EPA 635/R-03/008
www.epa.gov/iris

TOXICOLOGICAL REVIEW

OF

CYCLOHEXANE

(CAS No. 110-82-7)

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

August 2003

U.S. Environmental Protection Agency
Washington, DC

DISCLAIMER

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use. Note: This document may undergo revisions in the future. The most up-to-date version will be made available electronically via the IRIS Home Page at http://www.epa.gov/iris.

CONTENTS — TOXICOLOGICAL REVIEW OF CYCLOHEXANE (CAS No. 110-82-7)

LIST OF TABLES

LIST OF FIGURES

FOREWORD

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

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

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

AUTHORS, CONTRIBUTORS, AND REVIEWERS

CHEMICAL MANAGER

Colette S. Hodes, Ph.D.
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
Washington, DC

AUTHORS

Colette S. Hodes, Ph.D.
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
Washington, DC

Andrea Pfahles-Hutchens
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
Washington, DC

Elizabeth H. Margosches, Ph.D.
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
Washington, DC

Martha G. Price, Ph.D.
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
Washington, DC

REVIEWERS

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

INTERNAL EPA REVIEWERS

Angela Auletta, Ph.D.
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
Washington, DC

Karen Hogan
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

Susan Rieth, M.P.H.
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

Meena Sonawane
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
Washington, DC

EXTERNAL PEER REVIEWERS

Raymond B. Baggs, Ph.D., D.V.M.
University of Rochester Medical Center
Division of Laboratory Animal Medicine
Rochester, NY

Ronald David Hood, Ph.D.
Department of Biological Sciences
The University of Alabama
Tuscaloosa, AL

Anna M. Fan, Ph.D.
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency
Oakland, CA

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

1. INTRODUCTION

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

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

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

Development of these hazard identification and dose-response assessments for cyclohexane has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidelines that were used in the development of this assessment

may include the following: *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986a), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a), Draft Revised *Guidelines for Carcinogen Assessment* (U.S. EPA, 1999), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), (proposed) *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 1998b, 2000a), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000b); *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000c), and *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000d).

The literature search strategy employed for this compound was based on the CASRN and at least one common name. At a minimum, the following databases were searched: RTECS, HSDB, TSCATS, CCRIS, GENE-TOX, DART/ETIC, EMIC, TOXLINE, CANCERLIT, and MEDLINE. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. The relevant literature was reviewed through March 2003.

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

Common synonyms for cyclohexane include hexahydrobenzene, hexamethylene, and hexanaphthene (Merck Index, 1996). Some relevant physical and chemical properties of cyclohexane are listed below.

CASRN	110-82-7	NIOSH, 1997
Empirical formula	C_6H_{12}	Merck Index, 1996
Molecular weight	84.2	Merck Index, 1996
Physical state	Liquid (20°C)	Merck Index, 1996
Color	Colorless	NIOSH, 1997
Odor	Solvent odor	Merck Index, 1996
Boiling point (°C)	80.7	Merck Index, 1996
Melting point (°C)	6.47	Merck Index, 1996
Log Kow	3.44	Hansch et al., 1995
Vapor pressure (at 25°C)	97 mm Hg	Chao et al., 1983
Water solubility (at 25°C)	55 mg/L	Verschueren, 1996
Explosive Limits	LEL = 1.3%	NIOSH, 1997
	UEL = 8%	
Conversion factors	1 ppm = 3.44 mg/m^3	NIOSH, 1997
	1 mg/m^3 = 0.291 ppm	

The primary use of cyclohexane is in the production of nylon. A total of 55% is used to produce adipic acid and 26% is used to formulate caprolactam, both of which are then used to generate nylon. Another 13% is exported and the remaining 6% is used in solvents, insecticides, and plasticizers (Kavaler, 1998). The United States accounts for about one-third of the world's consumption of cyclohexane, or about 1 billion gallons per year (Eastman and Mears, 1995). In 1991, the total U.S. production of cyclohexane was 3.55×10^8 gallons. Cyclohexane is present in all crude oils in concentrations ranging from 0.1 to 1.0%, and it is also found in gasoline formulations (Eastman and Mears, 1995). It has also been detected in volcanic emissions and in

plant volatiles (Graedel, 1978). The general population is primarily exposed to cyclohexane through the inhalation of ambient air due to its presence in gasoline vapors. The average concentration of cyclohexane in the exhaust of six cars was 82 ppb (Blake et al., 1993).

In the ambient atmosphere, cyclohexane is expected to exist solely in the vapor phase (Bidleman, 1988), based on a measured vapor pressure of 97 mm Hg at 25°C (Chao et al., 1983). Vapor-phase cyclohexane is degraded by reacting with photochemically-produced hydroxyl radicals (Atkinson, 1989), with an estimated half-life of 45 hours. Cyclohexane is expected to have moderate mobility in soils, based on an estimated K_{oc} value of 160, determined by a structure estimation method that uses molecular connectivity indices (Meylan et al., 1992). Volatilization from water surfaces is expected to be an important fate process, based on its Henry's Law constant of 0.15 atm m^3/mol (Bocek, 1976). Estimated volatilization half-lives for a model river and model lake are 1 hour and 3.6 days, respectively (Meylan and Howard, 1991).

3. TOXICOKINETICS RELEVANT TO ASSESSMENTS

3.1. ABSORPTION

Cyclohexane is rapidly absorbed into the blood via the lungs, gastrointestinal tract, and skin. At higher doses, some cyclohexane is expired unchanged due to preferential partitioning to alveoli rather than the blood, where it has low solubility (see Section 3.4.).

Several occupational monitoring studies of workers exposed via inhalation demonstrate rapid uptake by the human body (Brugnone et al., 1980; Mutti et al., 1981; Perbellini and Brugnone, 1980; Yasugi et al., 1994). Perbellini and Brugnone (1980) found strong correlations between cyclohexane levels in factory air and alveolar air, and between alveolar air and blood concentrations in 22 shoe factory workers during hours 4–8 of their work shift. Breathing space air concentrations ranged from 17 to 2,484 mg/m^3. The exposed factory workers exhibited mean levels of cyclohexane in alveolar air that corresponded to 78% of the workplace concentration. Blood levels ranged from 29 to 367 μg/L (mean 158 μg/L), corresponding to 53 to 78% of alveolar concentration.

Yasugi et al. (1994) assessed a cohort of 33 female workers exposed 8 hours per day for at least 1 year to glue solvent containing by volume up to 83% cyclohexane, 16% toluene, and 0.9% hexane. Measured by personal monitors, the geometric mean and maximum concentrations of cyclohexane in breathing zone air were 27 and 274 ppm, respectively. Toluene and hexane levels were 2.8 ppm (maximum 11 ppm) and 1.4 ppm (maximum 12 ppm), respectively. Cyclohexane concentrations in blood samples collected at the end of the workweek's last shift correlated significantly ($p < 0.01$) with exposure. Serum analyses of various liver and kidney functions gave normal results. The analyses included total protein, blood urea nitrogen, creatinine, uric acid, total cholesterol, triglycerides, HDL-aminotransferase, aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transpeptidase, alkaline phosphatase, leucine aminopeptidase, and lactate dehydrogenase.

Mutti et al. (1981) measured the total lung uptake of eight shoe factory workers during a 4-hour exposure period. Workplace air contained from 52.7 ± 7.1 to 266.5 ± 11.2 mg/m^3

cyclohexane. Results indicate that 34% of the alveolar cyclohexane and 23% of the total respiratory intake was absorbed into the pulmonary blood. The total mean intake and uptake were calculated at 354 ± 12 mg and 81.2 mg cyclohexane, respectively.

Brugnone et al. (1980) studied industrial exposure to cyclohexane in different factories. Alveolar air and breathing zone air samples (n = 108) were collected simultaneously during the work shift. The ratio of alveolar air (Ca) to workplace air (Ci) was high and gave a linear correlation (r = 0.98). Alveolar retention (defined as Ci – Ca) rose constantly during hours 4 to 8 of the work shift. A linear correlation (r = 0.82) was found between workplace air concentration and alveolar retention.

Uptake has also been demonstrated in occupational settings by examining urinary metabolites of cyclohexane (see Section 3.4.1.) (Governa et al., 1987; Mraz et al., 1994; Perbellini et al., 1980, 1987; Yasugi et al., 1994; Yuasa et al., 1996).

Absorption of cyclohexane via the lungs, gastrointestinal tract, and skin has been described in several animal species. [^{14}C]Cyclohexane administered to rats either by oral gavage or by intravenous injection was rapidly absorbed and distributed to the tissues (RTI, 1984). Savolainen and Pfaffli (1980) and Zahlsen et al. (1992) repeatedly exposed rats via inhalation and found cyclohexane in blood, brain, fat, and other tissues. Rabbits exposed orally or via inhalation excreted cyclohexane metabolites in their urine (Treon et al., 1943a, b; Elliott et al., 1959).

Naruse (1984) exposed mice to varying amounts of an adhesive containing approximately 7.5, 13, or 19 g cyclohexane in a closed chamber. Air levels of cyclohexane stabilized after 1 hour, producing concentrations of 8,000, 14,000, or 17,500 ppm, respectively. Corresponding blood concentrations were determined to be 27, 69, and 122 µg/mL, respectively, and were correlated (r = 0.99) with the total dose introduced into the exposure chamber (Table 3-1).

Cyclohexane is absorbed through the skin surface. Iyadomi et al. (1998) measured the time course of dermal absorption in male WBN/ILA-Ht hairless rats. Solvent chambers glued to abdominal skin allowed contact over a 6.28 cm^2 area. Cyclohexane (1 mL) was injected into the

chamber, and carotid blood samples (n = 8) were drawn from 5 minutes to 4 hours after initiation of exposure. Arterial cyclohexane concentration increased rapidly up to 30 min, peaking in about 1 hour at approximately 0.24 μmol/L. Thereafter, blood levels decreased in a linear fashion. A rough blood clearance time was calculated as 400 minutes.

3.2. DISTRIBUTION

Perbellini et al. (1985) and Gargas et al. (1989) used different experimental methods to determine physiologically based partition coefficients for cyclohexane in human cadaver and rat tissues (Table 3-2). For volatile compounds such as cyclohexane, the air-to-blood and the blood-to-tissue concentration ratios factor significantly in blood uptake and subsequent distribution to the tissues. Not surprisingly for a nonpolar organic compound, cyclohexane partitions preferentially to lipid-rich tissues such as fat, liver, and brain. The values obtained for muscle tissue differ between the two studies.

Table 3-1. Uptake and disappearance of cyclohexane in mouse blood after inhalation exposure

Cage dose[a]	Measured levels[b]	Exposure time		Post-exposure time			
		30 minutes	1 hour	30 minutes	1 hour	2 hours	3 hours
7.5 g	AIR ppm (g/m^3)		8,000 (27.5)				
	BLOOD µg/mL	7	27	7 (25%)	4 (15%)	1 (4%)	
13 g	AIR ppm (g/m^3)		14,000 (48.2)				
	BLOOD µg/mL	41	69	9 (13%)	5 (7%)	2 (3%)	
19 g	AIR ppm (g/m^3)		17,500 (60.2)				
	BLOOD µg/mL	71	122	16 (13%)	8 (7%)	3 (2%)	1

[a] Cage dose, cyclohexane grams in the chamber, calculated from Table I and Figure 8 of Naruse (1984).
[b] Measured levels in AIR calculated from Figure 8 of Naruse (1984).

Sikkema et al. (1994) found that cyclohexane preferentially accumulated in a microbial phospholipid bilayer membrane (liposome) and calculated the partition coefficient for the bilayer membrane to the potassium phosphate buffer system to be 498.

Cyclohexane has been detected in human milk in 5 out of 12 samples collected in urban areas of the United States. The fat content of mothers' milk, approximately 3.8%, would promote partition (Pellizzari et al., 1982). No other studies addressing the distribution of cyclohexane in humans were found.

Table 3-2. Tissue, saline, blood, and oil/air partition coefficients for cyclohexane

Tissue/substance	Perbellini et al. (1985)[a]	Gargas et al. (1989)
0.9% saline	-	< 0.01 (approximated)
blood	1.3 ± 0.1	1.41 ± 0.14 (human) 1.39 ± 0.09 (rat)
lung	2.7 ± 0.1	-
heart	5.8 ± 1.0	-
kidney	7.2 ± 1.0	-
liver	10.8 ± 0.9	7.88 ± 0.59 (rat)
brain	10.7 ± 1.4	-
muscle	10.5 ± 0.7	1.03 ± 0.17 (rat)
fat	260 ± 11.0	235 ± 4 (rat)
olive oil	293 ± 11.0	293 ± 2

[a] Two male cadavers, ages 30 and 40, cause of death, heart attack.

Existing studies provide information on the distribution of cyclohexane in rats. Rats exposed repeatedly for 1 week to 300, 1,000, or 2,000 ppm cyclohexane exhibited body burdens in fat and brain that reflect the ratios of the partition coefficients for these tissues. After 2 weeks of exposure, however, the cyclohexane concentrations in fat increased disproportionately (Table 3-3). The body burden in perirenal fat was proportional ($r > 0.95$) to exposure levels and an order of magnitude higher than that found in brain (Savolainen and Pfaffli, 1980).

Zahlsen et al. (1992) studied the distribution of cyclohexane in male Sprague-Dawley rats up to 3 days of exposure (12 hrs/day) to a mean level of 100.4 ppm (range: 96–107 ppm). The mean concentration in tissues was relatively steady during the 3 days of exposure except in fat, where mean levels increased daily (Table 3-4). The high concentration of cyclohexane in kidney cannot be explained by its solubility in kidney tissues alone. The authors concluded that cyclohexane toxicokinetics are complex and cannot be extrapolated from solubility properties alone.

Table 3-3. Cyclohexane burdens in rat cerebrum and perirenal fat after repeated exposure

Dose	Fat (nmol/g tissue)		Cerebrum (nmol/g)		Fat:Brain Ratio	
	1 week	2 weeks	1 week	2 weeks	1 week	2 weeks
300 (ppm)	538 ± 43	483 ± 106	14 ± 8	8 ± 2	38	60
1,000 (ppm)	2340 ± 592	1706 ± 199	101 ± 28	21 ± 10 [a]	23	81
2,000 (ppm)	3542 ± 658	2748 ± 806	150 ± 31	55 ± 7 [a]	24	50

[a] differs from the first week value at $p < 0.001$.
Source: Adapted from Savolainen and Pfaffli, 1980.

Seventy-two hours after a single intravenous dose of 10 mg/kg [^{14}C]cyclohexane or a single oral dose of 200 mg/kg to adult male Fischer 344 rats, the concentration of radioactivity in adipose was 16 times greater than that in blood. At higher oral doses (1,000 or 2,000 mg/kg), the adipose tissue-to-blood ratio of radioactivity approximately 45. Although radioactivity in adipose tissues was primarily cyclohexane (79–84%), in muscle, liver and skin, only 2–18% of the ^{14}C was identified as cyclohexane. Cyclohexane, cyclohexanol, and cyclohexanone were present in all tissues (RTI, 1984).

Table 3-4. Concentration of cyclohexane in rat tissues after repeated exposure

Tissue	Exposure Levels (µmol/kg) (% change from previous day)				Post Exposure (µmol/kg)
	Day 1	Day 2	Day 3	Mean	Day 3 + 12 hours
Blood	4.0 ± 0.3	4.4 ± 0.1	4.1 ± 0.9	4.2	0.1 ± 0.1
Liver	22.6 ± 3.0	22.3 ± 2.9	26.4 ± 1.7	23.8	0.5 ± 0.4
Brain	31.7 ± 2.2	33.6 ± 3.2	34.7 ± 1.1	33.3	2.0 ± 2.5
Kidney	86.5 ± 2.0	100.1 ± 10.3	99.4 ± 13.0	95.3	1.3 ± 0.1
Fat	417 ± 66	475 ± 27 (14%)	482 ± 17 (1.5%)	-	169.17

- = not calculated by study authors, values still increasing.
Source: Adapted from Zahlsen et al. (1992).

3.3. METABOLISM

Metabolic studies of the microsomal mixed-function oxidase (monooxidase) system in liver confirm hydroxylation of cyclohexane to cyclohexanol. Cyclohexanol is the primary metabolite of cyclohexane; however, lesser amounts of cyclohexanone and 1,2-cyclohexane-diol have been identified. Cyclohexyl metabolites are conjugated to glucuronides for excretion, but at high doses sulfate conjugation may occur. Information on the metabolic pathways of cyclohexane is insufficient. No human studies of cyclohexane metabolism were found in the literature.

A metabolic adaptation in mice to repeated exposures of cyclohexane was observed by Naruse (1984). Immediately following a 1-hour exposure period to 14,000 ppm cyclohexane, blood levels averaged 69 μg/mL and cleared within 3 hours. However, after 120 days of repeated exposure (1 hr/day, 6 days/wk), blood levels measured immediately after exposure dropped to 30 μg/mL, clearing within 2 hours.

Evidence suggestive of mixed-function oxidase activation in rats exposed to cyclohexane was observed by Savolainen and Pfaffli (1980). Male Wistar rats were exposed repeatedly (6 hrs/day, 5 days/wk for 1–2 weeks) to cyclohexane vapor (300, 1,000, or 2,000 ppm) and the body burden was examined (Table 3-3). After the first week of exposure, a linear correlation ($r = 0.99$) was found between brain and fat cyclohexane levels. By the end of the second week, cyclohexane levels had decreased in both tissues, indicating metabolic adaptation. Elimination of cyclohexane from the brain was particularly enhanced when compared to body fat. This changed the relationship between tissue concentrations to one described best by an exponential function.

In these experiments, measures of brain metabolism, RNA, glutathione, and glutathione peroxidase activity were not affected by cyclohexane exposure. However, the cyclohexane dose increased, brain azoreductase activity decreased significantly and was still well below control levels after a recovery period. Activation of the mixed-function oxidase system has been found to inhibit azo reduction (Klaassen et al., 1986). The authors suggested that although increased

blood circulation in the brain compared to fatty tissue enhances cyclohexane elimination from the brain, the activation of a liver mixed-function oxidase system is the primary vehicle for decreasing the cyclohexane concentration in the body as a whole.

