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TOXICOLOGICAL REVIEW

OF

n-HEXANE

(CAS No. 110-54-3)

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

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U.S. Environmental Protection Agency
Washington, DC

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FOREWORD

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

In Section 6, *Major Conclusions in the Characterization of Hazard and Dose Response,* EPA has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose response by addressing knowledge gaps, uncertainties, quality of data, and scientific controversies. The discussion is intended to convey the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's IRIS Hotline at 202-566-1676.

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LIST OF ABBREVIATIONS AND ACRONYMS

1SD	One standard deviation
ACGIH	American Conference of Governmental Industrial Hygienists
ADJ	Adjusted for continuous exposure
AIC	Akaike's Information Criterion
API	American Petroleum Institute
ATSDR	Agency for Toxic Substance and Disease Registry
BAEP	Brainstem auditory evoked potentials
BEI	Biological exposure index
BMC	Benchmark concentration
BMCL	95% lower bound on the benchmark concentration
BMD	Benchmark dose
BMDL	95% lower bound on the benchmark dose
BMDS	Benchmark dose software
BMR	Benchmark response
BRRC	Bushy Run Research Center
CA	Chromosomal aberrations
CASRN	Chemical Abstracts Service registry number
CHL	Chinese hamster lung
CHO	Chinese hamster ovary
CI	Confidence interval
CIIT	Chemical Industry Institute of Toxicology
CMAP	Compound muscle action potential
CNS	Central nervous system
CYP450	Cytochrome P450
DCV	Distribution of conduction velocity
DL	Distal latency
DOF	Degree of freedom
EMG	Electromyography
ENM	Electroneuromyography
EP	Evoked potentials
EPA	U.S. Environmental Protection Agency
EPL	Experimental Pathology Laboratories
ERG	Electroretinogram
EROD	Ethoxyresorufin O-deethylase
FM	Farnsworth-Munsell
FOB	Functional observation battery
GD	Gestation day
HEC	Human equivalent concentration
HSDB	Hazardous Substances Data Bank
IRDC	International Research and Development Corporation
IRIS	Integrated Risk Information System

LC_{50}	Median lethal concentration
LD_{50}	Median lethal dose
LDH	Lactate dehydrogenase
LOAEL	Lowest-observed-adverse-effect level
MAP	Motor nerve action potential
MCV	Motor nerve conduction velocity
MDL	Minimum detection limit
MN	Micronucleus
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
NCE	Nonchromatic erythrocytes
NSC	National Safety Council
NCTR	National Center for Toxicological Research
NF	Neurofilament
NL	Nested logistic
NOAEL	No-observed-adverse-effect level
NTP	National Toxicology Program
OR	Odds ratio
PBTK	Physiologically based toxicokinetic
PCE	Polychromatic erythrocyte
PND	Postnatal day
PNS	Peripheral nervous system
ppm	Parts per million
PROD	Pentoxyresorufin O-dependentylase
RfC	Reference concentration
RfD	Reference dose
RvR	Rai and van Ryzin
SCE	Sister chromatid exchange
SCV	Sensory nerve conduction velocity
SD	Standard deviation
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEM	Standard error of the mean
SEP	Somatosensory evoked potential
SNAP	Sensory nerve action potential
TLV	Threshold limit value
TWA	Time weighted average
UF	Uncertainty factor
UPDRS	Unified Parkinson Disease Rating Scale
VEP	Visual evoked potential
WBC	White blood cell

1. INTRODUCTION

This document presents background information and justification for the Integrated Risk Information System (IRIS) Summary of the hazard and dose-response assessment of n-hexane. 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 toxicity values are 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. 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. It is expressed in units of mg/kg-day. 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 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 to better facilitate their use: (1) generally, the slope factor is the result of application of a low-dose extrapolation procedure and is presented as the risk per mg/kg-day of oral exposure; (2) the unit risk is the quantitative estimate in terms of either risk per g/L drinking water or risk per g/m^3 continuous airborne exposure; and (3) the 95% lower bound and central estimate on the estimated concentration of the chemical substance in drinking water or air presents 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 n-hexane has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). The United States Environmental Protection Agency (EPA) guidelines that were used in the development of this assessment includes the following: *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), *Guidelines for carcinogen risk assessment* (U.S. EPA, 2005a), *Supplementary Guidance*

for Assessing Susceptibility from Early-Life Exposure to Carcinogens (U.S. EPA., 2005b), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), *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, 2000a), *Science Policy Council Handbook: Risk Characterization* (U.S. EPA, 2000b), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000c), and *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002).

The literature search strategy employed for this compound was based on the Chemical Abstracts Service registry number (CASRN) and at least one common name. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document.

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

n-Hexane (CASRN 110-54-3) is a straight-chain, fully saturated hydrocarbon with six carbon atoms also referred to as hexane and hexyl hydride. The chemical is also referred to by trade names such as Skellysolve B and NCI-C60571. Some physical and chemical properties of n-hexane are shown below (HSDB, 2005; ATSDR, 1999).

Chemical formula	C_6H_{14}
Molecular weight	86.18
Melting point	–95 ºC
Boiling point	69 ºC
Density	0.66 g/mL (at 20 ºC)
Water solubility	9.5 mg/L (at 25 ºC)
Log K_{OW}	3.29
Log K_{OC}	2.9
Vapor pressure	150 mm Hg (at 25 ºC)
Henry's Law constant	1.69 atm-m^3/mol
Conversion factor	1 ppm = 3.5 mg/m^3; 1 mg/m^3 = 0.28 ppm (at 25 °C, 760 mm Hg)

n-Hexane is a solvent that has many uses in the chemical and food industries, either in pure form or as a component of the mixture commercial hexane. Highly purified n-hexane is primarily used as a reagent for chemical or chromatographic separations. Commercial hexane is a mixture that contains approximately 52% n-hexane; the balance is made up of varying amounts of structural isomers and related chemicals, such as methylpentane and methylcyclopentane.

Mixtures containing n-hexane are also used in processes for the extraction of edible fats and oils in the food industry, as cleaning agents in textile and furniture manufacturing, and in the printing industry. n-Hexane is the solvent base for many commercial products, such as glues, cements, paint thinners, and degreasers (NSC, 2003; ATSDR, 1999).

The chemical is a minor constituent of crude oil and natural gas and, therefore, represents a variable proportion of different petroleum distillates. For example, n-hexane comprises about 11.6% of unleaded gasoline and about 2% of JP-4 aviation fuel (ATSDR, 1993a, b).

3. TOXICOKINETICS RELEVANT TO ASSESSMENTS

3.1. ABSORPTION

No oral exposure studies evaluating absorption of n-hexane in humans or laboratory animals are available. However, absorption following oral exposure has been suggested by the identification of n-hexane and its metabolites in expired air, serum, and urine (Baelum et al., 1998; Ono et al., 1981; Krasavage et al., 1980). For example, increased levels of n-hexane in exhaled air and a major metabolite of n-hexane (2,5-hexanedione) in urine were observed following exposure of human volunteers to n-hexane (0.3 and 1.0 mg/minute for 60 minutes) by a gastric feeding tube (Baelum et al., 1998). Krasavage et al. (1980) showed increased levels of 2,5-hexanedione in serum of rats exposed to n-hexane by gavage. In addition, neurotoxicity observed following oral exposure of rats to n-hexane also suggests oral absorption of the chemical (Ono et al., 1981; Krasavage et al., 1980).

There is also limited evidence in humans inferring absorption following inhalation exposure to n-hexane. Mutti et al. (1984) measured n-hexane in the inhaled and expired air of 10 workers who routinely breathed in solvent vapors during their shift at a shoe factory. n-Hexane concentrations in breathing zone air (8-hour time weighted average [TWA] median concentration of 69 parts per million [ppm] or 243 mg/m^3) were monitored with personal monitors. The authors noted that other structural isomers and related chemicals of n-hexane (2-methylpentane, 3-methylpentane, cyclohexane, and n-heptane) were also present in the breathing air. Samples (inhaled and exhaled air) were collected simultaneously for 5 minutes; the last 100 mL of the tidal volume represented alveolar air. Alveolar uptake was determined by the following equation:

$$D = kC_{i(TWA)}V_aRt$$

where D = alveolar uptake or dose (mg), k = factor converting ppm to mg/L (3.5×10^{-3}), $C_{i(TWA)}$ = time weighted average of breathing zone levels of n-hexane (ppm), V_a = alveolar ventilation, R = pulmonary retention coefficient, and t = time (minutes). Alveolar retention (difference between inhaled and alveolar concentrations of n-hexane) was approximately 25%. The authors stated that the absorption rate, taking into account both retention and alveolar ventilation, was approximately 17%. Further evidence for absorption following inhalation exposure of n-hexane was suggested by the presence of metabolites of n-hexane in the urine, which was monitored at the beginning and end of each shift, and 15 hours after exposure.

Veulemans et al. (1982) studied the respiratory uptake and elimination of n-hexane in six healthy male volunteers. Subjects were exposed at rest to 360 or 720 mg/m^3 (102 or 204 ppm) of n-hexane for 4 hours and to 360 mg/m^3 (102 ppm) under various levels of exercise. A 2-week rest period was allowed between experiments. Inhaled and exhaled air were monitored (for up to 4 hours after exposure), and blood samples were collected. The authors reported an approximate 22% and 24% retention of n-hexane from inhaled air (360 and 720 mg/m^3, respectively) at rest. The corresponding absorption rates were calculated as 0.84 and 1.59 mg/minute, respectively. Absorption rates for n-hexane (360 mg/m^3) also increased with increasing physical activity.

3.2. DISTRIBUTION

Following inhalation exposure, n-hexane is absorbed into the circulation and transported to the liver, the major site of metabolism. In the liver, n-hexane is metabolized to various metabolites that are then distributed in the blood to various organs and tissues, including the liver, kidney, and brain. Several inhalation studies in humans and animals demonstrate the distribution of n-hexane.

Perbellini et al. (1985) reported partition coefficient values (olive oil: air, human blood: air, and human tissues: air) for n-hexane. The following values were presented: olive oil: air, 146; blood: air, 0.80; and 1.0, 2.8, 5.0, 5.0, 3.0, 5.2, and 104 for lung, heart, muscle, brain, kidney, liver, and fat: air, respectively. These values are similar to those reported in some tissues from F344 rats (2.9 for muscle, 5.2 for liver, and 159 for fat [Gargas et al., 1989]). In addition, in vitro blood: air (2.13) and breast milk:air (4.66) partition coefficients for humans (eight volunteers) were used to calculate a milk: blood partition coefficient of 2.10 (Fisher et al., 1997).

