting stumbling, sliding, or tumbling) was recorded for each animal. Exposure was then continued until animals expired and the time of death was reported. After incapacitation, death occurred very quickly (in 1.9-19.7 minutes). Prior to death, animals exhibited clinical signs of respiratory distress, agitation, and convulsions. Ocular effects were not noted.

Mortality and clinical signs have also been reported in other species. Groups of 50 mice, 20 guinea pigs, and 5 rabbits were exposed to 2,279 ppm (5,225 mg/m^3) of acrolein vapor for 13, 25, and 27 minutes, respectively, until the animals died (Salem and Cullumbine, 1960). In addition, the same species were exposed to 2,019 ppm (4,624 mg/m^3) of an acrolein aerosol for 13, 24, and 26 minutes, respectively, until the animals died. Initial exposure to both forms of acrolein caused increased activity which was attributed to compound-related irritation. Respiration then slowed and animals convulsed just prior to death. Of the nine aldehydes tested, acrolein had the highest relative toxicity.

Beeley et al. (1986) examined the effects of acute acrolein exposure in female New Zealand rabbits. Animals (18/group) were exposed to 375 or 489 ppm (860 or 1,121 mg/m^3) for 15 minutes. Animals were sacrificed at 3 days post-exposure, and lung and trachea were removed and examined for histopathological changes. Five animals in the 860 mg/m^3 exposure group and 8 animals in the 1,121 mg/m^3 exposure group died during the 3 day post-exposure period. The surviving animals exhibited edema, necrosis of the lung parenchyma, and damage to the bronchial linings of the large airways. Acute inflammatory reactions were found in conjunction with areas of necrosis.

To assess the potential of acrolein to impair escape, a signal avoidance task was developed in which a baboon's ability to escape from a chamber containing the noxious gas was monitored (Kaplan, 1987). Male juvenile baboons (1/group) were exposed to 12, 25, 95, 100, 250, 505, 1,025, or 2,780 ppm (28, 57, 218, 229, 573, 1,158, 2,350, or 6,374 mg/m^3) for 5 minutes. After exposure, animals were allowed to exit the chamber by depressing a lever. Escape time, i.e., the time it took for the animal to select the correct lever and exit the chamber, was measured. Acrolein exposure did not inhibit escape time. However, irritant effects of the gas were noted at each concentration tested, and the severity of the effects increased with increasing concentration. Irritant effects manifested from blinking and closing of the eyes and rubbing the nose/eyes at lower concentrations to salivation, nasal discharge, violent shaking of the head, and nausea at higher concentrations. However, the exposures at which the more

serious effects occurred were not reported by the study authors. Animals exposed to 2,350 and 6,374 mg/m^3 acrolein expired after 24 and 1.5 hours, respectively. Severe pulmonary edema and hemorrhage were the significant histological changes observed in these two animals.

An acute oral LD_{50} was reported as 29 mg/kg in Sprague-Dawley rats administered acrolein by gavage (Bioassay Systems Corp., 1981c); male rats were somewhat more sensitive with an LD_{50} of 25 mg/kg compared to females with an LD_{50} of 33 mg/kg. In contrast, LD_{50} s of 10.3 (males) and 11.8 mg/kg (females) were reported in another gavage study with acrolein with a stated purity of 97% (Microbiological Assoc., 1989). In male CD-1 mice the LD_{50} was 14 mg/kg (Bioassay Systems Corp., 1981d). In female mice, the LD_{50} was determined to be 18 mg/kg (Bioassay Systems Corp., 1981e). The acute dermal LD_{50} in New Zealand white rabbits was 231 mg/kg with females somewhat more sensitive (223 mg/kg) than males (240 mg/kg) (Bioassay Systems Corp., 1981f).

4.2.2. Sensory Irritation

Alterations in respiratory function have been used as an indicator of sensory irritation. Murphy et al. (1963) exposed male guinea pigs (n=10) to 0.6 ppm (1.4 mg/m^3) acrolein for 2 hours. The study authors reported that expiratory flow resistance and tidal volume increased and respiratory rate decreased. These adverse responses were rapid and reached a maximum within 30 to 60 minutes. In a second experiment, male guinea pigs were exposed to 0.1, 0.2, 0.35, 0.6 or 1 ppm (0.2, 0.5, 0.8, 1.4, or 2.3 mg/m^3) for 2 hours. Respiratory flow resistance during inspiration and expiration was significantly increased and respiratory rates decreased at levels of 0.35 to 1 ppm (0.8 to 2.3 mg/m^3). The study authors also reported that several drugs (atropine, aminophylline, isoproterenol, and epinephrine) partially or completely reversed increased flow resistance. Statistically significant increases in respiratory resistance and tidal volume coupled with decreases in respiration rate and minute volume were observed in guinea pigs exposed to 17 ppm (40 mg/m^3) for 60 minutes (Davis et al., 1967).

One measure of the potency of a sensory irritant is the exposure concentration at which respiratory rate is depressed by 50% (RD_{50}). Table 1 shows RD_{50}s for mice and rats. A comparison of rat and mouse values indicates that mice are more sensitive than rats to sensory irritation. Respiratory rate depression following acrolein exposure recovers rapidly, usually within 10 minutes (Cassee et al., 1996a; Nielsen et al., 1984; Steinhagen and Barrow, 1984). However, the recovery rate decreases as acrolein concentration increases. Cassee et al. (1996a) reported that 24 hours after exposure of Wistar rats to 1.7, 11.1 and 31.9 ppm (3.9, 25.4, and 73 mg/m^3), breathing patterns were comparable to pre-exposure values, indicating that the effect was not persistent. The decrease in breathing frequency was maximal between 1 and 3 minutes of exposure with desensitization occurring only with the two lower concentrations. Kane and Alarie (1977) reported that 4 daily consecutive 3-hour exposures to 0.5 and 1.7 ppm (1.1 and 3.9 mg/m^3) caused further decreases in respiratory rate, which suggests that animals become sensitized to the irritant effect. However, when animals were exposed to 0.17 ppm (0.39 mg/m^3) acrolein 3 hr/day for 3 days and then exposed to 0.44-11.2 ppm (1.0-26.7 mg/m^3) acrolein for 10 minutes, there was a decrease in response compared to controls, i.e., the control RD_{50} was 1.7 ppm (3.9 mg/m^3) compared to 3 ppm (6.8 mg/m^3) in pre-exposed animals.

TABLE 1. RD_{50}s for Rats and Mice

Species	RD_{50}	Reference
F-344 Rats (male)	6.0 ppm (13.7 mg/m^3)	Babiuk et al., 1985
Wistar Rats (male)	4.6 ppm (10.5 mg/m^3)	Bergers et al., 1996
Wistar Rats (male)	9.2 ppm (21.7 mg/m^3)	Cassee et al., 1996b
Swiss Webster Mice (male)	1.7 ppm (3.9 mg/m^3)	Kane and Alarie, 1977
Ssc:CF-1 Mice (male)	2.9 ppm (6.6 mg/m^3)	Nielsen et al., 1984
B6C3F1 Mice (male)	1.41 ppm (3.2 mg/m^3)	Steinhagen and Barrow, 1984
Swiss Webster Mice (male)	1.03 ppm (2.4 mg/m^3)	

Davis et al. (1967) examined the respiratory irritant effect of acrolein in normal and tracheotomized guinea pigs. Groups of normal and tracheotomized guinea pigs were exposed to 17 ppm (39 mg/m^3) acrolein for one hour. Normal animals exhibited clinical signs of sensory irritation, i.e., depressed respiratory rate as described by Murphy et al. (1963). However, tracheotomized animals did not exhibit respiratory rate depression. A similar finding was reported by Kane and Alarie (1977). Davis et al. (1967) theorized that tracheotomized animals lacked receptors for irritant responses that were present in the intact animal.

To further understand the mechanism through which acrolein elicits its irritant effect, Lee et al. (1992) examined the effect of capsaicin treatment of the cervical vagi followed by acrolein exposure in rats. Capsaicin treatment selectively blocked C-fiber afferent nerves and inhibited the respiratory rate depression normally observed during acrolein exposure. In addition, bilateral vagotomy also inhibited the respiratory rate depression. These results are consistent with a mode of action in which acrolein activates C-fiber afferent nerves.

Since acrolein exposure in the workplace is usually concurrent with other chemicals, particularly aldehydes, studies have been undertaken to examine the effects of acrolein exposure with pre-exposure and co-exposure to other chemicals. Babiuk et al. (1985) examined the effects of pre-exposure to 15 ppm (34 mg/m^3) formaldehyde 6 hr/day, for 9 days followed by exposure to acrolein for 10 minutes on the 10th day. The study authors reported that the RD_{50} in pre-exposed animals increased to 29.6 ppm (68.1 mg/m^3) compared to 6 ppm (13.8 mg/m^3) in the controls. This would suggest that pre-exposure to lower concentrations of sensory irritants desensitizes animals to sensory irritation effects of acrolein. However, co-exposure to acrolein with other aldehyde sensory irritants, acetaldehyde and formaldehyde, resulted in a more pronounced decrease in respiratory rate in male Wistar rats than exposure to acrolein only (Cassee et al., 1996a). Groups of four rats were exposed to a mixture of the three at concentrations which were expected to result in a decrease in breathing frequency (DBF) between 10 and 35% for each. The observed DBF for the mixture was more pronounced than the DBF for each chemical separately, but was less than the sum of the DBFs for the single

chemicals. Model prediction indicated that the combined effect was consistent with a competition for a common receptor, i.e., the trigeminal nerve.

The clinical signs and sensory irritation reported in the above mentioned animal studies indicate that the respiratory system is a principal target following acute exposure to acrolein. Further studies provide additional evidence. Kilburn and McKenzie (1978) exposed Syrian golden hamsters to 6 ppm (14 mg/m^3) acrolein for 4 hours, which caused a > 50% exfoliation of ciliated cells in the bronchi. The cells were pale and swollen at 24 and 48 hours post-exposure. The basal lamina was indented or penetrated by proliferating basal cells. After 96 hours there were areas of irregular epithelium with early stratification and hyperplasia. There was no recruitment of polymorphonuclear leukocytes (PMN) to the trachea or intrapulmonary airways; however, acrolein administered absorbed on carbon or simultaneous with carbon was chemotactic for PMN leukocytes. Formaldehyde behaved similarly to acrolein.

Acrolein has been reported to deplete the neuropeptides calcitonin-gene related peptide (CGRP) and substance P in the trachea of rats (Springall et al., 1990). Female Wistar rats exposed to 22, 81 or 249 ppm (51, 186, or 571 mg/m^3) for 10 minutes exhibited a dose-dependent decrease in these two sensory neuropeptides. The study authors suggested that the neuropeptide decrease could be responsible for the observed vasodilation and bronchoconstriction that follows irritant exposure. Roemer et al. (1993) reported that respiratory tract cell proliferation in male Sprague-Dawley rats occurred following an acute 6-hour exposure to 0.2 and 0.6 ppm (0.46 and 1.4 mg/m^3) acrolein.

Bronchial hyperresponsiveness following acrolein exposure has also been reported. Leikauf (1991) and Leikauf et al. (1989a) exposed guinea pigs to 0.31-1.26 ppm (0.71-2.9 mg/m^3) acrolein for 2 hours and determined bronchial responsiveness with an acetylcholine challenge up to 24 hours after exposure. The effective dose of acetylcholine sufficient to double specific resistance (ED_{200}) was decreased at all post-exposure times. The authors interpreted these results as suggestive evidence that asthmatics may be predisposed to an asthmatic attack following acrolein exposure. In addition, thromboxane B_2, the inactive form of the potent vasoconstrictor thromboxane A_2, and prostaglandin $F_{2\alpha}$ were increased immediately after exposure, and neutrophils were increased 24 hours after exposure. In a subsequent study, Leikauf et al. (1989b) reported that acrolein exposure resulted in an increase in leukotriene C4 (LTC4) in bronchoalveolar lavage fluid in guinea pigs. It was also determined that hyperresponsiveness to acetylcholine following acrolein exposure could be abated if guinea pigs were pretreated with 5-lipooxygenase inhibitors and leukotriene receptor antagonists, which suggests that the sulfidopeptide leukotrienes play a causal role in acrolein-induced bronchial hyperresponsiveness.

Acute exposure of Swiss-Webster mice to acrolein (0.3 or 0.6 μg/ml; 300 or 600 mg/m^3) decreased pulmonary compliance, pulmonary resistance, tidal volume and respiratory frequency (Watanabe and Aviado, 1974); pretreatment with a beta-adrenergic blocking agent indicated that lung effects were not mediated through adrenergic receptors. Similarly, chronic exposure (30 minutes, daily, 5 weeks) to a lower concentration reduced pulmonary compliance.

4.2.3. Other Effects

Antibacterial Defenses: Several studies have assessed the effects of acrolein exposure on pulmonary antibacterial defenses. Jakab (1977) exposed Swiss CD-1 mice (18-24 animals/group) to 1-2 ppm (2.3-4.6 mg/m^3) acrolein for 4 or 24 hours following a 0.5-hour bacterial challenge to *Staphylococcus aureus* and *Proteus mirabilis*. After 24 hours of exposure, there was a statistically significant increase in the number of surviving bacteria (both *S. aureus* and *P. mirabilis*) in exposed animals compared to controls. In a second study, Astry and Jakab (1983) exposed female Swiss mice (6/group) to 0.5, 3, 6.2, 7.5, or 9 ppm (1.1, 6.8, 14.2, 17.2, or 20.6 mg/m^3) for 8 hours following a 45-minute bacterial challenge to *S. aureus*. Exposure to 0, 0.5, 3.0, 6.2, 7.6, and 9.1 ppm (0, 1.1, 6.8, 14.2, 17.2, and 20.6 mg/m^3) resulted in survival of 3.2, 5.0, 12.8, 33.9, 35, and 40% of bacteria, respectively. The study authors reported significantly greater percent of surviving bacteria at exposures ≥ 3 ppm (6.8 mg/m^3). Exposure to 0.09 ppm (0.21 mg/m^3) acrolein for 3 hours following exposure to *Klebsiella pneumonia* had no effect on percent bacteria killed compared to controls in female CD1 mice (Aranyi et al., 1986). These studies suggest that acrolein exposure can inhibit pulmonary antibacterial defenses.

Cardioinhibitory Effects: Egle and Hudgins (1974) examined possible cardioinhibitory effects of acrolein exposure in male Wistar rats. Animals (6-11/group) were exposed to concentrations of acrolein ranging from 4-2,181 ppm (9.2-5,000 mg/m^3) for 1 minute. Animals were assessed for changes in blood pressure and heart rate. The principal effects observed were significant increases in blood pressure and heart rate with exposure concentration, with statistically significant increases in heart rate occurring at exposures ≥ 50 mg/m^3. However, exposure to 1,100 and 2,200 ppm (2,500 and 5,000 mg/m^3) acrolein generally caused a decrease in heart rate. Intravenous studies in Wistar rats with several aldehydes indicated that the relative pressor potency of acrolein was higher than that of formaldehyde, acetaldehyde and proprionaldehyde.

Biochemical Changes: Biochemical changes have also been reported following inhalation exposure to acrolein. Alabert et al. (1971) found significant alterations in NAD/NADH ratios in liver, lung, and brain of rats exposed to high concentrations of acrolein. Murphy (1965) reported that liver alkaline phosphatase and tyrosine transaminase activities were increased 3.1- and 3.6-fold, respectively, in male Holtzman rats exposed to 8 ppm (18.3 mg/m^3) acrolein for 4 hours; a dose-response relationship was also observed upon injection of acrolein. Cassee et al. (1996b) examined changes in the nasal epithelium of male Wistar rats exposed to 0, 0.25, 0.67, or 1.40 ppm (0, 0.45, 1.2, or 2.5 mg/m^3) acrolein by nose only exposure for 6 hours. No effects on cell proliferation or treatment-related lesions were observed for this duration of exposure. Likewise, non-protein sulfhydryl levels were similar to controls. However, exposure to 0.67 or 1.4 ppm (1.2 or 2.5 mg/m^3) acrolein significantly decreased GSH reductase activity in a dose-dependent manner.

Glutathione and P450 Levels: When male rats were given a single i.p. dose of acrolein (89 μmoles/kg) and sacrificed at 30 min, 4 and 24 hours, hepatic GSH was decreased 51% only at the 4-hour period (Witz, 1989). Levels returned to normal at 24 hours. However, cytochrome P450 levels were 61-71% of controls at 24 hours. Walk and Hausmann (1989) found that acute

inhalation exposure of rats to acrolein (0.7 to 4 ppm; 1.6 to 9.2 mg/m^3) resulted in a decrease in the total glutathione (GSH and GSSG) pool of nasal and olfactory epithelia and in the trachea and lungs. These decreases were accompanied by complex changes in GSH enzyme activities. After a 4-hour exposure of rats to acrolein (1 to 15 ppm; 2.3 to 34.5 mg/m^3), a dose-dependent decrease in the total GSH pool was observed in nasal olfactory and respiratory epithelia (Hausmann and Walk, 1989). Activities of GSH peroxidase, GSH reductase, and GSH transferase increased slightly in olfactory epithelium, but decreased in respiratory epithelium as exposures increased.

Eye Irritation: Eye lesions were reported in New Zealand white rabbits when acrolein was placed on the everted lower lids and examined for different time periods up to 7 days post-exposure (Bioassay Systems Corp., 1981a).

Skin Irritation: Acrolein was determined to be a skin irritant after 0.5 ml was place on intact and abraded skin of six male New Zealand white rabbits with erythema and edema scored after 24 and 72 hours (Bioassay Systems Corp., 1981b).

4.3. PRECHRONIC AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS--ORAL AND INHALATION

4.3.1. Noncancer Toxicity

Acrolein, like other aldehydes, is a known sensory irritant (Lyon et al., 1970; Cassee et al., 1996a,b) producing both nasal and eye irritation. Breathing frequency which is depressed upon initial exposure has been shown in Wistar rats to partially or fully recover during post-exposure. Sensory irritation and depressed breathing frequency are regarded as defense mechanisms for penetration to the lower respiratory tract. Acrolein was the most potent of 15 saturated and unsaturated aldehydes in sensory irritation potential as measured by the reflex decrease in respiratory rate in B6C3F1 and Swiss-Webster mice (Steinhagen and Barrow, 1984). The relationship of the RD_{50} and other structure-activity properties of acrolein in relation to other sensory irritants has been documented by Alarie et al. (1998).

4.3.1.1. Inhalation Studies

Several studies have found that subacute exposure of guinea pigs, rats, and mice to acrolein causes pulmonary inflammation, decreases in respiratory rate, and nasal lesions, effects also seen upon acute exposure. The effects of inhaled acrolein on laboratory animals are shown in Table 2.

TABLE 2. Effects of Inhaled Acrolein on Laboratory Animals

Species	Exposure Duration	Concentration (ppm)	Principal Effects	Reference
Rat				
Wister, male	6 h/day for 3 days	0, 0.25, 0.67, and 1.4	Nasal necrosis of respiratory epithelium and increased proliferation up to 0.67 ppm; 1.4 ppm group not evaluated.	Cassee et al. (1996b)
S-D, male	6 h/day 5 days/wk for 3 weeks	0, 0.1, 1.0, and 3.0	No effect on macrophage killing of inhaled *K. pneumonia.*	Sherwood et al. (1986)
S-D, male	6 h/day 5 days/wk for 3 weeks	0, 0.1, 1.0, and 3.0	Nasal exfoliation, erosion necrosis of respiratory epithelium and squamous metaplasia at 3 ppm; no effects on lungs or in local pulmonary antibody responsiveness to *L. monocytogenes.*	Leach et al. (1987)
F-344, male	6 h/day 5 days/wk 62 days (except for weekends for 12.4 weeks)	0, 0.4, 1.4, and 4.0	1. High mortality at 4 ppm. 2. Increase in lung collagen at 1.4 and 4 ppm ($p<0.05$). 3. Elastin content in 4 ppm group twice controls. 4. Bronchial necrosis and pulmonary edema at 4 ppm. 5. Parenchymal restriction at 0.4 ppm and obstructive lesions at 4.0 ppm. 6. No cytogenic or sperm abnormalities	Kutzman (1981); Kutzman et al. (1985); Costa et al. (1986)
Dahl, female (selected for susceptibility or resistance to salt-induced hypertension)	6 h/day 5 days/wk for 61-63 days (excluding weekends for 12.4 weeks)	0, 0.4, 1.4, and 4.0	1. All susceptible 4 ppm rats died after 11 days and 60% of resistant rats survived to end of study. 2. Lungs of susceptible rats had severe airway necrosis with edema and hemorrhage but only proliferative changes with resistant rats. 3. No differences in histopath between rat groups at lower doses. 4. No effect of exposure on blood pressure changes.	Kutzman et al. (1984, 1986)
SPF-OFA, male	Not explicitly stated, but up to 77 days	0, 0.55	1. Decrease in alveolar macrophage. 2. No effects on reproductive potential.	Bouley et al. (1975, 1976)
S-D, male	7 h/day for 3 consecutive days	1.7	1. Olfactory degeneration in all exposed rats. 2. Ulceration of respiratory epithelium in 4/10.	Teredesai and Stinn (1989)

Species	Exposure Duration	Concentration (ppm)	Principal Effects	Reference
S-D, male	6 h/day for 1 or 3 days	0, 0.2, and 0.6	Proliferative nasal and tracheal cells in epithelia at both concentrations.	Roemer et al. (1993)
Mouse				
Swiss-Webster, male	6 h/day for 5 days	1.7	1. Lesions of moderate severity in respiratory epithelium except for severe squamous metaplasia. 2. Lesions (ulceration and necrosis) of moderate severity in olfactory epithelium with squamous metaplasia mild. 3. Incomplete recovery after 72 hours.	Buckley et al. (1984)
Swiss, female	4 h/day for 4 days	2.5	Coexposure to acrolein and carbon black increased pulmonary killing of *P. mirabilis* and impaired elimination of *L. monocytogenes*. Killing of *S. aureus* was suppressed on first post-exposure day, but returned to normal on seventh day.	Jakab (1993)
CDI, female	3 h/day for 5 consecutive days	0.1	Decreased ($p<0.01$) in percent killing of *S. zooepidemicus* and *K. pneumonia.*	Aranyi et al. (1986)
White albino, male	6 h/day, for one 5-day period or 6h/day for two 5-day periods	various	1. Lung lesions (but no mortality) in mice exposed for two 5-day periods (concentration unknown). 2. LC_{50} of 66 ppm in group exposed for 6 hours. 3. 91% mortality in mice exposed to 50 ppm for 5 days.	Philippin et al. (1970)
FVB/N, male	6 h/day 5 days/wk for 3 weeks	3.0	Acrolein-induced excessive macrophage accumulation was associated with mucus hypersecretion.	Borchers et al. (1999b)
Guinea Pig, Rabbit				
Guinea pigs	7.5 h/day 2 consecutive days	1.6	1. Pulmonary inflammation. 2. Prolonged increase in airway sensitivity to substance P.	Turner et al. (1993)
Rabbits, New Zealand, female	15 min	375 and 489	1. Mortality at both concentrations. 2. Extensive lung damage at both concentrations.	Beeley et al. (1986)

Species	Exposure Duration	Concentration (ppm)	Principal Effects	Reference
Multiple				
S-D rat, male and female Beagle dogs, male Princeton or Hartley guinea pigs, male and female Squirrel monkeys, male	8 h/day 5 days/wk for 6 weeks	0.7 or 3.7	1. No concurrent controls. 2. No nasal histopathology. 3. Excessive salivation and eye irritation in dogs and monkeys at 3.7 ppm. 4. Chronic lung inflammatory changes and occasional emphysema in all animals at 0.7 ppm.	Lyon et al. (1970)
	24 h/day for 90 consecutive days	0.22, 1.0, and 1.8	1. Ocular and nasal discharges in dogs and monkeys at 1 ppm; severe at 1.8 ppm. 2. Squamous metaplasia of trachea in all monkeys at 1.8 ppm. 3. Two dogs at 1.8 ppm had confluent bronchopneumonia. 4. Evidence of pulmonary inflammation (guinea pigs at 1 ppm) and fecal liver necrosis (rats and guinea pigs at 1 ppm). 5. Nonspecific inflammatory changes in a variety of tissues in both rats and guinea pigs at 1.8 ppm.	
Syrian golden hamsters, male and female Wister rats, male and female Dutch rabbits, male and female	6 h/day 5 days/wk for 13 weeks	0, 0.4, 1.4, and 4.9	1. Mortality in rats at 4.9 ppm. 2. Necrotizing rhinitis in rats at 4.9 ppm and squamous metaplasia at 1.4 ppm with neutrophilic infiltration. 3. Lungs of hamsters unaffected. Severe nasal lesions in hamsters at 4.9 ppm, and tracheal hyperplasia in all female hamsters at 4.9 ppm. 4. Nasal and tracheal lesions similar to rat and hamster in rabbits at 4.9 ppm; no nasal lesions at lower doses.	Feron et al. (1978)

RATS: Male Wistar rats (5-6/group) were exposed 6 hr/day, for 3 consecutive days, in a nose-only exposure chamber to acrolein at concentrations of 0, 0.25, 0.67, or 1.4 ppm (0, 0.6, 1.5, or 3.2 mg/m^3) (Cassee et al., 1996b). Variation in exposure concentration was 13%. Rats were examined for nasal lesions (6 levels of the nasal tract examined) immediately after the last exposure. **Histopathology:** After one 6-hour exposure, no treatment-related histopathological lesions were found in any of the treatment groups. Only the histopathology of the 0.25 and 0.67 ppm (0.6 and 1.5 mg/m^3) groups were reported following three days of exposure; effects at 1.4 ppm (3.2 mg/m^3) were not reported. After 3 days, slight to moderate effects were observed from acrolein exposure in two of the four histopathology categories evaluated. In the category for disarrangement, necrosis, thickening and desquamation in the respiratory/transitional epithelium, 4/5 animals exposed to 0.25 ppm (0.6 mg/m^3) were observed to have slight effects (characterized as mainly disarrangement) and 1/5 developed a moderate level of effect. In the 0.67 ppm (1.5 mg/m^3) group, 3/6 were classified as slightly affected and 3/6 rats developed a moderate degree of response. For rhinitis, 1/5 of the 0.25 ppm (0.6 mg/m^3) rats developed a moderate response, and only 1/6 of the 0.67 ppm (1.5 mg/m^3) rats had a response and it was scored as a slight response. For the other two categories, single cell necrosis or atrophy of the olfactory epithelium, no effects were observed in either the 0.25 or 0.67 ppm (0.6 or 1.5 mg/m^3) group. **Proliferation:** After one 6-hour exposure, no treatment-related proliferative effects were found in any of the treatment groups. A proliferative response was defined as basal cell proliferation and/or an increased number of mitotic figures in respiratory/transitional epithelium. After 3 days, 3/5 male rats at 0.25 ppm (0.6 mg/m^3) developed a slight focal proliferative response and 2/5 showed no response. In the 0.67 ppm (1.5 mg/m^3) group, 2/6 rats developed a slight response and 4/6 developed a moderate response. The concentrations of acrolein associated with the proliferation indices were considerably lower than those of formaldehyde and acetaldehyde. Cell proliferation data was expressed as the number of positive-stained cells per millimeter basement membrane. Proliferative effects were not reported for the 1.4 ppm (3.2 mg/m^3) exposure group. **Enzymatic Changes**: Among biotransformation enzymes measured in homogenates of nasal tissue, glutathione S-transferase activity was significantly depressed in the 1.4 ppm (3.2 mg/m^3) exposure group ($p<0.01$) while formaldehyde dehydrogenase and aldehyde dehydrogenase activities were significantly increased ($p<0.05$). No changes were reported in the other dose groups, or for glutathione peroxidase activity in any of the dose groups. Non-protein sulfhydryl (NPSH) depletion was not observed in this study. No biochemical effects were observed in olfactory tissue. The LOAEL in this study is 0.25 ppm (0.6 mg/m^3). The duration-adjusted LOAEL is 0.25 ppm x 6/24 x 3/7 = 0.03 ppm or 0.07 mg/m^3.

In a study designed to evaluate the effect of acrolein on bacterial defense systems, male Sprague-Dawley rats were exposed to 0.1, 1.0 or 3.0 ppm (0.23, 2.3 or 6.9 mg/m^3) acrolein at 6 hr/day, 5 days/week for 3 weeks (Sherwood et al., 1986). No change was noted in the clearance of ^{35}S-*Klebsiella pneumonia* at any of the concentrations. Alveolar macrophage lysozyme and 5'-nucleotidase of acrolein-exposed rats were significantly increased at all exposure concentrations ($p<0.05$ at the low and intermediate concentration, and $p<0.01$ at the high concentration), while alkaline phosphatase showed a non-statistically significant increase. Phagocytosis was significantly increased at the low and intermediate concentrations ($p<0.01$), but not at the 3.0 ppm (6.9 mg/m^3). However, these changes had no apparent effect on macrophage killing of inhaled bacteria and were not indicative of extreme chemical toxicity.

Four groups of 40 male Sprague-Dawley rats were exposed by inhalation to target concentrations of 0, 0.1, 1.0, and 3.0 ppm (0, 0.23, 2.3 and 6.9 mg/m^3) acrolein 6 hr/day, 5 days/week for 3 weeks (Leach et al., 1987). Mean body weights were lower in the high-dose group, although differences were not statistically significant. There were no statistically significant effects of acrolein on immune responsiveness as measured by a hemolytic plaque assay performed on lung-associated lymph node cells. The ability of spleen- and lung-associated lymph nodes to respond to the T cell mitogen, PHA, and the B cell mitogen, STM, as well as resistance to infection by *L. monocytogenes* were not affected by acrolein exposure. Microscopic examination of the nasal turbinates of the high-dose group revealed acrolein-induced exfoliation, erosion and necrosis of the respiratory epithelium as well as squamous metaplasia. No effects were reported in the lungs of the high-dose group or at any location at the lower concentrations.

Kutzman (1981) and Kutzman et al. (1985) exposed male Fischer 344 rats (50/group) via inhalation to acrolein at 0, 0.4, 1.4, or 4.0 ppm (0, 0.9, 3.2 or 9.2 mg/m^3) 6 hr/day, 5 days/week for 62 exposure days (consecutive weekdays, except for weekends, for 12.4 calender weeks) to principally relate lung function with lung pathology. The duration-adjusted concentrations were 0, 0.07, 0.25 and 0.9 ppm (0, 0.16, 0.57, and 2.0 mg/m^3). Of the 50 animals/group, 24 were assessed for pulmonary function, 8 for pathology only, 10 for cytology, and 8 for reproductive function. Ten males and 8 females served as controls. Eight females per group were exposed to assess reproductive potential; weight gain and mortality were also evaluated. There was no histopathology for females. Cytological endpoints included sister chromatid exchanges (SCE) and cell proliferation kinetics. All examinations (with the exception of the cytology studies) were measured 6 days after final exposure to reduce the effect of acute exposure upon results. This recovery period undoubtedly allowed for compensatory changes. Sperm was examined for morphological abnormalities. Histopathology was performed on lung, peribronchial lymph node, nasal turbinates, brain, kidney, liver, spleen, testes, and heart (8 male rats from each dose group except 3 only from the 4.0 ppm or 9.2 mg/m^3 group). Of the 24 animals/group examined for pulmonary function, the right lung was subsequently used for biochemical analyses and the left lung processed for pathological examination.

Mortality (32/57) was observed only in males at the highest concentration, with many displaying severe acute bronchopneumonia. Body weights were significantly lower in the high-dose males and females during the first 10 days after which they gained weight; females never achieved their starting weight throughout the study. Lung hydroxyproline per mg protein (as an index of lung collagen) was increased 113 and 137% above controls ($p<0.05$) in the 1.4 and 4.0 ppm (3.2 and 9.2 mg/m^3) groups, respectively. Lung elastin per mg protein did not change significantly in the two lower dose groups but was increased to 174% of control levels ($p<0.05$) in the group exposed to 4.0 ppm (9.2 mg/m^3). Histologically, the 4.0 ppm (9.2 mg/m^3) surviving animals demonstrated bronchiolar epithelial necrosis and sloughing, bronchiolar edema with macrophages, and focal pulmonary edema. Rats from the 0.4 and 1.4 ppm (0.9 and 9.2 mg/m^3) groups did not exhibit pulmonary lesions attributable to acrolein exposure. Changes in the non-respiratory organs appeared incidental. The severity of the pulmonary lesions was scored for the left lung with a concentration-related increase in severity noted. No adverse histopathology was noted in other tissues examined. The only finding in the nasal turbinates was an apparent dose-

dependent increase in submucosal aggregates. In addition, no cytogenetic nor sperm abnormalities were observed nor was there any treatment-related effect on reproductive performance. In this latter aspect of the study, exposed male rats were mated with unexposed females for 6 days and also exposed females were mated with unexposed males.

Pulmonary function testing and the morphometric and compositional analyses in the male Fisher 344 rats (24 rats/exposure group on the sixth post-exposure day) from the Kutzman et al. (1985) studies was reported by Costa et al. (1986). Results indicated that at 0.4 ppm (0.9 mg/m^3), parenchymal tissue density was significantly increased (+15%) along with significantly increased maximal expiratory flow volume (MEFV), together inferring some degree of parenchymal restriction. Lung composition was similar to controls. The animals in the 1.4 ppm (3.2 mg/m^3) group did not differ functionally from controls. Parameters measured in the 4 ppm (9.2 mg/m^3) group, however, suggested obstructive lesions causing impaired ventilation in both the small and large airways. Internal surface areas of the lung were elevated (6 to 29%) in all exposure groups indicating hyperinflation ($p<0.001$). While the diffusing capacity for carbon monoxide was elevated significantly ($p<0.001$) in all exposure groups compared to controls, when normalized for lung volume these increase largely disappeared. The investigators conjectured that the functional effects from the restrictive lesions (0.4 ppm; 0.9 mg/m^3) and obstructive lesions at 4 ppm (9.2 mg/m^3) canceled in the 1.4 ppm (3.2 mg/m^3) group. Based upon an adequate number of animals evaluated by acceptable methodology at a time point at which acute effects are minimized, these data support the level of 0.4 ppm (0.9 mg/m^3) as a LOAEL associated with minimal effects, with more substantial lung damage occurring at elevated concentrations. Since only a single time point was evaluated, it is difficult to gauge the role of adaptation in the effects observed.

Female Dahl rats (which are derived from the Sprague-Dawley rat) that have been selected for either susceptibility (DS) or resistance (DR) to salt-induced hypertension were exposed to filtered air at 0.4, 1.4, and 4.0 ppm (0.9, 3.2 and 9.2 mg/m^3) acrolein (Kutzman et al., 1984, 1986). Ten DS and 10 DR rats/group were exposed 6 hr/day, 5 days/week for 61-63 days (consecutive weekdays, except for weekends, for 12.4 calender weeks). A 0.4% NaCl commercial diet was provided during non-exposure hours. Animals were necropsied one week after final exposure or 13.3 weeks after the first exposure. All of the DS rats exposed to 4.0 ppm (9.2 mg/m^3) acrolein died within the first 11 days of exposure, while 60% of the DR animals survived to the end of exposure. Neither dose-dependent blood pressure changes or altered behavioral characteristics were evident following acrolein exposure. Measures of lung connective tissue, hydroxyproline and elastin, as well as several serum chemistry parameters, alkaline phosphatase, phosphorus, SGOT and SGPT were significantly increased ($p<0.05$) in the DR rats following exposure to 4.0 ppm (9.2 mg/m^3) acrolein. There was a marked difference in the pulmonary pathology observed in DS and DR rats exposed to 4.0 ppm (9.2 mg/m^3) acrolein. The lungs of the DS rats exhibited severe airway epithelial necrosis with edema and hemorrhage, while surviving high-dose DR rats developed primarily a proliferative change. These included collections of intra-alveolar macrophages with foamy cytoplasm, terminal bronchiolar hyperplasia, squamous metaplasia of tracheal epithelium and terminal bronchial epithelium, as well as interstitial pneumonitis in 4/6 survivors. Pathologic changes in the two lower dose groups were similar, but less severe. Collections of intra-alveolar macrophages with foamy

cytoplasm were present in 7/10 DS rats and 5/10 DR rats in the 0.4 and 1.4 ppm (0.9 and 3.2 mg/m^3) and were adjacent to acutely damaged terminal bronchioles. Differences between the DS and DR groups at the 2 lower doses were minimal and not dose-dependent. Reasons for the difference in susceptibility of DS and DR rats to 4.0 ppm (9.2 mg/m^3) acrolein are unclear.

Bouley et al. (1975, 1976) exposed male SPF OFA rats (110/group) via inhalation to 0 and 0.55 ppm (0 and 1.3 mg/m^3) acrolein. Daily length of exposure and duration of the exposure were not explicitly stated, although measurements were reported for exposures up to 77 days. Body weights decreased to slightly less than 80% of controls by 60 days of exposure. Signs of nasal irritation (sneezing) were consistently observed in exposed rats between the 7th and 21st day of exposure. Sneezing subsequently disappeared despite continuing exposure. Exposed rats also exhibited a significant decrease in the number of alveolar macrophages. No differences were noted in liver weight after 22 days of exposure although liver/body weight ratios were decreased in exposed animals after day 15. Lung/body weight ratios were unchanged after day 15 or 32, but were significantly elevated ($p<0.002$) after 77 days. There was no effect on hepatic alcohol dehydrogenase after 15 days of exposure. Serum alkaline phosphatase was unchanged at days 15, 32 and 77. On the other hand, serum acid phosphatase was increased on day 15 ($p<0.001$), but not on days 32 and 77. An LD_{50} inhaled dose of *Salmonella enteritidis* resulted in a higher death rate in treated animals than controls at 18 days, but not at 63 days. Results were negative in a reproduction study involving 21 females and 3 males. In this portion of the study, mating was started on the 4th day after exposure was initiated and female rats were sacrificed on the 26th day after exposure began. There were no significant differences between control and exposed animals in the number of pregnant animals or number and mean weight of foetuses. While a large number of animals were exposed in the study and numerous measurements were made, use of only one exposure concentration and lack of histopathology greatly limit the usefulness of this study.

In an abstract, Teredesai and Stinn (1989) reported that exposure of male Sprague-Dawley rats to 1.7 ppm (3.9 mg/m^3) acrolein for 7 hr/day for 3 successive days caused ulceration of the respiratory epithelium in 4/10 rats and olfactory degeneration in all rats. Proliferative responses in nasal and tracheal epithelia were observed in male Sprague-Dawley rats exposed at levels of 0.2 and 0.6 ppm (0.5 and 1.4 mg/m^3) acrolein for 6 hr/day on 1 or 3 successive days (Roemer et al., 1993); significant cell proliferative changes were also noted with formaldehyde alone, but at 2 ppm (4.6 mg/m^3) and higher.

MICE: Male Swiss-Webster mice were exposed via inhalation 6 hr/day for 5 consecutive days to 1.7 ppm (3.9 mg/m^3) acrolein, the estimated concentration resulting in a 50% decrease in respiration (RD_{50}) (Buckley et al., 1984). Eight to 10 animals were sacrificed for pathologic examination immediately post exposure and an approximately equal number were sacrificed 72 hours later. The nasal region was sectioned at 5 levels for examination. Changes were labeled as none, slight, minimal, moderate or severe. For respiratory epithelium, exfoliation, inflammation, erosion, ulceration and necrotic changes were considered to be moderate. Squamous metaplasia was considered severe. For olfactory epithelium, ulceration and necrosis were considered to be moderate while squamous metaplasia and serous exudate mild. No effects were reported in the lungs. Recovery after 72 hours was minimal to moderate, suggesting that

the recovery period was insufficient for complete repair of lesions. During single exposures to acrolein, the reflex decrease in respiration was virtually eliminated by tracheal cannulation, providing additional evidence that the critical site for irritant effects of acrolein is the nasal region rather than the deep lung (Kane and Alarie, 1977).

The effect of coexposure to acrolein and carbon black upon lung defenses was evaluated by Jakab (1993). Female Swiss mice were exposed using a nose-only inhalation chamber, 4 hr/day for 4 days to carbon black (10 mg/m^3), acrolein (2.5 ppm; 5.7 mg/m^3), or the two combined. Twenty-four animals per group were assayed for resistance to *Staphylococcus aureus* and *Proteus mirabilis* at 1, 4, and 7 days post-exposure. For *Listeria monocytogenes*, 30 animals/group were utilized, with 6 animals/group sacrificed at 3, 6, 10, and 13 days post exposure. For influenza virus, 30 mice/group were used and 6 mice/group were sacrificed at 3, 6, 8, and 11 days post exposure with an additional group of six lavaged for quantitative cell counts and determination of lung lavage albumin concentrations. *S. aureus* was used for the alveolar macrophage (AM) surveillance phagocytic system, *P. mirabilis* for the dual phagocytic system composed of AMs and inflammatory polymorphonuclear leucocytes (PMNs), *Listeria monocytogenes* for the lymphokine-mediated arm of the acquired cellular immune response, and the influenza A virus for the cytotoxic T-cell mediated effector mechanism of cellular immunity.

Intrapulmonary killing of *S. aureus* was suppressed on the first day post-exposure to acrolein, with a return to normal by day 7. Coexposure enhanced pulmonary killing of *P. mirabilis,* which correlated with a significant increase in accessory phagocytic PMNs recovered from the lungs. Elimination of *L. monocytogenes* and influenza A virus from the lungs was impaired. Exposure to acrolein or carbon black alone had no effect upon lung defenses. Effects noted were likely due not only to the ability of carbon black to carry acrolein into the deep lung, but ingestion of particles by macrophages resulting in enhanced cellular penetration of acrolein. In an earlier study, Astry and Jakab (1983) found that an underlying viral pneumonia in mice compounded the pulmonary toxicity of 3 or 6 ppm (6.9 or 12.8 mg/m^3) acrolein in that antibacterial (challenge with *S. aureus*) defense mechanisms were suppressed.

Aranyi et al. (1986) exposed female CD1 mice via inhalation to 0.10 ± 0.22 ppm (0.23 ± 0.50 mg/m^3) acrolein, 3 hr/day for 5 consecutive days. To evaluate resistance to infection, the animals were simultaneously challenged with *Streptococcus zooepidemicus* for measurements of mortality and ^{35}S-*Klebsiella pneumonia* (noncapsulated) for determination of *in vivo* bacteriocidal activity of alveolar macrophages. A non-significant increase in mortality from 6/140 among controls to 11/140 in exposed mice was recorded. The percent bacteria killed showed a small, but significant decrease from 84.3 to 76.6 ($p < 0.01$).

Philippin et al. (1970) examined the inhalation effects of acrolein in mice exposed for 6 hours and for 2 weeks (6 hours/day). Groups of white albino male mice were exposed (1) for 6 hours, (2) for two 5-day periods (not known if consecutive) at 6 hr/day, and (3) for one 5-day period. At the conclusion of the two 5-day exposures (47 mice/group), there was no mortality at 6, 15, and 25 ppm (2.6, 34, and 58 mg/m^3); there was 91% mortality when 34 mice were exposed to 50 ppm (116 mg/m^3) for 5 consecutive days. There was no mortality at the lowest concentration tested (31 ppm; 71 mg/m^3) in the 6-hours-only exposure group. The acute LC_{50}

was determined to be 66 ppm (152 mg/m^3). Primary lung lesions observed in the groups (15 mice each examined for histopathology) exposed for two 5-day periods and sacrificed 24 hours after the last exposure were atelectasis and inflammatory responses with edema.

Borchers et al. (1998) obtained evidence that exposure of male Sprague-Dawley rats to 3 ppm (6.9 mg/m^3) acrolein for 6 hr/day, 5 days/week for 2 weeks were associated with mucus hypersecretion in isolated tracheal preparations from increases in MUC5ac gene expression. These investigators (Borchers et al., 1999b) later exposed FVB/N male mice to 3.0 ppm (6.9 mg/m^3) acrolein for 6 hr/day, 5 days/week for 3 weeks and found a significant and persistent increase in macrophages in bronchoalveolar lavage fluid and evidence that acrolein-induced excessive macrophage accumulation is associated with mucus hypersecretion.

MULTI-SPECIES: Groups of 15 Sprague-Dawley rats, 7-8/sex; Princeton or Hartley guinea pigs, 7-8/sex; 2 male purebred beagle dogs; and 9 male squirrel monkeys (*Saimiri sciurea)* were exposed to acrolein, 8 hr/day, 5 days/week for 6 weeks at concentrations of either 0.7 or 3.7 ppm (1.6 or 8.4 mg/m^3) (Lyon et al., 1970). According to the first author (Lyon, 2001), there were no concurrent controls in this study; control data (including histopathology) were obtained at a different time point. Histopathological examinations were stated to have been carried out on all dogs and monkeys and about one-half of the rats and guinea pigs. Nasal histopathology was not conducted. No deaths occurred and all animals appeared to be normal during exposure to 0.7 ppm (1.6 mg/m^3) acrolein. Lung sections from all animals exposed to 0.7 ppm (1.6 mg/m^3) showed chronic inflammatory changes and occasional emphysema. These changes were more prominent in dogs and monkeys. The inflammatory changes, consisting of interstitial infiltration of round cells, were mild and ranged from focal to diffuse, and while some infiltrates were peribronchial in distribution, no alteration of the respiratory epithelium or of the peribronchial smooth muscle was noted. In the 3.7 ppm (8.4 mg/m^3) exposure groups, dogs and monkeys salivated excessively and blinked frequently during the first week, and during the next four weeks the dogs experienced continued eye irritation. Two monkeys died, but it is unclear if these deaths were related to exposure because the condition of the monkeys upon arrival was not discussed. Nonspecific inflammatory changes were noted in sections of lung, liver, and kidney from all species. Focal calcification of renal tubular epithelium was noted in some of the rats and monkeys. Significant morphological changes, considered by the investigators to be related to acrolein exposure, consisted of squamous metaplasia and basal cell hyperplasia of the trachea of dogs and monkeys, and necrotizing bronchitis and bronchiolitis with squamous metaplasia of the lungs from 7 of the 9 monkeys, including 2 that died early in the study. The lung changes were commonly present in the bronchi rather than the bronchioles; the necrosis of the bronchial mucosa was associated with varying degrees of repair and regeneration of the epithelium. Bronchopneumonia was noted in dogs. There was no mention of any histopathological effects in control animals. Although data from control animals was under-reported, it appears that 0.7 ppm (1.6 mg/m^3) was associated with lung injury in all species evaluated.

Lyon et al. (1970), using identical group sizes, species (Sprague-Dawley rats, Hartley- or Princeton-derived guinea pigs, beagle dogs and squirrel monkeys) and strains as described above in the 6-week study also carried out 90-day continuous inhalation exposures (24 hr/day) at concentrations of 0, 0.21, 0.23, 1.0, and 1.8 ppm (0, 0.5, 0.5, 2.3, and 4.1 mg/m^3). The two lower

exposures were combined as one experiment at 0.22 ppm (0.5 mg/m^3). The same histopathological protocol was followed. All animals appeared normal during the 0.22 ppm (0.5 mg/m^3) exposure.

Monkeys and Dogs: Two monkeys died as a result of apparent infections. Histopathology demonstrated inflammatory effects in the eyes of dogs and monkeys, although no detail regarding number of animals or degree of inflammation was given. Ocular and nasal discharges were reported in the dogs and monkeys exposed to 1.0 ppm (2.3 mg/m^3), the latter keeping their eyes closed for extended periods. Morphological changes observed in tracheas of monkeys and lungs of dogs were considered related to exposure. At 1.0 ppm (2.3 mg/m^3) focal inflammatory reactions were reported in the lungs, liver, and kidneys of dogs. At 1.8 ppm (4.1 mg/m^3) the dogs and monkeys experienced severe irritation as evidenced by excessive salivation and ocular discharge. All monkeys in the 1.8 ppm (4.1 mg/m^3) group showed squamous metaplasia and 6/9 monkeys presented with basal cell hyperplasia of the trachea. The lungs from the two dogs at this concentration showed confluent bronchopneumonia. Lungs from 2/4 dogs in the 0.22 ppm (0.5 mg/m^3) group demonstrated moderate emphysema and focal splenic hemorrhage. The other two dogs showed hyperplasia of the thyroid. It is not clear if these observations from the 0.22 ppm (0.5 mg/m^3) group were treatment-related since there was no discussion of the condition of the control dogs. The investigators, however, did consider the lung effects in dogs (at all exposure levels) to be treatment-related.

Rats and Guinea Pigs: While weight gain was significantly ($p<0.005$) lower in rats exposed to 1.0 and 1.8 ppm (2.3 and 4.1 mg/m^3), no statistically significant differences in weight gain were noted in the other three species. In the 1 ppm (2.3 mg/m^3) groups, guinea pigs showed various degrees of pulmonary inflammation and occasional focal liver necrosis while rats (3/9) had occasional pulmonary hemorrhage and focal liver necrosis. In the 1.8 ppm (4.1 mg/m^3) exposure groups nonspecific inflammatory changes were observed in sections of brain, heart, lung, liver, and kidney of all animals.

Given the similarities in lung effects across species seen during the repeated and continuous exposures as well as in other studies, 1.0 ppm (2.3 mg/m^3) can be considered a LOAEL.

Feron et al. (1978) exposed four equal groups, each consisting of 20 Syrian golden hamsters, 12 Wistar rats, and 4 Dutch rabbits (equal numbers of each sex) to 0, 0.4, 1.4, and 4.9 ppm (0, 0.9, 3.2, and 11 mg/m^3) acrolein, 6 hr/day, 5 days/week for 13 weeks in whole-body exposure chambers. Duration-adjusted values are 0, 0.07, 0.25, and 0.9 ppm (0, 0.16, 0.57, and 2.0 mg/m^3). Histopathology was performed on all major organs/tissues, including three transverse sections of the nasal cavity.

Rats: Of the three species, rats seemed to be the most sensitive to the effects of acrolein. Mortality (6/24) occurred in the 4.9 ppm (11 mg/m^3) group and animals kept their eyes closed. No adverse clinical observations were reported for the other concentration groups. Hematological and serum enzyme levels were within the normal range. Body weight gain was significantly ($p<0.001$) depressed at 4.9 ppm (11 mg/m^3) and at 1.4 ppm (3.2 mg/m^3) ($p<0.05$).

The decrease in weight gain appeared related to decreased food consumption. Of several rats that died, hemorrhage, perivascular and alveolar edema of the lung were seen. Focal broncho-pneumonia, bronchitis, hyper- and metaplasia of the bronchial and bronchiolar epithelium, increased numbers of mucous-producing cells in the bronchioles, macrophage accumulation, and focal interstitial pneumonitis were observed in surviving rats in the 4.9 ppm (11 mg/m^3) group. Incidence of nasal lesions was not reported for any of the exposure groups. Only one male rat of the 0.4 ppm (0.9 mg/m^3) group showed any evidence of histopathological effects (metaplastic and inflammatory changes of slight severity) in the nasal tract. Incidence data in the 1.4 ppm (3.2 mg/m^3) group were not reported. Squamous metaplasia and neutrophilic infiltration (moderate severity) of the nasal mucosa were observed in the 1.4 ppm (3.2 mg/m^3) group. In the 4.9 ppm (11 mg/m^3) group, necrotizing rhinitis was occasionally seen in the dorsomedial part of the nasomaxillary region, with normal epithelium being partly replaced by stratified squamous epithelium, and in some cases showing keratinization. Neutrophil infiltration was invariably observed, but substantial neutrophilic exudation was seen in only a few animals. The trachea of rats in the 4.9 ppm (11 mg/m^3) group was described as "severely damaged," with nodules of granulation tissues protruding into the lumen. Given the apparent concentration-related increase in severity of nasal lesions (i.e., slightly to severely affected), it is reasonable to consider 0.4 ppm (0.9 mg/m^3) as a minimal LOAEL (i.e., an exposure level close to the expected NOAEL). Even though only 1/12 rats at this concentration demonstrated minimal metaplastic and inflammatory changes, these effects were consistent with the pathology demonstrated at the higher concentrations in which severity was increased. The duration-adjusted LOAEL is 0.4 ppm (0.9 mg/m^3) x 6/24 x 5/7 = 0.07 ppm (0.16 mg/m^3).

Hamsters: There was one death among the hamsters, but it was not related to treatment. Body weight gain was depressed only in the 4.9 ppm (11 mg/m^3) group and there was no evidence of decreased food intake. Lungs were unaffected by exposure. Only minimal inflammatory changes were seen in the nasal cavity at 1.4 ppm (3.2 mg/m^3); the nasal lesions observed in the 4.9 ppm (11 mg/m^3) group were similar (severe) to those seen in the rat. Hyperplasia and metaplasia in the trachea occurred in a few males and all females at 4.9 ppm (11 mg/m^3). In females at 4.9 ppm (11 mg/m^3), there were statistically significant increases in red blood cell packed cell volume, hemoglobin (Hb) content, and in numbers of lymphocytes accompanied by a decrease in the number of neutrophilic leucocytes. All serum enzyme activities were within normal ranges. The NOAEL based on nasal lesions is 0.4 ppm (0.9 mg/m^3) with a LOAEL of 1.4 ppm (3.2 mg/m^3).