Two studies describe cyclohexane metabolism *in vivo* (Elliott et al., 1959; RTI, 1984). Elliott et al. (1999) studied the quantitative metabolism of single gavage doses of [^{14}C]cyclohexane (0.3 to 400 mg/kg) administered to adult chinchilla doe rabbits. The experiment apparatus allowed for the capture of expired air and urine from the rabbits. Recovery of the radioactivity was about 95%. Only [^{14}C]-labeled carbon dioxide and unchanged cyclohexane were detected in expired air. The concentration of unchanged cyclohexane in expired air increased with the dose. In urine, the metabolites detected were primarily cyclohexanol with lesser *trans*-1,2-cyclohexane-diol formation. Cyclohexanol and the diol were both excreted as glucuronides.

The authors did not explain the presence of [^{14}C]carbon dioxide, because the dicarboxylic (adipic, succinic, maleic, malonic, or oxalic) acids produced in the ring schism were not detected. Carbon dioxide has not been detected in other more recent studies of cyclohexane metabolism in mammals. Carbon dioxide is a well-established metabolite of *n*-hexane (Battershill et al., 1987), but *n*-hexane contamination was not reported in the current study.

Oral administration of cyclohexanol resulted in the same two metabolites as did cyclohexane: cyclohexanol and the *trans*-1,2-cyclohexane-diol. Further, when cyclohexanone and cyclohex-1-enyl acetate were administered, both were converted to cyclohexanol. All cyclohexane derivatives were conjugated to glucuronides (Elliott et al., 1959). Perbellini and Brugnone (1980) observed cyclohexanol and cyclohexanone in rat urine following cyclohexane exposure.

A definitive study of the absorption, distribution, metabolism, and excretion of [^{14}C]cyclohexane following a single intravenous dose (10 mg/kg) or single oral doses (100, 200, 1,000, or 2,000 mg/kg) to adult male Fischer 344 rats was carried out under the auspices of the National Institute of Environmental Health Sciences (RTI, 1984). After an oral dose of 200

mg/kg [^{14}C]cyclohexane, five unidentified metabolites in blood accounted for more than half of the radioactivity, regardless of sampling time. Another radioactive metabolite in blood was cyclohexanol, present at levels two to three times higher than cyclohexanone. The highest blood concentrations of cyclohexanol and cyclohexanone occurred 2 hours after dosing. During the first 12 hours, cyclohexanone levels accounted for 10% of the blood ^{14}C but decreased to 1–3% within 24 hours. Cyclohexane was also detected as a minor blood constituent.

3.4. EXCRETION

Inhaled cyclohexane is excreted primarily via expiration from the lungs. A small portion partitions to and is excreted in the urine. Metabolites of cyclohexane are conjugated, primarily to glucuronides and possibly to sulfates, and excreted in the urine.

A number of occupational health and monitoring studies of leather (shoe and luggage) factory workers exposed to cyclohexane provide evidence of alveolar and urinary excretion. Perbellini and Brugnone (1980) detected evidence of cyclohexane expiration in some shoe factory workers, but did not identify it as such (see Section 3.1.1.).

Mutti et al. (1981) calculated that approximately 10% of an absorbed dose of cyclohexane was expired after exposure, while most of the dose was retained. Expiration was initially rapid (11.2 minute half-life) for the first hour, followed by a slower component (115.3 minute half-life) thereafter. The total lung uptake during a 4-hour exposure period and the total alveolar expiration during a 6-hour postexposure period for eight factory workers was calculated as 81.2 mg (mean) and 9 mg (mean) cyclohexane, respectively. The authors suggested that alveolar excretion of cyclohexane in most occupational settings is low and fluctuates rapidly in response to environmental concentration changes. At higher inhalation doses, some cyclohexane is expired unchanged due to preferential partitioning to the alveoli rather than to blood, where it has low solubility (see Section 3.1.1.).

Ghittori et al. (1987) proposed that, according to Henry's Law, a small portion of cyclohexane must be excreted in the urine in its unchanged form. Unreacted cyclohexane in the

blood should reach a steadystate equilibrium with alveolar air and glomerular filtrate. Measuring cyclohexane in the urine of 43 human subjects, the authors calculated the urine-to-air partition coefficient for cyclohexane (0.9) and found a relationship between environmental cyclohexane levels and urinary cyclohexane levels using the following regression equation:

$y = ax+b.$

where: y = urinary cyclohexane concentration (nmol/mL)
x = time-weighted breathing zone cyclohexane concentration (ppm)

The result with correlation coefficient is:

$$y = 0.05\ x + 8.26 \quad (r = 0.89)$$

Perbellini et al. (1980) detected urinary cyclohexanol at a mean level of 1.4 ± 1.6 mg/L in shoe factory workers exposed to a leather adhesive containing nine solvents, including cyclohexane (456 ± 464 mg/m^3). Yuasa et al. (1996) found that the urinary concentration of cyclohexanol in 18 female workers at a luggage factory that used a cyclohexane-based (76%) solvent ranged from 0.12 to 1.51 mg/L over an 18-month period. Breathing space cyclohexane levels ranged from 5 to 211 ppm. There was a strong correlation between the cyclohexane exposure in personal air and urinary cyclohexanol.

Mutti et al. (1981) detected cyclohexanol and cyclohexanone in workers' urine, accounting for no more than 0.5 to 1% of the calculated absorbed dose. The level of excretion was poorly related to the level of exposure and showed wide scatter at higher occupational concentrations. Similarly, Perbellini and Brugnone (1980) determined that urinary cyclohexanol levels accounted for only 0.1 to 0.2% of the absorbed cyclohexane. In 22 workers, the mean level of urinary cyclohexanol was 2.24 µg/L (range: 0.27–7.18), and the mean excretion rate was 0.92 µg/minute (range: 0.05–3.23).

Two studies of shoe factory workers who were continually exposed to leather adhesive and cleaning solvent vapors containing cyclohexane suggest that urinary excretion of

cyclohexanol is rapid and declines to low levels by the morning following the exposure event. Governa et al. (1987) detected cyclohexanol (0.23 ± 0.68 mg/L; range: 0.1–3.80 mg/L) in about 20% of urine samples collected at 9 a.m. from workers (n = 40). Perbellini et al. (1987) analyzed urine samples collected from three workers before the start and at the end of the work shift for an entire workweek. All morning samples were < 0.4 mg/L cyclohexanol, whereas levels in afternoon samples (approximately 1–4 mg/L) increased proportionally to the mean occupational exposure levels.

Perbellini and Brugnone (1980) determined that urinary cyclohexanol levels correlated with environmental and alveolar cyclohexane concentrations and that urinary cyclohexanol excretion rates correlated with alveolar cyclohexane. The correlation between blood cyclohexane levels and urinary cyclohexanol levels and excretion rate were weaker. Measurements were taken during the last 4 hours of an 8-hour work shift.

Occupational health monitoring by Yasugi et al. (1994) of 33 female workers exposed to cyclohexane vapors evaluated urinary metabolites of cyclohexane. Urine was collected on the fourth or fifth day of the workweek at shift's end and again the next morning. Analysis showed the presence of cyclohexanol and cyclohexanone. The mean level of cyclohexanol in urine was 875.7 ± 2.86 μg/g. More than 90% was conjugated as glucuronide, the remainder was unbound. Some unconjugated cyclohexanone was also detected. No sulfate conjugates were detected. Both urinary cyclohexanol and cyclohexanone concentrations correlated with exposure levels. Quantitative estimation indicated that only 1% of the absorbed cyclohexane was excreted in the urine as cyclohexanol by the end of the work shift. Cyclohexanol was still present in urine 16 hours after exposure (95.8 ± 2.86 μg/g). A rough biological half-life of 5 hours was calculated assuming a single compartment, suggesting that clearance from the body is relatively rapid.

Treon et al. (1943a, b) demonstrated that oral gavage of young white rabbits at one-half the lethal cyclohexane dose (2.88 g/kg) was followed by an increase in organic conjugated compounds in urine that lasted 48 hours. Other rabbits, repeatedly exposed to cyclohexane vapors (6–8 hours/day, 5 days/week for 2–26 weeks) at levels ranging from 434 to 18,565 ppm (1.46 to 62.5 mg/L), exhibited an increase in glucuronic acid and conjugated organic sulfate in the urine. At an exposure level of 434 ppm for 26 weeks, the excretion of glucuronic acid was

elevated (225 mg/day), but it returned to normal (59 mg/day) when dosing was discontinued. Approximately 50% of the cyclohexane dose was recovered as glucuronides. In the lowest-dose group (434 ppm), excretion of organic sulfate was similar to controls but increased at higher doses until a maximum response was reached. Sulfate excretion returned to normal levels on termination of exposure.

When the dose of cyclohexane administered to rabbits was an order of magnitude lower, there was no evidence of sulfate conjugation. The radioactivity from an oral gavage dose of [^{14}C]cyclohexane was almost completely recovered in urine and expired air within 2 days after dosing. At low doses (0.3 mg/kg), around 85% of the radioactivity was recovered in urine as glucuronide conjugates of cyclohexanol and *trans*-1,2-cyclohexanol. At high doses, the amount in urine decreased to about 50% since the entire cyclohexane dose was not metabolized. Up to 38% was expired unchanged in air. While most of the metabolized cyclohexane (80–90%) was excreted in the urine, radioactivity in expired carbon dioxide increased to about 15% as the dose increased. At all doses, a small amount of radioactivity (< 5%) was detected in the feces and tissues (Elliott et al., 1959).

Studies in mice (Naruse, 1984) and rats confirm that when exposure to cyclohexane is discontinued, cyclohexane levels in the body drop rapidly. In rats, cyclohexane levels in perirenal fat are high, but no cyclohexane was detected in other tissues 2 weeks after a 14-day exposure period (Savolainen and Pfaffli, 1980). In rats dosed for 3 days, perirenal fat levels were reduced by two-thirds only 12 hours after exposure ceased. Other tissues contained 6% or less of their respective exposure values (Zahlsen et al., 1992).

In the RTI (1984) study (see Sections 3.2. and 3.3.), a comparison of the intravenous and oral route of dosing in adult male Fischer 344 rats found that after intravenous dosing with 10 mg/kg [^{14}C]cyclohexane, approximately 80% of the radioactivity was expired the first day - 54% within the first hour. Slightly more than 1% of the dose was expired on the second and third days. Urinary metabolites constituted 14% of the dose; more than 80% of the metabolites were excreted during the first day. The ratio of the radioactivity expired to the radioactivity excreted in urine was approximately 6.2 to 1. After oral dosing, the amount expired was proportional to the dose. Twelve to 29% of the radioactivity was excreted in the urine, where levels were

inversely proportional to the dose. With doses of 100 or 200 mg/kg [^{14}C]cyclohexane, the ratio of the radioactivity expired to that excreted in urine was 2 to 1, and at doses of 1,000 or 2,000 mg/kg the ratio was 5 to 1 and 6.5 to 1, respectively.

Independent of dose vector, cyclohexane accounted for 93–99% of the expired ^{14}C. Less than 1% was expired as cyclohexanol and cyclohexanone. In the urine, cyclohexane, cyclohexanol, and cyclohexanone separately represented less than 0.1% of the excreted ^{14}C. The authors suggest that the remainder of the urinary ^{14}C was conjugated. The level of ^{14}C in feces was not significant. No significant amounts of [^{14}C]carbon dioxide were detected, and there did not appear to be any substantial retention of radioactivity in any tissue. The body half-life for ^{14}C was estimated at 10 to 15 hours (RTI, 1984).

4. HAZARD IDENTIFICATION

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

There are very few epidemiologic studies on cyclohexane alone. Most of the occupational studies reported in the literature indicate that cyclohexane is only one of several solvents used in the workplace. In most of these studies, it is suspected that other solvents such as *n*-hexane or toluene comprise the majority of the workplace exposures and are responsible for adverse health effects, including neurotoxicity and spontaneous abortion (Agnesi et al., 1997; Lee et al., 1998; Mutti et al., 1981). Notwithstanding, two small studies (Yasugi et al., 1994; Yuasa et al., 1996) have been conducted with workers who were exposed primarily to cyclohexane. Although very few adverse health effects associated with cyclohexane exposure were reported, definitive conclusions cannot be drawn due to certain limitations within both studies.

Thirty-three women were exposed to cyclohexane in a Japanese factory where glue is applied to surfaces by automated sprayers (Yasugi et al., 1994) . The two glues used in the factory contained at least 75% cyclohexane. The workers included in the study were exposed to the glue solvents for at least 1 year. They provided a urine sample after 5–6 hours of exposure and blood and urine samples after the 8-hour work shift. They were also administered a questionnaire on subjective symptoms experienced within the last 3 months both at home and at work. Personal monitors indicated that 274 ppm (27 ppm geometric mean) was the highest level of cyclohexane measured.

The exposed subjects were divided into low (< 5–13 ppm) and high (15–274 ppm) exposure groups with 17 and 16 workers, respectively. They were compared to 10 controls using chi-squared tests. Hematology and serum biochemistry parameters were analyzed. At the $p < 0.05$ significance level, no differences were found in the hematology or serum biochemistry parameters in liver and kidney function. In the questionnaire, there were no significant differences ($p > 0.05$) in the subjective symptoms experienced at work either individually or in

combination. There was also no difference in sister chromatid exchange rate between exposed and nonexposed workers.

Given the small number of women in the survey, the even smaller number of control subjects, and the relatively low exposure levels, it is not surprising that effects were not reported in this study. In addition, the report does not contain any definitions of the subjective symptoms or the results of these tests or any specific results of the hematology and biochemistry tests. The results are therefore difficult to interpret.

In another Japanese study, neurophysiological effects were analyzed in female luggage factory workers exposed to glue containing 75% cyclohexane, 12% toluene, and 0.9% *n*-hexane (Yuasa et al., 1996). Prior to the start of the study, *n*-hexane was the primary solvent used at the plant, but it was gradually discontinued and replaced with cyclohexane in 1992. Therefore, several of the participants had past exposures to *n*-hexane; 12 of 18 women in the first study year (at a length of n-hexane exposure time of 0.3–20 years) and 8 of 9 in the second study year. The workers were exposed for approximately 8 hours/day. In the first study year (April through July 1993), the women had been employed for 0.4–2.6 years; the shortest time between exposure of any of the workers to *n*-hexane and the first study was 0.7 years. Due to changes in job assignment, only 9 women in the first study year participated in the second observation period (July 1994). The 18 control subjects consisted of medical students and clerical workers, and were significantly different from the study subjects.

Air monitoring indicated that cyclohexane levels ranged from 5 to 211 ppm (geometric mean, 28 ppm; median, 46 ppm), although it is unclear whether these levels were observed in the first or second study year. Concentrations prior to the first study period were much higher (6–720 ppm; geometric mean, 77 ppm). Based on the exposure data, high (> 100 ppm) and low (< 100 ppm) exposure groups were formed for the first study period.

In the first study year, workers participated in biological monitoring (urinary cyclohexanol), a neurophysiological study (nerve conduction velocity), and a subjective symptom survey (fatigue, headache, etc.). Urinary cyclohexanol measurements ranged from 0.12 to 8.23 mg/L and were highly correlated to ambient cyclohexane levels in the wokplace.

The symptom survey did not indicate any significant difference in subjective symptoms between exposed workers and controls except for general fatigue ($p < 0.1$). The symptom survey was not administered in the second year.

In the neurophysiological examination, the nerve conduction velocity measurements of the exposed workers when compared to controls were not significantly different ($p < 0.05$), nor were they significant when high- ($n = 7$) and low- ($n = 11$) exposure groups were compared to controls. In the second year, only 9 of the original 18 exposed workers were included in the study. A significant improvement in several of the nerve conduction velocity tests was noted in these employees between the first and second years. Therefore, the authors concluded that *n*-hexane affected the measurements in the first study and that the workers recovered by the second period.

As previously mentioned, there are many limitations to this study and the results should be interpreted carefully. The small number of participants, especially in the second year, limits the reliability of the data. Also, the controls were not chosen from unexposed workers at the same plant and were not matched well to the subjects. Past exposures to other solvents and length of past exposure was not taken into consideration, even though it varied widely among participating workers.

4.2. PRECHRONIC AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL AND INHALATION

No adequate oral prechronic studies and no chronic studies of any exposure route were located for cyclohexane. However, two unpublished, 90-day inhalation toxicity studies were conducted with cyclohexane in mice and rats (DuPont HLR, 1996a, b). These studies were later summarized and published as parts of Malley et al. (2000).

Exposure concentrations for the 90-day study of mice were selected based on the results of a 2-week range-finding study and knowledge of the explosive properties of cyclohexane. As summarized in DuPont HLR (1996a), the range-finding study (concentrations of 3,000, 6,000,

and 9,000 ppm [10,329, 20,658, and 30,987 mg/m^3]) showed that the two higher concentrations decreased the response to an alerting stimulus during exposure. The response to an auditory stimulus was evaluated prior to the initiation of exposure to cyclohexane, during exposure to cyclohexane and during the time required to clear the chambers. Groups of animals were observed for normal, diminished, or hyperresponsive altering behavior in response to an auditory stimulus. In addition, mice exposed to 30,987 mg/m^3 displayed sporadic incidences of jumping and/or slow circling behavior. It was reported that male mice had compound-related increases in relative lung weights in the 20,658 and 30,987 mg/m^3 groups. Female mice in the same groups had significantly increased absolute and relative liver weights (DuPont HLR, 1996a).

In DuPont HLR (1996a), male and female Crl:CD-1 BR mice were exposed by whole-body inhalation to cyclohexane vapor at concentrations of 0, 500, 2,000, or 7,000 ppm (0, 1,721, 6,886, or 24,101 mg/m^3) for 6 hours/day, 5 days/week for 90 days. Initially, there were 20/sex/concentration for the control and high-concentration groups and 10/sex/concentration for the low- and intermediate-concentration groups. Ten mice per sex from the control and high-concentration groups were allowed a 1-month recovery period prior to sacrifice.

There were no treatment-related deaths. A few mice in each group died due to blood sampling errors. There were no treatment-related effects on body weight, body weight gain, or food consumption. Clinical signs and response to an alerting stimulus during exposure exhibited a dose-response relationship. Inhalation exposure of mice to 24,101 mg/m^3 produced clinical signs of hyperactivity and marked central nervous system stimulation, which persisted for a short period after the end of each daily exposure. The clinical signs of toxicity included: hyperactivity, circling, jumping/hopping, excessive grooming, kicking of rear legs, standing on hind legs, and occasional flipping behavior. These signs were evident by the fourth exposure and persisted throughout the remaining exposures. The clinical observations of response to an alerting stimulus varied as it was diminished in some instances and it could not be assessed due to hyperactivity at other periods. During the recovery period, no central nervous system stimulation was observed.