No studies investigating the distribution of n-hexane following oral exposure in humans or laboratory animals are available. However, one human study evaluating the distribution of n-hexane following inhalation exposure is available. Veulemans et al. (1982) measured blood levels of n-hexane at various intervals for 4 hours after exposure (resting and during physical activity) to n-hexane (102 and 204 ppm for 4 hours). Blood levels of n-hexane rapidly decreased to approximately half the steady state exposure values within the first 10 minutes and reached steady state levels by 100 minutes. The average half life for n-hexane in blood was 1.5–2 hours.

Studies in laboratory animals indicate that n-hexane is distributed to a variety of tissues following inhalation exposure. Bus et al. (1979) monitored n-hexane in the blood of pregnant F344 rats that were exposed via inhalation to 1000 ppm (3520 mg/m^3) n-hexane (purity not stated) for 6 hours on gestation day (GD) 20. n-Hexane was measured in maternal blood, liver,

kidney, brain, and fetuses using gas chromatography-mass spectrometry at 0, 1, 2, 4, and 8 hours after exposure. Maximum tissue concentrations of n-hexane were observed immediately after cessation of exposure. Average concentrations of n-hexane in blood and tissues are presented in Table 3-1.

Table 3-1. Concentration of n-hexane in blood and tissues of pregnant F344 rats immediately after a 6-hour exposure to 1000 ppm n-hexane

Tissue	Concentration (µg/g wet weight or µg/mL)
Blood	0.45 ± 0.11
Liver	0.85 ± 0.13
Kidney	6.33 ± 0.75
Brain	0.04 ± 0.00
Fetus	0.61 ± 0.14

Source: Bus et al., 1979.

Bus et al. (1981) administered a single exposure or five daily 6-hour exposures of 1000 ppm n-hexane to male F344 rats (three/group) and observed the appearance of n-hexane in blood, liver, kidney, brain, and sciatic nerve. In the single dose experiment, concentrations of n-hexane in blood, liver, kidney, brain, and sciatic nerve were 0.50 ± 0.1 µg/mL, and 1.23 ± 0.14, 5.8 ± 0.3, 3.0 ± 0.11, and 46 ± 10 µg/g, respectively. However, levels of n-hexane were undetectable in blood after 1 hour and in liver, kidney, and brain after 4 hours.

Bus et al. (1982) also monitored the distribution of radioactivity in a range of tissues for up to 72 hours after exposing male F344 rats to a single 6-hour exposure to either 0, 500, 1000, 3000, or 10,000 ppm n-hexane (95.5% pure) containing 11.8–54.9 µCi/mmol [1,2-^{14}C]-n-hexane (Table 3-2).

Table 3-2. Tissue distribution of radioactivity in male F344 rats 72 hours after a 6-hour inhalation exposure to various concentrations of [1,2-^{14}C]-n-hexane

Tissue	Exposure (ppm)			
	500	1000	3000	10,000
	Tissue concentration (nmol-equivalents/g wet weight or mL)			
Liver	63.5 ± 0.9	90.9 ± 3.0	313.8 ± 34.6	189.0 ± 10.1
Lung	58.4 ± 0.7	85.3 ± 13.1	176.9 ± 9.5	118.1 ± 2.8

Tissue	Exposure (ppm)			
	500	1000	3000	10,000
	Tissue concentration (nmol-equivalents/g wet weight or mL)			
Kidney	69.2 ± 3.4	88.1 ± 6.6	242.2 ± 6.6	135.4 ± 5.5
Testes	33.5 ± 0.9	48.6 ± 1.4	ND	67.8 ± 5.4
Brain	24.6 ± 0.7	33.1 ± 2.2	ND	57.5 ± 3.3
Sciatic nerve	53.0 ± 1.9	84.8 ± 27.7	ND	ND
Blood	27.8 ± 1.3	23.5 ± 2.2	ND	79.0 ± 7.3

ND = Not determined.

Source: Bus et al., 1982.

The authors stated that since the n-hexane was labeled at the C-1 and C-2 positions, it was likely that the radioactivity found in the various tissues was due to incorporation by metabolism of 2-hexanone, but it was possible that residual tissue radioactivity was due to binding of reactive metabolites to macromolecules, which is supported by in vitro studies showing 2,5-hexanedione forming Schiff bases and/or pyrrole derivatives with lysine amino groups (Sanz et al., 1995; DeCaprio et al., 1988 , 1982; Boekelheide, 1987; Lapadula et al., 1986; Anthony et al., 1983a, b; Graham et al., 1982b).

Similarly, after a single 6-hour exposure of n-hexane to male F344 rats (0, 500, 1000, 3000, or 10,000 ppm), n-hexane was detected in blood, liver, kidney, sciatic nerve, testis, brain, and lung (Baker and Rickert, 1981) (Table 3-3).

Table 3-3. Apparent steady state concentration of n-hexane concentrations in male F344 rats after 6 hours inhalation exposure to [1,2-^{14}C]-n-hexane

Tissue	Exposure (ppm)			
	500	1000	3000	10,000
	Tissue concentration (nmol-equivalents/g wet weight or mL)			
Liver	2.6 ± 0.9	6.7 ± 0.3	22.8 ± 2.3	72.4 ± 4.8
Lung	3.1 ± 0.2	8.8 ± 0.7	27.7 ± 2.9	89.3 ± 11.6
Kidney	7.0 ± 1.0	22.0 ± 2.0	41.4 ± 4.2	54.4 ± 1.8
Testes	3.5 ± 0.8	20.0 ± 2.0	27.3 ± 2.4	53.9 ± 6.7
Brain	1.8 ± 0.0	19.1 ± 2.3	36.1 ± 2.8	54.2 ± 1.6
Sciatic nerve	12.0 ± 1.0	48.0 ± 5.0	130.3 ± 17.4	430.5 ± 59.4
Blood	1.3 ± 0.2	2.2 ± 0.2	8.4 ± 0.8	20.9 ± 0.3

Data are means ± standard error of the mean (SEM) (n = 3).

Source: Baker and Rickert, 1981.

3.3. METABOLISM

n-Hexane is principally metabolized in the liver. As shown in Figure 3-1, n-hexane is initially hydroxylated by the action of mixed function oxidases to form either 1- or 3-hexanol in a detoxification pathway or 2-hexanol in a bioactivation pathway. Through the bioactivation pathway, 2-hexanol is converted to 2-hexanone and 2,5-hexanediol. Both of these metabolites are then further metabolized to 5-hydroxy-2-hexanone, 2,5-hexanedione, and 4,5-dihydroxy-2-hexanone. 2,5-Hexanedione is believed to be the major toxic metabolite produced in humans following acid hydrolysis of urine samples prior to analysis by gas chromatography (Perbellini et al., 1981).

Evidence that the liver is the primary location for the initial hydroxylation step for bioactivation of n-hexane comes from the measurement of hydroxylating activity in isolated microsomes from liver, lung, brain, and the extensor digitorum longus and soleus skeletal muscles (Crosbie et al., 1994). Microsomes were incubated with n-hexane in the presence of reduced nicotinamide adenine dinucleotide phosphate (NADPH) and the rates of production of the primary alcohols, 1-, 2-, and 3-hexanol, were compared. Liver microsomes produced significantly more 2-hexanol (1104 ± 205 pmol/minute-mg protein) than microsomes from lung, brain, and soleus and extensor digitorum longus skeletal muscles (132 ± 25, 3 ± 2, 4 ± 1, 28 ± 5 pmol/minute-mg protein, respectively). Similarly, in liver microsomes, 2-hexanol production occurred at a much faster rate than that of 1- or 3-hexanol. In contrast, the production of 1-hexanol appeared to occur at a much faster rate in microsomal preparations from the lung.

Metabolism of n-hexane in humans primarily forms 2,5-hexanedione. Perbellini et al. (1981) identified this metabolite along with 2,5-dimethylfuran, γ-valerolactone, and 2-hexanol in the urine of 41 shoe workers exposed to 11–250 mg/m^3 commercial hexane (a mixture containing n-hexane). The mean concentrations, determined following acid extraction of the urine, were 5.4 ± 4.9, 3.7 ± 4.1, 3.3 ± 2.7, and 0.19 ± 0.26 mg/L, respectively. n-Hexane exposure correlated well both with total metabolites (r = 0.7858) and with 2-hexanol (r = 0.6851) and 2,5-hexanedione (r = 0.6725) individually.

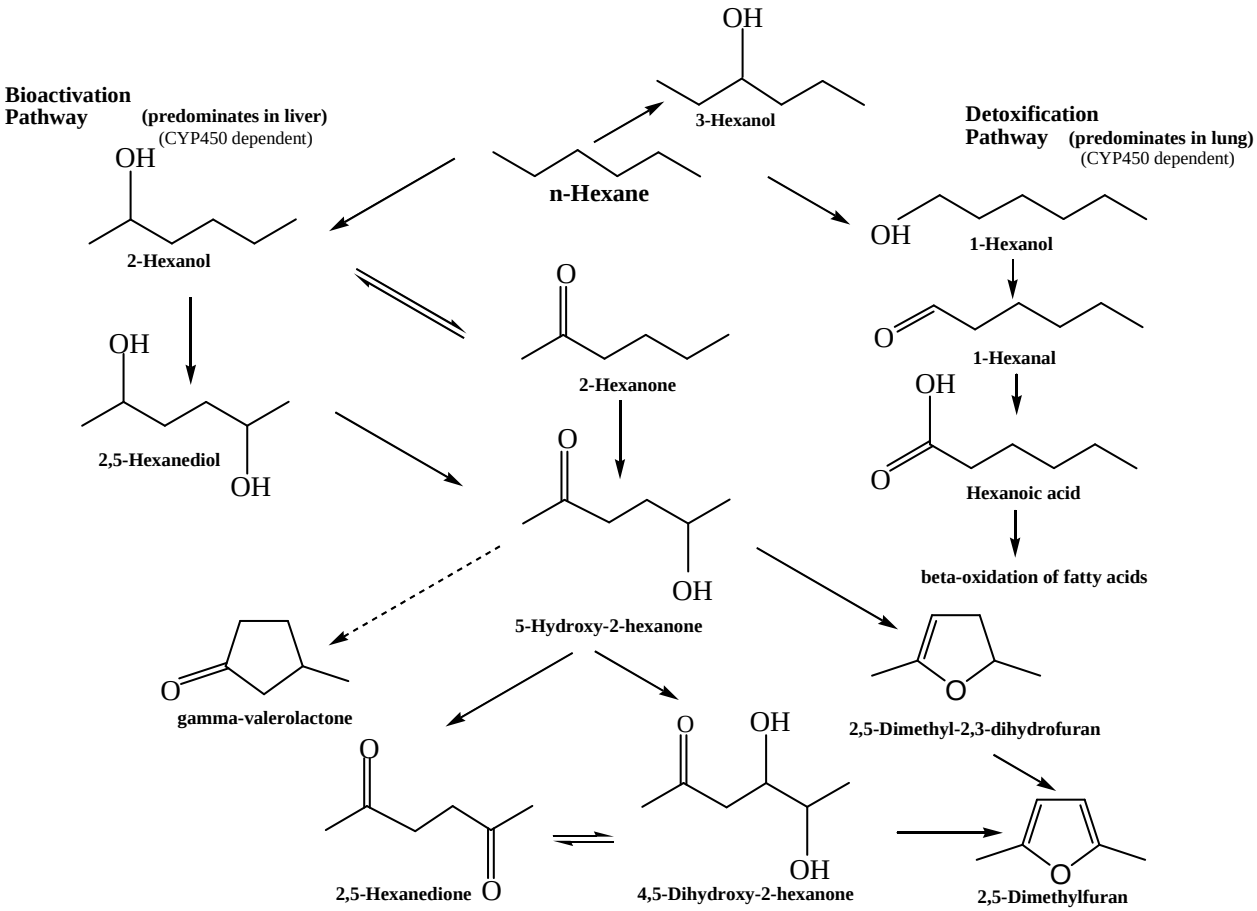


Figure 3-1. Biotransformation of n-hexane.