Rabbits: Body weight gain (males and females combined) was significantly depressed (<0.05) in only the 4.9 ppm (11 mg/m^3) group. Decreased weight gain appeared to be related to diminished food intake. No effects were detected in the nasal region in the low- and mid-dose groups. Nasal lesions in the 4.9 ppm (11 mg/m^3) group was similar to those in the rat, but less severe. Tracheal effects were seen only in the high-dose group, primarily hyperplasia and metaplasia.

Based on the severity of respiratory tract lesions in the rat compared to diminished responses in the rabbit and hamster in the 4.9 ppm (11 mg/m^3) groups, the rat is considered the most sensitive species of the three with a minimal LOAEL for nasal lesions of 0.4 ppm (0.9

mg/m^3).

GUINEA PIGS: Turner et al. (1993) found that exposure of guinea pigs for 7.5 hours per day for 2 consecutive days to 1.6 ppm (3.7 mg/m^3) acrolein resulted in pulmonary inflammation and epithelial damage. Even after 28 days post-exposure there was a prolonged increase in airway sensitivity to aerosolized substance P, a sensitivity which may have been enhanced by an acrolein-induced reduction in neutral endopeptidase in bronchoalveolar lavage fluid.

RABBITS: In a study designed to evaluate the effects of corticosteroids on mortality and lung histopathology of female New Zealand rabbits exposed to acrolein, animals (18/group) were exposed to 375 and 489 ppm (862 and 1125 mg/m^3) for 15 minutes (Beeley et al., 1986). Although treatment with methylprednisolone reduced mortality (no significant differences between the two groups), there was no evidence of an improvement in lung histopathology (hemorrhagic necrosis, edema).

4.3.1.2. Oral Administration

RATS: Arumugam et al. (1997) demonstrated that acrolein treatment results in severe depletion of liver cytosolic GSH. In a study that clearly shows acrolein-induced damage to cellular function, Arumugam et al. (1999b) exposed male Wistar rats, 5 animals/group, daily for 45 days to distilled water or acrolein in distilled water (2.5 mg/kg BW) via intubation. The authors did not specify as to whether the dosing was continuous or 5 days/week. Observation of clinical symptoms and histopathology were not part of the protocol. Electron microscopic examination revealed a loss of mitochondrial lamellae of the cristae in the treated livers compared to normal architecture in controls. This was accompanied by a 41% decrease in mitochondrial GSH ($p<0.001$) as well as the activities of the citric acid cycle enzymes, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, malate dehydrogenase, succinate dehydrogenase, NADH dehydrogenase, cytochrome c oxidase ($p<0.001$), and levels of cytochrome a, b, c_1, and c. The decreases in the activities of the citric acid cycle enzymes ranged from 30 to 56%. The activities of GSH peroxidase and superoxide dismutase were increased significantly ($p<0.001$). Because GSSG was unchanged, GSH depletion was presumed to result from conjugation with acrolein. Superoxide anion radicals generated by mitochondria under physiological and pathological conditions are converted to hydrogen peroxide by superoxide dismutase. The authors state that hydrogen peroxide is cleared from mitochondria only by GSH peroxidase, and if the GSH cofactor has been depleted due to conjugation with acrolein, the resulting higher peroxide levels may lead to increased lipid peroxidation, mitochondrial damage, and the observed decreased enzyme activities.

Limitations of the study for evaluation of chronic effects are the (1) use of a 45-day exposure period rather than a longer one, (2) use of only one dose level, and (3) lack of histopathology of the stomach which would have ascertained if intubation of acrolein damaged stomach lining. Given the significant depletion of citric acid cycle enzymes, longer-term exposure to acrolein could compromise an animal's ability to survive.

Administration of acrolein in water by oral gavage at 0.05, 0.5, and 5.0 mg/kg to male and female Sprague-Dawley rats (30/sex/group) daily, 5 days/week for 13 weeks did not result in any significant observed toxic effects (Bioassay Systems Corp., 1981g). No mortality or clinical, hematological, or urinalysis effects were ascribed to treatment. Histopathological results were similar to controls. Histotopathology was conducted on 12 rats/group at the 5.0 mg/kg/day dose level and controls only. This study provided the dose selection used by Parent et al. (1992c) in the chronic gavage study described below.

Parent et al. (1992c) administered acrolein in water daily via gavage to Sprague-Dawley rats, 70/sex/group, at dose levels of 0, 0.05, 0.5, and 2.5 mg/kg BW. There was no indication in the report that the "daily" dosing was limited to 5 days/week. Dosing volume was 10 ml/kg. Ten animals from each group were sacrificed after one year and the remainder after two years. An extensive array of tissues was examined microscopically, including stomach tissue. Although it was not explicitly stated that both the glandular stomach and forestomach were examined, it is unlikely that both parts of the stomach were not examined. Daily observations were made and various clinical, hematological and urinary parameters were measured after 3, 6, 12, and 18 months of treatment and immediately prior to termination. There were no significantly increased incidences of microscopic lesions in the treated rats, whether neoplastic or non-neoplastic. Food consumption and body weights were unaffected by treatment. With the exception of a statistically significant depression of creatinine phosphokinase (creatine kinase) levels at all dose levels and at most time intervals (except 12 months), clinical chemistry parameters, hematology and urinalysis measurements were unaffected by treatment. The most definitive responses reported were treatment-related increases in early cumulative mortality. Data were provided in the form of survival curves. Among high-dose males, survival was significantly reduced after one year ($p<0.05$), and marginally reduced among mid-dose males (p value not reported). Among high-dose males, a trend test for survival during the first year indicated a highly significant ($p=0.003$) decrease; however, the statistical differences are nullified when the survival data for two years are included in the analysis. Survival among females during the first year corresponded closely to those obtained for males. A statistically significant decrease in survival ($p<0.05$) was reported in the high-dose group, while a decrease in survival in the mid-dose group was marginally significant (p value not reported). A highly significant trend toward reduced survival ($p<0.001$) in the high-dose group was also reported. Unlike responses in males, the significant associations between dosing and survival persisted in females through the end of the study. After two years, a statistically significant reduction in survival was noted based on four different statistical tests for the mid-dose group and in 3/4 statistical tests in the high-dose group (p values not reported). Although the differences in survival were statistically significant in females after two years, it should be noted that the differences were relatively small. No differences in survival were seen in the low-dose groups. There was no apparent cause cited for the early mortalities. There were 11 confirmed accidental deaths due to gavage error and 17 possible. However, even after censoring these data early mortality remained. There was no information or discussion in the report relating to forestomach hemorrhage as a possible cause. No obvious dose-related clinical symptoms were observed.

MICE: In a study designed to evaluate the potential carcinogenicity of acrolein (Parent et al., 1991b), Swiss albino CD-1 mice (70/sex/group) were dosed via gavage (acrolein in

distilled water and stabilized with hydroquinone) with 0, 0.5, or 2.0 mg/kg-day for 18 months. A separate group (75/sex) was similarly dosed at 4.5 mg/kg-day. All animals were sacrificed at 18 months. The primary effect was increased mortality ($p<0.05$) only in high-dose males of the 4.5 mg/kg-day group; mortality in mid- and low-dose groups was less than controls. There were no dose-related adverse histopathological or clinical findings.

Pretreatment of male Charles River mice with high oral doses of L-ascorbic acid, L-cysteine, and an alpha adrenergic-blocking agent gave a high degree of protection against the lethality of orally-administered acrolein administered once after which animals were followed for 72 hours (Sprince et al., 1979).

RATS and MICE: In a 13-week daily gavage study of acrolein (in 0.5% methyl cellulose) conducted for the National Toxicology Program (NTP), 10 F344 rats/sex/dose were administered 0.75, 1.25, 2.5, 5.0, and 10 mg acrolein/kg; 10 B6C3F1 mice/sex/dose were administered 0, 1.25, 2.5, 5.0, 10 and 20 mg/kg. Dose volume was 5 ml/kg for rats and 10 ml/kg for mice. Treatment resulted in similar dose-related effects in both sexes of rats: hemorrhage and necrosis and chronic-active inflammation of the forestomach and glandular stomach and secondary changes associated with acrolein-induced mortality in high-dose animals (NTP, 1995; Pathology Working Group, 1997). Hemorrhage of the glandular stomach was also confirmed in 5 and 10 mg/kg males and 10 mg/kg females. Abnormal breathing and nasal/eye discharge were among the clinical findings in high-dose rats. Nearly all high-dose animals died or were removed from study because of gastrointestinal toxicity. Forestomach squamous epithelial hyperplasia was observed in male rats at 2.5 mg/kg and higher (no-observed-adverse-effect level, NOAEL, of 1.25 mg/kg-day) and in females at 1.25 mg/kg and higher (NOAEL of 0.75 mg/kg-day).

There were no clinical signs of toxicity in mice. The forestomach lesions in mice were similar to those in the rat. Glandular stomach lesions were only seen in the 10 and 20 mg/kg males and in the 20 mg/kg females. Statistically significant increases in absolute and relative liver weights were seen in male mice at 10 mg/kg without attendant hepatic histopathology. Forestomach squamous epithelial hyperplasia was observed in one male mouse at the lowest dose of 1.25 mg/kg (i.e., no NOAEL for the male mice), and in female mice at 2.5 mg/kg-day and higher (NOAEL of 1.25 mg/kg-day).

DOGS: Six male and 6 female beagle dogs/group were administered acrolein (0.1% aqueous) in gelatin capsules at doses of 0, 0.1, 0.5, and 1.5 mg/kg-day, 7 days/week for 53 weeks (Parent et al., 1992a). At week 4, the high dose was increased to 2 mg/kg-day. Blood and biochemical measurements were made at pretest and at 3-month intervals. At termination, all dogs were subjected to full necropsy and histological examination. Body weights and food consumption were not significantly affected by treatment. A primary effect noted was a dose-dependent increase in the frequency of vomiting shortly after dosing which can limit the dose retained. The frequency decreased with time indicating adaptation. Serum albumin, calcium and total protein levels were significantly depressed (p values not given) in high-dose animals throughout the study. Measurements for the other exposure groups were not listed. Some variability in red blood cell parameters and coagulation times were noted, but the significance of

these effects was not obvious. It was reported that clinical signs, with the exception of vomiting, were evenly distributed among groups. At termination, gross necropsy indicated vascular congestion and mucosal reddening of the gastrointestinal tract of both males and females, but it is unclear if these effects were treatment-related. While the study was well-designed and the methodology adequately reported, incomplete reporting of results limits its usefulness in a quantitative dose-response assessment.

4.3.1.3. Dermal Administration

The toxicity of acrolein dissolved in water + ethanol in rabbits was evaluated by dermal application of 7, 21, and 63 mg acrolein/kg-day, 5 days/week for 3 consecutive weeks according to a FIFRA study design (Bioassay Systems Corp., 1982a). Observations included slight to significant reduction in body weight, moderate to severe skin irritation, and histopathologic lesions in skin and lungs.

4.3.2. Cancer Assessment

4.3.2.1. Inhalation Exposure

Feron and Kryusse (1977) exposed groups of 36 Syrian golden hamsters of both sexes to acrolein vapor at measured levels of 0 and 4.0 ppm (0 and 9.2 mg/m^3), 7 hr/day, 5 days/week for 52 weeks. Six animals per group were sacrificed at 52 weeks and the remainder at 81 weeks. Overall mortality was 38% in exposed hamsters and 33% in controls. Histological changes were observed in the anterior half of the nasomaxillary turbinates, consisting of epithelial metaplasia, but not hyperplasia. In addition, exposure resulted in abnormal behavior and growth retardation. The only respiratory tract tumor observed was a small tracheal papilloma in an acrolein exposed female. The exposure period for this study was short for a cancer bioassay and sacrifice at 81 weeks may have been insufficient to allow for latency.

In a study by Le Bouffant et al. (1980), 20 female Sprague-Dawley rats/group were exposed to 8 ppm (18.3 mg/m^3), 1 hr/day, 5 days/week for either 10 or 18 months. No tumors or metaplasias were reported. Use of only one exposure concentration and less than lifetime exposure duration limits inferences that can be drawn from this study.

4.3.2.2. Oral Administration

There have been three long-term cancer bioassays by the oral route: male F-344 rats via drinking water (Lijinsky and Reuber, 1987); CD-1 mice via drinking water (Parent et al., 1991b); and Sprague-Dawley rats via drinking water (Parent et al., 1992c).

Male Fischer 344 rats (20/group) were administered acrolein in the drinking water at concentrations providing average daily doses of 0, 1.9, 5.0, or 12.5 mg/day, 5 days/week for 104-124 weeks (Lijinsky and Reuber, 1987). On the remaining 2 days, tap water was provided. High-dose animals stopped drinking the solution before the other groups. Drinking water solutions were prepared weekly and stored at unspecified refrigerator temperatures until

dispensed. Each cage of four rats was given a measured amount (80 ml) of drinking water over the span of the study. The daily dose per kg BW could not be calculated from the data given.[2] The maximum tolerated dose was not determined. Major organs and tissues were reported as being examined histopathologically (if there were any non-proliferative lesions they were not reported). One group of 20 females also received the highest dose on the same schedule as the males. Adrenocortical tumors (5/20) and hyperplastic nodules of the adrenal cortex (2/20) were found only in females in the high concentration group. The increased incidence of adrenocortical tumors was considered by the authors to be marginally significant as judged by the Fisher's exact test (p=0.091) and significant for adrenocortical tumors plus hyperplastic nodules (p=0.022). According to the authors, this type of tumor is rare in untreated female Fischer 344 rats; there was one reported in concurrent controls. The historical incidence is approximately 4.8% based on the findings of Solleveld et al. (1984) for untreated female F-344 rats allowed to die naturally. Significant increases in tumor incidence were not found in male rats. There was no treatment-related mortality. Lijinsky and Reuber (1987) also exposed rats to acrolein diethylacetal, acrolein oxime, and allyl alcohol, agents that can be expected to be hydrolyzed to acrolein in the stomach acids, with negative results. A reevaluation of the tumors in this study (Parent et al., 1992c) is described in Section 4.7.

Lijinsky and Reuber (1987) also exposed hamsters to acrolein, but the does proved to be too toxic to complete the cancer bioassay. A single, 1 mg dose via gavage in corn oil killed all of the animals within a few hours; hamsters reportedly drank too little water to make the study feasible.

Four groups of 70-75 male and 70-75 female Swiss albino CD-1 mice, eight weeks of age, were administered 0, 0.5, 2.0, or 4.5 mg acrolein/kg BW via gavage in deionized water daily for 18 months, followed by sacrifice of survivors at the end of the treatment period (Parent et al., 1991b). Dosing levels were chosen based on a range-finding study demonstrating severe stomach lesions at higher dosing levels. Body weight gains were decreased and mortality increased in males, especially at the high dose. All mice killed at the end of treatment, as well as those found dead or moribund, were necropsied. Tissues from major organs were examined histologically. No treatment-related increase in tumor frequency was observed. The study was near lifetime duration for mice and the maximum tolerated dose (MTD) appeared to be achieved. Thus, acrolein appears unlikely to be carcinogenic in mice by gavage.

Parent et al. (1992c) also gavaged 560 Sprague-Dawley rats about 6 weeks of age (70/sex/group) daily with 0, 0.05, 0.5 and 2.5 mg acrolein/kg in water (10 ml/kg). Ten rats/sex/group were sacrificed at 1 year for various clinical measurements. The remainder of the animals were treated for 102 weeks followed by sacrifice. Dosing solutions were prepared daily from stock solutions (prepared daily) and analyzed weekly by gas chromatography. Stability studies indicated losses at <10% after storage for 3 hours at room temperature. The only statistically significant changes noted in treated animals were consistent depression of creatine

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[2] Parent et al. (1992c), assuming that each of the four rats/cage drank the same amount of water, estimated a daily dose of 50 mg/kg BW at the highest concentration, which exceeds the LD_{50} for rats. This suggests a lower rate of intake or that acrolein in solution may not have been as stable in solution as reported.

phosphokinase levels (significance unknown) and consistent increases in early cumulative mortalities in both males and females. There was no significantly increased incidence of either neoplastic or non-neoplastic microscopic lesions in treated rats. Analyses of survival took into account confirmed and possible accidental deaths (28 total). Decreased survival of high- and mid-dose males during the first year was highly and marginally significant, respectively; however, this trend did not persist into the second year. Unlike survival in male rats, decreased survival in females during the first year persisted until the end of the second year. Based upon results of this two-year exposure in which mortality indicated a maximum tolerated dose (MTD) was achieved, it can be concluded that there was no evidence for carcinogenicity in an adequately designed and conducted study. While the doses/kg BW used in this study are most likely much lower than those used by Lijinsky and Reuber (1987), Parent et al. have raised concerns about the conclusions reported in the Lijinsky and Reuber (1987) study, and that dose levels may have been lower than the original authors assumed (see Section 4.7).

4.3.2.3. Injection Studies

The earliest reported study investigating the potential carcinogenicity of acrolein was reported by Steiner et al. (1943). Fifteen female partly-inbred albino mice received subcutaneous injections (0.2 mg/kg) of acrolein weekly for 24 weeks. No sarcomas developed at the site of injection. The use of only one dose level and a small number of animals limits any conclusions.

4.3.2.4. Initiation and Promotion Studies

Cohen et al. (1992) exposed 30 male Fischer 344 rats/group to acrolein, 2 mg/kg by i.p. injection twice weekly as part of a larger initiation/promotion study. All groups were sacrificed 53 weeks from the start of the study. No increases in tumor incidence were reported in groups exposed to acrolein alone for either 6 or 21 weeks (severe toxicity occurred during the 21 week study). Exposure to acrolein for 6 weeks followed by administration of uracil (3% by weight) for an additional 20 weeks resulted in the induction of 18 papillomas and one carcinoma, a significantly greater incidence ($p<0.05$) than following exposure to uracil alone (8/30). While it appears that acrolein may have some tumor initiating capability, it should be noted that the incidence of papillomas and nodular hyperplasias combined, was significantly greater in the uracil only group compared with the group initiated with acrolein ($p<0.05$).

A group of 15 "S" strain mice (sex and age unspecified) received 10 weekly skin applications of a 0.5% solution of acrolein in acetone at a total dose of 12.6 mg/animal (Salaman and Roe, 1956). Starting 25 days after the first application of acrolein, the mice received weekly skin applications of 0.17% croton oil for 18 weeks; for the second and third applications the concentration was reduced to 0.085%. When croton oil and acrolein were administered together, each compound was given alternately at 3 or 4 day intervals. Tumor incidence was evaluated at the end of the croton oil treatment. Four skin papillomas were reported in 4 of 19 control animals that received croton oil alone. A total of 3 papillomas were noted in 2 of the 15 mice treated with acrolein and croton oil. The data suggest that acrolein lacks potential for initiation of skin tumors. Small numbers, however, limit any definitive conclusions.

Feron and Kryusse (1977) exposed groups of 30 male and 30 female Syrian golden hamsters, about 6 weeks of age, to 0 or 4 ppm (0 or 9.2 mg/m^3) acrolein for 52 weeks, together with either weekly intratracheal installations of 0.175 or 0.35% benzo[a]pyrene (BP) in 0.9% saline, or subcutaneous injections of 0.0675% *N*-nitrosodiethylamine (DENA) in saline once every 3 weeks (total dose, 2 μL/animal). The experiment was terminated at 81 weeks, and all survivors were killed and autopsied. An increased incidence of papillomas, adenomas, adenocarcinomas and squamous-cell carcinomas of the respiratory tract were found in acrolein-exposed male and female hamsters treated with BP and DENA. Exposure to acrolein vapor alone resulted in only one respiratory tumor (female).

Conclusions that can be drawn from the Feron and Kryusse (1977) inhalation and initiation/promotion studies are limited because of the use of only one dose level, although they did report toxic responses at concentrations of 9.2 mg/m^3. Because respiratory tract tumors typically occur in hamsters administered BP or DENA, the evidence is insufficient to suggest that acrolein is a cofactor in carcinogenesis.

4.4. REPRODUCTIVE/DEVELOPMENTAL STUDIES-ORAL AND INHALATION

***In vivo*:**

Kutzman (1981) exposed female and male Fischer 344 rats (8/group) via inhalation to acrolein at 0, 0.4, 1.4, or 4.0 ppm (0, 0.9, 3.2 or 9.2 mg/m^3) 6 hr/day, 5 days/week for a total of 62 exposure days. The duration of exposure was 12.4 weeks and the animals were evaluated 13.3 weeks after initiation of exposure. Exposed and control male rats were mated with unexposed females for 6 days and also exposed females were mated with unexposed and exposed males. Parameters evaluated were corpora lutea, viable embryos, early and late deaths, and pre-implantation losses. There were no treatment-related effects on reproductive performance.

The only other inhalation reproduction study reported to date was performed by Bouley et al. (1976). Three male and 21 female SPF OFA rats were exposed continuously to acrolein at a measured concentration of 1.3 mg/m^3 (0.6 ppm) and then mated on the fourth day of exposure. Exposures were continued for an additional 22 days when the females were sacrificed. The exposure did not cover the entire period of spermatogenesis. No significant differences in the number of and mean weight of fetuses (no data given) were reported. While the results were negative, the minimal results reporting limits conclusions that can be drawn from this study.

Claussen et al. (1980) intravenously injected New Zealand white rabbits on day 9 of gestation with 3, 4.5, or 6 mg/kg acrolein. Embryolethal effects increased in a dose-dependent manner, but few malformations were noted. After direct injection into the rabbit embryos at doses of 10, 20 and 40 μL, resorptions and malformations increased in a dose-dependent manner. The highest dose by both routes showed that direct embryo injection of acrolein induced malformation at doses 50-60 times lower than those inducing embryolethal effects via intravenous injection.

In a two-generation gavage study, four groups of 30 male and 30 female Sprague-Dawley rats were gavaged daily with 70 doses of acrolein at levels of 0, 1, 3, or 6 mg/kg in a dosing

volume of 5 ml/kg (Parent et al., 1992b). Rats within each dosing group (F_0 generation) were then assigned to a 21-day period of cohabitation. Dosing continued for females through cohabitation, gestation, and lactation. A similar regime was carried out for F_1 generation offspring, resulting in F_2 generation pups. Mortality was significant (at 6 mg/kg) in both males and female of the F_0 and F_1 generations with the pattern continuing with F_1 mid-dose animals, most of the latter showing signs of respiratory distress and histopathological lesions in the lungs and stomach. Reproductive parameters (i.e., mating performance and fertility indices) were unaffected. Erosions of glandular mucosa and hyperplasia/hyperkeratosis of the forestomach were the most frequent stomach lesions observed. Significant depressions in body weight gains were noted in the high-dose groups and achieved statistical significance in the mid-dose animals on several occasions. No treatment-related gross or microscopic effects were observed in the reproductive tissues of any of the F_0 or F_1 animals. There were no statistically significant differences among the groups in the number of F_1 litters with gross abnormalities for the pups (F_2) during lactation or gross lesions identified in the pups at necropsy. The data provide evidence that acrolein is not a selective reproductive toxicant, but does produce toxicological effects at doses as low as 3 mg/kg-day.

The respiratory effects in the Parent et al. (1992b) gavage study raises the question about possible reflux and/or regurgitation and aspiration of gavage solution, which can occur with volatile or highly irritating chemicals. There is also the possibility of incorrect tube placement, or esophageal or gastric perforation. The authors note the development of stomach lesions suggesting that the dose was being delivered to the stomach, but provide no further discussion of the possible amount of chemical that may have been aspirated to the lungs. In light of studies indicating no systemic distribution (Parent et al. 1996b, 1998), the respiratory effects noted in this gavage study may be due to aspiration of gavage solution.

Pregnant New Zealand white rabbits (20/sex/group) were dosed via gavage with 0, 0.1, 0.75, or 2.0 mg/kg-day for days 7 through 19 of presumed gestation and subjected to caesarean sectioning on day 29 (Parent et al., 1993). Three deaths were observed, but were considered a result of misdosing or aspiration. Transient effects on feed consumption and body weight gains were noted. Resorptions were elevated in the high-dose group, but the effect was not statistically significant. Fetal malformations were distributed evenly among the groups and were consistent with historical control data. Higher doses in a range-finding study (0, 0.5, 1.0, 2.0, 4.0 and 6.0 mg/kg-day) produced high incidences of maternal mortality (at 4 and 6 mg/kg), spontaneous abortion, resorption, clinical signs, gastric ulceration, and/or sloughing of the gastric mucosa. Thus, acrolein was not found to be a developmental toxicant or teratogen at maternally nontoxic doses.

***In vitro*:**

Rat conceptuses were explanted from the uterus on day 10.5 of gestation, transferred to culture bottles and treated with acrolein at concentrations ranging from 100 to 250 μM (Schmid et al., 1981). Slight, but statistically significant inhibition of growth was reported at 100 and 150 μM. A concentrations of 200 μM resulted in drastic inhibition of growth and differentiation and no gross structural defects, but 250 μM completely arrested differentiation and growth. These findings indicate that acrolein is lethal to embryos in a narrow dose range, but has no teratogenic

potential.

Slott and Hales (1986), however, reported 100% mortality in embryos cultured in a standard medium containing an acrolein concentration of 140 μM, and teratogenic effects at 80-120 μM. In a serum-free medium acrolein was 100% lethal to embryos at 20 μM and was teratogenic in the range of 5-15 μM. The EC_{50} for malformations in the serum medium was 137 μM, whereas that for embryolethality was 115 μM. In a further study, Slott and Hales (1987a) reported that acrolein induced 64 and 100% mortality at acrolein concentrations of 120 and 160 μM, respectively. At concentrations of 80 and 120 μM, 50 and 100% of the embryos were malformed, respectively. In addition, both concentrations of acrolein produced growth retardation manifested by significant decreases in the yolk sac diameter, crown-rump and head lengths, number of somites, and morphological score. Concurrent exposure to 100 or 500 μM GSH markedly protected embryos against all of these effects, but GSH addition 2 hours after the beginning of acrolein exposure offered little protection. Because addition of GSH resulted in little change in concentration in the yolk sack or embryos, protection was believed to be primarily due to interaction between acrolein and GSH in the culture medium. The embryotoxicity of acrolein, on the other hand, was significantly enhanced by addition of glutathione sulfoxamine (10 or 100 μM), an inhibitor of GSH synthesis (Slott and Hales, 1987b).

Stahlmann et al. (1985) tested acrolein in a mouse limb bud culture system. Concentrations of acrolein between 3 and 10 mg/ml (56 and 178 μM) induced a significant impairment of limb bud differentiation with explants from 12 day old mouse embryos. Scapula and paw skeleton were more affected than ulna and radius. With limbs from 11-day-old embryos similar effects were reported at even lower concentrations. A contact time of 20-40 minutes was sufficient to induce abnormal development.

Mirkes et al. (1984) evaluated the role of acrolein in cyclophosphamide (CP) teratogenesis in a culture medium containing day 10 rat embryos. The dechloro derivative of cyclophosphamide (D-CP), breaks down upon activation to acrolein and dechlorophosphamide mustard (D-PM). D-CP was teratogenic and resulted in decreases in growth parameters, whereas D-PM did not. When embryos were exposed to acrolein alone (0.45 to 18 μM), all concentrations produced abnormal flexion in some embryos, but effects did not resemble those induced by D-CP. Complete lethality was produced at 8.9 μM. This suggests that the high reactivity of acrolein limits its entry to sensitive sites, but when D-CP is transported into the cell, yielding acrolein, teratogenicity can result.

Using the embryo culture, Hales (1989) reported that while phosphoramide mustard and acrolein are both teratogenic, they had differing effects on developing limbs, indicating different targets and/or mechanisms of action.

No evidence of acrolein-induced teratogenicity was found in chicken eggs treated on day 3 with acrolein at doses of 0.001-10 μmol and examined on day 14 of incubation. The LD_{50} was estimated to be 0.05 μmol (Kankaanpää et al., 1979). In a similarly designed study, however, Korhonen et al. (1983) reported malformation in chicks at doses of 0.05 μmol/egg. Chibber and Gilani (1986) also reported increases in malformations in chicks at doses as low as 0.001 mg/egg

(0.02 μmol) when dosed at 48 hours of incubation and examined on day 13.

In summary, acrolein can induce teratogenic and embryotoxic effects if administered directly to the embryos or fetuses, but had no selective reproductive, developmental or teratogenic effects in a gavage study in the rat. In the rabbit, there were no teratogenic effects following iv administration. An inhalation study found no effect of acrolein on reproductive performance in exposed male and female rats. The high reactivity of acrolein may limit its ability to reach critical sites in the developing embryo.

4.5. OTHER STUDIES

This section focuses upon the results of *in vitro* tissue and cell culture experiments, including the genotoxic potential of acrolein and its conjugates in *Salmonella.*

4.5.1. *In Vitro* Toxicity

Heart: Perfusion of rat hearts with 0.01-0.03 mM acrolein led to cessation of beating within 15 minutes; no lesions were detected, but creatine kinase was reported to be inactivated (Sklar et al., 1991). Rat neonatal myocytes were unaffected by exposure to 0.01 mM acrolein, but stopped beating within 2 hours during exposure to 0.05 mM acrolein with accompanying cell lysis and release of lactic dehydrogenase (Toraason et al., 1989). While acrolein was shown to act as an inhibitor of mitochondrial electron transport, the effective concentrations for a 50% inhibition (0.39-0.80 mM) are probably too great to invoke a direct action on electron transport as a primary mechanism for cardiotoxicity of acrolein (Biagini et al., 1990).

Pulmonary Cells: Patel and Block (1993) observed that acrolein exposure results in alterations in plasma membrane-dependent transport in cultured pulmonary endothelial cells, leading to decreased availability of precursor amino acids used in GSH and protein synthesis. Joseph et al. (1994) reported that acrolein at concentrations of 5-50 μM resulted in disruption of actin cytoskeletal fibers in cultured pulmonary artery endothelial cells. The damage was postulated as possibly due to cross-linking of sulfhydryl groups.

Survival of human alveolar macrophages was significantly decreased following 24 hours exposure to acrolein concentrations of 25 μM or greater (Li et al., 1997). Incubation of type II alveolar macrophages cultured for 24 hours with acrolein led to a near-zero adenosine triphosphate (ATP) concentration at 50 μM and a significant increase ($p<0.001$) in LDH at an acrolein concentration of 25 μM. These effects were considerably muted when lung slices were used (Monteil et al., 1999).

Cytotoxicity of acrolein, as measured by the decrease in colony forming efficiency (CFE) of cultured human bronchial fibroblasts, was not observed at 1 μM acrolein, but CFE decreased to less than 50% following 7-8 days incubation at 3 μM (Krokan et al., 1985); intracellular thiol content was decreased and inhibited the DNA repair enzyme O^6-methylguanine-DNA methyltransferase, but had no effect on activity of uracil-DNA glycosylase. Cell survival was significantly decreased at lower acrolein levels than those that reduced thiol levels. Grafström et

al. (1988) and Grafström (1990) also reported that less than 3 μM acrolein was required to decrease-colony forming efficiency 50% in human bronchial epithelial cells. The 1-hour LD50 determined by trypan blue exclusion (a measure of cell permeability) was about 20 μM. A small, but significant increase in single strand breaks and DNA protein cross-links occurred at a concentration of 30 μM acrolein. Fibroblasts (origin not stated) derived from patients with Xeroderma pigmentosum (XP) were more sensitive to the cytotoxic effects of acrolein than were cells from normal individuals (Curren et al., 1988).

Acrolein enhanced responsiveness of human bronchi sections to carbachol following 20 minutes exposure at 0.1 μM (Marthan et al., 1996). After 60 minutes responsiveness was depressed, indicating a toxic effect. The effects appeared to follow a time-concentration C x T relationship, with a maximum response at a C x T (μM x min) of slightly less than 10 and a depressed response at a C x T of 60.

Liver: Acrolein induced a rapid dose-related depletion of GSH in rat hepatocyte cultures at concentrations of 25-500 μM after 2 hours (Zitting and Heinonen, 1980); at 500 μM, recovery did not occur and the integrity of cell membranes was impaired. Similarly, Silva and O'Brien (1989) reported that five minutes exposure to 25 μM acrolein resulted in an approximately 25% decrease in viability of cultured rat hepatocytes, while 50 μM resulted in a greater than 50% decrease (significance levels not given).

Several studies have evaluated the effects of inhibiting aldehyde dehydrogenase (ALDH) on the toxicity of acrolein. Oxidation of acrolein by hepatic ALDH is a detoxification reaction (Rikans, 1987). ALDH metabolizes acrolein to the less reactive acrylic acid. Silva and O'Brian (1989) showed that incubating rat hepatocytes with inhibitors of ALDH resulted in an increased toxicity and greater depletion of GSH. The administration of ALDH inhibitors, cyanamide or disulfiram, caused substantial inhibition of acrolein oxidation by the hepatic mitochondrial and cytosolic low Km ALDHs (Rikans, 1987). A significant increase in lipid peroxidation ($p<0.01$), and a depletion of GSH ($p<0.04$) occurred within 5 minutes of exposure to 100 μM acrolein to cultured liver cells (Watanabe et al., 1992). Dogterom et al. (1988) observed an increase in acrolein toxicity evidenced by increased cell death following disulfiram inhibition of ALDH and exposure of cultured rat liver cells to 0.4 mM acrolein, but there was an unexplained decrease in lipid peroxidation. Acrolein alone can inhibit ALDH. Incubation of rat liver hepatocytes with 30 μM acrolein resulted in an irreversible inhibition of high affinity ALDH with a 91 and 33% reduction in mitochondrial and cytosolic ALDH activities (Mitchell and Petersen, 1988). N-acetylcysteine protected against acrolein-induced toxicity in isolated hepatocytes, possibly by maintaining sulfhydryl levels (Dawson et al., 1984).

Brain: Recent evidence has established that increased lipid peroxidation is intimately involved in the pathogenesis of Alzheimer's disease and represents a marker of oxidative stress (Calingasan et al., 1999). Lovell et al. (2001) obtained evidence in brains (10) obtained from Alzheimer's patients at autopsy (8 age-matched controls) that acrolein is increased in brains of Alzheimer's patients. In hippocampal neuron cultures, acrolein was neurotoxic in a time- and concentration-dependent manner and disrupted calcium homeostasis.

Acrolein was found to be a potent inhibitor of ADP-induced mitochondria state 3 and calcium-induced respiration in whole brain mitochondria obtained from adult male Sprague-Dawley rats. Acrolein did not affect basal levels of state 3 respiration, did not alter activity of complexes I-V or mitochondrial calcium transporter activity, and did not induce cyctochrome c release (Picklo and Montine, 2001). Inhibition was prevented by GSH and N-acetylcysteine. These results were similar to those obtained using isolated rat hepatic mitochondria in which phosphate and glutamate transport were inhibited (Zollner, 1973). In isolated mitochondria from rat heart, acrolein did inhibit complex II-linked state 3 and uncoupled respiration (Biagini et al., 1990).

Following a 24-hour exposure of acrolein to cultured neuroblastoma cells, the concentrations of acrolein required to induce a 50% change in cytotoxic endpoints from controls were as follows: sloughed cells (1 μM), neurite formation (7.6 μM), viability of sloughed cells (5.3 μM), total cell number (580 μM), and viability of harvested neuroblastoma cells (30 μM) (Koerker et al., 1976). Neuronal survival was decreased to about 50% following 24-hour exposure to 600 μM acrolein and less than 25% following 48-hour exposure (Smith et al., 1990b).

Treatment of hippocampal cultures taken from gestation day 18 rat embryos with acrolein led to a time- and concentration-dependent decrease in cell survival as well as a concentration-dependent increase in intracellular calcium (Lovell et al., 2000). When cortical neuron or astrocyte cultures were similarly treated, there was an impairment of glutamate uptake.

Skin fibroblasts: A one-hour exposure of xeroderma pigmentosum cells to acrolein caused depletion of GSH and free protein thiols to a quantitative extent similar to that in normal skin fibroblasts without causing changes in the thiol redox state (Dypbukt et al., 1993).

Tumor cells: Acrolein was shown to be highly cytotoxic in two lung carcinoma cell lines and in a glioblastoma cell line (Rudra and Krokan, 1999); in one of the lung carcinoma cell lines, toxicity was partially reversed by vitamin E. Acrolein was shown to reduce AP-1 activation in human lung adenocarcinoma cells of the A549 cell line (Biswal et al., 2000). There was also an elevation in CYP2E1 and a >9-fold elevation in redox-related gene activity. In an earlier study of the A549 cell line (Horton et al., 1997), acrolein's ability to alter the proliferation of cells *in vitro* was dependent on cell density and total cell number.

Transformed cells: Four concentrations of acrolein failed to induce malignant transformation in C3H/10T1/2 mouse embryo fibroblasts (Bioassay Systems Corp., 1982c).

Immunotoxicity: Topical administration of acrolein to the shaved skin of female guinea pigs (15) was shown not to result in positive skin reactions; positive controls were used (Susten and Breitenstein, 1990).

Miscellaneous: Survival of human umbilical artery cells was unchanged by exposure to 10 μM acrolein, but reduced to 17 and 11% of controls by 50 and 100 μM acrolein (Pino and Lyles, 1995). Inhibition of P450 in rat microsomal preparations by acrolein has been

demonstrated by Gurtoo et al. (1981). Myeloperoxidase isolated from human neutrophils was shown to convert L-threonine into acrolein (Anderson et al., 1997); activated neutrophils required the myeloperoxidase-H_2O_2-chloride system to produce acrolein in high yield. It was suggested that activated phagocytes have the potential to cause tissue damage at sites of inflammation.

Toxicity of conjugates: The toxicity of acrolein-thiol conjugates as well as acrolein mercapturates to human lung adenocarcinoma A549 cells was examined by Ramu et al. (1996). These conjugates were incubated with cells following a 2-hour exposure of cells treated with diethyl maleate (DEM) to deplete GSH. There was a dose-dependent inhibition of cell growth following treatment with acrolein, S-3-oxopropyl N–acetyl cysteine and its sulfoxide, with the sulfoxide also resulting in plasma membrane damage. A 24-hour (but not 2-hour) exposure of cells to S-3-oxopropyl GSH also resulted in growth inhibition. Pretreatment with DEM increased the inhibition of cell growth seen with acrolein. Quantitative, pH, and rate considerations suggested that β-elimination of acrolein was not the sole mechanism of toxicity of S-3-oxopropyl N–acetyl cysteine and its sulfoxide. Acrolein itself has been demonstrated to inhibit NF-κB activation of A549 cells, consistent with formation of acrolein-NF-κB conjugates (Horton et al., 1999). NF-κB is a transcription factor controlling a number of genes, including those involved in proliferation and apoptosis.

Perry et al. (1995) evaluated the toxicity of several acrolein derivatives to A549 cells. No significant toxicity was observed with S-3-hydroxypropyl N-acetyl cysteine or S-3-oxopropylGSH. S-3-oxopropyl N-acetyl cysteine caused growth inhibition that was reversed by GSH and N-acetyl cysteine.

Eisenbrand et al. (1995) speculated that acrolein-GSH could potentially function as transport molecules for 2-alkenals, such as acrolein, if they reach tissues low in GSH and GST. These investigators found that, in the absence of GSH, acrolein-GSH conjugate decomposed slowly into aldehyde and GSH. The toxicological importance of GSH lies in its role as a substrate in detoxifying conjugation reactions catalyzed by GSH transferase and as a substrate for GSH peroxidase, which protects against membrane damage (Zitting and Heinonen, 1980). Incorporation of the GST isozyme P1-1 (Pi class) into Hep G2 cells was found to increase their resistance to acrolein toxicity, suggesting that GSTs may play a role in cellular detoxication (Berhane et al., 1994). Comparison of the specific activities of GST A1-1 (alpha class), M1-1 (Mu class), and P1-1 indicated that the rate of reaction was in the order of A1-1< M1-1< P1-1.

4.5.2. Intraperitoneal/Intragastric/Intravenous Toxicity

Intraperitoneal administration of acrolein (0.5 to 6 mg/kg BW) to male F344 rats was found to result in urinary bladder hyperplasia; hyperplasia was not observed upon intragastric administration at lethal levels (Sakata et al., 1989). The nephrotoxicity of a 1:1 acrolein-GSH adduct in the rat was examined by Horvath et al. (1992). Male Sprague-Dawley rats were given the adduct intravenously at 0.5 or 1 mmole/kg. In addition to gross and histologic changes in the kidney, glucosuria and proteinuria, an elevation in serum urea nitrogen was observed. The nephrotoxicity was inhibited by acivicin, a γ-glutamyltranspeptidase inhibitor, indicating that

metabolism through the first step in the renal mercapturic acid synthesis pathway is required.

4.5.3. Genotoxicity

4.5.3.1. DNA Adduct Formation, Sister Chromatid Exchange and DNA-Protein Cross-links

Munsch et al. (1973) found that acrolein (2×10^{-5} to 8×10^{-4}M) inhibited partially purified regenerating rat liver DNA polymerase, but not DNA polymerase I from *E. coli*. The site of action was at the -SH groups and was presumed to oxidize the -SH groups. In a confirmatory study, Munsch et al. (1974) observed that (^{3}H) acrolein was bound to regenerating rat liver DNA polymerase 10 to 20 times more than to *E. coli* DNA polymerase I, the latter having no -SH groups at its active center. Acrolein has also been demonstrated to inhibit transcriptional activity of isolated liver nuclei from male Wistar rats and bacterial RNA polymerase (Moulé et al., 1971).

Acrolein at concentrations of 5, 15, and 20 μM, but not lower doses, induced significant increases in sister chromatid exchanges in cultured human lymphocytes (Wilmer et al., 1986). Acrolein was reported to induce formation of deoxyguanosine adducts at concentrations of 10 μM, but not 4 or 7 μM, in *Salmonella* tester strain TA104 (Foiles et al., 1989). Lack of response at the lower doses suggests the presence of a saturable repair mechanism. Increasing adduct formation with dose was seen in tester strain TA100 at concentrations of 4 mM and greater. The responses are in agreement with increases in mutagenicity at about the same doses in these two tester strains. Using the same methodology, deoxyguanosine adduct formation increased progressively at concentrations ranging from 0.1 to 1 mM in Chinese hamster ovary cells (CHO) (Foiles et al., 1990). In studies by Chung et al. (1984), reaction of acrolein with deoxyguanosine or DNA under physiological conditions led to the formation of cyclic 1,N^2-propanodeoxyguanosine and its adducts (Chung et al., 1984). Smith et al. (1990c) were able to determine the structure of an adduct formed from calf thymus DNA following acrolein exposure as 1,N^6-propanodeoxyadenosine. Several putative adducts were observed in DNAs isolated from acrolein-treated human fibroblasts. One of these adducts was tentatively identified as the cyclic 1,N^2-hydroxypropanodeoxyguanosine product, 3-(2′-deoxyribosyl)-5,6,7,8-tetrahydro-8-hydroxypyrimido[1,2-*a*]purine-10-one (Wilson et al., 1991). Chenna et al. (1992) reported that reaction of acrolein with thymidine resulted in one major product, N^3-(3″-oxopropyl)thymidine. Reaction of acrolein with 2-deoxyuridine under physiological conditions formed N^3-(3″-oxopropyl)-2′-deoxyuridine. This product was reduced to give N^3-(3″-hydroxypropyl)-2′-deoxyuridine (Chenna and Iden, 1993). At neutral pH, acrolein reacts with guanosine and cytosine and adenine derivatives to yield several cyclic adducts (Sodum and Shapiro, 1988).

Using a ^{32}P-postlabeling method, Nath et al. (1996) found evidence of DNA-acrolein adducts (1,N^2-propanodeoxyguanosine) in liver DNA of unexposed humans and untreated F344 rats, suggesting that they may be prevalent background lesions. Using the same technique, Nath and colleagues (Penn et al., 2001) found this adduct in aortic DNA of white leghorn cockerels exposed to 1 or 10 ppm (2.3 or 23 mg/m^3) acrolein for 6 hours. When cockerels were exposed to

1 ppm (2.3 mg/m^3) acrolein for 6 hr/day for 8 weeks to examine arteriosclerotic plague formation potential, there was no effect of exposure on plaque development.

DNA-protein cross-links were increased between calf thymus DNA and histone at acrolein concentrations of about 25 μM, based upon graphic data, although numbers were not reported (Kuykendall and Bogdanffy, 1992). Costa et al. (1997) reported significant ($p<0.05$) increases in DNA-protein cross-links in human Burkitt's lymphoma cells exposed 4 hours at concentrations greater than 150 μM acrolein. It is uncertain if the differences in sensitivity reported are due to differences in methodology or cell type. Kozekov et al. (2001) cross-linked two DNA strands with the principal adduct of acrolein.

Inhalation exposure of male F344 rats to 2 ppm (4.6 mg/m^3) acrolein for 6 hours did not cause detectable DNA-protein cross-linking in the nasal respiratory mucosa whereas cross-linking was observed under *in vitro* conditions (Lam et al., 1985). It was hypothesized that acrolein reacted preferentially with sulhydryl-containing nucleophiles.

DNA single strand breaks were induced by acrolein in cultures of Namalva cells, a human lymphoblastic cell line poor in deactivating enzymes and low in GSH and in GST activity, at much lower concentrations than needed in primary rat hepatocytes (Eisenbrand et al., 1995). Acrolein caused a higher extent of DNA single-strand breaks (SSB) in XP cells (which normally have < 5% of excision repair capacity of normal cells) than normal cells (Dypbukt et al., 1993). Exposure to acrolein followed by incubation in fresh medium resulted in continued formation of DNA SSB in normal cells without further accumulation in XP cells.

4.5.3.2. Mutagenic Effects of Acrolein in *Drosophila melanogaster*

Effects of acrolein on somatic mutations in *Drosophila* are shown in Table 3. Vogel and Nivard (1993) reported positive effects only with inhalation at toxic exposure levels, and not in feeding studies of larvae. Sierra et al. (1991), on the other hand, reported positive effects in feeding studies under similar conditions. Reasons for the difference in findings are uncertain, although they could be due to the use of 48-hour cultures by Vogel and Nivard (1993) compared with 72-hour cultures by Sierra et al. (1991).

Effects of acrolein on sex-linked recessive lethals (SLRLs) in *Drosophila* are shown in Table 4. No effects of acrolein were reported for SLRL induction in feeding studies, but highly significant increases were noted in injection studies at high mM concentrations (Sierra et al., 1991). No effect on percent lethals, either by injection (200 ppm) or feeding (3,000 ppm) was observed in Canton-S wild-type males (Zimmering et al., 1985). Mutations in excision repair deficient *Drosophila* (*mus201*) induced a greater incidence of SLRLs than in repair efficient females. Based upon statistical analysis to evaluate hypermutability, it was concluded by the authors that acrolein induces lesions that are partially repaired by excision repair mechanisms. Since cyclic adducts can be repaired by excision mechanisms (Vogel, 1989), and the only acrolein-derived lesions reported to date are cyclic adducts (Foiles et al., 1989; Smith et al., 1990b; Wilson et al., 1991), it appears that at least some of the SLRLs are derived from cyclic adducts. The response of *Drosophila* to acrolein with *mus308* mutation, which is thought to play

a role in repair of cross-linking adducts, was no different than normal mice (Commendador et al., 1992; Barros et al., 1994a). These results thus provide additional support for the likelihood that cyclic adducts are the predominant forms induced by acrolein.

Barros et al. (1994b) tested the effects of metabolic modification upon induction of SLRLs. Diethyl maleate, a GSH-depleting agent, induced an increase in SLRLs in feeding studies with acrolein-exposed *D. melanogaster (Berlin K and Muller-5* strains). Phenobarbital, a cytochrome P450 inducing agent, eliminated response to acrolein via injection. Iproniazid and 1-phenylimidazole, potent inhibitors of cytochrome P450 oxidative enzymes of *Drosophila*, had no effect on SLRL induction by injection of acrolein. These results support the hypothesis that acrolein is a direct mutagen. Moreover, acrolein is deactivated by enzymatic activity induced by phenobarbital. The results also indicate that sensitivity to acrolein by the oral route is relatively low. This may be a function of its reactivity, with little reaching the reproductive organs by way of food.

TABLE 3. Effects of acrolein on somatic mutations in *Drosophila melanogaster*

Sex	Conc (mM)	Dose Method	Endpoint	% Spots	Average clone size	Reference
both	0	food	eye spots	4.2	2.9	Vogel and Nivard (1993)
both	10	food	eye spots	3.0 (-)	2.4	
both	20	food	eye spots	4.1 (-)	3.8	
both	80	food	eye spots	4.0 (-)	2.1	
both	0	inhalation	eye spots	4.7	4.1	
both	500 ppm	inhalation	eye spots	8.9 (+)	4.1	
both	1,000 ppm	inhalation	eye spots	4.4	4.0	
both	2,000 ppm	inhalation	eye spots	lethal	-----	
male	0	food	eye spots	1.4	5.0	Sierra et al. (1991)
male	5	food	eye spots	5.5 (+)	4.1	
male	10	food	eye spots	3.8 (+)	4.7	
male	20	food	eye spots	11.6 (+)	5.7	
female	0	food	eye spots	3.3	7.0	
female	5	food	eye spots	7.4 (+)	11.2	
female	10	food	eye spots	7.4 (+)	4.4	
female	20	food	eye spots	16.6 (+)	6.9	
both	0	food	wing spots	17.5	1.3	
both	5	food	wing spots	21.1 (-)	1.6	
both	10	food	wing spots	29.3 (+)	1.8	
both	20	food	wing spots	29.6 (+)	3.0	

A statistical analysis was conducted according to Frei and Wurgler (1988): +,S positive; -, inactive

TABLE 4. Effects of acrolein in the induction of sex-linked recessive lethal (SLRL) mutations in *D. melanogaster* exposed for 5 hr (feeding) and by injection

Concentration (mM)	Dose Method	Stage of Meiosis	Other Treatments	Percent Lethals	Reference
0.00 (broods pooled)	food	post	none	0.5	Sierra et al. (1991)
0.50	food	post	none	0.33	
1.00	food	post	none	0.16	
2.50	food	post	none	0.15	
5.00	food	post	none	0.16	
10.00	food	post	none	0.50	
5.00 (24-hr exposure)	food	post	none	0.19	
0.00	injection	post	none	0.17	
2.00	injection	post	none	0.31	
3.00	injection	post	none	0.39*	
3.00	injection	post	none	0.92***	
5.00	injection	post	none	0.61***	
5.00	injection	post	none	1.01***	
7.00	injection	post	none	0.60***	
7.00	injection	post	none	0.34	
0.00	injection	post	*mus*201	0.35	Barros et al. (1994a)
2.00	injection	post	*mus*201	0.82**	
3.00	injection	post	*mus201*	0.99***	
5.00	injection	post	*mus*201	1.06***	
0.00	injection	post	*mus*308	0.24	
1.00	injection	post	*mus*308	0.41*	
2.00	injection	post	*mus*308	0.25	
3.00	injection	post	*mus*308	1.41**	
5.00	injection	post	*mus*308	0.31	
0.00	injection	post	DEM	0.08	Barros et al. (1994b)
0.00	injection	pre	DEM	0.09	
3.00	injection	post	DEM	0.50*	
5.00	injection	post	DEM	0.46*	
5.00	injection	pre	DEM	0.14	
7.00	injection	post	DEM	0.20	
0.00	injection	post	PB	0.27	
0.00	injection	pre	PB	0.10	
3.00	injection	post	PB	0.30	
3.00	injection	pre	PB	0.03	
5.00	injection	post	PB	0.18	

Concentration (mM)	Dose Method	Stage of Meiosis	Other Treatments	Percent Lethals	Reference
5.00	injection	pre	PB	0.00	
0.00	injection	post	PHI + IPR	0.10	
0.00	injection	pre	PHI + IPR	0.12	
0.30	injection	post	PHI + IPR	0.18	
0.30	injection	pre	PHI + IPR	0.17	
0.50	injection	post	PHI + IPR	0.36*	
0.50	injection	pre	PHI + IPR	0.13	
0.70	injection	post	PHI + IPR	0.37*	
0.70	injection	pre	PHI + IPR	0.17	

*mus*201 maternal excision repair deficiency
*mus*308 hypersensitive to cross-linking agents
PB (phenobarbital): Induces xenobiotic metabolism
DEM (diethylmaleate): Glutathione-depleting agent
PHI (1-phenylimidazole): Inhibitor of cytochrome P450
IPR (ipronazid): Inhibitor of cytochrome P450
* ($p<0.05$), **($p<0.01$), ***($p<0.001$)

4.5.3.3. Tests for Gene Mutation in Mammalian Cell Cultures

Although acrolein has been shown to induce DNA adducts in a variety of cell types as well as mutagenesis in *Drosophila* and microorganisms under certain conditions, there is limited information regarding the ability of acrolein to induce mutations in normal mammalian cells. Acrolein was shown to be highly mutagenic to human fibroblast cells that were deficient in DNA repair (cells from xeroderma pigmentosum patients). While a positive dose-response was observed between 0.2 and 0.8 μM acrolein in the repair deficient cells, acrolein did not induce an increase in the mutant frequency of normal fibroblasts (Curren et al., 1988). Acrolein was also mutagenic in V79 cells deficient in DNA repair (Smith et al., 1990a). Normal V79 cells were not tested. *In vitro* chromosomal studies of acrolein have produced weakly positive findings in Chinese hamster ovary (CHO) cells (Au et al., 1980) and in cultured human lymphocytes (Wilmer et al., 1986). Chromosomal aberrations were not detected in CHO cells either in the presence or absence of metabolic activation (Bioassay Systems Corp., 1982d) or in rat bone marrow cells (Bioassay Systems Corp., 1982e). More recently Parent et al. (1991a) failed to detect mutagenic effects of acrolein using the sensitive Chinese hamster ovary hypoxanthine-guanine phosphoribosyl transferase (HGPRT) forward mutation assay system both with and without exogenous activation, even at toxic dose levels. These results confirmed earlier findings in which acrolein was found not to induce mutations at the HGPRT locus in CHO cells (Bioassay Systems Corp., 1982b). Kawanishi et al. (1998) conducted a molecular analysis using *supF* shuttle vector plasmids for the spectrum of mutations that acrolein may induce in human fibroblast cells. The majority of the mutations were base substitutions (76%) followed by deletions and insertions (24%). Single base substitutions were most frequently found (46%), multiple base substitutions accounted for 18%, and tandem (two adjacent) base substitutions

were 12%. Of the base substitutions, G:C to T:A transversions accounted for 44% of the total and G:C to A:T transitions for 24%.