The relative liver weights increased in male and female mice in the 24,101 mg/m^3 group, mean relative liver weights (percent of body weight [standard deviation], males: 4.822 [0.313]

vs. controls 4.151 [0.411]; females: 4.726 [0.307] vs. controls 4.272 [0.388]). At the end of the recovery period, no significant changes in liver weights were observed. The erythrocyte mass was increased, but the cause of this change is unknown although it may have been due to the bleeding schedule. Mice in the 6,886 mg/m^3 group showed hyperactivity late in the exposure period and sedative effects that were apparent through most of the exposure period.

Exposure concentrations were selected for a similarly designed 90-day inhalation toxicity study of cyclohexane in male and female Sprague-Dawley rats based on the results of a 2-week range-finding study and knowledge of the explosive properties of cyclohexane. In this range-finding study, as reported in DuPont HLR (1996b), concentrations of 3,000, 6,000, and 9,000 ppm (10,329, 20,658, and 30,987 mg/m^3) were tested. The two higher concentrations decreased the response to an alerting stimulus during exposure. The body weights of the rats exposed to 30,987 mg/m^3 were lower than the controls and there was a higher incidence of mitotic figures in hepatocytes from the high-concentration group and 20,658 mg/m^3 males (DuPont HLR, 1996b).

In the 90-day inhalation toxicity study ((DuPont HLR, 1996b; Malley et al., 2000) in male and female Sprague-Dawley rats (20/sex/concentration for control and high-concentration groups and 10/sex/concentration for low- and intermediate-concentration groups) were exposed by whole-body inhalation to cyclohexane vapor at concentrations of 0, 500, 2,000, or 7,000 ppm (0, 1,721, 6,886, or 24,101 mg/m^3) for 6 hours/day, 5 days/week for 90 days. At the end of 90 days, 10 rats/sex/concentration were sacrificed. After a 1-month recovery period, 10 remaining rats/sex from the control and high-dose groups were sacrificed and their tissues were examined for histologic changes.

There were no treatment-related deaths and there were no significant differences in body weight among the control and the treatment groups. The most common clinical observation was diminished alerting responses in the chamber during exposure at 6,886 and 24,101 mg/m^3. This effect was characterized as transient and was not observed immediately after removing the animals from the chamber. Rats in the 1,721 mg/m^3 group did not show this central nervous system effect.

The only other treatment-related effect was increased relative liver weights in high-concentration males at 90 days and after the 1-month recovery period. Both males and females in the 24,101 mg/m^3 group had hepatocellular hypertrophy, which was considered the cause of the enlarged livers. Decreases in the activity of some serum enzymes related to hepatic function were statistically significant. Significant decreases in aspartate aminotransferase, sorbitol dehydrogenase, lactate dehydrogenase and creatine phosphokinase were detected, but the mean values generally did not exhibit a dose-response relationship. Although increases in such enzyme activities can indicate tissue damage, decreases in these enzyme activities generally are not considered biologically significant effects.

The mean relative liver weights in high-concentration males were significantly higher than those of controls at the 90-day and 1-month recovery terminations (percent of body weight [standard deviation], day 95: 4.001 [0.265] vs. control 3.649 [0.214]; day 123: 4.009 [0.313] vs. control 3.767 [0.240]). Gross observations showed large livers in 10/10 and 4/10 males at 24,101 mg/m^3 at 90 days and after 1-month recovery period, respectively. Centrilobular hepatocellular hypertrophy was observed microscopically in 9/10 males and 5/10 females in the 24,101 mg/m^3 group at the 90-day sacrifice but not after the 1-month recovery period. The study authors considered the hepatic enlargement to be an adaptive response and not an adverse effect. On the other hand, in the absence of long-term exposure data, the hepatic enlargement and the incomplete reversibility of the effect during the recovery period may indicate that there could be a progression of liver effects to frank toxicity with longer exposure to cyclohexane.

In summary, 90-day inhalation toxicity studies (Malley et al., 2000) were conducted with cyclohexane in mice (DuPont HLR, 1996a) and rats (DuPont HLR, 1996b). In mice, hyperactivity and diminished response to an alerting stimulus was observed at 6,886 and 24,101 mg/m^3. In rats, diminished response to an alerting stimulus was also observed at 6,886 and 24,101 mg/m^3. Relative liver weights were increased in both rats and mice treated with 24,101 mg/m^3 cyclohexane. In the absence of pathological changes in the liver, it cannot be determined whether these changes are the first signs of a potential liver toxicity that would only become apparent with long-term exposure to cyclohexane.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

No adequate studies of reproductive or developmental toxicity of oral exposure to cyclohexane were located. Unpublished reports of two-generation reproduction toxicity in rats and prenatal developmental toxicity in rats and rabbits exposed to cyclohexane by inhalation were submitted by industry (DuPont HLR, 1997a, b, c) and later summarized and published (Kreckmann et al., 2000).

A two-generation reproduction inhalation toxicity study of rats was conducted with cyclohexane involving the production of one set of litters in each generation (DuPont HLR, 1997a; Kreckmann et al., 2000). Male and female Crl:CD BR rats (Sprague-Dawley strain, 30/sex/concentration) were exposed by whole body inhalation to cyclohexane vapor at concentrations of 0, 500, 2,000 or 7,000 ppm (0, 1,721, 6,886, or 24,101 mg/m^3). Following 10 weeks of exposure (generally 6 hours/day for 5 days/wk, excluding holidays), the animals were bred within their respective treatment groups and allowed to deliver and rear their offspring until weaning. Pregnant females were exposed daily, 6 hours/day, during days 0–20 of gestation. As specified in the protocol, they were not exposed from day 21 of gestation until day 4 of lactation. At day 5 of lactation, daily exposure of dams was resumed. Neonates were not directly exposed to cyclohexane. At weaning, F1 rats were randomly selected to produce the next generation and exposed to cyclohexane as described above. At least 11 weeks after weaning, the F1 rats were bred to produce the F2 litters.

It was reported that the high concentration was based on a pilot developmental toxicity study demonstrating that maternal body weight and food consumption were reduced at 6,000 ppm (20,658 mg/m^3) and above. The explosive hazard of cyclohexane under pressure limited the high concentration tested to 7,000 ppm (24,101 mg/m^3), corresponding to approximately 60% of the lower explosive limit. The remaining concentrations were selected by equal spacing on a log scale. The purity of cyclohexane was reported to be greater than 99.9%.

Clinical observations during exposure showed a diminished response or no response to a sound stimulus beginning at exposure 15 in animals exposed to 6,886 or 24,101 mg/m^3. Specifically, females showed a diminished or absent altering response during exposure to 8,886

or 24,101 mg/m^3 cyclohexane. Because the premating animals were exposed 5 days/week, this would be approximately day 19 of the exposure period. The sedation was characterized as transient and was no longer apparent shortly after the rats were removed from the chamber. The animals in these two groups also showed salivation, stained perioral area, and wet chin. These clinical signs may be related to the sedation.

For the P1 generation, there were no compound-related reductions in body weight or food consumption for the males. Mean body weight for the P1 females in the 24,101 mg/m^3 group was significantly reduced by day 64 of the premating period (94% of the control). The authors stated that although the mean body weight was significantly reduced as compared with controls for the gestation and lactation periods, body weight gain was similar to that of controls during these periods, indicating that the differences were probably due to the preexisting weight deficits established during the premating period. For the F1 generation, body weight was significantly reduced throughout the study for F1 males in the 24,101 mg/m^3 group and throughout the premating, gestation, and lactation periods for F1 females in the 24,101 mg/m^3 group (92% of control at the end of premating). The authors stated that, as with the P1 generation females, the lower body weights during gestation and lactation of the F1 females were probably a continuation of the body weight deficits established during the premating period as reflected in generally similar body weight gains between treated and control females in the later two periods.

There were no significant differences in mating, fertility or gestation indices, implantation efficiency, or gestation length in either the P1 or the F1 generation. There were no dose-related trends in the mean number of implantation sites, mean number of pups/litter, or any survival indices for both F1 and F2 litters (sex ratio, percent born alive, 0–4 day viability, lactation index, and litter survival). The mean pup weight was significantly reduced from postpartum day 7 through the remainder of the 25-day lactation period for F1 and F2 litters (see Tables 5-1 and 5-2 in Section 5.2.1.). The method of statistical analysis in the report was an analysis of covariance with litter size and sex ratio as covariates, followed when significant with linear contrast of the least square means. No compound-related effects on organ weights, gross observations, or microscopic findings were found.

In summary, the reproductive toxicity study concluded that the inhalation exposure of rats to 24,101 mg/m^3 cyclohexane vapor produced significant reductions in body weights in P1 and F1 females and F1 males, significant reduction in pup weights from lactation days 7 to 25 for F1 and F2 litters, and the clinical observation of diminished or absent response to a sound stimulus while in the exposure chamber. At the 6,886 mg/m^3 level, the only cyclohexane effect was the subjective clinical observation that rats as a group had a diminished response to a sound stimulus while in the exposure chamber. Based on the reduced rat pup weights during lactation in the two generations tested, the NOAEL for developmental effects in this reproductive toxicity study was 6,886 mg/m^3.

Inhalation developmental toxicity studies of cyclohexane (Kreckmann et al., 2000) were conducted using rats (DuPont HLR, 1997b) and rabbits (DuPont HLR, 1997c).

Pregnant rats (Crl:CD:BR strain, 25/concentration) were exposed by whole-body inhalation to test material vapor at concentrations of 0, 500, 2,000, or 7,000 ppm (0, 1,721, 6,886, or 24,101 mg/m^3) for 6 hours/day on days 6–15 of gestation. In addition to the standard control group, a pair-fed control group was included; this group received an amount of food equal to the cumulative average amount of food consumed by the high-concentration group on the corresponding gestation day. Maternal body weights were recorded on days 0, 6–15, and 21; food consumption was recorded daily, and clinical signs were recorded daily on days 0–6 and 16–21 and twice daily on days 6–15. Dams were sacrificed on day 21, and the fetuses were weighed, sexed, and examined for external and skeletal abnormalities; one-half of the fetuses were examined for visceral and head abnormalities (DuPont HLR, 1997b; Kreckmann et al., 2000).

Maternal toxicity was evident in the mid- and high-concentration groups. Mean maternal body weight gain was significantly reduced during the treatment period in both groups (approximately 69% of control for the 24,101 mg/m^3 group), and food consumption was significantly reduced during the treatment period for the 24,101 mg/m^3 group. Mean body weight gain was also significantly reduced in the pair-fed control group. At 24,101 mg/m^3 there was a significant increase in the clinical sign "stain chin." While the incidence of salivation was also increased, the difference was not statistically significant. The source of the stained fur was

presumed to be salivation. These signs lasted 10–15 minutes after exposure but were not observed prior to or during exposure. Animals in the 6,886 and 24,101 mg/m^3 groups exhibited a diminished response or no response to a sound stimulus while in the chambers during exposure, indicating a transient sedative effect. Necropsy revealed no gross lesions. On the basis of these results, the LOAEL for maternal toxicity in rats was 6,886 mg/m^3, and the NOAEL was 1,721 mg/m^3.

No evidence of statistically significant developmental toxicity was presented for rats at any dose level. There were no significant differences between control and treatment groups in the number of resorptions, the number of live fetuses, average fetal weight, or sex ratios of pups. There were also no statistically significant differences in the number of external, skeletal, visceral, or head abnormalities in pups. The total incidence of fetal malformations was four fetuses from four litters in the 24,101 mg/m^3 group, one fetus in one litter in the 6,886 mg/m^3 group, none in the 1,721 mg/m^3 group, two fetuses in two litters from pair-fed controls, and none in the *ad libitum*-fed control group. In general, finding one defective fetus in all four litters is of greater concern than an observation of four such fetuses in one litter (Hood, 1996). However, the malformations in the four high-dose fetuses were of different types, malformations were observed in fetuses from two litters from the pair-fed controls, and no other signs of developmental toxicity were noted. The authors of the study did not comment on these data but stated that statistical analyses were only conducted on individual endpoints, which would miss this increase in the total incidence of fetal malformations. They concluded, "No compound-related effect on the incidence of fetal malformations was observed" (DuPont HLR, 1997b). The study authors stated that the NOAEL for developmental toxicity was the highest dose tested, 24,101 mg/m^3.

In a study designed similarly to the rat study, pregnant New Zealand white rabbits (20/concentration) were exposed by whole-body inhalation to cyclohexane vapor at concentrations of 0, 500, 2,000, or 7,000 ppm (0, 1,721, 6,886, or 24,101 mg/m^3) for 6 hours/day on gestation days 6–18 (DuPont HLR, 1997c; Kreckmann et al., 2000).

In the control, 1,721, and 24,101 mg/m^3 groups, all 20 females had litters. There were no treatment-related deaths. Measurements of body weight and weight gain, food consumption,

clinical observations, and postmortem findings did not show any treatment-related effects. In the 6,886 mg/m^3 group, 17 females were pregnant and one aborted the litter before the end of gestation. No statistically significant differences were found between control and treatment groups in pregnancy rate, abortion rate, total resorption rate, the mean number of implantations per litter, or the mean number of live fetuses per litter.

There was a statistically significant decrease in the mean number of corpora lutea for females in the 6,886 and 24,101 mg/m^3 groups, which had a mean number of 8.9 corpora lutea in 17 litters and 8.8 in 20 litters, respectively. While the 1,721 mg/m^3 group had a mean number of 8.9 corpora lutea in 20 litters, it was not significantly different from the control value (10.2 in 20 litters). However, the study authors did not judge the decrease in the mean number of corpora lutea to be biologically significant. First, the decreased means were within the range of control data (historical control data in the report, Appendix M), which showed that 17 studies conducted between 1990 and 1995 had a mean value of 8.9 $\pm$ 1.1; the maximum was 10.9 and the minimum was 7.0. Secondly, the number of corpora lutea for the concurrent control group was near the high end of that range. Most importantly, because ovulations and implantation occurred prior to exposure to the test substance, the decrease in the mean number of corpora lutea for treated groups was not considered to be compound related.

There was a significant trend in sex ratio (number of males/total number of pups), with the ratios being higher for the 6,886 and 24,101 mg/m^3 groups (0.59 and 0.54, respectively, compared with the control ratio of 0.48). However, the authors concluded that this trend appeared to be of unknown significance because of the disparity between the ratios for the 1,721 mg/m^3 (0.42) and the 6,886 mg/m^3 (0.59) groups. No true dose-response for the differences in the sex ratios was found, and the values generally fell within the historical control values (0.40–0.56).

Among the fetuses, there were no significant differences between control and treatment groups in early, late, or total resorptions, fetal weight, malformations, or variations. No significant differences were observed between control and treatment groups in any measures of developmental toxicity. Therefore, the NOAEL for maternal and fetal effects in rabbits was 24,101 mg/m^3, the highest concentration tested.

In summary, inhalation developmental toxicity studies of cyclohexane were conducted using rats (DuPont HLR, 1997b) and rabbits (DuPont HLR, 1997c) and later summarized and published as parts of Kreckmann et al., 2000. Inhalation exposure to concentrations of 6,886 and 24,101 mg/m^3 cyclohexane resulted in maternal toxicity in CD rats, as demonstrated by a significant reduction in body weight gain. There was no evidence of developmental toxicity in rat pups at the highest concentration tested, 24,101 mg/m^3. Therefore, the LOAEL for maternal toxicity in rats was 6,886 mg/m^3, and the NOAEL was 1,721 mg/m^3. The NOAEL for developmental toxicity in rat pups was 24,101 mg/m^3 (DuPont HLR, 1997b; Kreckmann et al., 2000). Inhalation exposure to the highest concentration of 24,101 mg/m^3 cyclohexane resulted in no evidence of maternal or developmental toxicity in rabbits (DuPont HLR, 1997c; Kreckmann et al., 2000).

4.4. OTHER STUDIES

4.4.1. Acute Toxicity

Acute oral lethality of cyclohexane was apparently affected by age in rats; LD_{50} values were 8, 39, and 16.5 mL/kg in 14-day-old, young adult, and older adult rats, respectively (Kimura et al., 1971). The minimum lethal dose after a single gavage exposure in white rabbits (strain not specified) was between 5,500 and 6,000 mg/kg; body weight loss, diarrhea, increased respiratory rate, conjunctival congestion, and lethargy were observed in rabbits administered cyclohexane at or above 1,000 mg/kg (specific dose levels not reported) (Treon et al., 1943a). In mice, an oral LD_{50} of 813 mg/kg has been reported (NIOSH, 2000). No mortality, change in body weight gain, or gross pathological changes were observed in a group of five male and five female rats (unspecified strain) up to 2 weeks after a single gavage exposure to 5,000 mg/kg cyclohexane, but clinical signs suggestive of central nervous system involvement included transient depression, salivation, and soft feces (HLA, 1982a).

Similarly, no mortality, change in body weight gain, or gross pathological changes were observed in a group of five male and five female rats (unspecified strain) up to 2 weeks after a single inhalation exposure to 21,250 ppm cyclohexane for 4 hours, but clinical signs suggestive

of central nervous system involvement included tremors, hyperactivity, rapid respiration, ataxia, and prostration (HLA, 1982b). Cyclohexane did not produce significant upper airway irritation in mice after a single inhalation exposure to 21,750 ppm, as indicated by no exposure-related change in respiration rate (HLA, 1982c). Trembling, "disturbed" equilibrium, or complete recumbency were reported in mice, guinea pigs, rabbits, and cats exposed by inhalation to 18,000 ppm cyclohexane for 30 or fewer minutes (Flury and Zernik, 1931).

4.4.2. Neurological Studies

An acute operant behavior study of cyclohexane by inhalation in rats and a 90-day inhalation neurotoxicity study of cyclohexane in the rat were conducted by DuPont HLR (1996c, d), Christoph et al. (2000), and Malley et al. (2000). Similar to observations in previously discussed studies, there was no evidence of neurotoxicity or impaired response caused by inhalation exposure to cyclohexane beyond the subjective clinical observation of a diminished response to an alerting stimulus at the time of exposure in rats in the current study.

