SCREENING-LEVEL HAZARD CHARACTERIZATION OF HIGH PRODUCTION VOLUME CHEMICALS

CHEMICAL CATEGORY NAME
CHLOROBENZENES

SPONSORED CHEMICALS

Monochlorobenzene [9th C.I. Name: Benzene, chloro-]	**CAS No. 108-90-7**
1,2-Dichlorobenzene [9th C.I. Name: Benzene, 1,2-dichloro-]	**CAS No. 95-50-1**
1,3-Dichlorobenzene [9th C.I. Name: Benzene, 1,3-dichloro-]	**CAS No. 541-73-1**
1,2,3-Trichlorobenzene [9th C.I. Name: Benzene, 1,2,3-trichloro-]	**CAS No. 87-61-6**

SUPPORTING CHEMICALS

1,4-Dichlorobenzene [9th C.I. Name: Benzene, 1,4-dichloro-]	**CAS No. 106-46-7**
1,2,4-Trichlorobenzene [9th C.I. Name: Benzene, 1,2,4-trichloro-]	**CAS No. 120-82-1**

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INTERIM

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SCREENING LEVEL HAZARD CHARACTERIZATION OF HIGH PRODUCTION VOLUME CHEMICALS

The High Production Volume (HPV) Challenge Program[1] is a voluntary initiative aimed at developing and making publicly available screening-level health and environmental effects information on chemicals manufactured in or imported into the United States in quantities greater than one million pounds per year. In the Challenge Program, producers and importers of HPV chemicals voluntarily sponsor chemicals; sponsorship entails the identification and initial assessment of the adequacy of existing toxicity data/information, conducting new testing if adequate data do not exist, and making both new and existing data and information available to the public. Each complete data submission contains data on 18 internationally agreed to "SIDS" (Screening Information Data Set[1,2]) endpoints that are screening-level indicators of potential hazards (toxicity) for humans or the environment.

The Environmental Protection Agency's Office of Pollution Prevention and Toxics (OPPT) is evaluating the data submitted in the HPV Challenge Program on approximately 1,400 sponsored chemicals. OPPT is using a hazard-based screening process to prioritize review of the submissions. The hazard-based screening process consists of two tiers described below briefly and in more detail on the Hazard Characterization website[3].

Tier 1 is a computerized sorting process whereby key elements of a submitted data set are compared to established criteria to "bin" chemicals/categories for OPPT review. This is an automated process performed on the data as submitted by the sponsor. It does not include evaluation of the quality or completeness of the data.

In Tier 2, a screening-level hazard characterization is developed by EPA that consists of an objective evaluation of the quality and completeness of the data set provided in the Challenge Program submissions. The evaluation is performed according to established EPA guidance[2,4] and is based primarily on hazard data provided by sponsors. EPA may also include additional or updated hazard information of which EPA, sponsors or other parties have become aware. The hazard characterization may also identify data gaps that will become the basis for a subsequent data needs assessment where deemed necessary. Under the HPV Challenge Program, chemicals that have similar chemical structures, properties and biological activities may be grouped together and their data shared across the resulting category. This approach often significantly reduces the need for conducting tests for all endpoints for all category members. As part of Tier 2, evaluation of chemical category rationale and composition and data extrapolation(s) among category members is performed in accord with established EPA[2] and OECD[5] guidance.

The screening-level hazard characterizations that emerge from Tier 2 are important contributors to OPPT's existing chemicals review process. These hazard characterizations are technical documents intended to support subsequent decisions and actions by OPPT. Accordingly, the documents are not written with the goal of informing the general public. However, they do provide a vehicle for public access to a concise assessment of the raw technical data on HPV chemicals and provide information previously not readily available to the public. The public, including sponsors, may offer comments on the hazard characterization documents.

The screening-level hazard characterizations, as the name indicates, do not evaluate the potential risks of a chemical or a chemical category, but will serve as a starting point for such reviews. In 2007, EPA received data on uses of and exposures to high-volume TSCA existing chemicals, submitted in accordance with the requirements of the Inventory Update Reporting (IUR) rule. For the chemicals in the HPV Challenge Program, EPA will review the IUR data to evaluate exposure potential. The resulting exposure information will then be combined with the screening-level hazard characterizations to develop screening-level risk characterizations[4,6]. The screening-level risk characterizations will inform EPA on the need for further work on individual chemicals or categories. Efforts are currently underway to consider how best to utilize these screening-level risk characterizations as part of a risk-based decision-making process on HPV chemicals which applies the results of the successful U.S. High Production Volume Challenge Program and the IUR to support judgments concerning the need, if any, for further action.

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[1] U.S. EPA. High Production Volume (HPV) Challenge Program; http://www.epa.gov/chemrtk/index.htm.
[2] U.S. EPA. HPV Challenge Program – Information Sources; http://www.epa.gov/chemrtk/pubs/general/guidocs.htm.
[3] U.S. EPA. HPV Chemicals Hazard Characterization website (http://www.epa.gov/hpvis/abouthc.html).
[4] U.S. EPA. Risk Assessment Guidelines; http://cfpub.epa.gov/ncea/raf/rafguid.cfm.
[5] OECD. Guidance on the Development and Use of Chemical Categories; http://www.oecd.org/dataoecd/60/47/1947509.pdf.
[6] U.S. EPA. Risk Characterization Program; http://www.epa.gov/osa/spc/2riskchr.htm.

SCREENING LEVEL HAZARD CHARACTERIZATION
Chlorobenzenes Category

Introduction

The sponsor, The Synthetic Organic Chemical Manufactueres Association's (SOCMA) Chlorobenzene Producers Association (CPA), submitted a Test Plan and Robust Summaries to EPA for the chlorobenzenes category on March 18, 2002. EPA posted the submission on the ChemRTK Web site on April 2, 2002 (http://www.epa.gov/chemrtk/pubs/summaries/chlrbnzs/c13650tc.htm). EPA comments on the original submission were posted to the website on November 13, 2002. Public comments were also received and posted to the website. The sponsor submitted revised and final documents on February 7, 2003, which were posted to the ChemRTK website on March 21, 2003. The chlorobenzenes category consists of the following four chemicals and two supporting chemicals:

Sponsored Chemicals	
Monochlorobenzene [9th C.I. name: benzene, chloro-]	CAS No. 108-90-7
1,2-Dichlorobenzene [9th C.I. name: benzene, 1,2-dichloro-]	CAS No. 95-50-1
1,3-Dichlorobenzene [9th C.I. name: benzene, 1,3-dichloro-]	CAS No. 541-73-1
1,2,3-Trichlorobenzene [9th C.I. name: benzene, 1,2,3-trichloro-]	CAS No. 87-61-6

Supporting Chemicals	
1,4-Dichlorobenzene [9th C.I. name: benzene, 1,4-dichloro-]	CAS No. 106-46-7
1,2,4-Trichlorobenzene [9th C.I. name: benzene, 1,2,4-trichloro-]	CAS No. 120-82-1

This screening-level hazard characterization is based primarily on the review of the test plan and robust summaries of studies submitted by the sponsor under the HPV Challenge Program. In preparing the hazard characterization, EPA considered its own comments and public comments on the original submission as well as the sponsor's responses to comments and revisions made to the submission. Structures of the sponsored and supporting chemicals are included in the appendix. The screening-level hazard characterization for environmental and human health toxicity is based largely on SIDS endpoints and is described according to established EPA or OECD effect level definitions and hazard assessment practices.

Category and Supporting Chemicals Justification

The category consists of mono-, di- and tri-chlorobenzenes. All four category members and the two supporting chemicals have a benzene ring in which one, two or three hydrogen atoms are replaced by chlorine atoms. The two category members 1,2- and 1,3-dichlorobenzene and supporting chemical 1,4-dichlorobenzene, are isomers – the placement of the chlorines on the benzene ring being in the *ortho*, *meta*, and *para* positions, respectively. Likewise, the supporting chemical, 1,2,4-trichlorobenzene, is an isomer of the category member, 1,2,3-trichlorobenzene with the difference being a chlorine in the *para* position rather than the *meta* position. Based on structural similarities and similarity in physical-chemical and environmental fate properties and toxicity, the chemicals in this category are grouped and evaluated together. Data for the tested category members are extrapolated to provide estimates of similar properties for the untested members.

1,2-Dichlorobenzene and 1,4-dichlorobenzene have been assessed under the OECD High Production Volume Chemicals Programme and the evaluations published by the United Nations Environmental Programme (UNEP): http://www.chem.unep.ch/irptc/sids/OECDSIDS/95501.pdf and http://ecb.jrc.it/esis/index.php?PGM=hpv

Summary-Conclusion

The log K_{ow} values of chlorobenzene category members indicate that their potential to bioaccumulate is expected to be low. The chlorobenzene category members are not readily biodegradable, indicating that they have the potential to persist in the environment.

The evaluation of available toxicity data for fish, aquatic invertebrates and aquatic plants indicates that the potential acute hazard of the chlorobenzenes category members is generally moderate; the exception being that toxicity of 1,2,3-trichlorobenzene in algae was high. A trend of increasing toxicity with increasing chlorine substitution is observed for fish, daphnia and algae.

The acute oral toxicity of members of the chlorobenzenes category to rats is moderate to low and the acute dermal toxicity to rats and rabbits and acute inhalation toxicity to rats and mice is low. Repeated exposures resulted in decreased body weight gain at high doses and major effects on liver, kidney, spleen, thymus and thyroid. Liver effects included increased liver weights, increased incidence of hepatic necrosis, hepatocellular degeneration, hepatic cytoplasmic alterations and increased levels of bilirubin, cholesterol, increased activity of liver enzymes and mild to moderate porphyria. Increased kidney weights with necrosis or degeneration of the proximal tubular epithelium were seen in kidneys. Decreases in absolute and relative spleen weights and splenic lymphoid depletion were observed. Effects on hematological parameters seen for the supporting chemical, 1,4-dichlorobenzene, included decreases in hemoglobin, hematocrit and erythrocytes counts. Thymic lymphoid depletion and depletion of colloid density in the thyroid were seen. The category members did not show any treatment-related effect on reproductive parameters. 1,2-Dichlorobenzene was slightly fetotoxic to pups in a two-generation reproductive study showing decreased postnatal weights of pups from high-dose animals (both F_1 and F_2) and mid-dose animals (F_1). Exposure to 1,2,3-trichlorobenzene showed no effects on reproductive organs of rats at high doses. Several developmental toxicity studies concluded that members of the chlorobenzene category did not show any embryotoxic or fetotoxic effects. At high doses, there was an increased incidence of delayed ossification of centra of the cervical vertebrae following exposure of rats to monochlorobenzene. The chlorobenzenes category members did not induce gene mutation in bacteria or chromosomal aberrations *in vitro*. When tested *in vivo*, all members of the category showed increased numbers of micronucleated erythrocytes in mice. There was some but not clear evidence of carcinogenicity of monochlorobenzene male rats. Carcinogenic effects of chlorobenzene were not observed in female rats or male or female mice. There was no evidence of carcinogenicity of 1,2-dichlorobenzene in male or female rats or mice. The supporting chemical, 1,4-dichlorobenzene, produced clear evidence of carcinogenicity in male and female mice and male rats, but there was no evidence of carcinogenicity for female rats.

The potential health hazard of the chlorobenzene category members is moderate based on repeated-dose toxicity except for 1,3-dichlorobenzene which showed high toxicity based on effects on liver, thyroid and pituitary at low doses. Some but not clear evidence of carcinogenicity of monochlorobenzene and clear evidence of carcinogenicity of 1,4-dichlorobenzene have been observed.

No data gaps have been identified under the HPV Challenge Program.

1. Physical-chemical Properties and Environmental Fate

A summary of physical-chemical properties and environmental fate data submitted is provided in Table 1. For the purpose of the screening-level hazard characterization, the review and summary of these data was limited to the octanol-water partition coefficient and biodegradation endpoints as indictors of bioaccumulation and persistence, respectively.