4.5.3.4. Tests for Gene Mutation in Bacterial Cells

Results are summarized in Table 5. In tests for frameshift mutagens without metabolic activation (TA98 and TA1538), TA98 gave some positive responses while TA1538 was negative. The only positive response for TA98 with S-9 activation was reported by Claxton (1985). With this exception, metabolic activation generally resulted in negative responses in all strains. Tests for base repair and point mutations (TA100, TA104 and TA1535) were positive in some tests with TA100, in most tests with TA104, but not with TA1535. TA104 has been reported to be more sensitive to carbonyl compounds (Marnett et al., 1985). Among strains sensitive to cross-linking (TA102, TA2638, WP2 and *Escherichia coli* HB101), TA2638 and HB101 were positive in the only study reported for each strain, while TA102 and WP2 strains were negative. The *Escherichia coli* strains JTG10 and AR1157, which are lacking in GSH synthetase, are sensitive to induction of mutations as well as induction of cytotoxicity at very low concentrations.

VanderVeen et al. (2001) has shown that when acrolein reacts with guanine residues in *S. typhimurium* to form 8-hydroxypropanodeoxyguanosine, the latter was not mutagenic in *S. typhimurium*. Acrolein was similarly nonmutagenic in *E. coli* (Yang et al., 2001).

It is clear from the studies reported that acrolein is highly reactive and cytotoxic. Acrolein has been shown to be mutagenic in some test systems within a narrow range of concentrations. Sensitivity to mutational effects is increased by GSH depleting agents and decreased by addition of metabolic activation, indicating that acrolein is a direct acting agent. While acrolein is capable of alkylating DNA and DNA bases (Maccubbin et al., 1990) and is known to inhibit purified DNA methylase activity from liver and bladder (Cox et al., 1988), it may never reach the target tissues of whole animals other than those at the site of insult. Even in the *in vitro* assays cited, acrolein is so reactive that special techniques must generally be employed to reduce cytotoxicity to induce positive effects. Parent et al. (1996b) have suggested that the reactivity of acrolein precludes its reaching target cells at a sufficient concentration to initiate the carcinogenic process.

TABLE 5. Tests for gene mutation in bacterial systems

Species/Strain	Result[a] +S9	Result[a] -S9	Test Type	Reference
Salmonella typhimurium TA98	-	-	Reverse mutation	Basu and Marnett (1984)
	+	+		Claxton (1985)
	-	-		Haworth et al. (1983)
	-	-		Florin et al. (1980)
	-	+		Lijinsky and Andrews (1980)
	-	-		Loquet et al. (1981)
	-	±		Parent et al. (1996b)
Salmonella typhimurium TA100	-	-		Basu and Marnett (1984)
	toxic	+		Eder et al. (1993)
	-	-		Florin et al. (1980)
		+		Foiles et al. (1989)
	±	-		Haworth et al. (1983)
	-	-		Lijinsky and Andrews (1980)
	-	-		Loquet et al. (1981)
	-	+		Lutz et al. (1982)
	-	+		Parent et al. (1996b)
Salmonella typhimurium TA102	-	-		Jung et al. (1992)
	-	-		Parent et al. (1996b)
	-	-		Watanabe et al. (1998)
Salmonella typhimurium TA104	0	+		Foiles et al. (1989)
	-	+		Hoffman et al. (1989)
	0	+		Marnett et al. (1985)
	-	-		Parent et al. (1996b)
Salmonella typhimurium TA1535	-	-		Florin et al. (1980)
	-	±		Hales (1982)
	-	-		Haworth et al. (1983)
	-	-		Lijinsky and Andrews (1980)
	-	-		Loquet et al. (1981)
	-	-		Parent et al. (1996b)
Salmonella typhimurium TA1537	-	-		Florin et al. (1980)
	-	-		Haworth et al. (1983)
	-	-		Lijinsky and Andrews (1980)
	-	-		Parent et al. (1996b)
Salmonella typhimurium TA1538	-	-		Basu and Marnett (1984)
	-	-		Lijinsky and Andrews (1980)
	-	-		Parent et al. (1996b)

Species/Strain	Result[a] +S9	-S9	Test Type	Reference
Salmonella typhimurium TA2638	0	+		Watanabe et al. (1998)
*Salmonella typhimurium his*D3052/*nop*KM101	-	-		Basu and Marnett (1984)
Salmonella typhimurium TA1535	0	-	SOS (*umu*) induction assay	Benamira and Marnett (1992)
Escherichia coli PQ37	0	+	SOS repair	Eder et al. (1993)
Escherichia coli HB101pUC13	0	+	DNA-histone cross-links	Kuykendall and Bodanffy (1992)
Escherichia coli WP2 *uvr*A	-	±	Reverse mutation	Hemminki et al. (1980)
	±	-		Parent et al. (1996b)
Escherichia coli WP2/pKM101	0	-		Watanabe et al. (1998)
Escherichia coli WP2 *uvrA*/pKM101	0	-		Watanabe et al. (1998)
Escherichia coli JTG10	0	+		Nunoshiba and Yamamoto (1999)
Escherichia coli AB1157	0	+		
Escherichia coli WP2	0	-		Aikawa and Miwa (1993)
Escherichia coli WP2(*urv*)A155	0	-		
Escherichia coli ZA159(*uvrB*)	0	-		

[a] +, ≥2 x background rate or statistically significant ($P < 0.05$); ±, equivocal; -, negative; 0, not tested.

4.5.4. Mechanistic Studies

A number of *in vitro* and *in vivo* studies demonstrated that acrolein has the potential to: (1) perturb the environments of human and laboratory animal cells in which GSH plays an important role, (2) suppress host defense mechanisms, and (3) elicit pro-inflammatory processes.

GSH depletion in isolated rat hepatocytes incubated with 0.25-0.5 mM acrolein caused lipid peroxidation and impaired integrity of cell membranes (Zitting and Heinonen, 1980). Depletion of GSH at 3-25 μM acrolein has also clearly been established in cultured endothelial cells (Patel and Block, 1993), and in human bronchial epithelial cells at 3 μM acrolein (Grafström et al., 1988). There was a dose-related decrease in plasma membrane surface -SH groups in human polymorphonuclear leukocytes and rat pulmonary alveolar macrophages when incubated at acrolein concentrations from 1 to 1000 μM (Witz et al., 1987). GSH protects cells by removing reactive metabolites such as electrophilic carbonium ions. Thus, GSH depletion deprives the cell of its natural defense against ubiquitous reactive metabolites and leaves the thiol groups in critical proteins vulnerable to attack by oxidation, cross-linking, and the formation of mixed disulfides or covalent adducts. For example, cellular constituents of the antioxidant defense system, including ascorbic acid, α-tocopherol, GSH peroxidase, and catalase in rat lung were decreased following inhalation exposure of male Wistar rats to 1 or 2 ppm (2.3 or 4.6 mg/m^3) acrolein (Arumugam et al., 1999a). This led to enhanced lipid peroxidation,

which produced extensive lung damage as indicated by elevated levels of the biochemical markers – angiotensin converting enzyme, LDH, protein, and lactate in the bronchioalveolar lavage.

In vivo exposure resulted in GSH depletion in nasal respiratory mucosa (McNulty et al., 1984). This is likely due to the highly reactive nature of acrolein, which reacts by virtue of its allylic function with GSH and similar compounds (Zitting and Heinonen, 1980). Meacher and Menzel (1999), using cultured adult rat type II alveolar cells, demonstrated with a fluorogenic reagent that the depletion of GSH by 1-5 μmol/L of acrolein follows the nonenzymatic rate constant for the forward reaction. In addition, rates of GSH depletion by other alkenals and alkanals correlated with LD50 values for each compound, leading the authors to conclude that structure-activity relationships are useful for predicting toxicity of aldehydes.

Adams and Klaidman (1993) reported that acrolein and its GSH adduct glutathionyl-propionaldehyde can directly induce oxygen radical formation *in vitro*. The enzymes, xanthine oxidase and ALDH, were found to interact with this adduct to produce $O_2 \cdot^-$ and HO·. Acrolein was also oxidized by xanthine oxidase to produce acroleinyl radical $O_2 \cdot^-$.

It would appear that when a reactive chemical, such as acrolein, comes in contact with a cell, its first site of attack is the plasma membrane. Srivastava et al. (1992) have, in fact, reported that in *in vitro* studies, acrolein interaction at low concentration inhibited rat liver plasma membrane enzymes (i.e., ATPases) to varying degrees and attacked membrane surface proteins, suggesting at least a superficial change could lead to changes in ion transport and membrane potential. Pompella et al. (1991), on the other hand, determined that alkylation of macromolecules by acrolein is not a major factor in liver cell injury. Although acrolein was observed to rapidly bind to cytosolic soluble proteins and membrane-bound thiols *in vitro*, acrolein avoided membrane-bound thiols *in vivo*, even after GSH depletion. Gurtoo et al. (1981) have obtained convincing evidence that acrolein binds to cytochrome P450 resulting in its denaturation.

The role of acrolein in suppressing host defense mechanisms is also an area of increasing research interest. Using cultured human alveolar macrophages, Li et al. (1997) demonstrated that acrolein *in vitro* inhibited the release of the cytokines IL-1β, TNF-α, and IL-12, and induced apoptosis and necrosis in human alveolar macrophages. Subsequently, Li and Holian (1998) provided preliminary information that inhibition of the transcription factor for many cytokine genes, NF-κB, may be responsible for the inhibition of cytokine release as well as acrolein induced apoptosis in alveolar macrophages. Most recently, Li et al. (1999) found that acrolein inhibited phosphorylation of the principal regulator of NF-κB. The activated form of NF-κB is of relevance to genes encoding cytokines involved in immune and proinflammatory responses, including viral genomes such as human immunodeficiency virus, type 1 (Müller et al., 1993). NF-κB also plays a central role in expression of adhesion molecules in human vascular endothelial cells (Collins et al., 1995). Since acrolein *in vitro* acts as an inhibitor of NF-κB activity, immunomodulation by acrolein should be regarded as an area for further investigation, particularly at environmental levels.

Acrolein also has been demonstrated to inhibit in a dose-dependent manner the *in vitro* synthesis of prostaglandin E_2 in rat resting and zymosan-stimulated alveolar macrophages (Grundfest et al., 1982). This resulted in a relative increase in release of thromboxane B_2, the inactive form of the potent vasoconstrictor, thromboxane A_2. GSH protected the macrophages from acrolein-induced changes in arachidonic acid metabolism. Acrolein was found to increase bronchial reactivity to intravenously administered acetylcholine in guinea pigs with a maximum at 2-4 hour postexposure. Upon bronchoalveolar lavage, thromboxane B_2 and prostaglandin $F_{2\alpha}$ was shown to be increased immediately after exposure followed by an influx of neutrophils 24 hours later (Leikauf, 1991). Prostaglandin $F_{2\alpha}$ has been demonstrated to increase bronchial reactivity in asthmatics (Mathe et al., 1973). Evidence suggests that acrolein-induced bronchial hyperresponsiveness may be the result of damage to epithelial cells (Costa et al., 1986).

Using freshly isolated rat tracheal smooth muscle myocytes, Hyvelin et al. (2000, 2001) found that acrolein modulates the Ca^{++} signaling pathway by increasing production of inositol triphosphate and does not directly affect the muscarinic cholinoceptor or inositol triphosphate receptor sensitivity. This extends previous work (Ben-Jebria et al., 1993, 1994) in which it was reported that acrolein exposure increased the reactivity of human bronchial and rat tracheal rings to muscarinic agents in a dose-dependent manner.

The mode of action whereby acrolein produces nasal irritation in Fischer 344 rats has been investigated by Morris et al. (1999). At 20 ppm (46 mg/m^3) for 50 minutes, acrolein induced vasodilation and plasma protein extravasation into nasal tissues; both responses were inhibited by capsaicin. Vasodilation, but not protein extravasation, was also elevated over controls at 2, 5, and 10 ppm (4.6, 11.4, and 22.9 mg/m^3). Inhibition by capsaicin was regarded as evidence of C-fiber involvement. While there was evidence of tachykinin release, substance P and neurokinin were not thought to be involved. On the other hand, exposure of female Wistar rats to 22, 81, and 249 ppm (50.4, 185.5, and 570.2 mg/m^3) acrolein for 10 minutes resulted in a significant decrease in nerves of the trachea immunoreactive for substance P (less so for calcitonin gene-related peptide) with the effect spreading further down the respiratory tract with increasing dose (Springall et al., 1990). There appeared to be no evidence of nerve damage.

It is clear that GSH plays a major role in acrolein toxicity. The depletion of GSH and the formation of acrolein GSH adducts resulting in an increase in reactive oxygen species is undoubtedly a major factor in the induction of toxic and mutagenic effects. Although membrane binding, inhibition of regulatory proteins, and modulation of cytokine release at the gene transcription level have been demonstrated, their importance at low levels of exposure is still uncertain.

Bronchitis, asthma, and cystic fibrosis, marked by inflammation and mucus hypersecretion, can be caused or exacerbated by airway pathogens or irritants including acrolein, an aldehyde present in tobacco smoke. To determine whether acrolein and inflammatory mediators alter mucin gene expression, steady-state mRNA levels of two airway mucins, MUC5AC and MUC5B, were measured (by RT-PCR) in human lung carcinoma cells (NCI-H292). MUC5AC mRNA levels increased after >/=0.01 nM acrolein, 10 μM prostaglandin E2 or 15-hydroxyeicosatetraenoic acid, 1.0 nM tumor necrosis factor-alpha

(TNF-alpha), or 10 nM phorbol 12-myristate 13-acetate (a protein kinase C activator). In contrast, MUC5B mRNA levels, although easily detected, were unaffected by these agonists, suggesting that irritants and associated inflammatory mediators increase mucin biosynthesis by inducing MUC5AC message levels, whereas MUC5B is constitutively expressed. When transcription was inhibited, TNF-alpha exposure increased MUC5AC message half-life compared with control level, suggesting that transcript stabilization is a major mechanism controlling increased MUC5AC message levels. Together, these findings imply that irritants like acrolein can directly and indirectly (via inflammatory mediators) increase airway mucin transcripts in epithelial cells (Borchers et al., 1999a).

4.6. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS AND MODE OF ACTION—ORAL AND INHALATION

4.6.1. Oral Administration

No human studies are available regarding exposure by the oral route. Several animal studies are available.

Gavage studies (13 weeks) in F344 rats and B6C3F1 mice (NTP, 1995) demonstrated dose-response increases in gastrointestinal effects. Hemorrhage, necrosis, and chronic inflammation of the forestomach and glandular stomach with increased mortality was observed in the high-dose groups of both species (10 mg/kg-day in rats; 20 mg/kg-day in mice), and in both males and females. Hemorrhage of the glandular stomach was also seen at the next two lower dose levels in males and the next lower level in females. When Sprague-Dawley rats were administered acrolein in water by gavage at much lower doses for 2 years (Parent et al., 1992c), early mortality occurred. However, there was no gross or histopathological evidence of stomach lesions or histopathological evidence in other organs. Mortality occurred at a much lower dose (0.5 mg/kg-day) and early mortality was significant even after correction for gavage error. When this protocol was applied to CD-1 mice (Parent et al., 1991b), the primary effect was mortality in high-dose animals (4.5 mg/kg-day), again without any adverse clinical or histopathological findings.

Wistar rats were gavaged with 2.5 mg/kg-day for a period of 45 days, but clinical observation and histopathology were not part of the protocol (Arumugam et al., 1999b). At the end of dosing, there was significant reduced activities of citric acid cycle enzymes and cytosolic and mitochondrial GSH in the liver, as well as oxidative damage to mitochondrial membrane integrity. These effects may contribute to an increased mortality due to progressive mitochondrial damage over time.

Early mortality observed in the F344 and Sprague-Dawley rats is considered to be the critical effect that occurred at the lowest dose level (i.e., in the Parent et al., 1992c study). At higher dose levels in F344 rats and B6C3F1, gastrointestinal damage accompanies increased mortality.

Reasons for no reported observations of stomach lesions in Sprague-Dawley female rats

at the highest dose (2.5 mg/kg) of the Parent et al. (1992c) study compared with forestomach squamous epithelial hyperplasia observed in female F344 rats in the NTP (1995) study at 1.25 mg/kg-day are not readily apparent, but may relate to differences in strain sensitivity or vehicle. The vehicle dose volume was 5 ml/kg in the NTP (1995) study and 10 ml/kg in the Parent et al. (1992c) study, and there may have been reduced local gastric mucosal irritation and pathology by virtue of dilution. There were also differences in the vehicle solution and, possibly, the stability of the dosing solutions. Parent et al. (1992c) conducted stability studies on acrolein in water, and monitored the stability of their dosing solutions (reporting losses of less than 10% for 3 hours at room temperature). They used a stabilizing agent, 0.25% hydroquinone, in the stock solution, and prepared dosing solutions daily. The NTP (1995) study used a dose vehicle of 0.5% methylcellulose in deionized water, and no information was available on stability or stabilizing agents.

For the mouse results, there is a similar divergence between the absence of reported forestomach lesions in the CD-1 mice at 4.5 mg/kg in the Parent et al. (1991b) study compared with effects observed in female B6C3F1 at 2.5 mg/kg in the NTP (1995) study. Species differences and dose volume again may have accounted for the observed differences in response. Dose volume in the NTP (1995) study for mice was 10 ml/kg, and was unspecified in the Parent et al. (1991b) study.

An explanation for the early cumulative mortality in the absence of other significant effects is not provided by Parent et al. There is mention of the significant decrease in creatinine phosphokinase. Creatinine phosphokinase (CPK), also referred to as creatine kinase (CK), is a widespread enzyme that catalyzes the reversible oxidation of creatine (by adenosine triphosphate (ATP)) to creatine phosphate. CK occurs as three different isoenzymes, each composed of two polypeptide chains, B (brain derived) and M (muscle derived). Skeletal muscle and cardiac muscle have a very high CK content but different isozyme ratios, with very low percentage (less than 5%) of CK-MB in skeletal muscle and a higher percentage (20-30%) of CK-MB in heart. Brain, prostate, thyroid, gut and lung have predominantly CK-BB; plasma has predominantly CK-MM with less than 6% CK-MB. Usually, the heart is the only tissue in which the amount of CK-MB exceeds 5%. Serum CK is a very sensitive indicator of target tissue damage, with elevated serum levels within 4-6 hours post injury. If not progressive, CK serum levels decline to normal within 24 hours. Illness of the nervous system, heart, or musculature can also produce elevated serum CK levels (Hayes, 1994). Low serum CK levels have been associated with impaired energy metabolism or reduced skeletal muscle function from phosphate depletion (Brautbar et al., 1983), connective tissue disease including rheumatism (Wei et al., 1981; Lee et al., 2000) or alcoholic liver disease (Nanji and Blank, 1981).

The research demonstrating acrolein's high reactivity, low systemic distribution, toxicity at the point of entry, pronounced decreases in citric acid cycle enzymes and in liver GSH, depression of serum CK levels, and increased mitochondrial damage in the Wistar rat are suggestive of pathologies that could potentially be responsible for early mortality. In the absence of gastrointestinal histopathology, one could postulate that there was sufficient subclinical gastrointestinal toxicity to interfere with normal metabolic processes and possibly absorption of essential nutrients sufficient to lead to early mortality. Further research is needed

to support a more definitive understanding of acrolein's mode of action.

4.6.2. Inhalation Exposure

Acute Exposures:

In the few clinical studies that have examined the effects of low-level acrolein exposure, it is clear that measured levels considerably lower than 1 ppm (2.3 mg/m^3) elicit subjective complaints of eye and nasal irritation and a decrease in the respiratory rate (Weber-Tschopp et al., 1977; Sim and Pattle, 1957). Such effects should be considered adverse based upon longer-term studies in laboratory animals at higher concentrations that have demonstrated more severe nasal lesions as well as pronounced adverse effects on lung function leading to lethality.

Acrolein was reported by male and female volunteers (53) as causing eye irritation beginning at concentrations of 0.09 ppm (0.21 mg/m^3) and higher when they were exposed for 35 minutes to slowly increasing concentrations from zero to a specified amount (0.09 - 0.60 ppm), which was then held constant for 5 more minutes. Investigators reported nasal irritation at concentrations of 0.26 ppm (0.6 mg/m^3) and higher, and a decrease in respiratory rate at 0.6 ppm (1.4 mg/m^3) (Weber-Tschopp et al., 1977). In an inhalation study by Sim and Pattle (1957), male volunteers (12) reported 0.8 ppm (1.9 mg/m^3) acrolein for 10 minutes as extremely irritating. It was not clear how the acrolein was administered in the latter study, by mask or in a chamber.

Signs of respiratory distress and irritation were noted in rats exposed to as low as 4.8 ppm (11 mg/m^3) for one hour (Ballantyne et al., 1989). These clinical indicators were not observed when rats were exposed to levels of 0.25 to 1.4 ppm (0.6 to 3.2 mg/m^3) for 6 hours or 6 hr/day for 3 days (Cassee et al., 1996b). Nor were there any acrolein-induced histopathological nasal lesions after 6 hours of exposure. Other exposure studies of laboratory animals involved much higher concentrations with expected results of lethality associated with respiratory distress.

The limited information from studies with human volunteers suggests that levels below 1 ppm (2.3 mg/m^3) can be expected to elicit subjective signs of nasal and eye irritation and affect the breathing rate. The limited human data as well as data from animal studies at higher concentrations and longer durations suggest that clinical symptoms of distress (and histopathological lesions in the case of laboratory animals) become more pronounced as exposure increases.

Long-Term Exposures:

No chronic exposure human or laboratory animal studies are available.

Two 90-day animal studies and four 60-day or more exposure studies have been reported. Feron et al. (1978) dosed groups of Syrian golden hamsters, Wistar rats, and Dutch rabbits 30 hours/week for 90 days to 0, 0.4, 1.4, and 4.9 ppm (0, 0.9, 3.2, and 9.2 mg/m^3) acrolein in whole-body exposure chambers. At the highest dose, mortality occurred in rats, while ocular and nasal irritation, growth depression and histopathologic changes in the respiratory tract were seen in all three species. At the intermediate dose, squamous metaplasia and neutrophilic infiltration of the

nasal mucosa was seen in the rat, whereas in hamsters, minimal inflammatory changes were seen in the nasal cavity. No effects were detected in the nasal region in the mid and low-dose rabbits. Slight inflammatory effects were reported in the nasal mucosa of one of 12 rats in the 0.4 ppm (0.9 mg/m^3) group. Thus, the LOAEL (minimal) for rats, the most sensitive species, was 0.4 ppm (0.9 mg/m^3) for slight inflammatory changes of the nasal mucosa. The NOAEL for hamsters was determined to be 0.4 ppm (0.9 mg/m^3), and the LOAEL was 1.4 ppm (3.2 mg/m^3) based on inflammatory changes in the nasal cavity. The NOAEL for rabbits was determined to be 1.4 ppm (3.2 mg/m^3) with a LOAEL of 4.9 ppm (9.2 mg/m^3).

Additional evidence in support of a minimal LOAEL of 0.4 ppm (0.9 mg/m^3) from Feron et al. (1978) is provided by the studies of Kutzman and colleagues (Kutzman, 1981; Kutzman et al., 1985; Costa et al., 1986) and Cassee et al. (1996b). Kutzman and colleagues exposed male Fischer 344 rats (50/group) via inhalation to acrolein at 0, 0.4, 1.4, or 4.0 ppm (0, 0.9, 3.2 or 9.2 mg/m^3) 6 hr/day, 5 days/week for 62 exposure days (consecutive weekdays, except for weekends, for 12.4 calender weeks). When rats were evaluated on the 6th day postexposure, some evidence of functional deficits was found at 0.4 ppm (0.9 mg/m^3) and more substantial damage at the highest concentration (4 ppm; 9.2 mg/m^3). The Cassee et al. (1996b) 3-day nose-only study in the rat reported slight nasal effects at lower concentrations (0.25 ppm; 0.6 mg/m^3) than in the Feron et al. (1978) whole-body inhalation study. The Cassee et al. (1996b) study was designed to evaluate the severity of effects from mixtures versus single chemical exposure, and used a higher resolution analysis to detect any interactions. The observed effects at lower levels in the Cassee et al. (1996b) study may be due to the higher resolution analysis of the nasal tract, i.e., six levels of sampling compared to only three by Feron et al. (1978). Alternatively, the nose-only exposure chamber may have delivered more dose or had a different dosimetric distribution to the nasal epithelium as compared to exposure in the whole-body chambers used by Feron et al (1978). In a whole body chamber, rats may bury their noses in their fur during daytime sleeping postures resulting in the animals receiving less exposure than assumed. Because the Feron et al. (1978) study was much longer in duration, it is also possible that some adaptation to the irritant effects of acrolein occurs with increasing duration, or that cessation of exposure for 2 days each week provided a period during which partial recovery from nasal effects might occur. Collectively, the principal study and supporting studies (Kutzman, 1981; Kutzman et al., 1985; Costa et al., 1986; Feron et al., 1978; Cassee, 1996b) provide support for a minimal LOAEL of 0.4 ppm (0.9 mg/m^3).

Lyon et al. (1970) exposed rats, guinea pigs, dogs, and monkeys to 0, 0.22, 1.0 and 1.8 ppm (0, 0.5, 2.3, and 4.1 mg/m^3) acrolein for 24 hr/day for 90 days. A LOAEL of 1 ppm (2.3 mg/m^3) could be derived based upon inflammation in several organs of one or more of the species; however, there was a principal deficiency in this study because of the absence of concurrent control groups, making it unclear whether or not the changes were directly related to an exposure to acrolein.

Acrolein is highly reactive and can induce toxicity in a variety of ways. An increase in reactive oxygen species resulting from reaction with and depletion of glutathione is considered to be the primary mechanism of toxicity (Zitting and Heinonen, 1980; Arumugam et al., 1999a). Reactions with cell membrane proteins and inhibition of regulatory proteins may also play a role.

As a result of acrolein's high degree of reactivity during inhalation, deposition occurs primarily in the nasal mucosa with the accompanying pathological effects. As concentrations increase, penetration and toxicity occur deeper within the respiratory system. Effects in other organs such as the liver were occasionally reported (Lyon et al., 1970), but only at concentrations higher than those affecting the respiratory system, and the mechanism(s) for the effects are uncertain given acrolein's high reactivity. Therefore, the nasal mucosa is considered to be the critical target site, with a minimal LOAEL of 0.4 ppm (0.9 mg/m^3) in the most sensitive species, the rat (Feron et al., 1978). The data were not sufficient to derive a NOAEL.

4.7. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CHARACTERIZATION

Under the Draft Revised Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1999), the potential carcinogenicity of acrolein cannot be determined because the existing "data are inadequate for an assessment of human carcinogenic potential for either the oral or inhalation route of exposure."

There are no adequate human studies of the carcinogenic potential of acrolein. Collectively, experimental studies provide inadequate evidence that acrolein causes cancer in laboratory animals. Specifically, two inhalation bioassays in laboratory animals are inadequate to make a determination because of protocol limitations. Two gavage bioassays failed to show an acrolein-induced tumor response in two species of laboratory animals. The finding of suggestive evidence of an extra-thoracic tumorigenic response in a drinking water study in female rats was not supported in a reanalysis of the data by an independently-convened pathology working group. Questions were also raised about the accuracy of the reported levels of acrolein in the drinking water from this study. A skin tumor initiation-promotion study was negative, and the findings from an intraperitoneal injection study were of uncertain significance. Although acrolein has been shown to be capable of inducing sister chromatid exchange, DNA cross-linking and mutations under certain conditions, its highly reactive nature and the lack of tumor induction at portals of entry make it unlikely that acrolein reaches systemic sites at biologically-significant exposure levels. The observations of positive mutagenic results in bacterial systems occurred at high concentrations near the lethal dose.

This evaluation replaces the cancer assessment for acrolein added to the IRIS data base in 1988. Under the Risk Assessment Guidelines of 1986 (EPA/600/8-87/045) applied at that time, acrolein was classified as a possible human carcinogen (Category C). The 1988 classification for acrolein was based on the increased incidence of adrenal cortical adenomas in female rats and carcinogenic potential of an acrolein metabolite, its mutagenicity in bacteria, and its structural relationship to probable or known human carcinogens. The updated cancer characterization considered new study results and reevaluated previous studies.

Human Studies:

Ott et al. (1989) reported a series of nested case-control studies in relation to various work areas, specific chemicals, and chemical activity groups. An odds ratio of 2.6 for nonlymphocytic leukemia was found for workers who had exposure to acrolein during employment. The small number of cases (3) and the likelihood of exposure to other chemicals, however, provide inadequate evidence of acrolein-induced leukemia or of the carcinogenic potential of acrolein.

Laboratory Animal Studies:

Two cancer bioassays failed to show an increase in tumor incidence when rats (Parent et al., 1992c) and mice (Parent et al., 1991b) were administered acrolein by gavage. In both studies the maximum tolerated dose was demonstrated by a significant increase in mortality.

Although administration of acrolein in drinking water to female F344 rats (Lijinsky and Reuber, 1987) resulted in an elevation of adrenocortical tumors (only in females) over 104-124 weeks (total dose=115 mmoles), the increase was only significant when the tumors were combined with hyperplastic nodules. This incidence of adrenal lesions appeared to exceed the historical control range for female F344 rats reported by Goodman et al. (1979) and Solleveld et al. (1984). However, because of the difference in findings between the Parent et al. (1992c) and Lijinsky and Reuber (1987) studies, an independent pathology working group (PWG) was convened to reevaluate the cortical tumors reported by Lijinsky and Reuber (1987). According to the PWG (cited in Parent et al., 1992c), the "slightly elevated incidence of pheochromocytomas (3/20; 15%) in the treated females were well within limits for historical controls (3/34; 9%) and were of no biological significance," and "it is the opinion of the PWG that there is no evidence of any carcinogenic effect of acrolein on the adrenal glands of female rats in this study." The PWG noted that the slides evaluated were taken from archived tissue blocks because the original slides for the high-dose females were not available for re-examination and only one of the original control slides was available. Parent et al. (1992c) identify additional weaknesses in the Lijinsky and Reuber (1987) studies that brings into question the dose levels and the overall conclusions. They reexamined the Lijinsky and Reuber (1987) reported intake levels, and calculated an estimated daily dose of 50 mg/kg BW for the high-dose group under the assumption that each of the four rats/cage in the group drank an equal share of the 80 ml delivered in the drinking water container. This dose, however, exceeds the LD_{50} for rats, and would have been ingested for five days a week for 132 weeks. Parent et al. (1992c) suggest that the acrolein in the drinking water solution might not have been as stable as Lijinsky and Reuber (1987) assumed, or that intake levels were lower than reported. An additional question was raised as to why Lijinsky and Reuber (1987) observed no increases in adrenal tumors from comparable studies with the acrolein parent compounds – diethylacetal, acrolein oxime, and allyl alcohol – compounds that are expected to be hydrolyzed to acrolein in the stomach acids.

Evidence that acrolein may have some tumor-initiating activity was shown in the study by Cohen et al. (1992). Intraperitoneal injection of acrolein, 2 mg/kg BW for either 6 or 21 weeks into male Fischer 344 rats did not induce cancer, but 6 weeks treatment with acrolein, followed by 20 weeks of uracil in the diet induced urinary bladder papillomas in 18 of 30 rats

compared with 8 of 30 administered uracil alone. A caveat in this study is that the incidence of nodular
hyperplasias (considered to be precursors to papillomas) was considerably lower in the acrolein pretreated group (10 of 30) compared with the solvent control/uracil only group (21 of 30). When the incidence of nodular hyperplasias and papillomas were combined, there were no significant differences between the two groups. Acrolein was too toxic to evaluate its tumor promoting potential, and the impact of its cytotoxicity on conclusions about its tumor initiating potential can not be determined from this study alone.

Based upon the (1) negative findings in 2/3 oral exposure studies that evaluated the carcinogenic potential of acrolein, (2) questionable findings in one study with positive results (Lijinsky and Reuber, 1987), and (3) uncertainty about the significance of the i.p. study for initiating potential (Cohen et al., 1992), the oral exposure data is considered inadequate to determine acrolein's carcinogenic potential.

Acrolein did not produce a carcinogenic response in two inhalation studies, one in hamsters (Feron and Kryusse, 1977) and one in rats (Le Bouffant et al., 1980). The use of only one exposure concentration and less than lifetime exposure duration limits inferences that can be drawn from these studies about the carcinogenic potential of acrolein from an inhalation exposure.

Genotoxicity Studies:

In vitro, acrolein has been shown to induce DNA adducts in a variety of cell types as well as mutagenesis in *Drosophila* and microorganisms under certain conditions, but there is only limited information regarding the ability of acrolein to induce mutations in normal mammalian cells. In mammalian cell *in vitro* assays, acrolein has been shown to induce sister chromatid exchange, DNA cross-linking, and binding to DNA polymerase. Even in the *in vitro* assays, acrolein is so reactive that special techniques must generally be employed to reduce cytotoxicity and induce positive effects. While mutagenic activity has occasionally been shown, positive results generally occurred only in a narrow, near lethal, dose range.

There have been conflicting results reported in the literature for *in vitro* mutagenicity. In a series of Ames assays, Parent et al. (1996b) proposed an explanation for the conflicting data by considering the presence or absence of non-DNA nucleophiles from the S9 activation mixture, in the test chemical solution, or in the plating solutions. They suggested that, in the presence of non-DNA nucleophiles, acrolein will rapidly and indiscriminately react with any available species and not reach the DNA target.

Mode of Action:

Acrolein and its GSH adduct directly induce oxygen radical formation *in vitro* (Adams and Klaidman, 1993) that could induce DNA damage. Extensive lung damage due to lipid peroxidation after inhalation exposure of rats to 1 or 2 ppm (2.3 or 4.6 mg/m^3) acrolein was demonstrated by Arumugam et al. (1999a); also antioxidant levels were significantly decreased. The highly reactive nature of acrolein, however, and the studies supporting the lack of systemic distribution of acrolein suggest that acrolein is not likely to reach potential target sites at a

sufficient concentration to initiate a carcinogenic process in mammalian species. According to Beauchamp et al. (1985), acrolein administered by the inhalation route is retained primarily in the upper respiratory tract because of its reactivity. Some evidence for systemic uptake following oral exposure was noted by Draminski et al. (1983), however, the large doses used (10 mg/kg) would be expected to induce cellular damage, which may allow some absorption. Tissues at the site of contact are, therefore, expected to be most highly exposed, and no evidence of tumor induction in the respiratory tract, skin or gastrointestinal tract has been reported. Studies by Parent et al. (1996a, 1998) indicate little systemic distribution to tissues.

4.8. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

4.8.1. Possible Childhood Susceptibility

The results from animal studies indicate that ingested or inhaled acrolein does result in adverse developmental or teratogenic effects. The only indication from case histories, clinical studies or epidemiology studies of an increased susceptibility of children to acrolein toxicity is for children who have respiratory conditions that are marked by inflammation and mucus hypersecretion such as bronchitis, asthma, or cystic fibrosis.

A number of epidemiological and clinical studies support an association between air pollutants and increased prevalence of respiratory symptoms and emergency room visits (Leikauf, 2002). Some air pollutants are asthmagens, i.e., they can induce asthma and evoke asthma symptoms through immunologic mechanisms. Others do not induce asthma, but can augment the symptoms and exacerbate asthma. More research is needed to fully characterize the antigenic or asthma inducing potential of acrolein. Acrolein is one of the 33 Hazardous Air Pollutants (HAPs) of greatest concern for exposure and health effects, and one of the compounds that does have the potential to exacerbate asthma (TRI, 2003; Leikauf, 2002). *In vitro* mechanistic studies also indicate that irritants like acrolein can directly and indirectly (via inflammatory mediators) increase airway mucin transcripts in epithelial cells (Borchers et al., 1999a). Bronchial hyperresponsiveness and increases in inflammatory mediators following acrolein exposure have also been reported in a number of animal studies (Leikauf, 1991; Leikauf et al., 1989a).

Because children have higher rates of asthma compared to adults, and children tend to have more severe asthma symptoms due to their relatively smaller airways, children may have an increased susceptibility to adverse effects from an agent that can exacerbate asthma.

4.8.2. Possible Gender Differences

There are no human data and only limited, equivocal animal data on gender differences in response to acrolein.

No sex-related differences in toxicological responses to acrolein were reported in dogs exposed for up to 53 weeks orally to 2 mg/kg-day acrolein (Parent et al., 1992a). Parent et al. (1992c) found that female Sprague-Dawley rats intubated daily with 2.5 mg/kg acrolein had a

statistically significant ($p < 0.001$) decrease in survival over a two-year period compared to male rats, which showed some evidence of dose-related mortality during the first year of treatment, but not the second. On the other hand, the Parent et al. (1991b) study in mice showed increased mortality in high-dose males only.

Sex-related differences were absent in rats, guinea pigs, monkeys and dogs exposed 40 hours/week for 6 weeks, or continuously for 90 days to inhaled concentrations of acrolein up to 3.7 ppm (8.5 mg/m^3) (Lyon et al., 1970). Feron et al. (1978) reported no sex differences in responses among rats, hamsters and rabbits exposed to inhaled acrolein 30 hours/week for 13 weeks. LC_{50} values were nearly identical for male and female Sprague-Dawley rats following 1- and 4-hour inhalation exposures to acrolein (Ballantyne et al., 1989). Kutzman et al. (1985), however, reported that 32 of 57 male Fischer 344 rats exposed 6 hours/day, 5 days/week for 12.4 weeks to 4.9 ppm (9.2 mg/m^3) inhaled acrolein died compared with none of 8 exposed females.

4.8.3. Other

As noted in Section 4.5, depletion of GSH increases sensitivity to acrolein cytotoxicity and the induction of mutations. Also, male Wistar rats intubated with acrolein for 45 days had decreased GSH levels leading to mitochondrial damage in liver. Individuals with metabolic defects, such as decreased ability to synthesize GSH, would be expected to be more sensitive to the toxicity of acrolein. Differences in cytochrome P450 activity may affect sensitivity in humans, although this possibility has not been tested.

As discussed in section 4.8.1, acrolein is a respiratory irritant that can exacerbate asthma. Individuals who are asthmatics or who suffer from chronic bronchitis or other chronic pulmonary diseases are considered to be at an increased risk of respiratory symptoms from acrolein inhalation exposure.

Inhalation studies in Sprague-Dawley rats selected for either susceptibility (DS) or resistance (DR) to salt-induced hypertension reported a marked difference in the pulmonary pathology observed in DS and DR rats exposed to the highest dose (4.0 ppm; 9.2 mg/m^3) of acrolein. The lungs of the DS rats exhibited severe airway epithelial necrosis with edema and hemorrhage, while surviving high-dose DR rats developed primarily a proliferative change. Pathologic changes in the two lower dose groups were similar, but less severe. Differences in other respiratory measures between the DS and DR groups at the two lower doses were minimal and not dose-dependent. Reasons for the difference in susceptibility of DS and DR rats at the high dose of acrolein are unclear (Kutzman et al., 1984, 1986). These results suggest that people with hypertension may be more sensitive to respiratory effects from high exposures to acrolein.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

5.1.1. Choice of Principal Study and Critical Effect--with Rationale and Justification

There are no chronic human studies suitable for dose-response assessment.

Long-term studies with Sprague-Dawley rats and CD-1 mice (Parent et al., 1991b; 1992c), dogs (Parent et al., 1992a), and Fisher-344 rats and $B6C3F_1$ mice (NTP, 1995) indicate that mortality and stomach lesions are the predominant effects from oral exposure. The two-year gavage study with the Sprague-Dawley rat (Parent et al., 1992c) is considered to be the most suitable for developing an RfD. This study was a lifetime study that used an adequate number of animals (70/sex/group), compared with only 10/sex in the 13-week NTP (1995) study. Parent et al. (1992c) reported a statistically significant increase in mortality for female Sprague-Dawley rats over the two-year span of the study at doses as low as 0.5 mg/kg-day. Based on this reported mortality as the critical effect, the frank effect level (FEL) in rats was determined to be 0.5 mg/kg-day, and the NOAEL to be 0.05 mg/kg-day. The FEL is defined as "a level of exposure or dose which produces irreversible, adverse effects at a statistically- or biologically-significant increase in frequency or severity between those exposed and those not exposed" (IRIS, 2003).

The NTP (1995) 13-week gavage study provides supporting evidence that treatment causes early mortality. The NTP doses were higher than in the Parent et al. (1992c) study, and mortality was accompanied by the occurrence of observable glandular stomach and forestomach lesions. The stomach lesions observed at doses as low as 0.75 mg/kg-day in mice were not observed in the Parent et al. (1992c) study. Reasons for no reported observations of stomach lesions in Sprague-Dawley female rats at the highest dose (2.5 mg/kg) of the Parent et al. (1992c) study compared with forestomach squamous epithelial hyperplasia observed in female F344 rats in the NTP (1995) study at 1.25 mg/kg-day are not readily apparent, but may relate to differences in strain sensitivity or vehicle. The vehicle dose volume was 5 ml/kg in the NTP (1995) study, and 10 ml/kg in the Parent et al. (1992c) study for rats, and there may have been reduced local gastric mucosal irritation and pathology by virtue of dilution. There were also differences in the vehicle solution and, possibly, the stability of the dosing solutions. Parent et al. (1992c) conducted stability studies on acrolein in water, and monitored the stability of their dosing solutions (reporting losses of less than 10% for 3 hours at room temperature). They used a stabilizing agent, 0.25% hydroquinone, in the stock solution, and prepared dosing solutions daily. The NTP (1995) study used a dose vehicle of 0.5% methylcellulose in deionized water, and no information was available on stability or stabilizing agents.

For the mouse results, there was a similar divergence between the absence of reported forestomach lesions in the CD-1 mice at 4.5 mg/kg in the Parent et al. (1991b) study compared with effects observed in female B6C3F1 at 2.5 mg/kg in the NTP (1995) study. Species differences and dose volume again may have accounted for differences in response. Dose

volume in the NTP (1995) study for mice was 10 ml/kg, and was unspecified in the Parent et al. (1991b) study.

The Parent et al. (1992a) dog study (6 animals/sex/group) was deemed unsuitable as a principal study because of uncertain amounts of retained dose following vomiting from administration of acrolein. Dogs were administered acrolein (0.1% aqueous) in gelatin capsules at doses of 0, 0.1, 0.5, and 1.5 mg/kg-day, 7 days/week for 53 weeks to 6 beagle dogs/group. After four weeks the high-dose was increased to 2.0 mg/kg-day. At termination all dogs were subjected to full necropsy and histological examination. Body weights and food consumption were not significantly affected by treatment. The most commonly reported effect was a dose-dependent increase in the frequency of vomiting. The incidence, however, decreased greatly with duration of treatment. Observed treatment-related lesions on gross necropsy included vascular congestion and mucosal reddening of the gastrointestinal tract. The results of this study are difficult to evaluate. Although there were some alterations in blood parameters, they were unsupported by pathology evaluation. Some of the clinical parameters may have been changed as a result of vomiting. Moreover, adaptation appears to occur, as noted by the decreased vomiting with duration of exposure. Lack of changes in food consumption and body weight also suggest that any effects noted were mild.

A rat study by Arumugam et al. (1999b) provides support and a plausible explanation for the mortality increases reported in the Parent et al. (1992c) study. Arumugam et al. (1999b) exposed male Wistar rats, 5 animals/group, daily to acrolein via intubation (2.5 mg/kg BW) for 45 days. Damage to mitochondria, through loss of mitochondrial lamellae of the cristae, was demonstrated along with a decrease in the availability of GSH, a substrate for glutathione peroxidase, and a decrease in activities of citric acid cycle enzymes, resulting in decreased energy production in liver cells. The duration of the study was less than subchronic in duration and included only a single dose level. Also, the incidence of mortality, if any, was not reported in this study. These results indicate that at least some uptake occurs from the oral route, however, the stomach was not examined by light microscopy.

With regard to the relevance of gavage bolus dose to human exposures, the concentration of the administered dose can affect the time course and degree of severity of toxicity at the point of entry. Rats have both a forestomach and a glandular stomach, while humans have only a glandular stomach. The glandular stomach is more resistant than the forestomach to pH changes and irritation. The residence time in the forestomach (of approximately 2 hours) is sufficiently long compared to the reaction time for toxicity with airway tissue observed in inhalation studies (i.e., microseconds) so that the dose to the glandular stomach may be much lower than that to the forestomach (TERA, 1998). The dog is a better model for glandular stomach toxicity than the rat; however, Parent et al. (1992a) administered acrolein (0.1% aqueous) in gelatin capsules to beagle dogs, so the dose concentration to the glandular tissue is not known. In lieu of studies that provide data on glandular stomach toxicity, the Parent et al. (1992c) rat study remains the most suitable choice.

5.1.2. Methods of Analysis

The development of a RfD was based upon a NOAEL for mortality as determined from the Parent et al. (1992c) study, and the application of uncertainty factors. A benchmark dose approach was unsuitable for RfD development because the data in the Parent et al. (1992c) study were presented graphically, with statistical evaluation at one- and two-year time points, but no numerical values. Moreover, the NOAEL derived from the Parent et al. (1992c) study and used as the basis for the RfD is from a statistically significant increase in mortality, a frank effect. A benchmark dose analysis would not be appropriate when the dose-response is for early cumulative mortality.

5.1.3. RfD Derivation—Including Application of Uncertainty Factors (UFs)

The NOAEL for mortality of 0.05 mg/kg-day from the Parent et al. (1992c) study was used as the point of departure for calculating the RfD. A total uncertainty factor of 100 was applied to this point of departure: 10 for interspecies extrapolation (UF_A) and 10 for susceptible human subpopulations (UF_H).

A default UF_A of 10 was applied to account for interspecies differences between laboratory animals and humans. No information was available to support a change from the default.

A default UF_H of 10 was applied for intraspecies uncertainty to account for human variability and sensitive subpopulations, i.e., to account for human variability in the severity or range of response from any given acrolein exposure amongst different individuals.

A UF_D was not applied because the data base for acrolein was considered complete. The available oral data base includes chronic toxicity studies in the rat and mouse, an oral reproductive toxicity study in Sprague-Dawley rats and an oral developmental toxicity study in New Zealand white rabbits. The findings from the oral reproductive and developmental toxicity studies are supported by an inhalation reproductive toxicity study of acrolein in Fisher 344 rats that revealed no reproductive or developmental effects. Acrolein's high reactivity at the point of contact and the evidence for minimal systemic distribution of acrolein obviates the need for additional repeat dose studies.

The RfD is based on a NOAEL from a chronic study, which obviates the need for an uncertainty factor for LOAEL to NOAEL extrapolation or for subchronic to chronic extrapolation.

Application of a total uncertainty factor of 100 to the NOAEL of 0.05 mg/kg-day results in a reference dose (RfD) of 5×10^{-4} mg/kg-day.

5.1.4. Previous Oral Assessment

A RfD for acrolein was not previously available on IRIS.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

5.2.1. Choice of Principal Study and Critical Effect

Studies on the effects of chronic exposure to inhaled acrolein are unavailable. In the previous IRIS assessment, the principal study was the Feron et al. (1978) study, and the Kutzman (1981) and Kutzman et al. (1985) studies were cited as co-principal. The Kutzman studies (along with Costa et al., 1986) are considered supporting because of the reported lack of adverse histopathology in either the nose or lung in rats exposed to 0.4 ppm (0.9 mg/m^3) on the sixth day of postexposure, as well as some evidence of a functional pulmonary deficit (parenchymal restriction) at this concentration

In the current assessment, the Feron et al. (1978) study was considered the most suitable study for the development of a RfC. Based upon the results of slight nasal effects in 1 of 12 rats, a minimal LOAEL of 0.4 ppm (0.9 mg/m^3) is derived. In this study, 6 Wistar rats/sex/concentration, 10 Syrian golden hamsters/sex/concentration, and 2 Dutch rabbits/sex/concentration were exposed 6 hr/day, 5 days/week for 13 weeks to 0, 0.4, 1.4, or 4.9 ppm (0, 0.9, 3.2, or 11 mg/m^3) acrolein in a whole-body exposure chamber. Incidence data were not reported, but histopathological changes in the nasal cavity, lung, larynx, and trachea were graded as slightly, moderately, or severely affected. Hematological parameters were unaffected by acrolein in rats. Body weight gain was significantly inhibited at the high dose in rats, and less so at the intermediate concentration, but food consumption appeared to be decreased in these groups as well. At the intermediate concentration, both male and female rats showed significantly retarded weight gain ($p<0.05$). Three male and 3 female rats died during exposure at the highest dose. No other deaths considered to be treatment-related were reported in any of the species or exposure groups.

Histopathologic changes described as "slightly affected" were found in the nasal cavity of 1 of 12 rats exposed to 0.4 ppm (0.9 mg/m^3). Severity increased at the higher levels of exposure. No nasal lesions were reported in other species at 0.4 ppm (0.9 mg/m^3). The severity of nasal lesions was concentration-related in all 3 species, most clearly so in the rat. In the 4.9 ppm (11 mg/m^3) groups of all 3 species, slightly to markedly increased lesions were reported in the nasal cavity and trachea; moderate to marked effects were seen in the bronchi and lungs of rats and rabbits (but not hamsters). Based upon the concentration-related severity of lesions, the rat is clearly the most sensitive species, with hamsters and rabbits intermediate in sensitivity.

Although the Feron et al. (1978) study was adequately designed, the incidence of nasal lesions for treated groups was not reported. However, grading of histopathology allowed determination of NOAELs, LOAELs and FELs for the 3 species, determination of the critical target site, and a comparison of sensitivity among the 3 species tested. Other limitations of this study include: (1) an exposure duration of 3 months rather than lifetime, (2) histopathological examination of only three sections of the nasal cavity, (4) lack of characterization of the type of nasal lesions by sex, and (5) only 6 rats/sex were exposed.

Nasal and point of contact effects have been observed in clinical studies with short-term exposure. Exposure to acrolein, at levels as low as 0.09 ppm (0.21 mg/m^3) for 5 minutes, may elicit subjective complaints of eye irritation with increasing concentrations leading to more extensive eye, nose and respiratory symptoms (Weber-Tschopp et al., 1977).

Studies by Kutzman and colleagues (Kutzman, 1981; Kutzman et al., 1985; Costa et al., 1986) support the Feron et al. (1978) results, with additional evidence of lung deficits on day six post-exposure in male rats following 62 total days of exposure spread over 12.4 weeks. The nasal region had only minimal evidence of submucosal lymphoid aggregates at 0.4 ppm (0.9 mg/m^3). Although the degree of involvement increased to moderate at higher concentrations, more extensive damage to the nasal epithelium was not observed. The absence of extensive damage may have been partly due to adaptation that might have occurred during the 6 days from the last day of exposure to evaluation.

Additional support for acrolein's respiratory effects and association with increased mortality is provided by Kutzman et al. (1984). Dahl rats (derived from the Sprague-Dawley rat) that were either susceptibility (DS) or resistance (DR) to salt-induced hypertension had increased mortality (100% and 40%, respectively) when exposed in whole body inhalation chambers to the highest dose of 0.4, 1.4, and 4.0 ppm (0.9, 3.2, and 9.2 mg/m^3) acrolein dose levels. Dose-response increases in the severity of epithelial lesions occurred in both species with the DS rats being more sensitive, and demonstrating a different pathological response at the high-dose.