Source: Adapted from Soriano et al., 1996; Couri and Milks, 1982.

The time course of n-hexane metabolism in humans was determined by van Engelen et al. (1997). Volunteers were exposed to approximately 60 ppm n-hexane for 15.5 minute intervals in the morning and afternoon. 2,5-hexanedione blood levels peaked between 16.2 and 19.8 minutes after the start of exposure (no difference was found between morning and afternoon exposures).

Other studies indicate that 2,5-hexanedione levels identified in the urine of humans may be an artifact of the extraction method used (dos Santos et al., 2002; Fedtke and Bolt, 1986). Specifically, studies indicate that hydrolysis of urinary conjugates with acid may lead to the conversion of 4,5-dihydroxy-2-hexanone to 2,5-hexanedione. It is also possible that acid hydrolysis could result in the release of 2,5-hexanedione that is bound to protein amino groups by a reversible imino bond. For example, dos Santos et al. (2002) observed increased levels (approximately 10 times higher) of urinary 2,5-hexanedione in acidified urine samples from 52 Brazilian shoe workers exposed to n-hexane compared to samples from exposed workers that were not subjected to acid hydrolysis (0.94 mg/L after acid hydrolysis versus 0.09 mg/L without hydrolysis).

Fedtke and Bolt (1986) used the acidification pretreatment procedure to detect a small amount of 2,5-hexanedione in the urine of 12 subjects who had not been exposed to n-hexane. The range of values was 0.12–0.78 mg/L with an arithmetic mean of 0.45 ± 0.20 mg/L. The authors speculated that the low levels of 2,5-hexanedione detected in the urine might be explained by the metabolism of endogenously produced n-hexane rather than environmental exposure. Studies in humans and laboratory animals have shown that rodents exhale alkanes as metabolic products of lipid peroxidation of liver phospholipid fatty acids (Vaz and Coon, 1987; Gelmont et al., 1981; Kivits et al., 1981). Fedtke and Bolt (1986) also investigated (in a single urine sample) the effect of acid hydrolysis on formation of 2,5-hexanedione. A pH range of 3–7 had no effect on the amount of 2,5-hexanedione liberated, but a pH below 3 (down to 0.1) increased the amount of 2,5-hexanedione released.

A study in rats suggested that 2-hexanol may be the major metabolite of n-hexane following inhalation exposure. Fedtke and Bolt (1987) exposed three male Wistar rats/group to mean concentrations of 0, 50 ± 3, 102 ± 6, 248 ± 6, 504 ± 20, 1003 ± 74, or 3074 ± 96 ppm n-hexane for 8 hours. The authors collected urine samples during and after exposure (8, 16, 24, 32, 40, and 48 hours postexposure). The formation of n-hexane metabolites was dependent on exposure concentrations up to approximately 300 ppm. Formation of 1-, 2-, and 3-hexanol and 2-hexanone was evident during exposure, but had ceased by 8 hours postexposure. Levels of 2,5-hexanedione and 4,5-dihydroxy-2-hexanone were initially low and the metabolism of n-hexane to these metabolites had ceased by 16 and 40 hours postexposure, respectively. The

primary metabolite formed in rats following inhalation exposure was 2-hexanol (approximately twice all other metabolites observed), followed by 4,5-dihydroxy-2-hexanone. These metabolites together accounted for about 90% of the total metabolites formed. The level of 4,5-dihydroxy-2-hexanone was approximately 10 times higher than 2,5-hexanedione (calculated by subtraction of the concentration of free 2,5-hexanedione measured without acid hydrolysis from the concentration of 2,5-hexanedione measured following complete acid hydrolysis).

Krasavage et al. (1980) exposed male COBS, CD(SD) BR rats to single gavage doses of 0, 6.6, 13.2, and 46.2 mmol/kg n-hexane and evaluated peak 2,5-hexanedione levels in serum. The peak serum concentrations of 2,5-hexanedione measured at each dose were 24, 44, and 53 µg/mL, respectively.

Bus et al. (1979) observed the metabolism of n-hexane in the fetus and maternal tissues of pregnant F344 rats that had been administered a single 6-hour exposure of 0 or 1000 ppm n-hexane on GD 12 or 20. n-Hexane and its metabolites, 2,5-hexanedione and 2-hexanone, were detected in the liver, kidney, brain, blood, and the developing fetus at time points up to 18 hours after exposure. The metabolism of n-hexane to 2,5-hexanedione and 2-hexanone was rapid (Table 3-4). 2,5-hexanedione was the major metabolite observed in all maternal tissues evaluated and in the fetus. Levels reached peak concentrations in these tissues at 4 hours postexposure.

Table 3-4. Metabolism of n-hexane following a 6-hour exposure of pregnant F344 rats on gestation day 20

Tissues	Time after exposure (hours)[a]						
	0	**1**	**2**	**4**	**8**	**12**	**18**
Maternal							
Liver							
n-Hexane	0.85 ± 0.13	0.33 ± 0.06	0.15 ± 0.04	ND	ND	ND	ND
2-Hexanone	0.16 ± 0.03	0.06 ± 0.00	0.03 ± 0.00	0.03 ± 0.01	0.01 ± 0.00	ND	ND
2,5-HD	0.56 ± 0.03	0.75 ± 0.04	0.74 ± 0.04	1.30 ± 0.30	0.85 ± 0.08	0.36 ± 0.06	0.11 ± 0.02
Kidney							
n-Hexane	6.33 ± 0.75	3.16 ± 0.97	1.15 ± 0.15	0.67 ± 0.27	ND	ND	ND
2-Hexanone	1.04 ± 0.31	0.32 ± 0.01	0.14 ± 0.02	0.08 ± 0.03	0.03 ± 0.00	ND	ND
2,5-HD	1.29 ± 0.10	0.73 ± 0.04	1.22 ± 0.24	1.37 ± 0.07	0.80 ± 0.03	0.24 ± 0.06	0.07 ± 0.03
Brain							
n-Hexane	0.04 ± 0.00	ND	ND	ND	ND	ND	ND

Tissues	Time after exposure (hours)[a]						
	0	1	2	4	8	12	18
2-Hexanone	0.69 ± 0.13	0.29 ± 0.02	0.11 ± 0.01	0.03 ± 0.01	0.01 ± 0.00	ND	ND
2,5-HD	2.41 ± 0.30	1.79 ± 0.10	3.10 ± 0.34	3.61 ± 0.40	2.07 ± 0.09	0.29 ± 0.01	0.16 ± 0.01
Blood							
n-Hexane	0.45 ± 0.11	0.30 ± 0.05	0.13 ± 0.02	0.04 ± 0.01	ND	ND	ND
2-Hexanone	0.70 ± 0.10	0.30 ± 0.05	0.10 ± 0.01	0.04 ± 0.01	0.01 ± 0.00	ND	ND
2,5-HD	1.06 ± 0.27	0.93 ± 0.12	1.51 ± 0.21	1.73 ± 0.30	0.74 ± 0.09	0.33 ± 0.03	0.14 ± 0.04
Fetus							
n-Hexane	0.61 ± 0.14	0.31 ± 0.12	ND	ND	ND	ND	ND
2-Hexanone	0.51 ± 0.08	0.18 ± 0.00	0.10 ± 0.01	0.03 ± 0.00	0.01 ± 0.00	ND	ND
2,5-HD	1.17 ± 0.15	0.97 ± 0.16	1.24 ± 0.09	1.67 ± 0.16	0.80 ± 0.05	0.29 ± 0.07	0.07 ± 0.01

[a] Values are µg/mL or µg/g wet weight ± SEM.
ND = Not detected.

Source: Bus et al., 1979.

The kinetics of the metabolism of n-hexane has also been investigated in vitro using microsomal preparations from the liver and lung of male Sprague-Dawley rats (Toftgard et al., 1986). The concentrations of the metabolic products formed and the reaction velocities were determined. The kinetic data were plotted using an Eadie-Scatchard transformation. An Eadie-Scatchard transformation is a plot of velocity/substrate concentration on the y-axis against velocity on the x-axis. It is used to estimate the K_m and V_{max} for an enzyme. The estimated parameters for n-hexane hydroxylation in the liver and lung are presented in Table 3-5.

The values for 1- and 2-hexanol suggested that a two-enzyme system is responsible for the metabolism of n-hexane to these metabolites in liver tissue. The lower a K_m value, the higher the affinity of an enzyme for a substrate. The data indicate that one of the metabolic enzymes has a high affinity for n-hexane as a substrate, while the other has a lower affinity. The metabolite of greatest interest in the liver is 2-hexanol because of its conversion to 2,5-hexanedione, a toxicologically active metabolite. The enzyme represented by K_{m1} in Table 3-5 with a K_m of 6.0 µM is primarily responsible for the production of 2-hexanol. The second enzyme system (K_{m2}) involved in the production of 2-hexanol has a K_m of 1,100 µM and thus a far lower affinity for n-hexane than the first system. This suggests that the first system is likely to play the major role in the production of 2-hexanol in the liver. The production of 1-hexanol in the liver also appears to involve two enzymes with considerably different affinities for the substrate. The enzyme represented by K_{m1} with a K_m of 0.4 µM has a greater affinity for hexane

than the enzyme represented by K_{m2} with a K_m of 300 µM.