Octanol-water Partition Coefficient

Monochlorobenzene (CAS No. 108-90-7)
Log K_{ow}: 2.84 (measured)

1,2-Dichlorobenzene (CAS No. 95-50-1)

Log K_{ow}: 3.43 (measured)

1,3-Dichlorobenzene (CAS No.541-73-1)
Log K_{ow}: 3.38 (measured)

1,4-Dichlorobenzene (CAS No. 106-46-7, supporting chemical)
Log K_{ow}: 3.39 (measured)

1,2,3-Trichlorobenzene (CAS No. 87-61-6)
Log K_{ow}: 3.93 (estimated)

1,2,4-Trichlorobenzene (CAS No. 120-82-1, supporting chemical)
Log K_{ow}: 3.93 (estimated)

Biodegradation

Monochlorobenzene (CAS No. 108-90-7)
In two modified MITI tests using activated sludge as inoculum, 0 – 15% of monochlorobenzene degraded after 28 days.
Monochlorobenzene is not readily biodegradable.

1,2-Dichlorobenzene (CAS No. 95-50-1)
In a MITI (1) test using activated sludge as inoculum, 0% of 1,2-dichlorobenzene degraded after 28 days.
1,2-Dichlorobenzene is not readily biodegradable.

1,3-Dichlorobenzene (CAS No.541-73-1)
In a modified MITI test using an activated sludge as inoculum, 0% of 1,3-dichlorobenzene degraded after 28 days.
1,3-Dichlorobenzene is not readily biodegradable.

1,4-Dichlorobenzene (CAS No. 106-46-7, supporting chemical)
In a modified Zahn-Wellens test for inherent biodegradability using activated sludge as inoculum, 20% of 1,4-dichlorobenzene degraded after 20 days. 1,4-Dichlorobenzene is considered not readily biodegradable.
1,4-Dicholrobenzene is not readily biodegradable.

1,2,3-Trichlorobenzene (CAS No. 87-61-6)
In a MITI (1) test using activated sludge as inoculum, the supporting chemical, 1,2,4-trichlorobenzene degraded 0% after 14 days. Based on extrapolation of data for other category members, plus limited biodegradation data from the supporting chemical, 1,2,4-trichlorobenzene, 1,2,3-trichlorobenzene is considered not readily biodegradable.
1,2,3-Trichlorobenzene is readily not biodegradable.

Conclusion: The log K_{ow} values of chlorobenzene category members indicate that their potential to bioaccumulate is expected to be low. The chlorobenzene category members are not readily biodegradable, indicating that they have the potential to persist in the environment.

Table 1. Summary of Physicochemical Properties and Environmental Fate Data						
Endpoints	**Monochlorobenzene (108-90-7)**	**1,2-Dichlorobenzene (95-50-1)**	**1,3-Dichlorobenzene (541-73-1)**	**1,4-Dichlorobenzene (supporting chemical) (106-46-70**	**1,2,3-Trichloro-benzene (87-61-6)**	**1,2,4-Trichloro-benzene (supporting chemical) (120-82-1)**
Melting Point (°C)	**-45.2 (m)**	**-17 (m)**	**-25.5 (m)**	**53.5 (m)**	**52.6 (m)**	**17 (m)**
Boiling Point (°C)	**132.1 (m)**	**180.5 (m)**	**173 (m)**	**174 (m)**	**221 (m)**	**213 (m)**
Vapor Pressure (hPa at 25°C)	**11.7 (m)**	**1.3 (m)**	**1.8 (m)**	**0.8 (m)**	**1.33 (m) (40 °C)**	**0.36 (m) (70 °C)**
Log K_{ow}	**2.84 (m)**	3.28 (e); **3.43 (m)**	3.28 (e); **3.38 (m)**	3.28 (e); **3.39 (m)**	3.93 (e)	3.93 (e)
Water Solubility (mg/L at 20°C)	**210 (m)**	**145 (m) (25 °C)**	**100 (m)**	**60 (m)**	17.4 (e)	49 (e)
Direct Photodegradation	**Direct photolysis will not contribute to degradation**					
Indirect (OH^-) Photo-degradation Half-life ($t_{1/2}$)	6 h (e)	320 h (e)	16 d (e)	–	37.7 d (e)	–
Stability in Water (Hydrolysis) ($t_{1/2}$)	–	**35.5 d (pH 3) 35.4 d (pH 7) 45.4 d (pH 11) (m)**	Unable to measure or estimate	–	Unable to measure or estimate	–
Fugacity (Level III Model)						
Air (%)	25.5	12.4	13.1	13.1	6.45	6.27
Water (%)	31.1	19	20.2	19.1	11.9	12
Soil (%)	43.1	67.9	66	67	79.1	79.4
Sediment (%)	0.26 (e)	0.72 (e)	0.75 (e)	0.8 (e)	2.55 (e)	2.4 (e)
Biodegradation at 28 days (%)	**0 – 15 (m)** Not Readily Biodegradable	**0 (m)** Not Readily Biodegradable	**0 (m)** Not Readily Biodegradable	**20 (m)[1]** Not Readily Biodegradable	No Data 0 Not Readily Biodegradable (RA)	**0 (m)** Not Readily Biodegradable

(m) = measured data (i.e., derived from testing); (e) = estimated data (i.e., derived from modeling); (RA) = Read Across; [1]Inherent biodegradation test.

2. Environmental Effects – Aquatic Toxicity

A summary of aquatic toxicity data submitted for SIDS endpoints is provided in Table 2. The table also indicates where data for tested category members are read-across (RA) to untested members of the category. For the chlorobenzene category, data are available for each member for most of the aquatic toxicity endpoints.

Acute Toxicity to Fish

Monochlorobenzene (CAS No. 108-90-7)
Rainbow trout (*Salmo gairdneri*) were exposed to nominal concentrations of monochlorobenzene at 0.03, 1.8, 3.2, 5.8, 10, 18, 32 or 100 mg/L under static conditions 96 hours. Mortality occurred within 20 hours after exposure and was 100% at ≥ 18 mg/L, 45% at 10 mg/L and 0% at ≤ 5.8 mg/L. Due to limitations in the methodology used to test this chemical, at EPA's request the sponsor supported the toxicity value with a 96-hour EC_{50} value for this endpoint estimated using ECOSAR (13.55 mg/L). The modeled results are corroborate the measured results; therefore, the measured data were accepted for the purpose of characterizing hazard to daphnia.
96-h LC_{50} = 10.4 mg/L
96-h LC_{50} = 13.55 mg/L (estimated)

1,2-Dichlorobenzene (CAS No. 95-50-1)
Fathead minnows (*Pimephales promelas*; 20/concentration) were exposed to measured concentrations of 1,2-dichlorobenzene at 2.09, 3.32, 5.86, 7.77 and 18.0 mg/L under flow-through conditions for 96 hours. All fish exposed to 18 mg/L died within 24 hours and those exposed to 2.09, 5.56 and 7.77 showed signs of toxicity including death (1/20, 4/20 and 5/20, respectively).
96-h LC_{50} = 9.47 mg/L

1,3-Dichlorobenzene (CAS No. 541-73-1)
Fathead minnows (*P. promelas*; 20/concentration) were exposed to nominal concentrations of 1,3-dichlorobenzene at 5.54, 8.52, 13.1, 20.1 and 30.9 mg/L under flow-through conditions for 96 hours. The corresponding measured concentrations were 1.71, 3.37, 4.62, 5.76 and 14.4 mg/L. The fish showed signs of toxicity including death at ≥ 1.7 mg/L. Mortality was 100% at 14.4 mg/L and occurred within 5 hours of treatment.
96-h LC_{50} = 8.03 mg/L

1,2,3-Trichlorobenzene (CAS No. 87-61-6)
Fathead minnows (*P. promelas*) were exposed to nominal concentrations of 1,2,3-trichlorobenzene at 1.1, 1.6, 2.5, 3.9 and 6.0 mg/L for flow-through conditions for 96 hours. The corresponding measured concentrations were 0.69, 0.96, 1.5, 2.2 and 3.5 mg/L. The fish showed signs of toxicity at ≥ 0.96 mg/L. Mortality was 100% at 3.5 mg/L and occurred within 72 hours of treatment. No deaths were seen at ≤ 1.5 mg/L.
96-h LC_{50} = 2.4 mg/L

Acute Toxicity to Aquatic Invertebrates

Monochlorobenzene (CAS No. 108-90-7)
Water fleas (*Daphnia magna*) were exposed to measured concentrations (not provided in robust summary) of monochlorobenzene under static conditions for 24 hours. The submitted study duration deviates from the standard 48-hour daphnid toxicity study duration. The sponsor supported the 24-h toxicity value with a 48-hour EC_{50} value for this endpoint estimated using ECOSAR (15.38 mg/L). The modeled results are indicate lower toxicity than the measured 24-hour results; therefore, the measured 24-hour data were accepted for the purpose of characterizing hazard to daphnia.
24-h EC_{50} = 4.3 mg/L
48-h EC_{50} = 15.4 mg/L (estimated)

1,2-Dichlorobenzene (CAS No. 95-50-1)
D. magna were exposed to measured concentrations (not provided in robust summary) of 1,2-dichlorobenzene under static conditions for 24 hours. The submitted study duration deviates from the standard 48-hour daphnid toxicity study duration. The sponsor supported the 24-h toxicity value with a 48-hour EC_{50} value for this endpoint estimated

using ECOSAR (6.345 mg/L). The modeled results indicate lower toxicity than the 24-hour results; therefore, the measured 24-hour data were accepted for the purpose of characterizing hazard to daphnia.
24-h EC_{50} = 0.78 mg/L
48-h EC_{50} = 6.5 mg/L (estimated)

1,3-Dichlorobenzene (CAS No. 541-73-1)
D. magna were exposed to measured concentrations (not provided in robust summary) of 1,3-dichlorobenzene under static conditions for 48 hours.
48-h EC_{50} = 4.2 mg/L

1,2,3-Trichlorobenzene (CAS No. 87-61-6)
(1) *D. magna* were exposed to measured concentrations (not provided in robust summary) of 1,2,3-trichlorobenzene under static conditions for 24 hours.
24-h EC_{50} = 0.35 mg/L

(2) *D. magna* were exposed to nominal concentrations (not provided in robust summary) of 1,2,3-trichlorobenzene under static conditions for 48 hours.
48-h EC_{50} = 2.71 mg/L

(3) Mysid shrimp (*Mysidopsis bahia*) were exposed to nominal concentrations of 1,2,3-trichlorobenzene at 0.89, 1.4, 2.1, 3.3 and 5.0 mg/L under flow- through conditions for 96 hours. Corresponding measured concentrations were 0.12, 0.12, 0.21, 0.35 and 0.57 mg/L.
96-h EC_{50} = 0.35 mg/L

Toxicity to Aquatic Plants

Monochlorobenzene (CAS No. 108-90-7)
Green algae (*Pseudokirchneriella subcapitata*) were exposed to measured concentrations (taken at equilibrium) of monochlorobenzene at 6.5, 14.3, 23.3, 29.6, 37.8, 45.0 and 63.0 mg/L for 96 hours.
96-h EC_{50} (growth) = 12.5 mg/L

1,2-Dichlorobenzene (CAS No. 95-50-1)
Green algae (*P. subcapitata*) were exposed to measured concentrations (taken at equilibrium) of 1,2-dichlorobenzene at 0.88, 2.75, 4.99, 6.38, 10.66, 17.55 and 21.19 mg/L for 96 hours.
96-h EC_{50} (growth) = 2.2 mg/L

1,2,3-Trichlorobenzene (CAS No. 87-61-6)
Green algae (*P. subcapitata*) were exposed to measured concentrations (taken at equilibrium) of 1,2-dichlorobenzene at 0.26, 0.45, 1.09, 1.5, 1.79, 1.95 and 2.94 mg/L for 96 hours
96-h EC_{50} (growth) = 0.9 mg/L

Conclusion: The evaluation of available toxicity data for fish, aquatic invertebrates and aquatic plants indicates that the potential acute hazard of the chlorobenzenes category members is generally moderate; the exception being that toxicity of 1,2,3-trichlorobenzene in algae was high. A trend of increasing toxicity with increasing chlorine substitution is observed for fish, daphnia and algae.