An acute operant behavior study of cyclohexane by inhalation in rats examined the effects of 6-hour exposures on schedule-controlled operant behavior in Crl:CD:BR rats (DuPont HLR, 1996c; Christoph et al., 2000). Rats were exposed to 0, 500, 2,000, or 7,000 ppm (0, 1,721, 6,886, or 24,101 mg/m^3) in a chamber. Behavioral toxicity was detected at the high dose of 24,101 mg/m^3 only. No effects were seen in control animals or at exposure concentrations of 1,721 or 6,886 mg/m^3. At 24,101 mg/m^3, a transient decrease in the mean fixed-ratio rate of responding was apparent from the analysis of variances over the day before exposure, the day of exposure, and the day after exposure. None of the other recorded parameters showed statistically significant effects. Fixed-ratio pause duration showed a slight, nonsignificant increase after exposure, which is difficult to interpret since prior to exposure the mean fixed-ratio pause duration for the two high-dose groups was considerably lower than for the other groups.

Although no effect was detected in the study, subtle effects may have been missed by the protocol. Positive control data for amphetamine sulfate and chlorpromazine on schedule controlled operant behavior were submitted with the study. Although this is a well-conducted study, these data do not demonstrate evidence that the experimental paradigm could detect both

increases and decreases in response rates in all four of the relevant parameters, demonstrating the low sensitivity of this type of acute testing in practice.

In the 90-day inhalation neurotoxicity study in rats (DuPont HLR, 1996d; Malley et al., 2000), the neurotoxicity screening battery included a functional observational battery, motor activity, and neuropathology. Twelve male and female Crl:CD BR rats per group were exposed to concentrations of 0, 500, 2,000, or 7,000 ppm (0, 1,721, 6,886, or 24,101 mg/m^3) of cyclohexane for 6 hours/day, 5 days/week for approximately 90 days. All rats were evaluated using motor activity and functional observational battery assessments prior to exposure to establish baseline measurements. The tests were conducted again on weeks 4, 8, and 13; however, the precise timing of the tests relative to exposure was not stated. Observations of responsiveness to an alerting stimulus were made during exposure. Rats were also observed for postexposure clinical signs. Following testing, six rats/gender/group were sacrificed, perfused, and examined grossly. Histopathology was conducted on the control and high-dose groups.

Positive control data indicate that the equipment and procedures were capable of detecting effects that may be seen in this type of neurotoxicity study. No compound-related effects were found on food consumption, body weight, or body weight gain at any exposure concentration. Clinical observations included a dose-related effect on alerting response noted while rats were in the exposure chamber. Rats in the group exposed to 6,886 mg/m^3 exhibited a normal alerting response during 4 exposure sessions, a diminished response during 32 sessions, and no response during 35 sessions. Rats in the group exposed to 24,101 mg/m^3 had a diminished response in 3 exposure sessions and no response in 68 sessions. These effects were interpreted as a compound-related sedative effect. There were no compound-related effects on alerting during exposure to 1,721 mg/m^3.

Immediately following exposure, female rats in the 6,886 and 24,101 mg/m^3 exposure groups and males in the 24,101 mg/m^3 group had an increased incidence of stained chin. Although this clinical observation was dose-related, the toxicological significance is not clear. This observation was characterized as transient by the study authors.

Functional observational battery assessments were conducted at some time prior to motor activity testing. No significant treatment-related effects were found for either sex in any of the 34 functional observational battery parameters evaluated at any of the exposure concentrations. There were no compound-related effects on forelimb or hind limb grip strength or hind limb foot splay.

Motor activity was measured in 10-minute intervals for a session total of 60 minutes, using automated Coulbourn activity monitors. Data were evaluated as the group mean total motor activity counts and the mean duration of activity. The 6,886 mg/m^3 male group showed significant effects such as decreased duration of movement during the second 10-minute interval at week 8. At week 13, there was a decrease in duration of movement and mean number of movements over the third and fourth interval, and there was an overall decrease in mean duration and mean number of movements. However, these effects were not dose-related.

Of the 12 rats used for functional observational battery and motor activity, 6 rats/sex of the high-concentration (24,101 mg/m^3) and control groups were randomly selected for perfusion and neuropathology. Brain and spinal cord sections were processed by routine neuropathological techniques for paraffin embedment. Sections of sciatic and tibial nerves, dorsal and ventral roots, and dorsal root ganglia were processed, embedded, and stained using standard procedures. Microscopic evaluation revealed no morphological differences from control rats. There were no compound-related microscopic observations in this study.

In summary, no statistically significant compound related effects on functional observational battery, motor activity, or neuropathology measures were found following exposure to any concentration (1,721, 6,886, and 24,101 mg/m^3) tested in the 90-day study of rats (DuPont HLR, 1996d; Malley et al., 2000).

4.4.3. Genotoxicity

In comparison with controls, cyclohexane did not alter the number of revertant colonies with or without exogenous metabolic activation in *Salmonella*/microsome tests using five strains of *Salmonella typhimurium* (HLA, 1982d; McCann et al., 1975; Mortelmans et al., 1986).

Cyclohexane also did not alter the number of benzo(a)pyrene-induced revertants in *S. typhimurium* strain TA100 (Maron et al., 1981). The incidence of forward mutations was increased in cultured L5178Y mouse lymphoma cells in the presence of exogenous metabolic activation, but the increase was not dose-related at exposure levels up to that which inhibited cell growth (HLA, 1982e). A second study showed no increase in mouse lymphoma cell forward mutations either in the presence or the absence of exogenous metabolic activation (Litton Bionetics, 1982). No increase in the number of sister chromatid exchanges either in the presence or the absence of exogenous metabolic activation was observed in cultured Chinese hamster ovary cells at exposure levels up to that which inhibit cell growth (HLA, 1982f). The rate of DNA synthesis in cultured human lymphocytes, as measured by [^{3}H]thymidine uptake, was not affected by cyclohexane either in the presence or the absence of exogenous metabolic activation (Perocco et al., 1983). Equivocal results were obtained in *E. coli* in the DNA cell binding assay (an assay of the binding of DNA to cellular protein mediated by active carcinogenic chemicals) (Kubinski et al., 1981). No significant increase in chromosome structural aberration frequency was observed in bone marrow cells of male or female rats exposed by inhalation for 5 consecutive days to levels of cyclohexane up to 1,000 ppm (Litton Bionetics, Inc., 1981).

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

Existing human studies are inadequate to determine the toxicity of cyclohexane in humans. There have been two small studies published recently in which workers were exposed primarily to cyclohexane. Although very few adverse health effects associated with cyclohexane exposure were reported, definitive conclusions cannot be drawn due to limitations of both studies. No chronic studies of toxicity of cyclohexane in animals were located.

In a well-conducted two-generation study of rats (DuPont HLR, 1997a; Kreckmann et al., 2000), cyclohexane exposure of dams was associated with low pup weights in both the F1 and F2 litters. Pup weights were significantly reduced from lactation days 7 through 25. Body weights in adult P1 and F1 females and F1 males were also significantly reduced. Rats in both the two-generation study (DuPont HLR, 1997a; Kreckmann et al., 2000) and a developmental

toxicity study (DuPont HLR, 1997b) exhibited maternal toxicity in the form of decreased body weight. On the other hand, rabbits in DuPont HLR (1997c) did not exhibit signs of maternal toxicity at the same doses (Kreckmann et al., 2000).

In the 90-day inhalation toxicity studies of rats and mice (DuPont HLR, 1996a, b; Malley et al., 2000), the animals exhibited liver changes, including increased relative liver weights, hepatocellular hypertrophy, and changes in liver enzyme profiles. The study authors argued that these liver changes were adaptive responses, associated only with the high dose and were not indicative of cyclohexane toxicity. However, not all of the changes were reversible in male rats. In the absence of longer-duration studies (6 months, 1 year, or lifetime), it cannot be concluded that these liver changes were not the first indications of systemic toxicity.

Observations indicating central nervous system effects have been noted in many of the studies. Acute studies in rats, mice, guinea pigs, rabbits, and cats exposed by inhalation showed trembling, sedation, and other neurological effects. In 90-day inhalation studies (Malley et al., 2000), diminished response to an alerting signal was observed in rats while in the exposure chambers (DuPont HLR, 1996b) while hyperactivity and diminished response to an alerting response were observed in mice (DuPont HLR, 1996a). However, in an acute study (DuPont HLR, 1996c; Christoph et al., 2000) and a 90-day study of neurotoxicity (DuPont HLR, 1996d; Malley et al., 2000) in adult rats, no statistically significant changes were detected. In the 90-day neurotoxicity study, as in other studies of that duration, clinical observations of a diminished response to an alerting stimulus were detected.

No proposed mechanisms of action were found to explain the observed toxic effects. Extrapolating from *in vitro* studies of membrane disruption by cyclohexane, it has been postulated that cyclohexane and other solvents may cause central nervous system effects via disruption of ion balances or membrane proteins in neurons (Naskali et al., 1993, 1994).

Studies at the cellular and subcellular level report effects of cyclohexane on membrane functions, enzyme kinetics, and metabolic regulation of the cell. In several studies, Naskali et al. (1993, 1994) and Tähti and Naskali (1992) demonstrated *in vitro* that cyclohexane disturbs ATPase-dependent astrocytic regulation of ion balance in the neuronal environment. Initial

studies using whole-brain reaggregate or granule cell cultures established that cyclohexane treatment produced concomitant changes in the neural membrane fluidity and integral enzyme activity. Subsequently, the activity of astrocytic membrane-bound ATPase was measured using isolated neural membranes from primary astrocyte cultures of neonatal Sprague-Dawley rats. Incubation with 3, 6, or 9 mM cyclohexane caused significant enzyme inhibition in a dose-dependent manner (up to 18% of control activity at 9 mM concentration). ATPase inhibition was greater than previously found in whole-brain or granule cell cultures. The level of inhibition caused by cyclohexane was similar to the inhibition caused by hexane but much smaller than that caused by other industrial solvents such as benzene or toluene. The authors suggested that astrocytes are very sensitive to cyclohexane and other solvents that affect the central nervous system.

In vitro, cyclohexane impaired the activity of phospholipid liposomes and reconstituted cytochrome c oxidase proteoliposomes from *Escherichia coli* (Sikkema et al., 1994). Partitioning of cyclohexane from the membrane surface to the bilayer center caused swelling and increased fluidity in a concentration-dependent manner, disrupting the proton motive force (the electrical potential) and the pH gradient across the membrane and increasing the permeability to ions and low molecular weight compounds. The cumulative result was inhibition of cytochrome c oxidase activity and dissipation of the energy-transducing properties of the membrane. The authors suggested that affecting the proton motive force across the membrane is a critical part of the effects of cyclohexane on membranes and membrane-embedded proteins. The results support the findings of Uribe et al. (1990) on intact yeast cells and isolated mitochondria (*Saccharomyces cerevisiae*), where cyclohexane disrupted the permeability barrier of the inner mitochondrial membrane.

4.6. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CHARACTERIZATION

Cancer studies in humans and inhalation or oral carcinogenicity assays using animals were not located. The genotoxicity studies that were performed using cyclohexane are generally negative. Under EPA's *Proposed Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1999),

the available information on cyclohexane would be evaluated as "Data are inadequate for an assessment of human carcinogenic potential."

4.7. SUSCEPTIBLE POPULATIONS

4.7.1. Possible Childhood Susceptibility

No specific data were located that address the relative sensitivity of children and adults to the toxic effects of cyclohexane. A two-generation inhalation reproductive study of rats (DuPont HLR, 1997a; Kreckmann et al., 2000) that examined the effect of cyclohexane exposure on animals exposed in utero through sexual maturity found decreased pup weights during the lactation period in both first- and second-generation offspring. Two well-conducted inhalation studies in rats (DuPont HLR, 1997b) and rabbits (DuPont HLR, 1997c) showed no evidence of developmental abnormalities in utero at doses that caused maternal toxicity (evidenced by reduced body weights) in rats (Kreckmann et al., 2000).

People of all ages are probably exposed to cyclohexane in the ambient air. In addition, nursing children may be exposed to cyclohexane in mothers' milk. In one very limited study, cyclohexane has been detected in 5 of 12 samples of human milk taken from urban areas across the United States, but no information about potential exposures of the women donating the samples is available (Pellizzari et al., 1982). There is also a lack of adequate data from animal studies to evaluate the potential toxic effects of oral exposure to cyclohexane.

Although no neurotoxicity or impaired response caused by the inhalation of cyclohexane was found in acute (DuPont HLR, 1996c; Christoph et al., 2000) and 90-day neurotoxicity tests of adult rats (DuPont HLR, 1996b; Malley et al., 2000), clinical observations of changed responses to an alerting signal were seen in adult rats and mice; therefore, developmental neurotoxicity is an area of concern for childhood susceptibility where a data gap exists.

4.7.2. Possible Gender Differences

The limited occupational studies available provide no data to suggest that gender differences in toxicity might occur as a result of exposure to cyclohexane. In animal studies, some gender differences were noted. In 90-day studies, both female and male mice exhibited significantly increased relative liver weights (DuPont HLR, 1996a; Malley et al., 2000). Although both male and female rats exhibited hepatocellular hypertrophy, only male rats exhibited significantly increased relative liver weights (DuPont HLR, 1996d; Malley et al., 2000).

Females in general or pregnant females in particular may be more susceptible than males to decreased body weights after inhalation exposure to cyclohexane. A two-generation inhalation reproductive study of rats (DuPont HLR, 1997a; Kreckmann et al., 2000) examined the effect of cyclohexane exposure and found significantly decreased adult body weights in first- and second-generation treated females (P1 and F1) but only in F1 adult males. The deficit in the females' body weight occurred in the premating phase of the study, and body weight gain was not significantly different than controls' during gestation or lactation. In prenatal developmental toxicity studies, inhalation exposure also produced decreased body weight in rat dams but no similar responses in rabbits (DuPont HLR, 1997b, c; Kreckmann et al., 2000).

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

No adequate studies for the derivation of an RfD were located. Available information was inadequate for a route-to-route extrapolation from the inhalation pathway to the oral pathway.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

5.2.1. Choice of Principal Study and Critical Effect—with Rationale and Justification

There are no adequate human studies and no chronic or lifetime animal studies available to determine the RfC. The two-generation reproduction inhalation toxicity study of rats (DuPont HLR, 1997a; Kreckmann et al., 2000) was chosen as the principal study. In this study, one set of litters was produced in each generation. Cyclohexane exposure of dams was associated with low pup weights—the chosen critical effect—in the F1 and F2 litters. Pup weights were significantly reduced from lactation days 7 through 25 (Tables 5-1 and 5-2), and this was chosen as the critical effect. Significant decreases in body weight in young animals can be associated with developmental delays and lifelong mental and physical deficiencies (U.S. EPA, 1991; Hood, 1996).

Table 5-1. Mean pup weights (G)—F1 generation

Maternal Group Concentration (mg/m^3)	0	1,721	6,886	24,101
Day 0	6.7	6.7	6.7	6.6
Day 4 preculling	11.0	11.0	11.2	10.6
Day 4 postculling	11.0	11.0	11.3	10.6
Day 7	16.2	16.2	16.3	15.1 [a]
Day 14	30.0	29.9	29.7	26.5 [a]
Day 21	48.5	48.5	48.3	43.1 [a]
Day 25	67.5	67.8	68.3	62.2 [a]

[a] Statistically significant difference from control ($p \leq 0.05$).
Source: Adapted from DuPont HLR, 1997a.

Table 5-2. Mean pup weights (G)–F2 generation

Maternal Group Concentration (mg/m^3)	0	1,721	6,886	24,101
Day 0	6.4	6.6	6.3	6.3
Day 4 preculling	10.8	10.8	10.1	10.2
Day 4 postculling	10.9	10.8	10.1	10.1
Day 7	16.3	16.0	15.3	14.3 [a]
Day 14	31.0	30.2	28.9	26.2 [a]
Day 21	50.0	48.3	46.4	42.8 [a]
Day 25	69.3	67.1	65.6	61.3 [a]

[a] Statistically significant difference from control ($p \leq 0.05$).
Source: Adapted from DuPont HLR, 1997a.

Adult P1 and F1 females and F1 males also had significantly reduced body weights. In adults, however, reduced body weights were less severe (generally less than 10% difference from controls) and in some cases appeared reversible, as affected females increased body weight gains over controls in later stages of the study (DuPont HLR, 1997a; Kreckmann et al., 2000).

The two-generation and developmental toxicity studies of rats exhibited maternal toxicity in the form of decreased body weight, and decreased body weight gain in the developmental study (DuPont HLR, 1997a, b; Kreckmann et al., 2000).

Other effects noted in the principal study (decreased body weights in adult males and females) and in the developmental study of rats (decreased body weights and body weight gains in dams) and the 90-day rodent inhalation exposure studies (increased relative liver weights, hepatocellular hypertrophy, and changes in liver enzymes) were not associated with pathological changes in the liver. The clinical observation of diminished response to a sound stimuli while in the exposure chamber, noted in most 90-day animal studies as the most sensitive endpoint, added information to the qualitative assessment of the toxicity of cyclohexane but does not provide data of the quality necessary for the quantitative estimation of an RfC (U.S.EPA, 1994b). These are subjective observations (the observers know which treatment group they are observing), and are made on a per group basis rather than an individual test animal basis (only a few animals in the exposure chamber are visible when the chamber is hit with the rod to produce an alerting stimulus) (Malley et al., 2000).

Guidance for extra-respiratory effects of category 3 gases (U.S. EPA, 1994b) was used for the cyclohexane assessment. Category 3 gases typically induce extra-respiratory effects because they are considered nonreactive (i.e., they have a low propensity for dissociation or metabolism to reactive forms) in the respiratory tract and have relatively low water solubility, which would promote rapid partitioning into the bloodstream and transport away from respiratory tissues. Cyclohexane is classified as a category 3 gas for this assessment on the basis of toxicological data from repeated exposure studies. The studies indicate that cyclohexane is not appreciably reactive in biological tissues since no histological effects were observed in either upper or lower respiratory tissues in animals at exposure concentrations that caused systemic effects (DuPont HLR, 1997a, b; Kreckmann et al., 2000; Malley et al., 2000). Cyclohexane is considered to be insoluble in water, and it is absorbed readily into the bloodstream from inhalation exposures.