Respiratory distress and irritation were observed by Cassee et al. (1996b) following a 3-day inhalation exposure of male Wistar rats to acrolein via nose-only inhalation at levels lower than 0.4 ppm (0.9 mg/m^3). Cassee et al. (1996b) examined the nasal effects of inhalation exposure of formaldehyde, acetaldehyde, and acrolein on male Wistar rats (5-6/group) exposed 6 hr/day, for 3 consecutive days, in a nose-only exposure chamber to acrolein at concentrations of 0, 0.25, 0.67, or 1.40 ppm (0, 0.6, 1.5, or 3.2 mg/m^3). The Cassee et al. (1996b) study was designed to evaluate the severity of effects from mixtures versus single chemical exposure, and analyzed six levels of the nasal tract for histopathological and biochemical changes immediately after the last exposure. After one 6-hour exposure, no treatment-related histopathological lesions were found in any of the treatment groups. After 3 days, 4/5 animals exposed to 0.25 ppm (0.6 mg/m^3) were observed to have slight effects (characterized as mainly disarrangement) and 1/5 developed a moderate level of effect. In the 0.67 ppm (1.5 mg/m^3) group, 3/6 were classified as slightly affected and 3/6 rats developed a moderate degree of response. The LOAEL in this study is 0.25 ppm (0.6 mg/m^3).

The occurrence of lesions at lower doses in the Cassee et al. (1996b) study than used in the Feron et al. (1978) study may be: (1) a consequence of nose-only exposure where, unlike whole-body exposure, the animals cannot minimize exposure by burying their noses in their fur, so that animals receive a full and uninterrupted dose; or (2) due to a higher resolution evaluation from the use of extended sectioning (6 sections) of the nasal tract compared to only 3 in the Feron et al. study.

Cassee et al. (1996b) does not discuss the persistence or reversibility of the observed

histopathological changes in the low-dose group with exposures greater than 3 days (e.g., adaptive response). An adaptive response in nonprotein sulfhydryl levels after 3 days of exposure was observed and is discussed. It is possible that an adaptative response to the irritant effects of acrolein occurs over time. Conversely, cessation of exposure for 2 days each week in the Feron et al. (1978) study might have provided a period during which partial recovery from nasal effects could occur. Because the Feron et al. (1978) study was much longer in duration, it is possible that some adaptation to the irritant effects of acrolein occurs with increasing duration, or that cessation of exposure for 2 days each week provides a period during which partial recovery from nasal effects might have occurred.

The rationale for the choice of the Feron et al. (1978) study over the Cassee (1996b) study includes: (1) the higher number of test animals [12 (6/sex) vs. 6 male only]; (2) the longer duration [5 days/week for 13 weeks vs. 3 days]; (3) the testing of multiple species and both sexes in the Feron et al. (1978) study; and (4) the better characterization of multiple endpoints and the dose-response. Feron et al. (1978) evaluated many different end points and demonstrated dose-response for all 3 dose groups in all 3 species tested. The Feron et al. (1978) study also evaluated a dose-response over a 12-fold increase from low- to high-dose. The Cassee (1996b) study used about a 6-fold increase in dose level from low- to high-dose. Collectively, the principal study and supporting studies (Kutzman, 1981; Kutzman et al., 1985; Costa et al., 1986; Feron et al., 1978; Cassee, 1996b) provide support for a minimal LOAEL of 0.4 ppm (0.9 mg/m^3) (i.e., a LOAEL close to the expected NOAEL).

5.2.2. Methods of Analysis

The nasal cavity is considered the most sensitive target site for the pathological effects of acrolein, in part because it is the first point of contact in inhalation exposures. A benchmark dose approach was not possible because nasal pathology incidence data were not provided. Therefore, the approach used to derive the RfC was the determination of a LOAEL with application of uncertainty factors.

5.2.3. RfC Derivation

The endpoint used to derive the RfC was based upon the results in the Feron et al. (1978) study, which identified a minimal LOAEL of 0.4 ppm (0.92 mg/m^3) based on evidence of nasal histopathology in the Wistar rat (1/12). The LOAEL was adjusted from the dosing regimen of 0.9 mg/m^3 for 6 hr/day, 5 days/week for 13 weeks to a continuous exposure as follows:

$$\begin{aligned} LOAEL_{ADJ} &= 0.9\ mg/m^3 \times 6/24 \times 5/7 \\ &= 0.16\ mg/m^3 \end{aligned}$$

A Regional Gas Dose Ratio (RGDR) for a Category 1 gas with extrathoracic respiratory effects was then derived using a calculated ventilation rate of 0.20 m^3/day for an average Wistar rat (average of male and female ventilation rates), and a default value of 20 m^3/day for humans

along with default extrathoracic region surface area values of 15.0 cm^2 for the rat, and 200 cm^2 for humans (U.S. EPA, 1994b). The resulting equation is as follows:

$$RGDR = \frac{\text{Ventilation rate (rat) / surface area (rat)}}{\text{Ventilation rate (human) / surface area (human)}}$$

$$= \frac{0.20/15.0}{20/200}$$

$$= 0.14$$

Applying the RDGR of 0.14 to the adjusted LOAEL of 0.16 mg/m^3 yields a LOAEL dosimetically adjusted to a human equivalent concentration (HEC) of 0.02 mg/m^3.

The $LOAEL_{HEC}$ was used as the point of departure for calculating the RfC. A total uncertainty factor of 1,000 was applied to this point of departure: 3 ($10^{1/2}$) for extrapolation from animal to humans (UF_A), 10 for intrahuman variability (UF_H), 10 for subchronic to chronic duration (UF_S), and 3 ($10^{1/2}$) for use of a minimal LOAEL (UF_L).

A UF_A of 3 ($10^{1/2}$) was used for interspecies extrapolation, since this factor embodies two areas of uncertainty: pharmacokinetics and pharmacodynamics. In this assessment, the pharmacokinetic component was addressed by the calculation of the human equivalent concentration (HEC) according to the procedures in the RfC methodology (U.S. EPA, 1994b). Accordingly, only the pharmacodynamic area of uncertainty remains as a partial factor for interspecies uncertainty ($10^{0.5}$ or approximately 3).

A default UF_H of 10 was applied to for intraspecies uncertainty to account for human variability and sensitive subpopulations, i.e., to account for human variability in the severity or range of response from any given acrolein exposure amongst different individuals.

A UF_S of 10 was applied for adjustment from subchronic to chronic duration because the principal study involved a 13-week dosing period and because there are insufficient inhalation data to preclude an increase in severity (or incidence) with an increase in exposure duration from subchronic to chronic.

A UF_L of 3 ($10^{1/2}$) was applied for use of a minimal LOAEL of 0.4 ppm (0.9 mg/m^3) in lieu of a NOAEL. Although the severity of the nasal effect at the 0.4 ppm (0.9 mg/m^3) level was minimal and in only 1 of 12 animals in the Feron et al. (1978) study, a 3-day study in the male Wistar rat by Cassee et al. (1996b) also reported slight nasal effects in the respiratory/transitional epithelium from a nose-only inhalation exposure at 0.25 ppm (0.6 mg/m^3). With the Cassee et al. (1996b) results and the observed increase in the severity of the effects in the Feron et al. (1978) study as dose increases, the 0.4 ppm (0.9 mg/m^3) was designated a minimal LOAEL instead of a NOAEL.

A UF_D was not applied because the data base for acrolein was considered complete. The available inhalation data base includes subchronic toxicity studies in multiple species, and an inhalation reproductive toxicity study of acrolein in Fisher 344 rats that revealed no reproductive or developmental effects. Acrolein's high reactivity at the point of contact and the evidence for minimal systemic distribution of acrolein obviates the need for additional studies of repeat-dose toxicity or reproductive/developmental toxicity.

Application of a total uncertainty factor of 1,000 (3 x 10 x 10 x 3) to the $LOAEL_{HEC}$ of 0.02 mg/m^3 yields a RfC of 2×10^{-5} mg/m^3.

5.2.4. Previous Inhalation Assessment

The RfC of 2×10^{-5} mg/m^3 derived in this assessment is the same as the value entered on IRIS in 1991. The previous RfC was based on squamous metaplasia and neutrophilic infiltration of nasal epithelium as reported in the subchronic rat inhalation studies of Kutzman (1981) and Feron et al. (1978), and application of a total UF of 1,000.

5.3. CANCER ASSESSMENT

A dose-response assessment for carcinogenicity is precluded because there is inadequate evidence to establish a link between exposure to acrolein and cancer.

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

Acrolein is a colorless to yellowish flammable liquid at room temperature with a disagreeable, choking odor. It is extremely acrid and irritating to mucous membranes. Acrolein and its derivatives are used as an intermediate in the synthesis of acrylic acid for making acrylates, and of DL-methionine, an essential amino acid. It is used as a herbicide and to control algae aquatic weeds and molluscs in recirculating process systems, growth of microorganisms in liquid fuel, growth of algae in oil fields, and the formation of slime in paper manufacture. It is also used to promote protein cross-linking in leather tanning, and as a tissue fixative for histological preparations.

Acrolein is released to the air as a result of manufacturing processes, through incomplete combustion of petroleum fuels, as a component of cigarette smoke, and as a photooxidation product of hydrocarbon pollutants (ATSDR, 1990). Combustion of fuels represents the major source of emissions of acrolein to the atmosphere.

Inhaled acrolein is retained primarily in the upper respiratory tract (Egle, 1972) because of its high solubility and reactivity. No direct evaluations of uptake via oral administration have

been reported. Draminski et al. (1983) identified a low level of acrolein derived conjugates in the urine of rats following oral dosing, and Arumugam et al. (1999b) reported toxicological effects in the liver of rats exposed by daily intubation (acrolein in water) for 45 days. These results indicate that at least some uptake occurs from the oral route; however, the stomach was not examined by light microscopy.

The main pathway of metabolism for acrolein is the addition of GSH to the activated double bond followed by processing to mercapturic acid. A second pathway is that of epoxidation of the double bond followed by attack on the epoxide by glutathione. A third pathway is addition of water to acrolein to form 3-hydroxypropionaldehyde, which can be further metabolized and ultimately incorporated into normal metabolic pathways (Parent et al., 1998).

Data are not available to evaluate the toxicological effects in humans from chronic exposure to acrolein. Acute duration studies (Weber-Tschopp et al., 1977; Esterbauer et al., 1991) have documented that acrolein can cause pronounced eye and nasal irritation. Inhalation studies in laboratory animals indicate that the principal target sites for acrolein toxicity are the nasal membranes (Feron et al., 1978) and the lung (Lyon et al., 1970; Kutzman, 1981; Kutzman et al., 1985), i.e., the initial sites of contact. When acrolein was administered to laboratory animals by gavage, the principal sites affected were the stomach (Parent et al., 1992a; NTP, 1995) and liver (Arumugam et al., 1999b).

At present the carcinogenicity of acrolein cannot be determined by the inhalation route because of a lack of human data and lack of adequate chronic bioassays in laboratory animals. For oral exposures, two chronic oral bioassays, one with rats (Parent et al., 1992c) and one with mice (Parent et al., 1991b), reported negative results. Marginally positive effects were reported in one other chronic oral study in rats (Lijinsky and Reuber, 1987), but these results were questioned following reevaluation of the tissues at a later date by a pathology work group. Questions were also raised about the validity of the assumptions that supported the reported dose and uptake levels in the Lijinsky and Reuber (1987) study. A weak tumor initiating effect was reported in an intraperitoneal injection study (Cohen et al., 1992), but results were negative in a skin tumor initiation study (Salaman and Roe, 1956).

Because of acrolein's reactivity, toxicity can be induced by more than one mode of action. A major mode of action, however, has been shown to be related to depletion of GSH. Reaction of acrolein with GSH deprives the cell of its natural defense against reactive oxygen species (Arumugam et al., 1999a,b). Moreover, the acrolein GSH adduct has been shown *in vitro* to directly induce oxygen radical formation (Adams and Klaidman, 1993).

Based upon EPA's draft revised guidelines for carcinogen risk assessment (U.S. EPA, 1999), the "data are inadequate for an assessment of human carcinogenic potential by either the inhalation or oral routes of exposure."

6.2. DOSE RESPONSE

Quantitative estimates of the noncancer risk from either oral or inhalation routes of exposure were developed from animal data since no adequate human data are available. A RfD of 5×10^{-4} mg/kg-day was derived from a study in Sprague-Dawley rats (Parent et al., 1992c) based upon a NOAEL (with increased mortality as the critical effect) of 0.05 mg/kg-day, and adjusted by a 10-fold interspecies uncertainty factor and a 10-fold uncertainty factor for intraspecies (human) variability in sensitivity for response to acrolein.

Confidence in the principal study is medium. Several supporting studies involving other species also indicated that mortality increases sharply with an elevated dose. The research demonstrating acrolein's high reactivity, low systemic distribution, toxicity at the point of entry, pronounced decreases in serum creatinine phosphokinase (creatine kinase), citric acid cycle enzymes, and liver GSH; and increased mitochondrial damage in the Wistar rat are suggestive of interference with normal metabolic processes or possibly absorption of essential nutrients sufficient to lead to early mortality, although further research is needed to support a definitive mode of action. In the NTP (1995) study there were glandular stomach and forestomach lesions at higher doses that likely played a role in the observed mortality. Confidence in the data base is judged high with chronic exposure studies in two species. Moreover, two studies (Parent et al., 1992b; Parent et al., 1993) provide evidence that reproductive and developmental effects are not critical endpoints although only one species was tested for reproductive effects (rat) and for developmental effects (rabbit). While the possibility of some transport of acrolein or a metabolite of acrolein to systemic sites remains, the critical target sites are at the point of contact, e.g., the respiratory system, the gastrointestinal tract, mucous membranes, and skin. The high reactivity of acrolein and the lack of significant systemic distribution obviates the need to examine reproductive/developmental effects in a second species. The overall confidence in this RfD assessment is medium-to-high; a variety of studies across different durations of exposure and in several different laboratory animal species has been consistent in demonstrating that in the absence of mortality there are no clear indications of adverse effects.

A RfC of 2×10^{-5} mg/m^3 was derived from the results of a 13-week inhalation study with rats, hamsters and rabbits (Feron et al., 1978). The critical endpoint was lesions in the upper respiratory tract and lung. A minimal LOAEL of 0.4 ppm (0.9 mg/m^3) was based on lesions of slight severity in the nasal epithelium of rats following 13 weeks exposure at this level. Severity of the lesions increased with exposure concentration. This 0.4 ppm (0.9 mg/m^3) concentration was a NOAEL for hamsters and rabbits in the same study, although at the 4.9 ppm (11 mg/m^3) level severity of nasal lesions was similar across all 3 species.

The RfC was derived by duration adjusting the LOAEL of 0.9 mg/m^3 from 30 hour/week exposure to continuous exposure of 0.16 mg/m^3. Applying an RGDR for a Category 1 gas of 0.14 (U.S. EPA, 1994b) to convert dose/unit surface area of the extrathoracic region in the rat to that in humans, resulted in an equivalent human concentration (HEC) for continuous exposure of 0.02 mg/m^3. A total UF of 1,000 was applied (3 for interspecies extrapolation of a dosimetrically adjusted dose, 10 for intrahuman variability, 10 for subchronic to chronic extrapolation, and 3 for use of a minimal LOAEL). Support for the use of a minimal LOAEL is

provided by NOAELs for 2 of the 3 species tested in the same study at the same dose level. The resulting adjustment of the minimal $LOAEL_{HEC}$ concentration of 2×10^{-2} mg/m^3 by a total uncertainty factor of 1,000 yields a RfC of 2×10^{-5} mg/m^3.

The confidence in the principal study is judged medium. Although the principal study (3 species) was adequately designed and examined a wide range of endpoints, it had several shortcomings: (1) only 3 sections of the nasal cavity were examined, (2) there was low sample size, and (3) a lack of incidence data. Support for the minimal LOAEL is provided by subchronic studies in 2 other species (rabbit and hamster) and a 3-day study (Cassee et al., 1996b) in the rat in which nasal lesions of similar type and severity were observed. The primary limitation in the data base is the lack of a chronic inhalation study and the attendant uncertainty relating to incidence/severity of nasal lesions at subchronic/chronic exposure levels lower than 0.4 ppm (0.9 mg/m^3). The high reactivity of acrolein at the point of contact, the lack of significant systemic distribution demonstrated in studies with the dog and rat, and the lack of effects in oral studies lessens the priority for an evaluation of reproductive/developmental endpoints in a two-generation inhalation study. Additional evaluation of immunological endpoints is warranted especially focusing on potential contribution to asthma or compromise in respiratory response. Thus, confidence in the data base is judged low to medium. Overall, confidence in the RfC is judged medium.

As stated previously, the data are inadequate for an assessment of the human carcinogenic potential from exposure to acrolein that would precede any evaluation of a cancer dose-response.

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Number 43. Published under the joint sponsorship of the United Nations Environment Programme, the International Labour Organization, and the World Health Organization. World Health Organization, Geneva. Available online at: http://www.inchem.org/documents/cicads/cicads/cicad43.htm

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APPENDIX A: SUMMARY OF EXTERNAL PEER REVIEW COMMENTS AND DISPOSITION

The support document and IRIS summary for acrolein have undergone both internal peer review performed by scientists within EPA and a more formal external peer review performed by scientists in accordance with EPA guidance on peer review (U.S. EPA, 1998b, 2000a). Comments made by the internal reviewers were addressed prior to submitting the documents for external peer review and are not part of this appendix. The three external peer reviewers were tasked with providing written answers to general questions on the overall assessment and on chemical-specific questions in areas of scientific controversy or uncertainty. The reviewers made a number of editorial suggestions to clarify specific portions of the text. These changes were incorporated in the document as appropriate and are not discussed further. A summary of significant comments made by the external reviewers and EPA's response to these comments follows:

(1) General Questions for Peer Reviewers

A. Are you aware of any other data/studies that are relevant (i.e., useful for the hazard identification or dose-response assessment) for the assessment of the adverse health effects, both cancer and noncancer, of this chemical?

Comments: One reviewer identified a number of pharmacokinetic and mechanistic studies that should be reviewed and cited. The other reviewers were not aware of additional studies.

Response: The studies identified were reviewed and discussed in the Toxicological Review.

B. For the RfD and RfC, has the most appropriate critical effect been chosen (i.e., that adverse effect appearing first in a dose-response continuum)? Points relevant to this determination include whether or not the choice follows from the dose-response assessment, whether the effect is considered adverse, and if the effect and the species in which it is observed in a valid animal model for humans.

Comments: One reviewer requested any additional information on the cause of the early mortality in the Parent et al. (1992c) study be discussed. A second reviewer agreed with the selection of the Parent et al. (1992c) study and the critical effect. The third reviewer did not answer the question regarding the RfD. For the RfC, one reviewer agreed with the choice of the Feron et al. (1978) study and nasal effects as appropriate choices given the totality of the data base, but recommended that the low dose be considered a NOAEL and not a minimal LOAEL. Another reviewer also agreed with the choice of critical study and effect, but was concerned with the low dose as a minimal LOAEL. A third reviewer also agreed with the choices and indicated a clear dose-response in the principal study.

Response: The mortality data of Parent et al. (1992c) was re-examined and some minor additional detail was provided, although the cause of the early mortality was still not clear. Given the weight-of-evidence that inhalation exposure causes nasal effects and the clear indication of nasal effects given in the shorter-term nose-only study by Feron and colleagues (Cassee et al., 1996b) at an even lower concentration, the low dose in the Feron et al. (1978) study was categorized as a minimal LOAEL.

C. Have the noncancer assessments been based on the most appropriate studies? These studies should present the critical effect/cancer (tumors or appropriate precursor) in the clearest dose-response relationship. If not, what other study (or studies) should be chosen and why?

Comments: One reviewer thought the NTP (1995) gavage study should be used as the principal study or at least co-critical with the Parent et al. (1992c) gavage study given the "serious difficulties" (i.e., early mortality) with the latter study. A second reviewer thought the use of the Parent et al. (1992c) study was appropriate for the oral RfD considering the decrease in survival. The third reviewer provided no comments on the RfD portion of this question. With respect to the RfC portion, two reviewers felt that use of the Feron et al. (1978) study was appropriate. The third reviewer also felt the principal study was appropriate, but suggested that the results of Kutzman et al. (1985) and Costa et al. (1986) provide a more technically defensible basis for the LOAEL.

Response: Kutzman et al. (1985) and Costa et al. (1986) were re-considered and judged to provide support for the findings of Feron et al. (1978). The text was revised accordingly. EPA recognized the NTP (1995) gavage study as an important study; however, EPA continued to call it a "supportive" study rather than a co-principal study because it does not directly alter the quantitative determination of the reference dose. The text, however, was revised to further emphasize the importance of the NTP (1995) study results showing that acrolein causes early mortality and increased incidence of glandular stomach and forestomach lesions in the F344 strain.

D. Studies included in the RfC under the heading "Supporting/Additional studies" are meant to lend scientific justification for the designation of critical effect by including any relevant pathogenesis in humans, any applicable mechanistic information, any evidence corroborative of the critical effect, or to establish the comprehensiveness of the data base with respect to various endpoints (such as reproductive/developmental toxicity studies). Should other studies be included under the "Supporting/Additional" category? Should some studies be removed?

Comments: One reviewer suggested that Kutzman et al. (1984) be evaluated as a supporting study since lung pathology was evident at 0.4 ppm (0.9 mg/m^3). A second reviewer focused on the IRIS summary and suggested all studies, except for Feron et al. (1978) be moved from section I.B.2 and that the Cassee et al. (1996b) study be discussed more fully. The third reviewer suggested that the results of the 13-week gavage study conducted by Bioassay Systems

Corporation be included in the IRIS summary; the description of additional studies in the RfC was comprehensive.

Response: The Kutzman et al. (1984) study was re-examined and included in the IRIS summary in the Additional Studies section. A description of the Kutzman (1991), Kutzman et al. (1985) and Costa et al. (1986) studies was kept in section I.B.2. of the IRIS summary because the results were supportive of those of Feron et al. (1978) as were the results of Cassee et al. (1996b), the discussion of which was revised to relate better to the findings of Feron et al. (1978).

E. For the noncancer assessments, are there other data that should be considered in developing the uncertainty factors or the modifying factor? Do you consider that the data support the use of different (default) values than those proposed?

Comments: One reviewer recommended that the wording of the uncertainty factor text be revised to make it consistent with previous discussions on IRIS and to consider an uncertainty factor of 3 to reflect an incomplete data base (i.e., a lack of adequate reproductive and a second species developmental oral toxicity study). A second reviewer had no substantive comments in this area. The third reviewer suggested that if mortality, without a mechanistic explanation as to why it occurred, is retained as the critical effect, then a modifying factor of 10 should be included; this reviewer considered the uncertainty factors for the RfC appropriate.

Response: The uncertainty factor section was revised for consistency with past discussions. Although the criteria established for a complete data base call for a second species oral study for developmental effects, the nature of the data base suggests that a second study is not needed. The suggestion to include a modifying factor of 10 because of mortality was also judged not necessary. The observation in the Wistar rat that oral dosing results in substantial decreases in the activities of citric acid cycle enzymes, perturbs mitochondrial membrane integrity and decreases GSH, provides a plausible basis for why longer-term dosing could result in mortality.

F. Do the confidence statements and weight-of-evidence statements present a clear rationale and accurately reflect the utility of the studies chosen, the relevancy of the effects to humans, and the comprehensiveness of the data base? Do these statements make sufficiently apparent all the underlying assumptions and limitations of these assessments? If not, what needs to be added?

Comments: For the RfD, one reviewer rated the data base as medium-to-high, and the overall confidence in the RfD as high based upon the breadth of endpoints examined in the principal study. The reviewer suggested adding text to the confidence statement that the principal and supporting studies include chronic exposure in two species, but noting the absence of a second species developmental toxicity. A third reviewer rated the study confidence as low based upon deficiencies in the Parent et al. (1992c) study including lack of reporting of mortality incidence data and clinical signs data, plus no reasonable explanation for the treatment related mortality; the data base as low-to-medium; and the RfD as low-to-medium. For the RfC, two

reviewers agreed with the confidence ratings. One reviewer rated the study as low-to-medium based upon low sample size, inadequately quantified histopathological data, and acute data that does not support the study results. This reviewer rated the data base as low because of the absence of chronic data or developmental or reproductive studies, and the overall confidence in the RfC as low-to-medium.

Response: The Agency agrees that the confidence in the principal study would be higher if there was a clearer basis for the early mortality, although the supporting studies add confidence to the validity of this endpoint. Confidence in the principal study was thus rated as medium. The confidence in the data base was considered high due to the variety of studies at different durations and in different species. Although only one species was tested for reproductive and developmental effects, the critical target sites (discussed in Section 4) are at the point of contact (e.g., the respiratory system, the gastrointestinal tract, mucous membranes, and skin) and there is little evidence of systemic distribution. The high reactivity of acrolein and the lack of significant systemic distribution obviates the need to examine reproductive/developmental effects in a second species. The overall confidence in the RfD was thus considered medium-to-high. The text was revised to include the above rationale. For the RfC, the Agency agrees that the confidence in the principal study would be higher had the histopathology been more highly resolved and the sample sizes increased. The study was, however, in 3 species and examined a wide range of endpoints, and thus was rated medium. The data base was rated low-to-medium because of the absence of a chronic study. The absence of inhalation studies for reproductive or developmental effects was considered of less import because of the results from oral studies, and the rationale that there is little acrolein systemic distribution because of its high reactivity with tissues at the portal of entry. The confidence in the overall RfC was therefore medium.

(2) Chemical-specific Comments

A. Given the consistent nature of the irritative effects of acrolein across species, it is reasonable to focus upon 0.09 ppm (eye irritation) in the Weber-Tschopp et al. (1977) study as basis for the threshold level of concern for acute effects described in section 4.6.2 in the Toxicological Review? If not, why not?

Comments: One reviewer had no objection to using the wording "a threshold level of concern," but recommended reconciling some discrepancies in the discussions in sections 4.1 and 4.6.2. A second reviewer mistakenly concluded that discussion of this study was related to development of the RfC, and felt the focus on a 'threshold level of concern' was inappropriate. The third reviewer objected to using this study because (1) the results were published in German, (2) the English translation was difficult to understand, (3) number of subjects was low, (4) not clear how close the actual concentrations were to the target concentrations, and (5) the study was 25 years old.

Response: Given the comments from the external peer reviewers, the discussion was revised to remove the quantitative calculations and the wording 'threshold level of concern.' The study was, however, retained and placed in context of identifying an approximate level of

exposure above which subjective complaints would be expected during acute exposures. There is no reason to discount a study published in German with a relatively large number of subjects (53, 42, and 46 individuals in the three experiments, respectively) who elicited subjective complaints. The English translation provided by the Chemical Manager was considered sufficient to judge the merits of the study. The measured levels of acrolein in this study were within ± 10% of target concentrations.

B. Histopathological evidence of nasal lesions in Wistar rats (Feron et al., 1978) exposed to 0.4 ppm (0.9 mg/m^3) for 13 weeks was chosen as the critical effect for RfC derivation although the Cassee et al. (1996b) study in the Wistar rat indicated nasal lesions, albeit slight, at 0.25 ppm (0.6 mg/m^3) after only 3 days. How can this apparent inconsistency be explained? Each study indicated that effects increased in severity with increasing concentration.

Comments: One reviewer did not see any inconsistency because the duration-adjusted LOAEL from the Feron et al. (1978) study was 0.07 ppm (0.16 mg/m^3) compared to 0.06 ppm (0.14 mg/m^3) for the Cassee et al. (1996b) study. It was also indicated that some adaptation could have taken place during the two days per week in the Feron et al. (1978) study that exposure did not take place or that severity increases with increasing exposure duration. A second reviewer suggested that the apparent inconsistency may relate to nose-only (Cassee et al., 1996b) versus whole-body (Feron et al., 1978) exposure in relation to stress. Also, given the daytime exposures and the normal sleeping position of the animals, the animals may have inhaled less than the measured concentrations if they kept their noses buried in their fur. The third reviewer felt that the Cassee et al. results were confounding and suggested they be reconciled.

Response: The results of the Feron et al.(1978) and Cassee et al. (1996b) studies were considered not to be confounding. The points raised by the second reviewer were considered sufficient to explain why animals in the Cassee et al. (1996b) study had nasal effects at effects lower than the minimal ones noted in the Feron et al. (1978) study. The comparison of the duration-adjusted LOAELs as a basis was considered less than satisfactory in that the calculation for the Cassee et al. (1996b) study did not include a 3/7 (exposure days/weekdays) factor in the derivation; thus the duration-adjusted LOAEL including this factor would have resulted in 0.03 ppm (0.07 mg/m^3), approximately half that of the similar value (0.07 ppm or 0.16 mg/m^3) calculated for Feron et al. (1978). The discussions were therefore revised in both the Toxicological Review and IRIS summary to reflect the comments raised by the reviewers.

C. Evidence of restrictive lung function at 0.4 ppm (0.9 mg/m^3) was found in male F344 rats by Costa et al. (1986) who measured function 6 days <u>post-exposure</u>. Should this study thus be elevated to a co-principal study along with the Feron et al. (1978) study? Or should it stand as the principal study, with a derivation of a new RfC based on the lung as a critical effect? Or would it be more useful to derive RfCs based on both nasal lesions and lung function and present both values? [Note: a LOAEL(HEC) for the thoracic region = 0.3 mg/m^3].

Comments: One reviewer felt that the Costa et al. (1986) publication should serve as the primary study for deriving a RfC. The pulmonary function parameters at 0.4 ppm (0.9 mg/m^3) were statistically significant relative to controls and the increases in internal lung surface area and the correlated increase in diffusing capacity for carbon monoxide were found to increase in a dose-dependent manner. Use of this study "provides quantifiable effects that appear technically more defensible than the 'slightly affected' nasal tissue of a single rat in the 0.4 ppm (0.9 mg/m^3) Feron et al. (1978) study." A second reviewer would not use the Costa et al. (1986) study as a co-principal study because: (1) lung function measurements can be highly variable, (2) it is not known if the increase in MEFV was transient and reversible, (3) there was no confirmatory histopathological evidence, (4) tidal volume, breathing frequency, and pulmonary resistance did not differ between the 0.4 ppm (0.9 mg/m^3) group and controls, and (5) Costa et al. (1986) themselves noted that the interpretation of the pulmonary function tests are limited in the absence of other supporting pathologic or functional data. The third reviewer felt that there are too many unresolved issues to utilize the Costa et al. (1986) as a principal or co-principal study.

Response: The Agency agrees that the Costa et al. (1986) study of pulmonary function should not serve as a principal or co-principal study. However, the results of the study do provide more substantial support for 0.4 ppm (0.9 mg/m^3) as a minimal LOAEL based on the Feron et al. (1978) study alone. Costa et al. (1986) did show that 0.4 ppm (0.9 mg/m^3) resulted in a significant ($p<0.001$) increase in internal lung surface and diffusing capacity for carbon monoxide compared to controls using state-of-the-art measurement techniques with acceptable standard deviations for the parameters measured. The number of animals used (24) in each exposure group provided an acceptable number for comparison purposes with controls. As a result, this study was considered a supporting study. Although the animals in this study were those evaluated for other purposes in Kutzman (1981) and Kutzman et al. (1985) the findings (i.e., no effects at 0.4 ppm or 0.9 mg/m^3) are described under additional studies in the IRIS summary.

of exposure (MOE) for the overall U.S. population (all seasons) and the following subpopulations: all infants (<1 year), non-nursing infants (<1 year), children (1–6 years), children (7–12 years), females (13–19 years), females (13–50 years), males (13–19 years), males (>20 years), and seniors (>55 years). In this refined Tier 2 analysis, all evaluated population subgroups had an exposure equal to 0% of the aRfD with a corresponding MOE of >1 million at the 95th percentile.

Foliar application use (pome fruit). Tomen has conducted an acute dietary exposure Tier 1 analysis with Dietary Exposure Evaluation Model (DEEM) using proposed tolerance of 1 ppm, 100% crop treated and no adjustment of processing factor for the overall U.S. populations and the following subpopulations: all infants, nursing infants (<1 year), non-nursing infants (<1 year), children (1–6 years), children (7–12 years), and females (13–50 years). The results of Tier 1 analysis from foliar use of pome fruit indicated that the highest exposure never exceeds 5.42% of the aRfD at the 95th percentile.

The chronic reference dose (cRfD) of 0.097 mg/kg bwt/day (chronic NOAEL with a 100–fold uncertainty factor) was used to assess chronic dietary exposure.

Seed treatment use. Bayer's chronic dietary analysis estimated the percent of the cRfD and corresponding MOE for the overall U.S. population (all seasons) and the following subpopulations: all infants (<1 year), non-nursing infants (<1 year), children (1–6 years), children (7–12 years), females (13–19 years), females (13–50 years), males (13–19 years), males (>20 years), and seniors (>55 years). In this analysis, all evaluated population subgroups had an exposure equal to 0% of the cRfD. The corresponding MOE was >1 million.

Foliar application use. Tomen has conducted a chronic Tier 1 analysis and the results indicated that the highest exposure never exceeds 8.7% of the cRfD at the 95th percentile.

i. *Food. See* above discussion.

ii. *Drinking water.* For drinking water, the models SCI-GROW (ground water), and generic expected environmental concentration (GENEEC) (surface water), were selected to calculate the potential exposure of TM–444 in drinking water. Both short-term (acute) and long-term (chronic) exposures were estimated with respect to foliar uses on apples and pears. The predicted ground water concentrations for foliar application of apples and pears were 1.17 and 1.30 μ/L, respectively. The highest estimated acute and chronic exposures from surface water were 9.10 and 3.07 μ/L, respectively. Based on the standard exposure scenarios for drinking water (70kg adult- 2L/day; 10 kg child- 1L/day), the potential human exposure and risk can be estimated. Using the acute (0.60 mg/kg/day) and chronic (0.097 mg/kg/day) reference doses (RfD), the human risk from exposure to TM-444 in drinking water is estimated. The risk to adults and children from ground water exposure ranged from 0.006 to 0.019% of the acute RfD and from 0.038 to 0.134% of the chronic RfD; from surface water, the estimated risk ranged from 0.039% to 0.152% of the acute RfD and 0.081 to 0.316% of the chronic RfD respectively.

2. *Non-dietary exposure.* Clothianidin is currently not registered for use on any residential non-food site. Therefore, residential exposure to clothianidin residues will be through dietary exposure only.

D. Cumulative Effects

There is no information available to indicate that toxic effects produced by clothianidin are cumulative with those of any other compound.

E. Safety Determination

1. *U.S. population.* Using the conservative exposure assumptions described above and based on the completeness of the toxicity data, it can be concluded that total aggregate exposure to clothianidin from all proposed uses will be less than 9% of the RfD for the overall U.S. population. All evaluated population subgroups had an exposure less than 9% of the RfD. EPA generally has no concerns for exposures below 100% of the RfD, because the RfD represents the level at or below which daily aggregate exposure over a lifetime will not pose appreciable risks to human health. Thus, Arvesta believes that it can be concluded that there is a reasonable certainty that no harm will result from aggregate exposure to clothianidin residues.

2. *Infants and children.* In assessing the potential for additional sensitivity of infants and children to residues of clothianidin, the data from developmental toxicity studies in both the rat and rabbit, a two-generation reproduction study in rats and a developmental neurotoxicity study in rats have been considered.

The developmental toxicity studies evaluate potential adverse effects on the developing animal resulting from pesticide exposure of the mother during prenatal development. The reproduction study evaluates effects from exposure to the pesticide on the reproductive capability of mating animals through two generations, as well as any observed systemic toxicity.

The developmental neurotoxicity studies evaluate the neurobehavioral and neurotoxic effects on the developing animal resulting from the exposure of the mother. FFDCA section 408 provides that EPA may apply an additional uncertainty factor for infants and children based on the threshold effects to account for prenatal and postnatal effects and the completeness of the toxicity data base. Based on the current toxicological data requirements the toxicology data base for clothianidin relative to prenatal and postnatal development is complete, including the developmental neurotoxicity study. None of the studies indicated the offsprings to be more sensitive. All effects were secondary to severe maternal toxicity. The RfD for clothianidin was calculated using the NOAEL of 9.7 mg/kg bw/day from the two–year chronic/oncogenicity study. This NOAEL is lower than the NOAEL from the two–generation reproduction study, the developmental studies, and the developmental neurotoxicity study. Moreover, using a toxicologically justified UF of 100, the RfD for a non-oncogenic clothianidin was established at a level 0.097 mg/kg/day, a value that offers a measure of safety that is the highest among the other alternative compounds for control of apple and pear pests.

F. International Tolerances

No CODEX maximum residue levels (MRL's) have been established for residues of clothianidin on any crops at this time.

[FR Doc. 03–32205 Filed 12–30–03; 8:45 am]

BILLING CODE 6560–50–S

ENVIRONMENTAL PROTECTION AGENCY

[OW–FRL–7605–2]

National Recommended Water Quality Criteria for the Protection of Human Health

AGENCY: Environmental Protection Agency.

ACTION: Notice of availability.

SUMMARY: Pursuant to section 304(a) of the Clean Water Act (CWA), the Environmental Protection Agency (EPA) is announcing the availability of updated national recommended water quality criteria for the protection of human health for the following fifteen pollutants: chlorobenzene; cyanide; 1,2-dichlorobenzene; 1,4-dichlorobenzene; 1,1-dichloroethylene; 1,3-

dichloropropene; endrin; ethylbenzene; hexachlorocyclopentadiene; lindane; thallium; toluene; 1,2-transdichloroethylene; 1,2,4-trichlorobenzene; and vinyl chloride.

The criteria are based on EPA's 2000 methodology for deriving human health water quality criteria and supercede criteria for these chemicals that the Agency published before this notice.

EPA's recommended section 304(a) water quality criteria are guidance to States and authorized Tribes in adopting water quality standards for protecting human health. They are also a scientific basis for developing controls of discharges or releases of pollutants. They are guidance to EPA for promulgating Federal regulations under CWA section 303(c), when such action is necessary.

Under the CWA and its implementing regulations, States and authorized Tribes are to adopt water quality criteria to protect designated uses (*e.g.*, public water supply, recreational use, industrial use). EPA's recommended human health water quality criteria do not substitute for the CWA or regulations, nor are they regulations themselves. Thus, EPA's recommended criteria do not impose legally binding requirements. States and authorized Tribes have the discretion to adopt, where appropriate, other scientifically defensible water quality standards that differ from these recommendations.

ADDRESSES: Copies of documents specifically referenced in this notice and scientific views received are in Docket ID No. OW–2002–0054. Materials in the public docket are available for public viewing at the Water Docket in the EPA Docket Center, (EPA/DC) EPA West, Room B102, 1301 Constitution Ave., NW., Washington, DC. The EPA Docket Center Public Reading Room is open from 8:30 a.m. to 4:30 p.m., Monday through Friday, excluding legal holidays. The telephone number for the Public Reading Room is (202) 566–1744, and the telephone number for the Office of Water Docket is (202) 566–2426. A reasonable fee will be charged for copies. An electronic version of the public docket is available through EPA's electronic public docket and comment system, EPA Dockets, at *http://www.epa.gov/edocket/*. Once in the system, select "search," then key in the appropriate docket identification number.

FOR FURTHER INFORMATION CONTACT: Cindy Roberts, Health and Ecological Criteria Division (4304T), U.S. EPA, Ariel Rios Building, 1200 Pennsylvania Ave., NW., Washington, DC 20460; (202) 566–1124; *roberts.cindy@epa.gov.*

SUPPLEMENTARY INFORMATION:

General Information

A. Interested Entities

Entities potentially interested in today's notice are those that produce, use, or regulate chlorobenzene; cyanide; 1,2-dichlorobenzene; 1,4-dichlorobenzene; 1,1-dichloroethylene; 1,3-dichloropropene; endrin; ethylbenzene; hexachlorocyclopentadiene; lindane; thallium; toluene; 1,2-transdichloroethylene; 1,2,4-trichlorobenzene; and vinyl chloride. Categories and entities interested in today's notice include:

Category	Examples of interested entities
States, Authorized Tribes, and Jurisdictional Governments.	NPDES Authorized States, Tribes and Jurisdictions.
Industry	Industries discharging pollutants to surface waters or to publically-owned treatment works discharging pollutants to surface waters.
Municipalities	Publically-owned treatment works discharging pollutants to surface waters.

This table is not intended to be exhaustive, but rather provides a guide for readers regarding entities likely to be interested in this notice. This table lists the types of entities that EPA is now aware could potentially be interested in this notice. Other types of entities not listed in the table could also be interested.

B. How Can I Get Copies of the National Recommended Water Quality Criteria for the Protection of Human Health and Other Related Information?

1. *Docket.* EPA has established an official public docket for this notice under Docket ID No. OW–2002–0054. The official public docket consists of the documents specifically referenced in this notice, any public scientific views received, and other information related to this announcement. Although a part of the official docket, the public docket does not include Confidential Business Information (CBI) or other information whose disclosure is restricted by statute. The official public docket is the collection of materials that is available for public viewing at the Water Docket in the EPA Docket Center, (EPA/DC) EPA West, Room B102, 1301 Constitution Ave., NW., Washington, DC. The EPA Docket Center Public Reading Room is open from 8:30 a.m. to 4:30 p.m., Monday through Friday, excluding legal holidays. The telephone number for the Public Reading Room is (202) 566–1744, and the telephone number for the Office of Water Docket is (202) 566–2426. A reasonable fee will be charged for copies.

2. *Electronic Access.* You may access this Federal Register document electronically through the EPA Internet under the "Federal Register" listings at *http://www.epa.gov/fedrgstr/*.

An electronic version of the public docket is available through EPA's electronic public docket and comment system, EPA Dockets. You may use EPA Dockets at *http://www.epa.gov/edocket/* to view scientific views submitted by the public, access the index listing of the contents of the official public docket, and to access those documents in the public docket that are available electronically. Although not all docket materials may be available electronically, you may still access any of the publicly available docket materials through the docket facility identified in section B.1. Once in the system, select "search," then key in the appropriate docket identification number.

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I. Background Information

A. What Are Human Health Water Quality Criteria?

Human health water quality criteria are numeric values that describe ambient water concentrations that protect human health from the harmful effects of pollutants in ambient water. These criteria are developed under CWA section 304(a) and are based solely on data and scientific judgments about the relationship between pollutant

concentrations and environmental and human health effects. Human health water quality criteria do not reflect consideration of economic impacts or the technological feasibility of meeting the chemical concentrations in ambient water.

CWA section 304(a)(1) requires EPA to develop and publish and, from time to time, revise criteria for water quality that accurately reflect the latest scientific knowledge. EPA's recommended section 304(a) water quality criteria provide guidance to States and authorized Tribes in adopting water quality standards for protection of human health and can be used as a scientific basis for developing controls of discharges or releases of pollutants. The criteria also provide guidance to EPA when promulgating Federal regulations under CWA section 303(c), when such action is necessary.

B. How Is the 2000 Human Health Methodology Used?

In November 2000, EPA published the revised *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (2000)* (EPA–822–B–00–004, October 2000; hereafter referred to as the "2000 Human Health Methodology"). Before this, the Agency developed recommended human health water quality criteria using the 1980 *Guidelines and Methodology Used in the Preparation of Health Effects Assessment Chapter of the Consent Decree Water Criteria Documents* (45 FR 79347, called the "1980 Methodology"). The 2000 Human Health Methodology incorporates significant scientific advances that have occurred over the last two decades, particularly in the areas of cancer and noncancer risk assessments (using new information, procedures, and published Agency guidelines), exposure assessments (using new studies on human intake and exposure patterns, and new Agency guidelines), and methodologies to estimate bioaccumulation in fish. EPA will use the 2000 Human Health Methodology to develop new section 304(a) water quality criteria for additional pollutants and to revise existing section 304(a) water quality criteria. The 2000 Human Health Methodology is an important part of EPA's efforts to improve the quality of the Nation's waters and strengthen the overall scientific basis of water quality criteria. Furthermore, the 2000 Human Health Methodology will help States and authorized Tribes address their unique water quality issues and make risk management decisions to protect human health consistent with CWA section 303(c). The 2000 Human Health Methodology provides a detailed means for developing water quality criteria, including systematic procedures for evaluating cancer risk, noncancer health effects, human exposure, and bioaccumulation potential in fish.

C. How Does EPA Use Its Recommended Water Quality Criteria?

Water quality standards generally consist of designated uses (*e.g.*, public water supply, recreational use, industrial use), water quality criteria to protect those uses, a policy for antidegradation (that maintains and protects existing uses and water quality conditions), and general policies for application and implementation of water quality standards. As part of the water quality standards triennial review process defined in CWA section 303(c)(1), States and authorized Tribes are responsible for maintaining and revising water quality standards. Section 303(c)(1) requires States and authorized Tribes to review and, if appropriate, modify their water quality standards at least once every three years. EPA's recommended section 304(a) water quality criteria may form the basis for Agency decisions, both regulatory and non-regulatory, until they are superseded by EPA's publication of new or revised section 304(a) water quality criteria. These recommended water quality criteria are used in the following ways:

(1) as guidance to States and authorized Tribes in adopting water quality standards,

(2) as guidance to EPA in promulgating Federal water quality standards,

(3) to interpret a State's narrative water quality standard (in the absence of a State adopted numeric standard) in order to establish National Pollutant Discharge Elimination System (NPDES) water quality-based permit limits, and

(4) for all other purposes of CWA section 304(a).

Two distinct purposes are served by the section 304(a) water quality criteria. The first is as guidance to the States and authorized Tribes in the development and adoption of water quality criteria that will protect designated uses for their waters. The second is as guidance for promulgation of Federal water quality criteria for States and authorized Tribes, when such action is necessary under the terms of the CWA.

D. What Is the Relationship Between 304(a) Criteria and Your State or Tribal Water Quality Standards?

States and authorized Tribes must adopt water quality criteria that protect designated uses pursuant to CWA section 303(c)(2)(A). Protective criteria are based on a sound scientific rationale and must contain sufficient parameters or components to protect the designated uses. Water quality criteria may be expressed in either narrative or numeric form. States and authorized Tribes may use one of four approaches when adopting water quality criteria:

(1) Establish numerical values based on section 304(a) recommended water quality criteria,

(2) Modify the section 304(a) recommended water quality criteria to reflect site-specific conditions,

(3) Use other scientifically defensible methods to derive protective water quality criteria, and

(4) Establish narrative water quality criteria where numeric criteria cannot be determined or to supplement numeric water quality criteria.

EPA encourages States and authorized Tribes to use EPA's section 304(a) water quality criteria as guidance when adopting water quality standards consistent with CWA section 303(c) and the Federal regulations at 40 CFR part 131.

E. May States and Authorized Tribes Adopt Water Quality Criteria Based on Local Conditions?

EPA encourages States and authorized Tribes to develop and adopt water quality criteria to reflect local and regional conditions. In the 2000 Human Health Methodology, EPA published default values for risk level, fish intake, drinking water intake, and body weight for use by EPA, States or authorized Tribes in deriving human health water quality criteria. EPA believes these default values result in water quality criteria that protect the general population. States and authorized Tribes may also use these default values for their own water quality criteria, or they may use other values more representative of local conditions if they have data supporting the alternative values.

F. How Does the Review and Approval of State and Tribal Water Quality Standards Affect Water Quality Criteria Adopted by States and Authorized Tribes?

In 2000, EPA published new regulations addressing its review and approval of water quality standards adopted by States and authorized Tribes (see 65 FR 24642; April 27, 2000.) Under the new regulations, (codified at 40 CFR 131.21(c)–(f)), State or authorized Tribal water quality standards that were adopted by law or regulation before May 30, 2000, are in

effect for CWA purposes unless superseded by replacement Federal water quality standards (see 40 CFR 131.21(c)). However, under the new regulation, State or authorized Tribal water quality criteria adopted into State or Tribal law or regulation on or after May 30, 2000, are in effect for CWA purposes only after EPA approves any new or revised water quality standards. Therefore, new or revised water quality criteria adopted by States or authorized Tribes would not take effect for CWA purposes until after EPA approves them.

II. Human Health Water Quality Criteria Revisions

A. What Are the Criteria Revisions?

Today, EPA is announcing the availability of national recommended water quality criteria for the protection of human health for the following fifteen pollutants: Chlorobenzene; cyanide; 1,2-dichlorobenzene; 1,4-dichlorobenzene; 1,1-dichloroethylene; 1,3-dichloropropene; endrin; ethylbenzene; hexachlorocyclopentadiene; lindane; thallium; toluene; 1,2-transdichloroethylene; 1,2,4-trichlorobenzene; and vinyl chloride. The updated criteria are based on EPA's new methodology for deriving human health water quality criteria (i.e., the 2000 Human Health Methodology), and they supercede criteria previously published by the Agency.

These criteria represent partial updates of the section 304(a) water quality criteria, as described in both the draft Methodology revisions and the **Federal Register** notice that accompanied the final Methodology (65 FR 66444; November 3, 2000). EPA believes that updating a limited number of components for which there are available data or improved science (i.e., a partial update) is a reasonable and efficient way to more frequently publish revised section 304(a) water quality criteria. EPA has also described its process for publishing revised criteria [see *National Recommended Water Quality Criteria—Correction* (64 FR 19781; or EPA 822–Z–99–001) or the **Federal Register** notice for the final Methodology (65 FR 66444)].

Because recalculation of these fifteen criteria resulted in significant changes, EPA issued a **Federal Register** notice soliciting scientific views on the criteria on December 27, 2002 (67 FR 79091). This **Federal Register** Notice was issued in accordance with the published process for revising section 304(a) water quality criteria. EPA considered the scientific views received in response to the December 27, 2002, **Federal Register** notice. All criteria concentrations in this Notice are the same as those published in the December 27, 2002 (67 FR 79091), with the exception of the criterion for protecting human health from consumption of organism only for cyanide. (See section B, response to Scientific view b, Incidental ingestion should be considered when deriving human health water quality criteria for toxic pollutants with a low BCF.) Table II–1 presents the updated criteria, as well as the components used in their derivation (*e.g.*, bioconcentration factor, relative source contribution).

TABLE II–1.—REVISED HUMAN HEALTH WATER QUALITY CRITERIA

Priority pollutant	CAS No.	Human health water quality criteria for consumption of:		Components
		Water + organism (ug/L)	Organism only (ug/L)	
Thallium	7440280	0.24	0.47	RfD = 6.8E–5, BCF = 116 (RfD listed is for thallium (I) sulfate 7446–18–6), RSC = 20%, FI = 17.5.
Cyanide	57125	140	*140	RfD = 2E–2, BCF = 1, RSC = 20%, FI = 17.5.
Chlorobenzene	108907	130	1,600	RfD = 2E–2, BCF = 10.3, RSC = 20%, FI = 17.5.
1,1-Dichloroethylene	75354	330	7,100	RfD = 5E–2, RSC = 20%, BCF = 5.6, FI = 17.5.
1,3-Dichloropropene	542756	0.34	21	*q1 = 0.1, BCF = 1.9, FI = 17.5.
Ethylbenzene	100414	530	2,100	RfD = 1E–1, BCF = 37.5, RSC = 20%, FI = 17.5.
Toluene	108883	1,300	15,000	RfD = 2E–1, BCF = 10.7, RSC = 20%, FI = 17.5.
1,2-Trans-Dichloro-ethylene	156605	140	10,000	RfD = 2E–2, BCF = 1.58, RSC = 20%, FI = 17.5.
Vinyl Chloride	75014	0.025	2.4	*q1 = 1.4 (LMS exposure from birth), BCF = 1.17, FI = 17.5.
1,2-Dichlorobenzene	95501	420	1,300	RfD = 9E–2, BCF = 55.6, RSC = 20%, FI = 17.5.
1,4-Dichlorobenzene	106467	63	190	ADI = 1.34E–2, (ADI for 1,2-DCB used), BCF = 55.6, RSC = 20%, FI = 17.5.
Hexachlorocyclo-pentadiene	77474	40	1,100	RfD = 6E–3, BCF = 4.34, RSC = 20%, FI = 17.5.
1,2,4-Trichloro-benzene	120821	35	70	RfD = 1E–2, BCF = 114, RSC = 20 %, FI = 17.5.
gamma-BHC (Lindane)	58899	0.98	1.8	RfD= 3E–4, BCF = 130, RSC= 20%, FI = 17.5.
Endrin	72208	0.059	0.060	RfD = 3E–4, BCF = 3970, RSC = 20%, FI = 17.5.