Table 2. Summary of Environmental Effects – Aquatic Toxicity Data						
Endpoints	**Mono-chlorobenzene (108-90-7)**	**1,2-Di-chlorobenzene (95-50-1)**	**1,3-Di-chlorobenzene (541-73-1)**	**1,4-Di-chlorobenzene (supporting chemical) (106-46-7)**	**1,2,3-Tri-chlorobenzene (87-61-6)**	**1,2,4-Tri-chlorobenzene (supporting chemical) (120-82-1)**
Fish 96-h LC_{50} (mg/L)	**10.4 (m)** 13.55 (e)	**9.47 (m)**	**8.03 (m)**	**—****	**2.4 (m)**	**—****
Aquatic Invertebrates 48-h EC_{50} (mg/L)	**4.3 (m) (24-h)** 15.4 (e)	**0.78 (m) (24-h)** 6.4 (e)	**4.2 (m)**	**—****	**2.71 (m)**	**—****
Aquatic Plants 96-h EC_{50} (mg/L) (growth)	**12.5 (m)**	**2.2 (m)**	No Data 1.6 (RA)	**1.6 (m)**	**0.9 (m)**	**—****

(m) = measured data (i.e. derived from testing); (e) = estimated data (i.e. derived from modeling—ECOSAR); [1]Data available for 24-h duration only; (RA) = Read Across; — indicates endpoint not addressed for this chemical; ** indicates endpoint not necessary for supporting chemical

3. Human Health Effects

A summary of health effects data submitted for SIDS endpoints is provided in Table 3. The table also indicates where data for tested category members are read-across (RA) to untested members of the category.

Acute Oral Toxicity

Monochlorobenzene (CAS No. 108-90-7)
(1) Sprague-Dawley rats (5/sex/dose) were administered a single oral dose of monochlorobenzene at 250– 4000 mg/kg-bw and observed for 14 days. All deaths occurred within 3 days of dosing. Transient ataxia, labored breathing, prostration and hyperpnea (hyperventilation) were observed.
LD_{50} = 1540 mg/kg-bw

(2) Fischer 344 rats (5/sex/dose) were administered a single oral doses of monochlorobenzene at 250, 500, 1000, 2000 or 4000 mg/kg-bw and observed for 14 days. The mortality was as follows: none at 250 mg/kg-bw, 1 male at 500 mg/kg-bw, 2 females at 1000 mg/kg-bw, 1 male at 2000 mg/kg-bw and 3 males and 4 females at 4000 mg/kg-bw. All deaths occurred within 3 days of dosing. Transient ataxia, labored breathing, prostration and hyperpnea (hyperventilation) were observed. LD_{50} was not calculated.
LD_{50} = 2000 – 4000 mg/kg-bw

(3) B6C3F1 mice (5/sex/dose) were administered a single oral dose of monochlorobenzene at 250, 500, 1000, 2000 or 4000 mg/kg-bw and observed 14 days. The mortality was as follows: 3 males and 2 females at 250mg/kg-bw, 1 male at 500 mg/kg-bw, 5 males and 2 females at 1000 mg/kg-bw and all animals at 2000 and 4000 mg/kg-bw. Most deaths occurred within 4 days of dosing. The LD_{50} was not calculated but appears to be < 250 mg/kg-bw.
LD_{50} < 250 mg/kg-bw

***1,2-Dichlorobenzene* (*CAS No. 95-50-1*)**
Guinea pigs (10 mixed sex/dose) were administered a single oral dose of 1,2-dichlorobenzene at 800 or 2000 mg/kg-bw and observed for 14 days. All animals at 800 mg/kg-bw survived and those at 2000 mg/kg-bw died.
800 < LD_{50} < 2000 mg/kg-bw

1,3-Dichlorobenzene (CAS No. 541-73-1)
Sprague-Dawley rats (5/sex/dose) were administered a single dose of 1,3-dichlorobenzene at 631, 794, 1000, 1260 and 1580 mg/kg-bw and observed for 14 days. At 1580 mg/kg-bw all rats died within 1 day. Signs of toxicity

included lethargy, weakness, ocular discharge and collapse. Hemorrhagic lungs, liver kidneys and spleen discoloration and acute gastrointestinal tract inflammation were seen at necropsy.
LD_{50} = 1100 mg/kg-bw

1,2,4-Trichlorobenzene (CAS No.120-82-1, supporting chemical)
CFE rats (4/sex/dose) and CF No. 1 mice were administered 1,2,4–trichlorobenzene via gavage at various doses (not provided) and observed for10 days. Deaths occurred within 5 days of dosing for rats and within 3 days of dosing for mice. All survivors did not show any abnormalities at necropsy.
LD_{50} = 756 mg/kg-bw

Acute Dermal Toxicity

Monochlorobenzene (CAS No. 108-90-7)
New Zealand White rabbits (2 males and 1female) were dermally administered monochlorobenzene at 5010 or 7940 mg/kg-bw and observed for 14 days. (No other information was provided.)
LD_{50} > 7940 mg/kg-bw

1,4-Dichlorobenzene (CAS No. 106-46-7, supporting chemical)
Sherman rats (10/dose, sex distribution not provided) were dermally administered 1,4-dichlorobenzene in xylene up to 6000 mg/kg-bw on shaved backs and observed for 14 days or until all survivors had recovered form signs of toxicity.
LD_{50} = 6000 mg/kg-bw

1,3-Dichlorobenzene (CAS No. 541-73-1)
New Zealand White rabbits (4, males and females-sex distribution not provided) were dermally administered 1.3-dichlorobenzene at 2000 mg/kg-bw. No signs of toxicity were noted.
LD_{50} > 2000 mg/kg-bw

1,2,4-Trichlorobenzene (CAS No. 120-82-1, supporting chemical)
Rats (strain unspecified) were dermally administered undiluted 1,2,4-trichlorobenzene to shorn dorso-lumbar skin under occlusive conditions for 24 hours and observed for 10 days. Deaths occurred within 5 days of exposure.
LD_{50} = 6139 mg/kg-bw

Acute Inhalation Toxicity

Monochlorobenzene (CAS No. 108-90-7)
(1) Sprague-Dawley rats (12 males/concentration) were exposed to monochlorobenzene vapor at concentrations ranging form 2000 – 3500 ppm (approximately 9.2 – 16.1 mg/L) for 6 hours and observed for 14 days.
LC_{50} = ~ 13.9 mg/L

(2) Mice (25 females/concentration) were exposed to monochlorobenzene vapor at concentrations ranging form 1400 – 3000 ppm (approximately 6.4 – 13.8 mg/L) for 6 hours and observed for 14 days.
LC_{50} = ~ 8.8 mg/L

(3) Sprague-Dawley rats (6 males) were exposed to monochlorobenzene via inhalation at 39.7 mg/L. All animals died within 3.75 hours. Signs of toxicity were tremors, labored breathing and collapse. Slight lung congestion was noted at necropsy.
LC_{50} < 39.7 mg/L

***1,2-Dichlorobenzene* (*CAS No. 95-50-1*)**
(1) Sprague-Dawley rats (12/concentration) were exposed to 1,2-dichlorobenzene vapor at concentrations ranging from 1000 to 2000 ppm (approximately 6.0 – 12.0 mg/L) for 6 hours and observed for 14 days.
LC_{50} = 9.38 mg/L

(2) Mice (strain not specified, 25/concentration) were exposed to 1,2-dichlorobenzene vapor (concentrations not provided) for 6 hours and observed for 14 days.
LC_{50} = 7.43 mg/L

1,4-Dichlorobenzene (CAS No. 106-46-7, supporting chemical)
Sprague-Dawley rats (5/sex) were exposed 1,4-dichlorobenzene vapor at 6 mg/L for 4 hours and observed for 14 days. All animals survived the exposure. Signs of toxicity included lacrimation, salivation, nasal discharge, labored breathing, gasping, tremors, ano-genital staining and chromodacryorrhea.
LC_{50} > 6 mg/L

Repeated-Dose Toxicity

Monochlorobenzene (CAS NO. 108-90-7)
(1) In a National Toxicology Program (NTP) study, Fisher 344 rats (10/sex/dose) were administered monochlorobenzene via gavage at 0, 62, 125, 250, 500 or 750 mg/kg-bw/day, 5 days/week for 13 weeks. Survival was decreased at 500 and 750 mg/ kg-bw/day. Body weight gains were marginally to moderately depressed at 500 and 750 mg/ kg-bw/ day. Dose-dependent hepatocellular necrosis was observed at 250, 500 and 750 mg/ kg/ day. Nephrotoxicity and lymphoid or myeloid depletion of the spleen, bone marrow, and thymus were observed at 500 and 750 mg/ kg/ day. Renal necrosis or degeneration was noted in one male at 250 mg/kg-bw/day. Scattered changes in urinary and clinical chemistry, hematology, organ weight, and porphyrin metabolism parameters were also observed at 500 and 750 mg/ kg/ day.
http://ntp.niehs.nih.gov/?objectid=0706D0A9-EA0B-A103-B5B2CA2F7B2B4B38
LOAEL = 250 mg/kg-bw/day (based on liver and renal effects)
NOAEL = 125 mg/kg-bw/day

(2) In a National Toxicology Program (NTP) study, B6C3F1 mice (10/sex/dose) were administered monochlorobenzene via gavage at 0, 62, 125, 250, 500 or 750 mg/kg-bw/day, 5 days/week for 13 weeks. Survival was decreased at 250, 500 and 750 mg/kg/day. Final body weights were lower in males at 250 mg/kg-bw/day and in females at 250 and 500 mg/kg-bw/day. Dose-dependent hepatocellular necrosis was observed at 250, 500 and 750 mg/kg-bw/day. Nephrotoxicity, thymic necrosis, and lymphoid or myeloid depletion of the thymus, spleen and bone marrow were also observed at 250, 500, or 750 mg/kg-bw/day.
http://ntp.niehs.nih.gov/?objectid=0706D0A9-EA0B-A103-B5B2CA2F7B2B4B38
LOAEL = 250 mg/kg-bw/day (based on mortality and multiple organ effects)
NOAEL = 125 mg/kg-bw/day

(3) Beagle dogs (16/sex/dose) were administered monochlorobenzene orally via capsule at 0, 0.025, 0.050 or 0.250 mL/kg-bw/day (0, 27.25, 54.5 or 272.5 mg/kg-bw/day), 5 days/week for 93 days. Mortality was seen only at the high dose. Clinical signs before death in these animals included decreased appetite and activity, anorexia, body weight loss, cachexia and coma. A number of clinical chemistry parameters were also altered at the high dose. There were gross and/or microscopic changes in the liver, kidney, gastrointestinal tract and hematopoetic tissue of the high-dose dogs that died and, less extensively, in the high-dose dogs that were euthanized at the end of the exposure period. The four high-dose dogs that died or were euthanized during the study showed increased relative liver, kidney, adrenal and heart weight and slightly increased relative thyroid weight.
LOAEL = 272.5 mg/kg-bw/day (based on mortality and changes in multiple organs/tissues)
NOAEL = 54.5 mg/kg-bw/day

(4) Beagle dogs (6/sex/concentration) were exposed to monochlorobenzene via inhalation at 0.79, 1.59 and 2.06 mg/L/day 6 hours/day, 5 days/week for 6 months. All treated animals had abnormal stool during weeks 7 – 11. This also was evident in controls at week 11. Emesis occurred at the mid and high doses groups during weeks 8 – 10 and 17 – 29. The relative liver weights in the mid- and high-dose females were increased relative to controls and the relative adrenal weights in the mid- and high-dose males were decreased with respect to controls. No treatment-related changes were seen during gross and microscopic examinations.
LOAEL = 1.59 mg/L/day (based on changes in relative liver and adrenal weights)
NOAEL = 0.79 mg/L/day

1,2-Dichlorobenzene (CAS No. 95-50-1)
(1) Sprague-Dawley rats (10/sex/dose) were administered 1,2-dichlorobenzene via gavage at 25, 100 and 400 mg/kg-bw/day for 90 days. At 400 mg/kg-bw/day, males exhibited decreased mean body weight and absolute and relative spleen weight, and increased absolute and relative kidney and liver weight and relative heart, lung, brain and testes weight. An increase in erythrocytes versus control was noted in high-dose males. Males also had increased erythrocytes, serum ALT, BUN and serum total bilirubin. In females at this dose, total food consumption was increased during weeks 11 – 13 and increased absolute and relative kidney and liver weight and decreased absolute spleen weight were observed. Serum total bilirubin was also increase in these females. Hepatic degeneration, hypertrophy and necrosis of liver were observed in high-dose males and females. At 100 mg/kg-bw/day, male rats exhibited increased absolute and relative liver weight and increased ALT. Females at this dose had increased absolute and relative liver weight and absolute kidney weight.
LOAEL = 100 mg/kg-bw/day (based on increased liver weight in males and females and kidney weight in females)
NOAEL = 25 mg/kg-bw/day