Table 3-5. Apparent kinetic parameters for n-hexane hydroxylation in rat liver and lung microsomes

Tissue parameter	Product formed		
	1-hexanol	2-hexanol	3-hexanol
Liver			
K_{m1} (µM)	0.4	6	ND
V_{max1} (nmoles/mg-min)	0.09	1	ND
K_{m2} (µM)	300	1100	290
V_{max2} (nmoles/mg-min)	1.2	4.6	0.5
Lung			
K_m (µM)	9	50	65
V_{max}(nmoles/mg-min)	2.2	1.3	0.2

ND = No data.

Source: Toftgard et al., 1986.

The liver data for the production of 3-hexanol suggest that there is only one enzyme involved in the metabolism of n-hexane to this product. The affinity of this enzyme for n-hexane is similar to the low affinity enzyme system responsible for the production of 1-hexanol. The authors concluded that there were at least four enzymes involved in the metabolism of n-hexane to 1-, 2-, and 3-hexanol, in the liver but could not identify these enzymes from the kinetic data. The K_m and V_{max} values indicate that 1- and 2-hexanol are the favored hydroxylation products in the liver. The reaction requirement for NADPH suggests that these enzymes may be cytochrome P450 (CYP450) isozymes.

The Eadie-Scatchard plots for lung microsomes suggest that a single enzyme is responsible for the hydroxylation of n-hexane to 1-, 2-, and 3-hexanol in this tissue. The kinetic parameters for each of the lung metabolites are presented in Table 3-5. Based on the low K_m, and accompanying V_{max} , 1-hexanol is the favored product in the lungs. The enzymes responsible for the formation of 2-hexanol and 3-hexanol have similar affinities for n-hexane.

CYP450 enzymes catalyze the initial steps (either detoxification or bioactivation) involving hydroxylation in the metabolism of n-hexane. Specifically, the enzymes responsible for the metabolism of n-hexane have been investigated in vivo. Nakajima et al. (1991) characterized the CYP450 enzymes that are induced following exposure to n-hexane in male

Wistar rats. The authors used phenobarbital, n-hexane, 2-hexanone, and 2,5-hexanedione to induce different CYP450s to which they also raised monoclonal antibodies. The enzyme activities of the CYP450 isozymes 2E1, 2C2/6, 1A1/2, and 2B1/2 were measured indirectly by benzene aromatic hydroxylase activity, toluene side chain oxidation, ethoxyresorufin O-deethylase (EROD) activity, and pentoxyresorufin O-depentylase (PROD) activities, respectively. There was increased activity of benzene aromatic hydroxylase in liver microsomes from n-hexane-treated rats, indicating the induction of CYP2E1. Conversely, there was no increase in PROD or EROD activities in microsomal preparations from n-hexane-treated rats compared to control preparations, indicating that n-hexane did not specifically induce CYP2A1/2 or CYP2B1/2. 2,5-hexanedione induced CYP2E1 and, to some extent, CYP2B1/2, suggesting that more than one CYP450 species may be involved in the overall conversion of n-hexane to its metabolic products. n-Hexane and 2-hexanone increased CYP2E1 to a similar extent when measured in an immunoinhibition assay of toluene side-chain oxidation. In addition, 2-hexanone induced CYP2B1/2 to a lesser extent than phenobarbital treatment.

Iba et al. (2000) demonstrated in in vivo studies that CYP2E1 may be involved in the metabolism of n-hexane to 2,5-hexanedione. CYP2E1 knockout mice and control mice were administered daily intraperitoneal injections of n-hexane (200 mg/kg) for up to 21 days. CYP2E1 knockout and control mice had similar urinary levels of 2,5-hexanedione on day 10 of administration (6.1 and 4.3 µg/mL in the CYP2E1 knockout and control mice, respectively). Levels of 2,5-hexanedione continued to increase in control mice on days 14 and 21 (22.9 and 16.1 µg/mL) but not in CYP2E1 knockout mice. These data indicate that CYP2E1 may be involved in the metabolism of n-hexane to 2,5-hexanedione following prolonged daily exposures.

In vitro studies also indicate the involvement of CYP450 enzymes in n-hexane metabolism (specifically the CYP2B1 enzyme), primarily leading to the formation of 2- and 3-hexanol (bioactivation pathway). Toftgard et al. (1986) evaluated the role of the phenobarbital-inducible CYP450 isozymes (CYP2B1 and 2B2) and β-naphthoflavone-inducible CYP450 isozyme (CYP1A1) in n-hexane hydroxylation. Specific isozyme preparations were isolated from rat livers after exposure to the appropriate inducer and the isozymes involved in the production of hexanols were identified. Production of 1-, 2-, and 3-hexanol was measured relative to time and expressed as nmol metabolite/minute-nmol enzyme protein (turnover number). The enriched CYP2B1 preparation produced 2-, 3-, and 1-hexanol with a turnover ratio of approximately 30:10:1. The CYP2B2 turnover ratio was similar to CYP2B1 (20:8:1 for 2-, 3-, and, 1- hexanol, respectively). Turnover numbers were highest for CYP2B1 and lowest

for CYP2A1. CYP2B1/2 primarily produced 2-hexanol, whereas CYP2A1 primarily produced 3-hexanol. All three isozymes had low turnover numbers for production of 1-hexanol.

Although Toftgard et al. (1986) did not unequivocally demonstrate the identities and numbers of the CYP450 isozymes involved in the hydroxylation of n-hexane in the liver, the isozyme turnover data in combination with the kinetic data presented above support the hypothesis that 2-hexanol is the primary n-hexane metabolite in rat liver. In addition, Toftgard et al. (1986) used antibodies to inhibit these isozymes to evaluate the role of each isozyme in n-hexane metabolism. Anti-CYP2B1 inhibited the formation of 2- and 3-hexanol but not 1-hexanol. Anti-CYP1A1 had little antagonistic effect on the formation of any of the metabolic products.

Crosbie et al. (1997) used metyrapone, a specific inhibitor of CYP2B1/2 to monitor the appearance of 1-, 2-, and 3-hexanol in liver and lung microsomes from male Wistar rats that were incubated with n-hexane. The inhibitor did not affect the amounts of 1-hexanol produced by liver and lung microsomes, but 2-hexanol levels were reduced by 33% in liver microsomes and by 74% in lung microsomes. 3-hexanol levels were reduced by 31% in liver microsomes (not statistically significant compared to preparations without inhibitor) and by 92% in lung microsomes. This near complete abolition of the 3-hexanol-producing activity of n-hexane-incubated lung microsomes suggests that CYP2B1 is important for n-hexane hydroxylation and detoxification in this organ. The partial reductions of 2-hexanol production in metyrapone-treated liver and lung microsomes suggests the partial involvement of CYP2B1 in hydroxylation for bioactivation.

Several studies have demonstrated that the presence of other chemicals may affect the metabolism of n-hexane. Van Engelen et al. (1997) examined the effects of coexposure to methyl ethyl ketone on the toxicokinetics of n-hexane in human volunteers. Subjects (four to five persons) were exposed to 60 ppm n-hexane for 15.5 minutes with or without coexposure to 200 or 300 ppm methyl ethyl ketone. Each subject served as their own control by being exposed on the same day to n-hexane or methyl ethyl ketone alone and to the mixture of both solvent vapors. Methyl ethyl ketone had no effect on the concentration-time course for exhaled n-hexane, but the concentration-time course for 2,5-hexanedione appearance in serum was threefold lower after coexposure to methyl ethyl ketone. The authors suggested that one of the intermediate steps in the conversion of n-hexane to 2,5-hexanedione may have been inhibited by coexposure with methyl ethyl ketone.

Studies in laboratory animals also demonstrate the effect of coexposure to other solvents on n-hexane metabolism. Robertson et al. (1989) demonstrated that 1.87 mL/kg methyl ethyl

ketone given by gavage to male F344 rats 4 days prior to a single 6-hour inhalation exposure to 1000 ppm n-hexane increased the concentration of 2,5-hexanedione in blood, sciatic nerve, and testis up to 10-fold. 2,5-Dimethylfuran was also detected in increased quantities as a result of coexposure to methyl ethyl ketone.

Shibata et al. (1990a) monitored the appearance of 2-hexanol, 2,5-hexanedione, and 2,5-dimethylfuran in the urine for up to 48 hours after the start of a single 8-hour exposure of six male Wistar rats/group to either 2000 ppm n-hexane alone or 2000 ppm n-hexane containing either 200, 630, or 2000 ppm methyl ethyl ketone. Both total (free and conjugated) and free n-hexane metabolite levels were decreased in urine of rats exposed to a mixture of n-hexane and methyl ethyl ketone (significant in the 2000 ppm n-hexane plus 2000 ppm methyl ethyl ketone exposure group).

Shibata et al. (1990b) also demonstrated lower concentrations of 2,5-hexanedione and 2-hexanone in the serum of rats coexposed to 2000 ppm n-hexane and 2000 ppm methyl ethyl ketone compared to rats receiving n-hexane alone. The area under the serum concentration curve for 2,5-hexanedione was 109.35 µg/mL/24 hours in rats exposed to n-hexane alone compared to 23.7 µg/mL/24 hours in rats coexposed to n-hexane and methyl ethyl ketone.

Iwata et al. (1983) treated five male Wistar rats/group with a single 8-hour inhalation exposure of either 1000 ppm n-hexane, 1000 ppm n-hexane plus 1000 ppm toluene, 1000 ppm n-hexane plus 1000 ppm methyl ethyl ketone, or fresh air. The authors evaluated the levels of 2,5-dimethylfuran, 2-hexanone, 2-hexanol, 2,5-hexanedione, and γ-valerolactone in urine (samples were acid-hydrolyzed) following exposure to either n-hexane alone or to solvent mixtures. The total concentrations of metabolites decreased by approximately one-sixth following coexposure to n-hexane and toluene and one-fourth following coexposure to n-hexane and methyl ethyl ketone.

In a more recent study, Cardona et al. (1996) reported the effects of acetone on n-hexane metabolism and elimination. These authors analyzed the relationship between exposure to these solvents and the concentrations of free and total (samples were acid-hydrolyzed) 2,5-hexanedione in the urine. Environmental monitoring and urinary samples were obtained from 87 workers in the shoe industry in Spain. Environmental concentrations of n-hexane, toluene, and acetone were monitored at each subject's workplace for the final 2–4 hours (averaging 200 minutes) of a work shift. Urine and alveolar (exhaled) air samples were collected 15 minutes after the end of the work shift. The median concentrations of n-hexane, toluene, and acetone in the workplace were 47 mg/m^3 (range of 4–652 mg/m^3), 57 mg/m^3 (range of 12–683 mg/m^3), and 109 mg/m^3 (70 cases evaluated; range of 1–1826 mg/m^3), respectively. The level of

free 2,5-hexanedione in the urine of exposed workers was about 12% of total urinary 2,5-hexanedione. Total urinary 2,5-hexanedione concentration was significantly correlated (p<0.001) with environmental n-hexane exposure (r = 0.936) and n-hexane in exhaled air (r = 0.7435). Acetone was statistically significantly correlated (p<0.001) with the ratios of total or free 2,5-hexanedione and atmospheric n-hexane concentrations (r = 0.6459 and 0.6965, respectively). In addition, there was significant correlation (p<0.001; r = 0.96626 and 0.94217, respectively) between total and free 2,5-hexanedione concentrations and n-hexane and acetone environmental exposures, cutaneous absorption (glove use), interaction of n-hexane and acetone, and day of the week.