RfD = reference dose; q1* = cancer potency factor; ADI = allowable daily intake; BCF = bioconcentration factor;
RSC = relative source contribution; FI = fish intake

*This recommended water quality criterion is expressed as total cyanide, even though the IRIS RfD we used to derive the criterion is based on free cyanide. The multiple forms of cyanide that are present in ambient water have significant differences in toxicity due to their differing abilities to liberate the CN-moiety. Some complex cyanides require even more extreme condition than refluxing with sulfuric acid to liberate the CN-moiety. Thus, these complex cyanides are expected to have little or no 'bioavailability' to humans. If a substantial fraction of the cyanide present in a water body is present in a complexed form (*e.g.*, $Fe_4[Fe(CN)_6]_3$), this recommended criterion may be over conservative.

EPA received much support for revising criteria based on partially updated components of the criteria equations as a way of increasing the frequency of scientific improvements to the nationally recommended criteria. For EPA to consider a water quality criterion revision based on a partial update to be acceptable, the components being used in the update should be comprehensive (*e.g.*, a revised reference dose or cancer dose-response assessment), stand alone, and be based on new national or local data. The recalculation of all fifteen water quality criteria integrates the updated national default freshwater/estuarine fish consumption rate of 17.5 grams/day. Thirteen of the criteria were calculated using a previously-determined relative source contribution (RSC) value from the national primary drinking water standards for the same chemicals. EPA also incorporated into the recalculations

a new cancer potency factor (q1*) for 1,3-dichloropropene and vinyl chloride, and a new reference dose (RfD) for 1,1-dichloroethylene, hexachlorocyclopentadiene, and lindane. These values were already published in the Agency's Integrated Risk Information System (IRIS). Both an RfD and q1* are available in IRIS for 1,3-dichloropropene and vinyl chloride. Because it resulted in more protective criteria, EPA used the q1* to derive the criteria in these cases rather than the RfD.

We derived the water quality criteria presented here with bioconcentration factors (BCFs) or field-measured bioaccumulation factors (BAFs) based on the 1980 Methodology. These values are consistent with those used to promulgate human health water quality criteria for priority toxic pollutants in rules such as the 1992 National Toxics Rule and the 2000 California Toxics Rule.

B. What Are EPA's Responses to the Scientific Views Received on the Criteria Revisions?

This section summarizes the scientific views received in response to the December 27, 2002, **Federal Register** Notice. It also presents EPA's responses to the scientific views.

1. 2000 Human Health Methodology

a. Support application of EPA's new methodology for deriving human health water quality criteria.

Scientific View—One submitter expressed support of EPA's application of the new human health methodology, including using more current estimates of daily fish intake, relative source contribution (for noncarcinogenic effects), and updated toxicological data.

Response—EPA acknowledges and appreciates the submitter's support.

b. Incidental ingestion should be considered when deriving human health water quality criteria for toxic pollutants with a low BCF.

Scientific View—One submitter indicated that EPA should consider acute and chronic effects from incidental ingestion of water when deriving human health water quality criteria associated with the consumption of "organisms only" for toxic pollutants with a low BCF. It is possible to exceed the RfD based on chronic toxicity when incidental ingestion occurs at the criterion concentration established for protecting human health for consumption of organisms only. Before finalizing the criteria revisions, EPA should compare the potential for acute toxicity from incidental ingestion of acutely toxic substances to the threshold for acute toxicity. The submitter uses cyanide as an example of a chemical for which acute and chronic effects from incidental ingestion of water should be considered as we develop human health water quality criteria.

Response—In developing the 2000 Human Health Methodology, EPA reviewed estimates of incidental water ingestion rates averaged over time. Based on this review, EPA generally believes that the averaged amount is negligible and will not impact the chemical criteria values that represent both drinking water and fish ingestion, unless (as indicated in the 2000 Methodology) the chemical exhibits minimal or no bioaccumulation potential.

EPA expects that the cyanide criterion for consumption of organisms only established based on the 2000 Human Health Methodology is generally protective of human health. However, cyanide is an acutely toxic substance (with a low bioaccumulation potential), and the resulting criterion of 16,000 ug/L derived for consumption of organism only may not protect humans from acutely toxic effects. Thus, EPA considers it prudent health policy to establish the criterion concentration for consumption of organisms only at the same level as the value for protecting human health for consumption of water and organisms (140 ug/L). The EPA's IRIS RfD that we used to derive the criterion is based on free cyanide. If a substantial fraction of the cyanide present in a water body is present in a complexed form (*e.g.*, $Fe_4[Fe(CN)_6]_3$), this recommended criterion may be overly conservative. State and authorized Tribes, however, have the discretion to modify section 304(a) criteria to reflect site-specific conditions.

c. Future updates of human health water quality criteria should consider additional exposure routes.

Scientific view—A submitter supported EPA's plans to include additional exposure routes resulting from recreational activities (*e.g.*, dermal, inhalation).

Response—EPA appreciates the submitter's support. As stated in the published draft methodology revisions (65 FR 66444; November 3, 2000) and in *Response to Peer Review Comments on Draft Revisions to the Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health* (EPA–822–R–00–009, August 2000), EPA acknowledges that the potential for inhalation and dermal exposures exist, and an approach to account for them in the context of developing individual water quality criteria is appropriate. EPA intends to refine the 2000 Human Health Methodology in the future to incorporate guidance on inhalation and dermal exposures.

d. National default BCFs and BAFs should not be used in the derivation of water quality criteria.

Scientific view—A submitter stated that the 15 proposed human health water quality criteria are based, in part, on using national default BCFs or BAFs without demonstrating that a statistically and ecologically significant correlation exists between the compound in the water column and levels found in fish tissues. The submitter uses methylmercury as an example of a chemical for which that correlation has not yet been demonstrated. As a consequence, the submitter strongly objects to the use of BCFs or BAFs in deriving the criteria. The submitter further stated that EPA should notify States and authorized Tribes not to adopt the revised criteria into State or Tribal standards until they can confirm a statistically significant (and important) relationship between water column concentrations and fish tissue concentrations.

Response—Using national default BCFs for water quality criteria began in 1980 and is necessary to ensure that criteria related to human ingestion of fish and shellfish will be protective of the consumer human populations who eat them. The BCF values determined for the water quality criteria represented the best scientific information available at the time. BCFs for nonionic organic chemicals that were determined from Veith *et al.* (1979) are based on a statistically significant correlation between experimentally determined chemical concentrations in water and fish tissues. We describe in detail the scientific basis for applying this data in the 1980 Ambient Water Quality Criteria National Guidelines (45 FR 79347).

EPA recognizes that many scientific advances have occurred in the area of bioaccumulation since it published the 1980 Methodology. As a result, EPA has revised the bioaccumulation portion of the 1980 Methodology to reflect the current state of science and to improve accuracy in assessing bioaccumulation for setting 304(a) criteria. EPA's *Methodology for Deriving Ambient Water Quality Criteria for Protection of Human Health (2000)* (65 FR 66444; hereafter referred to as the "2000 Methodology") contains the revised procedures for incorporating bioaccumulation in ambient water quality criteria (AWQC) and a summary of the key changes. EPA will publish more detailed information on the BAF

methodology in the near future (*Technical Support Document Volume 2: Development of National Bioaccumulation Factors*). We developed the approaches to deriving bioaccumulation factors and applying them in AWQC presented from a process that included extensive review from EPA's Science Advisory Board, peer review workshops, and stakeholder meetings (65 FR 6644).

EPA's framework deriving bioaccumulation factors is designed to account for chemical, biological and ecological attributes. For example, we provide separate procedures for deriving national BAFs depending on the type of chemical (*i.e.*, nonionic organic, ionic organic, inorganic and organometallic). More specifically, EPA's framework recognizes that the derivation of BAFs for organometallic chemicals differs in several ways from procedures for organic chemicals. For example, there are no generic bioaccumulation models that can be used to predict BAFs for organometallic chemicals as a whole; therefore, EPA's preferred approach for deriving national BAFs for such chemicals is to use empirical field data.

EPA took this approach in deriving draft national BAFs for methylmercury (see *Water Quality Criterion for the Protection of Human Health: Methylmercury* (EPA–823–R–01–001, January 2001)). We found the empirically-derived draft methylmercury BAFs to be variable, reflecting the influences of various biotic factors and abiotic factors on methylmercury bioaccumulation that were not well understood at that time. EPA acknowledged that these factors resulted in uncertainty as to the ability of the BAFs to accurately predict bioaccumulation of methylmercury across the waters of the United States. However, in this same document, EPA noted that this is not the case for other highly bioaccumulative pollutants (*i.e.*, non-organometallics). For such pollutants, EPA has methods that improve the predictive capability of empirically-derived or model-predicted BAFs.

When it conducts a full re-evaluation of the human health water quality criteria for the chemicals included in this Notice, EPA will evaluate the best available evidence concerning BAF values. EPA will develop national BAF values to the extent possible given the best available data at the time. Where derivation of National BAFs is not possible, EPA's 2000 Methodology encourages States and authorized Tribes to derive BAFs that are specific to regions or waterbodies as appropriate.

e. Scientific validity of using cancer potency factors or RfDs to define thresholds of unacceptable adverse effects is questionable.

Scientific view—One submitter questioned the scientific validity of using cancer potency factors or RfDs to define thresholds of unacceptable adverse effects. EPA should explicitly address the "scientific gray area" that exists between human health effects and RfDs and a benchmark dose or the lowest observed effect level on which an RfD might be based.

Response—As discussed in *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (2000)* (EPA–822–B–00–004, October 2000), human health water quality criteria are designed to minimize the risk of adverse effects to humans from chronic (lifetime) exposure to substances through the ingestion of drinking water and eating fish from surface waters.

The water quality criteria are based on chronic health effects data (both cancer and noncancer). However, the criteria also are intended to protect against adverse effects not only for the general population over a lifetime of exposure, but also for special populations (*e.g.*, sports fishers, children, elderly) who have an increased risk of receiving a dose that would elicit adverse effects due to their high water- or fish-intake rates or their biological sensitivities. Neither the benchmark dose nor a lowest observed effects level represent a "threshold" for response in the human or animal populations. Instead, those values typically are associated with a small proportional response level for the populations in question. EPA acknowledges the possibility that other populations might be more sensitive than those examined.

The Agency fully documents the derivation of its cancer potency factors and RfDs in IRIS. Those values were derived using the Agency guidelines for risk assessment, extensive peer review, and the best available information at the time the values were developed. The Agency continues to review and update the human health effects data in IRIS to ensure it considers the most current literature. That process, however, takes time. The IRIS Web (*http://www.epa.gov/iris/*) site describes EPA's policy on the "scientific gray areas" that reflect the use of uncertainty factors to cover certain types of data gaps.

2. EPA Should Adopt a Fish Tissue-Based Criteria in Lieu of the Proposed Water Column Criteria

Scientific view—EPA should derive fish tissue criteria, rather than water column concentrations, for the 15 compounds to avoid the scientific deficiencies related to the inappropriate use of BCFs and BAFs. Compliance monitoring and site-specific adjustments also are simplified when criteria are based on fish-tissue measurements in lieu of water column criteria. The submitter also requested a table of the intermediate fish tissue levels used in (or derived from) the calculation of the proposed water column criteria.

Response—For the most part, EPA has published water column concentrations as their recommended water quality criteria values for protection of human health. The recent exception being the fish tissue concentration for methylmercury (see 66 FR 1344, January 8, 2001). When the new methylmercury criterion was published, EPA withdrew its previous ambient human health water quality criteria for mercury as the recommended section 304(a) water quality criteria. At that time, EPA also recognized that this approach differed from the traditional water column criteria approach and suggested ways to relate the fish and shellfish tissue criterion to concentrations of methylmercury in the water column. We must relate tissue concentrations to water column concentrations in order to use the criterion to establish discharge limits for point sources. Fish tissue criteria can be developed and potentially simplify compliance monitoring and site-specific adjustments, yet this does not eliminate the need to develop BAFs.

Using national BAFs is a scientifically valid approach to deriving national water quality criteria. EPA encourages States and authorized Tribes to develop BAFs based on field-measured data from local/regional fish, whenever possible, when developing their own water quality standards.

The 15 revised human health criteria do not incorporate BAFs, a component of the new methodology; rather, the revised criteria are based on previously-developed BCFs. Thus, we have not estimated intermediate fish tissue concentrations.

3. EPA Should Provide All Numeric Factors Used in the Derivation of the Proposed Criteria

Scientific view—One submitter stated that EPA should provide information and references for all components needed to calculate the proposed criteria, including K_{ow} values and food chain multipliers.

Response—EPA included all basic parameters necessary for deriving the criteria in the December 27, 2002,

Federal Register notice announcing the proposed revisions (67 FR 79091). These parameters include: BCFs, fish consumption rate, body weight, reference dose or cancer potency factor, and relative source contribution. You can find information relevant to the derivation of these basic parameters (*e.g.*, K_{ow} values used in the derivation of BCFs) in other data sources such as EPA's criteria documents.

The revised human health criteria EPA developed use the BCF values derived from the 1980 Ambient Water Quality Criteria National Guidelines (45 FR 79347). We did not use food chain multipliers in the 1980 Methodology and, therefore, did not use them in deriving the proposed criteria. Rather, the proposed criteria rely on previously-derived BCFs which may have been derived from lab or field studies. Even though these BCFs emphasize bioconcentration, in some instances they may reflect trophic level transfers but not through the use of food chain multipliers.

4. EPA Should Publish All Proposed Changes to the Human Health Water Quality Criteria in the **Federal Register**

Scientific view—One submitter stated that EPA should publish all proposed changes to the human health water quality criteria in the **Federal Register**. In this way, dischargers and other affected parties will be aware of upcoming changes that will affect permits and other activities.

Response—EPA described its process for publishing revised criteria in *National Recommended Water Quality Criteria—Correction* (64 FR 19781; or EPA 822–Z–99–001) and the **Federal Register** notice for the final methodology (65 FR 66444). EPA specifically stated that, when making minor revisions to existing criteria based on new information about individual components of the criteria, the Agency will publish the recalculated criteria directly as the Agency's national recommended water quality criteria. This is a reasonable and efficient way to more frequently publish revised section 304(a) criteria. Based on this approach, EPA partially revised 83 national recommended water quality criteria for the protection of human health. EPA published these updated national recommended water quality criteria in a compilation entitled *National Recommended Water Quality Criteria: 2002* (EPA–822–02–047).

EPA also revised 15 more national recommended water quality criteria for the protection of human health. Although the revision of these criteria represent a partial update of the section 304(a) criteria, EPA decided to solicit scientific views on the criteria because applying the new methodology resulted in significant changes (67 FR 79091; December 27, 2002).

5. The Criteria Compilation Should Clearly Articulate That the Recommended Criteria Are Available for States To Use, as Appropriate, in Adopting Their Water Quality Criteria

Scientific view—A submitter stated that the 2000 Human Health Methodology encourages States to use local fish consumption rates to establish site-specific criteria rather than default fish consumption rates. However, without site-specific fish consumption rates, States cannot develop the most accurate criteria. Therefore, the criteria compilation should clearly articulate that States are not required to adopt EPA's recommended criteria, but that EPA's recommended criteria are available, as appropriate, when adopting criteria.

Response—CWA section 304(a)(1) requires EPA to develop and publish criteria for water quality that accurately reflect the latest scientific knowledge. Under this authority, EPA publishes national criteria that are recommendations to States and authorized Tribes in adopting water quality standards. These criteria are based on national default parameters, such as fish ingestion rates. Nevertheless, as stated in the *National Recommended Water Quality Criteria:* 2002 (EPA–822–02–047) compilation, "State and Tribal decision-makers have the discretion to adopt approaches on a case-by-case basis that differ from this guidance when appropriate." In addition, the 2002 compilation document explains that:

> "States and authorized Tribes have four options when adopting water quality criteria for which EPA has published section 304(a) criteria. They can: (1) Establish numerical values based on recommended section 304(a) criteria; (2) adopt section 304(a) criteria modified to reflect site-specific conditions; (3) adopt criteria derived using other scientifically defensible methods; or (4) establish narrative criteria when numeric criteria cannot be determined (40 CFR 131.11)."

Thus, EPA clearly stated that States and authorized Tribes are not required to adopt EPA national recommended water quality criteria, and that States and authorized Tribes have the discretion to derive criteria based on site-specific considerations such as local fish consumption rates.

6. Vinyl Chloride

a. The proposed human health water quality criteria for vinyl chloride are too low.

Scientific view—A submitter indicated that improper methods, overly conservative assumptions, and data quality deficiencies result in the proposed human health water quality criteria for vinyl chloride being too low.

Response—In deriving the water quality criteria for vinyl chloride, EPA applied the 2000 Human Health Methodology. In developing this methodology, EPA solicited and incorporated input from many sources, including the EPA Science Advisory Board, several peer review workshops, and the public. EPA believes that the resulting methodology accurately reflects the latest scientific knowledge on the kind and extent of all identifiable effects on health and welfare that can be expected when pollutants are present in any body of water. Thus, the human health water quality criteria for vinyl chloride accurately reflect the relationship between vinyl chloride concentrations and human health effects.

The recommended water quality criteria for vinyl chloride are guidance for States and authorized Tribes to establish water quality standards. State and Tribal decision-makers have the discretion to adopt approaches on a case-by-case basis that differ from this guidance when appropriate.

b. EPA should use a central estimate as a point of departure in deriving vinyl chloride criteria.

Scientific view—Two submitters stated that the revised vinyl chloride human health water quality criteria for consumption of water and organism and consumption of organisms only are too low because EPA used overly conservative assumptions in their derivation. Risk-specific doses derived based on linear low-dose extrapolations using the lower 95 percent confidence limit on a dose associated with a 10 percent extra risk, or, LED_{10}, as the point of departure should not be used to derive criteria. Rather, risk-specific doses based on a central estimate, such as a dose associated with a 10 percent extra risk, or ED_{10}, should be used as a point of departure.

EPA's rationale for using the LED_{10} as the point of departure for model-based dose-response extrapolations in the 1996 proposed guidelines for carcinogen risk assessment is very weak. EPA did not hear the advice from peer review workshops on benchmark dose and the proposed cancer guidelines

recommending the use a of central estimate (ED_{10}) point of departure.

EPA's decision to use an LED_{10}, as opposed to an ED_{10}, in deriving revised human health criteria for vinyl chloride is inconsistent with EPA's Guidelines for Ensuring and Maximizing the Quality, Objectivity, Utility, and Integrity of Information Disseminated by the Environmental Protection Agency and the Information Quality Act (IQA). EPA's science policy decision to use the LED_{10}, instead of the ED_{10}, introduces significant uncertainty in the risk assessment that underlies the water quality criteria derivations, which is in violation of the IQA. The submitter requested that we correct this information.

Response—The 2000 Human Health Methodology includes toxicological and exposure assessment parameters derived from scientific analysis, science policy, and risk management decisions, including the 1986 cancer guidelines [see *Guidelines for Carcinogen Risk Assessment* (51 FR 33992)] and principles from the 1999 draft revised cancer guidelines [see *1999 Guidelines for Carcinogenic Risk Assessment—Review Draft* (NCEA–F–0644, July 1999)]. These principles arise from scientific discoveries about cancer made in the last 15 years and from EPA policy supporting full characterization of hazard and risk for both the general population and potentially sensitive groups like children.

In particular, EPA's 1999 draft revised cancer guidelines gave a rationale for selecting point of departures (PODs). For quantitative modeling of dose-response relationships in the observed range, the guidelines recommend calculating the lower 95 percent confidence limit on a dose associated with an estimated 10 percent increased tumor or relevant non-tumor response (LED_{10}). The estimate of the LED_{10} is used as the point of departure (POD) for low-dose extrapolation. This standard point of departure (LED_{10}) is adopted as a matter of science policy to remain as consistent and comparable across different studies. It is also a convenient comparison point for noncancer endpoints. The rationale for using the LED_{10} is that a 10 percent response is at or just below the limit of sensitivity for discerning a statistically significant tumor response in most long-term rodent studies and is also within the observed range for other toxicity studies. Using the lower limit takes experimental variability and sample size into account. Note that use of the lower 95 percent confidence limit on the ED_{10} implies that, given the experimental parameters (e.g., sample size, variation in response) of the study being used, there is a five percent chance or less that the "true" ED_{10} would be lower than the LED_{10}. For well-conducted studies with large numbers of animals, relatively close dose spacing, and little inherent variability in the animal responses, LED_{10} values will be close to the central estimate of the ED_{10} value. For studies that include smaller numbers of animals, wider dose spacing, and more variable responses in replicates at the same dose, the LED_{10} value will be further removed from the ED_{10} value. It is part of EPA's science policy to use the lower bound of a 95 percent confidence interval around a preferred value (e.g., central estimate of the ED_{10}) as a point of departure to ensure that the criterion will be adequately protective, that is, that the experimental uncertainty is small (a few percent or less). The EPA's IRIS cancer assessment of vinyl chloride uses the LED_{10} as the POD. EPA's *Guidelines for Ensuring and Maximizing the Quality, Objectivity, Utility, and Integrity of Information Disseminated by the Environmental Protection Agency* (EPA/260R–02–008, October 2002) indicated that EPA intends to specify the central estimate of human health risk when it is available. The ED_{10} (central estimate) for vinyl chloride is not presented in IRIS. More recent IRIS entries do include the central estimate, but this was not the policy at the time vinyl chloride was completed. The requirement for its inclusion was instituted in the 2003 Standard Operating Procedures for IRIS.

c. The vinyl chloride MCL is a more appropriate benchmark level.

Scientific view—A submitter indicated that the current maximum contaminant level (MCL) for vinyl chloride of two parts per billion (ppb) which was developed under the Safe Drinking Water Act (SDWA) is a more appropriate benchmark level.

Response—The human health water quality criteria developed under CWA section 304(a) are based solely on data and scientific judgments about the relationship between pollutant concentrations and environmental and human health effects. Unlike the MCLs, the criteria do not consider economic impacts or the technological feasibility of meeting the chemical concentrations in ambient water. Thus, MCLs are not considered counterparts to water quality criteria.

d. The vinyl chloride water quality criterion for consumption of organisms should only be based on incidental ingestion of non-potable, recreational waters.

Scientific view—A submitter stated that the revised vinyl chloride human health criteria for potable water was derived based on the assumption that people would drink two liters of surface water each day over a lifetime. Thus, surface water is effectively considered a public water supply. However, if the intended use of the water quality criteria is to set NPDES limits for potable waters not being used as public water supplies, then the water consumption assumption is overly conservative. Such waters serve only as recreational or occasional use water bodies, so that a value for incidental water ingestion would be more appropriate. For regulatory consistency, the water quality criteria for vinyl chloride for potable water supplies should be the same as the MCL.

Response—As required by CWA section 304(a), EPA develops water quality criteria that reflect the latest scientific knowledge on effects of pollutants on human health. States and authorized Tribes use the Agency's recommended section 304(a) water quality criteria to adopt enforceable water quality standards, including designating uses of a water body consistent with CWA section 101(a) (*e.g.*, public water supply, fishing, recreation). In developing the 2000 Human Health Methodology, we made assumptions about exposure to contamination from consuming surface waters of the U.S. Our assumptions ensure that, if criteria are met in a water body designated with the uses specified in section 101(a), people can safely consume water from that water body. In order to ensure this, it is necessary to assume that all of the consumed water is taken from water bodies at the criteria level (*i.e.*, contaminated to the maximum safe level).

The designated use inherent in the submitter's example is drinking water (potable water), even though the particular water body might not be used that way at the moment. Thus, the main issue in the view relates to the State's (or authorized Tribe's) assignment of designated use, not to numeric values for the national ambient water quality criteria for vinyl chloride.

Again, the human health water quality criteria developed under CWA section 304(a) are based solely on data and scientific judgments on the relationship between pollutant concentrations and environmental and human health effects. Unlike the MCLs, the criteria do not consider economic impacts or the technological feasibility of meeting the chemical concentrations in ambient water. MCLs are not counterparts to water quality criteria.

e. EPA's BCF for vinyl chloride is overstated and its water quality criterion

for consumption of organisms should only be based on incidental ingestion of non-potable, recreational waters.

Scientific view—One submitter stated that EPA derived its vinyl chloride human health criterion for consumption of organisms only using a bioconcentration factor (BCF) of 1.17. The submitter believes that this BCF is overstated because:

(1) This value is based on the assumption of equilibrium conditions between water and an organisms tissue, which is not the case because the compound is highly metabolized;

(2) the high volatility of vinyl chloride would contribute to its depuration during processing or cooking;

(3) the portions of the fish most likely to contain the compound, (*e.g.*, skin and fat) are not typically consumed by humans; and

(4) cooking would result in further off-gasing or destruction of the chemical.

Thus, we expect the potential for humans consuming aquatic organisms to be exposed to vinyl chloride to be negligible. Moreover, vinyl chloride does not biomagnify, and higher tropic level organisms consumed by humans would not contain elevated levels of vinyl chloride. EPA should derive its vinyl chloride criteria for consumption of organisms only based on exposure from incidental ingestion of non-potable recreational waters only.

Response—In updating its human health water quality criteria for vinyl chloride, EPA used the BCF derived from the 1980 Ambient Water Quality Criteria National Guidelines (45 FR 79347). The submitter is correct that, if a contaminant is readily metabolized in fish, the actual BCF might be less than estimated using the $KLED_{ow}$ method. EPA thanks the submitter for the information and will consider it when the Agency comprehensively updates the vinyl chloride criterion document to incorporate the BAF derivation procedures described in the 2000 Human Health Methodology.

C. Where Other Views Submitted?

We received a number of views on criteria that EPA was not revising, or the views expressed were not related to the science supporting the criteria derivations. EPA did not prepare responses addressing these views.

Dated: December 23, 2003.

Geoffrey H. Grubbs,

Director, Office of Science and Technology.

[FR Doc. 03–32211 Filed 12–30–03; 8:45 am]

BILLING CODE 6560–50–P

ENVIRONMENTAL PROTECTION AGENCY

[FRL–7604–7]

RIN 2040–ACXX

Preliminary Effluent Guidelines Program Plan for 2004/2005

AGENCY: Environmental Protection Agency (EPA).

ACTION: Notice of preliminary effluent guidelines plan; request for comments.

SUMMARY: Today's notice presents and invites comment on EPA's preliminary Effluent Guidelines Program Plan for 2004/2005. Under the Clean Water Act (CWA), EPA establishes technology-based national regulations, termed "effluent guidelines," to reduce pollutant discharges from industrial facilities to waters of the United States. Section 304(m) of the Clean Water Act (CWA) requires EPA to publish an Effluent Guidelines Program Plan every two years. Today's notice has three purposes. First, it presents the results of EPA's annual review of the effluent guidelines that EPA has promulgated under CWA section 304(b). Second, it solicits public comment on the preliminary Effluent Guidelines Program Plan. Third, it describes and solicits comment on the analytical framework that EPA has employed to date in performing the annual review for 2003 and in developing today's preliminary Effluent Guidelines Program Plan. EPA had articulated an early form of this evolving analytical framework in the draft Strategy for National Clean Water Industrial Regulations, which EPA hopes to finalize concurrently with the Effluent Guidelines Program Plan in 2004.

DATES: EPA must receive comments on the preliminary Effluent Guidelines Program Plan for 2004/2005 by February 17, 2004. EPA will conduct a public meeting on Wednesday, January 28, 2004, from 9 a.m. to 12 p.m. Eastern Standard Time. For information on the location of the public meeting, see **ADDRESSES** section.

ADDRESSES: You can submit comments electronically, by mail, or through hand-delivery/courier. Please mail comments to the Water Docket, Environmental Protection Agency, Mail Code: 4101 T, 1200 Pennsylvania Avenue, NW., Washington, DC 20460 or submit them electronically to *http://www.epa.gov/edocket/*. For more information on submitting comments, see section I.C. EPA will hold an informational public meeting for interested stakeholders in the EPA East Building, Room 1153 (also known as the "Great Room" or the "Map Room"), 1201 Constitution Avenue, NW., Washington, DC. For more information on the details and location of the public meeting, see section I.F.

FOR FURTHER INFORMATION CONTACT: Mr. Carey A. Johnston at (202) 566–1014 or *johnston.carey@epa.gov*, or Mr. Tom Wall at (202) 566–1060 or *wall.tom@epa.gov*.

SUPPLEMENTARY INFORMATION:

How Is This Document Organized?

The outline of the preliminary Effluent Guidelines Program Plan for 2004/2005 follows.

I. General Information
II. Legal Authority
III. What Are Effluent Guidelines?
IV. What Requirements Apply to This Effluent Guidelines Program Plan Effort?
V. What Is the Purpose of Today's **Federal Register** Notice?
VI. 2003 Annual Review of Effluent Guidelines That EPA Has Promulgated Under CWA Section 304(b)
VII. What Will Be the Focus of EPA's 2004 Annual Review?
VIII. Identification of and Schedule for Possible Categories for Potential New Effluent Guidelines
IX. Request for Comment and Information

I. General Information

A. Regulated Entities

Today's preliminary Effluent Guidelines Program Plan for 2004/2005 does not contain regulatory requirements, nor will the final plan do so. Rather, today's preliminary Effluent Guidelines Program Plan describes the current status of the effluent guidelines planning process, presents the results of the Agency's annual review of the effluent guidelines EPA has already promulgated for industrial categories, and identifies industrial categories that EPA expects to investigate further for the possible development or revision of effluent limitations guidelines.

B. How Can I Get Copies of This Document and Other Related Information?

1. Docket

EPA has established an official public docket for this action under Docket ID No. OW–2003–0074. The official public docket consists of the documents specifically referenced in this action, any public comments received, and other information related to this action. Although a part of the official docket, the public docket does not include information claimed as Confidential Business Information (CBI) or other information whose disclosure is restricted by statute. The official public docket is the collection of materials that

Exhibit H

Summary of Reasons Supporting Proposed Revision to the Alabama Department of Environmental Management's Administrative Code for Division 6 (Water Division)

335-6-10-.05(2)

This rule is being revised to update a reference to federal regulations (40 CFR 136.3) that describes which tests and analytical procedures are acceptable for determining compliance or noncompliance with water quality criteria. The previous date of 1990 will be replaced with 2003 to reference the most current federal regulations.

Rule 335-6-10-.07
Table 1, Toxic Pollutant Criteria
Appendix A

This rule is being revised to update the Department's toxic pollutant criteria to be consistent with criteria established by EPA.

These revisions involve:

- Updating the water hardness-dependent equations for metals to include EPA-recommended conversion factors and new parameter values.
- Updating Table 1, Toxic Pollutant Criteria, to reflect aquatic life criteria values revised by EPA and to include criteria values for tributyltin (TBT) which EPA has recently added to its recommended water quality criteria for priority pollutants.
- Updating Appendix A, to reflect new parameter values revised by EPA. These parameters are used in equations to derive the Department's human health criteria for carcinogenic and non-carcinogenic toxic pollutants.
- Updating Table 1 and Appendix A to include an additional column identifying each toxic pollutant by its unique Chemical Abstracts Service (CAS) number. This will increase the effectiveness of these tables and help eliminate potential confusion due to the variety of pseudonyms for each chemical species.

The proposed revisions to Rule 335-6-10-.07 will make the Department's toxic pollutant criteria consistent with EPA's recommended criteria as reflected in its guidance document entitled "National Recommended Water Quality Criteria: 2002", as well as the fifteen (15) recommended water quality criteria EPA revised and published in the Federal Register on December 27, 2002, and criteria for tributyltin (TBT) which EPA also published in the Federal Register on December 27, 2002. The revisions EPA has made to its recommended water quality criteria reflect the latest scientific knowledge concerning toxic chemicals and are based on substantial data and extensive peer review.

335-6-10-.08

This rule is being revised to clarify the language regarding the waste treatment requirements for municipal facilities that have effluent concentration limitations more stringent than secondary treatment. The proposed revision is being made to be consistent with EPA guidance.

335-6-10-.09(7)(5)

This rule is being revised to clarify the minimum stream flow used when establishing effluent limitations in waters with the Agricultural and Industrial Water Supply use classification. The proposed revision is intended to make Rule 335-6-10-.09(7)(5) consistent with the regulations contained in Chapter 335-6-6 concerning the National Pollutant Discharge Elimination System.

Chapter 1 Water Quality Standards

1.1 Water Quality Standards Program

The Water Quality Standards Program at the Alabama Department of Environmental Management (ADEM) has been very active since the last 305(b) Report was submitted in April of 2004. ADEM's Water Quality Standards (WQS) Program, consisting of the Water Quality Criteria (Rule 335-6-10) and Water Use Classifications for Interstate and Intrastate Waters (Rule 335-6-11) has been the subject of significant changes over the last year. The subject regulations, which govern our water quality program have been amended twice within a 12-month period and are once again undergoing public review as a result of additional proposed changes. Nutrient criteria development for Alabama reservoirs, new bacteriological criteria for coastal waters, and revision of the state's toxic criteria have been the primary focus of water quality standards development within ADEM's WQS Program over the past year. The section that follows provides a brief summary of the subject rules. The Department believes the recent changes to the WQS Program is a direct reflection of our ongoing commitment to restore, maintain, and protect the physical, chemical, and biological integrity of Alabama's waters.

For information pertaining to Water Quality Standards, contact Mr. Stan Shirley in ADEM's Montgomery Office at (334) 274-4250 or sls@adem.state.al.us

1.2 Water Quality Rule Changes

On April 20, 2004, the State of Alabama adopted regulations that became effective May 27, 2004. Highlights of the rule changes are listed below.

- Amendment of ADEM Administrative Code Rule 335-6-10-.11 to establish lake-specific, nutrient criteria (expressed as chlorophyll a targets) for 11 additional reservoirs within Alabama, including Claiborne Lake, Dannelly Lake, Lake Harding, Point A Lake, Gantt Lake, Warrior Lake, Oliver Lake, Holt Lake, Lake Tuscaloosa, Bankhead Lake, and Lewis Smith Lake.

- Amendment of ADEM Administrative Code Rule 335-6-11-.02 to add the Public Water Supply use classification to Whitesides Mill Lake on the western border of the Talladega National Forest. This change was adopted to provide classification for this reservoir for use as a water supply source for the city of Anniston.

- Amendment of ADEM Administrative Code Rule 335-6-10-.09 to replace fecal coliform with enterococci as the bacterial indicator to use when monitoring recreational coastal waters for bacterial contamination and the presence of potential human pathogens. This rule

revision fulfills the requirements mandated by the federal Beaches Environmental Assessment and Coastal Health (BEACH) A2ct. In marine water studies conducted by EPA, it was determined that enterococcus was the most effective bacterial indicator to use as the basis for bacteriological criteria in coastal waters since it is best suited for indicating potential pathogens associated with fecal pollution.

On December 3, 2004, the State of Alabama adopted regulations that became effective January 15, 2005. Highlights of the rule changes are listed below.

- Amendment of ADEM Administrative Code Rule 335-6-10-.05 to update a reference to federal regulations (40 CFR 136.3) that describes which tests and analytical procedures are acceptable for determining compliance or noncompliance with water quality criteria.

- Amendment of ADEM Administrative Code Rule 335-6-10-.07 to change certain factors in the hardness-dependent equations for calculating aquatic life criteria for certain metals and to clarify that the criteria are expressed as the dissolved fraction of the metal in the water column. The adopted revisions make the Department's toxic pollutant criteria consistent with EPA's recommended criteria.

- Amendment of ADEM Administrative Code Rule 335-6-10-.07 to update reference doses, cancer potency factors, and bioconcentration factors used in the calculation of certain human health toxic pollutant criteria. The adopted revisions make the Department's toxic pollutant criteria consistent with EPA's recommended criteria as reflected in its guidance document entitled "National Recommended Water Quality Criteria: 2002", as well as the fifteen (15) recommended water quality criteria EPA revised and published in the Federal Register on December 27, 2002, and criteria for tributyltin (TBT) which EPA also published in the Federal Register on December 27, 2002.

- Amendment of ADEM Administrative Code Rules 335-6-10-.07 and 335-6-10-.09 to clarify the minimum stream flow used when establishing effluent limitations in waters with the Agricultural and Industrial Water Supply use classification. The adopted revision makes Rule 335-6-10-.09(7)(5) consistent with the regulations contained in Chapter 335-6-6 concerning the National Pollutant Discharge Elimination System.

- Amendment of ADEM Administrative Code Rule 335-6-10-.08 to clarify the language regarding the waste treatment requirements for municipal facilities that have effluent concentration limitations more stringent than secondary treatment.

On August 12, 2005, the State of Alabama adopted regulations that became effective September 21, 2005. Highlights of the rule changes are listed below.

- Amendment of ADEM Administrative Code Rule 335-6-10.11 to establish lake-specific nutrient criteria (expressed as chlorophyll a targets) for 5 additional reservoirs within Alabama, including Inland Lake, Lake Jackson, Coffeville Lake, Demopolis Lake, and Gainesville Lake.

- Amendment of ADEM Administrative Code Rule 335-6-10.11-.02 to correct typographical

Exhibit J

United States
Environmental Protection
Agency

Office of Water
Office of Science and Technology
(4304T)

2006

National Recommended Water Quality Criteria

NATIONAL RECOMMENDED WATER QUALITY CRITERIA FOR PRIORITY TOXIC POLLUTANTS

	Priority Pollutant	CAS Number	Freshwater CMC (µg/L)	Freshwater CCC (µg/L)	Saltwater CMC (µg/L)	Saltwater CCC (µg/L)	Human Health For Consumption of: Water + Organism (µg/L)	Human Health For Consumption of: Organism Only (µg/L)	FR Cite/ Source
1	Antimony	7440360					5.6 B	640 B	65FR66443
2	Arsenic	7440382	340 A,D,K	150 A,D,K	69 A,D,bb	36 A,D,bb	0.018 C,M,S	0.14 C,M,S	65FR31682 57FR60848
3	Beryllium	7440417					Z		65FR31682
4	Cadmium	7440439	2.0 D,E,K,bb	0.25 D,E,K,bb	40 D,bb	8.8 D,bb	Z		EPA-822-R-01-001 65FR31682
5a	Chromium (III)	16065831	570 D,E,K	74 D,E,K			Z Total		EPA820/B-96-001 65FR31682
5b	Chromium (VI)	18540299	16 D,K	11 D,K	1,100 D,bb	50 D,bb	Z Total		65FR31682
6	Copper	7440508	13 D,E,K,cc	9.0 D,E,K,cc	4.8 D,cc,ff	3.1 D,cc,ff	1,300 U		65FR31682
7	Lead	7439921	65 D,E,bb,gg	2.5 D,E,bb,gg	210 D,bb	8.1 D,bb			65FR31682
8a	Mercury	7439976	1.4 D,K,hh	0.77 D,K,hh	1.8 D,ee,hh	0.94 D,ee,hh		0.3 mg/kg J	62FR42160 EPA823-R-01-001
8b	Methylmercury	22967926							
9	Nickel	7440020	470 D,E,K	52 D,E,K	74 D,bb	8.2 D,bb	610 B	4,600 B	65FR31682
10	Selenium	7782492	L,R,T	5.0 T	290 D,bb,dd	71 D,bb,dd	170 Z	4200	62FR42160 65FR31682 65FR66443
11	Silver	7440224	3.2 D,E,G		1.9 D,G				65FR31682

NATIONAL RECOMMENDED WATER QUALITY CRITERIA FOR PRIORITY TOXIC POLLUTANTS

	Priority Pollutant	CAS Number	Freshwater CMC (µg/L)	Freshwater CCC (µg/L)	Saltwater CMC (µg/L)	Saltwater CCC (µg/L)	Human Health For Consumption of: Water + Organism (µg/L)	Human Health For Consumption of: Organism Only (µg/L)	FR Cite/ Source
12	Thallium	7440280					0.24	0.47	68FR75510
13	Zinc	7440666	120 D,E,K	120 D,E,K	90 D,bb	81 D,bb			65FR31682
							7,400 U	26,000 U	65FR66443
14	Cyanide	57125	22 K,Q	5.2 K,Q	1 Q,bb	1 Q,bb			EPA820/B-96-001
							140 jj	140 jj	57FR60848 68FR75510
15	Asbestos	1332214					7 million fibers/L I		57FR60848
16	2,3,7,8-TCDD (Dioxin)	1746016					5.0E-9 C	5.1E-9 C	65FR66443
17	Acrolein	107028					190	290	65FR66443
18	Acrylonitrile	107131					0.051 B,C	0.25 B,C	65FR66443
19	Benzene	71432					2.2 B,C	51 B,C	IRIS 01/19/00 &65FR66443
20	Bromoform	75252					4.3 B,C	140 B,C	65FR66443
21	Carbon Tetrachloride	56235					0.23 B,C	1.6 B,C	65FR66443
22	Chlorobenzene	108907					130 Z,U	1,600 U	68FR75510
23	Chlorodibromomethane	124481					0.40 B,C	13 B,C	65FR66443
24	Chloroethane	75003							

NATIONAL RECOMMENDED WATER QUALITY CRITERIA FOR PRIORITY TOXIC POLLUTANTS

	Priority Pollutant	CAS Number	Freshwater CMC (µg/L)	Freshwater CCC (µg/L)	Saltwater CMC (µg/L)	Saltwater CCC (µg/L)	Human Health For Consumption of: Water + Organism (µg/L)	Human Health For Consumption of: Organism Only (µg/L)	FR Cite/ Source
25	2-Chloroethylvinyl Ether	110758							
26	Chloroform	67663					5.7 C,P	470 C,P	62FR42160
27	Dichlorobromomethane	75274					0.55 B,C	17 B,C	65FR66443
28	1,1-Dichloroethane	75343							
29	1,2-Dichloroethane	107062					0.38 B,C	37 B,C	65FR66443
30	1,1-Dichloroethylene	75354					330	7,100	68FR75510
31	1,2-Dichloropropane	78875					0.50 B,C	15 B,C	65FR66443
32	1,3-Dichloropropene	542756					0.34 C	21 C	68FR75510
33	Ethylbenzene	100414					530	2,100	68FR75510
34	Methyl Bromide	74839					47 B	1,500 B	65FR66443
35	Methyl Chloride	74873							65FR31682
36	Methylene Chloride	75092					4.6 B,C	590 B,C	65FR66443
37	1,1,2,2-Tetrachloroethane	79345					0.17 B,C	4.0 B,C	65FR66443
38	Tetrachloroethylene	127184					0.69 C	3.3 C	65FR66443
39	Toluene	108883					1,300 Z	15,000	68FR75510
40	1,2-Trans-Dichloroethylene	156605					140 Z	10,000	68FR75510

NATIONAL RECOMMENDED WATER QUALITY CRITERIA FOR PRIORITY TOXIC POLLUTANTS

	Priority Pollutant	CAS Number	Freshwater CMC (µg/L)	Freshwater CCC (µg/L)	Saltwater CMC (µg/L)	Saltwater CCC (µg/L)	Human Health For Consumption of: Water + Organism (µg/L)	Human Health For Consumption of: Organism Only (µg/L)	FR Cite/ Source
41	1,1,1-Trichloroethane	71556					z		65FR31682
42	1,1,2-Trichloroethane	79005					0.59 B,C	16 B,C	65FR66443
43	Trichloroethylene	79016					2.5 C	30 C	65FR66443
44	Vinyl Chloride	75014					0.025 C,kk	2.4 C,kk	68FR75510
45	2-Chlorophenol	95578					81 B,U	150 B,U	65FR66443
46	2,4-Dichlorophenol	120832					77 B,U	290 B,U	65FR66443
47	2,4-Dimethylphenol	105679					380 B	850 B,U	65FR66443
48	2-Methyl-4,6-Dinitrophenol	534521					13	280	65FR66443
49	2,4-Dinitrophenol	51285					69 B	5,300 B	65FR66443
50	2-Nitrophenol	88755							
51	4-Nitrophenol	100027							
52	3-Methyl-4-Chlorophenol	59507					U	U	
53	Pentachlorophenol	87865	19 F,K	15 F,K	13 bb	7.9 bb			65FR31682
54	Phenol	108952					0.27 B,C	3.0 B,C,H	65FR66443
55	2,4,6-Trichlorophenol	88062					21,000 B,U	1,700,000 B,U	65FR66443
56	Acenaphthene	83329					1.4 B,C	2.4 B,C,U	65FR66443
							670 B,U	990 B,U	65FR66443

NATIONAL RECOMMENDED WATER QUALITY CRITERIA FOR PRIORITY TOXIC POLLUTANTS

	Priority Pollutant	CAS Number	Freshwater CMC (µg/L)	Freshwater CCC (µg/L)	Saltwater CMC (µg/L)	Saltwater CCC (µg/L)	Human Health For Consumption of: Water + Organism (µg/L)	Human Health For Consumption of: Organism Only (µg/L)	FR Cite/ Source
57	Acenaphthylene	208968							
58	Anthracene	120127					8,300 B	40,000 B	65FR66443
59	Benzidine	92875					0.000086 B,C	0.00020 B,C	65FR66443
60	Benzo(a)Anthracene	56553					0.0038 B,C	0.018 B,C	65FR66443
61	Benzo(a)Pyrene	50328					0.0038 B,C	0.018 B,C	65FR66443
62	Benzo(b)Fluoranthene	205992					0.0038 B,C	0.018 B,C	65FR66443
63	Benzo(ghi)Perylene	191242							
64	Benzo(k)Fluoranthene	207089					0.0038 B,C	0.018 B,C	65FR66443
65	Bis(2-Chloroethoxy)Methane	111911							
66	Bis(2-Chloroethyl)Ether	111444					0.030 B,C	0.53 B,C	65FR66443
67	Bis(2-Chloroisopropyl)Ether	108601					1,400 B	65,000 B	65FR66443
68	Bis(2-Ethylhexyl)Phthalate[X]	117817					1.2 B,C	2.2 B,C	65FR66443
69	4-Bromophenyl Phenyl Ether	101553							
70	Butylbenzyl Phthalate[W]	85687					1,500 B	1,900 B	65FR66443
71	2-Chloronaphthalene	91587					1,000 B	1,600 B	65FR66443
72	4-Chlorophenyl Phenyl Ether	7005723							

NATIONAL RECOMMENDED WATER QUALITY CRITERIA FOR PRIORITY TOXIC POLLUTANTS

	Priority Pollutant	CAS Number	Freshwater CMC (µg/L)	Freshwater CCC (µg/L)	Saltwater CMC (µg/L)	Saltwater CCC (µg/L)	Human Health For Consumption of: Water + Organism (µg/L)	Human Health For Consumption of: Organism Only (µg/L)	FR Cite/ Source
73	Chrysene	218019					0.0038 B,C	0.018 B,C	65FR66443
74	Dibenzo(a,h)Anthracene	53703					0.0038 B,C	0.018 B,C	65FR66443
75	1,2-Dichlorobenzene	95501					420	1,300	68FR75510
76	1,3-Dichlorobenzene	541731					320	960	65FR66443
77	1,4-Dichlorobenzene	106467					63	190	68FR75510
78	3,3'-Dichlorobenzidine	91941					0.021 B,C	0.028 B,C	65FR66443
79	Diethyl Phthalate[W]	84662					17,000 B	44,000 B	65FR66443
80	Dimethyl Phthalate[W]	131113					270,000	1,100,000	65FR66443
81	Di-n-Butyl Phthalate[W]	84742					2,000 B	4,500 B	65FR66443
82	2,4-Dinitrotoluene	121142					0.11 C	3.4 C	65FR66443
83	2,6-Dinitrotoluene	606202							
84	Di-n-Octyl Phthalate	117840							
85	1,2-Diphenylhydrazine	122667					0.036 B,C	0.20 B,C	65FR66443
86	Fluoranthene	206440					130 B	140 B	65FR66443
87	Fluorene	86737					1,100 B	5,300 B	65FR66443
88	Hexachlorobenzene	118741					0.00028 B,C	0.00029 B,C	65FR66443

NATIONAL RECOMMENDED WATER QUALITY CRITERIA FOR PRIORITY TOXIC POLLUTANTS

	Priority Pollutant	CAS Number	Freshwater CMC (µg/L)	Freshwater CCC (µg/L)	Saltwater CMC (µg/L)	Saltwater CCC (µg/L)	Human Health For Consumption of: Water + Organism (µg/L)	Human Health For Consumption of: Organism Only (µg/L)	FR Cite/ Source
89	Hexachlorobutadiene	87683					0.44 B,C	18 B,C	65FR66443
90	Hexachlorocyclopentadiene	77474					40 U	1,100 U	68FR75510
91	Hexachloroethane	67721					1.4 B,C	3.3 B,C	65FR66443
92	Ideno(1,2,3-cd)Pyrene	193395					0.0038 B,C	0.018 B,C	65FR66443
93	Isophorone	78591					35 B,C	960 B,C	65FR66443
94	Naphthalene	91203							
95	Nitrobenzene	98953					17 B	690 B,H,U	65FR66443
96	N-Nitrosodimethylamine	62759					0.00069 B,C	3.0 B,C	65FR66443
97	N-Nitrosodi-n-Propylamine	621647					0.0050 B,C	0.51 B,C	65FR66443
98	N-Nitrosodiphenylamine	86306					3.3 B,C	6.0 B,C	65FR66443
99	Phenanthrene	85018							
100	Pyrene	129000					830 B	4,000 B	65FR66443
101	1,2,4-Trichlorobenzene	120821					35	70	68FR75510
102	Aldrin	309002	3.0 G		1.3 G		0.000049 B,C	0.000050 B,C	65FR31682 65FR66443
103	alpha-BHC	319846					0.0026 B,C	0.0049 B,C	65FR66443
104	beta-BHC	319857					0.0091 B,C	0.017 B,C	65FR66443

NATIONAL RECOMMENDED WATER QUALITY CRITERIA FOR PRIORITY TOXIC POLLUTANTS

	Priority Pollutant	CAS Number	Freshwater CMC (µg/L)	Freshwater CCC (µg/L)	Saltwater CMC (µg/L)	Saltwater CCC (µg/L)	Human Health For Consumption of: Water + Organism (µg/L)	Human Health For Consumption of: Organism Only (µg/L)	FR Cite/ Source
105	gamma-BHC (Lindane)	58899	0.95 K		0.16 G		0.98	1.8	65FR31682 68FR75510
106	delta-BHC	319868							
107	Chlordane	57749	2.4 G	0.0043 G,aa	0.09 G	0.004 G,aa	0.00080 B,C	0.00081 B,C	65FR31682 65FR66443
108	4,4'-DDT	50293	1.1 G,ii	0.001 G,aa,ii	0.13 G,ii	0.001 G,aa,ii	0.00022 B,C	0.00022 B,C	65FR31682 65FR66443
109	4,4'-DDE	72559					0.00022 B,C	0.00022 B,C	65FR66443
110	4,4'-DDD	72548					0.00031 B,C	0.00031 B,C	65FR66443
111	Dieldrin	60571	0.24 K	0.056 K,O	0.71 G	0.0019 G,aa	0.000052 B,C	0.000054 B,C	65FR31682 65FR66443
112	alpha-Endosulfan	959988	0.22 G,Y	0.056 G,Y	0.034 G,Y	0.0087 G,Y	62 B	89 B	65FR31682 65FR66443
113	beta-Endosulfan	33213659	0.22 G,Y	0.056 G,Y	0.034 G,Y	0.0087 G,Y	62 B	89 B	65FR31682 65FR66443
114	Endosulfan Sulfate	1031078					62 B	89 B	65FR66443
115	Endrin	72208	0.086 K	0.036 K,O	0.037 G	0.0023 G,aa	0.059	0.060	65FR31682 68FR75510
116	Endrin Aldehyde	7421934					0.29 B	0.30 B,H	65FR66443

NATIONAL RECOMMENDED WATER QUALITY CRITERIA FOR PRIORITY TOXIC POLLUTANTS

	Priority Pollutant	CAS Number	Freshwater CMC (µg/L)	Freshwater CCC (µg/L)	Saltwater CMC (µg/L)	Saltwater CCC (µg/L)	Human Health For Consumption of: Water + Organism (µg/L)	Human Health For Consumption of: Organism Only (µg/L)	FR Cite/ Source
117	Heptachlor	76448	0.52 G	0.0038 G,aa	0.053 G	0.0036 G,aa	0.000079 B,C	0.000079 B,C	65FR31682 65FR66443
118	Heptachlor Epoxide	1024573	0.52 G,V	0.0038 G,V,aa	0.053 G,V	0.0036 G,V,aa	0.000039 B,C	0.000039 B,C	65FR31682 65FR66443
119	Polychlorinated Biphenyls PCBs:			0.014 N,aa		0.03 N,aa	0.000064 B,C,N	0.000064 B,C,N	65FR31682 65FR66443
120	Toxaphene	8001352	0.73	0.002 aa	0.21	0.002 aa	0.00028B,C	0.00028 B,C	65FR31682 65FR66443

Footnotes:

A This recommended water quality criterion was derived from data for arsenic (III), but is applied here to total arsenic, which might imply that arsenic (III) and arsenic (V) are equally toxic to aquatic life and that their toxicities are additive. In the arsenic criteria document (EPA 440/5-84-033, January 1985), Species Mean Acute Values are given for both arsenic (III) and arsenic (V) for five species and the ratios of the SMAVs for each species range from 0.6 to 1.7. Chronic values are available for both arsenic (III) and arsenic (V) for one species; for the fathead minnow, the chronic value for arsenic (V) is 0.29 times the chronic value for arsenic (III). No data are known to be available concerning whether the toxicities of the forms of arsenic to aquatic organisms are additive.