(2) In a National Toxicology Program study, Fischer 344 rats (10/sex/dose) were administered 1,2-dichlorobenzene via gavage at 0, 30, 60, 125, 250 or 500 mg/kg-bw/day, 5 days/week for 90 days. Survival was decreased in males and females at 500 mg/kg-bw/day. Dose-related increases in relative liver weight were noted in both sexes at 125, 250 and 500 mg/kg-bw/day. The only hematologic changes considered notable were slight decreases in hemoglobin and hematocrit in the 500 mg/kg male and female rats and in red blood cell counts in the 500 mg/kg male rats. Centrolobular necrosis of the liver, hepatocellular degeneration and depletion of lymphocytes in the thymus and spleen was observed in both sexes at 500 mg/kg-bw/day. At 250 mg/kg-bw/day, necrosis of individual hepatocytes was observed in both sexes. Minimal hepatocellular necrosis was observed in a few rats at a dose of 125 mg/kg. Renal tubular degeneration was observed in male rats at 500 mg/kg/bw/day.
http://ntp.niehs.nih.gov/?objectid=0706B89C-F481-CA0A-7AECAB0C45EE3FCA
LOAEL = 125 mg/kg-bw/day (based on increases in liver weight in both sexes and liver necrosis)
NOAEL = 60 mg/kg-bw/day

(3) In a National Toxicology Program study, B6C3F1 mice (10/sex/dose) were administered 1,2-dichlorobenzene via gavage at 0, 30, 60, 125, 250 or 500 mg/kg-bw/day, 5 days/week for 13 weeks. At 500 mg/kg-bw/day, morality and decreased body weight and body weight gains were observed in both sexes. Relative liver weight was increased in both sexes. No marked hematological changes were observed in mice. Centrolobular necrosis of the liver, hepatocellular degeneration and depletion of lymphocytes in the thymus and spleen was observed in both sexes at 500 mg/kg-bw/day. At 250 mg/kg-bw/day, necrosis of individual hepatocytes was observed in both sexes. Multifocal mineralization of the myocardial fibers of the heart and skeletal muscle were seen in mice at 500 mg/kg/bw/day. http://ntp.niehs.nih.gov/?objectid=0706B89C-F481-CA0A-7AECAB0C45EE3FCA
LOAEL = 250 mg/kg-bw/day (based on liver pathology in males only)
NOAEL = 125 mg/kg-bw/day

(4) Rats (strain not specified; 20/sex/concentration) were exposed to 1,2-dichlorobenzene via inhalation at nominal concentrations of 65 and 106 ppm (measured concentrations were 49 ppm [~ 0.3 mg/L] and 93 ppm [~ 0.57 mg/L], respectively) 7 hours/day, 5 days/week for 6 – 7 months. At 93 ppm, decreased average body weight was observed in males. There were no effects on mortality, growth, organ weights, gross appearance, behavior or gross and microscopic examination of tissues.
LOAEL ~ 0.57 mg/L (based on decreased body weight)
NOAEL ~ 0.3 mg/L

1,3-Dichlorobenzene (CAS No. 541-73-1)
Sprague-Dawley rats (10/sex/dose) were administered 1,3-dichlorobenzene via gavage at doses of 0, 9, 37, 147 and 588 mg/kg-bw/day for 90 days. No mortality or clinical signs were noted in the treated groups.
At 588 mg/kg-bw/day, average daily water consumption was increased and body weights were decreased in both sexes. Increased relative kidney and liver weights were seen in both sexes and increased relative brain and testes weights were also seen in males. At 147 mg/kg-bw/day, decreased relative brain and increased relative liver weight were noted in females and increased relative kidney and liver weights were found in males. A variety of blood chemistry changes were observed at $\geq$ 147 mg/kg-bw/day; consistent effects among sexes and across doses were not clearly apparent. Hepatic inflammation, hepatocellular cytoplasmic alterations, hepatocellular necrosis was observed at all doses. Mild to minimal cytoplasmic vacuolization in the pars distalis of the pituitary was noted in

60, 60, 100 and 100% of males only (vs. 20% in controls) at 9, 37, 147 and 588 mg/kg-bw-day, respectively. Depletion of colloid density in the thyroid was seen in 65, 90, 89 and 94% of treated animals (vs. 15% in controls) at 9, 37, 147 and 588 mg/kg-bw/day, respectively
LOAEL = 9 mg/kg-bw/day (based on effects on liver, thyroid and pituitary)
NOAEL = Not established

1,4-Dichlorobenzene (CAS No. 106-46-7, supporting chemical)
(1) In two National Toxicology Program studies, Fischer 344 rats (10/sex/dose) were administered 1,4-dichlorobenzene via gavage, 5 days/week for 13 weeks. In the first study, rats were dosed with 300 – 1500 mg/kg-bw/day. Because histological changes were observed in the kidney of male rats at all doses, a second 13-week study was performed at doses of 37.5, 75, 150, 300 and 600 mg/kg-bw/day. Survival was decreased in males at 1200 or 1500 mg/kg-bw/day and in females at 1500 mg/kg-bw/day. Weight gain was decreased in males at ≥ 300 mg/kg-bw/day and in females at 1200 or 1500 mg/kg-bw/day. Liver weight to brain weight ratios were increased ≥ 900 mg/kg-bw/day for both males and females. The kidney weight to brain weight ratio was increased in males receiving doses of ≥ 600 mg/kg-bw/day. Doses of 1200 or 1500 mg/kg-bw/day produced degeneration and necrosis of hepatocytes, hypoplasia of the bone marrow, lymphoid depletion of the spleen and thymus, and epithelial necrosis of the nasal turbinates in males and females. Renal tubular cell degeneration was observed in males receiving ≥ 300 mg/kg-bw/day in the first study, but only slight changes were seen at 300 mg/kg-bw/day in the second study. Slight but statistically significant decreases in the hematocrit, red blood cell count and hemoglobin level were seen in all males at 300 – 1200 mg/kg-bw/day. No clear hematologic changes were observed in females. Minimal changes in clinical chemistry parameters were observed, including increased serum cholesterol levels at ≥ 600 mg/kg-bw/day in males and ≥ 900 mg/kg-bw/day in females; reduced serum triglycerides at ≥ 300 mg/kg-bw/day in males. Blood urea nitrogen was increased slightly in males at ≥ 900 mg/kg-bw/day. Urinary porphyrins were increased slightly in males at 1200 or 1500 mg/kg-bw/day and females at 1200 mg/kg-bw/day. However these increases were modest and the study authors considered them indicative of a mild porphyrinuria rather than hepatic porphyria. Liver porphyrins were not increased at any dose. http://ntp.niehs.nih.gov/?objectid=0707D46D-9C42-21DF-D577330353775DE0
LOAEL = 300 mg/kg-bw/day (decreased weight gain in males, effects on kidneys in males and hematological changes)
NOAEL = 150 mg/kg bw/day

(2) In two National Toxicology Program studies, B6C3F1 mice (10/sex/dose) were administered 1,4-dichlorobenzene via gavage, 5 days/week for 13 weeks. The doses for the first study were 600 – 1800 mg/kg-bw/day. Survival was decreased in male and female mice receiving doses of ≥1500 mg/kg-bw/day and body weight gain was decreased at all doses. Hepatocellular degeneration was observed in both sexes at all doses and the liver weight to brain weight ratio was increased at ≥ 900 mg/kg-bw/day. Serum cholesterol levels were increased in males at ≥ 900 mg/kg-bw/day, whereas serum protein and triglycerides were increased at ≥ 1500 mg/kg-bw/day. The study authors concluded that these relatively modest clinical chemistry changes probably reflect the hepatic effects of 1,4-dichlorobenzene. White blood cell count was reduced significantly in males at ≥ 600 mg/kg-bw/day and females at ≥ 1000 mg/kg-bw/day. Hepatic porphyria was not found at any dose. Because hepatic effects were seen at all doses in the first study, a second 13-week study was performed at doses of 0, 84.4, 168.8, 337.5, 675 and 900 mg/kg-bw/day. In this study, hepatocellular cytomegaly was observed in both sexes at ≥ 675 mg/kg-bw/day, but not at 337.5 mg/kg-bw/day. Renal damage was not observed in either 13-week study. http://ntp.niehs.nih.gov/?objectid=0707D46D-9C42-21DF-D577330353775DE0
LOAEL = 675 mg/kg-bw/day (based on effect on liver)
NOAEL = 337.5 mg/kg-bw/day

1,2,3-Trichlorobenzene (CAS No. 87-61-6)
Sprague-Dawley rats (10/sex/concentration) were administered 1,2,3-trichlorobenzene via the diet at 1, 10, 100 or 1000 ppm (corresponding to average 0.105, 1.04, 9.8 and 95.5 mg/kg-bw/day) daily for 90 days . At 1000 ppm, a decrease in body weight gain and an increase in the liver to body weight and kidney to body weight ratios were seen in males. Mild to moderate histopathological changes in the liver and thyroid were observed in males and females, with effects more pronounced in males. Microscopic changes in the liver were mild to moderate increases in cytoplasmic volume and larger than normal nuclei of hepatocytes. Changes in the thyroid included mild to moderate reduction in follicular size and colloid density. Below 1000 ppm the study authors did not consider changes in weight gain and organ-to-body weight rations to be treatment-related.
LOAEL ~ 95.5 mg/kg-bw/day (based on decreased body weight gain, increased liver- and kidney-to-body weight ratios and microscopic changes in the liver and thyroid)
NOAEL ~ 9.8 mg/kg-bw/day

1,2,4-Trichlorobenzene (CAS No. 120-82-1, supporting chemical)
Sprague-Dawley rats (5/sex/concentration) were administered 1,2,4-trichlorobenzene via the diet at 1, 10, 100 and 1000 ppm (corresponding to ~0.07, 0.78, 7.8 and 82 mg/kg-bw/day for males and ~0.11, 1.4, 15 and 101 mg/kg-bw/day for females) for 90 days. There was no effect on body weight. Relative liver and kidney weights of males were increased with respect to control only at the highest dose. Animals treated with 1000 ppm had marked changes in the liver characterized by aggregated basophilia and widespread midzonal vacuolization due to fatty infiltration. Mild to moderate changes in the thyroid included reduction in follicular size and colloid density. Higher activities of hepatic microsomal aniline hydroxylase and aminopyrene demethylase activities were also observed at 1000 ppm.
LOAEL = 82/101 mg/kg-bw/day (males/females; based on effects on liver, kidney and thyroid)
NOAEL = 7.8/15 mg/kg-bw/day (males/females)

Reproductive Toxicity

Monochlorobenzene (CAS No. 108-90-7)
In a two-generation reproductive toxicity study, Sprague-Dawley rats (30/sex/dose) were exposed to monochlorobenzene via inhalation at 0, 50, 150 or 450 ppm (~ 0.23, 0.70 or 2.11 mg/L, respectively) for 6 hours/day, 7 days/week. The exposure period for F_0 and F_1 generations was for approximately 10 weeks of premating, during mating (males and females), 0 – 20 days of gestation and 4 – 21 days of lactation (females). No mortalities were observed in the adult generations. Mean food consumption and body weights for adults in both generations were comparable at all doses. Mating and fertility indices for males and females were unaffected by treatment. In the F_1 and F_2 litters, pup and litter survival for all treated groups was comparable to controls. In the F_2 litters, a slight decrease in pup survival index (days 0 – 4) was seen in offspring from high-dose animals. The study authors did not consider this to be treatment-related as it was predominantly due to loss of litters from two dams (one dam lost 12/15 pups and another lost all 10). Increases in absolute and relative liver weight were observed in F_0 and F_1 adults exposed to 150 or 450 ppm. An increase in the incidence of small flaccid testes and dilated renal pelvis was observed in high-dose F_0 and Fl males. There was an increase in the incidence of dilated renal pelvis in F_0 (dose- related) and F_1 males. In the F_1 generation, two females at 150 or 450 ppm had dilated renal pelvis versus none in controls. Microscopically, minimal to mild hepatocellular hypertrophy was noted in the mid- and high-dose F_0 and F_1 males and in one high-dose F_0 female. F_0 and F_1 males in the 150 ppm group and F_1 males in the 450 ppm group exhibited unilateral degeneration and F_0 males of the 450 ppm group exhibited bilateral degeneration of varying degrees in the germinal epithelium of the testes.
LOAEL (systemic toxicity) ~ 0.7 mg/L/day (based on liver, kidney and testicular effects)
NOAEL (systemic toxicity) ~ 0.23 mg/L/day
NOAEL (reproductive toxicity) ~ 2.1 mg/L/day (based on no significant effects at the highest dose tested)