3.4. ELIMINATION

A single study in humans suggests elimination following oral exposure to n-hexane. Specifically, Baelum et al. (1998) collected urine from human volunteers immediately following exposure to n-hexane via a gastric feeding tube and at 1, 2, 3, and 4.5 hours postexposure. These samples contained 2,5-hexanedione at a mean concentration of 0.22 ± 0.10 μmols/L. No oral exposure studies in laboratory animals are available indicating elimination of n-hexane.

Several human inhalation studies have provided evidence for the elimination of n-hexane and metabolites following occupational and voluntary exposures to n-hexane. Imbriani et al. (1984) measured the amount of parent n-hexane in the urine of 30 shoe workers who were exposed to n-hexane in the workplace. The employees wore personal samplers that provided estimates of the amount of n-hexane in the air that ranged from 13 to 197 mg/m^3 (3.7–56 ppm). The median concentration of n-hexane in the urine was 4.8 μg/L, with an overall correlation coefficient of 0.84 for the 30 subjects.

Mutti et al. (1984) monitored 10 workers exposed to n-hexane in a shoe factory (8 hour TWA of 243 ppm n-hexane). Alveolar excretion of n-hexane accounted for 10% of the total uptake. Among the metabolites eliminated in urine (samples were collected pre-shift, end of shift, and the next morning and metabolites were measured following acid hydrolysis) were 2,5-hexanedione, 2,5-dimethylfuran, 2-hexanol, and γ-valerolactone. The authors indicated that end of shift 2,5-hexanedione levels were the best estimate of n-hexane exposure. Urinary 2,5-hexanedione excretion of approximately 3 mg/g creatinine was considered to indicate exposure to 50 ppm n-hexane.

In a follow-up study, Mutti et al. (1993) observed a weaker correlation between n-hexane exposure in the workplace and the amount of 2,5-hexanedione in the urine of exposed individuals. The study authors indicated that 2,5-hexanedione levels may build up during the

course of a workweek; therefore, urinary levels may not consistently reflect the ambient n-hexane exposure concentration.

Several other human studies support the assertion that 2,5-hexanedione levels in urine are the best estimate of n-hexane exposure in the workplace. For example, Ahonen and Schimberg (1988) documented 2,5-hexanedione excretion in the urine of four healthy female shoe workers who were exposed to varying amounts of n-hexane, along with acetone, toluene, and other solvents. n-Hexane and other organic solvent concentrations were measured in the breathing zone of the workers. Urine samples were collected from each worker during the experiment and the following weekend. Regression data between the 8-hour TWA concentration of n-hexane in the air and urinary 2,5-hexanedione for the three most heavily exposed workers at the different sampling times indicated that a 180 mg/m^3 (50 ppm) 8-hour TWA concentration of n-hexane in air would result in a urinary 2,5-hexanedione concentration of 10 ± 3 µmol/L.

Saito et al. (1991) correlated the amount of 2,5-hexanedione in urine with exposure of 50 individuals to n-hexane at various concentrations. Saito et al. (1991) performed acid hydrolysis to ensure that any conjugated urinary metabolites of 2,5-hexanedione, such as 4,5-dihydroxy-2-hexanone, were converted to 2,5-hexanedione prior to assay, giving a total value for the subject metabolite. Urinary concentrations of 2,5-hexanedione measured in this manner showed a good correlation with exposure to n-hexane (r = 0.973).

Cardona et al. (1993) analyzed working conditions and environmental exposure to solvents in 27 shoe factories in Italy and Spain and measured end-of-shift total 2,5-hexanedione concentrations in urine. They reported that urinary concentrations of 2,5-hexanedione tended to increase during the workweek, although a significant linear correlation was obtained between mean environmental concentrations of n-hexane and urinary concentrations of the metabolite. Concentrations of 2,5-hexanedione in end-of-shift urine ranged from 0.2 to 24.2 mg/L, with an arithmetic mean of 6.3 ± 4.9 mg/L. Variability in the correlation was thought to have been due to differing practices among the subjects in the use of protective clothing and rubber gloves. Percutaneous absorption of n-hexane was thought to have occurred in some cases.

Mayan et al. (2001) analyzed urine samples from 45 Portuguese shoe workers for total 2,5-hexanedione and correlated these values with measured amounts of n-hexane in workplace air. The urine samples, which were collected 1 hour before the end of the shift, had a geometric mean 2,5-hexanedione concentration of 2.68 mg/g creatinine. The individual values ranged from 0.6 to 8.5 mg/g creatinine and correlated (r = 0.85) with personal air sample n-hexane concentrations ranging from 6 to 70 ppm.

A similar study by the same research group in 111 shoe workers showed a positive

correlation between workplace n-hexane concentrations ranging from 5–70 ppm and total 2,5-hexanedione concentrations in urine of 0.12–14.25 mg/g creatinine (Mayan et al., 2002).

Dos Santos et al. (2002) evaluated the amounts of free and total 2,5-hexanedione in the urine of 52 Brazilian shoe workers and categorized the subjects according to the mean concentrations of n-hexane to which they were exposed during the course of their work. No numerical exposure data for n-hexane were provided in the report, but subjects (14) who applied glue with a paintbrush had higher concentrations of 2,5-hexanedione in their urine than individuals who used a glue handgun (total 2,5-hexanedione 1.5 versus 0.7 mg/L). Eleven subjects who worked under a fume hood had the lowest urinary 2,5-hexanedione concentrations (0.08 mg/L).

Prieto et al. (2003) monitored free and total 2,5-hexanedione and 4,5-dihydroxy-2-hexanone in the urine of 132 Spanish shoe workers who were exposed to n-hexane ranging from 4–709 mg/m^3 (1–200 ppm). Most subjects were exposed to other solvents during the course of their work, such as toluene, methyl ethyl ketone, other hexane isomers, heptane, acetone, and ethyl acetate. The amounts of total urinary 2,5-hexanedione gave the best correlation with levels of exposure to n-hexane ($r = 0.91$). Concentrations of the metabolite ranged from 0.3–32.46 mg/L.

Studies in animals are also available, suggesting the elimination of n-hexane metabolites in urine following exposure to n-hexane via inhalation. Bus et al. (1982) exposed F344 rats (three/group) to a single 6-hour exposure of either 500, 1000, 3000, or 10,000 ppm n-hexane (95.5% pure) containing 11.8–54.9 µCi/mmol [1,2-^{14}C]-n-hexane. More than 50% of the recovered radioactivity was expired as $^{14}CO_2$ or excreted in the urine. Similarly, Baker and Rickert (1981) administered a single 6-hour inhalation exposure of 500, 1000, 3000, or 10,000 ppm n-hexane to male F344 rats. Urinary elimination of metabolites during the 72-hour period following exposure included 2-hexanone, 2,5-hexanedione, 5-hydroxy-2-hexanone, 2-hexanol, and dimethylfuran. The total amounts of dimethylfuran and 2,5-hexanedione were higher in urine samples that were acid-hydrolyzed compared with untreated urine (Table 3-6). This change was probably caused by the dehydration or hydrolysis of conjugated metabolites to dimethylfuran or 2,5-hexanedione under the acidic conditions (Fedtke and Bolt, 1986).

Table 3-6. Metabolites excreted in urine during a 72-hour period following inhalation exposure to n-hexane in male F344 rats

Exposure concentration (ppm)	Metabolite	Total metabolites formed (µg)[a]		
		No treatment	Hydrolysis with β-glucuronidase	Hydrolysis with 3N HCl
500	2-Hexanone	0.4 ± 0.1	0.8 ± 0.0	1.5 ± 0.2
	Dimethylfuran	7.0 ± 4.0	14.3 ± 0.5	162.0 ± 2.0
	2,5-Hexanedione	4.0 ± 1.0	3.8 ± 0.2	9.9 ± 0.2
	5-Hydroxy-2-hexanone	3.3 ± 0.7	0.5 ± 0.1	2.3 ± 0.0
	2-Hexanol	ND	1.5 ± 0.3	ND
1000	2-Hexanone	1.3 ± 0.2	1.3 ± 0.1	4.0 ± 0.1
	Dimethylfuran	1.2 ± 0.2	86.2 ± 5.1	194.3 ± 60.2
	2,5-Hexanedione	3.3 ± 0.2	4.8 ± 0.6	72.4 ± 6.1
	5-Hydroxy-2-hexanone	2.9 ± 0.01	33.0 ± 2.2	5.8 ± 0.9
	2-Hexanol	ND	0.6 ± 0.1	ND
3000	2-Hexanone	8.6 ± 0.3	15.1 ± 1.0	10.1 ± 2.4
	Dimethylfuran	17.0 ± 2.0	357.4 ± 49.2	879.3 ± 231.1
	2,5-Hexanedione	44.4 ± 0.5	50.3 ± 4.3	222.4 ± 21.0
	5-Hydroxy-2-hexanone	41.5 ± 0.8	38.1 ± 2.4	45.4 ± 2.3
	2-Hexanol	1.3 ± 0.2	9.0 ± 3.1	3.5 ± 0.2

[a] Values are means for three animals ± SEM.
ND = Not detected.

Source: Baker and Rickert, 1981.

Frontali et al. (1981) exposed Sprague-Dawley rats (six to nine/group) to 500, 1000, 2500, or 5000 ppm n-hexane, 9–10 hours/day, 5 days/week for up to 30 weeks. Some animals were transferred to metabolic cages after exposure to permit the collection of overnight urine samples. Metabolites of n-hexane identified in β-glucuronidase and acid hydrolyzed urine samples included 2,5-dimethylfuran, γ-valerolactone, 3-hexanol, 2-hexanol, and 2,5-hexanedione.

Perbellini et al. (1982) studied the metabolic interaction between n-hexane and toluene in vivo in six male Wistar rats/group following intraperitoneal administration of either 200 mg/kg

n-hexane, 200 mg/kg n-hexane plus 200 mg/kg toluene, or 200 mg/kg toluene alone. Amounts of n-hexane metabolites obtained in 24-hour urine samples were lower in animals receiving n-hexane mixed with toluene (Table 3-7).