5.2.2. Methods of Analysis—Benchmark Concentration Analysis

Adjusted exposure concentrations were calculated from all experimental exposure concentrations. Prior to adjustment, all concentrations were converted from ppm to mg/m^3 assuming cyclohexane acted as an ideal gas at 25°C and 760 mm Hg pressure, using the following the example equation (U.S. EPA, 1994b):

$$BMCL\ (mg/m^3) = BMCL\ (ppm) \times MW\ (g/mole)/\ 24.45\ (L/g/mole)$$

$$BMCL\ (mg/m^3) = BMCL\ (ppm) \times 84.2/24.45$$

$$BMCL\ (mg/m^3) = BMCL\ (ppm) \times 3.443$$

Exposure concentrations for developmental and reproductive toxicity repeated-exposure studies were duration-adjusted to provide estimated equivalent continuous exposure levels (U.S. EPA, 1994b). Dams were exposed to cyclohexane in chambers for 6 hours/day, 7 days/week (DuPont HLR, 1997a, b; Kreckmann et al., 2000). Therefore, experiment values were multiplied by a factor of 6/24 (or 1/ 4) to estimate 24-hour equivalent continuous exposure levels.

For extra-respiratory effects of category 3 chemicals, the guidance indicates that human equivalent concentration (HEC) values are obtained from each adjusted exposure concentration following the example equation (U.S. EPA, 1994b):

$$BMCL_{[HEC]}\ (mg/m^3) = BMCL_{[ADJ]}\ (mg/m^3) \times (H_{b/g})_A/(H_{b/g})_H,$$

where

$BMCL_{[HEC]}$ = the BMCL (or other exposure concentration) expressed in mg/m^3, dosimetrically adjusted for differences between humans and animals in absorptivity of cyclohexane into blood;

$BMCL_{[ADJ]}$ = the BMCL (or other exposure concentration) expressed in mg/m^3, adjusted for exposure schedule to estimate equivalent continuous exposure concentration if appropriate; and

$(H_{b/g})_A/(H_{b/g})_H$ = the ratio of blood/gas partition coefficients of cyclohexane for the animal value to human value.

U.S. EPA (1994b) guidance indicates that the default value of the $(H_{b/g})_A/(H_{b/g})_H$ ratio should be set equal to 1 if the blood:air partition coefficient data are not available for either humans or animals or if the value is greater than 1. Only one animal blood:air partition coefficient was located (rat heparinized blood 1.39 ± 0.09 [Gargas et al., 1989]). Two averaged human values were located in the literature (human heparinized blood 1.41 ± 0.14, Gargas et al., 1989 and 1.3 ± 0.1, Perbellini et al., 1985). Although the ratio of the rat value to the averaged human values would be marginally greater than 1, a calculated value is not included since the available animal and human values cannot be distinguished statistically. Therefore, the default value of 1 was used and HEC values for cyclohexane were set equal to the duration adjusted exposure concentrations expressed in mg/m^3.

Reduced F2 pup weight gain during lactation from days 7 to 25 was modeled as the critical effect (DuPont HLR, 1997a; Kreckmann et al., 2000). All of the endpoints examined for the basis of RfC calculation were weight related. Weight-related endpoints are attractive for benchmark concentration (BMC) analyses because observations are not typically omitted by design on any animals (contrary to, say, histopathology; even pups are ordinarily weighed prior to any examination). Consequently, a model can be established throughout the range of dose response. Because such a model gives a point of departure that is not obliged to coincide with an experimental dose and is sensitive to the numbers of animals in a study, BMC analysis was chosen as the basis for RfC calculation. Presuming this model to be parallel to human response is implicit in using a BMC.

Because weight-related endpoints are continuous, however, decisions must be made regarding the magnitude of change that will be associated with the BMC. None of the pup weight endpoints for the multigeneration study has a typical magnitude identified as a level of change for benchmark consideration. Consequently, following the recommendations of U.S.

EPA's *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000c), change was measured in units of standard deviations from the control mean. If, for example, the bottom 1–2% of the weight gain distribution among controls is regarded as a plausible magnitude of change (whatever that weight gain is), then a shift downward of the mean weight gain by an interval of one standard deviation from the control mean weight gain would shift about 10% of the population into that range. In all instances, the benchmark response (BMR) was taken as one standard deviation change from the control value. The BMC limit (BMCL) was taken as the lower limit of a one-sided 95% confidence interval on the BMC.

Pup weights were available at all three test doses and control. All data were modeled as their HEC values; the BMC and BMCL values are also in terms of their HECs. U.S. EPA's Benchmark Dose Software (BMDS) Version 1.30 was used to establish a model and estimate a BMC. Although individual pup data were available to help describe litter parameters, modeling was carried out on the basis of litter averages because the current version of BMDS only accommodates nested quantal data and the mean responses were used for benchmark comparisons. The empirical curve of the data was not monotonic increasing, and thus a limited number of continuous models was examined.

The model finally chosen was quadratic with constant variance for the F2 generation for pup weight gain from days 7 to 25. Details appear in the tables and figures of Appendix B (Tables B-1 through B-3, Figure B-1). This model yielded a $BMC_{(1sd)}$ of 5,250.05 mg/m^3 and a $BMCL_{(1sd)}$ of 1,822.48 mg/m^3 (the lowest $BMCL_{(1sd)}$ of the models for the F1 and F2 pup weight gain).

Maternal body weight gain was among the endpoints examined, and it was found to decrease in a statistically significant manner in the developmental study (DuPont HLR, 1997b; Kreckmann et al., 2000) as well as in the multigeneration reproduction study (DuPont HLR, 1997a; Kreckmann et al., 2000). Species, strain, and nominal administered doses were the same in the two studies, although the dosing regimen was not comparable. As with the pup weight endpoints for the multigeneration study, neither of the maternal weight endpoints considered for the developmental study (absolute body weight was also examined) has a typical magnitude

identified as a level of change for benchmark consideration. Consequently, the BMR was taken as one standard deviation change from the control mean.

Maternal weight gain from gestation days 7 to 17 and maternal body weight on gestation day 17 were modeled for the developmental study using benchmark dose analyses of HEC values for all test concentrations. The $BMC_{(1sd)}$ for the former, based on an unrestricted linear model with constant variance, is 3,153.34 mg/m^3 and its $BMCL_{(1sd)}$ is 2,500.82 mg/m^3. The latter had a $BMC_{(1sd)}$ of 6,654.66 mg/m^3 and a $BMCL_{(1sd)}$ of 4,437.79 mg/m^3, also based on an unrestricted constant variance linear model. These results are within the range of the values from the pup data of the reproductive toxicity study.

5.2.3. RfC Derivation—Including Application of Uncertainty Factors (UFs)

A factor of 3 (equivalent to approximately $10^{1/2}$) was applied to account for interspecies differences between humans and laboratory test animals. The factor for interspecies differences has two components: pharmacokinetics and pharmacodynamics. In this assessment the pharmacokinetic component was addressed by the calculation of the human equivalent concentration (HEC) according to the RfC methodology for a category 3 gas (U.S. EPA, 1994a, 1994b, 2002). Accordingly, only the pharmacodynamic area of uncertainty remains as a partial factor for interspecies uncertainty.

A factor of 10 was used to account for intraspecies variation among humans. Although the RfC is based on a sensitive lifestage (developing offspring), the uncertainty factor is appropriate because of the lack of any information on the range of responses in humans exposed to cyclohexane.

A factor of 10 was also applied to account for database deficiencies. There is a lack of long-term or chronic studies of animals in the data base available for deriving the RfC (U.S. EPA, 1994b). The subjective clinical observation of altered response to an alerting stimulus by adult mice and rats increases concern for developmental neurotoxicity, although specific neurotoxicity testing of adult rats did not reveal significant changes (DuPont HLR, 1996a, b, c, d; Christoph et al., 2000; Malley et al., 2000). Similarly, the increased liver size detected in

mice and rats in 90-day studies (Malley et al., 2000), although not accompanied by pathological changes in necropsy, may be early indications of changes that might progress to frank liver toxicity with long-term exposure.

Consistent with EPA practice (U.S. EPA, 1991, 1996), an additional uncertainty factor was not used to account for the extrapolation from endpoints in less-than-chronic studies to chronic effects since developmental toxicity (reduced pup body weight during lactation) was used as the critical effect. The developmental period is recognized as a sensitive lifestage where exposure during critical developmental time windows may induce effects not caused by lifetime adult exposure.

The resulting RfC calculated with the HEC $BMCL_{(1sd)}$ of 1,822.48 mg/m^3 is 6 mg/m^3:

$$RfC = 1{,}822.48\ mg/m^3 / 300 = 6\ mg/m^3$$

5.3. CANCER ASSESSMENT

No adequate cancer or chronic studies were located. No data were located regarding the existence of an association between cancer and cyclohexane exposure in humans. The are no adequate animal studies of cancer or of chronic duration by any exposure route. The genotoxicity studies that have been performed are generally negative. Under EPA's draft cancer guidelines (U.S. EPA, 1999), data are inadequate for an assessment of human carcinogenic potential.

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

Humans are likely to be exposed to cyclohexane through inhalation due to its presence in gasoline vapors and crude oils as well as the use of purified cyclohexane in solvents, insecticides, and plasticizers.

Cyclohexane is rapidly absorbed into the bloodstream via the lungs, gastrointestinal tract, and skin. At higher inhalation doses, some cyclohexane is expired unchanged due to preferential partitioning to the alveoli rather than to blood, where it has low solubility. In human cadaver and animal *in vivo* studies, cyclohexane partitioned to the lipid-rich areas in the body, including fat, brain, and liver. Cyclohexane has been detected in 5 of 12 samples of human milk taken from across the United States, but no information about potential exposures of the women donating the samples is available. In studies of workers, cyclohexane was excreted primarily via expiration from the lungs and secondarily in urine.

Existing human occupational studies are inadequate to determine the toxicity of cyclohexane in humans. No chronic toxicity studies of cyclohexane in animals were located.

In a well-conducted two-generation study of rats, cyclohexane exposure of dams was associated with low pup weights during lactation in the F1 and F2 generations (DuPont HLR, 1997a; Kreckmann et al., 2000). This effect was chosen as the critical effect for calculating the RfC. There is concern that if there is increased susceptibility of young animals, developmental neurotoxicity could occur, but no testing was located.

The two-generation (DuPont HLR, 1997a) and developmental (DuPont HLR, 1997b) toxicity studies of rats both demonstrated maternal toxicity in the form of decreased body weight (Kreckmann et al., 2000). Although only statistically significant at the highest dose tested, mid-dose dams contributed to the downward trend in body weight used in the benchmark dose analyses.

In the 90-day inhalation toxicity studies (Malley et al., 2000) of rats (DuPont HLR, 1996b) and mice (DuPont HLR, 1996a), animals exhibited liver changes including increased relative liver weights, hepatocellular hypertrophy, and changes in liver enzyme profiles. The authors stated that the changes were reversible adaptive responses associated only with the high dose and not indicative of cyclohexane toxicity (Malley et al., 2000). However, not all of the changes were reversible in male rats. In the absence of longer-duration studies (6 months, 1 year, or lifetime), it cannot be concluded that these liver changes are not first indications of potential liver toxicity that would become apparent with longer exposure periods.

Clinical observations of an altered response to an alerting stimulus were noted in many of the subchronic studies. In the 90-day rat study, authors (DuPont HLR, 1996b; Malley et al., 2000) report transient sedation with diminished response to an alerting signal was observed while the rats were in the exposure chamber. In the 90-day study of mice, both hyperactivity and transient sedation were observed. However, these are subjective observations (the observers know which treatment group they are observing), and are made on a per group basis not an individual test animal basis (only a few animals in the exposure chamber are visible when the chamber is hit with the rod to produce an alerting stimulus). Therefore while helpful in the qualitative characterization of the toxicity of cyclohexane, the observations do not yield data of adequate quality for use in quantitative assessment. Furthermore, in both acute (DuPont HLR, 1996c; Christoph et al., 2000) and 90-day (DuPont HLR, 1996d; Malley et al., 2000) neurotoxicity studies in adult rats, no effects beyond the clinical observations of diminished response to an alerting stimulus were detected.

6.2. DOSE-RESPONSE

The RfC of 6 mg/m^3 was derived by dividing the HEC $BMCL_{(1sd)}$ of 1822.48 mg/m^3 by the product of uncertainty factors of 300, as described in Section 5.2. The benchmark dose is the preferred approach because it incorporates data for all exposure concentrations tested instead of only the NOAEL. Other effects noted in the principal study (decreased body weights in adult males and females), the developmental study of rats (decreased body weights and body weight gains in dams), and the 90-day rodent inhalation exposure studies (increased liver weights,

hepatocellular hypertrophy, and changes in liver enzymes) may reflect transient changes and appear less severe than the reduced pup weight during lactation.

Confidence in the principal inhalation study (DuPont HLR, 1997a; Kreckmann et al., 2000) is high because it uses an adequate number of study animals and exposure levels to evaluate an adequate set of endpoints. Confidence in the remainder of the inhalation toxicity data base is low to moderate because although it is comprised of a number of well-designed 90-day toxicity, neurotoxicity, and developmental toxicity animal bioassays, no data were available for long-term or lifetime exposures or for developmental neurotoxicity. The database included some evidence suggestive of neurological effects in occupationally-exposed humans, but these subjects were exposed to mixtures of chemicals, including those more clearly demonstrated to have such effects (*n*-hexane and toluene). Adult rats and mice exhibited altered responses to an alerting stimulus at the mid-level and high doses tested in subchronic studies, indicating the possibility of neurotoxicity. However, the observations were subjective (the observers knew what dose group they were watching), the observations were not on an individual animal basis, and no significant effects were detected in the neurotoxicity test battery conducted on adult rats. Therefore, confidence in the RfC is low to moderate, reflecting primarily the lack of chronic duration exposure and a lack of developmental neurotoxicity testing.

7. REFERENCES

Agnesi, R; Valentiri, F; Mastrangelo, G. (1997) Risk of spontaneous abortion and maternal exposure to organic solvents in the shoe industry. Int Arch Occup Environ Health 69(5):311–316.

Atkinson, R, ed. (1989) Journal of physical chemistry reference data monograph 1. American Chemical Society, New York, NY.

Battershill, JM; Illing, HPA; Shillaker, RO; et al. (1987) n-Hexane. Health and Safety Exec Toxicity Rev 18. London:Her Majesty's Stationery Office (HMSO).

Bidleman, TF. (1988) Atmospheric processes. Environ Sci Technol 22:361–367.

Blake, NJ; Penkett, SA; Clemitshaw, KC; et al. (1993) Estimates of atmospheric hydroxyl radical concentrations from the observed decay of many reactive hydrocarbons in well-defined urban plumes. J Geophys Res 98:2851–2864.

Bocek, K. (1976) Relations among activity coefficients, partition coefficients and solubilities. Experientia Suppl 23:231–40.

Brugnone, F; Perbellini, L; Gaffuri, E; et al. (1980) Biomonitoring of industrial solvent exposures in workers' alveolar air. Arch Occup Environ Health 47(3):245–61.

Chao, J; Lin, CT; Chung, TH. (1983) Vapor pressure of coal chemicals. J Phys Chem 12:1033–1063.

Christoph, GR; Kelly, DP; Krivanek, N. (2000) Acute inhalation exposure to cyclohexane. And schedule-controlled operant performance in rats: comparison to d-amphetamine and chlorpromazine. Drug Chem Toxicol 23(4):539-53.

DuPont HLR. (1996a) 90-Day inhalation toxicity study with cyclohexane in mice, with cover letter dated 8/16/96. Submitted by Chemical Manufacturers Association, Cyclohexane Panel; E.I. du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine to U.S. EPA under TSCA Section 4. U.S. EPA Document No. 44631. Fiche No. OTS0558870.

DuPont HLR. (1996b) 90-Day inhalation toxicity study with cyclohexane in rats, with cover letter dated 11/18/96. Submitted by Chemical Manufacturers Association, Cyclohexane Panel; E.I. du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine to U.S. EPA under TSCA. Section 4. U.S. EPA Document No. 44634. Fiche No. OTS0558873.

DuPont HLR. (1996c) Acute operant behavior study of cyclohexane by inhalation in rats, final report, with cover letter dated 2/16/96. Submitted by Chemical Manufacturers Association, Cyclohexane Panel; E.I. du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine to U.S. EPA under TSCA Section 4. U.S. EPA Document No. 44622. Fiche No. OTS0558850.

DuPont HLR. (1996d) 90-Day inhalation neurotoxicity study with cyclohexane in rats, with cover letter dated 8/16/96. Submitted by Chemical Manufacturers Association, Cyclohexane Panel; E.I. du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine to U.S. EPA under TSCA Section 4. U.S. EPA Document No. 44631. Fiche No. OTS0558869.

DuPont HLR. (1997a) Reproductive and fertility effects with cyclohexane inhalation multigeneration reproduction study in rats, with cover letter dated 4/18/97. Submitted by Chemical Manufacturers Association, Cyclohexane Panel; E.I. du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine to U.S. EPA under TSCA Section 4. U.S. EPA Document No. 44640. Fiche No. OTS0558881.

DuPont HLR. (1997b) Inhalation developmental toxicity study of cyclohexane in rats, with cover letter dated 1/17/97. Submitted by Chemical Manufacturers Association, Cyclohexane Panel; E.I. du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine. Submitted to U.S. EPA under TSCA Section 4. U.S. EPA Document Number 44637. Fiche No. OTS0558877.

DuPont HLR. (1997c) Inhalation developmental toxicity study of cyclohexane in rabbits, with cover letter dated 6/17/97. Submitted by Chemical Manufacturers Association, Cyclohexane Panel; E.I. du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine. Submitted to U.S. EPA under TSCA Section 4. U.S. EPA Document No. 44641. Fiche No. OTS0558883.

Eastman, AD; Mears, DE. (1995) Hydrocarbons (C_1-C_6). In: Kirk-Othmer encyclopedia of chemical technology, vol. 13. 4th ed. New York: John Wiley and Sons; pp. 812–837.

Elliott, TH; Parke, DV; Williams, RT. (1959) Studies in detoxification: 79. the metabolism of cyclo-^{14} C-hexane and its derivatives. Biochem J 72:193–200.

Flury, F; Zernik, F. (1931) Schadliche Gase. Springer, Berlin. As cited in Fassett, DW; Irish, DD, eds. Patty's industrial hygiene and toxicology, Vol. II. New York: Interscience Publishers; pp. 1208–1211.

Gargas, ML; Burgess, RJ; Voisard, DE; et al. (1989) Partition coefficients of low-MV volatile chemicals in various liquids and tissues. Toxicol Appl Pharmacol 98:87–89.