1,2-Dichlorobenzene (CAS No. 95-50-1)
Sprague-Dawley rats (30/sex/dose) were exposed to 1,2-dichlorobenzene via inhalation at 50, 150 and 400 ppm (corresponding to ~0.3, 0.9 and 2.4 mg/L/day) of 6 hours/day, 7 days/week during 10 weeks (premating and mating). Pregnant females were then exposed during gestation (19 days) and lactation (another 23 days). Males were exposed for an additional 3 weeks post-mating. A similar exposure regimen was followed for the F_1 generation. At 50 ppm, mean body weights of adults were decreased during the pre-mating period; however, mean weight gain over the entire pre-mating interval for both generations was comparable to controls. At 400 ppm, mean weekly weights for F_0 and F_1 adults during the pre-mating interval were decreased and continued to be depressed

through to termination. Mean weight gains over the entire pre-mating interval were decreased for males in both generations and for F_0 females. Maternal weight gain during the gestation/lactation interval was comparable to controls at all doses. A slight increase in absolute and relative liver weight in both the F_0 and F_1 adults was observed at 50 ppm. Mean absolute and relative liver weights were increased in F_0 adult males and females at 150 ppm and both generations at 400 ppm; in F_1 adults at 150 ppm, only relative liver weights were increased. Males of both generations had increased relative kidney weights at 150 and 400 ppm. No effects of treatment on reproductive performance, fertility indices, gestation length and litter size were apparent at any dose in either the F_0 or F_1 generation. At 50 ppm, pup survival and growth rates were comparable to controls. At 150 ppm, pup survival indices were unaffected but F_1 mean pup weight at day 0 (birth) was markedly lower than control; however, they were comparable to controls for the remaining weighing intervals and for all F_2 litters. At 400 ppm, pup survival indices were generally comparable to controls for both generations. While the F_2 pup survival index for days 0 – 4 of lactation decreased, this was largely attributed to complete loss of a single litter. Excluding this litter, the pup survival index for day 0 – 4 was comparable to controls. Pup survival for days 4 – 21 of lactation was comparable to controls. Mean pup weights were markedly decreased on days 0, 14, 21 and 28 in F_1 litters and on days 14 and 21 in F_2 litters. No adverse effect of treatment was evident from the gross postmortem evaluations of adults and offspring. Histopathological examination showed treatment-related morphologic abnormalities in the liver and kidneys of the F_0 and F_1 adults. Hypertrophy of the central lobular hepatocytes was seen in almost all F_0 and F_1 adult males and females and numerous males and several females from the mid-exposure group. Kidneys showed dilated tubular lumens with intra-luminal granular casts, predominantly at the cortico-medullary junctions in several F0 and FI adult males from the mid- and high-exposure groups. Intra-cytoplasmic granules/droplets in the proximal convoluted tubular epithelium were seen in almost all F_0 and F_1 adult males for which the kidneys were examined microscopically. Based on mean severity, this kidney effect was dose-dependent. Several F_1 generation animals in all groups had findings suggestive of Sialodacryoadenitis viral (SDAV) infection. All males and females were noted as free of SDAV infection by week 41. The presence of SDAV did not appear to have adversely affected the study.
LOAEL (systemic toxicity) = ~ 0.3 mg/L/day (based on liver effects)
NOAEL (systemic toxicity) = Not established
NOAEL (reproductive toxicity) ~ 2.4 mg/L/day (based on no effects at highest dose tested)
LOAEL (offspring toxicity, F_1) ~ 0.9 mg/L/day (based on effect on pup weight)
NOAEL (offspring toxicity, F_1) ~ 0.3 mg/L/day
LOAEL (offspring toxicity, F_2) ~ 2.4 mg/L/day (based on effects survival and pup weight)
NOAEL (offspring toxicity, F_2) ~ 0.9 mg/L/day

1,3-Dichlorobenzene (CAS No. 541-73-1)
In the 90-day repeated-dose toxicity study in male and female Sprague-Dawley rats described previously, 1,3-dichlorobenzene was administered by gavage at 0 (corn oil), 9, 37, 147 or 588 mg/kg-bw/day for 90 days. High-dose males had markedly increased relative testes weights. There were no histopathological lesions observed to correlate the finding. There were no effects on any other reproductive organs in males or females.

1,4-Dichlorobenzene, (CAS No. 106-46-7, supporting chemical)
In a two-generation study, Sprague-Dawley rats (28/sex/dose) were exposed, via inhalation to measured concentrations of 0, 66.3, 211 and 538 ppm (50, 150 and 450 ppm nominal corresponding to ~ 0, 0.3, 0.9 and 2.7 mg/L/day) of 1,4-dichlorobenzene 6 hours/day, 7 days/week, 10 weeks prior to mating, during mating (males and females) through gestation and lactation (females). There was no effect of treatment on reproductive parameters (mating or fertility indices of males or females, gestational index, 7-, 14-, 21- or 28-day survival index, lactation index or number of copora lutea or implantation sites, including resorptions and live conceptuses) in either generation.
450 ppm: F_0 and F_1 males and F_1 females exhibited consistently reduced body weights and weight gains throughout the study. F_0 females had reduced body weights for the first week of exposure prior to mating and on gestational day 20. Gestational weight or weight gain of F_0 and F_1 dams was reduced on gestational days 0 – 20. Lactational weights of F_1 females were reduced on postnatal days 0, 4 and 7. Non-exposed male and female F_1 recovery animals exhibited consistently reduced body weights. Food consumption was reduced in F_1 males for 5 of the 11 exposure weeks and was reduced in F_0 and F_1 females and F_0 males during the first week and third week (F_0 females only) of exposure. Food consumption was reduced in recovery animals during the first 2 weeks of the recovery period. Clinical signs observed in F_0 and F_1 animals included unkempt appearance, tremors, twitches, hypoactivity, ataxia, salivation and periocular and perioral encrustation. F_0 and F_1 females had an increased incidence of urogenital wetness. There were no clinical observations in recovery animals. Liver and kidney weights of F_0 males and

females were increased (as well as relative liver, brain, tested and kidney weights of F_0 males and relative liver and kidney weights of F_0 females). An increased incidence of hepatocellular hypertrophy was observed in F_0 males and females and increased incidences of hydronephrosis, hyaline droplet nephropathy[1], tubular proteinosis, granular cast formation, renal tubular cell hyperplasia and interstitial nephritis were observed in F_0 males. Exposure-related histological findings in F_1 adults were similar to those of F_0 adults. The mean number of F_2 live born pups per litter was reduced. F_1 and F_2 litter size (but not sex ratio) was reduced on lactational day 4. Pups from F_1 and F_2 litters exhibited reduced body weights per litter during lactation. There was an increase in the incidence of stillborn pups (F_2) and postnatal deaths on days 0 – 4 (both F_1 and F_2 pups).
150 ppm: Sporadic reductions in body weights and weight gains of F_0 and F_1 animals were observed at this concentration. F_0 and F_1 males had increased absolute and/or relative kidney and liver weights and F_0 females had increased relative liver weights. An increased incidence of nephrosis was observed in F_0 and F_1 males. Histological findings in the liver were similar to those of males treated with 450 ppm (with the exception of no renal cell hyperplasia and the additional finding of renal interstitial fibrosis). No histological alterations were noted in the liver. There were no effects of treatment on the pups.
50 ppm: Absolute and/or relative kidney weights of F_0 and F_1 males and liver weights of F_0 males were increased. There was an increased incidence of nephrosis in F_0 males, with similar histological findings as in the 150 ppm males. F_1 males did not exhibit nephrosis, but had an increased incidence of hyaline droplet formation in the kidneys. No histological alterations were noted in the liver. There were no effects of treatment on the pups.
LOAEL (systemic toxicity) ~ 0.3 mg/L/day (based on kidney and liver effects)
NOAEL (systemic toxicity) = Not established
NOAEL (reproductive toxicity) ~ 2.7mg/L/day (based on no effects at highest dose tested)
LOAEL (offspring toxicity) ~ 2.7 mg/L/day (based on survival and effect on body weight)
NOAEL (offspring toxicity, F_1 and F_2) ~ 0.9 mg/L/day

1,2,3-Trichlorobenzene (CAS No. 87-61-6)
In the repeated-dose toxicity study in Sprague-Dawley rats described previously, there was no effect of treatment on histology or weights of the ovaries, uterus, vagina, testes, prostate or epididymides.

1,2,4-Trichlorobenzene (CAS No. 120-82-1, supporting chemical)
In a two-generation study, male and female rats (strain not specified) were exposed to 25, 100 or 400 ppm 1,2,4-trichlorobenzene orally via drinking water during pre-mating (90 days) and then continuously until day 32 of the F_2 generation. The mean doses, calculated based on water intake, for F_0 females were 8.3, 28.0 and 133 mg/kg-bw/day for at 29 days of age and 3.7, 14.8 and 53.6 mg/kg-bw/day at 83 days of age and for F_0 males were 8.5, 27.6 and 133.6 mg/kg-bw/day at 29 days of age and 2.5, 8.9 and 33.0 mg/kg-bw/day at 83 days of age. Food intake was increased in F_0 high-dose males at 29 days and water intake was decreased in high-dose males and females at 83 days of age. At 400 ppm, slight increases in the weight of the left adrenal gland (the only one weighed) of males and females. Fertility, growth, viability, locomotor activity and blood chemical analyses of two generations of rats were not affected at any dose tested.
LOAEL (systemic toxicity) ~ 33/54 mg/kg-bw/day (male/female; based on increased adrenal weight)
NOAEL (systemic toxicity) ~ 9 /15 mg/kg-bw/day (male/female)
NOAEL (reproductive toxicity) ~ 33/54 mg/kg-bw/day (male/female; based on no effects at the highest dose tested)

Developmental Toxicity

Monochlorobenzene (CAS No. 108-90-7)
(1) Pregnant Fischer 344 (30-32/dose) rats were exposed to monochlorobenzene vapor via inhalation at 75, 210 or 590 ppm (~ 0.35, 0.98 or 2.76 mg/L/day) for 6 hours/day during days 6 – 15 of gestation. No maternal deaths or

[1] The presence of nephropathy in association with the hyaline droplet accumulation in male rats suggests that the nephropathy in the males is occurring by an $alpha_{2u}$-globulin-mediated mechanism which is male rate-specific and not considered relevant to humans. EPA's Risk Assessment Forum has outlined the key events and the data that are necessary to demonstrate this mode of action ($Alpha_{2u}$-Globulin: Association with Chemically Induced Renal Toxicity and Neoplasia in the Rat, EPA/625/3-91/019F). One of the key events, $alpha_{2u}$-globulin accumulation, has not been demonstrated. Therefore, the nephropathy is assumed to be relevant to human health and it is concluded that a NOAEL for nephropathy in male rats was not established.

abnormal clinical signs were noted. At 590 ppm, animals lost weight during the first 3 days of exposure. Absolute and relative liver weights of dams at 590 ppm were slightly, but significantly (significance not provided) greater than control at necropsy. No adverse effects were noted in mean litter size or incidence of implantations. With the exception of a cleft palate in one fetus at 75 ppm, the malformations observed were similar to the controls and were within the historical incidences for controls. An increased incidence of some minor skeletal variants in fetuses from treated animals was noted. A higher incidence of delayed ossification of centra of the cervical veterbrae was seen at 75 ppm and 590 ppm, but not 210 ppm. A higher incidence of bilobed centra of the thoracic vertebrae and a lower incidence of cervical spurs were noted at 590 ppm. The study authors concluded the skeletal changes observed at the highest concentration were indicative of a slight delay in skeletal development, but none of the variants were considered to be indicative of a specific teratogenic response.
LOAEL (maternal toxicity) ~ 2.76 mg/L/day (based on decreased body weight and increased liver weight)
NOAEL (maternal toxicity) ~ 0.98 mg/L/day
LOAEL (developmental toxicity) ~ 0.35 mg/L/day (based on delayed ossification, cleft palate)
NOAEL (developmental toxicity) = Not established