Table 3-7. n-Hexane metabolite levels in urine of Wistar rats coexposed to n-hexane and toluene

Metabolite	Treatment	
	n-hexane (200 mg/kg)[a]	n-hexane (200 mg/kg) and toluene (200 mg/kg)[a]
2-Hexanol	230.42 ± 145.83	88.74 ± 54.16[b]
2,5-Hexanedione	138.97 ± 58.91	72.93 ± 41.06[b]
2,5-Dimethylfuran	91.57 ± 35.83	32.50 ± 11.94[c]
γ-Valerolactone	47.67 ± 20.71	16.70 ± 11.56[c]

[a] Data are means ± SD.
Significantly different from n-hexane: [b] $p<0.005$, [c] $p<0.01$, as calculated by the authors.

Source: Perbellini et al., 1982.

3.5. PHYSIOLOGICALLY BASED TOXICOKINETIC MODELS

A series of reports by Perbellini and coworkers described the construction of a physiologically based toxicokinetic (PBTK) model for the distribution of n-hexane and its metabolites among eight functionally described compartments of the body (Perebellini et al., 1990, 1986, 1985). The compartments represent (1) the site of intake (lungs), (2) highly vascularized tissues, (3) a muscle group, (4) a fat group, (5) a metabolizing center, and (6) three other compartments important in the kinetics of metabolism (biotransformation, water, and urinary compartments). The scheme, displayed in Figure 3-2, shows the catabolism of n-hexane, with the production of 2,5-hexanedione and its subsequent transfer to the water and urinary compartments. It is assumed that the chemical instantly establishes a balance between alveolar air and venous blood and that the chemical is in equilibrium with each tissue compartment. However, in the model the liver is theoretically considered to be the only site at which metabolites of n-hexane are formed, and all rate constants are assumed to be first order (Perbellini et al., 1986).

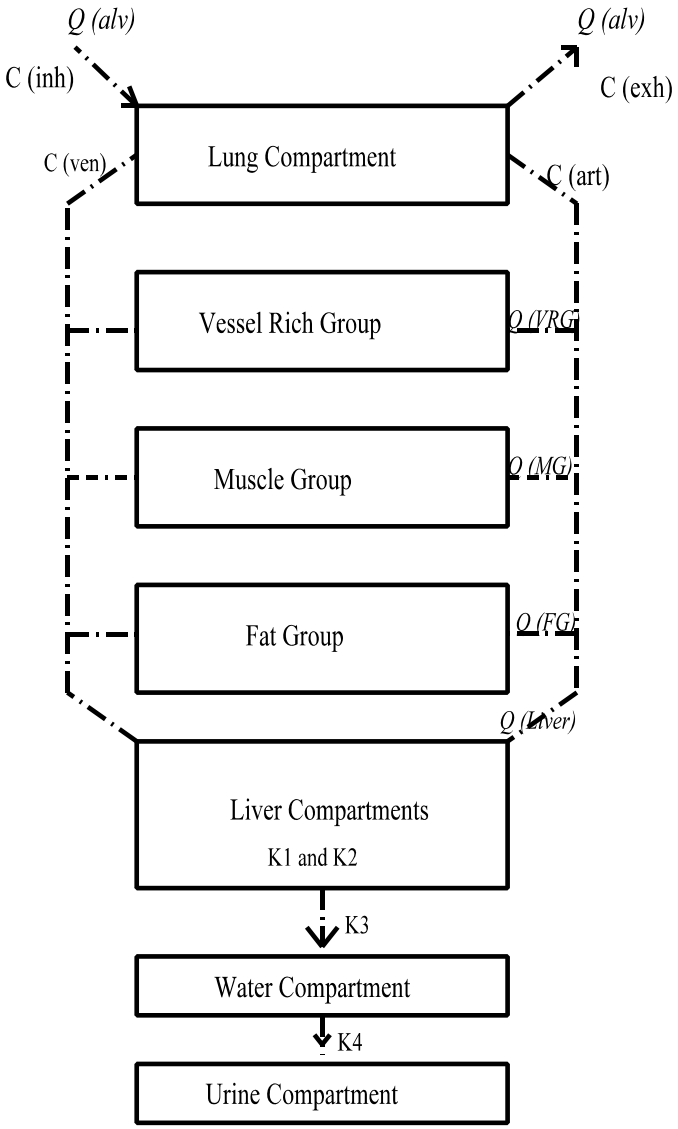


Figure 3-2. Physiologically based toxicokinetic model of the distribution of n-hexane in the body and the urinary excretion of 2,5-hexanedione.

Source: Perbellini et al., 1986.

Perbellini and coworkers obtained much of their data on tissue volumes and blood flows from the scientific literature (Perbellini et al., 1985; Mapleson, 1973). This information was combined with experimental data on the partition of n-hexane between air and various media and extracts of human tissue obtained at autopsy (Perbellini et al., 1985). At the core of the PBPK model was a series of differential equations that described: (1) the concentration of n-hexane reaching the lungs in venous blood; (2) the rate of change of n-hexane in the pulmonary compartment and the rate of change of the arterial blood n-hexane concentration; (3) the rate of change of n-hexane in the liver and the resulting amount of hepatic 2,5-hexanedione; (4) the amount of 2,5-hexanedione in the water compartment; and (5) the rate of change of 2,5-hexanedione in the urine. Solving the set of differential equations simultaneously for various hypothetical exposure scenarios allowed the model to be tested against experimental data on n-hexane and 2,5-hexanedione concentrations that had been reported for human volunteers by Veulemans et al. (1982). Key findings from this comparison were that when modeling hypothetical human exposures (approximately 100 ppm for 4 hours) the resulting n-hexane concentration in the venous blood (176 μg/L) was similar to the measured concentration in human volunteers (207 ± 32 μg/L) (Veulemans et al., 1982). The concentration of n-hexane declined rapidly at cessation of exposure, with the model showing close agreement to the charted experimental data (Perbellini et al., 1986). The latter was best described by the regression equation:

$$C_v \text{ (mg/L)} = Ae^{-bt}$$

with values for the constants of 0.114 (mg/L) for A, and 0.0074 $(\text{min})^{-1}$ for b. The half-life for n-hexane in the blood was 94 minutes (Veulemans et al., 1982).

The key utility of the model was to simulate occupational conditions that had been found in factories where n-hexane-containing products are used and where exposure is constant. When the hypothetical exposure duration was extended to 8 hours, the concentration of n-hexane in the fat compartment was shown to follow an upward trend, though with broad fluctuations, representing the interval between shifts. Similarly, the concentration of 2,5-hexanedione in urine displayed peaks and troughs without dropping to negligible levels until a time point after the last exposure representing the latter part of the weekend. Perbellini et al. (1990) drew attention to the persistence of n-hexane in the fat compartment and reported a half life of 64 hours in this tissue group. This half-life suggests that accumulated n-hexane in fat could not be completely excreted by the start of the following workweek, and that near to complete excretion of n-hexane

in fat would require more than 10 days of no further exposure.

Perbellini et al. (1990) used their approach to evaluate the likely impact on the biological exposure index (BEI) of the 50 ppm threshold limit value (TLV) proposed for n-hexane for 1988–1989 by the American Conference of Governmental Industrial Hygienists (ACGIH, 2003). As simulated by the model, urinary concentrations of 2,5-hexanedione ranged from 2.4 to 2.9 mg/L before the start of the first shift of the work week and from 3.3 to 4.3 mg/L on the morning of the following work days. Fisher et al. (1997) used a generic human lactation PBTK model that was developed using published human and animal PBTK model parameters to simulate the transfer of 19 volatile chemicals, including n-hexane, from a nursing mother to her infant during breast feeding. The model was used to estimate the amount of chemical that would be transferred during a given nursing schedule, assuming resumed occupational exposure after childbirth and maternity leave. Specifically, the five-compartment model of Ramsey and Andersen (1984) was adapted by the incorporation of a milk compartment that changed in volume in response to a nursing infant. For n-hexane, rodent tissue solubility and allometrically scaled metabolic rate constants available in the published literature were used to estimate human tissue metabolic parameters for the model. Blood:air and milk:air partition coefficients were determined by running the model for a simulated maternal exposure at the TLV of 50 ppm n-hexane. This simulation predicted the amount of chemical that would be ingested by an infant over a 24-hour period. The amount of n-hexane ingested by an infant was 0.052 mg (Fisher et al., 1997). The Fisher et al. (1997) model does not specifically address target tissues or extrapolate between species or routes and has not been validated. n-Hexane levels in breast milk have not been quantified for measured exposures to n-hexane. The authors suggested that the absence of exposure and toxicokinetic data on lactational transfer of chemicals such as n-hexane to nursing infants is a disadvantage of this model.

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS—EPIDEMIOLOGY AND CASE REPORTS

4.1.1. Oral Exposure

No studies were identified that address the toxic effects of n-hexane in humans via the oral route.

4.1.2. Inhalation Exposure

4.1.2.1. *Subchronic Exposure*

No subchronic exposure studies were identified that addressed the toxic effects of n-hexane in humans via the inhalation route.

4.1.2.2. *Chronic Exposure*

Beall et al. (2001) conducted a nested case control study evaluating the relationship between the occurrence of intracranial tumors among employees at a petrochemical plant and exposure to agents, including ionizing radiation, methylene chloride, acrylonitrile, vinyl chloride, formaldehyde, n-hexane, and various other chlorinated, halogenated, volatile, and aromatic hydrocarbons and nitroso compounds. The workers were also exposed to organometallic and elemental metallic catalysts. The study authors selected subjects from approximately 2595 plant workers. The workers were mailed questionnaires that evaluated work history in the plant, and a total of 12 cases of intracranial tumors was identified that had been diagnosed among respondents after they had been hired at the plant. All cases were confirmed by review of medical records and pathology specimens by four neuropathologists. Six of these cases, all of which were men, had primary brain cancers or gliomas (two astrocytomas, two oligodendrogliomas, and two glioblastomas). Six cases had benign intracranial tumors, of which two were diagnosed as vestibular schwannomas (observed in one man and one woman), two as meningiomas (both in men), and two pituitary adenomas (observed in one man and one woman). Ten healthy controls were matched to each case by gender, birth year (± 2 years), race, and a start date for work in the building complex that preceded the tumor diagnosis date for the matched case. The median length of employment at the facility was 16.8 years for cases and 10.9 years for controls.