Ghittori, S; Imbriani, M; Pezzagno, G; et al. (1987) The urinary concentration of solvents as a biological indicator of exposure: proposal for the biological equivalent exposure limit for nine solvents. Am Ind Hyg Assoc J 48(9):786–790.

Governa, M; Calisti, R; Coppa, G; et al. (1987) Urinary excretion of 2,5-hexanendione and peripheral polyneuropathies in workers exposed to hexane. J Toxicol Environ Health 20(3):219–228.

Graedel, TE. (1978) Chemical compounds in the atmosphere. New York: Academic Press; p. 99.

Hansch, C; Leo, A; Hoekman, D, eds. (1995) Exploring QSAR: hydrophobic, electronic, and steric constants. Washington, DC: American Chemical Society; p. 23.

HLA (Hazleton Laboratories America, Inc.). (1982a) Acute oral toxicity study in rats: cyclohexane. Final report. Submitted by Phillips Petroleum Co. to U.S. EPA under TSCA Section 4. U.S. EPA Document No. 40-8623065. Fiche No. OTS0527456.

HLA. (1982b) Acute inhalation toxicity test: cyclohexane. Final report. Submitted by Phillips Petroleum Co. to U.S. EPA under TSCA Section 4. U.S. EPA Document No. 40-8623065. Fiche No. OTS0527456.

HLA. (1982c) Respiratory tract irritancy study in mice: cyclohexane. Final report. Submitted by Phillips Petroleum Co. to U.S. EPA under TSCA Section 4. U.S. EPA Document No. 40-8623065. Fiche No. OTS0527456.

HLA. (1982d) *Salmonella typhimurium* mammalian microsome plate incorporation assay: cyclohexane. Final report. Submitted by Phillips Petroleum Co. to U.S. EPA under TSCA Section 4. U.S. EPA Document No. 40-8623065. Fiche No. OTS0527456.

HLA. (1982e) Mouse lymphoma forward mutation assay: cyclohexane. Final report. Submitted by Phillips Petroleum Co. to U.S. EPA under TSCA Section 4. U.S. EPA Document No. 40-8623065. Fiche No. OTS0527456.

HLA. (1982f) *In vitro* sister chromatid exchange in Chinese hamster ovary cells. Final report. Submitted by Phillips Petroleum Co. to U.S. EPA under TSCA Section 4. U.S. EPA Document No. 40-8623065. Fiche No. OTS0527456.

Hood, RD, ed. (1996) Handbook of developmental toxicology. New York: CRC Press.

Iyadomi, M; Higaki, Y; Ichiba, M; et al. (1998) Evaluation of organic solvent-induced inflammation modulated by neuropeptides in the abdominal skin of hairless rats. Indust Health 36(1):40–51.

Kavaler, AR, ed. (1998) Chemical marketing reporter. New York: Schnell Publishing; June 8. p. 53.

Kimura, ET; Ebert, DM; Dodge, PW. (1971) Acute toxicity and limits of solvent residue for sixteen organic solvents. Toxicol Appl Pharmacol 19:699–704.

Klaassen, CD; Amdur, MO; Doull, J. (1986) Casarett and Doull's toxicology. New York: Macmillan.

Kreckmann, KH; Baldwin, JK; Roberts,LG; et al. (2000) Inhalation developmental toxicity and reproduction studies with cyclohexane. Drug Chem Toxicol 23(4):555-73.

Kubinski, H; Gutzke, GE; Kubinski, ZO. (1981) DNA-cell-binding (DCB) assay for suspected carcinogens and mutagens. Mutat Res 89:95–136.

Lee, DH; Park, IG; Kim, JH; Lee, YH; Kim, D; Kang, S-K. (1998) Neurobehavioral changes in shoe manufacturing workers. Neurotoxicol Teratol 20(3):259–263.

Litton Bionetics, Inc. (1981) Mutagenicity evaluation of certified cyclohexane in the rat bone marrow cytogenetic assay. Draft report. Submitted by American Petroleum Institute to U.S. EPA under TSCA Section FYI. U.S. EPA Document No. FYI-AX-1081-0142. Fiche No. OTS0000142.

Litton Bionetics, Inc. (1982) Mutagenicity evaluation of certified cyclohexane in the mouse lymphoma forward mutation assay. Final report. Submitted by American Petroleum Institute to U.S. EPA under TSCA Section FYI. U.S. EPA Document No. FYI-AX-1081-0142. Fiche No. OTS0000142.

Malley, LA; Bamberger, JR; Stadler, JC; et al. (2000) Subchronic toxicity of cyclohexane in rats and mice by inhalation exposure. Drug Chem Toxicol. 23 (4):539-53.

Maron, D; Katzenellenbogen, J; Ames, BN. (1981) Compatibility of organic solvents with the Salmonella/microsome test. Mutat Res 88:343–350.

McCann, J; Choi, E; Yamasaki, E; Ames, BN. (1975) Detection of carcinogens as mutagens in the Salmonella/microsome test: assay of 300 chemicals. Proc Natl Acad Sci 72: 5135–5139.

The Merck Index (1996), 12th edition; Budavari, S, ed.; Whitehouse Station, NJ: Merck & Co., Inc.

Meylan, WM; Howard, PH. (1991) Bond contribution method for estimating Henry's Law constants. Environ Toxicol Chem 10:1283–1293.

Meylan, WM; Howard, PH; Boethling, RS. (1992) Molecular topology/fragment contribution method for predicting soil sorption coefficients. Environ Sci Technol 26:1560–1567.

Mortelmans, K; Haworth, S; Lawlor, T; et al. (1986) Salmonella mutagenicity tests: II. results from testing of 270 chemicals. Environ Mutagen 8(Suppl 7):1–119.

Mraz, J; Galova, E; Nohava, H; Vitkova, D. (1994) Markers of exposure to cyclohexanone, cyclohexane, and cyclohexanol: 1,2 and 1,4-cyclohexanediol. Clin Chem 40(7):1466–1468.

Mutti, A; Falzoi, M; Lucertini, S; et al. (1981) Absorption and alveolar excretion of cyclohexane in workers in a shoe factory. J Appl Toxicol 1(4):220–223.

Naruse, M. (1984) Effects on mice of long-term exposure to organic solvents in adhesives. Nagoya Med J 28:183–210.

Naskali, L; Engelke, M; Tahti, H; et al. (1993) The effects of selected organic solvents on rat synaptosomal membrane fluidity and integral enzyme activities. Neurosci Res Commun 13(1):27–35.

Naskali, L; Oksanen, H; Tahti, H. (1994) Astrocytes as targets for CNS effects of organic solvents *in vitro*. Neurotoxicol 15(3):609–612.

National Research Council. (1983) Risk assessment in the federal government: managing the process. Washington, DC: National Academy Press.

NIOSH (National Institute for Occupational Safety and Health). (1997) Pocket guide to chemical hazards. U.S. Department of Health and Human Services, Washington, DC.

NIOSH. (2000) Registry of Toxic Effects of Chemical Substances. Cincinnati, OH.

Pellizzari, ED; Hartwell, TD; Harris, BSH; et al. (1982) Purgeable organic compounds in mother's milk. Bull Environ Contam Toxicol 28:322–328.

Perbellini, L; Brugnone, F. (1980) Lung uptake and metabolism of cyclohexane in shoe factory workers. Int Arch Occup Environ Health 45(3):261–269.

Perbellini, L; Brugnone, F; Pavan, I. (1980) Identification of the metabolites of n-hexane, cyclohexane, and their isomers in men's urine. Toxicol Appl Pharmacol 53(2):220–229.

Perbellini, L; Brugnone, F; Caretta, D; Maranelli, G. (1985) Partition coefficients of some industrial aliphatic hydrocarbons 5 carbon 7 carbon in blood and human tissues. Br J Ind Med 43(3):162–167.

Perbellini, L; Brugnone, F; Gaffuri, E. (1987) Urinary metabolite excretion in the exposure to technical hexane: biological monitoring of exposure to chemicals. In: Ho, MH; Dillon, HK, eds. Organic compounds. New York: John Wiley and Sons; pp. 197–205.

Perocco, P; Bolognesi, S; Alberghini, W. (1983) Toxic activity of seventeen industrial solvents and halogenated compounds on human lymphocytes cultured *in vitro*. Toxicol Lett 16:69–75.

RTI (Research Triangle Institute). (1984) Adsorption, distribution, metabolism and excretion of cyclohexane. Submitted under contract to National Institute of Environmental Health Sciences. U.S. EPA/OTS. Document No. 40-8423127. Research Triangle Park, NC.

Savolainen, H; Pfaffli, P. (1980) Burden and dose-related neurochemical effects of intermittent cyclohexane vapour inhalation in rats. Toxicol Lett 7(1):17–22.

Sikkema, J; De Bont, JAM; Poolman, B. (1994) Interactions of cyclic hydrocarbons with biological membranes. J Biol Chem 269(11):8022–8028.

Tähti, H; Naskali, L. (1992) The effects of organic solvents on neural membrane integral protein tested in neural cell cultures. Neurosci Res Commun 10(2):71–77.

Treon, JF; Crutchfield, WE; Kitzmiller, KV. (1943a) The physiological response of rabbits to cyclohexane, methylcyclohexane, and certain derivatives of these compounds: I. oral administration and cutaneous application. J Ind Hyg Toxicol 25:199–214.

Treon, JF; Crutchfield, WE; Kitzmiller, KV. (1943b) The physiological response of animals to cyclohexane, methylcyclohexane, and certain derivatives of these compounds. J Ind Hyg Toxicol 25:323–347.

Uribe, S; Rangel, P; Espainola, G; Aguirre, G. (1990) Effects of cyclohexane, an industrial solvent, on the yeast *Saccharomyces cerevisiae* and on isolated yeast mitochondria. Appl Environ Microbiol 56(7):2114–2119.

U.S. EPA (Environmental Protection Agency). (1986a) Guidelines for the health risk assessment of chemical mixtures. Federal Register 51(185):34014–34025.

U.S. EPA. (1986b) Guidelines for mutagenicity risk assessment. Federal Register 51(185):34006–34012.

U.S. EPA. (1988) Recommendations for and documentation of biological values for use in risk assessment. EPA 600/6-87/008, NTIS PB88-179874/AS, February 1988. Available from National Technical Information Service, Springfield, VA.

U.S. EPA. (1991) Guidelines for developmental toxicity risk assessment. Federal Register 56(234):63798–63826.

U.S. EPA. (1994a) Interim policy for particle size and limit concentration issues in inhalation toxicity: notice of availability. Federal Register 59(206): 53799.

U.S. EPA. (1994b) Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry. EPA/600/8-90/066F.

U.S. EPA. (1995) Use of the benchmark dose approach in health risk assessment. EPA/630/R-94/007.

U.S. EPA. (1996) Guidelines for reproductive toxicity risk assessment. Federal Register 61(212):56274–56322.

U.S. EPA. (1998a) Guidelines for neurotoxicity risk assessment. Federal Register 63(93):26926–26954.

U.S. EPA. (1998b) Science policy council handbook: peer review. Prepared by the Office of Science Policy, Office of Research and Development, Washington, DC. EPA 100-B-98-001.

U.S. EPA. (1999) Guidelines for carcinogen risk assessment [review draft]. NCEA-F-0644, July. Risk Assessment Forum, Washington, DC.

U.S. EPA. (2000a) Science policy council handbook: peer review. Second edition. Prepared by the Office of Science Policy, Office of Research and Development, Washington, DC. EPA 100-B-00-001.

U.S. EPA. (2000b) Science policy council handbook: risk characterization. Prepared by the Office of Science Policy, Office of Research and Development, Washington, DC. EPA 100-B-00-002.

U.S. EPA. (2000c) Benchmark dose technical guidance document. External review draft, EPA/630/R-00/001, October, Office of Research and Development, Risk Assessment Forum, Washington, DC.

U.S. EPA. (2000d) Supplementary guidance for conducting health risk assessment of chemical mixtures. Office of Research and Development, Risk Assessment Forum, Washington, DC. EPA/630/R-00/002.

U.S. EPA. (2002) A review of the reference dose and reference concentration processes. December 2002. U.S. Environmental Protection Agency, Risk Assessment Forum, Washington, DC. EPA /630/P-02/002F

Verschueren, K, ed. (1996) Handbook of environmental data on organic chemicals. 3rd ed. New York: Van Nostrand Reinhold; p. 565–567.

Yasugi, T; Kawai, T; Mizunuma, K; et al. (1994) Exposure monitoring and health effect studies of workers occupationally exposed to cyclohexane vapor. Int Arch Occup Environ Health 65(5):343–350.

Yuasa, J; Kishi, R; Eguchi, T; et al. (1996) Concentrations of trichloroethylene and its metabolites in blood and urine after acute poisoning by ingestion. Occup Environ Med 53(3):174–179.

Zahlsen, K; Eide, I; Nilsen, AM; Nilsen, OG. (1992) Inhalation kinetics of C6 to C10 aliphatic, aromatic and naphthenic hydrocarbons in rat after repeated exposures. Pharmacol Toxicol 71(2):144–149.

APPENDIX A: SUMMARY OF EXTERNAL PEER REVIEW COMMENTS AND DISPOSITION

APPENDIX A: SUMMARY OF EXTERNAL PEER REVIEW COMMENTS AND DISPOSITION

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

(1) General Comments

The three external reviewers offered editorial comments, all of which have been incorporated into the text when feasible. Substantive scientific comments are addressed below.

A. Comment: Are additional data/studies recommended for inclusion?

One reviewer recommended an additional study, based on the abstract by Gupta, KP; Mehrotra, NK. (1990) Mouse skin ornithine decarboxylase induction and tumor promotion by cyclohexane. Cancer Lett 51:227–233.

Response: The study was retrieved and reviewed. Although the reviewer thought the study detailed evidence that cyclohexane might have tumor promoting capabilities the study concentrates on the induction of an enzyme associated with skin irritation (ornithine decarboxylase) and only secondarily measures skin tumor promotion activity of cyclohexane when applied after known chemical initiators and promoters. The cancer-promotion activity of cyclohexane on mouse skin is not clearly demonstrated by this study. It appears that the continual irritation caused by cyclohexane could cause the observed increase in tumors when applied three times a week for months to shaved albino mouse skin, but this is not discussed by the study authors. The mouse skin could have been dry and cracked, and skin wounding has long been known to promote skin cancer, but no mention of the skin condition is made. Furthermore, it appears there was no statistical analysis of the tumor data. The methodology also appears deficient because there is no indication of randomization of the study animals or

coding of skin samples ("blind" reading). The tumor classification, if conducted, was not presented. There is a general statement that most tumors were benign. Although some control groups were used, the most appropriate control of parallel treatment (initiator, promoter, and acetone treatment in place of cyclohexane) was not used. Because of the shortcomings outlined, the study does not add to the understanding of potential systemic carcinogenicity of cyclohexane and was not added to the IRIS summary or supporting documentation.

B. Comment: For the RfD and RfC, has the most appropriate critical effect been chosen (i.e., that adverse effect appearing in a dose-response continuum)?

All reviewers agreed with EPA's selection of reduced pup weight as the critical effect for the RfC. One reviewer was concerned about the concept of liver changes as adaptive. The reviewer also stated concern that the central nervous system effects - as the most sensitive, although transient - might indicate a potential risk to humans. The reviewer believes changes occur but the lack of sensitivity of the currently available neurotoxicological testing methods makes these changes undetectable.

Response: The language in the toxicological review and IRIS summary has been clarified to convey that insufficient data are available to negate the potential liver toxicity and neurotoxicity associated with chronic exposures to cyclohexane.

C. Comment: Has the noncancer assessment been based on the most appropriate study? This study should present the critical effect in the clearest dose-response relationship. If not, what other study (or studies) should be chosen and why?

All reviewers agreed with the conclusions reached by EPA on the selection of the most appropriate study for the critical effect from the existing data base for cyclohexane. One reviewer listed all the studies in the toxicological review and all NOAELs and LOAELs from the studies and then stated that EPA's conclusion was "reasonable."

Response: EPA considers reduced pup weight during lactation an adverse developmental effect. When evaluating the critical effect for cyclohexane, EPA considered effects from all available subchronic and systemic toxicological studies of adequate quality. The reduced rat pup weight in the reproductive toxicity study and the dam weight seemed to be the most relevant toxic

endpoints in humans and illustrate some dose-response relationship. Reduced pup weight could lead to lifelong developmental delays and deficiencies, whereas in some cases, adult weight loss showed reversibility. As a result, no substantial changes are proposed to the current IRIS file as a consequence of these comments. Wording was changed in the IRIS documents to make it clearer that the potential for chronic liver effects or neurotoxicity in humans could not be ruled out by the available animal studies.

D. Comment: Are there other data that should be considered in developing the uncertainty factors (UFs) or the modifying factor? Do you consider that the data supports the use of different (default) values other than those proposed?

The reviewers agreed with the UFs applied by EPA. One reviewer stated that the UFs were reasonable to provide a "health-protective" RfC.

E. Comment: Do the confidence statements and the weight-of-evidence statements present a clear rationale and accurately reflect the utility of the studies chosen, the relevancy of the effects to humans, and the comprehensiveness of the data? Do these statements make sufficiently apparent all the underlying assumptions and limitations of these assessments? If not, what needs to be added?

Two reviewers agreed with the confidence statements and weight-of-evidence statements. One reviewer did not directly comment.

F. Comment: Do you agree with the methods of analysis and the benchmark dose (BMD) methodology/calculations that were used to evaluate dose-response data for the chosen critical effects?

All reviewers agreed with the BMD methodology applied in order to derive an RfC.

(2) Chemical-specific Comments

A number of effects suggestive of adverse changes to liver and central nervous system were observed in animals following subchronic repeated inhalation exposures at comparable or lower exposure levels than the pup weight effects (identified as the critical effects in the data base of

inhalation studies), but these effects did not show a clear, toxicologically relevant continuum of severity and/or marked progression of response with increasing dose nor were there any treatment-related corroborative gross pathologies or histopathological lesions. In the absence of data from chronic exposure studies, these effects may be considered either adaptive, minimal, or of uncertain relevance to effects in humans from chronic exposures.