(2) In the first study, pregnant New Zealand White rabbits (30/dose) were exposed to monochlorobenzene vapor via inhalation at 75, 210 or 590 ppm (~ 0.35, 0.98 or 2.76 mg/L/day) for 6 hours/day during days 6 – 18 of gestation. There was an increase in absolute and relative liver weights at 210 and 590 ppm. Exposure to all concentrations resulted in a variety of malformations in all groups at incidences slightly higher than historical controls, an increased incidence of extra ribs (a variation) in the high dose animals (113 in 26 litters vs. 79 in 24 control litters), head/facial abnormalities in one fetus at 75 ppm and another at 590 ppm and heart defects were seen in one fetus at 210 ppm and two fetuses (in two litters) at 590 ppm. To determine if these malformations were true effects of treatment, the study was repeated at concentrations of 10, 30, 75 and 590 ppm. In the second study, liver weight was increased in dams was seen at 590 ppm. The observed increase in percentage of implantations (61%) at 590 ppm was within the historical control range (19-67%). There were seven fetuses in one litter with ablepharia (missing eyelid) in the 590 ppm group. This anomaly was not observed in the first study. Skeletal examinations revealed an increase in the incidence of extra ribs in the 10 ppm group (94 in 23 litters vs. 72 in 21 control litters). This alteration was considered a skeletal variant.
LOAEL (maternal toxicity) ~ 0.98 mg/L/day (based on effect on liver)
NOAEL (maternal toxicity) ~ 0.35 mg/L/day
NOAEL (developmental toxicity) ~ 2.76 mg/L/day (based on no effects at the highest dose tested)

1,2-Dichlorobenzene (CAS No. 95-50-1)
(1) Fischer 344 rats (30-32/dose) were administered 1,2-dichlorobenzene via inhalation at 0, 100, 200 and 400 ppm (~ 0, 0.6, 1.2, 2.4 mg/L, respectively) for 6 hours/day during days 6 – 15 of gestation. Mean body weights of females were reduced during gestation at 400 ppm and mean body weight gains at all concentrations were depressed on days 6 – 8, 12 – 15 and 6 – 20 of gestation. Food consumption was slightly depressed during the first 3 exposure days. An increase in absolute and relative liver weight was seen at 400 ppm and an increase in relative liver weight was noted at 100 ppm. Skeletal evaluation revealed a marked increase in the occurrence of spurs on the first lumbar vertebrae at 200 ppm only. Delayed ossification of cervical vertebral centra was markedly increased at 400 ppm. At 400 ppm, the other malformations included single cases of coarcted and retroesophageal aortic arch, unilateral testicular agenesis, polydactyly, cervical ribs and microphthalmia.
LOAEL (maternal toxicity) = 0.6 mg/L/day (based on effects on body weight and liver weights)
NOAEL (maternal toxicity) = Not established
LOAEL (developmental toxicity) = 2.4 mg/L (based on delayed ossification and malformations)
NOAEL (developmental toxicity) = 1.2 mg/L

(2) Female New Zealand White rabbits were exposed to 1,2-dichlorobenzene vapor via inhalation at 0, 100, 200 and 400 ppm (~ 0, 0.6, 1.2, 2.4 mg/L) for 6 hours/day during gestation days of 6 – 18. Does lost weight during the first 3 days of exposure at all concentrations. Total weight gain over days 6 – 28 was less in treated animals than controls. The ratio of male/female offspring was different in the 200 ppm group. None of the malformations in treated animals were at a higher statistical frequency (p = not provided) than controls.
LOAEL (maternal toxicity) = 0.6 mg/L/day (based on effects on body weight)
NOAEL (maternal toxicity) = Not established
NOAEL (developmental toxicity) = 2.4 mg/L/day (based on no effects at the highest dose tested)

1,4-Dichlorobenzene (CAS No. 106-46-7, supporting chemical)
New Zealand White rabbits (28-30/dose) were exposed to 1,4-dichlorobenzene vapor via inhalation at 0, 100, 300 and 800 ppm (~ 0, 0.59, 1.77, 4.72 mg/L/day) for 6 hours/day during days 6 – 16 of gestation. The post-exposure period was 11 days. Does exposed to 800 ppm gained less weight than controls on days 6 – 8 of gestation. There was an increase in the percentage of implantations undergoing resorption and the percentage of litters with resorptions at 300 ppm, but not at 800 ppm. The numbers of litters, corpora lutea/dam, implantation sites/dam, fetuses/litter, resorptions/litter, litters totally resorbed and resorptions/litters with resorptions were not different from control in any of the treated groups. Fetal sex ratio, fetal weight and crown-rump length of treated animals were not different from controls.
LOAEL (maternal toxicity) = 4.72 mg/L/day (based on effect on body weight gain)
NOAEL (maternal toxicity) = 1.77 mg/L/day
NOAEL (developmental toxicity) = 4.72 mg/L/day (based on no effects at the highest dose tested)

1,2,3-Trichlorobenzene (CAS No. 87-61-6)
Female Sprague-Dawley rats (13/dose, 14/control) were administered 1,2,3-trichlorobenzene via gavage at 0, 150, 300 and 600 mg/kg-bw/day on days 6 – 15 of gestation. The post-exposure period was 7 days. No clinical signs of toxicity were observed. An increase in relative liver weight was observed at 600 mg/kg-bw/day. Aminopyrine N-demethylase (APDM) activity was increased in high-dose animals. Hemoglobin concentrations were slightly decreased at 300 or 600 mg/kg-bw/day. Mild changes in the thyroid were noted at 300 and 600 mg/kg-bw/day. The changes consisted of a reduction of follicle size, which was often accompanied by angular collapse. There was no effect on pregnancy rate, number of resorptions, litter size and fetal weight. No visceral and skeletal abnormalities were seen in pups.
LOAEL (maternal toxicity) = 300 mg/kg-bw/day (based on effect on hemoglobin and changes in thyroid)
NOAEL (maternal toxicity) = 150 mg/kg-bw/day
NOAEL (developmental toxicity) = 600 mg/kg-bw/day (based on no effects at the highest dose tested)

Genetic Toxicity – Gene Mutation

In vitro
Monochlorobenzene (CAS No. 108-90-7)
Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538 were exposed to monochlorobenzene at 0.02 – 1.28 µL/plate in the presence and absence of metabolic activation. DMSO was the solvent control; other appropriate positive controls were also used. There was no increase in the average number of revertant colonies in any of the tested strains with or without metabolic activation. The controls responded appropriately. Cytotoxicity was apparent at concentration of 1.28 µL/plate in all strains.
Monochlorobenzene was not mutagenic in this assay.

(2) In several other assays, monochlorobenzene was tested up to 3333 µL/plate or 5500 µg/plate and did not induce positive response in tested strains of *S. typhimurium* or *Escherchia coli.*
Monochlorobenzene was not mutagenic in these assays.

1,2-Dichlorobenzene (CAS No. 95-50-1)
S. typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538 were exposed to 1,2-dichlorobenzene at 0.02 – 2.56 µL/plate in the presence and absence of metabolic activation. DMSO was the solvent control; other appropriate positive controls were also used and responded appropriately. Cytotoxicity was observed at 1.28 µL/plate in most strains, except 0.32 µL/plate was toxic to strain TA1537 in the absence of metabolic activation and 2.56 µL/plate was required to produce toxicity to strains TA98, TA1537 and TA100 in the presence of metabolic activation. There was no increase in the average number of revertant colonies in any of the tested strains with or without metabolic activation.
1,2-Dichlorobenzene was not mutagenic in this assay.

(2) In several other assays, 1,2-dichlorobenzene was tested up the concentrations of 13,000 µg/plate in *S. typhimurium* strains and *E. coli WP2(trp-, uvRA* in the presence and absence of metabolic activation. None of the assays showed increase in revertant colonies.
1,2-Dichlorobenzene was not mutagenic in these assays.

1,3-Dichlorobenzene (CAS No. 541-73-1)
(1) *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 were exposed to 1,3-dichlorobenzene at 0.02 – 2.56 µL/plate in the presence and absence of metabolic activation. DMSO was the solvent control; other appropriate positive controls were also used and responded appropriately. Cytotoxicity was apparent at a concentration of 1.28 µL/plate in all strains. There was no increase in the average number of revertant colonies in any of the tested strains with or without metabolic activation.
,3-Dichlorobenzene was not mutagenic in these assays.

(2) In several other assays, 1,3-dichlorobenzene was tested up to 2000 µg/plate and did not induce positive response in the tested strains of *S. typhimurium* or *E. coli.*
1,3-Dichlorobenzene was not mutagenic in these assays.

1,2,3-Trichlorobenzene (CAS No. 87-61-6)
S. typhimurium strains TA 98, TA100, TA1535 and TA1537 were exposed to 1,2,3-trichlorobenzene at 3.3, 10, 33.3, 100 and 333.3 µg/plate in the presence and absence of metabolic activation. DMSO was the solvent control; other appropriate positive controls were also used and responded appropriately. Concentrations of 100 and 333.3 µg/plate were toxic to all strains in the absence and presence of metabolic activation, respectively. There was no increase in the average number of revertant colonies in any of the tested strain with or without metabolic activation.
1,2,3-Trichlorobenzene was not mutagenic in this assay.

Genetic Toxicity – Chromosomal Aberrations

In vitro
Monochlorobenzene (CAS No. 108-90-7)
Chinese hamster ovary (CHO) cells were exposed to monochlorobenzene at 125, 250 and 500 µg/mL in the presence and absence of metabolic activation for 24 or 48 hours. DMSO was the negative control. Cytotoxicity was seen at 500 µg/mL without metabolic activation and > 500 µg/mL with metabolic activation. There was no increase in the frequency of aberrant cells.
Monochlorobenzene did not induce chromosomal aberrations in this assay.

1,4-Dichlorobenzene (CAS No. 106-46-7, supporting chemical)
CHO cells were exposed to 1,4-dichlorobenzene at 50, 100 and 150 µg/mL without metabolic activation and 25, 50 and 100 µg/mL with metabolic activation. Positive and negative controls responses were appropriate. A precipitate was noted at 150 µg/mL. There was an increase in the frequency of aberrant cells at doses of 25 – 150 µg/mL.
1,4-Dichlorobenzene did not induce chromosomal aberrations in this assay.

1,2,3-Trichlorobenzene (CAS No. 87-61-6)
CHO cells were exposed to 1,2,3-trichlorobenzene at 15.7 – 125 µg/mL with and without metabolic activation for 24 or 48 hours. The cytotoxic concentration was > 125 µg/mL. There was no increase in the frequency of aberrant cells.
1,2,3-Trichlorobenzene did not induce chromosomal aberrations in this assay.

1,2,4-Trichlorobenzene (CAS No. 120-82-1, supporting chemical)
CHO cells were exposed to 1,2,4-trichlorobenzene at 31.3 – 125 µg/mL with and without metabolic activation for 24 or 48 hours. The cytotoxic concentration was 125 µg/mL. There was no increase in the frequency of aberrant cells.
1,2,4-Trichlorobenzene did not induce chromosomal aberrations in this assay.

In vivo
Monochlorobenzene (CAS No. 108-90-7)
(1) B6C3F1 male mice (5/dose) were administered monochlorobenzene intraperitoneally for 3 days at doses of 128.8, 257.5 and 515 mg/kg-bw/day. Animals were sacrificed at 24, 48 and 72 hours, bone marrow was aspirated and slides were prepared, stained and evaluated for the presence of micronuclei. There was no increase in micronuclei at any dose level. Positive, negative and solvent controls responded appropriately.
Monochlorobenzene did not induce chromosomal aberrations in this assay.

(2) NMRI mice were administered intraperitoneal doses of monochlorobenzene (total dose of 225, 450, 675 and 900 mg/kg-bw) in two doses 24 hours apart. Animals were sacrificed 30 hours after the first injection. The femora were removed and the marrow was suspended in serum. Two smears per femur were prepared. One thousand polychromatic erythrocytes (PCEs) per smear were examined for the presence of micronuclei. The number of micronucleated cells/1000 PCEs were significantly (significance was not provided) different from control at all doses. Although the sponsor concluded that inappropriate statistical analysis were used, when compared to the positive control data, EPA concluded that monochlorobenzene was positive in this assay.
Monochlorobenzene induced chromosomal aberrations in this assay.