Work histories were obtained from company records or interviews, the latter providing information about complete work history, exposures encountered, extent of hands-on work at each job, and incidence of certain other nonoccupational factors that may be related to risk of occurrence of brain cancers and intracranial tumors (exposure to diagnostic irradiation, use of anticonvulsant and ototoxic drugs, history of head trauma, seizures, meningitis, use of cellular phones and radiation badges, amateur radio operation, pesticide application, furniture refinishing, and history of hearing loss). Exposure information was obtained from company accounting records that detailed hours worked on projects during each year of employment and self-reported workplace exposure to chemicals of interest. The authors compared cases and controls with respect to self-reported exposure to chemicals of interest, project-based work histories indicating the potential use of chemicals of interest, and self-reported exposure to any of the other nonoccupational factors that may be related to the risk of brain cancers. Conditional regression was used and maximum likelihood estimates of odds ratios (ORs) with a 95% confidence interval (CI) were reported.

The authors showed that the OR for self-reported exposure to n-hexane was statistically significantly elevated (OR, infinity), with a CI of 1.4 to infinity (6 cases and 26 controls evaluated) for gliomas. The OR for potential exposure to n-hexane based on job-related exposure estimates was 2.3 (CI, 0.4–13.7; four cases and 26 controls evaluated) for gliomas. Analyses by duration indicated a statistically significantly elevated OR of 16.2 (CI, 1.1–227.6; two cases and two controls evaluated) for potential long-term exposure to n-hexane (>48 months) for gliomas. No relationship was found between exposure to n-hexane and the occurrence of intracranial tumors.

Sanagi et al. (1980) compared peripheral nervous system (PNS) function in n-hexane-exposed and unexposed workers. Fourteen employees working in the mixing and drying jobs (during the study period) at a factory producing tungsten carbide alloy for 1–12 years (average of 6.2 years) served as the n-hexane exposed group. In addition, a group of five workers who had been mixers in the past (exposure for 1–16 years with an average of 5.2 years), but who were not engaged in these jobs at the time of the study, were classified as exposed in the past. Fourteen workers from the same factory who were not exposed to any solvents served as controls in this study. All subjects were males under 50 years of age and free of metabolic diseases and lifestyle factors that may affect the PNS. Twenty-two breathing zone monitoring samples taken twice a year over a 2-year period indicated an 8-hour TWA of 58 ppm for n-hexane and 39 ppm for acetone. No other solvent vapors were detected. Medical examinations consisted of interviews, questionnaires, clinical neurological examinations, and neurophysiological testing. The

questionnaire was comprised of 23 questions concerning neurological symptoms. Three questions regarding hearing deficit, vision disturbance, and writing deficit were answered with either present or absent. The remaining 20 questions were answered with always, sometimes, or absent. The neurological exams and neurophysical tests were conducted by the study authors without knowledge of participants' study group status.

Overall, no individual worker had obvious signs of PNS damage. However, compared to unexposed workers, exposed workers as a group reported a statistically significant increased incidence of headache, hearing deficit, dysesthesia in limbs, and muscle weakness (specific muscles not indicated) as reported on a questionnaire (Table 4-1). The authors classified these symptoms as either persistent or persistent and transient.

Sanagi et al. (1980) also carried out a number of neurological tests to identify exposure-related neurological signs of n-hexane toxicity (Table 4-2). No objective neurological symptoms related to muscle strength by manual testing, muscle wasting, or muscle tone were reported. Statistically significant exposure-related deficits in muscle strength (as determined by jump test on one foot) and reduced vibration sensation of the radial processes (determined by the tuning fork test for vibration sensation) in the exposed group (average group values) were observed compared to controls.

Table 4-1. Persistent and transient neurological symptoms following occupational exposure to n-hexane in a tungsten carbide alloy factory

Symptom	Incidence of symptoms (%)		
	Exposed	Exposed in the past	Controls
Headache	86[a]	60	43
Heaviness in head	71	40	43
Vertigo/dizziness	50	60	38
Anosmia/dysnosia	46	20	14
Vision disturbance[b]	57	40	43
Double vision	36	20	21
Tinnitus	36	40	29
Hearing deficit[b]	71[a]	20	14
Dysphagia	29	0	29
Dysarthria	14	40	21

Pain in neck/arm	71	80	43
Lumbago	54	60	50
Arthrodynia	36	20	29
Muscle pain	50	20	14
Sensitivity to cold	8	20	14
Limb dysesthesia	29[a]	40	0
Limb numbness	21	40	0
Stiff shoulders	64	100	64
Tired arms	57	40	36
Tired legs	79	80	46
Muscle weakness	29[a]	40	0
Writing impairment[b]	14	40	36
Unsteady gait	21	20	7

[a] Statistically significant from controls (p<0.05).
[b] Symptoms classified as persistent, no data are presented as persistent and transient.

Source: Sanagi et al., 1980.

Table 4-2. Results of neurological tests in control subjects and those occupationally exposed to n-hexane in a tungsten carbide alloy factory

Test (units)	Exposed group[a]	Controls[a]
Muscle strength		
Grip power (kg)	45.3 ± 2.9	44.9 ± 5.2
Jumping on one foot (cm)	21.3 ± 3.6[b]	26.0 ± 6.2
Vibration sensation		
Radial processes (s/16s)	13.8 ± 2.4[b]	15.4 ± 1.6
Medial malleoli (s/16s)	12.2 ± 2.1	13.4 ± 2.0
Position sense		
Barrany's test (cm)	0.8 ± 0.4	0.7 ± 0.5
Mann's test (%)	21	0
Coordination skills		
Knee slapping (times/15s)	24.8 ± 4.8	24.5 ± 2.8

Floor tapping (times/15s)	39.9 ± 7.7	42.6 ± 6.0

[a] Values are means ± SD.
[b] Statistically significant versus controls ($p<0.05$).

Source: Sanagi et al., 1980.

Neurophysiological findings indicated a slowing of MCV in the posterior tibial nerve, with delayed recovery in exposed groups compared to controls (Table 4-3). These findings are consistent with the neurological signs and subject-reported symptoms generally associated with n-hexane exposure.

Table 4-3. Nerve stimulation in control subjects and those occupationally exposed to n-hexane in a tungsten carbide alloy factory

Test (units)	Right median and ulnar nerves[a]		Right posterior tibial nerve[a]	
	Exposed group	Controls	Exposed group	Controls
Motor nerve conduction velocity (m/s)	57.3 ± 3.4	57.5 ± 3.2	46.6 ± 2.3[b]	48.3 ± 2.1
Muscle action potential ratio (%)	97.2 ± 5.2	100.3 ± 5.0	90.1 ± 7.4	88.9 ± 11.8
Residual latency (ms)	2.26 ± 0.46	2.19 ± 0.32	2.55 ± 0.48[b]	2.21 ± 0.34
Conduction velocity of slow fibers (m/s)	48.5 ± 4.5	49.9 ± 4.4	38.6 ± 2.2	39.1 ± 1.5
Distal sensory conduction velocity (m/s)	66.4 ± 6.9	65.2 ± 5.9	42.6 ± 5.0	41.7 ± 3.9
Mixed nerve conduction velocity (m/s)	72.5 ± 3.4	71.3 ± 3.8	59.1 ± 3.4	60.2 ± 3.3

[a] Values are means ± SD.
[b] Significantly different from controls ($p<0.05$).

Source: Sanagi et al., 1980.

Mutti et al. (1982a) monitored MCV in a group of 95 shoe factory workers exposed to a hydrocarbon mixture containing n-hexane, cyclohexane, methyl ethyl ketone, and ethyl acetate, and compared them to 52 unexposed workers from the same factory. Gender, age, and employment time were similar in the exposed and referent groups. Both groups were free of individuals suffering from diseases affecting the PNS. Neurological symptoms occurred more frequently among the exposed than the unexposed workers. These included statistically significant increases in the frequency of self-reported sleepiness, dizziness, weakness in the limbs, paresthesia (burning or tingling sensation in limbs), and hypoesthesia (partial loss of sensation and/or diminished sensibility). Electroneurographic measurements identified a statistically significant increased motor nerve action potential (MAP) duration and decreased

MCV in the median and ulnar nerves in exposed workers compared with unexposed workers. Exposed workers were divided into two groups (mild- and high-exposure) based on hydrocarbon exposure in their main jobs. The two groups were determined by exposure score, taking into account time in job and hygienic effect (defined as the ratio between the measured concentration of n-hexane and the proposed 1979 ACGIH TLV values for n-hexane). The TWA for n-hexane of the 108 breathing zone samples taken was 243 mg/m^3 (69 ppm) in the mildly exposed group and 474 mg/m^3 (134 ppm) in the highly exposed group. The MCV of the median nerve and the MAP duration of the ulnar nerve were related to hydrocarbon exposure. The median for the hygienic effect was 0.81 and 1.91 for the mild and highly exposed groups, respectively. The authors stated that estimates of past exposure concentrations were most likely underestimated and hygienic effects were higher due to industrial improvements implemented prior to the study.

Mutti et al. (1982b) also compared 15 women in a shoe factory and 15 healthy, age-matched female workers at four other shoe factories with no known exposure to neurotoxic substances or metabolic diseases affecting the PNS. Exposure times ranged from 2 to 8 years. The breathing zone TWA of organic solvents was measured 36 times at each workplace over 3 years. Over 50% of the samples exceeded the ACGIH TLV for technical grade hexane (a mixture containing n-hexane, cyclohexane, methyl ethyl ketone, and ethyl acetate) and was occasionally as high as 5000–7000 mg/m^3 (1422–1990 ppm). The median value for n-hexane was 448 mg/m^3 (127 ppm), and the median hygienic effect was 1.24. The study began 3 months after industrial hygiene improvements had been made to the factory (solvent levels decreased to trace amounts) and continued for 6 more months. The authors stated that the subjects were considered as past-exposed and therefore any detectable toxicity could be considered as a chronic effect. The results of neurophysiological examinations of the peroneal, ulnar, and median nerves in exposed subjects showed a significant reduction in the maximal MCV and the distal sensory nerve conduction velocity (SCV) compared with controls. There was also increased latency in the somatosensory evoked potential (SEP) of exposed workers compared with the unexposed workers. The distal SCV and the latency in the proximal segment of the tibial nerve were negatively correlated, reflecting a peripheral neuropathy in which increased signal latency and reduced conduction velocities were apparent in the same nerve. In addition, the SEP was flatter in the exposed group than in the referent group, indicating a block in central conduction and suggesting the possibility of n-hexane effects on the central nervous system (CNS). Indeed, studies in rats (Schaumburg and Spencer, 1976) showed that neurofilament (NF)-filled axonal swellings developed in the subterminal regions of the longest axons in the PNS of rats exposed to n-hexane for 45 days.