B This criterion has been revised to reflect The Environmental Protection Agency's q1* or RfD, as contained in the Integrated Risk Information System (IRIS) as of May 17, 2002. The fish tissue bioconcentration factor (BCF) from the 1980 Ambient Water Quality Criteria document was retained in each case.

C This criterion is based on carcinogenicity of 10^{-6} risk. Alternate risk levels may be obtained by moving the decimal point (e.g., for a risk level of 10^{-5}, move the decimal point in the recommended criterion one place to the right).

D Freshwater and saltwater criteria for metals are expressed in terms of the dissolved metal in the water column. The recommended water quality criteria value was calculated by using the previous 304(a) aquatic life criteria expressed in terms of total recoverable metal, and multiplying it by a conversion factor (CF). The term "Conversion Factor" (CF) represents the recommended conversion factor for converting a metal criterion expressed as the total recoverable fraction in the water column to a criterion expressed as the dissolved fraction in the water column. (Conversion Factors for saltwater CCCs are not currently available. Conversion factors derived for saltwater CMCs have been used for both saltwater CMCs and CCCs). See "Office of Water Policy and Technical Guidance on Interpretation and Implementation of Aquatic Life Metals Criteria," October 1, 1993, by Martha G. Prothro, Acting Assistant Administrator for

Water, available from the Water Resource center, USEPA, 401 M St., SW, mail code RC4100, Washington, DC 20460; and 40CFR§131.36(b)(1). Conversion Factors applied in the table can be found in Appendix A to the Preamble- Conversion Factors for Dissolved Metals.

E The freshwater criterion for this metal is expressed as a function of hardness (mg/L) in the water column. The value given here corresponds to a hardness of 100 mg/L. Criteria values for other hardness may be calculated from the following: CMC (dissolved) = $\exp\{m_A [\ln(\text{hardness})] + b_A\}$ (CF), or CCC (dissolved) = $\exp\{m_C [\ln(\text{hardness})] + b_C\}$ (CF) and the parameters specified in Appendix B- Parameters for Calculating Freshwater Dissolved Metals Criteria That Are Hardness-Dependent.

F Freshwater aquatic life values for pentachlorophenol are expressed as a function of pH, and are calculated as follows: CMC = exp(1.005(pH)-4.869); CCC = exp(1.005(pH)-5.134). Values displayed in table correspond to a pH of 7.8.

G This Criterion is based on 304(a) aquatic life criterion issued in 1980, and was issued in one of the following documents: Aldrin/Dieldrin (EPA 440/5-80-019), Chlordane (EPA 440/5-80-027), DDT (EPA 440/5-80-038), Endosulfan (EPA 440/5-80-046), Endrin (EPA 440/5-80-047), Heptachlor (EPA 440/5-80-052), Hexachlorocyclohexane (EPA 440/5-80-054), Silver (EPA 440/5-80-071). The Minimum Data Requirements and derivation procedures were different in the 1980 Guidelines than in the 1985 Guidelines. For example, a "CMC" derived using the 1980 Guidelines was derived to be used as an instantaneous maximum. If assessment is to be done using an averaging period, the values given should be divided by 2 to obtain a value that is more comparable to a CMC derived using the 1985 Guidelines.

H No criterion for protection of human health from consumption of aquatic organisms excluding water was presented in the 1980 criteria document or in the *1986 Quality Criteria for Water*. Nevertheless, sufficient information was presented in the 1980 document to allow the calculation of a criterion, even though the results of such a calculation were not shown in the document.

I This criterion for asbestos is the Maximum Contaminant Level (MCL) developed under the Safe Drinking Water Act (SDWA).

J This fish tissue residue criterion for methylmercury is based on a total fish consumption rate of 0.0175 kg/day.

K This recommended criterion is based on a 304(a) aquatic life criterion that was issued in the *1995 Updates: Water Quality Criteria Documents for the Protection of Aquatic Life in Ambient Water*, (EPA-820-B-96-001, September 1996). This value was derived using the GLI Guidelines (60FR15393-15399, March 23, 1995; 40CFR132 Appendix A); the difference between the 1985 Guidelines and the GLI Guidelines are explained on page iv of the 1995 Updates. None of the decisions concerning the derivation of this criterion were affected by any considerations that are specific to the Great Lakes.

L The CMC = 1/[(f1/CMC1) + (f2/CMC2)] where f1 and f2 are the fractions of total selenium that are treated as selenite and selenate, respectively, and CMC1 and CMC2 are 185.9 μg/l and 12.82 μg/l, respectively.

M EPA is currently reassessing the criteria for arsenic.

N This criterion applies to total pcbs, (e.g., the sum of all congener or all isomer or homolog or Aroclor analyses.)

O The derivation of the CCC for this pollutant (Endrin) did not consider exposure through the diet, which is probably important for aquatic life occupying upper trophic levels.

P Although a new RfD is available in IRIS, the surface water criteria will not be revised until the National Primary Drinking Water Regulations: Stage 2 Disinfectants and Disinfection Byproducts Rule (Stage 2 DBPR) is completed, since public comment on the relative source contribution (RSC) for chloroform is anticipated.

Q This recommended water quality criterion is expressed as μg free cyanide (as CN)/L.

R This value for selenium was announced (61FR58444-58449, November 14, 1996) as a proposed GLI 303(c) aquatic life criterion. EPA is currently working on this criterion and so this value might change substantially in the near future.

S This recommended water quality criterion for arsenic refers to the inorganic form only.

NATIONAL RECOMMENDED WATER QUALITY CRITERIA FOR PRIORITY TOXIC POLLUTANTS

T This recommended water quality criterion for selenium is expressed in terms of total recoverable metal in the water column. It is scientifically acceptable to use the conversion factor (0.996- CMC or 0.922- CCC) that was used in the GLI to convert this to a value that is expressed in terms of dissolved metal.

U The organoleptic effect criterion is more stringent than the value for priority toxic pollutants.

V This value was derived from data for heptachlor and the criteria document provides insufficient data to estimate the relative toxicities of heptachlor and heptachlor epoxide.

W Although EPA has not published a completed criteria document for butylbenzyl phthalate it is EPA's understanding that sufficient data exist to allow calculation of aquatic criteria. It is anticipated that industry intends to publish in the peer reviewed literature draft aquatic life criteria generated in accordance with EPA Guidelines. EPA will review such criteria for possible issuance as national WQC.

X There is a full set of aquatic life toxicity data that show that DEHP is not toxic to aquatic organisms at or below its solubility limit.

Y This value was derived from data for endosulfan and is most appropriately applied to the sum of alpha-endosulfan and beta-endosulfan.

Z A more stringent MCL has been issued by EPA. Refer to drinking water regulations (40 CFR 141) or Safe Drinking Water Hotline (1-800-426-4791) for values.

aa This criterion is based on a 304(a) aquatic life criterion issued in 1980 or 1986, and was issued in one of the following documents: Aldrin/Dieldrin (EPA 440/5-80-019), Chlordane (EPA 440/5-80-027), DDT (EPA 440/5-80-038), Endrin (EPA 440/5-80-047), Heptachlor (EPA 440/5-80-052), Polychlorinated biphenyls (EPA 440/5-80-068), Toxaphene (EPA 440/5-86-006). This CCC is currently based on the Final Residue Value (FRV) procedure. Since the publication of the Great Lakes Aquatic Life Criteria Guidelines in 1995 (60FR15393-15399, March 23, 1995), the Agency no longer uses the Final Residue Value procedure for deriving CCCs for new or revised 304(a) aquatic life criteria. Therefore, the Agency anticipates that future revisions of this CCC will not be based on the FRV procedure.

bb This water quality criterion is based on a 304(a) aquatic life criterion that was derived using the 1985 Guidelines (*Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses*, PB85-227049, January 1985) and was issued in one of the following criteria documents: Arsenic (EPA 440/5-84-033), Cadmium (EPA-822-R-01-001), Chromium (EPA 440/5-84-029), Copper (EPA 440/5-84-031), Cyanide (EPA 440/5- 84-028), Lead (EPA 440/5-84-027), Nickel (EPA 440/5-86-004), Pentachlorophenol (EPA 440/5-86-009), Toxaphene, (EPA 440/5-86-006), Zinc (EPA 440/5-87- 003).

cc When the concentration of dissolved organic carbon is elevated, copper is substantially less toxic and use of Water-Effect Ratios might be appropriate.

dd The selenium criteria document (EPA 440/5-87-006, September 1987) provides that if selenium is as toxic to saltwater fishes in the field as it is to freshwater fishes in the field, the status of the fish community should be monitored whenever the concentration of selenium exceeds 5.0 μg/L in salt water because the saltwater CCC does not take into account uptake via the food chain.

ee This recommended water quality criterion was derived on page 43 of the mercury criteria document (EPA 440/5-84-026, January 1985). The saltwater CCC of 0.025 ug/L given on page 23 of the criteria document is based on the Final Residue Value procedure in the 1985 Guidelines. Since the publication of the Great Lakes Aquatic Life Criteria Guidelines in 1995 (60FR15393-15399, March 23, 1995), the Agency no longer uses the Final Residue Value procedure for deriving CCCs for new or revised 304(a) aquatic life criteria.

ff This recommended water quality criterion was derived in *Ambient Water Quality Criteria Saltwater Copper Addendum* (Draft, April 14, 1995) and was promulgated in the Interim final National Toxics Rule (60FR22228-222237, May 4, 1995).

gg EPA is actively working on this criterion and so this recommended water quality criterion may change substantially in the near future.

hh This recommended water quality criterion was derived from data for inorganic mercury (II), but is applied here to total mercury. If a substantial portion of the mercury in the water column is methylmercury, this criterion will probably be under protective. In addition, even though inorganic mercury is converted to

methylmercury and methylmercury bioaccumulates to a great extent, this criterion does not account for uptake via the food chain because sufficient data were not available when the criterion was derived.

ii This criterion applies to DDT and its metabolites (i.e., the total concentration of DDT and its metabolites should not exceed this value).

jj This recommended water quality criterion is expressed as total cyanide, even though the IRIS RFD we used to derive the criterion is based on free cyanide. The multiple forms of cyanide that are present in ambient water have significant differences in toxicity due to their differing abilities to liberate the CN-moiety. Some complex cyanides require even more extreme conditions than refluxing with sulfuric acid to liberate the CN-moiety. Thus, these complex cyanides are expected to have little or no 'bioavailability' to humans. If a substantial fraction of the cyanide present in a water body is present in a complexed form (e.g., $Fe_4[Fe(CN)_6]_3$), this criterion may be over conservative.

kk This recommended water quality criterion was derived using the cancer slope factor of 1.4 (LMS exposure from birth).

NATIONAL RECOMMENDED WATER QUALITY CRITERIA FOR NON PRIORITY POLLUTANTS

	Non Priority Pollutant	CAS Number	Freshwater CMC (µg/L)	Freshwater CCC (µg/L)	Saltwater CMC (µg/L)	Saltwater CCC (µg/L)	Human Health For Consumption of: Water + Organism (µg/L)	Human Health For Consumption of: Organism Only (µg/L)	FR Cite/Source
1	Alkalinity	--		20000 F					Gold Book
2	Aluminum pH 6.5 - 9.0	7429905	750 G,I	87 G,I,L					53FR33178
3	Ammonia	7664417	FRESHWATER CRITERIA ARE pH, Temperature and Life-stage DEPENDENT -- SEE DOCUMENT D SALTWATER CRITERIA ARE pH AND TEMPERATURE DEPENDENT						EPA822-R-99-014 EPA440/5-88-004
4	Aesthetic Qualities	--	NARRATIVE STATEMENT-- SEE DOCUMENT						Gold Book
5	Bacteria	--	FOR PRIMARY RECREATION AND SHELLFISH USES -- SEE DOCUMENT						Gold Book
6	Barium	7440393					1,000 A		Gold Book
7	Boron	--	NARRATIVE STATEMENT-- SEE DOCUMENT						Gold Book
8	Chloride	16887006	860000 G	230000 G					53FR19028
9	Chlorine	7782505	19	11	13	7.5	C		Gold Book
10	Chlorophenoxy Herbicide (2,4,5,-TP)	93721					10 A		Gold Book
11	Chlorophenoxy Herbicide (2,4-D)	94757					100 A,C		Gold Book
12	Chloropyrifos	2921882	0.083 G	0.041 G	0.011 G	0.0056 G			Gold Book
13	Color	--	NARRATIVE STATEMENT-- SEE DOCUMENT F						Gold Book

NATIONAL RECOMMENDED WATER QUALITY CRITERIA FOR NON PRIORITY POLLUTANTS

	Non Priority Pollutant	CAS Number	Freshwater CMC (µg/L)	Freshwater CCC (µg/L)	Saltwater CMC (µg/L)	Saltwater CCC (µg/L)	Human Health For Consumption of: Water + Organism (µg/L)	Human Health For Consumption of: Organism Only (µg/L)	FR Cite/Source
14	Demeton	8065483		0.1 F		0.1 F			Gold Book
15	Ether, Bis(Chloromethyl)	542881					0.00010 E, H	0.00029 E,H	65FR66443
16	Gases, Total Dissolved	--		NARRATIVE STATEMENT -- SEE DOCUMENT F					Gold Book
17	Guthion	86500		0.01 F		0.01 F			Gold Book
18	Hardness	--		NARRATIVE STATEMENT-- SEE DOCUMENT					Gold Book
19	Hexachlorocyclo-hexane-Technical	319868					0.0123	0.0414	Gold Book
20	Iron	7439896		1000 F			300 A		Gold Book
21	Malathion	121755		0.1 F		0.1 F			Gold Book
22	Manganese	7439965					50 A,O	100 A	Gold Book
23	Methoxychlor	72435		0.03 F		0.03 F	100 A,C		Gold Book
24	Mirex	2385855		0.001 F		0.001 F			Gold Book
25	Nitrates	14797558					10,000 A		Gold Book
26	Nitrosamines	--					0.0008	1.24	Gold Book
27	Dinitrophenols	25550587					69	5300	65FR66443
28	Nonylphenol	1044051	28	6.6	7.0	1.7			71FR9337
29	Nitrosodibutylamine,N	924163					0.0063 A,H	0.22 A,H	65FR66443
30	Nitrosodiethylamine,N	55185					0.0008 A,H	1.24 A,H	Gold Book

NATIONAL RECOMMENDED WATER QUALITY CRITERIA FOR NON PRIORITY POLLUTANTS

	Non Priority Pollutant	CAS Number	Freshwater CMC (µg/L)	Freshwater CCC (µg/L)	Saltwater CMC (µg/L)	Saltwater CCC (µg/L)	Human Health For Consumption of: Water + Organism (µg/L)	Human Health For Consumption of: Organism Only (µg/L)	FR Cite/Source
31	Nitrosopyrrolidine,N	930552					0.016 H	34 H	65FR66443
32	Oil and Grease	--	NARRATIVE STATEMENT -- SEE DOCUMENT F						Gold Book
33	Oxygen, Dissolved Freshwater	7782447	WARMWATER AND COLDWATER MATRIX -- SEE DOCUMENT N						Gold Book
	Oxygen, Dissolved Saltwater				SALTWATER – SEE DOCUMENT				EPA-822R-00-012
34	Diazinon	333415	0.17	0.17	0.82	0.82			71FR9336
35	Parathion	56382	0.065 J	0.013 J					Gold Book
36	Pentachlorobenzene	608935					1.4 E	1.5 E	65FR66443
37	pH	--		6.5 - 9 F		6.5 - 8.5 F,K	5 - 9		Gold Book
38	Phosphorus Elemental	7723140				0.1 F,K			Gold Book
39	Nutrients	--	See EPA's Ecoregional criteria for Total Phosphorus, Total Nitrogen, Chlorophyll *a* and Water Clarity (Secchi depth for lakes; turbidity for streams and rivers) (& Level III Ecoregional criteria)						P
40	Solids Dissolved and Salinity	--					250,000 A		Gold Book
41	Solids Suspended and Turbidity	--	NARRATIVE STATEMENT -- SEE DOCUMENT F						Gold Book
42	Sulfide-Hydrogen Sulfide	7783064		2.0 F		2.0 F			Gold Book
43	Tainting Substances	--	NARRATIVE STATEMENT-- SEE DOCUMENT						Gold Book
44	Temperature	--	SPECIES DEPENDENT CRITERIA -- SEE DOCUMENT M						Gold Book

NATIONAL RECOMMENDED WATER QUALITY CRITERIA FOR NON PRIORITY POLLUTANTS

	Non Priority Pollutant	CAS Number	Freshwater CMC (µg/L)	Freshwater CCC (µg/L)	Saltwater CMC (µg/L)	Saltwater CCC (µg/L)	Human Health For Consumption of: Water + Organism (µg/L)	Human Health For Consumption of: Organism Only (µg/L)	FR Cite/Source
45	Tetrachlorobenzene,1,2,4,5-	95943					0.97 E	1.1 E	65FR66443
46	Tributyltin (TBT)	--	0.46 Q	0.072 Q	0.42 Q	0.0074 Q			EPA 822-F-00-008
47	Trichlorophenol,2,4,5-	95954					1,800 B,E	3,600 B,E	65FR66443

Footnotes:

A This human health criterion is the same as originally published in the Red Book which predates the 1980 methodology and did not utilize the fish ingestion BCF approach. This same criterion value is now published in the Gold Book.

B The organoleptic effect criterion is more stringent than the value presented in the non priority pollutants table.

C A more stringent Maximum Contaminant Level (MCL) has been issued by EPA under the Safe Drinking Water Act. Refer to drinking water regulations 40CFR141 or Safe Drinking Water Hotline (1-800-426-4791) for values.

D According to the procedures described in the *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses*, except possibly where a very sensitive species is important at a site, freshwater aquatic life should be protected if both conditions specified in Appendix C to the Preamble- Calculation of Freshwater Ammonia Criterion are satisfied.

E This criterion has been revised to reflect EPA's q1* or RfD, as contained in the Integrated Risk Information System (IRIS) as of May 17, 2002. The fish tissue bioconcentration factor (BCF) used to derive the original criterion was retained in each case.

F The derivation of this value is presented in the Red Book (EPA 440/9-76-023, July, 1976).

G This value is based on a 304(a) aquatic life criterion that was derived using the 1985 Guidelines (*Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses*, PB85-227049, January 1985) and was issued in one of the following criteria documents: Aluminum (EPA 440/5-86-008); Chloride (EPA 440/5-88-001); Chloropyrifos (EPA 440/5-86-005).

H This criterion is based on carcinogenicity of 10^{-6} risk. Alternate risk levels may be obtained by moving the decimal point (e.g., for a risk level of 10^{-5}, move the decimal point in the recommended criterion one place to the right).

I This value for aluminum is expressed in terms of total recoverable metal in the water column.

J This value is based on a 304(a) aquatic life criterion that was issued in the *1995 Updates: Water Quality Criteria Documents for the Protection of Aquatic Life in Ambient Water* (EPA-820-B-96-001). This value was derived using the GLI Guidelines (60FR15393-15399, March 23, 1995; 40CFR132 Appendix A); the differences between the 1985 Guidelines and the GLI Guidelines are explained on page iv of the 1995 Updates. No decision concerning this criterion was affected by any considerations that are specific to the Great Lakes.

K According to page 181 of the Red Book:

For open ocean waters where the depth is substantially greater than the euphotic zone, the pH should not be changed more than 0.2 units from the naturally occurring variation or any case outside the range of 6.5 to 8.5. For shallow, highly productive coastal and estuarine areas where naturally occurring pH variations approach the lethal limits of some species, changes in pH should be avoided but in any case should not exceed the limits established for fresh water, i.e., 6.5-9.0.

L There are three major reasons why the use of Water-Effect Ratios might be appropriate. (1) The value of 87 μg/l is based on a toxicity test with the striped bass in water with pH= 6.5-6.6 and hardness <10 mg/L. Data in "Aluminum Water-Effect Ratio for the 3M Plant Effluent Discharge, Middleway, West Virginia" (May 1994) indicate that aluminum is substantially less toxic at higher pH and hardness, but the effects of pH and hardness are not well quantified at this time. (2) In tests with the brook trout at low pH and hardness, effects increased with increasing concentrations of total aluminum even though the concentration of dissolved aluminum was constant, indicating that total recoverable is a more appropriate measurement than dissolved, at least when particulate aluminum is primarily aluminum hydroxide particles. In surface waters, however, the total recoverable procedure might measure aluminum associated with clay particles, which might be less toxic than aluminum associated with aluminum hydroxide. (3) EPA is aware of field data indicating that many high quality waters in the U.S. contain more than 87 μg aluminum/L, when either total recoverable or dissolved is measured.

M U.S. EPA. 1973. Water Quality Criteria 1972. EPA-R3-73-033. National Technical Information Service, Springfield, VA.; U.S. EPA. 1977. Temperature Criteria for Freshwater Fish: Protocol and Procedures. EPA-600/3-77-061. National Technical Information Service, Springfield, VA.

N U.S. EPA. 1986. Ambient Water Quality Criteria for Dissolved Oxygen. EPA 440/5-86-003. National Technical Information Service, Springfield, VA.

O This criterion for manganese is not based on toxic effects, but rather is intended to minimize objectionable qualities such as laundry stains and objectionable tastes in beverages.

P Lakes and Reservoirs in Nutrient Ecoregion: II EPA 822-B-00-007, III EPA 822-B-01-008, IV EPA 822-B-01-009, V EPA 822-B-01-010, VI EPA 822-B-00-008 , VII EPA 822-B-00-009, VIII EPA 822-B-01-015, IX EPA 822-B-00-011, XI EPA 822-B-00-012, XII EPA 822-B-00-013, XIII EPA 822-B-00-014, XIV EPA 822-B-01-011; Rivers and Streams in Nutrient Ecoregion: I EPA 822-B-01-012, II EPA 822-B-00-015, III EPA 822-B-00-016, IV EPA 822-B-01-013, V EPA 822-B-01-014, VI EPA 822-B-00-017, VII EPA 822-B-00-018, VIII EPA 822-B-01-015, IX EPA 822-B-00-019, X EPA 822-B-01-016, XI EPA 822-B-00-020, XII EPA 822-B-00-021, XIV EPA 822-B-00-022; and Wetlands in Nutrient Ecoregion XIII EPA 822-B-00-023.

Q EPA announced the availability of a draft updated tributyltin (TBT) document on August 7, 1997 (62FR42554). The Agency has reevaluated this document and anticipates releasing an updated document for public comment in the near future.

NATIONAL RECOMMENDED WATER QUALITY CRITERIA FOR ORGANOLEPTIC EFFECTS

	Pollutant	CAS Number	Organoleptic Effect Criteria (μg/L)	FR Cite/Source
1	**Acenaphthene**	**83329**	**20**	**Gold Book**
2	Monochlorobenzene	108907	20	Gold Book
3	**3-Chlorophenol**	**--**	**0.1**	**Gold Book**
4	4-Chlorophenol	106489	0.1	Gold Book
5	**2,3-Dichlorophenol**	**--**	**0.04**	**Gold Book**
6	2,5-Dichlorophenol	--	0.5	Gold Book
7	**2,6-Dichlorophenol**	**--**	**0.2**	**Gold Book**
8	3,4-Dichlorophenol	--	0.3	Gold Book
9	**2,4,5-Trichlorophenol**	**95954**	**1**	**Gold Book**
10	2,4,6-Trichloropehnol	88062	2	Gold Book
11	**2,3,4,6-Tetrachlorophenol**	**--**	**1**	**Gold Book**
12	2-Methyl-4-Chlorophenol	--	1800	Gold Book
13	**3-Methyl-4-Chlorophenol**	**59507**	**3000**	**Gold Book**
14	3-Methyl-6-Chlorophenol	--	20	Gold Book
15	**2-Chlorophenol**	**95578**	**0.1**	**Gold Book**
16	Copper	7440508	1000	Gold Book
17	**2,4-Dichlorophenol**	**120832**	**0.3**	**Gold Book**
18	2,4-Dimethylpehnol	105679	400	Gold Book

NATIONAL RECOMMENDED WATER QUALITY CRITERIA FOR ORGANOLEPTIC EFFECTS

	Pollutant	CAS Number	Organoleptic Effect Criteria (μg/L)	FR Cite/Source
19	Hexachlorocyclopentadiene	77474	1	Gold Book
20	Nitrobenzene	98953	30	Gold Book
21	Pentachlorophenol	87865	30	Gold Book
22	Phenol	108952	300	Gold Book
23	Zinc	7440666	5000	45 FR79341

General notes:

1. These criteria are based on organoleptic (taste and odor) effects. Because of variations in chemical nomenclature systems, this listing of pollutants does not duplicate the listing in Appendix A of 40 CFR Part 423. Also listed are the Chemical Abstracts Service (CAS) registry numbers, which provide a unique identification for each chemical.

NATIONAL RECOMMENDED WATER QUALITY CRITERIA

Additional Notes:

1. Criteria Maximum Concentration and Criterion Continuous Concentration

The Criteria Maximum Concentration (CMC) is an estimate of the highest concentration of a material in surface water to which an aquatic community can be exposed briefly without resulting in an unacceptable effect. The Criterion Continuous Concentration (CCC) is an estimate of the highest concentration of a material in surface water to which an aquatic community can be exposed indefinitely without resulting in an unacceptable effect. The CMC and CCC are just two of the six parts of an aquatic life criterion; the other four parts are the acute averaging period, chronic averaging period, acute frequency of allowed exceedence, and chronic frequency of allowed exceedence. Because 304(a) aquatic life criteria are national guidance, they are intended to be protective of the vast majority of the aquatic communities in the United States.

2. Criteria Recommendations for Priority Pollutants, Non Priority Pollutants and Organoleptic Effects

This compilation lists all priority toxic pollutants and some non priority toxic pollutants, and both human health effect and organoleptic effect criteria issued pursuant to CWA §304(a). Blank spaces indicate that EPA has no CWA §304(a) criteria recommendations. For a number of non-priority toxic pollutants not listed, CWA §304(a) "water + organism" human health criteria are not available, but EPA has published MCLs under the SDWA that may be used in establishing water quality standards to protect water supply designated uses. Because of variations in chemical nomenclature systems, this listing of toxic pollutants does not duplicate the listing in Appendix A of 40 CFR Part 423. Also listed are the Chemical Abstracts Service CAS registry numbers, which provide a unique identification for each chemical.

3. Human Health Risk

The human health criteria for the priority and non priority pollutants are based on carcinogenicity of 10^{-6} risk. Alternate risk levels may be obtained by moving the decimal point (e.g., for a risk level of 10^{-5}, move the decimal point in the recommended criterion one place to the right).

4. Water Quality Criteria published pursuant to Section 304(a) or Section 303(c) of the CWA

Many of the values in the compilation were published in the California Toxics Rule. Although such values were published pursuant to Section 303(c) of the CWA, they represent the Agency's most recent calculation of water quality criteria and are thus the Agency's 304(a) criteria.

5. Calculation of Dissolved Metals Criteria

The 304(a) criteria for metals, shown as dissolved metals, are calculated in one of two ways. For freshwater metals criteria that are hardness-dependent, the dissolved metal criteria were calculated using a hardness of 100 mg/l as $CaCO_3$ for illustrative purposes only. Saltwater and freshwater metals' criteria that are not hardness-dependent are calculated by multiplying the total recoverable criteria before rounding by the appropriate conversion factors. The final dissolved metals' criteria in the table are rounded to two significant figures. Information regarding the calculation of hardness dependent conversion factors are included in the footnotes.

6. Maximum Contaminant Levels

The compilation includes footnotes for pollutants with Maximum Contaminant Levels (MCLs) more stringent than the recommended water quality criteria in the compilation. MCLs for these pollutants are not included in the compilation, but can be found in the appropriate drinking water regulations (40 CFR 141.11-16 and 141.60-63), or can be accessed through the Safe Drinking Water Hotline (800-426-4791) or the Internet

(http://www.epa.gov/waterscience/drinking/standards/dwstandards.pdf).

7. Organoleptic Effects

The compilation contains 304(a) criteria for pollutants with toxicity-based criteria as well as non-toxicity based criteria. The basis for the non-toxicity based criteria are organoleptic effects (e.g., taste and odor) which would make water and edible aquatic life unpalatable but not toxic to humans. The table includes criteria for organoleptic effects for 23 pollutants. Pollutants with organoleptic effect criteria more stringent than the criteria based on toxicity (e.g., included in both the priority and non-priority pollutant tables) are footnoted as such.

8. Gold Book

The "Gold Book" is Quality Criteria for Water: 1986. EPA 440/5-86-001.

9. Correction of Chemical Abstract Services Number

The Chemical Abstract Services number (CAS) for Bis(2-Chlorisoprpyl) Ether, has been revised in IRIS and in the table. The correct CAS number for this chemical is 108-60-1. The previous CAS number for this pollutant was 39638-32-9.

10. Contaminants with Blanks

EPA has not calculated criteria for contaminants with blanks. However, permit authorities should address these contaminants in NPDES permit actions using the States' existing narrative criteria for toxics.

11. Specific Chemical Calculations

A. Selenium

Aquatic Life

This compilation contains aquatic life criteria for selenium that are the same as those published in the proposed CTR. In the CTR, EPA proposed an acute criterion for selenium based on the criterion proposed for selenium in the Water Quality Guidance for the Great Lakes System (61 FR 58444). The GLI and CTR proposals take into account data showing that selenium's two prevalent oxidation states in water, selenite and selenate, present differing potentials for aquatic toxicity, as well as new data indicating that various forms of selenium are additive. The new approach produces a different selenium acute criterion concentration, or CMC, depending upon the relative proportions of selenite, selenate, and other forms of selenium that are present.

EPA is currently undertaking a reassessment of selenium, and expects the 304(a) criteria for selenium will be revised based on the final reassessment (63FR26186). However, until such time as revised water quality criteria for selenium are published by the Agency, the recommended water quality criteria in this compilation are EPA's current 304(a) criteria.

Appendices

Appendix A - Conversion Factors for Dissolved Metals

Metal	Conversion Factor freshwater CMC	Conversion Factor freshwater CCC	Conversion Factor saltwater CMC	Conversion Factor saltwater CCC[1]
Arsenic	1.000	1.000	1.000	1.000
Cadmium	1.136672-[(ln hardness)(0.041838)]	1.101672-[(ln hardness)(0.041838)]	0.994	0.994
Chromium III	0.316	0.860	--	--
Chromium VI	0.982	0.962	0.993	0.993
Copper	0.960	0.960	0.83	0.83
Lead	1.46203-[(ln hardness)(0.145712)]	1.46203-[(ln hardness)(0.145712)]	0.951	0.951
Mercury	0.85	0.85	0.85	0.85
Nickel	0.998	0.997	0.990	0.990
Selenium	--	--	0.998	0.998
Silver	0.85	--	0.85	--
Zinc	0.978	0.986	0.946	0.946

Appendix B - Parameters for Calculating Freshwater Dissolved Metals Criteria That Are Hardness-Dependent

Chemical	m_A	b_A	m_C	b_C	Freshwater Conversion Factors (CF)	
					CMC	CCC
Cadmium	1.0166	-3.924	0.7409	-4.719	1.136672-[(ln hardness)(0.041838)]	1.101672-[(ln hardness)(0.041838)]
Chromium III	0.8190	3.7256	0.8190	0.6848	0.316	0.860
Copper	0.9422	-1.700	0.8545	-1.702	0.960	0.960
Lead	1.273	-1.460	1.273	-4.705	1.46203-[(ln hardness)(0.145712)]	1.46203-[(ln hardness)(0.145712)]
Nickel	0.8460	2.255	0.8460	0.0584	0.998	0.997
Silver	1.72	-6.59	--	--	0.85	--
Zinc	0.8473	0.884	0.8473	0.884	0.978	0.986

Hardness-dependant metals' criteria may be calculated from the following:
CMC (dissolved) = $\exp\{m_A [\ln(\text{hardness})] + b_A\}$ (CF)
CCC (dissolved) = $\exp\{m_C [\ln(\text{hardness})] + b_C\}$ (CF)

Appendix C - Calculation of Freshwater Ammonia Criterion

1. The one-hour average concentration of total ammonia nitrogen (in mg N/L) does not exceed, more than once every three years on the average, the CMC (acute criterion) calculated using the following equations.

Where salmonid fish are present:

$$CMC = \frac{0.275}{\quad} + \frac{39.0}{\quad}$$

$$1 + 10^{7.204-pH} \qquad 1 + 10^{pH-7.204}$$

Or where salmonid fish are not present:

$$CMC = \frac{0.411}{1 + 10^{7.204-pH}} + \frac{58.4}{1 + 10^{pH-7.204}}$$

2A. The thirty-day average concentration of total ammonia nitrogen (in mg N/L) does not exceed, more than once every three years on the average, the CCC (chronic criterion) calculated using the following equations.

When fish early life stages are present:

$$CCC = \left(\frac{0.0577}{1 + 10^{7.688-pH}} + \frac{2.487}{1 + 10^{pH-7.688}} \right) \bullet \quad MIN\ (2.85,\ 1.45 \cdot 10^{0.028 \cdot (25-T)})$$

When fish early life stages are absent:

$$CCC = \left(\frac{0.0577}{1 + 10^{7.688-pH}} + \frac{2.487}{1 + 10^{pH-7.688}} \right) \bullet \quad 1.45 \cdot 10^{0.028 \cdot (25-MAX\ (T,7))}$$

2B. In addition, the highest four-day average within the 30-day period should not exceed 2.5 times the CCC.

Exhibit K

David A Ludder

From: Bethel.Heidi@epamail.epa.gov
Sent: Friday, December 15, 2006 10:15 AM
To: DavidALudder@comcast.net
Cc: Swietlik.William@epamail.epa.gov; Borum.Denis@epamail.epa.gov; Cruz.Luis@epamail.epa.gov; Pendergast.Jim@epamail.epa.gov
Subject: Re: National Recommended Water Quality Criteria: Acrolein and Phenol

Dear Mr. Ludder,

Thank you for your e-mail of December 5, 2006 (attached below) regarding the National Recommended Water Quality Criteria Table and the current criteria listed in the table for phenol and acrolein. As noted in your e-mail, criteria for these two pollutants have not been updated with the most current Integrated Risk Information System (IRIS) reference doses (RfDs). The current criteria values for acrolein are based on an Acceptable Daily Intake (ADI) value of 15.6 ug/kg/day (National Academy of Sciences, 1977). The values for phenol are based on an RfD of 6 x 10
-1 mg/kg-day (IRIS, 2/1/90). The updated IRIS RfDs are 3 x 10-1 mg/kg-day for phenol (IRIS, 9/30/02) and 5 x 10-4 mg/kg-day (IRIS,
6/3/03) for acrolein.

Criteria in the table are updated by the Office of Water on a periodic basis. The table was updated in 2002 and most recently in 2003 in a Federal Register Notice (FRN) (http://www.epa.gov/fedrgstr/EPA-WATER/2003/December/Day-31/w32211.htm).
The 2003 FRN included updates to fifteen pollutants. Due to process time lines for updating criteria (i.e. publication in public dockets, etc.), the most recent updates to the table do not include the current RfDs for phenol and acrolein. This in no way reflects a rejection of the IRIS published RfDs. The Office of Water fully accepts IRIS values as the Agency's most recent quantitative risk assessment values which have been reviewed and agreed upon by an interdisciplinary group of scientists representing various Program Offices within the Agency and represents an Agency wide consensus. States have the option, but are not required, to update ambient water quality criteria published by the Office of Water as current information becomes available on IRIS. Until the Office of Water updates criteria values, the criteria published in the table referenced are considered acceptable for use by the states.

For your future reference, a companion document to the table entitled National Recommended Water Quality Criteria: 2002, Human Health Criteria
Matrix(http://www.epa.gov/waterscience/criteria/hh_calc_matrix.pdf)
shows the parameters used for calculation of the ambient water quality criteria (AWQC) for all chemicals which were updated in 2002. In this matrix, it can be seen that the only parameter which changed in the calculation for both phenol and acrolein in the 2002 updates was the fish intake level, which increased from 6.5 g/day to 17.5 g/day. Page
19 of the document presents equations which may be used for the purpose of updating the ambient water quality criteria with the current RfDs.
Note that for the 15 chemicals updated in 2003, the parameter values included in the FRN supersede those in the 2002 matrix document.

Should you have any further questions about this matter, please contact me directly.

Sincerely,

Heidi Bethel

Heidi Bethel, Ph.D.
Environmental Scientist
(202) 566-2054

Postal Mail Address:
Do not use for FedEX or Courier
US EPA
Office of Water
Office of Science and Technology
Health and Ecological Criteria Division (4304T) 1200 Pennsylvania Avenue, NW Washington, DC 20460
http://epa.gov/waterscience/

----- Forwarded by William Swietlik/DC/USEPA/US on 12/05/2006 08:42 AM

Jim Pendergast/DC/US EPA/US
12/05/2006 08:05 AM

To
DavidALudder@comcast.net

cc
William Swietlik/DC/USEPA/US@EPA

Subject
Re: National Recommended Water Quality Criteria: Acrolein and Phenol(Document link: William Swietlik)

Dear Mr. Ludder:

Thank you for your note. I will forward it to William Swietlik, whose branch has the lead for the national criteria.

Jim Pendergast
Health and Ecological Criteria Division
US EPA
1200 Pennsylvania Ave, NW (MC 4304T)
Washington, DC 20004
202-566-0398 (phone)
202-566-0409 (fax)

David A Ludder

<DavidALudder@comcast.net>

12/05/2006 07:35 AM

To
Jim Pendergast/DC/USEPA/US@EPA

cc
Luis Cruz/DC/USEPA/US@EPA

Subject
National Recommended Water Quality Criteria: Acrolein and Phenol

Please respond to
DavidALudder@comcast.net

Dear Mr. Pendergast:

It appears that the current National Recommended Water Quality Criteria (2006) table at http://www.epa.gov/waterscience/criteria/nrwqc-2006.pdf does not reflect oral references doses (RfDs) in the Integrated Risk Information System for Acrolein http://www.epa.gov/iris/subst/0364.htm and Phenol http://www.epa.gov/iris/subst/0088.htm published on June 3, 2003 and September 30, 2002, respectively.

Does this fact reflect a deliberate rejection of the Acrolein and Phenol oral RfDs for use in developing recommended criteria or is it simply that human health-based recommended criteria are revised only periodically and have not been reviewed since the new RfDs for Acrolein and Phenol were published?

I note that the last published revision of human health-based recommended criteria was in December 2003 http://www.epa.gov/waterscience/criteria/humanhealth/15table-fs.htm. I also note that the recommended criterion for Phenol is footnoted as follows: "This criterion has been revised to reflect The Environmental Protection Agency=s q1* or RfD, as contained in the Integrated Risk Information System (IRIS) as of May 17, 2002."

LAW OFFICE OF
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9150 McDougal Court, Tallahassee, Florida 32312-4208
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DavidALudder@comcast.net

Exhibit L

BEFORE THE
ALABAMA ENVIRONMENTAL MANAGEMENT COMMISSION

IN THE MATTER OF:

PETITION TO AMEND ADEM ADMIN. CODE CHAP. 335-6-10, APPENDIX A

[Alabama League of Environmental Action Voters, Alabama Rivers Alliance, Inc., Black Warrior Riverkeeper, Inc., Cahaba River Society, Inc., Choctawhatchee Riverkeeper, Episcopal Diocese of Alabama - Task Force for the Stewardship of Creation, Flint River Conservation Association, Inc., Friends of the Little Cahaba River, Friends of the Locust Fork River, Friends of Hurricane Creek, Friends of Rural Alabama, Inc., Lake Watch of Lake Martin, Inc., Lookout Mountain Heritage Alliance, Inc., Mobile Baykeeper, North Alabama Citizens for Environment Protection (NACEP), Save Our Saugahatchee, Sierra Club, Inc. - Alabama Chapter, Southeastern Alabama Group - Sierra Club, Inc., and Wolf Bay Watershed Watch,

Petitioners]

_____/

PETITION TO AMEND ADEM ADMIN. CODE CHAP. 335-6-10, APPENDIX A
REFERENCE DOSE (ACROLEIN AND PHENOL)

LAW OFFICE OF
DAVID A. LUDDER
A Professional Limited Liability Company
9150 McDonald Court, Tallahassee, Florida 32312-4209
(850) 386-5672

I. INTRODUCTION

1. *Code of Alabama* § 41-22-8 and ADEM Admin. Code R. 335-2-2 authorize any person to petition the Environmental Management Commission to engage in rulemaking. These provisions are "intended to provide the members of the public with a mechanism for affecting the content of an agency's rules." Commentary to *Code of Alabama* § 41-22-8. This mechanism allows any person to induce the Environmental Management Commission "to engage in a reasoned reconsideration of the existing state of the law and to change it if, ... that seems appropriate." *Summary of Reasons Supporting Adoption of Rules on "Petitions for Rulemaking," ADEM Admin. Code Chapter 335-2-2* (quoting Bonfield, *The Iowa Administrative Procedure Act: Background, Construction, Applicability, Public Access to Agency Law, The Rulemaking Process*, 60 Iowa L. Rev. 731, 894-95 (1975)). Granting a petition for rulemaking does not mean or imply that the proposed rule will be adopted by the Commission; it only means that public comment on the proposed rule will be solicited and that a decision whether to adopt the proposed rule will be made later.

2. This Petition seeks to have the Environmental Management Commission amend ADEM Admin. Code Chap. 335-6-10, Appendix A to conform two pollutant (Acrolein and Phenol) toxicity values to values published by the U.S. Environmental Protection Agency in the Integrated Risk Information System database. Modifying the toxicity values for these pollutants will reduce the risk of adverse health effects that may be suffered by members of Petitioners from exposure to these pollutants in surface waters.

II. PETITIONERS

3. The Petitioners are:

Alabama League of Environmental Action Voters
P.O. Box 1987
Montgomery, Alabama 36102
(334) 221-5882

Alabama Rivers Alliance, Inc.
2027 2nd Avenue North, Suite A
Birmingham, Alabama 35203
(205) 322-6395

Black Warrior Riverkeeper, Inc.
712 37th Street South
Birmingham, Alabama 35222
(205) 458-0095

Cahaba River Society, Inc.
2717 7th Ave. South
Suite 205
Birmingham, AL 35233
(205) 322-5326

Choctawhatchee Riverkeeper
P.O. 6734
Banks, AL 36005
(334) 807-1365

Episcopal Diocese of Alabama
Task Force for Stewardship of Creation
521 20th Street North
Birmingham, Alabama 35203
(334) 365-8939

Flint River Conservation Association, Inc.
P.O Box 275
Brownsboro, Alabama 35741
(256) 427-5116

Friends of Hurricane Creek
P.O. Box 40836
Tuscaloosa, Alabama 35404
(205) 507-0867

Friends of the Little Cahaba River
1257 Montevallo Road SW
Leeds, Alabama 35094
(205) 699-5604

Friends of the Locust Fork River
P.O. Box 245
Hayden, Alabama 35079
(205) 466-3858

Friends of Rural Alabama, Inc.
145 Cross Creek Lane
Ashville, Alabama 35953
(205) 594-5943

Lake Watch of Lake Martin, Inc.
P.O. Box 72
Alexander City, Alabama 35011
(256) 825-9353

Lookout Mountain Heritage Alliance, Inc.
5460 East River Road
Mentone, Alabama 35984
(256) 634-4124

Mobile Baykeeper
300 Dauphin Street, Suite 200
Mobile, Alabama 36602
(252) 433-4229

North Alabama Citizens for Environment Protection (NACEP)
375 Ashley Drive
Grant, Alabama 35747
(256) 582-7240

Save Our Saugahatchee, Inc.
1960 Lee Road 65
Auburn, Alabama 36832
(334) 844-9212

Sierra Club, Inc. - Alabama Chapter
1330 21st Way South, Suite 110
Birmingham, Alabama 35205
205-221-7201

Southeastern Alabama Group - Sierra Club, Inc.
28 County Road 7759
Troy, Alabama 36081
(334) 670-6373

Wolf Bay Watershed Watch
P.O. Box 63
Elberta, Alabama 36530
(850) 457-8695

III. Statement of Interests

4. The Petitioners are membership organizations dedicated to the protection of human health and the environment. Members of Petitioners use and enjoy the surface waters of the State of Alabama for fishing and drinking. ADEM Admin. Code R. 335-6-10-.07 authorizes toxic pollutants to be present in surface waters. The U.S. Environmental Protection Agency has determined that Acrolein and Phenol are more toxic than indicated in ADEM Admin. Code Chap. 335-6-10, Appendix A. Exposure to Acroleion and Phenol in surface waters at levels authorized by ADEM Admin. Code Chap. 335-6-10, Appendix A presents an excessive risk of adverse health effects to the members of Petitioners.

IV. Proposed Amendment to ADEM Admin. Code Chap. 335-6-10, Appendix A

5. The specific language of the proposed amendment to ADEM Admin. Code Chap. 335-6-10, Appendix A is attached as Exhibit 1.

V. EVIDENCE, DATA, AND INFORMATION SUPPORTING PROPOSED AMENDMENT

6. ADEM Admin. Code R. 335-6-10-.07 establishes water quality criteria (maximum permissible pollutant levels) for toxic pollutants, including Acrolein and Phenol, in surface waters of the State of Alabama.

7. Water quality criteria for the above-listed toxic pollutants are expressed as equations in ADEM Admin. Code R. 335-6-10-.07. For waters classified Public Water Supply (PWS), where consumption of contaminated water and fish may occur, the equation for non-carcinogenic toxic pollutants is as follows:

$$conc.\ (mg/l) = (HBW \times RfD)/((FCR \times BCF) + WCR) \qquad \textbf{Eq. 16}$$

For all other waters, where consumption of contaminated fish may occur, the equation for non-carcinogenic toxic pollutants is as follows:

$$conc.\ (mg/l) = (HBW \times RfD)/(FCR \times BCF) \qquad \textbf{Eq. 17}$$

where:

HBW = human body weight, set at 70 kg
RfD = reference dose, in mg/(kg-day)
FCR = fish consumption rate, set at 0.030 kg/day
BCF = bioconcentration factor, in l/kg
WCR = water consumption rate, set at 2 l/day

8. ADEM Admin. Code R. 335-6-10-.07(1)(d)1.(iii) provides:

> The values used for the reference dose (RfD) shall be values available through the U.S. Environmental Protection Agency's Integrated Risk Information System (IRIS), and values used for the bioconcentration factor (BCF) shall be values contained in ambient water quality criteria documents published by the U.S. Environmental Protection Agency, except where other values are established pursuant to subparagraph (1)(g). The RfD and BCF values for specific pollutants are provided in Appendix A.[1]

---

[1] ADEM Admin. Code R. 335-6-10-.07(1)(g) provides:

9. The Integrated Risk Information System (IRIS) is a database containing, among other data and information, consensus and peer reviewed oral reference dose values for substances causing chronic non-carcinogenic health effects. *What is IRIS?* (undated) (Exhibit 2) and *U.S. EPA's Process for IRIS Assessment Development and Review* (April 2004) (Exhibit 3).

9. The reference dose (RfD) for Acrolein published in ADEM Admin. Code Chap. 335-6-10, Appendix A is 0.0156 mg/(kg-day). This value was identified by the U.S. Environmental Protection Agency as an "Acceptable Daily Intake" in 1980. *Ambient Water Quality Criteria for Acrolein* (EPA 440/5-80-016).

10. On June 3, 2003, the U.S. Environmental Protection Agency published a new reference dose (RfD) for Acrolein in the Integrated Risk Information System. The new reference dose is 0.0005 mg/(kg-day). *IRIS Data Summary for Acrolein* (Exhibit 4). The derivation of the new reference dose is more fully explained in *Toxicological Review of Acrolein*, EPA/635/R-03/003 (May 2003) (Exhibit 5). The proposed amendment to ADEM Admin. Code Chap. 335-6-10, Appendix A would modify the reference dose for Acrolein to 0.0005 mg/(kg-day).

> Numeric criteria may be computed by the Department from equations 16, 17, 18, and 19 using values for the reference dose (RfD), cancer potency factor (CPF), and bioconcentration factor (BCF) determined by the Department in consultation with the State Department of Public Health after review of information available from sources other than the U.S. Environmental Protection Agency's Integrated Risk Information System (IRIS) or ambient water quality criteria documents. Such criteria, or the RfD, CPF, and BCF values used to compute criteria, shall not be effective until adopted following established rulemaking procedures.

Petitioners do not believe that the Department determined alternative reference dose values for Acrolein and Phenol in consultation with the Department of Public Health.

11. The reference dose (RfD) for Phenol published in ADEM Admin. Code Chap. 335-6-10, Appendix A is 0.6 mg/(kg-day). This value was published by the U.S. Environmental Protection Agency in the Integrated Risk Information System on February 1, 1990.

12. On September 30, 2002, the U.S. Environmental Protection Agency published a revised reference dose (RfD) for Phenol in the Integrated Risk Information System. The revised reference dose is 0.3 mg/(kg-day). *IRIS Data Summary for Phenol* (Exhibit 6). The derivation of the revised reference dose is more fully explained in *Toxicological Review of Phenol*, EPA/635/R-02/006 (Sept. 2002) (Exhibit 7). The proposed amendment to ADEM Admin. Code Chap. 335-6-10, Appendix A would modify the reference dose for Phenol to 0.3 mg/(kg-day).

13. The Department has not initiated rulemaking to modify the reference dose for Acrolein and Phenol in part because the U.S. Environmental Protection Agency published *National Recommended Water Quality Criteria* (2006) (Exhibit 8) which continues to incorporate the older toxicity values for Acrolein and Phenol, rather than the new and revised reference dose values in the U.S. Environmental Protection Agency's Integrated Risk Information System. However, ADEM Admin. Code R. 335-6-10-.07(1)(d)1.(iii) mandates that the Department use reference dose values from the Integrated Risk Information System, not toxicity values used in the National Recommended Water Quality Criteria. Moreover, the U.S. Environmental Protection Agency explained:

> Criteria in the [National Recommended Water Quality Criteria] table are updated by the Office of Water on a periodic basis. The table was updated in 2002 and most recently in 2003 . . .. * * * Due to process time lines for updating criteria (i.e. publication in public dockets, etc.), the most recent updates to the table do not include the current RfDs for phenol and acrolein. This in no way reflects a rejection of the IRIS published RfDs. The Office of Water fully accepts IRIS values as the Agency's most recent quantitative risk assessment values which have been reviewed and agreed upon by an interdisciplinary group of scientists representing various Program Offices within the Agency and represents an Agency wide consensus.

Correspondence from Dr. Heidi Bethel, Health and Ecological Criteria Division, Office of Science and Technology, Office of Water, U.S. Environmental Protection Agency to David A. Ludder (Dec. 15, 2006) (Exhibit 9).

14. Revising the reference dose for Acrolein and Phenol in ADEM Admin. Code Chap. 335-6-10, Appendix A will reduce the authorized concentration of Acrolein and Phenol in Alabama's surface waters by 97% and 50%, respectively (Exhibit 10), enhance protection of human health, and conform the reference dose for Acrolein and Phenol to the level recommended in the U.S. Environmental Protection Agency's Integrated Risk Information System.

VI. Affected Persons

15. The health of members of Petitioners who consume fish and water from Alabama's waters will be better protected as a result of adoption of the proposed amendments.

16. 851,470 anglers live in Alabama. $858,124,876 in retail sales was generated by Alabama's anglers, which rippled through the economy to generate $1.7 billion in economic output for the state. The Alabama fishing industry supports nearly 20,000 jobs and those workers earned $412 million in salaries and wages. Fishing-related purchases in Alabama generated $16 million in state tax revenues and $42 million in federal income tax. *Report Shows Fishing's Mainstream Appeal and Broad Economic Impacts*, Alabama Department of Conservation and Natural Resources (Jan. 22, 2003) (http://www.outdooralabama.com/news/release.cfm?ID=112). The health of these anglers and the industry which supports them will be better protected as a result of adoption of the proposed amendments.

17. Pursuant to ADEM Admin. Code R. 335-6-10-.05, existing discharge permits shall be modified or reissued to limit the discharge of any pollutants causing or contributing to an exceedance of newly adopted water quality criteria. Compliance with the modified discharge limit

shall be required as soon as possible, but in no case later than three years after adoption of the revised water quality criteria. New permits issued to new dischargers must include discharge limitations necessary to achieve all applicable water quality criteria, including newly adopted criteria.