(3) Male B6C3F1 mice received a single intraperitoneal injection of corn oil or monochlorobenzene in corn oil at 312.5, 625 or 1250 mg/kg-bw following a subcutaneous implantation of a BrdUrd tablet 1 hour before injection. After 17 hours, mice were sacrificed, bone marrow cells from the femur were harvested, fixed and stained and 50 metaphase cells from each animal per treatment group were scored for the presence of chromosomal aberrations. There was no effect of treatment on the percentage of cells with abnormalities although the exposure period (17 hours) was less than the guideline required exposure of 24 hours. In similarly conducted second experiment, male mice were exposed to the doses of 250, 500 or 1000 mg/kg-bw and the cells were harvested from bone marrow at 36 hours. A positive trend was noted with a statistically significant increase in the percent of abnormal cells at 1000 mg/kg-bw ($p < 0.05$).
Monochlorobenzene induced chromosomal aberrations in this assay.

1,2-Dichlorobenzene (CAS No. 95-50-1)
(1) Male B6C3F1 mice were administered 1,2-dichlorobenzene via intraperitoneal injection at 150, 300 and 600 mg/kg-bw during 3 days of dosing. Animals were sacrificed at 48 hours after the third treatment, bone marrow was aspirated and slides were prepared, stained and evaluated for the presence of micronuclei. The incidence of micronucleated-PCEs per 1000 PCEs showed barely a positive trend ($p = 0.049$). The test was repeated using the 250 mg/kg-bw dose and was found to be negative by trend analysis ($p = 0.358$). Because of the relatively small increase in micronucleated-PCEs in the initial test and the lack of reproducibility, the overall result of this test was negative.
1,2-Dichlorobenzene did not induce chromosomal aberrations in this assay.

(2) NMRI mice were administered 1,2-dichlorobenzene in corn oil via intraperitoneal injection at 0, 187, 375, 562 and 750 mg/kg-bw in two doses given 24 hours apart. Animals were sacrificed 30 hours after the first injection, bone marrow was aspirated and slides were prepared, stained and evaluated for the presence of micronuclei. One thousand polychromatic erythrocytes (PCEs) per smear were examined for the presence of micronuclei. The number of micronucleated cells/1000 PCEs were markedly (significance was not provided) increased at all doses. Although the sponsor concluded that inappropriate statistical analysis were used, when compared to the positive control data, EPA concluded that monochlorobenzene was positive in this assay.
1,2-Dichlorobenzene induced chromosomal aberrations in this assay.

1,3-Dichlorobenzene (CAS No. 541-73-1)
(1) NMRI mice were administered 1,3-dichlorobenzene in corn oil via intraperitoneal injection at 0, 175, 350, 525 and 700 mg/kg-bw in two doses given 24 hours apart. Animals were sacrificed 30 hours after the first injection, bone marrow was aspirated and slides were prepared, stained and evaluated for the presence of micronuclei. There was a dose-related increase in the numbers of micronucleated cells per 1000 PCEs. Although an inappropriate statistical test was used to determine the significance, 1,3-dichlorobenzene was judged to be positive in this test.
1,3-Dichlorobenzene induced chromosomal aberrations in this assay.

1,4-Dichlorobenzene (CAS No. 106-46-7, supporting chemical)
NMRI mice were administered 1,4-dichlorobenzene in corn oil via gavage at 2500 mg/kg-bw. Animals were sacrificed 24, 48 or 72 hours after treatment. The incidence of micronucleated PCEs/l000 was not increased markedly and there was no dose response.. The positive control induced 12 micronucleated-PCEs/l000 PCEs. 1,4-dichlorobenzene, in corn oil, was also administered intraperitoneally twice at an interval of 24 hours. There was no difference between treated animals and control in the incidence of MN-PCE/1000.
1,4-Dichlorobenzene did not induce chromosomal aberrations in this assay.

1,2,3-Trichlorobenzene (CAS No. 87-61-6) and 1,2,4-Trichlorobenzene
Male NMRI mice were given intraperitoneal doses of 1,2,3-trichlorobenzene at 250, 500, 750 and 1000 mg/kg-bw (in two doses given 24 hours apart). The control group received corn oil only. Animals were killed 30 hours after the first injection. The femora were removed and the marrow was suspended in serum. Two smears per femur were prepared and 1000 PCEs per smear were examined for the presence of micronuclei. The numbers of micronucleated cells/1000 PCEs were increased in a dose-related manner.1,2,3-Trichlorobenzene was positive in the test.
1,2,3-Trichlorobenzene and 1,2,4-trichlorobenzene induced chromosomal aberrations in this assay.

Genetic Toxicity – Other

1,2-Dichlorobenzene (CAS No. 95-50-1)
CHO cells were exposed to 1,2-dichlorobenzene at concentrations of 5.9, 19.7 or 59 µg/mL without metabolic activation and 19.7, 59 or 197 µg/mL with metabolic activation in one test and 300, 400 or 500 µg/mL with metabolic activation in another test. The test substance did not induce sister chromatid exchange (SCE) in the absence of metabolic activation. In the first experiment with metabolic activation, concentrations of 19.7, 59.0 and 197.0 µg/mL induced 8.64, 8.98 and 8.90 SCEs per cell (compared to 7.26 in control). In a second experiment with metabolic activation, concentrations of 300, 400 and 500 µg/mL induced 10.68, 10.38 and 10.06 SCEs per cell (compared to 8.46 in control). The positive control induced appropriate responses in both experiments. The test for SCE was positive in the presence of metabolic activation.
1,2-Dichlorobenzene induced sister chromatid exchange in this assay.

1,4-Dichlorobenzene (CAS No. 106-46-7, supporting chemical)
CHO cells were exposed to 1,4-dichlorobenzene at 75, 100, 125 and 150 µg/mL without metabolic activation and 75, 100 and 125 µg/mL in one test with metabolic activation and 100, 125 and 150 µg/mL in another with metabolic activation. The solvent control was DMSO. Both positive controls induced approximately a 3-fold increase in SCEs. No treatment-related increase in SCEs was observed at doses of 75 – 150 µg/mL in the absence metabolic activation. A positive result at one concentration (75 µg/mL) was obtained in one test with metabolic activation. The test was repeated and the results at all three concentrations were similar to control. The overall result was negative. No toxicity or cell cycle delay was found. A precipitate was noted at 150 µg/mL.
1,4-Dichlorobenzene did not induce sister chromatid exchange in this assay.

Additional Information

Carcinogenicity

Monochlorobenzene (CAS No. 108-90-7)
(1) In a National Toxicology Program (NTP) study, F344N rats (50/sex/dose) were administered monochlorobenzene in corn oil via gavage at 60 or 120 mg/kg-bw/day, 5 days/week, for 103 weeks. Survival of high dose male rats was significantly (P=0.033) lower than that of the vehicle controls. No chlorobenzene-induced toxic lesions responsible for this reduction in survival were observed. Mean body weights of dosed rats were essentially the same or greater than those of the controls. Male rats exhibited a significant (P<0.05) increase in the incidence of neoplastic nodules of the liver. Increased incidences of hepatocellular carcinomas in male rats or of neoplastic nodules or hepatocellular carcinomas in female rats were not observed. No increased tumor incidences were observed in female rats.
Chlorobenzene administration increased the occurrence of neoplastic nodules of the liver in male F344/N rats, providing some but not clear evidence of carcinogenicity of chlorobenzene in male rats. Carcinogenic effects of chlorobenzene were not observed in female F344/N rats.

(2) In a National Toxicology Program (NTP) study, B6C3F1 mice were administered monochlorobenzene in corn oil via gavage at 30 or 60 mg/kg-bw/day (males) and 60 or 120 mg/kg-bw/day (females), 5 days/week, for 103 weeks. Mean body weights of dosed mice were essentially the same or greater than those of the controls. No increased tumor incidences were observed in female rats or in male or female mice.
Carcinogenic effects of chlorobenzene were not observed in female in male or female B6C3F$_1$ mice.

1,2-Dichlorobezene (CAS No. 95-50-1)
(1) In a National Toxicology Program (NTP) study, F344N rats (50/sex/dose) were administered 1,2-dichlorobenze in corn oil via gavage at 60 or 120 mg/kg-bw/day, 5 days/week, for 103 weeks. Survival of female rats was comparable to those of the corresponding vehicle controls, but survival of high-dose male rats was ($P<0.001$) shorter than that of the vehicle controls. In this group there were three accidental deaths and five deaths probably due to the gavage process; in addition aspiration of 1,2-dichlorobenzene in corn oil into the lungs may have been a contributing factor to the deaths of 12 high-dose male rats. Body weight was not affected in either sex or survival of mice or female rats. No compound-related nonneoplastic histological lesions were noted.
There was no evidence of carcinogenicity of 1,2-dichlorobenzene for male or female F344/N rats.

(2) In a National Toxicology Program (NTP) study, B6C3F1 mice were administered 1,2-dichlorobenzene in corn oil via gavage at 60 or 120 mg/kg-bw/day, 5 days/week, for 103 weeks. Survivals of males and females were comparable to those of the corresponding vehicle. The 120 mg/kg dose level of 1,2-dichlorobenzene did not affect body weight in mice of either sex. An increase in tubular regeneration in the kidney of high dose male mice was observed. No other compound-related nonneoplastic histological lesions were noted.
There was no evidence of carcinogenicity of 1,2-dichlorobenzene for male or female B6C3F$_1$ mice.

1,4-Dichlorobezene (CAS No. 106-46-7)
(1) In a National Toxicology Program (NTP) study, F344N rats (50/sex/dose) were administered 1,4-dichlorobenze in corn oil via gavage at 0, 150 or 300 mg/kg-bw/day (males) and 0, 300, 600 (females), 5 days/week, for 103 weeks. Survival of females was comparable to that of the vehicle controls; survival of high-dose males was significantly lower than that of the vehicle controls. Mean body weights of high-dose males were 5%-8% lower than those of vehicle controls after week 38, and those of high-dose females were 5%-7% lower than those of vehicle controls after week 55. Males had increased severity of nephropathy and epithelial hyperplasia of the renal pelvis, mineralization of the collecting tubules in the renal medulla and focal hyperplasia of renal tubular epithelium. There were increased incidences of nephropathy in both low- and high- dose females compared with vehicle controls. The incidence of tubular cell adenocarcinomas of the kidney was increased in males; one tubular cell adenoma was observed in a high-dose male rat. These malignant tumors are uncommon in male F344/N rats. There were no tubular cell tumors in females. There was a marginal increase in the incidence of mononuclear cell leukemia in males compared with that in vehicle controls.
1,4-dichlorobenzene produced clear evidence of carcinogenicity for male F344/N rats. There was no evidence of carcinogenicity for female F344/N rats.

(2) In a National Toxicology Program (NTP) study, B6C3F1 mice were administered 1,4-dichlorobenzene in corn oil via gavage at 0, 300 or 600 mg/kg-bw/day, 5 days/week, for 103 weeks. Survival of both sexes was comparable to that of the vehicle controls. Mean body weights were comparable to those of vehicle controls throughout the study. Incidences of nonneoplastic liver lesions in male and female mice were increased, including alteration in cell size (cytomegaly and karyomegaly), hepatocellular degeneration and individual cell necrosis. Increases in the incidences of nephropathy in male mice and renal tubular regeneration in female mice were seen. Increased the incidences of hepatocellular carcinomas in high-dose males and females and hepatocellular adenomas in dosed males and high- dose females were also seen. Hepatoblastomas were observed in four high-dose males but not in vehicle controls. This tumor has not occurred in 1,091 male vehicle control mice in NTP studies. An increase in thyroid gland follicular cell hyperplasia was observed in dosed males and there was a marginal positive trend in the incidence of follicular cell adenomas of the thyroid gland in females. Marginal increases were observed in the incidences of pheochromocytomas of the adrenal gland in males.
1,4-dichlorobenzene produced clear evidence of carcinogenicity for both male and female B6C3F$_1$ mice.