A. Comment: Do you agree with that conclusion? Is sufficient rational given to support that conclusion?

One reviewer agreed with the conclusions made by EPA that effects such as increases in enzyme levels and hepatomegaly were possibly adaptive responses and not considered as critical events clearly leading to potential adverse effects. Two other reviewers were in agreement with EPA that the observed effects on the liver and central nervous system should not be used as the critical effects in the data base of subchronic inhalation studies but thought wording should be added that made it clear that data gaps such as the lack of chronic and developmental neurotoxicity studies left concern for these endpoints. In particular, one reviewer objected to the word "adaptive" that had been used by study authors and referred to as one possible explanation by EPA.

Response: All three reviewers essentially agreed with EPA's decision not to use data on the liver, adult body weights, or central nervous system as the basis for the critical effect because these effects, taken either individually or in combination, did not show a clear, toxicological continuum of severity and/or marked progression of response with increasing dose or any treatment-related corroborative gross pathologies or histopathological lesions; as a result, they were considered to be either "adaptive," minimal, or of uncertain relevance to effects in humans from chronic exposures. For example, central nervous system-related effects were reported in animal studies, but they occurred only while the animals were in the exposure chamber. In the absence of histopathologies in nervous system tissues and the absence of other neurological effects in the acute and 90-day neurotoxicity studies, the observed effects were considered to be primarily transient responses with uncertain relevance to chronic effects in humans from long-term exposure to cyclohexane. Similarly, effects that may be associated with changes in the liver were also observed in animals but were lacking in any evidence of histopathology (for further
discussion, see Section 4.5.2). In the Toxicological Review of Cyclohexane, realizing the lack

of sensitivity of available toxicological test methods, data gaps were outlined (chronic studies and developmental neurotoxicity), and a UF of 10 was applied for data base deficiencies. As a result, no major changes are proposed to the current IRIS file as a consequence of these comments. Some language was changed in the IRIS documents to clarify EPA's conclusions and distinguish them from the study authors' conclusions.

A reviewer noticed a possible effect in the rat developmental toxicity study that had not been noted in the study or the Toxicological Review. A paragraph detailing the reviewer's observation was added.

OVERALL RECOMMENDATION

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

APPENDIX B: BENCHMARK DOSE CALCULATIONS

APPENDIX B: BENCHMARK DOSE CALCULATIONS

U.S. EPA's Benchmark Dose Software (BMDS) Version 1.30 was used to establish a model and estimate a benchmark concentration. Reduced F2 pup weight gain from days 7 to 25 was modeled as the critical effect (DuPont HLR, 1997c). Pup weights were available at all three test doses and control. Although individual pup data were available to help describe litter parameters, modeling was carried out on the basis of litter averages (HEC) because the current version of BMDS only accommodates nested quantal data and the mean responses were used for benchmark comparisons. The empirical curve of the data was not monotonic increasing, and thus a limited number of continuous models was examined. Although, for instance, higher-order polynomial models or a Hill model could have been tried, their shapes would not have given plausible fits or been readily interpretable. All models were evaluated using likelihood ratio goodness-of-fit tests. Selection among models was assisted by examining their Akaike Information Criteria (AIC).

The models examined using the F1 generation pup weight gain data included the linear with constant and heterogeneous variance, the quadratic with heterogeneous variance, and the power model with heterogeneous variance. None had restrictions on the coefficients. All instances except the power model were considered to adequately describe the data ($p>0.05$ on "model fit" test). The AIC of the quadratic model was 427.54. Although the least AIC was shown by the power model, its BMC was about 1,000 points greater than that of either linear model, owing to its unusual shape (while the power was 9.9, the slope was essentially zero), and the BMC limit (BMCL) computation failed. Between the two linear models, one with heterogeneous variance was favored by the goodness-of-fit comparisons, but the constant had the lower (426.71 versus 428.44) AIC. Nonetheless, BMDS currently is limited in the choices of variance models it provides, and the model of the variance it could incorporate into the benchmark model was inadequate to the form of heterogeneity in the data. Additionally, computation of the BMCL curve failed to use the heterogeneous variance model, so the constant variance model was selected for this data set. This model yielded a $BMC_{(1sd)}$ of 5755.67 mg/m^3 and a $BMCL_{(1sd)}$ of 4,117.51 mg/m^3. Results are shown in Tables B-1 and B-2.

Modeling also was carried out using the pup weight gain from days 7 to 25 in the F2 generation. These data appeared strictly decreasing. Linear, quadratic, and cubic constant variance models were examined; the constant variance assumption was not rejected in model

fitting, but the cubic model was an overfit model. The $BMC_{(1sd)}$ from the linear model was 6,042.91 mg/m^3 and the $BMCL_{(1sd)}$ was 4,165.97 mg/m^3. The $BMC_{(1sd)}$ and $BMCL_{(1sd)}$ values appear similar to those of the F1 generation. The $BMC_{(1sd)}$ from the quadratic model was 5,250.05 mg/m^3 and the $BMCL_{(1sd)}$ was 1,822.48 mg/m^3. Results are shown in Tables B-1 and B-2.

Dam weights of the P1 and F1 and absolute pup weights of F1 and F2 generations were also modeled. The results appear in Tables B-1 and B-2.

Table B-1. Summary of model outcomes, reproductive toxicity/multigenerational study (DuPont HLR, 1997a)[a]

Form of model	Assumptions on variance[a]	AIC[h]	Model fit?[b]	$BMC^{c}_{(1\,sd)}$	BMCL[c]
P1 - day71bw[e]					
linear	const. var.	866.97	yes	7,556.88	5,079.5
F1 - avg d25pupw[f]					
linear	const. var.	488.55	het. var.	6,113.36	4,303.23[d]
linear	het. var.	492.30	yes	6,078.26	failed
quadratic	het. var.	489.53	yes	6,166.96	5,176.92
F1 - pw d25-d7[g]					
linear	const. var.	426.71	het. var.	5,755.67	4,117.51[d]
linear	het. var.	428.44	yes	5,682.17	4,274.39
quadratic	het. var.	427.54	yes	6,058.50	5,053.81
power	het. var.	425.50	poor	6,021.64	5,016.80
F2 - avg d25pupw					
linear	const. var.	437.14	yes	5,815.77	4,053.03[d]
quadratic	const. var.	438.67	yes	4,687.20	1,751.17
F2 - pw d25-d7					
linear	const. var.	391.09	yes	6,042.91	4,165.97
quadratic	const. var.	392.70	yes	5,250.05	1,822.48[d]
cubic	const. var.	394.04	overfit	5,943.87	1,943.68

[a] The first column is the form of the model, the second is the assumptions on variance. const. = constant variance. het = heterogeneous; i.e., different variances for different groups. Details of models, including functional form and parameter estimates are shown in Table B-2.

[b] "Model fit?" designates a summary of several plausible ratio tests. See Table B-2 for additional considerations. "Het. var." indicates a recommendation was made to fit a model with heterogeneous variances. "Log(likelihood) for fit" is shown in Table B-2.

[c] BMC designates Benchmark Concentration; BMCL designates lower limit on Benchmark Concentration based on a 95% confidence limit obtained by profile likelihood methods. Parenthetically, the basis for the BMR (Benchmark Response) is identified as 1.0 standard deviation from the mean (1 sd). In a number of instances, the BMCL was provided at 1 sd, but no curve could be plotted because computation of the BMCL could not be completed at some other values. In particular, BMCL computation failed at all values for the linear, heterogeneous variance model for F1 average day 25 pup weight.

[d] Designates the recommended BMCL for this endpoint.

[e] day71bw = dam body weight on day 71 of study.

[f] avg d25pupw = average pup weight on day 25 after birth.

[g] pw d25-d7 = average pup weight gain from day 7 to day 25 after birth.

[h] AIC = Akaike Information Criterion.

Table B-2. Summary of model fits, reproductive toxicity/multigenerational study (DuPont HLR, 1997a) (continued)

Table B-2. Summary of model fits, reproductive toxicity/multigenerational study (DuPont HLR, 1997a)

Form of model	Assumptions on variance[a]	log (likelihood) for fit	df	p value	parameters (standard error)
P1- day 71 body weight					
linear: Y = beta_0 + beta_1*dose + e(ij)	const. var.	0.525	2	0.77	alpha = 488.53(63.069) beta_0 = 284.012(2.65785) beta_1 = -0.00292485(8.46298e-4)
F1 - avg d25 pup weight					
linear: Y = beta_0 + beta_1*dose + e(ij)	const. var.	3.265	2	0.1955	alpha = 32.6751(4.44652) beta_0 = 68.3207(0.723566) beta_1 = -0.000935036(2.30414e-4)
linear: Y = beta_0 + beta_1*dose + e(ij)	het. var.	4.799	2	0.09	-- (p<0.10)
quadratic: Y = beta_0 + beta_1*dose + beta_2*dose^2 + e(ij)	het. var.	0.028	1	0.87	-- nonmonotonic, not biol. plausible
F1 - pup weight d25-d7					
linear: Y = beta_0 + beta_1*dose + e(ij)	const. var.	3.952	2	0.14	alpha = 18.4303(2.50805) beta_0 = 51.9811(0.543421) beta_1 = -0.000745883(1.73048e-4)
linear: Y = beta_0 + beta_1*dose + e(ij)	het. var.	3.028	2	0.22	alpha = 2.1563e-5(3.13998e-4) rho = 3.47841(3.71037) beta_0 = 52.0808(0.561655) beta_1 = -0.000790673(1.626e-4)
quadratic: Y = beta_0 + beta_1*dose + beta_2*dose^2 + e(ij)	het. var.	0.122	1	0.73	alpha = 8.70447e-006(1.19975e-4) rho = 3.70326(3.51503) beta_0 = 51.3162(0.692133) beta_1 = 0.000729483(9.13198e-4) beta_2 = -2.38411e-7(1.41545e-7)

Form of model	Assumptions on variance[a]	log (likelihood) for fit	df	p value	parameters (standard error)
power: Y = control + slope * dose^power + e(ij)	het. var.	-0.08	1	<0.00001	-- (p<0.10)
F2 - average d25 pup weight					
linear: Y = beta_0 + beta_1*dose + e(ij)	const. var.	0.875	2	0.65	alpha = 47.7882(7.16376) beta_0 = 68.2523(0.979309) beta_1 = -0.00118865(3.01246e-4)
quadratic: Y = beta_0 + beta_1*dose + beta_2*dose^2 + e(ij)	const. var.	0.405	1	0.52	alpha = 47.5368(7.12606) beta_0 = 68.7824(1.24537) beta_1 = -0.0022747(0.00161119) beta_2 = 1.71476e-7(2.4992e-7)
F2 - pup weight d25-d7					
linear: Y = beta_0 + beta_1*dose + e(ij)	const. var.	1.055	2	0.59	alpha = 28.4843(4.26997) beta_0 = 52.1531(0.75607) beta_1 = -0.000883195(2.3258e-4)
quadratic: Y = beta_0 + beta_1*dose + beta_2*dose^2 + e(ij)	const. var.	0.663	1	0.42	alpha = 28.359(4.2512) beta_0 = 52.5272(0.961901) beta_1 = -0.00164977(0.00124445) beta_2 = 1.21033e-7(1.93038e-7)
cubic: Y= beta_0 + beta_1*dose + beta_2*dose^2 + beta_3*dose^3 + e(ij)	const. var.	approximately 0	0	NA	overfit! (and not at all plausible)

[a] het.var. means the variance is modeled as Var(i) = alpha*mean(i)^rho. ^ means raised to the power of.

Tables of Standardized Residuals (Tables B-3a – B-3f)

Table B-3a. F1: linear, constant variance model for average d25 pup weight

Dose (mg/m^3)	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	28	67.3	7.26	68.3	5.72	-4.96
430.3	26	67.8	4.60	67.9	5.72	-0.52
1,722	27	68.3	5.91	66.7	5.72	7.63
6,025	27	62.2	4.66	62.7	5.72	-2.14

Table B-3b. F1: linear, constant variance model for average pup weight gain d25-d7

Dose (mg/m^3)	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	28	51.1	5.58	52.0	4.29	-5.7.0
430.3	26	51.6	3.47	51.7	4.29	-0.20
1,722	27	52.0	4.11	50.7	4.29	8.24
6,025	27	47.1	3.61	47.5	4.29	-2.34

Table B-3c. F1: linear, heterogeneous variance model for average pup weight gain d25-d7

Dose (mg/m^3)	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	28	51.1	5.58	52.2	4.49	-6.07
430.3	26	51.6	3.47	51.9	4.44	-0.67
1,722	27	52.0	4.11	50.8	4.29	8.10
6,025	27	47.1	3.61	47.4	3.80	-1.43

Table B-3d. F1: quadratic, heterogeneous variance model for average pup weight gain d25-d7

Dose (mg/m^3)	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	28	51.1	5.58	51.3	4.33	-1.35
430.3	26	51.6	3.47	51.6	4.37	0.244
1,722	27	52.0	4.11	51.9	4.42	0.868
6,025	27	47.1	3.61	47.1	3.69	0.428

Table B-3e. F2: linear, constant variance model for average pup weight gain d25-d7

Dose (mg/m^3)	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	21	53.1	4.73	52.2	5.34	3.56
430.3	22	51.1	5.14	51.8	5.34	-2.72
1,722	22	50.3	5.76	50.6	5.34	-1.45
6,025	24	47.0	5.92	46.8	5.34	0.61

Table B-3f. F2: quadratic, constant variance model for average pup weight gain d25-d7

Dose (mg/m^3)	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	21	53.1	4.73	52.5	5.33	2.09
430.3	22	51.1	5.14	51.8	5.33	-3.0
1,722	22	50.3	5.76	50.0	5.33	0.975
6,025	24	47.0	5.92	47.0	5.33	-0.0643

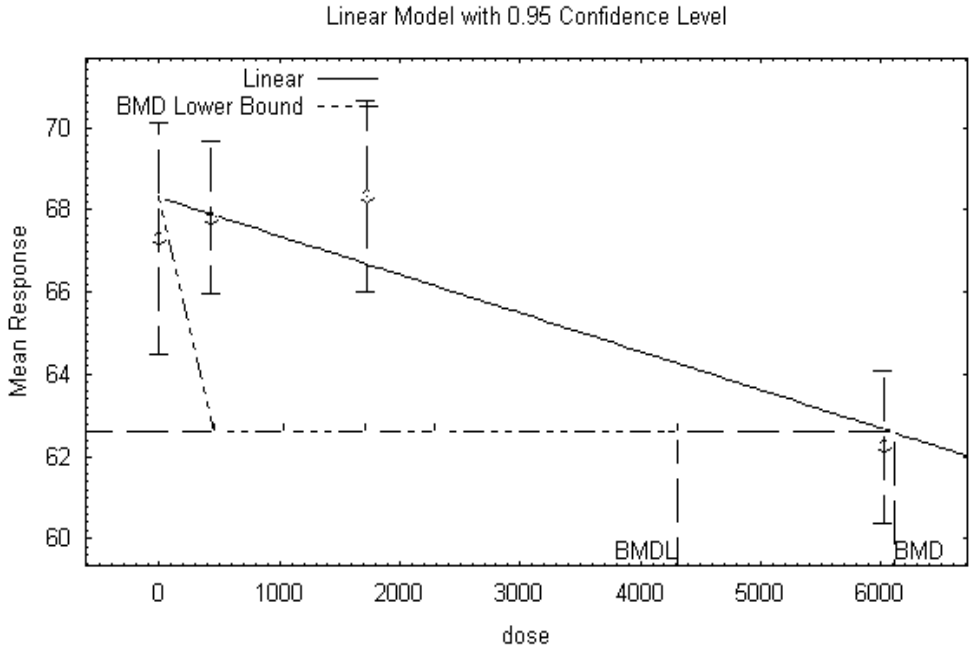


Figure B-1a. F1: linear, constant variance model for average d25 pup weight.