18. Existing facilities with permit discharge limitations or monitoring requirements for Acrolein or Phenol are listed in Exhibit 11. These existing facilities may be required to reduce their discharge of Acrolein or Phenol if necessary to achieve the revised water quality criteria resulting from adoption of the proposed amendment. Whether, and to what extent, such a reduction may be necessary will depend on site-specific considerations such as pollutant concentration, effluent flow, 7-day low flows of the receiving water that recurs once in 10 years, and mixing zone size. The cost of achieving compliance with the revised water quality criteria cannot be accurately estimated without knowledge of existing site-specific conditions including pollutant concentration, effluent flow, 7-day low flow of the receiving water that recurs once in 10 years, mixing zones, and treatment processes and capabilities.

19. Other existing facilities may discharge Acrolein or Phenol in quantities too low to have been a concern for achieving compliance with the present water quality criteria. These facilities do not have permit discharge limitations or monitoring requirements for Acrolein or Phenol and are not listed in Exhibit 11. The discharges from these facilities will have to be reevaluated to determine whether, and to what extent, discharge limitations and monitoring requirements may be necessary to achieve compliance with the revised water quality criteria resulting from adoption of the proposed amendment. The cost of achieving compliance with the revised water quality criteria cannot be accurately estimated without knowledge of existing site-specific conditions including pollutant concentration, effluent flow, 7-day low flow of the receiving water that recurs once in 10 years, mixing zone size, and treatment processes and capabilities.

VI. OTHER FACTORS FOR CONSIDERATION

20. There is no constitutional impediment to the adoption of the proposed amendment. The Department is authorized to adopt the proposed amendment by the following statutory provisions: *Code of Alabama* §§ 22-22-9, 22-22A-5, 22-22A-6, and 22-22A-8. *Code of Alabama* § 22-22-9 provides, in part, as follows:

> (f) It shall be the duty of the commission, after notice as provided in this subsection and after consideration of the purpose of this chapter, to establish such standards of quality for any waters in relation to their reasonable and necessary use as shall be in the public interest, recognizing that, because of variable factors and varied use of waters, no single standard of treatment and no single standard of quality are practical and that the degree of treatment of pollutants and other wastes must take into account the present and future uses, and such general policies relating to existing or proposed future pollution as it shall deem necessary to accomplish the purposes of this chapter and to modify, amend or cancel the same.

Code of Alabama § 22-22-9, also provides, in part, as follows:

> (h) It shall be the duty of the commission, and it shall have the authority, to adopt rules and regulations to carry out the provisions of this chapter and to accomplish the purpose of this chapter.

21. The proposed amendment will promote the expressed legislative intent and purposes of the Alabama Water Pollution Control Act which are stated as follows:

> Whereas the pollution of the waters of this state constitutes a menace to public health and welfare, creates public nuisances, is harmful to wildlife, fish and aquatic life and impairs domestic, agricultural, industrial, recreational and other legitimate beneficial uses of water, it is hereby declared to be the public policy of this state and the purpose of this chapter to conserve the waters of the state and to protect, maintain and improve the quality thereof for public water supplies, for the propagation of wildlife, fish and aquatic life and for domestic, agricultural, industrial, recreational and other legitimate beneficial uses; to provide for the prevention, abatement and control of new or existing water pollution; and to cooperate with other agencies of the state, agencies of other states and the federal government in carrying out these objectives.

Code of Alabama § 22-22-2. The proposed amendment will also promote the intent of the Alabama Environmental Management Act which is stated as follows:

> It is therefore the intent of the Legislature to improve the ability of the state to respond in an efficient, comprehensive and coordinated manner to environmental problems, and thereby assure for all citizens of the state a safe, healthful and productive environment.

Code of Alabama § 22-22A-2. In addition, the Alabama Department of Environmental Management was created to, among other things, "protect human health and safety." *Code of Alabama* § 22-22A-2(1).

22. The evidence, data, and information submitted with this petition is substantive, credible and relevant and reasonably supports the adoption of the proposed amendment.

23. On December 3, 2004 (over two years ago), the Commission adopted several amendments to ADEM Admin. Code Chap. 335-6-10, Appendix A. No amendment to the reference dose for Phenol (0.6 mg/(kg-day) was proposed or adopted. An amendment adding Acrolein and a toxicity value for Acrolein (0.0156 mg/(kg-day)) was proposed and adopted. The Summary of Reasons for the proposed amendment states that it "will make the Department's toxic pollutant criteria consistent with EPA's recommended criteria reflected in its guidance document entitled 'National Recommended Water Quality Criteria: 2002' . . .."

At least one Petitioner has not had a prior opportunity to present relevant evidence, data and information on the subject matter of the proposed amendment to the Environmental Management Commission. Lookout Mountain Heritage Alliance, Inc. was not incorporated until October 27, 2006 - well after the last amendments to ADEM Admin. Code Chap. 335-6-10, Appendix A (December 3, 2004).

The December 3, 2004 amendments to ADEM Admin. Code Chap. 335-6-10, Appendix A did not reflect the latest scientific knowledge concerning the toxicity of Acrolein and Phenol then available. Moreover, the December 3, 2004 amendments to ADEM Admin. Code Chap. 335-6-10,

Appendix A did not incorporate the reference dose values for Acrolein or Phenol in the U.S. Environmental Protection Agency's Integrated Risk Information System as required by ADEM Admin. Code R. 335-6-10-.07(1)(d)1.(iii). These scientific and legal errors deserve to be corrected - *for the protection of human health* - regardless of whether some of the Petitioners had an opportunity to present relevant evidence, data and information on the subject matter to the Commission in 2004.

24. Alternative means of obtaining the same or similar relief are not presently available and have not in the recent past been made available to all the Petitioners.

25. The proposed amendment will enhance the established program for the control of water pollution and promote the underlying policies of *Code of Alabama* § 22-22-1 *et seq.* and ADEM Admin. Code Div. 335-6.

VII. DISPOSITION OF PETITION

26. ADEM Admin Code R. 335-2-2-.06 provides:

> Disposition of Petition. Within sixty days after a petition is filed with the Commission in accordance with Rule 335-2-2-.04, the Commission shall do one of the following, provided however, that upon written notice to the petitioner, such sixty day period may be extended for not more than thirty days if the Commission's next regularly scheduled meeting is not within said sixty day period:
>
> (a) initiate rule-making proceedings in accordance with *Code of Alabama* 1975, §§ 22-22A-8 and 41-22-5, as amended; or
>
> (b) deny the petition in writing on the merits stating the reasons therefor.

See also Code of Alabama § 41-22-8.

Respectfully submitted,

David A. Ludder
Attorney for Petitioners

Address and Phone:
LAW OFFICE OF DAVID A. LUDDER
A Professional Limited Liability Company
9150 McDougal Court
Tallahassee, Florida 32312-4208
(850) 386-5671

CERTIFICATE OF SERVICE

I certify that the original of the foregoing Petition was hand delivered to:

W. Scott Phillips, Chairman
Alabama Environmental Management Commission
c/o Alabama Department of Environmental Management
1400 Coliseum Boulevard
Montgomery, Alabama 36110

Done this 20th day of April, 2007.

David A. Ludder

ENVIRONMENTAL MANAGEMENT COMMISSION

Alabama Department of Environmental
Management Building
Main Hearing Room
1400 Coliseum Boulevard
Montgomery, Alabama

June 29, 2007 11:00 a.m.

COMMISSION MEMBERS PRESENT:

W. SCOTT PHILLIPS, CHAIR

DR. KATHLEEN J. FELKER, VICE CHAIR

ANITA L. ARCHIE

DR. LAUREL G. GARDNER

DR. JOHN H. LESTER

SAM H. WAINWRIGHT, P.E.

COMMISSION MEMBERS NOT PRESENT:

KENNETH A. HAIRSTON, ESQUIRE

ALSO PRESENT:

ONIS "TREY" GLENN, III,
ADEM DIRECTOR

ROBERT TAMBLING, EMC LEGAL COUNSEL

DEBI THOMAS, EMC EXECUTIVE ASSISTANT

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MR. PHILLIPS: Good morning. I want to call the meeting of the Alabama Environmental Management Commission to order. I want to acknowledge that we do have a quorum, although we are missing Mr. Hairston.

Moving on to Agenda Item Number 1, Consideration of the Minutes of the Meeting on April the 20th, 2007. I'll entertain a motion from the Commission.

DR. LESTER: I move.

MR. PHILLIPS: I have a motion. Do I have a second?

DR. FELKER: Second.

MR. PHILLIPS: I have a motion and a second. I'll open it up for any discussion.

(No response.)

MR. PHILLIPS: There being no further discussion, all in favor of the motion, signify by signing aye.

(Commission members in favor of the motion so indicated.)

MR. PHILLIPS: All opposed, same sign.

(No response.)

MR. PHILLIPS: Motion carries. Thank you.

Agenda Item Number 2, we'll hear from the Director.

MR. GLENN: Thank you, Chairman Phillips and Commissioners. Welcome to Montgomery. I would like to first start with budget and legislative issues, and the state general fund budget that we've talked about several times over the past couple of meetings is now law. That included a $1.5 million increase for the Department, an increase over last year's budget. That increase did include the $800,000 for the steel dust money that we did not receive last year -- or part of it that we did

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1 not receive last year, so they gave
2 that to us this year. The budget also
3 included a few earmarks: $200,000 for
4 Environmental Justice, $300,000 for
5 e-government, along the lines of
6 efficiency and e-permitting and that
7 type of programs, and $350,000 for the
8 CAFO program. So there were some
9 earmarks in that budget. In addition
10 to ADEM's budget, there was $100,000
11 appropriated to the Department to our
12 ER program through the Emergency
13 Management Agency through a provision
14 of House Bill 395. So that's the good
15 news.
16 The bad news is, at least
17 the financial bad news, is also due to
18 actions at the legislature, we have an
19 increased financial burden beginning
20 next fiscal year of $1.3 million
21 because of actions taken there. So
22 that's the financial wrap-up.
23 The Uniform Environmental

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1 Covenants Act passed. We had
2 discussed that, and that was signed
3 into law. The Land Division is
4 currently preparing to implement that
5 act. The act, if you recall, as
6 originally introduced was amended. We
7 proposed some amendments to that act,
8 and that was amended to clarify our
9 responsibilities, number one, and
10 number two, include a mechanism -- a
11 funding mechanism so we could
12 implement that. So that occurred.
13 Another very notable act
14 that passed that does not directly
15 affect us -- but we will see some
16 change in activity because of it -- is
17 a bill lowering the tipping fee on
18 waste disposed of at Emelle passed.
19 And one would only assume that the
20 change which lowers the tipping fee
21 would increase the volume of waste
22 coming in there, which, of course,
23 will have an effect on our activities.

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1 But that's it for a very quick
2 legislative and budget wrap-up.
3 On to some administrative
4 items, you should have received a memo
5 from me last week outlining contracts
6 as well as potential rulemaking that
7 we propose -- we will possibly propose
8 in the coming months. That includes
9 some routine Air updates as well as
10 the Uniform Environmental Covenants
11 Act rules that we just mentioned. And
12 so you should have gotten those.
13 Now on to organizational
14 issues, just a real quick update on
15 the QMS, or the Quality Management
16 System implementation. We do continue
17 the implementation of the Quality
18 Management System that we've talked to
19 you about in an effort to better
20 fulfill two things really: Our
21 foundation of legislation and what it
22 tells us to do, and your charge given
23 to us in the 2004 Strategic Plan.

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1 Bottom line, if we're going to go from
2 a good organization to a great
3 organization, which is what you have
4 very clearly told us is your
5 expectation of us in the Department,
6 then we must put in place a
7 Department-wide mechanism that will
8 ensure the quality of the results that
9 we produce. And the real bottom line
10 of the Quality Management System, or
11 this mechanism, is to put in place a
12 Department-wide appropriate set of
13 controls -- very appropriate is the
14 key word here -- regarding the quality
15 of the outputs, whatever our outputs
16 are, whether it's permitting,
17 enforcement, compliance, outreach, or
18 other activities. So that's the focus
19 of the Quality Management System and
20 where we are to date on that.
21 Now, let me jump up to a
22 couple of Land issues real quick. The
23 Camp Sibert cleanup continues as of

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1 the week ending June 21st.
2 Unfortunately, 18 unexploded
3 liquid-filled ordinances were
4 recovered, and they are now being
5 stored in the interim holding
6 facility. This is -- if you recall,
7 this started several years ago --
8 really, much, much farther back than
9 that -- where we had a fear that there
10 was going to be a lot of unexploded
11 ordinances there that could
12 potentially harm people. And so that
13 cleanup is ongoing right now. As well
14 as the activities at Anniston Chem
15 Demil facility, they continue to
16 operate safely with continued 24/7
17 oversight by the Department. And they
18 are in the middle of the VX campaign
19 right now.
20 Now on to a couple of
21 Water issues. I hope you all went by
22 and saw the lobby board out front. As
23 you know, as you strive every meeting

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1 and as we strive every day, we work to
2 improve the water quality of the
3 impaired streams in Alabama. And the
4 lobby board display highlights a
5 project in the Flint River watershed
6 which had been identified as not
7 meeting water quality standards and,
8 therefore, proposed and put on the
9 Department's 303(d) List of Impaired
10 Waters in 1998.
11 Now, since that time,
12 ADEM has done three key things here:
13 We've, number one, worked with local
14 stakeholders to identify sources of
15 the impairments and put in place
16 actual fixes, best management
17 practices, to address that.
18 Number two, we conducted
19 local watershed tours, and educational
20 outreach activities were conducted and
21 completed and a way to raise the level
22 of awareness of environmental issues
23 and what the watershed meant to them

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1 and what they could do in that
2 watershed.
3 And lastly, we have
4 conducted water quality monitoring to
5 document the results and document the
6 actual water quality improvements such
7 that the good news on this program is
8 that we have proposed the removal of
9 this watershed from the 303(d) List of
10 Impaired Waters at this time. And
11 there are a lot of other activities
12 like that ongoing; I just wanted to
13 call your attention to that one.
14 We all know that we are
15 in a drought right now, so the
16 continued drought that we're involved
17 in raises the awareness and the fact
18 that water quality and drinking water
19 are becoming more and more of a
20 critical issue every single day for
21 us. So we are constantly working with
22 entities that we regulate or other
23 agencies on how we deal with the

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1 ongoing drought.
2 Lastly in the water
3 arena, fish tissue, we have recently
4 completed the annual fish tissue
5 sampling and are currently working
6 with our sister agencies on the
7 finalization and true implementation
8 of that sampling and that data. Just
9 a few stats on that: 515 fish were
10 collected this year from 39 different
11 locations. You have a map of those
12 locations as well as a summary of the
13 results. And the interesting thing to
14 note is samples collected from 27 out
15 of 39 locations did not exceed FDA or
16 EPA action levels. Fish from 12
17 different sites did exceed the action
18 level for mercury, only that for
19 mercury, not the other host of
20 elements that we look at. So 12 sites
21 did have exceeding limits of that.
22 And again, you have that data in your
23 package. And we anticipate news

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1 coming out from the Department of
2 Public Health very soon on that issue.
3 Okay. We can't leave out
4 Air. We've talked about Land and
5 Water. And you've probably seen it in
6 the clippings that you've received
7 about ozone standards, but on
8 June 20th, EPA proposed to strengthen
9 the National Ambient Air Quality
10 standards for ground-level ozone. The
11 existing standards set in 1997 is an
12 eight-hour standard of .08 parts per
13 million. Now, in practice, because of
14 rounding, an area meets the standard
15 if the ozone levels are .084 or lower.
16 So this time around, EPA proposes to
17 set a primary health-based standard to
18 a level within the range of .07 to a
19 .075. And you can see they're
20 addressing the rounding issue by going
21 out to one more significant digit
22 here. But at the same time they're
23 also requesting comments on a range of

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1 .06 to the current .084. So you can
2 see, we're at .084 right now. They
3 have identified .07, .075 as their
4 area they're looking at, but they're
5 accepting comments on a much broader
6 range than that. The timeline on this
7 is that they will take public comments
8 for 90 days following the publication
9 of the proposal in the register with a
10 statement that they will issue final
11 standards by March 12, 2008. So we'll
12 be hearing more about this and
13 implementing this as we go forward.
14 Now, my last thing is to
15 award some pollution prevention
16 awards. But before I do that, can I
17 answer any questions?
18 MR. PHILLIPS: Do we have
19 any questions from the Commission
20 members? Commissioner Archie?
21 MS. ARCHIE: Yes, I do. Can
22 you tell me a little bit about this
23 $1.3 million cost -- increase we're

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1 going to incur because of things at
2 the legislature?
3 MR. GLENN: Yes, ma'am.
4 That is mainly made up of the fact
5 that the legislature passed a pay
6 raise for all state employees, and
7 that's 3 and a half percent. And if
8 you look, we have approximately a
9 $30 million personnel budget; that's a
10 million dollars. That raise trickles
11 and affects fringes and other benefits
12 along with that, which turns into
13 approximately $1.3 million.
14 MS. ARCHIE: So can you
15 absorb that cost?
16 MR. GLENN: Well, the answer
17 to that is we did get an increase in
18 our general fund dollars. Now,
19 granted, that was tightened down a
20 little bit in the earmarks that we
21 received or what we would have
22 desired, but we did get an increase
23 there, which does help to offset some

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1 of the cost. And you have to also
2 consider that in the state system,
3 every year that goes by, all staff
4 that are eligible are evaluated. If
5 they're high performers, they're
6 eligible for a raise. So that is an
7 increased cost that we have too next
8 year. It probably puts us at
9 approximately $2 million, if you look
10 at the 1.3 added to that. So the
11 answer is, we did get a little more
12 general fund dollars. Our permit fees
13 will -- you know, we'll be looking at
14 an adjustment there for
15 cost-of-living-type issues. Our
16 federal funds are looking very
17 stagnant right now. They're looking
18 very flat. There may be only a few
19 areas where we have that. So we will
20 have to absorb that cost if we're
21 going to continue to do our business,
22 which is the key focus of the Quality
23 Management System, the key focus of an

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1 overarching look at the Department and
2 how we do our business such that we
3 can make sure that we're using every
4 single ounce of resources we have in
5 the best way we can possibly use them.
6 So the answer is, there's no choice
7 exactly, you know, and our desire is
8 to not affect the quality of service
9 that we provide. And so, therefore,
10 we have to all team up and work
11 together to potentially make a few
12 changes to make sure we can balance
13 that.
14 MR. PHILLIPS: Any other
15 questions from the Commission?
16 (No response.)
17 MR. PHILLIPS: Then thank
18 you, Director.
19 MR. GLENN: And if you will,
20 let me remain in Agenda Item 2 for
21 just a second, and, Chairman Phillips,
22 if you don't mind joining me down
23 here. And if you recall, every year

1 we give out a few pollution prevention
2 awards, and we're responsible for
3 awarding excellence in pollution
4 prevention. Today we have set aside
5 time to recognize and honor two
6 different facilities who are here with
7 us who have proved to exemplify and
8 exceed those standards that we all
9 feel are requirements that we have.
10 First, I want to point
11 out Lafarge. If Terry Bennett would
12 start making it up here, we would
13 appreciate that. In cooperation with
14 the Portland Cement Association and
15 the World Wildlife Federation, Lafarge
16 North America initiated various
17 programs to reduce CO2 emissions. The
18 Roberta Plant incorporated industrial
19 byproducts in its raw material feed.
20 Various plant-generated solid waste
21 was reintroduced into this process as
22 opposed to shipping offsite to a
23 landfill. Also, scrap tires were also

1 consumed as fuel, which offset 3 to
2 5 percent of the plant's coal
3 consumption -- excuse me, 5 to 6
4 percent. So today Terry Bennett is
5 here on behalf of Lafarge, and we
6 appreciate your efforts in this
7 achievement award.
8 (Applause.)
9 MR. GLENN: We also would
10 like to recognize Toyota Motor
11 Manufacturing of Alabama. Their
12 ultimate goal is to be a zero waste
13 facility. During 2006, they continued
14 to focus on the elimination of waste
15 streams that were currently processed
16 at the Covanta Waste to Energy Plant
17 in Huntsville. In 2005 they diverted
18 255 tons of plastic at a savings of
19 $22,000. And with their plant
20 expansion, new products were
21 introduced and they were able to
22 recycle and reuse some of these
23 materials in-house instead of sending

1 it to the waste to energy plant.
2 Along with the continuation of the
3 project from '05, re-using and
4 recycling initiatives in 2006, they
5 diverted another 197 tons at a savings
6 of over $28,000 to them. So we've got
7 Pamela Russell and Stephanie Deemer
8 here on behalf of Toyota Motor
9 Manufacturing to accept the 2006
10 Pollution Prevention Director's Award.
11 (Applause.)
12 MR. GLENN: Thank you. Is
13 there anything further from the
14 Commission?
15 MR. PHILLIPS: Do we have
16 anything else?
17 (No response.)
18 MR. PHILLIPS: Thank you,
19 Mr. Director.
20 MR. GLENN: Thank you.
21 MR. PHILLIPS: Moving on to
22 Agenda Item Number 3, Report From the
23 Commission Chair, I have a few things

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1 that I would like to share with the
2 Commission. First, I would like to
3 thank the Department for their support
4 of the development of a watershed
5 management plan for the Cole Spring
6 Branch watershed. It's in your book.
7 You'll notice that there were contract
8 amounts. But the Nature Conservancy
9 and the citizens of northeast Alabama,
10 I understand, are extremely thrilled
11 with that, and I want to thank the
12 Director for all of his work on that.
13 The next point that I
14 would like to bring out is it's been
15 brought to my attention that I may
16 have made a procedural error two
17 meetings ago relative to a motion that
18 was a little outside of Robert's Rules
19 of Order. I don't know whether that's
20 true yet or not, but I am looking into
21 it. I don't think it was a
22 significant motion, and it may not
23 have any bearing on any of the actions

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1 that we took. But I am going to work
2 with Robert between now and our next
3 meeting to make sure that that's the
4 case, and also look at how our other
5 business item really fares under our
6 Robert's Rules of Order. So I'll
7 bring that back to the Commission in
8 our August meeting.
9 And then finally, I want
10 to remind the Commission that this is
11 June. I know it's hard to believe.
12 We have an August meeting, and then we
13 have an October meeting. And just a
14 reminder, in that October meeting we
15 have elections. So I would like to
16 ask the committee chairs in our next
17 meeting in August, if you would, to be
18 prepared to give some committee
19 reports on the progress that you've
20 made over the course of the year based
21 on the input that I had asked you for
22 earlier. And then in October we'll be
23 prepared for elections, so I want you

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1 to be thinking about that.
2 And that's all I have.
3 Any questions from the Commission?
4 (No response.)
5 MR. PHILLIPS: No? Okay.
6 Thank you.
7 Moving to Agenda Item
8 Number 4, I want to note that we'll
9 consider the adoption of the proposed
10 amendments to the Division 6, the
11 Underground Storage Tank Regulations,
12 and we will hear from the Department.
13 SONJA MASSEY: Good morning.
14 My name is Sonja Massey. I'm Chief of
15 the Groundwater Branch in the Water
16 Division of ADEM. You have before you
17 for consideration proposed regulations
18 which will be necessary to implement
19 the Underground Storage Tank Program
20 requirements of the Federal Energy Act
21 of 2005.
22 This set of proposed
23 regulations will impose three primary

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1 requirements: First, it will require
2 that all new underground storage tanks
3 and associated piping have secondary
4 containment with interstitial
5 monitoring. Previously single wall
6 pipes and tanks were allowed, as long
7 as there was an approved method of
8 leak detection in place.
9 Second, all new dispenser
10 systems will be required to have under
11 dispenser containment. Releases from
12 under dispensers have been shown to be
13 one of the most frequent causes of new
14 releases since the first UST
15 regulations were issued in 1998,
16 second only to releases from piping.
17 Finally, the definition
18 of "motor fuel" is expanded to include
19 biodiesel or ethanol blended with
20 diesel or gasoline. This change will
21 clarify that the underground storage
22 of these alternative fuels when
23 blended with petroleum will be

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1 regulated under this program. And
2 I'll be glad to answer any questions
3 that you may have.
4 MR. PHILLIPS: Thank you.
5 Do we have any questions of the
6 Department?
7 (No response.)
8 MR. PHILLIPS: I'll
9 entertain a motion from the Commission
10 to adopt the proposed amendments to
11 Division 6.
12 MR. WAINWRIGHT: So move.
13 MR. PHILLIPS: I have a
14 motion. Do I have a second?
15 DR. FELKER: Second.
16 MR. PHILLIPS: I have a
17 motion and a second. Any further
18 discussion?
19 (No response.)
20 MR. PHILLIPS: All in favor
21 of the motion to adopt the proposed
22 amendments to Division 6 signify by
23 signing aye.

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1 (Commission members in favor of
2 the motion so indicated.)
3 MR. PHILLIPS: All opposed,
4 same sign?
5 (No response.)
6 MR. PHILLIPS: Motion
7 carries. Thank you.
8 While we are waiting to
9 sign the resolution here, I neglected
10 to recognize someone who is here with
11 us today, Mr. Taylor Nichols, from the
12 Governor's policy office. Mr. Nichols
13 is in the back of the room. Thank you
14 for joining us.
15 Thank you. Moving to
16 Agenda Item Number 5, I'll call on the
17 Rulemaking Committee Chair,
18 Commissioner Wainwright, to report on
19 the June 1st, 2007, Rulemaking
20 Committee meeting and the Committee's
21 recommendations regarding this
22 rulemaking petition. Commissioner
23 Wainwright?

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1 MR. WAINWRIGHT: Mr.
2 Chairman, we've had quite a bit of
3 response on this issue. I want to
4 thank Debi Thomas for keeping us all
5 informed. This is a tremendous amount
6 of paper, probably over 2000 sheets,
7 including a CD with probably another
8 thousand items in it. The Rulemaking
9 Committee recommended two motions,
10 which y'all have received. Motion
11 Number 1 is to adopt the
12 recommendation of the Rulemaking
13 Committee and deny the rulemaking
14 petition citing Paragraphs (d), (f)
15 and (g) of Rule 335-2-2-.05,
16 Consideration of the Petition as the
17 reasons for denial. Those reasons --
18 the reason given by the committee was
19 the adoption of the proposed rule
20 would impact the overall regulatory
21 scheme of the Department. That is
22 R. 335-2-2-.05(g).
23 Two other reasons were

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1 recommended by our attorney, and
2 they've been added. There's a lack of
3 substantive, credible and relevant
4 evidence, data and information that
5 would reasonably support the adoption
6 of a proposed rule at this time. In
7 addition, there appears to be
8 contradictory or conflicting evidence,
9 data or information that should be
10 investigated and addressed before the
11 Commission undertakes rulemaking in
12 this matter. That's
13 R. 335-2-2-.05(d). The second one,
14 because this matter has been referred
15 to the Department and a select
16 committee will be appointed to
17 consider these issues, alternative
18 means of obtaining the same similar
19 relief may be made available to the
20 petitioner. That's R. 335-2-2-.05
21 (f). And that is the motion to be
22 made by the Rulemaking Committee.
23 MR. PHILLIPS: That's the

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1 recommended motion?
2 MR. WAINWRIGHT: The
3 recommended motion.
4 MR. PHILLIPS: So I have a
5 motion -- do you want to hand me that
6 so I can read it? I've got about two
7 thousand pages in front of me. We
8 have a motion to adopt the
9 recommendation of the Rulemaking
10 Committee and deny the rulemaking
11 petition citing Paragraphs (d), (f)
12 and (g) --
13 MR. TAMBLING: Mr. Chair,
14 may I approach the podium?
15 MR. PHILLIPS: Yes.
16 MR. TAMBLING: Just for
17 purposes of clarification, those two
18 additional reasons are being
19 recommended by counsel here, by me,
20 today to the Commission. The
21 Rulemaking Committee has already acted
22 and made a recommendation and that was
23 based on (g). So my recommendation of

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1 adding (d) and (f) would be for the
2 full Commission to act on should it
3 choose to deny the rulemaking petition
4 at this point.
5 MR. PHILLIPS: Okay. So --
6 MR. TAMBLING: So
7 procedurally --
8 MR. PHILLIPS: Procedurally
9 the recommendation from the committee
10 recommended only (g)?
11 MR. TAMBLING: Only (g),
12 that's correct.
13 MR. PHILLIPS: And based on
14 your involvement or recommendation,
15 this motion has (d) and (f) added to
16 it?
17 MR. TAMBLING: Should the
18 Commission decide to deny the
19 rulemaking petition at this point, it
20 would be my recommendation that those
21 few paragraphs be added as reasons for
22 denial.
23 MR. PHILLIPS: Okay.

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1 MR. TAMBLING: I don't think
2 I can go back after the fact and
3 change the recommendation of the
4 Committee that's already been made.
5 MR. PHILLIPS: Okay. So,
6 Commissioner Wainwright, let me ask
7 you about your motion. Are you
8 recommending this motion as the
9 recommendation from the Rulemaking
10 Committee or as a motion from you?
11 MR. WAINWRIGHT: I'm making
12 the motion based on the recommendation
13 from the Rulemaking Committee.
14 MR. PHILLIPS: So --
15 MR. WAINWRIGHT: Before
16 Robert gets away, Mr. Chairman --
17 MR. PHILLIPS: Yes.
18 MR. WAINWRIGHT: Robert --
19 MR. TAMBLING: The second
20 reason for denial would only apply to
21 the second motion also, which would be
22 the alternative remedy available to
23 the petitioners via referral to a

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1 select committee.
2 MR. PHILLIPS: Okay. So,
3 Commissioner Wainwright, you wanted to
4 add something before you restated your
5 motion?
6 MR. WAINWRIGHT: Robert, I
7 would like for you to explain the
8 procedure we're going through with
9 regard to this denial and -- and the
10 further recommendation to pass it on
11 to the agency for rulemaking.
12 MR. TAMBLING: Let me
13 explain the recommendation that the --
14 and correct me if I'm wrong, Mr.
15 Wainwright -- that the petition for
16 rulemaking be denied. There's a
17 second motion -- or a second
18 recommendation that the matter be
19 referred to a select committee to be
20 appointed by the Director of the
21 Department. I'm just saying that if
22 you recommend denial of the petition,
23 that you cite an additional reason,

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1 which would be reason (d) which is
2 cited as probably -- I need to have it
3 in front of me. I need the actual
4 motion that's in front of the
5 Commission.
6 MR. PHILLIPS: Here you go.
7 MR. WAINWRIGHT: He can have
8 my copy.
9 MR. PHILLIPS: No. He'll
10 give it back.
11 MR. TAMBLING: Okay. As far
12 as motion -- the first motion is
13 concerned, I think the reasons for
14 denial that would apply in this
15 particular case would be Reason 1 and
16 Reason 3 as included in your
17 attachment. And I think procedurally
18 what the Commission needs to do is to
19 deal with the first motion; and if it
20 needs to be amended or -- the first
21 recommendation, the only
22 recommendation dealing with the first
23 motion, if it needs to be changed,

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1 then I think the Commission has the
2 authority to make that change by
3 amending the motion and adding these
4 reasons. I just want to assure that
5 procedurally we're on the same page,
6 and I don't want anybody to think that
7 I changed the Committee's
8 recommendation by myself by adding
9 these reasons.
10 DR. GARDNER: Robert, what
11 I'm hearing you say is that the motion
12 before the Commission includes only
13 the third reason listed?
14 MR. TAMBLING: Exactly, at
15 this point.
16 DR. GARDNER: And that if we
17 choose to act on that motion and deny
18 the petition, then we would need to
19 come back and amend to include the
20 other two reasons?
21 MR. TAMBLING: Well, I'm not
22 a parliamentarian, but I will tell you
23 that what I think you need to do is to

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1 go ahead and deal with the motion that
2 comes from the Committee, and then
3 amend it accordingly if you feel like
4 you need to. And if you need to
5 reject that motion and then come up
6 with a new motion, then I would
7 recommend that you do that.
8 DR. GARDNER: Okay. And if
9 we -- if we deal with the motion with
10 just the one reason for denial, at
11 what point prior to voting on that
12 motion or after voting on that motion
13 do we amend it?
14 MR. PHILLIPS: We would do
15 that during discussion on the motion.
16 DR. GARDNER: Okay.
17 MR. PHILLIPS: And if an
18 amendment is offered from the
19 Commission, then that would be
20 considered as a separate motion -- or
21 amendment. Okay?
22 All right. The only
23 questions or discussions we need to

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1 have at this point is relative to the
2 motion, so are there any other
3 discussion points relative to the
4 motion?
5 MR. WAINWRIGHT: Has it been
6 seconded?
7 MR. PHILLIPS: Well, not
8 yet. For clarification, we don't need
9 a second if there's some
10 misunderstanding of --
11 DR. GARDNER: The motion, as
12 I understand it, is including these
13 three reasons?
14 MR. PHILLIPS: The way he
15 has stated it, yes.
16 DR. GARDNER: It does?
17 MR. PHILLIPS: But what I'm
18 hearing Robert say is that we should
19 take this motion up without the two --
20 DR. GARDNER: Correct.
21 MR. PHILLIPS: -- that it
22 should come out as a recommendation
23 from the Committee.

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1 MR. TAMBLING: I just want
2 to -- I just want to clarify the way
3 Mr. Wainwright presented it wasn't
4 exactly the way I felt it needed to be
5 presented in the sense that when it
6 came out of the Rulemaking Committee,
7 only Paragraph (g), Reason Number 3,
8 was included as the reason for denial.
9 I am telling you that since that time,
10 I have reviewed that and I am
11 recommending to the Commission that
12 should you decide to deny this
13 petition, that you add as far as the
14 first motion is concerned, Reason
15 Number 1 to that.
16 MR. PHILLIPS: Okay. All
17 right. Sam, do you want to restate
18 your motion?
19 MR. WAINWRIGHT: Sure. May
20 I have that piece of paper? This was
21 prepared with all three on it, so
22 that's -- that's the problem.
23 The motion to adopt the

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1 recommendation of the Rulemaking
2 Committee and deny the rulemaking
3 petition citing Paragraph (g), yeah,
4 335-2-2-.05, Consideration of the
5 Petition as the reason for denial.
6 And that reason is the adoption of the
7 proposed rule would impact the overall
8 regulatory scheme of the Department.
9 That's R. 335-2-2-.05(g).
10 MR. PHILLIPS: Okay. So
11 that's the motion before the
12 Commission, dealing with only (g) as
13 the reason. Do I have a second?
14 DR. LESTER: I second it.
15 MR. PHILLIPS: I have a
16 second. Now, we will open the floor
17 for discussion. Do I have discussion?
18 MR. WAINWRIGHT: This would
19 be the point of making amendments?
20 MR. PHILLIPS: Yes,
21 absolutely.
22 DR. FELKER: Okay. Well, I
23 have some questions to ask. We're

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1 talking about the first case of
2 rulemaking which is for Acrolein and
3 Phenol?
4 MR. PHILLIPS: No, no. This
5 is the cancer risk.
6 DR. FELKER: Cancer risk,
7 okay. So we have a first motion which
8 would be to deny. And then, Mr.
9 Wainwright, there's a second motion
10 stated in the minutes for the
11 Rulemaking Committee meeting. Would
12 you -- is it time to explain that now
13 or not?
14 MR. WAINWRIGHT: Well,
15 that's what I was asking Robert to
16 explain, that this process is to
17 remove the rulemaking from the
18 Commission and place it within the
19 Department. We need to deny it as a
20 Commission, and then we need to refer
21 it to the Department for further
22 processing in the rulemaking
23 procedure.

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1 DR. FELKER: Okay. And then
2 the second motion deals with how it
3 will be referred to the Department?
4 MR. WAINWRIGHT: That's
5 correct.
6 DR. FELKER: Okay.
7 MR. PHILLIPS: Any further
8 discussion? Commissioner Gardner?
9 DR. GARDNER: I have a
10 question for the Department, and that
11 is, what precise manner would this
12 rulemaking affect the overall
13 regulatory scheme if we're supposed to
14 be considering that?
15 MR. PHILLIPS: Well, in the
16 merits of you deciding on this motion,
17 I'm assuming you want to understand
18 that?
19 DR. GARDNER: Correct.
20 MR. PHILLIPS: Department?
21 MR. GLENN: Give me one
22 moment.
23 MR. PHILLIPS: Absolutely.

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1 (Off-the-record discussion.)
2 MR. GLENN: It's always good
3 to -- you always rely on counsel, and
4 I'm going to also make sure that Mac
5 McIndoe is not too far behind me. If
6 y'all see him creeping back in the
7 rows, please let me know as soon as
8 possible.
9 The major effect that I
10 would see this would have on the
11 regulatory scheme of the Department
12 would be if the Commission were to
13 change this one variable in that
14 equation, we would need to go and look
15 at all the NPDES permitholders that
16 have something in their permit that
17 this impacts, recalculate, potentially
18 prepare new models to set new limits
19 for their permits, and then reissue
20 permits for those in accordance with
21 the calculations made pursuant to the
22 formula change.
23 MR. PHILLIPS: Not to

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1 diminish what you just said, but I'm
2 not sure that's quite what I heard
3 Commissioner Gardner asking.
4 DR. GARDNER: That is what
5 I'm asking.
6 MR. PHILLIPS: Is it what
7 you're asking? Okay.
8 DR. GARDNER: Yes. And a
9 further question would be, isn't that
10 something that you do every time
11 there's a rule change?
12 MR. GLENN: The answer to
13 that question is yes. When you make a
14 change that affects a standard, a
15 limit, a number, then the practice is
16 that we will go and evaluate that and
17 make any necessary changes to the
18 permits when they come up. So, yes,
19 we calculate -- we prepare models; we
20 calculate limits as a standard
21 practice here.
22 DR. GARDNER: And that is
23 something you do on a regular basis?

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1 MR. GLENN: Absolutely. And
2 my Water Division would probably say
3 amen on a way-too-regular basis.
4 MR. PHILLIPS: So did that
5 answer your question?
6 DR. GARDNER: It did.
7 MS. ARCHIE: Mr. Chair?
8 MR. PHILLIPS: Yes.
9 MS. ARCHIE: I just need to
10 make clear what we're voting on here,
11 okay? So are you telling me that --
12 you're telling me that the first
13 motion is only Number 3? To take up
14 the other two, which is (d) and (f),
15 we would have to come back and after
16 we voted on the original motion, come
17 back to it, because this is just --
18 MR. PHILLIPS: Let me
19 address that. The answer is no.
20 MS. ARCHIE: Okay.
21 MR. PHILLIPS: The answer is
22 now is the time if you want to amend
23 this motion based on anything that

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1 you've heard from Robert or any of the
2 other Commissioners or from the
3 Department to propose an amendment to
4 the motion. So if you want to amend
5 this motion by adding another reason,
6 now is the time.
7 MS. ARCHIE: Which would be
8 1 and 2?
9 MR. TAMBLING: Right.
10 MS. ARCHIE: At this time, I
11 would like to amend the motion.
12 MR. PHILLIPS: Okay. So we
13 have a motion to amend the original
14 motion by adding 1 and 2 --
15 MR. WAINWRIGHT: I'll second
16 it.
17 MR. PHILLIPS: -- is that
18 correct, Commissioner Archie?
19 MS. ARCHIE: Yes.
20 MR. PHILLIPS: Okay. So we
21 have that motion. Do I have a second?
22 MR. WAINWRIGHT:
23 (Indicating.)

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1 MR. PHILLIPS: I have a
2 second from Commissioner Wainwright.
3 Okay. Discussion on the amendment
4 motion? Any discussion on the
5 amendment motion?
6 MS. ARCHIE: Yes.
7 MR. PHILLIPS: Commissioner
8 Archie?
9 MS. ARCHIE: About this
10 select committee, who -- the
11 Department will determine who the
12 select committee will be?
13 MR. PHILLIPS: We haven't
14 taken that up in a motion at this
15 point. My understanding -- and
16 correct me if I'm wrong, Commissioner
17 Wainwright -- is that --
18 MR. WAINWRIGHT: That's
19 correct.
20 MR. PHILLIPS: -- if we
21 approve the motion to deny, then we
22 would move to that next motion and
23 would then take up that.

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1 MS. ARCHIE: Okay.
2 DR. FELKER: And then if we
3 wanted to amend the second part of the
4 motion, we would do so at that time?
5 Right now we're talking about the
6 first part of the motion, which is the
7 motion to deny?
8 MR. PHILLIPS: That's
9 correct. So before we vote on the
10 motion, this is the motion to amend by
11 adding Items 1 and 2 to the reasons
12 for denial of rulemaking petition.
13 Number 1 being there's a lack of
14 substantive, credible and relevant
15 evidence, data and information that
16 would be -- would reasonably support
17 the adoption of the proposed rule at
18 this time. In addition, there appears
19 to be contradictory or conflicting
20 evidence, data or information that
21 should be investigated and addressed
22 before the Commission undertakes
23 rulemaking in this matter, reference

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1 335-2-2-.05(d). And Number 2, because
2 this matter has been referred to the
3 Department and a select committee will
4 be appointed to consider these issues,
5 alternative means of obtaining the
6 same or similar relief may be made
7 available to the petitioner, reference
8 335-2-2-.05(f). Is that the motion?
9 MS. ARCHIE: Yes.
10 MR. PHILLIPS: Okay. Any
11 other discussion before we vote on
12 this motion?
13 DR. GARDNER: Yes.
14 MR. PHILLIPS: Commissioner
15 Gardner?
16 DR. GARDNER: Sorry.
17 MR. PHILLIPS: That's okay.
18 DR. GARDNER: The problem I
19 see with this second portion of the
20 motion is that we have not referred
21 this to the Department. We are
22 assuming that it will be referred and
23 that a select committee will be

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1 appointed to consider the issues. And
2 so to vote on something that has not
3 been approved yet, I think causes
4 quite a problem.
5 MR. PHILLIPS: Excellent
6 point.
7 MS. ARCHIE: I agree.
8 DR. FELKER: I agree and
9 think we need to vote on the first
10 motion and worry about the second
11 motion when we get to the second
12 motion.
13 MS. ARCHIE: Yes.
14 MR. PHILLIPS: So do I have
15 a recommendation --
16 DR. GARDNER: I move that we
17 -- can we do that --
18 MR. PHILLIPS: You can ask
19 for a --
20 DR. GARDNER: -- or ask for
21 guidance?
22 MR. TAMBLING: I had
23 recommended that you do that earlier.

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1 You probably don't remember, but I had
2 a discussion with counsel and the
3 Director on this. I agree with that
4 approach, given the way it's handled
5 so that we're just dealing with things
6 in order right now.
7 MR. PHILLIPS:
8 Commissioner --
9 MS. ARCHIE: At this time, I
10 would withdraw my motion.
11 MR. PHILLIPS: Okay.
12 DR. GARDNER: And I make a
13 motion that we amend the original
14 motion to include there's a lack of
15 substantial, credible and relevant
16 evidence, Number 1.
17 MR. PHILLIPS: So just
18 adding Number 1?
19 DR. GARDNER: Correct.
20 MR. PHILLIPS: Okay. I have
21 a motion on the floor to amend the
22 original motion by adding Item Number
23 1 as a reason. Do I have a second?

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1 MR. WAINWRIGHT: I'll
2 second. And, Mr. Chairman, I also
3 agree to the withdrawal.
4 MR. PHILLIPS: Thank you. I
5 have a motion and a second. Any
6 further discussion on this motion to
7 amend?
8 (No response.)
9 MR. PHILLIPS: Okay. No
10 further discussion --
11 DR. GARDNER: Well, I would
12 like to point something out. That I
13 have spent the last two weeks doing
14 research on this particular petition.
15 And the reality is that there is a
16 lack of evidence, data and information
17 on both sides of the board. There's a
18 great deal of circumstantial evidence,
19 but there is nothing specific or
20 concrete. So I would just like to put
21 that in the record.
22 MR. PHILLIPS: Thank you for
23 that, Commissioner Gardner.

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1 Do we have any other
2 comments from the floor before we vote
3 on this amendment?
4 (No response.)
5 MR. PHILLIPS: So no further
6 discussion on the amendment motion.
7 Okay. So we'll take a vote. All in
8 favor of the motion to amend the
9 original motion signify by signing aye
10 and raise your right hand.
11 (Commission members in favor of
12 the motion so indicated.)
13 MR. PHILLIPS: Motion
14 carries.
15 Now we'll take up the
16 original amended motion, which is to
17 deny the rulemaking petition with the
18 two reasons, Number 1 and Number 3.
19 Any further discussion on that motion?
20 DR. GARDNER: I'm sorry.
21 MR. PHILLIPS: That's okay.
22 Now is the time.
23 DR. GARDNER: I know.

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1 That's why I'm jumping in.
2 MR. PHILLIPS: Commissioner
3 Gardner?
4 DR. GARDNER: Well, just
5 this morning it occurred to me that
6 one of the angles that has not been
7 really approached with this issue is
8 looking at other states in the nation
9 as far as their economic prosperity
10 and where they stood with their cancer
11 risk levels. And so this morning I
12 did a very, very rapid search on the
13 internet and actually found three
14 different rankings. I can run through
15 that real quickly.
16 MR. PHILLIPS: Sure.
17 DR. GARDNER: The three
18 different sites that I found were the
19 U.S. Economic Freedom Index, the
20 Development Report Card for the States
21 from the Corporation for Enterprise
22 Development --
23 MR. PHILLIPS: Could I

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1 interrupt you for a minute? Would it
2 be relevant to us if we had that in
3 front of us as you went through it?
4 If it would, we'll get Debi to get us
5 some copies. If not --
6 MR. WAINWRIGHT: Mr.
7 Chairman, at this point in time we
8 won't have a rebuttal available.
9 MR. PHILLIPS: That's true.
10 But I'm just asking her if she would
11 like us to follow with her as she goes
12 along or if she just wants to provide
13 it.
14 DR. GARDNER: I guess my
15 question back would be, would the rest
16 the Commission like copies? It's
17 very, very brief.
18 DR. FELKER: Why don't you
19 go ahead and give it, and we'll get
20 copies later.
21 DR. GARDNER: Okay. And I'm
22 not saying that this is definitive, by
23 any means. The third one was the New

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1 Economy State Rankings, and it came
2 from rankings of 2006, 2002 and 2007.
3 The gist of what I discovered was that
4 of the top 10 states in the nation
5 based on these three different
6 rankings economically, the majority of
7 those states economically that were
8 ranked very high actually used the 10-6
9 number. The only reason I bring that
10 up is to point out that the economics
11 of this number seems to be in great
12 debate, and this is at least one
13 indication that these states have not
14 suffered economically because of
15 making the choice to go to 10-6.
16 MR. PHILLIPS: Okay. We
17 would just ask that you get us copies
18 of that, if you would.
19 Anyone else?
20 DR. FELKER: I would. I
21 have some comments that I wanted to
22 make. And I guess I was confused
23 because I wasn't really sure whether I

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1 should make them with the first motion
2 or the second motion, but I'm going to
3 go ahead with my comments. And
4 they're relevant, I guess, to both the
5 motions, even though right now we're
6 just considering the first one.
7 Okay. First of all, I
8 want to thank the petitioners for
9 presenting this rulemaking, and I want
10 to thank them for all the information
11 they've provided. You are commended
12 for your collection of relevant
13 materials, your timely submittals,
14 your assistance in providing the
15 Commissioners time to review the data,
16 and for your respectful interactions.
17 As the other
18 Commissioners know, I made comments at
19 the June 1st Rulemaking Committee
20 meeting. This issue was very
21 important to me, and I want to thank
22 you, Robert, for seeing that those
23 comments were read and submitted for

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1 the record. If anyone wants to see
2 those comments, they're available
3 publicly.
4 In addition to my
5 original comments, I have continued to
6 study this over the last month like
7 Dr. Gardner, and I have found the
8 following things: I found most
9 helpful a table provided by Mr. Glenn
10 to Mr. Phillips which was a
11 state-by-state comparison for each of
12 the 58 potential carcinogens. This
13 table demonstrates that for the final
14 concentration, regardless of the
15 various fractions used by each state,
16 that we are less stringent in the
17 final concentrations for most of the
18 carcinogens on the list. I found this
19 alarming, and I found it in conflict
20 with our Strategic Plan that we
21 approved. In general, I feel we need
22 to move in a different direction.
23 Interestingly, however, there are

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1 carcinogens on the list which are more
2 stringent than any other state in our
3 region. The most striking example is
4 arsenic. Other states have chosen to
5 use alternative methods to develop the
6 final concentration for arsenic, where
7 we have not. And we are far more
8 stringent than the states that we are
9 being compared to for that carcinogen
10 as well as several others that do and
11 several others that are on the list.
12 I also want to point out,
13 as I made in my original comments and
14 that's already been made, I believe,
15 by some of the other Commissioners,
16 that the choices for the formula are
17 not 10-4 to 10-6 as everyone thinks.
18 The choices for the formula are 10-5 to
19 10-7. We are not in the middle, but
20 actually at the lowest level of
21 protection. If any of the
22 Commissioners are unclear on this,
23 then Mr. Glenn will help us out with

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1 that, okay?
2 There have also been
3 suggestions that several of the other
4 factors in the formula need adjusting
5 as well. For example, we already set
6 the fish consumption at a higher rate
7 assuming that Alabamians eat more
8 fish, consume more fish than the
9 states around us. Even at that higher
10 level, we're still less stringent. So
11 looking at just one factor in the
12 formula does not necessarily result in
13 the desired effect that we're looking
14 -- or that the petitioners are looking
15 for. So looking at just one factor in
16 the formula has already proven to be
17 unsuccessful. There's also been some
18 doubt about the weight factor used.
19 It is not protective for smaller
20 individuals. Some of the states
21 around us have actually chosen higher
22 weight factors. For example,
23 Tennessee uses a higher weight factor

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1 than we do. This formula and all the
2 other factors have not been reviewed
3 by the Commission for 17 years. That
4 in and of itself I believe is cause
5 enough for the entire formula to be
6 reviewed again. The rulemaking before
7 us, whether it's Motion 1 or Motion 2,
8 only allows us to look at 10-6. It
9 doesn't allow us to look at the other
10 factors. And that, I am disappointed
11 in. It also does not allow for us to
12 evaluate alternative methods as the
13 states around us have done. And I
14 have indications -- I don't have it in
15 front of me -- that it's a
16 negotiation-type process with the EPA
17 where you say, this is what we want to
18 do, and they have to approve it. And
19 EPA is still open to doing that. So
20 that's another possibility for
21 handling the carcinogens on the list.
22 In summary, although we
23 have many unanswered questions, I feel

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1 we can do better than we are currently
2 doing. And I would like to see the
3 Commission to move in that direction
4 in some way. And that's my opinion.
5 And I open it up for any responses or
6 any other opinions.
7 MR. PHILLIPS: Thank you,
8 Commissioner Felker.
9 Any other discussion at
10 this time?
11 MS. ARCHIE: Yes.
12 MR. PHILLIPS: Commissioner
13 Archie?
14 MS. ARCHIE: For the record,
15 I agree with both Dr. Felker and Dr.
16 Gardner. I do agree that you cannot
17 look at that one factor and that it
18 needs to be opened up. You cannot
19 compare us to other states without
20 opening up everything. So I do agree
21 with her that we are looking at 10-6,
22 and we need to look at the other
23 factors that are in the equation. And

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1 maybe the amended motion should be
2 made to include that we are looking at
3 not only 10-6, but all factors that
4 affect that equation.
5 MR. PHILLIPS: Yeah. And
6 maybe that's for a later motion since
7 this one was to deny the rulemaking
8 petition.
9 DR. FELKER: It's hard to
10 discuss one of the motions without
11 discussing the other motion.
12 MR. PHILLIPS: Which is why
13 I'm trying to keep them straight.
14 DR. FELKER: Right. And
15 that's why our comments are kind of
16 overlapping.
17 MR. PHILLIPS: Not a
18 problem.
19 DR. FELKER: We all have
20 opinions obviously for different
21 reasons, but it's hard to separate the
22 two motions.
23 MR. WAINWRIGHT: The first

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1 motion, Mr. Chairman, the issue is
2 shifting the rulemaking from the
3 Commission to the agency.
4 MR. PHILLIPS: No, no, it's
5 not. The first motion is the denial
6 of the rulemaking petition.
7 MR. WAINWRIGHT:
8 (Inaudible.)
9 MR. PHILLIPS: That's to
10 come after your next motion, I would
11 assume. But at this point, the only
12 motion on the floor for the Commission
13 is the petition for rulemaking before
14 us, which is not the rule; it's a
15 petition to go to rulemaking. And you
16 have recommended in the motion before
17 this Commission to deny that petition
18 for rulemaking. It has been amended
19 to add an additional reason, and that
20 reason came from the Committee to
21 include Item 1 as a reason. So that's
22 the only motion on the floor at the
23 moment. Okay? Any other comments,

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1 discussion, thoughts?
2 MR. WAINWRIGHT: I've got
3 some on that Motion 2, but I would
4 like to go ahead and get Motion 1
5 taken care of.
6 MR. PHILLIPS: So with no
7 further discussion, I'll ask all in
8 favor of the motion, which is to deny
9 the petition for rulemaking as amended
10 by adding Item 1 to it, all in favor
11 signify by signing aye and holding up
12 your right hand.
13 (Commission members in favor of
14 the motion so indicated.)
15 MR. PHILLIPS: Motion
16 carries.
17 Debi, do you want us to
18 sign that, or do you want to wait?
19 MS. THOMAS: I just have one
20 order which includes both motions.
21 MR. PHILLIPS: All right.
22 Let's move to Motion 2, Commissioner
23 Wainwright.