Conclusion: The acute oral toxicity of members of the chlorobenzenes category to rats is moderate to low and the acute dermal toxicity to rats and rabbits and acute inhalation toxicity to rats and mice is low. Repeated exposures resulted in decreased body weight gain at high doses and major effects on liver, kidney, spleen, thymus and thyroid. Liver effects included increased liver weights, increased incidence of hepatic necrosis, hepatocellular degeneration, hepatic cytoplasmic alterations and increased levels of bilirubin, cholesterol, increased activity of liver enzymes and mild to moderate porphyria. Increased kidney weights with necrosis or degeneration of the proximal tubular epithelium were seen in kidneys. Decreases in absolute and relative spleen weights and splenic lymphoid depletion were observed. Effects on hematological parameters seen for the supporting chemical, 1,4-dichlorobenzene, included decreases in hemoglobin, hematocrit and erythrocytes counts. Thymic lymphoid depletion and depletion of

colloid density in the thyroid were seen. The category members did not show any treatment-related effect on reproductive parameters. 1,2-Dichlorobenzene was slightly fetotoxic to pups in a two-generation reproductive study showing decreased postnatal weights of pups from high-dose animals (both F_1 and F_2) and mid-dose animals (F_1). Exposure to 1,2,3-trichlorobenzene showed no effects on reproductive organs of rats at high doses. Several developmental toxicity studies concluded that members of the chlorobenzene category did not show any embryotoxic or fetotoxic effects. At high doses, there was an increased incidence of delayed ossification of centra of the cervical vertebrae following exposure of rats to monochlorobenzene. The chlorobenzenes category members did not induce gene mutation in bacteria and chromosomal aberrations *in vitro*. When tested *in vivo*, all members of the category showed increased numbers of micronucleated erythrocytes in mice. There was some but not clear evidence of carcinogenicity of monochlorobenzene male rats. Carcinogenic effects of chlorobenzene were not observed in female rats or male or female mice. There was no evidence of carcinogenicity of 1,2-dichlorobenzene in male or female rats or mice. The supporting chemical, 1,4-dichlorobenzene, produced clear evidence of carcinogenicity in male and female mice and male rats, but there was no evidence of carcinogenicity for female rats.

The potential health hazard of the chlorobenzene category members is moderate based on repeated-dose toxicity except for 1,3-dichlorobenzene which showed high toxicity based on effects on liver, thyroid and pituitary at low doses. Some but not clear evidence of carcinogenicity of monochlorobenzene and clear evidence of carcinogenicity of 1,4-dichlorobenzene have been observed.

Table 3. Summary of Human Health Data						
Endpoints	**Mono-chlorobenzene (108-90-7)**	**1,2-Di-chlorobenzene (95-50-1)**	**1,3-Di-chlorobenzene (541-73-1)**	**1,4-Di-chlorobenzene (supporting chemical) (106-46-7)**	**1,2,3-Tri-chlorobenzene (87-61-6)**	**1,2,4-Tri-chlorobenzene (supporting chemical) (120-82-1)**
Acute Oral Toxicity LD_{50} (mg/kg-bw)	**1540 – 4000 (rat) < 250 (mice)**	**> 800 – < 2000 (guinea pig)**	**1100**	**—****	No Data 756 (RA)	**756 (mice)**
Acute Dermal Toxicity LD_{50} (mg/kg-bw)	**> 7940**	No Data > 2000 (RA)	**> 2000**	**6000**	No Data 6139 (RA)	**6139**
Acute Inhalation Toxicity LC_{50} (mg/L)	**13.9 – < 39.7 (rat) 8.8 (mice)**	**9.4 (rat) 7.4 (mice)**	No Data 7.4 – 9.4 (RA)	**> 6**	No Data 7.4 (RA)	**—****
Repeated-Dose Toxicity NOAEL/LOAEL Oral (mg/kg-bw/day)	**(rat, mice) NOAEL =125 LOAEL = 250 (dog) NOAEL = 54.5 LOAEL = 272.5**	**(rat) NOAEL = 25 LOAEL = 100 (rat) NOAEL = 60 LOAEL = 125 (mouse) NOAEL = 125 LOAEL = 250**	**(rat) NOAEL = NE LOAEL = 9**	**(rat) NOAEL = 150 LOAEL = 300 (mice) NOAEL 338 LOAEL = 675**	**(rat) NOAEL = 9.8 LOAEL = 95.5**	**(rat) NOAEL = 8/15 (m/f) LOAEL = 82/101 (m/f)**
Repeated-Dose Toxicity NOAEL/LOAEL Inhalation (mg/L/day)	**(dog) NOAEL = 0.8 LOAEL = 1.6**	**(rat) NOAEL = 0.3 LOAEL = 0.6**	**—***	**—****	**—***	**—****
Reproductive Toxicity NOAEL/LOAEL Oral (mg/kg-bw/day) Systemic Toxicity Reproductive Toxicity	**—***	**—***	**—***	**—****		**NOAEL = 9/15 (m/f) LOAEL = 33/54 (m/f) 33/54 (m/f) hdt**
Reproductive Toxicity NOAEL/LOAEL Inhalation (mg/L/day) Systemic Toxicity Reproductive Toxicity	**(rat) NOAEL ~ 0.23 LOAEL ~ 0.7 NOAEL ~ 2.1 (hdt)**	**(rat) NOAEL = NE LOAEL ~ 0.3 NOAEL ~ 2.4 (hdt)**	No Data (rat) NOAEL = NE LOAEL ~ 0.3 NOAEL ~ 2.4 (hdt) (RA)	**(rat) NOAEL = NE LOAEL ~ 0.3 NOAEL ~ 2.7 (hdt)**	**—***	**—****

Table 3. Summary of Human Health Data						
Endpoints	**Mono-chlorobenzene (108-90-7)**	**1,2-Di-chlorobenzene (95-50-1)**	**1,3-Di-chlorobenzene (541-73-1)**	**1,4-Di-chlorobenzene (supporting chemical) (106-46-7)**	**1,2,3-Tri-chlorobenzene (87-61-6)**	**1,2,4-Tri-chlorobenzene (supporting chemical) (120-82-1)**
Developmental Toxicity NOAEL/LOAEL Oral (mg/kg-bw/day) **Maternal Toxicity** **Developmental Toxicity**	**—***	**—***	**—***	**—***	**(rat)** **NOAEL = 150** **LOAEL = 300** **NOAEL = 600 (hdt)**	**—****
Developmental Toxicity NOAEL/LOAEL Inhalation (mg/L/day) **Maternal Toxicity** **Developmental Toxicity**	**(rat)** **NOAEL = ~0.98** **LOAEL = ~ 2.8** **(rabbit)** **NOAEL = ~0.35** **LOAEL = ~0.98** **(rat)** **NOAEL = NE** **LOAEL = 0.35** **(rabbit)** **NOAEL = ~2.8** **(hdt)**	**(rat)** **NOAEL = NE** **LOAEL = ~ 0.6** **(rabbit)** **NOAEL = NE** **LOAEL = 1.2** **(rat)** **NOAEL = 1.3** **LOAEL = 2.4** **(rabbit)** **NOAEL = ~2.4** **(hdt)**	No Data (rat) NOAEL = NE LOAEL = ~ 0.6 (rabbit) NOAEL = NE LOAEL = 1.2 (rat) NOAEL = 1.3 LOAEL = 2.4 (rabbit) NOAEL = ~2.4 (hdt)	**(rabbit)** **NOAEL = 1.77** **LOAEL = 4.72** **(rabbit)** **NOAEL = 4.72** **(hdt)**	**—***	**—****
Genetic Toxicity – Gene Mutation *In vitro*	**Negative**	**Negative**	**Negative**	**—****	**Negative**	**—****
Genetic Toxicity – Chromosomal Aberrations *In vitro*	**Negative**	**Negative**	No Data Negative (RA)	**Negative**	**Negative**	**Negative**
Genetic Toxicity – Chromosomal Aberrations *In vivo*	**Positive**	**Positive** (NMRI mice) **Negative** (B6C3F1 mice)	**Positive**	**Negative**	**Positive**	**Positive**
Genetic Toxicity – Other SCE	**—***	**Positive**	**—***	**Negative**	**—***	**—****
Additional Information Carcinogenicity	**Some but not clear evidence**	**No evidence**	**—***	**Clear evidence**	**—***	**—****

Measured Data (bolded); RA = Read Across; NE = Not established; — indicates endpoint not addressed for this chemical; * indicates endpoint not included in the base data set for the HPV Challenge Program; ** indicates endpoint not necessary for supporting chemical; hdt = highest dose tested

4. Hazard Characterization

The log K_{ow} values of chlorobenzene category members indicate that their potential to bioaccumulate is expected to be low. The chlorobenzene category members are not readily biodegradable, indicating that they have the potential to persist in the environment.

The evaluation of available toxicity data for fish, aquatic invertebrates and aquatic plants indicates that the potential acute hazard of the chlorobenzenes category members is generally moderate; the exception being that toxicity of 1,2,3-trichlorobenzene in algae was high. A trend of increasing toxicity with increasing chlorine substitution is observed for fish, daphnia and algae.

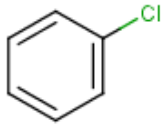
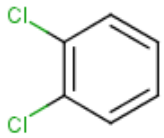
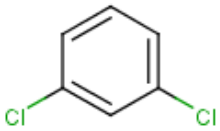
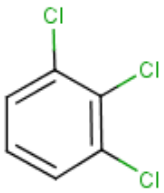
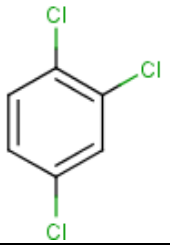
The acute oral toxicity of members of the chlorobenzenes category to rats is moderate to low and the acute dermal toxicity to rats and rabbits and acute inhalation toxicity to rats and mice is low. Repeated exposures resulted in decreased body weight gain at high doses and major effects on liver, kidney, spleen, thymus and thyroid. Liver effects included increased liver weights, increased incidence of hepatic necrosis, hepatocellular degeneration, hepatic cytoplasmic alterations and increased levels of bilirubin, cholesterol, increased activity of liver enzymes and mild to moderate porphyria. Increased kidney weights with necrosis or degeneration of the proximal tubular epithelium were seen in kidneys. Decreases in absolute and relative spleen weights and splenic lymphoid depletion were observed. Effects on hematological parameters seen for the supporting chemical, 1,4-dichlorobenzene, included decreases in hemoglobin, hematocrit and erythrocytes counts. Thymic lymphoid depletion and depletion of colloid density in the thyroid were seen. The category members did not show any treatment-related effect on reproductive parameters. 1,2-Dichlorobenzene was slightly fetotoxic to pups in a two-generation reproductive study showing decreased postnatal weights of pups from high-dose animals (both F_1 and F_2) and mid-dose animals (F_1). Exposure to 1,2,3-trichlorobenzene showed no effects on reproductive organs of rats at high doses. Several developmental toxicity studies concluded that members of the chlorobenzene category did not show any embryotoxic or fetotoxic effects. At high doses, there was an increased incidence of delayed ossification of centra of the cervical vertebrae following exposure of rats to monochlorobenzene. The chlorobenzenes category members did not induce gene mutation in bacteria and chromosomal aberrations *in vitro*. When tested *in vivo*, all members of the category showed increased numbers of micronucleated erythrocytes in mice. There was some but not clear evidence of carcinogenicity of monochlorobenzene male rats. Carcinogenic effects of chlorobenzene were not observed in female rats or male or female mice. There was no evidence of carcinogenicity of 1,2-dichlorobenzene in male or female rats or mice. The supporting chemical, 1,4-dichlorobenzene, produced clear evidence of carcinogenicity in male and female mice and male rats, but there was no evidence of carcinogenicity for female rats.

The potential health hazard of the chlorobenzene category members is moderate based on repeated-dose toxicity except for 1,3-dichlorobenzene which showed high toxicity based on effects on liver, thyroid and pituitary at low doses. Some but not clear evidence of carcinogenicity of monochlorobenzene and clear evidence of carcinogenicity of 1,4-dichlorobenzene have been observed.

5. Data Gaps

No data gaps have been identified under the HPV Challenge Program.

APPENDIX

Chlorobenzenes		
CAS No.	**Chemical Name**	**Structure**
SPONSORED CHEMICALS		
108-90-7	**Monochlorobenzene**	
95-50-1	**1,2-Dichlorobenzene**	
541-73-1	**1,3-Dichlorobenzene**	
87-61-6	**1,2,3-Trichlorobenzene**	
	SUPPORTING CHEMICALS	
106-46-7	**1,4-Dichlorobenzene**	
120-82-1	**1,2,4-Trichlorobenzene**