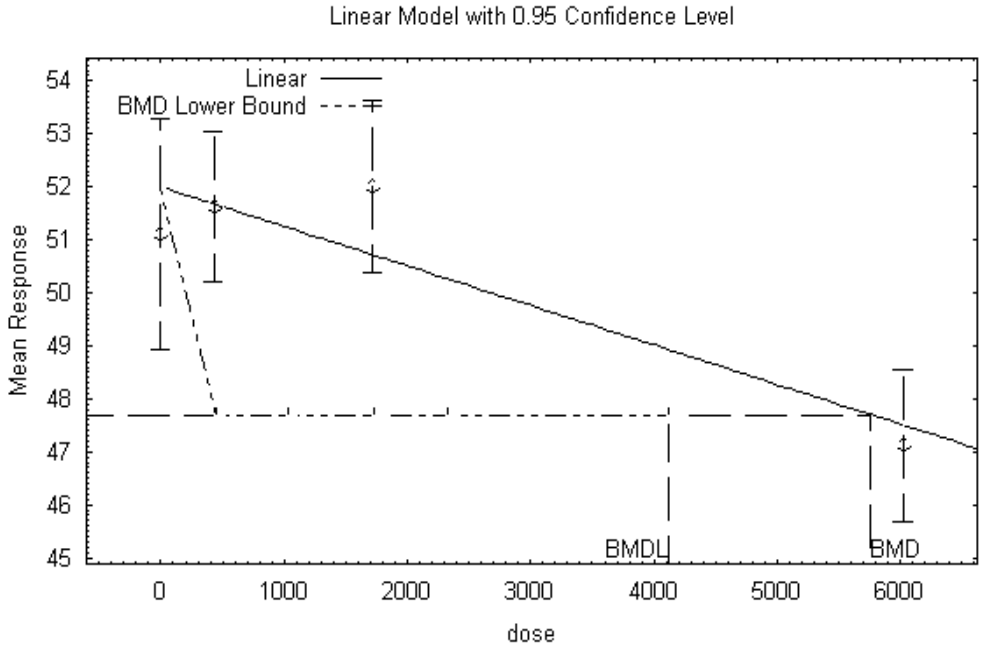
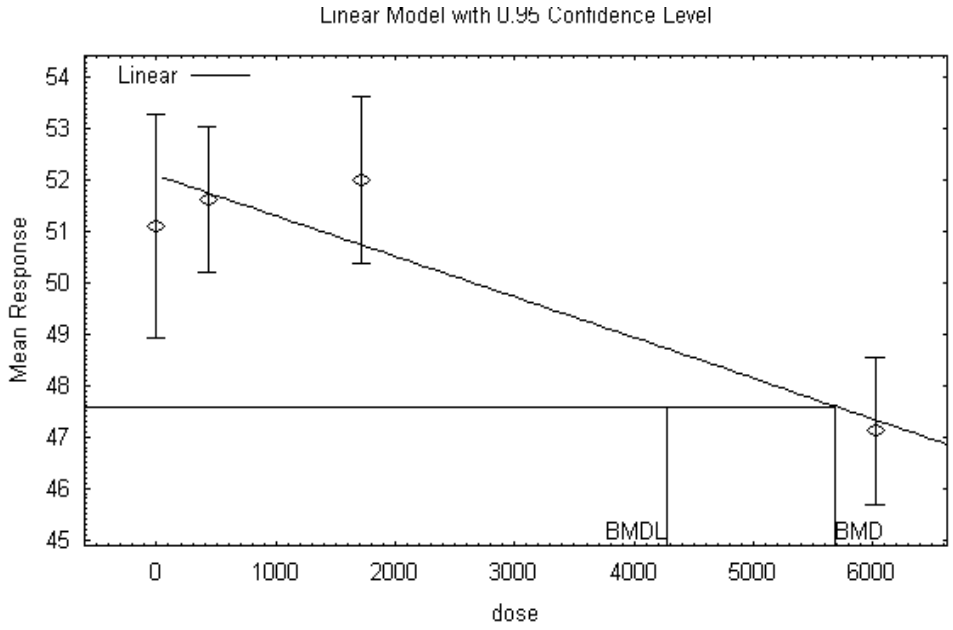
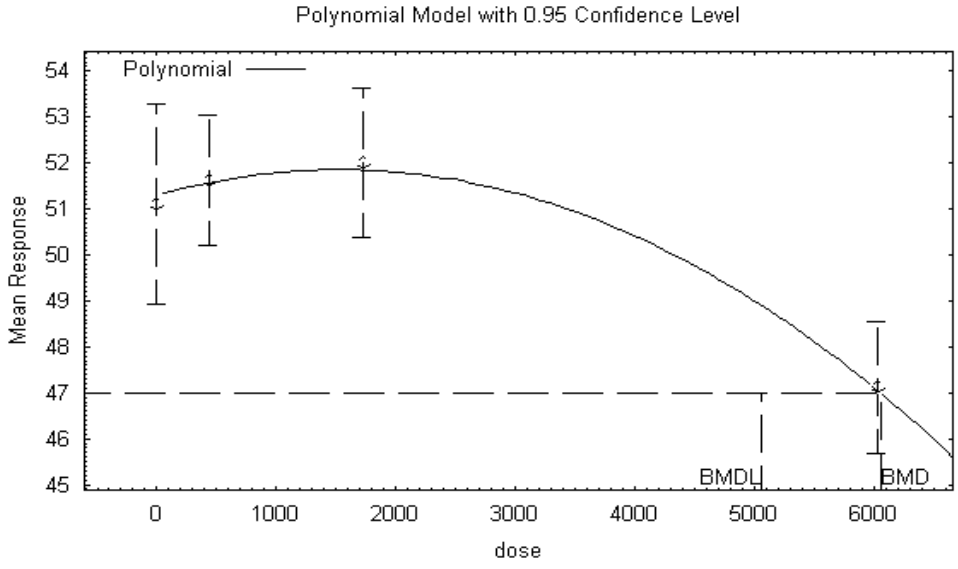


Figure B-1b. F1: linear, constant variance model for average pup weight gain d25-d7.



[a] BMCL computation failed for one or more point on the BMCL curve. The BMCL curve was not plotted.

Figure B-1c. F1: linear, heterogeneous variance model for average pup weight gain d25-d7[a].



[a] BMCL computation failed for one or more point on the BMCL curve. The BMCL curve was not plotted.

Figure B-1d. F1: quadratic, heterogeneous variance model for average pup weight gain d25-d7.

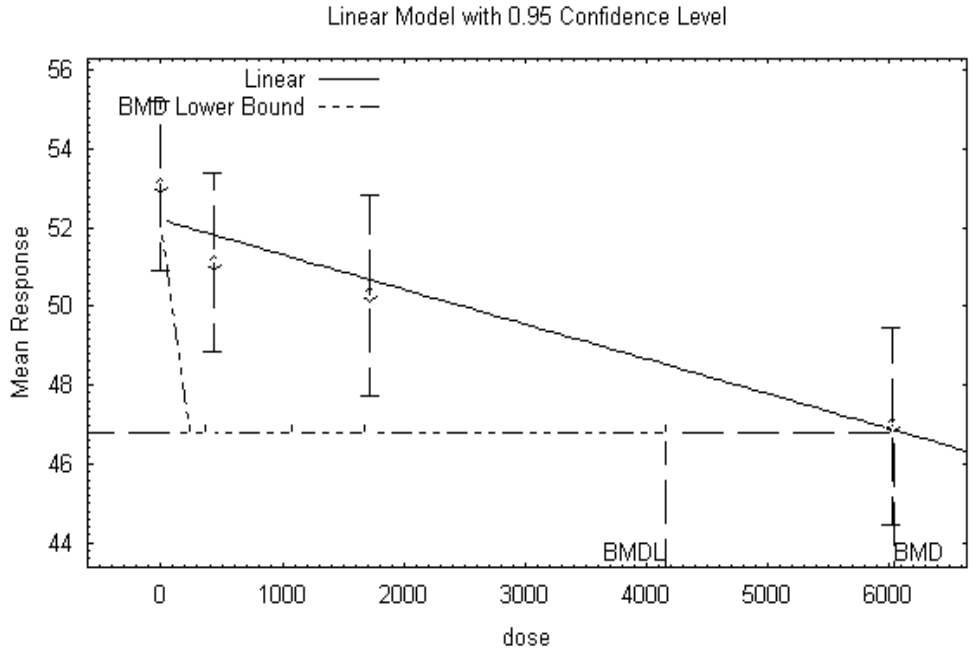


Figure B-1e. F2: linear, constant variance model for average pup weight gain d25-d7.

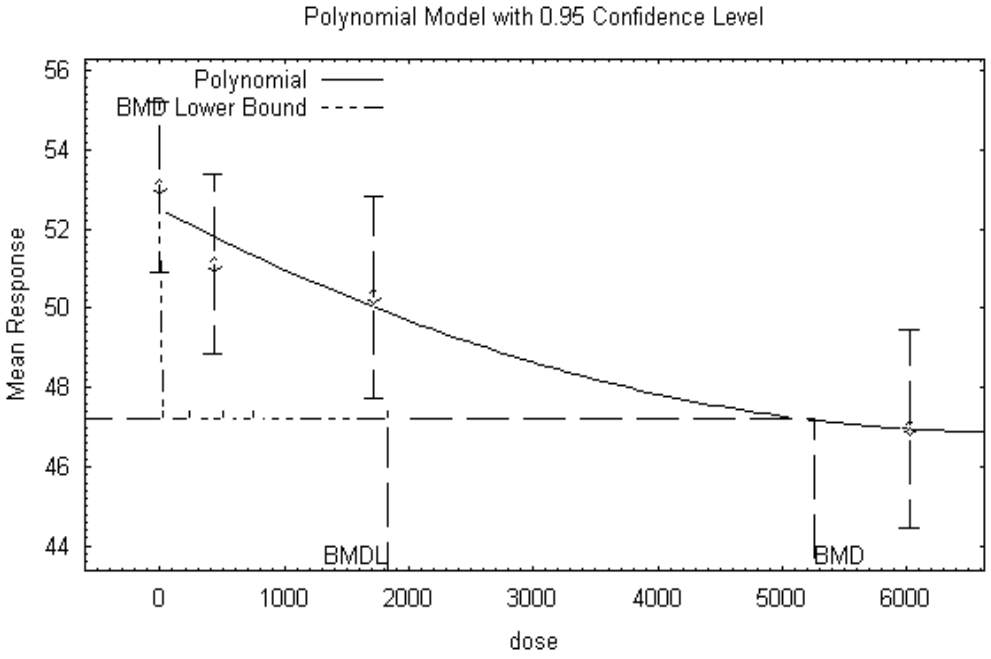


Figure B-1f. F2: quadratic, constant variance model for average pup weight gain d25-d7.

Tables B-4 - B-6 and Figure 2 refer to the results from the rat developmental study (DuPont HLR, 1997b).

Table B-4. Summary of model outcomes, developmental study

Form of model	Assumptions on variance[a]	AIC[g]	Model fit?[b]	$BMC^{c}_{(1\ sd)}$	$BMCL^{c}_{(1\ sd)}$
dams - bw gd 17-gd 7[e]					
linear	het. var.	503.64	const. var.	3,284.19	2,541.63
linear	const. var.	500.065	yes	3,153.34	2,500.82[d]
dams - gd 17 body wt[f]					
linear	const. var.	610.19	yes	6,654.66	4,437.79

[a] The first column is the form of the model, the second is the assumptions on variance. const. = constant variance. het = heterogeneous; i.e., different variances for different groups. Details of models, including functional form and parameter estimates are shown in Table B-5.

[b] "Model fit?" designates a summary of several plausible ratio tests. See Table B-5 for additional considerations. "Const. var." indicates a recommendation was made to fit a model with a constant variance. Log (likelihood) for fit is shown in Table B-5.

[c] BMC designates Benchmark Concentration; BMCL designates lower limit on Benchmark Concentration based on a 95% confidence limit obtained by profile likelihood methods. Parenthetically, the basis for the BMR (Benchmark Response) is identified as 1.0 standard deviation from the mean (1 sd).

[d] Designates the recommended BMCL for this endpoint.

[e] bw gd 17-gd 7 = average dam body weight gain from gestation day 7 to gestation day 17.

[f] gd 17 body wt = average dam weight gestation day 17.

[g] AIC = Akaike Information Criterion.

Table B-5. Summary of model fits, developmental study

Form of model	Assumptions on variance[a]	log(likelihood) for fit	df	p value	Parameters (standard error)
dams - bw gd 17-gd 7[b]					
linear: Y = beta_0 + beta_1*dose + e(ij)	het. var.	0.748	2	0.69	alpha = 6.47858(26.4514) rho = 0.671852(1.01509) beta_0 = 62.7782(1.42678) beta_1 = -0.00311345(4.16781e-4)
linear (same as above)	const. var.	0.807	2	0.67	alpha = 96.9084(14.5272) beta_0 = 62.7953(1.38954) beta_1 = -0.00312184(4.35222e-4)
dams - gd 17 body wt[c]					
linear (same as above)	const. var.	0.374	2	0.83	alpha = 334.004(50.0693) beta_0 = 333.049(2.57969) beta_1 = -0.00274631(8.0799e-4)

[a] het.var. means the variance is modeled as Var(i) = alpha*mean(i)^rho. ^ means raised to the power of.

[b] bw gd 17-gd 7 = average dam body weight gain from gestation day 7 to gestation day 17.

[c] gd 17 body wt = average dam weight gestation day 17.

Tables B-6a - B-6c. Standardized Residuals (DuPont HLR, 1997b)

Table B-6a. Linear, heterogeneous variance model for average dam weight gain, gd17-gd7

Dose (mg/m^3)	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	21	64.2	10.8	62.8	10.2	2.9
430.3	22	60.1	11.2	61.5	10.2	-2.72
1,722	23	57.2	8.51	57.4	9.92	-0.466
6,025	23	44.2	9.57	44.0	9.08	0.283

Table B-6b. Linear, constant variance model for average dam weight gain, gd17-gd7

Dose (mg/m^3)	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	21	64.2	10.8	62.8	9.84	2.98
430.3	22	60.1	11.2	61.5	9.84	-2.84
1,722	23	57.2	8.51	57.4	9.84	-0.476
6,025	23	44.2	9.57	44.0	9.84	0.339

Table B-6c. Linear, constant variance model for average dam body weight, gd17

Dose (mg/m^3)	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	21	332	23.4	333	18.3	-0.712
430.3	22	331	17.4	332	18.3	-1.15
1,722	23	330	14.8	328	18.3	2.5
6,025	23	316	18.5	317	18.3	-0.613

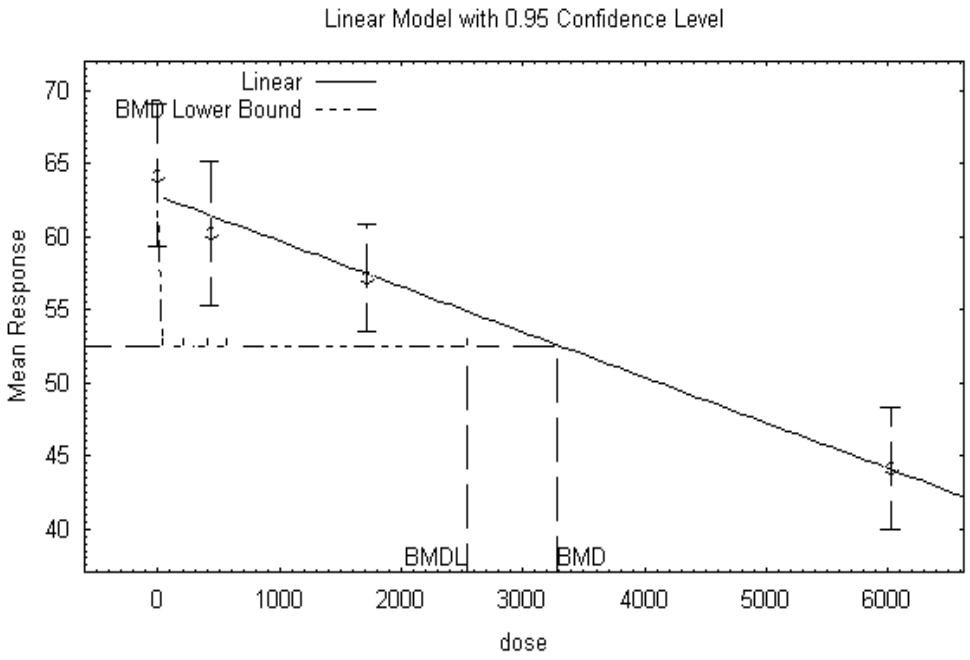


Figure B-2a. Linear, heterogeneous variance model for average dam weight gain, gd17-gd7. (DuPont HLR, 1997b)

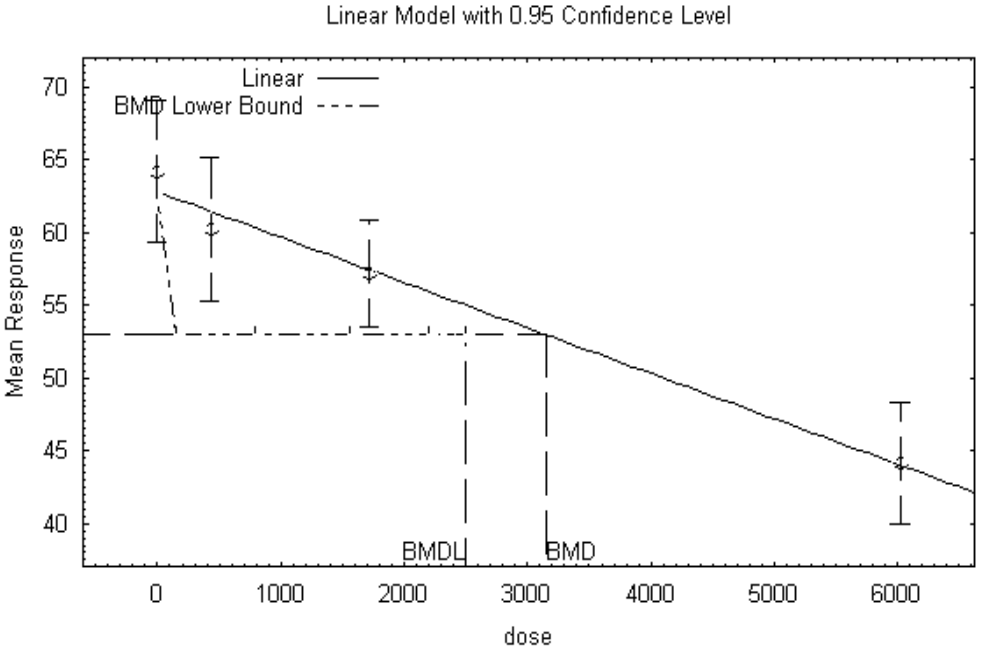


Figure B-2b. Linear, constant variance model for average dam weight gain, gd17-gd7. (DuPont HLR, 1997b)

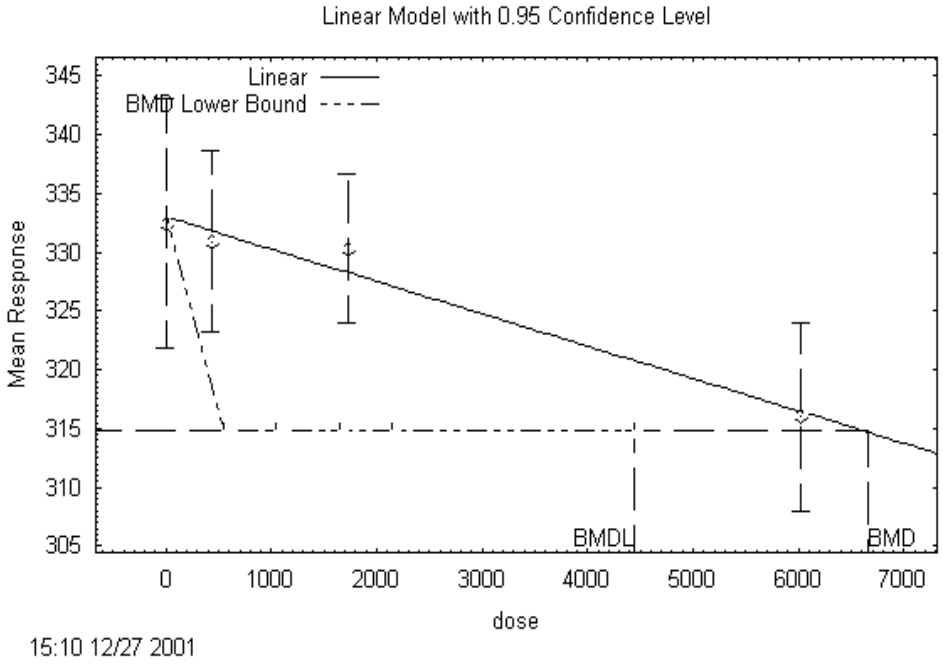


Figure B-2c. Linear, constant variance model for average dam body weight, gd17.