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1 MR. WAINWRIGHT: Motion 2,
2 that this issue be considered and
3 reviewed in accordance with Paragraph
4 (e) of Section 41-22-23, Code of
5 Alabama, 1975, by a select committee
6 as appointed by the Director and
7 approved by the Commission and that
8 the Committee's recommendations
9 thereafter refer to the Department for
10 rulemaking the next time Division 6,
11 Water Quality Classifications, is
12 reviewed for changes.
13 Mr. Chairman, this is the
14 motion that has been amended by the
15 attorneys and given to me. It's not
16 exactly the motion that was made by
17 the Committee.
18 MR. PHILLIPS: Well, it's
19 your motion, so --
20 MR. WAINWRIGHT: Well, I've
21 got a piece of paper here with the
22 thing written on it is what I'm
23 getting at.

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1 Robert, do you have the
2 motion that was made by the committee
3 so that we can amend it to fit this?
4 MR. TAMBLING: I don't.
5 Debi, do you have the original motion
6 that was made by the committee?
7 DR. FELKER: I have the
8 original motion.
9 MR. WAINWRIGHT: Let me read
10 the original motion and we will amend
11 it to fit this suggested motion.
12 Motion Number 2, to
13 recommend that the Commission refer
14 the request to the Department with a
15 recommendation that the issue be
16 considered for rulemaking the next
17 time Division 6, Water Quality Use
18 Classifications, of the rules is
19 reviewed for changes, and that the
20 issue be considered by a select
21 committee appointed by the Director
22 and approved by the Commission.
23 MR. PHILLIPS: Okay. That

1 is the motion on the floor. Do I have
2 a second?
3 DR. LESTER: I'll second it.
4 MR. PHILLIPS: I have a
5 motion and a second. And I'm going to
6 read the motion again for everybody
7 before we move to discussion. The
8 motion is to recommend that the
9 Commission refer this request to the
10 Department with a recommendation that
11 the issue be considered for rulemaking
12 the next time Division 6, which is the
13 Water Quality Use Classifications, of
14 the rules is reviewed for changes and
15 that the issue be considered by a
16 select committee appointed by the
17 Director and approved by the
18 Commission. That is the motion before
19 the Commission. I'll open it up for
20 discussion. And before we move to
21 discussion, as you saw on the last
22 motion, there's going to be a lot of
23 things going on, and if it follows

1 through to this one, I may step in and
2 just try to keep us focused on which
3 motion we're dealing with. So I'll
4 open it up for discussion.
5 MR. WAINWRIGHT: Mr.
6 Chairman, that motion is the motion
7 that was made by the recommendation of
8 the Rulemaking Committee, and it can
9 be amended to fit what we have written
10 for it.
11 MR. PHILLIPS: Okay.
12 Continued discussion?
13 DR. FELKER: I have two
14 items I would like to discuss. The
15 motion says that we will wait until
16 the next time that the Water Quality
17 Use Classification rules are reviewed.
18 I would like to see it happen sooner
19 than that. I don't want to wait until
20 that time.
21 DR. GARDNER: And I agree
22 with Dr. Felker.
23 DR. FELKER: I haven't made

1 an amendment yet. My second point is
2 it talks about a select committee
3 appointed by the Director, and I was
4 wondering if the Director could speak
5 to us now on the sort of select
6 committee he's thinking about.
7 MR. PHILLIPS: Okay. That's
8 your --
9 DR. FELKER: Those are my
10 two issues.
11 MR. PHILLIPS: That's your
12 two issues. So I'm going to ask the
13 Director, could you give us the sense
14 of what you believe and give us a time
15 frame idea around the next time Water
16 Quality Use Classifications --
17 MR. GLENN: Okay. Can you
18 bear with me for one moment?
19 MR. PHILLIPS: We most
20 certainly will.
21 (Off-the-record discussion.)
22 MR. GLENN: I'm going to
23 almost exactly quote that. As a

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1 reminder of what we discussed several
2 months ago on the Relative Source
3 Contribution, we indicated we were
4 planning on going by the end of the
5 calendar year with rulemaking because
6 that will be the next time that
7 Division 6 will be opened up like
8 this. But I think the words were
9 "this was a pretty big ticket item
10 that we would have to do a lot of work
11 on." As we discussed, all things have
12 come along procedurally with
13 rulemaking. So, therefore, if we do
14 that in coordination with the Relative
15 Source Contribution, that may add time
16 to it to, again, get everything
17 together. So that's --
18 MR. PHILLIPS: So give us a
19 sense, if you would or if you can --
20 if you can't, that's okay. If it adds
21 time, that infers to me that it would
22 be longer than the end of the year.
23 MR. GLENN: Yes, sir.

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1 MR. PHILLIPS: How much
2 longer?
3 MR. GLENN: That, I don't
4 know if I have a sense for.
5 (Off-the-record discussion.)
6 MR. GLENN: Again, just to
7 almost quote that, we could begin the
8 process almost immediately to review
9 that, but completing it would last
10 into next year. It would be into next
11 calendar year; hopefully in the
12 earlier part of next calendar year,
13 but into next calendar year.
14 DR. GARDNER: So would you
15 say within the year?
16 MR. GLENN: Within a year
17 from today.
18 DR. GARDNER: In June?
19 MR. GLENN: As the one who
20 is not doing all the work, let me --
21 (Off-the-record discussion.)
22 DR. GARDNER: He can address
23 that.

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1 MR. PHILLIPS: Yes, he can
2 speak to --
3 (Inaudible conversations all
4 at once.)
5 MR. McINDOE: I'm James
6 McIndoe with the Department's Water
7 Division. As I understand what the
8 Commission is considering with the
9 creation of this committee, once that
10 committee is in place, I think you
11 kind of have to let the process take
12 its course. And depending on the
13 members of that and what kind of
14 issues they want to get into, I think
15 the schedule is a little indeterminate
16 at this point.
17 DR. FELKER: Okay. I want
18 to make the point that I don't want to
19 see this drug out and, you know --
20 DR. GARDNER: And I second
21 that thought.
22 MR. GLENN: Can I address
23 your second question first and then

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1 get back to this first one?
2 DR. FELKER: Yes.
3 MR. GLENN: Because the
4 second question was obviously with the
5 discussions and decisions made at the
6 June 1st meeting, we as a Department
7 have been looking at if the Commission
8 were to refer this to the Department,
9 what would we envision. So let me
10 address that and see if that addresses
11 your timeline question for you. And I
12 put together a few notes on that.
13 Obviously this is your decision,
14 though, about the logistics of how we
15 do this and in being very specific in
16 how we do this, but we have looked at
17 what we think are the key things. I
18 feel there's some real critical keys
19 here to this process. Number one is
20 getting a very clear understanding
21 regarding what you expect out of the
22 process, and that's, I think, critical
23 for me as I represent the Department.

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1 Having a very clear and specific
2 charge and timeline for the group,
3 that's key to this issue, this panel.
4 And obviously it's no good if we don't
5 address the questions you have. So
6 those are some very key things.
7 I would envision the
8 group being made up of some key state
9 agencies plus others -- I'll get to
10 more specific notes on that -- where
11 we would task them to answer
12 questions, provide findings and
13 recommendations. And so let me give
14 you just my thoughts of a very
15 fast-paced look at an advisory panel.
16 Then I would like to conclude that
17 with if you choose to do that or if
18 you choose to go down this path, a
19 strong request for me, which would be
20 in order for this to happen, I do need
21 some things from you as the
22 Commission, such as, number one, a
23 very clear approval or agreement of

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1 this action plan that we're talking
2 about so that we've set a course and
3 we go by that. And then you'll see
4 from the action plan that I would also
5 request some names of individuals from
6 you that I may not have listed here
7 that you want us to address. And then
8 number two, list questions that you
9 want to have addressed. You've got,
10 as you've mentioned, thousands of
11 pages in front of you, and I'm sure
12 you each have a lot of questions, as
13 do I.
14 Let me give you a
15 potential timeline for this. By next
16 Thursday you could give me two lists
17 of names; individually send me two
18 list of names. One list of names
19 would be who you would envision to be
20 invited to serve on the advisory panel
21 in addition to the names that I will
22 list in a moment. And number two,
23 provide a list of who you envision --

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1 who would you like me to request a
2 statement of economic impact from --
3 if it's in addition to the list I
4 provide, I'll call and tell you -- so
5 that on the next day, Friday, July
6 6th, I would send invitations to serve
7 on an advisory panel to discuss the
8 implementation of this and to make
9 recommendations. I would think at a
10 minimum that advisory panel should be
11 composed of the Department of Public
12 Health, the Alabama Medicaid Agency,
13 the Department of Conservation and
14 Natural Resources, the Alabama
15 Development Office, and the Alabama
16 Department of Ag and Industries --
17 Agriculture and Industries. Those are
18 five sister state agencies that each
19 have very unique responsibilities.
20 MR. PHILLIPS: I'm going to
21 ask you because -- I know we'll get a
22 transcript, but I'm writing this down
23 so I don't miss it. But say them

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1 again.
2 MR. GLENN: Okay. And
3 again, when you -- if you confirm
4 this, I can give you any of this, but
5 this is very clearly a draft of my
6 notes. But Department of Public
7 Health, number one; Medicaid, number
8 two -- and excuse me, I'll just do the
9 short names -- Conservation and
10 Natural Resources, number three, DCNR;
11 ADO, the Alabama Development Office,
12 number four; and Ag and Industries,
13 number five. That would be, I think,
14 each one of those agencies, and
15 obviously, there are a lot of entities
16 who could be involved in this. But in
17 anticipation of your direction, the
18 smallest number of people that could
19 give us the biggest bang for the buck,
20 for lack of better words, is what I
21 was looking at.
22 MR. PHILLIPS: And the date
23 that you would request the list from

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1 us?
2 MR. GLENN: Yeah. If you
3 would like me -- and anyone else that
4 you ask that we put on this advisory
5 panel, I would ask that y'all give me
6 that by next Thursday, if you've got
7 names of who you'd like.
8 And again, I've put
9 together honestly a timeline. And
10 you'll see here my end date here; and
11 I apologize, because maybe going at it
12 in chronological order might be
13 backwards with this timeline. And we
14 can revisit anything that you want to
15 here.
16 Statements of economic
17 impact will be solicited from relevant
18 industry groups, the people whose
19 permits would actually change, or
20 industries who would actually change.
21 I would request them to provide that
22 information to me by August 10, 2007,
23 give them a little over a month to

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1 provide that information. The groups
2 that I would envision soliciting are
3 -- and, again, obviously, you know, we
4 don't have the authority to make
5 anyone doing anything, so this would
6 all be asking them very politely to
7 help us -- Manufacturers of Alabama,
8 and Business Council of Alabama -- and
9 there's 12 of these, so if you want to
10 jot them down, you can so you'll get
11 the gist of the group -- the Alabama
12 NFIB, the small business group, the
13 Coal Association, Farmers Federation,
14 Forestry Association, Pulp and Paper
15 Council, Rural Electric Association,
16 League of Municipalities, the Alabama
17 Association of County Commissioners,
18 the Alabama Power Company, and the
19 Alabama Electric Cities, and anyone
20 else that you request that we get a
21 statement of economic impact from.
22 There was a lot of
23 material sent recently. One of the

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1 things was a resolution highlighting
2 the cost of cancer and the cost of
3 treatment. And I would fully assume
4 that the most appropriate and the
5 one-stop shop for us to get all of
6 this information would be the
7 Department of Public Health. I would
8 envision that they would open up and
9 request information through whatever
10 means they can to provide the other
11 side of the cost, not the
12 implementation of the permitting
13 costs, but the health side of that.
14 And so that is why I did not
15 specifically list a whole lot of
16 medical agencies here, because I would
17 think that Dr. Williamson's group
18 would be the appropriate person to
19 coordinate that. But again, these are
20 my initial thoughts on that.
21 By Friday, July 13th --
22 that's two weeks from today -- I would
23 request that any questions that you

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1 have, that you want answered, would be
2 given to me, so I could have a list of
3 that so we could provide that.
4 That's, I think, an exhaustive list of
5 what I would ask of you as to the
6 timeline.
7 Then late July, early
8 August time frame, we would convene a
9 meeting of this panel, this advisory
10 panel, to communicate the background
11 information, including that panel's
12 charge, the timeline, and to provide
13 the questions posed by you, to give
14 them that, and to task them, request a
15 report from them outlining the
16 findings, opinions, recommendations,
17 answer to questions, whatever words
18 you want to use, by Friday, September
19 28th, 2007. That would be, I think,
20 about three weeks prior to your next
21 Commission meeting. I would
22 anticipate giving information to this
23 panel, you know, well in advance of

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1 the late July, early August meeting to
2 give them a heads-up so that we could
3 have very timely and appropriate and
4 effective conversations getting on the
5 same page in late July, early August.
6 Following the timeline on
7 down -- I'll restate a couple things
8 now -- Friday, August the 10th, we
9 would have requested the economic
10 impact statements to come in. The
11 next Friday, the 17th, I would have
12 compiled those and we would have
13 distributed them to the panel.
14 Obviously, you would get any of this
15 information that you would like to as
16 we go through this process. The next
17 Friday is your Commission meeting --
18 is your next scheduled Commission
19 meeting, which I know it's not
20 officially set yet, but we would
21 anticipate it being August the 24th,
22 2007. We would give you a full status
23 report at that meeting of where we

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1 are, and it would be more focused on
2 the logistics of where we are.
3 September 28th then is
4 the next key date, and that's when the
5 opinions and findings and
6 recommendations from the panel would
7 be requested to come in to me. I
8 would then like to have two weeks,
9 which would put us on Friday, October
10 the 12th, to collect that information
11 together and consolidate that
12 information to give that to you, of
13 course, with a full record of
14 everything that goes on, but to give
15 you a consolidated report of that by
16 Friday, October the 12th. That is
17 one week prior to the next
18 potentially-scheduled meeting, which I
19 know is not enough time for you to
20 digest that now five to 10,000 pages
21 of information, but it would at least
22 be a timeline that you could have in
23 front of you so if we needed to revise

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1 any task that you have given us,
2 assuming you do that at this meeting,
3 you would be prepared to do that at
4 the October meeting. That is just an
5 idea.
6 DR. GARDNER: Trey, I would
7 like to thank you for the obvious
8 thought that you and the Department
9 have put into this.
10 MR. GLENN: Well, thank you.
11 The anticipation was a fast -- this
12 is, I believe, the absolute as fast a
13 track as we could put it on. You
14 would be subject to a schedule like
15 I've set up. Obviously, somebody or
16 something might get left behind here.
17 Someone should they not be able to get
18 the information by the time frame
19 noted, that would not be included in
20 the final report that you would get.
21 So this is only as good as if people
22 can get this information to you.
23 Obviously you see that; that's the one

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1 characteristic that I would point out
2 in this.
3 It does not include the
4 one thing that you may all have in
5 your mind, and that is public
6 hearings. And that is because we
7 presume comments would continue to
8 come in. We've got a host of
9 comments, and I would only assume that
10 as our sister agencies go around and
11 try to collect information and form
12 their opinion, which is what I would
13 assume you're interested in, is their
14 just true opinion in this matter, I
15 would assume they would go out and
16 solicit input from whoever they feel
17 appropriate to form that opinion.
18 That's the only two things I'd point
19 out from that.
20 MR. PHILLIPS: I also want
21 to join in with Commissioner Gardner
22 and thank you for the obvious thought
23 that you've put into this, you and the

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1 Department, and for the hard work for
2 doing that based out of what came out
3 of the June 1 meeting. It's good to
4 see. I appreciate that. But just for
5 clarity's sake, just to make sure for
6 the Commission's benefit -- and
7 correct me if I'm wrong here, Robert
8 -- but the basis of this report is
9 really to provide information to us
10 that we could then use relative to any
11 additional action that may be required
12 that may or may not require
13 rulemaking; correct?
14 MR. GLENN: Yes, sir. And
15 if I could explain the rationale
16 behind that?
17 MR. PHILLIPS: Yes, please.
18 MR. GLENN: Okay. I think
19 the very clear, undisputed fact is
20 that we are discussing one variable,
21 as y'all point out, in an equation.
22 EPA has a range for that variable. We
23 are within that range. In addition to

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1 that, EPA has made it very clear that
2 the ultimate risk level, meaning what
3 happens, what comes out of that
4 formula, not that one variable in the
5 formula, the difference between 10-5
6 and 10-7 and 10-4 or 10-6. So we're
7 talking about a variable here, which
8 is what's in front of you compared to
9 the ultimate risk. And I have
10 provided a document before with a
11 methodology for deriving ambient water
12 quality criteria, which hopefully may
13 have cured up any sleep problems you
14 might have had. But that clearly
15 states that the EPA identifies that
16 the most critical subpopulation should
17 not have worse than a 10-4 risk. So,
18 therefore, given those two very clear
19 facts provided by the EPA -- there's a
20 range within it; there's an ultimate
21 number that they want to maintain --
22 then if we have confidence in the
23 other -- that the other variables of

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1 the formula are accurate or are
2 conservative, one of the two, then
3 using the 10-5 in the formula would
4 pass the 10-4 test, the ultimate test
5 the EPA has set up. And this is not
6 just an idea. This is very clearly
7 understood, because EPA has approved
8 what you as a Commission have done.
9 So that's the basis for saying that
10 this is a -- if there was some very
11 clear information to any of us where
12 like the Air rules that we mentioned
13 are going to go forward where you do
14 this or you lose primacy of the
15 program, that is a different and, I
16 would argue, maybe easier decision.
17 But this is not that, where I would
18 think what you need is information.
19 You need information from
20 professionals in this area, from other
21 entities that you might not have
22 available to you right now. And that
23 was the thought process behind what we

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1 set up.
2 DR. FELKER: And that's a
3 good point, Trey, in that we're
4 currently not doing -- we're following
5 EPA's guidelines; currently that's
6 what we're doing, and that's the point
7 that you just made. And the question
8 is, can we and do we want to do more,
9 and that's good.
10 And just to clarify what
11 you need from the Commissioners if we
12 were to proceed with this, in the very
13 short term to get the ball rolling,
14 you need a very clear list of our
15 concerns and questions to be addressed
16 and you need recommendations from
17 members to serve on your select
18 committee from us?
19 MR. GLENN: The panel as
20 well as a recommendation of anyone
21 else who you would think we would
22 solicit a statement of economic
23 interest from. The two different

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1 lists I mentioned, the list of mainly
2 industry and --
3 DR. FELKER: Right. They're
4 doing the economic interest portion.
5 MR. GLENN: And so if we
6 missed someone there that you'd like
7 us to solicit that from, then --
8 DR. FELKER: Right. What
9 about the rest of the issues that need
10 to be involved in the economic
11 portion?
12 MR. GLENN: Well, then I
13 would think that's the charge given to
14 the panel. That would be the charge
15 given to the panel. There's one key
16 piece of information that I believe
17 the petitioners have provided some
18 information last week or this week
19 about the economic statements and the
20 economic affects of this. This is the
21 one clear thing, that that piece of
22 information, technical information, is
23 not there in front of us. I think a

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1 few of you pointed that out today
2 already. And so I would just
3 anticipate that this would be
4 one piece of information that would be
5 helpful for at least some of those
6 agencies or panel members to look at.
7 MR. PHILLIPS: Okay. I
8 mean, I'll let you speak for yourself,
9 but I think her question is more
10 around that we have a charge as a body
11 to protect human health and the
12 environment. So I think the question
13 is, what about the environment side?
14 You've got DCNR on there, but --
15 DR. FELKER: Yeah. I would
16 like to see some environmentalists on
17 the panel, and I didn't hear any
18 mention of any.
19 (Applause.)
20 MR. GLENN: Thank you, I
21 think. We did. The Department of
22 Conservation and Natural Resources is
23 on the advisory panel.

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1 DR. FELKER: Would the
2 Commissioners be able to provide other
3 names?
4 MR. GLENN: That was my
5 strong request of you, because I mean,
6 there are --
7 DR. FELKER: Okay. Because
8 we discussed --
9 THE REPORTER: Wait. One at
10 a time, please.
11 DR. FELKER: Okay. You and
12 I discussed this before, and I thought
13 we -- I thought that's what we had
14 discussed.
15 MR. GLENN: And so let me
16 restate what I would request from you
17 as a Commission. I did this because
18 there are a host of industry groups or
19 environmental groups or interested
20 stakeholders out there. And if you
21 list all the different groups, they --
22 they number into the hundreds. And
23 so, therefore, I try to focus on the

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1 sister state agencies that had strong
2 responsibilities in this area, my
3 peers, and would recommend those to
4 you as some we should talk to. If you
5 want me to add other folks to that --
6 and I believe this is consistent with
7 how the Commission has done some
8 actions over the past several years.
9 And I would ask you to tell me who do
10 you feel strongly to add to that?
11 Again, this is just my proposed action
12 plan. If you would like for me to
13 pick people, I certainly can do that
14 too.
15 MR. PHILLIPS: I am going to
16 ask relative to that, just because of
17 past actions and for the new
18 Commissioners, we typically limit the
19 number of names that we provide to you
20 because very easily we can give you --
21 I mean, there's seven of us. If we
22 each give you 10 names, you've got 70
23 people, so it makes it a little

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1 unworkable. So I guess my question to
2 you relative to that is, what are you
3 trying to do in the way of that
4 advisory group size?
5 MR. GLENN: Well, I mean,
6 obviously, the smaller the group
7 balance is -- you want a small enough
8 group to where it is functional, but
9 you want enough people to represent
10 the interests, and y'all are the ones
11 who know what the interests are. And
12 if you feel comfortable that -- just
13 as an example, the Medicaid Agency
14 would represent, you know, 90 percent
15 of what your concern is, but there's
16 10 percent of your concern that you
17 think this private group might
18 represent; then you could say,
19 Department, please include this
20 private group. And so I would ask
21 that you limit your additional panel
22 members to one or two. I would
23 request that of you. But again,

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1 you're the ones that will dictate this
2 action plan.
3 MR. PHILLIPS: Okay. Any
4 further discussion from the Commission
5 or questions to Commissioner -- or
6 Director Glenn relative to his plan
7 that he's proposed to us?
8 DR. GARDNER: Yes.
9 MR. PHILLIPS: Commissioner
10 Gardner?
11 DR. GARDNER: Thank you.
12 You've given us a timeline on the
13 front end, and I know that
14 realistically that giving the timeline
15 on the back end of this is difficult.
16 But if we set a timeline for you as
17 far as end point -- because that's the
18 concern. We don't want to see this
19 drug out two years, three years, four
20 years, just dragged on and on and on.
21 We definitely want to see an end
22 point, and I know that you can't give
23 us an idea of that. But do you feel

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1 that a panel of this nature would be
2 able to work within the constraints of
3 a timeline handed to them?
4 MR. GLENN: The answer is
5 yes. I would feel that -- and again,
6 because the way I would set it up --
7 the way I would envision setting this
8 up is you set the end point. And I'm
9 assuming the end point of this is to
10 get you a host of information, new
11 information, hopefully very well
12 organized, very consolidated, that
13 represents a lot of different parties
14 that you might not have heard from to
15 date, get you that information -- and
16 I apologize also -- right prior to
17 your October meeting, which I know
18 does not allow you to make any
19 decision at the October meeting, but
20 it does allow you to look at, well,
21 what do I have and what do I need to
22 -- I didn't want any time to lapse
23 between you receiving a product and

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1 then you needing to retask the
2 Department to go and do something, and
3 that was the decision there. So, you
4 know, I would expect that -- I mean,
5 given the other work loads of
6 everyone, I don't know what that is,
7 so I don't know if this timeline is
8 reasonable. I would hesitate to say
9 you could do it any shorter than this
10 just because of scheduling and
11 calendars and everything else.
12 DR. GARDNER: Well, just
13 like I said, this is the front end.
14 From what I can see, this is the
15 beginning stage of the process, if I'm
16 understanding what you just outlined
17 for us.
18 MR. GLENN: And here's where
19 my very extremely strong request to
20 you as a Commission comes in, and that
21 is to please clarify what you desire
22 should you refer this to the
23 Department. I envision you referring

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1 to the Department to collect
2 information and give to you, which is
3 different from referring it to the
4 Department, collect information, then
5 go as a Department and make a policy
6 recommendation to you as a Commission
7 on whether your current standards need
8 to be changed.
9 MR. PHILLIPS: So for a
10 clarification, once again, for the
11 Commission, your approach, what you've
12 presented to us in this plan, is
13 really to give us a report, for lack
14 of a better word, of conclusions,
15 opinions, recommendations, I heard, of
16 action or actions that may be
17 something that the Commission wants to
18 consider? We will have that just
19 prior to our October meeting, so we
20 will most certainly know in our
21 October meeting whether we have it or
22 not.
23 MR. GLENN: Yes, sir.

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1 MR. PHILLIPS: And then
2 probably, I would think, by our
3 December meeting, we would have the
4 benefit of being a little more
5 thoroughly acquainted with that
6 document. And at that point, you
7 would look to us to say what it is we
8 would do moving forward? I'm trying
9 to say it as --
10 MR. GLENN: Well, yes, sir.
11 And I'm not putting the December time
12 frame on you.
13 MR. PHILLIPS: By all means,
14 but what --
15 MR. GLENN: But I think what
16 you said is very accurate because
17 anything further than that -- I mean,
18 obviously you recognize it's me and
19 the Department stepping outside of our
20 role that you've given us and stepping
21 into your role, and that is something
22 that I would never assume we would do
23 unless charged to do so by you.

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1 That's just -- I want that to be
2 clear.
3 MR. PHILLIPS: Okay. So
4 with that said, I wanted to clarify
5 that before I say this to the
6 Commission. When I read the motion,
7 the motion is, just to remind everyone
8 -- because it's been a little while --
9 to recommend that the Commission refer
10 this request to the Department with
11 the recommendation that the issue be
12 considered for rulemaking the next
13 time that Division 6 of the rules is
14 reviewed for the changes and that the
15 issue be considered by a select
16 committee appointed by the Director
17 and approved by the Commission.
18 MR. WAINWRIGHT: That's
19 correct.
20 MR. PHILLIPS: Now, that's
21 the motion. That doesn't necessarily
22 align with what we've just discussed.
23 DR. FELKER: Well, I would

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1 like to amend the motion, okay? I
2 would like to amend the motion to say
3 that we refer this to the Department
4 to a select committee appointed by the
5 Director with our input to begin
6 immediately; that selection of the
7 committee and the committee process
8 begin immediately, not wait until the
9 revision to start selecting the
10 committee, which is how -- which is
11 what could happen with the current
12 motion. Do you see what I'm saying?
13 So anybody can reword it any way they
14 want, but I would like the process as
15 outlined by Director Glenn to start,
16 and that we're not going to wait.
17 MR. PHILLIPS: And at this
18 point, they can't, because you've made
19 a motion to amend it --
20 DR. FELKER: Okay.
21 MR. PHILLIPS: -- so we're
22 going to deal with that first.
23 DR. FELKER: Okay. Good.

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1 MR. PHILLIPS: The motion to
2 amend -- let me make sure I have this
3 right -- is to refer this issue to the
4 Department and a select committee,
5 with our input, to begin immediately
6 in accordance with the plan as we have
7 heard it from Director Glenn before us
8 today. Is that correct?
9 DR. FELKER: Thank you, Mr.
10 Phillips. That's perfect.
11 MR. PHILLIPS: Do I have a
12 second?
13 MR. WAINWRIGHT: Mr.
14 Chairman --
15 MR. PHILLIPS: I have a
16 motion to amend on the floor.
17 MR. WAINWRIGHT: I
18 understand. But there's no motion
19 been made to --
20 DR. FELKER: I'm making the
21 motion.
22 MR. WAINWRIGHT: What my
23 point is, it's either a substitute

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1 motion or an amendment to the existing
2 motion.
3 MR. PHILLIPS: That is true.
4 Thank you.
5 MR. WAINWRIGHT: Is this a
6 substitute motion or is it an amended
7 motion that's amending this motion?
8 DR. FELKER: Robert?
9 MR. TAMBLING: It's the
10 original motion that came out of the
11 committee that he read.
12 MR. WAINWRIGHT: But what
13 she's doing --
14 DR. FELKER: But I'm trying
15 to decide at this point do I need to
16 amend the motion or substitute the
17 motion?
18 MR. TAMBLING: I think what
19 you did was substitute your motion for
20 his.
21 DR. FELKER: Okay. I'm
22 making a substitution then.
23 MR. PHILLIPS: All right.

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1 It's a substitute motion.
2 MR. WAINWRIGHT: Would you
3 restate the substitute motion then?
4 MR. PHILLIPS: The
5 substitute motion is to refer to the
6 Department this issue --
7 DR. GARDNER: For
8 rulemaking?
9 DR. FELKER: No.
10 DR. GARDNER: No.
11 MR. PHILLIPS: I -- I didn't
12 hear rulemaking. I'm going to say it
13 the way I heard it, and then she can
14 correct me if I missed something, and
15 then you can ask questions when we
16 comment.
17 DR. GARDNER: Okay.
18 MR. PHILLIPS: -- refer this
19 issue to the Department and to a
20 select committee as described with our
21 input to begin immediately and in
22 accordance with Director Glenn's
23 proposed plan as delivered to us

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1 today. That's what I heard.
2 DR. FELKER: And that is
3 what I said. And I think that Dr.
4 Gardner is really asking a question,
5 which is, and what if that's done,
6 then when would --
7 DR. GARDNER: Okay. I
8 second that motion, first of all.
9 DR. FELKER: Thank you.
10 DR. GARDNER: You're
11 welcome. And, yes, I am asking a
12 question.
13 MR. PHILLIPS: Before we go,
14 now, you've seconded that motion.
15 Okay. We have that motion. Everybody
16 understand it? We're only opening
17 this up for discussion on the
18 substitute motion. So now I'll open
19 it up for discussion. Commissioner
20 Gardner?
21 DR. GARDNER: My concern is
22 that there's no mention in the
23 substitute motion of it coming up for

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1 rulemaking. There's no motion of
2 rulemaking. We are discussing a
3 potential rule change in this select
4 committee.
5 DR. FELKER: And I think
6 that what Mr. Glenn said and what I
7 assumed was that after we got the
8 recommendations, we would decide as a
9 Department and a Commission -- the
10 Commission would decide to direct the
11 Department in the direction of
12 rulemaking or not.
13 (Off-the-record discussion.)
14 MR. GLENN: I'm sorry?
15 DR. FELKER: I think what I
16 assumed or thought I heard was that we
17 would do the committee, the study, get
18 all the answers, and then the
19 Commission take responsibility and
20 decide whether or not it's time for
21 rulemaking or not and direct the
22 Department accordingly?
23 MR. PHILLIPS: Could I

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1 propose just -- because I've used the
2 word twice "issue," and that may be
3 part of our problem here. I don't
4 know that it is. But maybe it's
5 "refer for consideration -- for future
6 consideration for rulemaking" --
7 DR. GARDNER: Thank you.
8 MR. PHILLIPS: -- of the --
9 whatever the quote is on the front --
10 to the Department, and then the rest
11 of the motion. Does that accomplish
12 what --
13 DR. GARDNER: Are you making
14 a motion to amend the substitute
15 motion?
16 DR. FELKER: It's really
17 very difficult trying to follow this
18 whole Robert's Rules thing.
19 MR. PHILLIPS: Well,
20 considering that you didn't -- I
21 didn't introduce that into your
22 motion; you just said "this issue,"
23 and so that's what I move forward; it

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1 would need to be an amendment to the
2 motion to clarify it.
3 DR. GARDNER: So are you
4 making that motion?
5 MR. PHILLIPS: No. I'm
6 telling you as the Chair what you
7 would need to consider. If you would
8 like to make that motion, you can.
9 DR. GARDNER: I would love
10 to make that motion. I have nothing
11 before me that I can read to --
12 MR. PHILLIPS: I can reread
13 it for you.
14 DR. GARDNER: If you would,
15 please.
16 MR. PHILLIPS: You would
17 make a motion to amend Dr. Felker's
18 substitute motion to include, instead
19 of "issue," refer for future
20 consideration of rulemaking.
21 DR. GARDNER: Okay. I make
22 an amended motion that instead of the
23 word "issue" that we say "to refer for

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1 future consideration of rulemaking."
2 MR. PHILLIPS: I have a
3 motion. Do I have a second?
4 DR. FELKER: I second.
5 MR. PHILLIPS: Do you have a
6 point of order?
7 DR. LESTER: Yes. Mr.
8 Chairman, she made a motion, but --
9 MR. PHILLIPS: She made a
10 substitute motion.
11 DR. LESTER: -- she didn't
12 get a second.
13 MR. PHILLIPS: She did get a
14 second.
15 DR. FELKER: I seconded it.
16 DR. LESTER: Oh, you
17 seconded it? Okay. I didn't hear
18 that second.
19 MR. PHILLIPS: It's on the
20 floor.
21 DR. LESTER: If she hadn't
22 got a second, then she can do it
23 herself.

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1 MR. PHILLIPS: She can do it
2 herself, you're right. But she has a
3 second.
4 So I have a motion. Do I
5 have a second for the amendment to the
6 substitute motion?
7 DR. FELKER: If I'm allowed
8 to, I'll second it. I don't know if I
9 am.
10 MR. PHILLIPS: You are.
11 DR. FELKER: Okay.
12 MR. PHILLIPS: So I have a
13 motion and a second. Any discussion
14 on the clarification language only?
15 (No response.)
16 MR. PHILLIPS: Okay. No
17 further discussion, all in favor of
18 clarifying the substitute motion,
19 signify by signing aye and raise your
20 right hand.
21 (Commission members in favor of
22 the motion so indicated.)
23 MR. PHILLIPS: All opposed?

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1 (No response.)
2 MR. PHILLIPS: Motion
3 carries. The substitute motion now is
4 amended to state that the Commission
5 will refer for consideration for
6 future rulemaking to the Department
7 and a select committee with our input
8 to begin immediately in accordance
9 with the plan as provided by Director
10 Glenn to the Commission today. That's
11 the substitute motion now. All right?
12 Any further discussion on the
13 substitute motion?
14 (No response.)
15 MR. PHILLIPS: Does that
16 motion -- let me ask the Department
17 just so that if there's anything we
18 need to know relative to that motion,
19 is there anything in that substitute
20 motion that we need clarification on?
21 Director Glenn?
22 MR. GLENN: Chairman
23 Phillips, I don't think there's

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1 anything you need clarification on.
2 There may be something I need
3 clarification on. Refer to the
4 Department for consideration of
5 rulemaking consists of --
6 DR. GARDNER: Future.
7 MR. GLENN: -- future,
8 excuse me, future --
9 MR. PHILLIPS: No. Refer
10 for consideration of future rulemaking
11 to the Department --
12 MR. GLENN: Okay.
13 MR. PHILLIPS: -- and a
14 select committee --
15 MR. GLENN: In accordance
16 with what I discussed?
17 MR. PHILLIPS: Your plan,
18 right.
19 MR. GLENN: The plan that I
20 discussed was based on just an
21 assumption, again, that we would
22 collect information and give it to the
23 Commission --

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1 MR. PHILLIPS: That's
2 correct.
3 MR. GLENN: -- to make
4 decisions about future rulemaking.
5 MR. PHILLIPS: So to me, I'm
6 hearing that what's being referred to
7 you and the committee is the
8 information and recommendations of
9 actions that we would need to consider
10 for that.
11 MR. GLENN: Okay. Thank
12 you.
13 MR. PHILLIPS: Is that
14 correct?
15 (Affirmative response by
16 Commissioners.)
17 MR. PHILLIPS: Okay. We
18 have a substitute motion on the floor.
19 Any discussion around that motion?
20 Any further discussion around that
21 motion?
22 (No response.)
23 MR. PHILLIPS: I'm just

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1 going to give you a moment.
2 MR. WAINWRIGHT: I wouldn't
3 dare.
4 DR. LESTER: Call for a
5 vote.
6 MR. PHILLIPS: Okay. With
7 no discussion, all in favor of the
8 substitute motion, signify by signing
9 aye and raise your right hand.
10 (Commission members in favor of
11 the motion so indicated.)
12 MR. PHILLIPS: Okay. Motion
13 carries. That was Agenda Item Number
14 5. Do you need a moment, Debi, to --
15 MS. THOMAS: May I let y'all
16 sign the signature page, and I'll have
17 to work with Mr. Tambling to get the
18 wording correct on that?
19 MR. PHILLIPS: Yes, you can.
20 MR. PHILLIPS: Well, let's
21 take a brief five-minute break. Be
22 back here in five minutes.
23 (Brief recess was taken.)

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1 MR. PHILLIPS: Okay. We are
2 going to reconvene now. I need the
3 room quiet.
4 Moving to Agenda Item
5 Number 6, I want to call on Rulemaking
6 Committee Chair Wainwright again to
7 report on his Rulemaking Committee
8 meeting and the recommendation
9 regarding the rulemaking petition.
10 Commissioner Wainwright?
11 MR. WAINWRIGHT: Mr.
12 Chairman, the second item that the
13 Rulemaking Committee took up was the
14 item that involved the Phenols, and it
15 was a second petition that was given
16 to us from -- just a moment and let me
17 find the minutes. It's the reference
18 dose of Acrolein and Phenol. The
19 recommendation of the committee was to
20 recommend to the full Commission that
21 it deny the petition for the rule
22 change citing Section 335-2-2-.05(g)
23 as the reason. The motion that we

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1 would like to make would be to adopt
2 the recommendation of the Rulemaking
3 Committee and deny the rulemaking
4 petition citing Paragraphs -- I
5 believe we would be citing Paragraph
6 (g) of the rule, 335-2-2-.05,
7 Consideration of Petition as the
8 reason for denial. That reason, (g),
9 is the adoption of the proposed rule
10 would impact the overall regulatory
11 scheme of the Department; that would
12 be R.335-2-2-.05(g).
13 Several other reasons
14 have been proposed by the attorney,
15 and they can be added as amendments.
16 But that would be my -- that would be
17 my motion and the reasons that was
18 given by the committee.
19 MR. PHILLIPS: Okay. Thank
20 you, Commissioner Wainwright. So the
21 motion is to deny the petition, refer
22 back to the Department?
23 MR. WAINWRIGHT: Not yet.

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1 MR. PHILLIPS: No? Okay.
2 Deny the petition with the (g) reason?
3 MR. WAINWRIGHT: Right.
4 MR. PHILLIPS: Okay. Do I
5 have a second?
6 DR. LESTER: Second.
7 MR. PHILLIPS: I have a
8 motion and a second. Do we need to
9 open this up for discussion?
10 Commissioner Gardner?
11 DR. GARDNER: My question to
12 the Department is the same as it was
13 with the first petition. Simply what
14 manner would the adoption of this
15 proposed rule impact the overall
16 regulatory scheme of the Department?
17 Is your answer basically the same as
18 it was previously?
19 MR. GLENN: Yes,
20 Commissioner Gardner, it is. And
21 there's a much smaller -- I say much;
22 there is a smaller universe of
23 affected individuals with this than

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1 the rulemaking being discussed in the
2 previous agenda item. And I'm going
3 to make sure I'm correct on that.
4 (Off-the-record discussion.)
5 MR. GLENN: Yes. The answer
6 is, yes, in a very similar manner.
7 MR. PHILLIPS: Any
8 additional discussion?
9 DR. GARDNER: If my
10 understanding of this petition is
11 correct, the numerical values that
12 we're looking at have been updated in
13 IRIS, which is, again if my
14 understanding is correct, what our
15 Code, our statutory authority,
16 whatever, requires us to follow when
17 making decisions regarding these two
18 items?
19 MR. PHILLIPS: Again, let's
20 ask the Department to address that.
21 MR. GLENN: Yes, I would be
22 happy to speak to that. And I guess
23 the easiest way to speak to that is to

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1 refer you to -- obviously you don't
2 have our Code here. Sorry. It takes
3 up a whole shelf. Let me make sure
4 I'm looking at this correctly.
5 Basically it's Page 10.9 -- I'm not
6 sure that's the official reference
7 here -- but (as read:) The values used
8 for the reference dose shall be the
9 values available through the U.S.
10 EPA's Integrated Risk Information
11 System (IRIS) -- that's the words
12 "shall be used" -- and the values for
13 bioconcentration factors shall be
14 contained, dot, dot, dot, except where
15 other values are established pursuant
16 to Subparagraph (1)(g). So that's
17 what our rules call for. My read of
18 that, I would probably read our rules
19 slightly different than what you
20 stated to say that you as a Commission
21 have told us in setting these permits,
22 use IRIS unless we tell you something
23 else. You've told us something else

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1 for this one. The something else that
2 you told us is actually consistent
3 with the National Recommended Water
4 Quality Criteria, which was, I think,
5 whatever it's based on, and that value
6 is different from the value in IRIS.
7 IRIS has a more stringent value.
8 DR. GARDNER: More stringent
9 and more updated, if I understand
10 correctly?
11 MR. GLENN: I can't speak to
12 that, because the decision made by the
13 Commission to adopt the current value
14 was made after the IRIS value -- the
15 IRIS report came out. And the current
16 National Recommended Water Quality
17 Criteria, which is what EPA tells us
18 they recommend, was published after
19 that IRIS value came out. So I can't
20 speak to the thought process that EPA
21 went through in setting their values
22 as they set them as far as the
23 timeline of that. That's just the

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1 timeline of the documents. I don't
2 know if -- does that answer your
3 question?
4 MR. PHILLIPS: Does that
5 answer your question, Commissioner
6 Gardner?
7 DR. GARDNER: It does.
8 MR. PHILLIPS: All right.
9 Any other discussion from the
10 Commission?
11 (No response.)
12 MR. PHILLIPS: No further
13 discussion, all --
14 MR. WAINWRIGHT: Mr.
15 Chairman, the --
16 MR. PHILLIPS: Commissioner
17 Wainwright?
18 MR. WAINWRIGHT: -- attorney
19 had recommended that we add another
20 reason for the rule change, which is
21 Item 1 in your attachment, if anyone
22 wants to amend the motion and add that
23 reason.

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1 MR. PHILLIPS: Okay.
2 Referring to your attachment,
3 Commissioner Wainwright is pointing
4 this out to us. Are you making this
5 as a motion, or are you just --
6 MR. WAINWRIGHT: I just want
7 the Commission to realize that he had
8 asked for it that this be added.
9 MR. PHILLIPS: So I'll ask
10 of the Commission, does anyone want to
11 amend the motion on the floor?
12 DR. FELKER: We're asking
13 for an amendment --
14 DR. GARDNER: He's asking if
15 anyone wants to amend the motion.
16 DR. FELKER: That Robert
17 gave --
18 MR. PHILLIPS: Right.
19 DR. FELKER: Okay. I'll
20 make that motion.
21 MR. PHILLIPS: I have a
22 motion to amend the previous motion --
23 DR. LESTER: I'll second it.

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1 MR. PHILLIPS: -- and a
2 second, to amend by adding Item Number
3 1, which is, the petition is not
4 supported by substantive, credible,
5 and relevant evidence, data and
6 information as would reasonably
7 support the proposed rule adoption,
8 amendment or repeal in the absence of
9 contradictory evidence, data or
10 information or other relevant factors,
11 evidence, data or information which
12 the Commission might legitimately
13 consider, reference 335-2-2-.05(d).
14 That's the motion to amend. Any
15 discussion on the motion?
16 DR. GARDNER: I don't think
17 that that applies in this case from
18 the studying that I have been going
19 over the last several weeks. As best
20 I can tell, there is newer data that
21 has been reviewed by the EPA. It's
22 posted on the IRIS website. Our
23 statutory authority says that we need

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1 to be following the IRIS data, and I
2 believe that's what we need to do.
3 MR. PHILLIPS: Okay. Any
4 other discussion on the amendment?
5 (No response.)
6 MR. PHILLIPS: No further
7 discussion -- and this is solely for
8 the amendment to the motion on the
9 floor -- all in favor of the amendment
10 to add as an additional reason the
11 reason that I just read, which is
12 Number 1 in your attachment, all
13 indicate with the sign of aye and
14 raise your right hand.
15 (Commission members in favor of
16 the motion so indicated.)
17 MR. PHILLIPS: All opposed?
18 (Commission member opposed to
19 the motion so indicated.)
20 MR. PHILLIPS: Motion
21 carries. So the amended motion is to
22 deny the petition for rulemaking
23 citing Section 335-2-2-.05(g) and (d)

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1 as reasons -- as the reasons. Any
2 discussion on the motion on the floor?
3 (No response.)
4 MR. PHILLIPS: No further
5 discussion, all in favor of the motion
6 signify by signing aye and raise your
7 right hand.
8 (Commission members in favor of
9 the motion so indicated.)
10 MR. PHILLIPS: All opposed?
11 DR. GARDNER: Aye.
12 MR. PHILLIPS: Motion
13 carries.
14 Do you have an order for
15 us?
16 MS. THOMAS: If you will
17 sign the signature page, I'll adjust
18 it.
19 (Off-the-record discussion.)
20 MR. PHILLIPS: Commissioner
21 Wainwright, anything else?
22 MR. WAINWRIGHT: The second
23 motion is to recommend that the

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1 Commission refer this request to the
2 Department with a recommendation that
3 the issue be considered for rulemaking
4 the next time Division 6, Water
5 Quality Use Classifications of the
6 rules is reviewed for changes.
7 MR. PHILLIPS: I have a
8 motion. Do I have a second?
9 DR. FELKER: Second.
10 MR. PHILLIPS: I have a
11 motion and a second. Discussion on
12 the motion?
13 DR. FELKER: I just have a
14 question for Trey. Trey, the time
15 frame on that is the same as you gave
16 earlier, the end of the year, the
17 beginning of the next year; is that
18 right?
19 MR. PHILLIPS: Director
20 Glenn?
21 MR. GLENN: Yes,
22 Commissioner. We clearly can evaluate
23 this and -- we can easily, I believe,

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1 evaluate this in coordination with the
2 Relative Source Contribution. And I
3 say that because I'm not sure what
4 else -- what other information there
5 is to gather that would not put us in
6 a position of running counter to the
7 policy that you have set for us to
8 follow or that directly has come from
9 the only other source that I would
10 look at to change and make a
11 recommendation to you, which would be
12 EPA. And I personally have had the
13 opportunity to speak to them, and they
14 have been very clear -- and it's
15 confusing, and as Dr. Gardner pointed
16 out, there's days of studies that
17 don't seem to add up with their
18 published numbers. But I have asked
19 the Region 4 staff directly, the
20 Regional Administrator, including
21 General Counsel there and the Water
22 Division individuals, what is their
23 recommendation to Alabama. They're

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1 clearly saying that's y'all's job to
2 make that decision, but what's their
3 recommendation? And they very clearly
4 said the National Recommended Water
5 Quality Criteria. So we can reask
6 those questions and go through that
7 very -- I think in a very short time
8 frame, but it would probably put us
9 back in front of you with a policy
10 decision that would challenge a
11 decision you had made previously on
12 setting limits that you've set in the
13 regulations. I mean, you say use IRIS
14 unless you say something different.
15 You've said something different. And
16 if I have no reason to challenge what
17 you've said differently or to bring up
18 to you what you've said differently,
19 then I feel you've spoken clearly to
20 us on this.
21 DR. FELKER: And all the
22 motion really says is just that it
23 will be re-evaluated at another time;

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1 right?
2 MR. GLENN: Right, and we'll
3 do that. I just wanted to walk you
4 through the process to see if you
5 wanted me to do anything different.
6 The other one that we talked about
7 obviously had a much more specific
8 timetable and outcome, and I want to
9 make sure that we're clear of what you
10 expect from the Department on this.
11 MR. WAINWRIGHT:
12 (Inaudible.)
13 DR. GARDNER: And I know
14 that I would like to see these because
15 there is -- you've got the United
16 States EPA, the national office,
17 saying one thing. Then you've got
18 Region 4 saying another. And so it is
19 very confusing.
20 MR. GLENN: Yes, it is.
21 DR. GARDNER: And when --
22 when you spoke with Region 4, did you
23 explain the confusion within our Code?

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1 MR. GLENN: Yes, I did have
2 the opportunity to do that. And they
3 referenced me to the national EPA
4 document that was published, the
5 criteria document, which does -- it is
6 confusing when you look at the dates
7 compared to the IRIS values. So they
8 referenced the national document. So
9 we will -- we'll revisit that and will
10 clearly, per your motion, do that in
11 coordination with the Relative Source
12 Contribution so it will be on a
13 parallel time track to that, and that
14 will be this calendar year.
15 MR. PHILLIPS: Any
16 additional discussion?
17 (No response.)
18 MR. PHILLIPS: There being
19 no further discussion, all in favor of
20 the motion signify by signing aye and
21 right hand.
22 (Commission members in favor of
23 the motion so indicated.)

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1 MR. PHILLIPS: Thank you.
2 All opposed, same sign?
3 (No response.)
4 MR. PHILLIPS: Motion
5 carries.
6 Do you have something for
7 us to sign?
8 MS. THOMAS: I had both
9 motions on one order. I can adjust to
10 include them on one order, or if you
11 want me to separate them, I can do
12 that. But as with the previous agenda
13 item, I can put them both on one.
14 MR. PHILLIPS: That's fine.
15 Just do it on one.
16 MS. THOMAS: Yes, sir.
17 Thank you.
18 MR. PHILLIPS: Moving to
19 Agenda Item Number 7, the Commission
20 will consider the recommendation of
21 the Administrative Law Judge to grant
22 the Joint Motion for Consent Order and
23 approve the proposed Consent Order.

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1 I'll entertain a motion from the
2 Commission.
3 DR. FELKER: I'll move.
4 MR. PHILLIPS: I have a
5 motion. Do I have a second?
6 DR. GARDNER: Second.
7 MR. PHILLIPS: I have a
8 motion and a second. Any further
9 discussion?
10 (No response.)
11 MR. PHILLIPS: All in favor
12 signify by signing aye.
13 (Commission members in favor of
14 the motion so indicated.)
15 MR. PHILLIPS: All opposed,
16 same sign.
17 (No response.)
18 MR. PHILLIPS: Motion
19 carries.
20 MS. THOMAS: I have two
21 places for you to sign.
22 MR. PHILLIPS: Moving on to
23 Agenda Item Number 8, I will note for

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1 the record the Petitioner's withdrawal
2 of his request for a hearing.
3 Agenda Item Number 9,
4 which is Other Business, before I move
5 to ask if there is any, I do want to
6 remind the Commission that I am going
7 to be looking into that relative to
8 our rules and Robert's Rules of Order,
9 and I will have a report back to you
10 in my Director's report at the next
11 meeting. So with that said, is there
12 any other business to come before the
13 Commission?
14 DR. GARDNER: Remind me what
15 you wanted from us before the next
16 meeting.
17 MR. PHILLIPS: In my
18 Director's report I had asked you to
19 just be prepared if you are a
20 committee chair to give us an update
21 relative to the goals and objectives
22 that you had set at the beginning of
23 the year and just be thinking about

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our election in October.
Okay. Any other business?
DR. LESTER: I move we adjourn.
MR. PHILLIPS: Can we move to the next agenda item first?
We do have one other agenda item, and it's really our future business meeting. And I just want to make sure that we all know that it is August the 24th, and I wanted to make sure that there are no conflicts that we need to discuss at this time.
If there are not, I will entertain a motion that we adjourn.
DR. FELKER: Motion made.
MR. PHILLIPS: I have a motion. Do I have a second?
DR. GARDNER: Second.
MR. PHILLIPS: All in favor?
(Commission members in favor of

the motion so indicated.)
MR. PHILLIPS: We're adjourned. Thank you.
(Meeting adjourned.)

(The foregoing AEMC Commission Meeting concluded and was adjourned at approximately 1:09 p.m., on June 29th, 2007.)

****
REPORTER'S CERTIFICATE
****

I, Karen Reagan Drinkard, Certified Court Reporter and Notary Public in and for the State of Alabama at Large, do hereby certify that I reported stenographically and then reduced to typewritten form by means of computer-aided transcription my stenographic notes of the foregoing Alabama Environmental Management Commission Meeting.

I further certify that the foregoing transcript is a true and correct transcript of the proceedings contained herein.

I further certify that I am neither of kin nor of counsel to the parties to said meeting, nor in any

manner interested in the results thereof.

Done this the 30th day of June, 2007.

Karen Reagan Drinkard, CCR
Reporter and Notary Public
State of Alabama at Large

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manner interested in the results thereof.

Done this the 30th day of June, 2007.

Karen Reagan Drinkard, CCR
Reporter and Notary Public
State of Alabama at Large

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