Governa et al. (1987) investigated the correlation between electrographic changes indicative of polyneuropathy and urinary excretion of metabolites indicative of exposure to n-hexane. Forty workers were randomly chosen from four small shoe factories. All workers handled a type of glue or solvent that contained over 50% n-hexane without protective equipment for about 7 hours/day. All subjects exhibited no more than mild or nonspecific symptoms of polyneuropathy and were free of other known risk factors for nervous system impairment. A urine sample was collected at the end of a shift, and then a neurophysiological examination (MCV, SCV, and associated distal latencies [DL]) was carried out the following day. Reference values were obtained from 41 unexposed individuals. A semiquantitative rating scale of the electroneuromyography (ENM) responses was used as a cumulative index score of the findings (Allen et al., 1975). The scale ranged from 0, where no ENM abnormalities were observed, to 10, where decreases in conduction velocities and increases in DL were indicative of impaired electrophysiological performance (Governa et al., 1987).

The urinary concentrations of cyclohexane, trichloroethylene, and n-hexane metabolites were measured in 40 workers, but only those for two of the five n-hexane metabolites were above minimum detection limits (MDLs): 2,5-hexanedione (mean, 6.80 mg/L) and γ-valerolactone (mean, 3.31 mg/L). Trichloroethylene was the only other chemical for which a significant portion of the workers had metabolite levels above the MDL. However, the trichloroethylene levels were much lower than those associated with the recommended occupational exposure limit. Urinary concentrations of the n-hexane metabolite, 2-hexanol, were below 0.1 mg/L in 29 of 40 workers. The results of the neurological exam found 26 workers with ENM results within normal limits (ENM score <4), 3 workers with increased DL with or without a decreased SCV or MCV (ENM score of 4 or 5), and 11 workers with these changes in DL and SCV and MCV values plus changes in MAPs in at least one muscle (ENM score ≥8). While length of exposure to n-hexane was unrelated to ENM scores, a statistically significant dose-response relationship for these scores was found for 2,5-hexanedione and γ-valerolactone. Looking at the utility of urinary 2,5-hexanedione concentrations as a screening device to detect significant ENM abnormalities (defined as ENM score >3), Governa et al. (1987) identified a threshold value of 7.5 mg/L as being closely related to the incidence of abnormalities. However, some variation from this relationship was apparent, because Governa et al. (1987) identified three workers with 2,5-hexanedione urinary concentrations of 3.0, 3.3, and 4.5 mg/L, all of whom displayed ENM changes.

Pastore et al. (1994) measured urinary 2,5-hexanedione in 20 asymptomatic workers with prolonged exposure to solvents containing n-hexane. These workers were free from known

diseases affecting the nervous system or from risk factors for alterations in nervous system function. Urine samples, taken at the end of the shift, were all in excess of the recommended ACGIH BEI of 5 mg/L 2,5-hexanedione, with a mean of 11.02 ± 4.5 (range 5.3 to 24.2) mg/L. The neurological findings in these workers were compared with those obtained during the previous 8 years in healthy adults of a similar age who were not occupationally exposed to any toxic substance. No significant anomalies were identified in neurological examinations or worker responses to questionnaires about neurophysiological problems. However, the results of electrographic evaluations showed significant decreases in the amplitude of sensory nerve action potential (SNAP) for the median, sural, and ulnar nerves. These results were unrelated to urinary 2,5-hexanedione levels. However, the SNAP amplitude for the sural and median nerves was significantly related to the number of years exposed to n-hexane. Adjusting for age did not alter these results. No differences were found in values of the SCV, MCV, compound muscle action potential, and F wave latency (a more precise indication of small variations in conduction) for the nerves evaluated.

Murata et al. (1994) studied the effects of solvent exposure on the autonomic nervous system and cerebellar function in shoe and leather workers exposed to n-hexane, xylene, and toluene. 2,5-hexanedione, hippuric acid, and methylhippuric acid concentrations in urine samples (taken the morning prior to electrophysiological examination) were determined. Urinary concentrations of 2,5-hexanedione were 0–3.18 (mean 1.39) mg/L; concentrations of hippuric acid were 0.05–2.53 (mean 0.41) g/g creatinine; and concentrations of methylhippuric acid were 0.10–0.43 (mean 0.19) g/g creatinine for occupationally exposed workers. In unexposed workers, the urinary concentration of 2,5-hexanedione was 0.1–0.8 g/g creatinine and hippuric acid was < 1.5 g/g creatinine; methylhippuric acid was not found. Exposure concentrations for n-hexane, xylene, or toluene were not reported by the study authors. The study subjects were free of known confounding factors related to nervous system function and were similar in their reported use of alcohol and tobacco. Exposed workers had worked in household factories for a period of 18–42 years (31 ± 6 years). Murata et al. (1994) measured the distribution of MCVs and SCVs of the median nerve and the variation in the electrocardiographic duration of the ventricular cardiac cycle (R-R interval) in 30 workers and in 25 healthy controls unexposed to solvents. The SCV and MCV of the median nerve were significantly slowed in exposed workers compared with unexposed. Variations in the R-R interval and the respiratory sinus arrhythmia component of the R-R interval also were significantly lower in the exposed group. The SCV in the forearm was significantly correlated to the variation in the Mayer sign wave arrhythmia component of the R-R interval. Duration of

exposure, concentration of urinary metabolites for solvent exposure, age, or alcohol consumption were not significantly related to any of these electrophysiological results. While the results imply that both the PNS and the autonomic nervous system were affected by solvent exposure, failure to identify a dose-response relationship and the mixture of solvents to which the workers were exposed led to equivocal results for n-hexane. Because the urine was collected more than 12 hours after exposure, the concentrations of metabolites in urine may have been an underestimation of the actual solvent exposure.

In a study of the same workers as those monitored by Murata et al. (1994), Yokoyama et al. (1997) evaluated 29 subjects and 22 healthy unexposed controls for postural sway frequency in order to assess subclinical cerebellar dysfunction. Subjects were male workers in shoe, sandal, and leather factories who routinely were exposed to n-hexane, xylene, and toluene during the course of their work. Postural balance was measured quantitatively using a strain-gauge-type force platform on which subjects were asked to stand for 60 seconds with their eyes open and then for 60 seconds with their eyes closed. Lengths of displacement of the body's center of pressure in the mediolateral and anteroposterior directions were used as indicators of the extent of postural sway in each direction. Mean urinary concentration of 2,5-hexanedione was 1.20 mg/L (range 0.41 to 3.06 mg/L), and the estimated mean level in workplace air was 40 ppm (range 13 to 100 ppm) n-hexane. The measurements of postural balance, specifically spinocerebellar afferent type of sway, showed a significant positive association with 2,5-hexanedione concentration in urine. The authors indicated that xylene could possibly inhibit the effects of n-hexane exposure on sway. Specifically, there was an inverse correlation between urinary methylhippuric acid from xylene exposure and vestibulocerebellar type of sway.

Passero et al. (1983) screened 654 workers in 44 shoe factories and 86 home shops during a period from 1973 to 1981. Evaluation by clinical and electrodiagnostic examination identified 184 workers with some degree of neurological abnormality. Of these 184 subjects, 9 had other neurological disorders (the authors reported the most common of which was radiculopathy due to intervertebral disc disease), 77 displayed minimal changes and were considered normal following repeated examination by the study authors, and 98 manifested overt polyneuropathy. The majority of the workplace solvent samples collected contained commercial hexane. The commercial hexane was determined to contain greater than 60% of total mass as hydrocarbons such as pentane, 2-methyl-pentane, 3-methyl-pentane, n-hexane, heptane, cyclopentane, cyclohexane, and methyl-cyclopentane. In 7 of 12 samples taken from workplaces of individuals with the most severe polyneuropathy, over 99% of the total solvent was composed of these hydrocarbons. No relationship was found between length of exposure and severity of

disease. In the cases of polyneuropathy, the neurological pattern showed an insidious onset of loss of distal motor and sensory function with marked reflex loss. General symptoms, such as nausea or vomiting, epigastric pain, and insomnia, preceded or accompanied the neuropathy. Clinical symptoms were weakness, paresthesia (burning or tingling sensation in limbs), and cramp-like pain with related motor impairment, hypoesthesia (partial loss of sensation and/or diminished sensibility), changes in tendon reflexes, and muscle trophism and tone. These symptoms were usually confined to distal portions of the limbs and occurred with varying degrees of intensity depending on the extent of exposure. All 98 polyneuropathy cases exhibited abnormal MAPs, regardless of severity. The occurrence of fibrillations, positive waves, fasciculations, and slowing of MCV increased with disease severity. Several of the most affected cases exhibited CNS involvement with alterations in electroencephalogram or spasticity in the lower limbs and increased deep tendon reflexes. The clinical course of these 98 cases was followed for up to 8 years. Except for the most severe cases, patients improved slowly when removed from the affected environment. However, deterioration continued for some even after exposure ceased.

A group of 122 cases of polyneuropathy among workers in 72 shoe factories was evaluated for severity of neurological impairment in relation to duration of exposure to a mixture of solvents (Abbritti et al., 1976; Cianchetti et al., 1976). Every worker with polyneuropathy was questioned about work experience; type of chemical material used on the job; specific job function performed at onset of disease, and in the years previous to onset of disease; symptoms; and the order of appearance and evolution of symptoms. All patients were given an electromyographic examination and were determined to be free of lifestyle or medical conditions such as diabetes and alcoholism that would cause neurological impairment. None of the subjects had a history of exposure to other chemicals that might cause neuropathy, such as lead, arsenic, carbon disulfide, or drugs such as sulfonamides. The workers were divided into three groups based on severity in the reduction of the MCV of the peroneal nerve. Group I had a maximum MCV of less than 35 m/s; Group II had an MCV of 35–44 m/s; and Group III had a maximum MCV of 45 m/s or greater. No quantitative air measurements were taken, but samples of five glues and two cleaners from five factories in which 20 cases worked were analyzed for several solvents of interest. Six of these samples contained at least 40% n-hexane, with other solvents, such as pentane, 2-methylpentane, 3-methylpentane, toluene, and cyclohexane, usually present. No correlation was identified between severity of neuropathy and length of employment in the factory.

Sobue et al. (1978) identified 93 cases of polyneuropathy among 1662 shoe workers

screened. These workers were divided into three groups according to the presence of (1) sensory symptoms only (53 subjects), (2) sensorimotor symptoms (32 subjects), or (3) sensorimotor symptoms with amyotrophy (eight subjects). Follow-up of a subgroup of workers continued for up to 18 months. All 93 cases were engaged in operations that used pastes consisting of at least 70% n-hexane. Air concentrations of n-hexane in the workrooms ranged from 500 to 2500 ppm. The degree of neurological disorder was related to hygienic conditions in the workplace. A further reduction in MCV was noted among some of the workers with the most severe polyneuropathy, even after removal from the solvent exposure. In addition to signs of sensory disturbance that are typical of peripheral neuropathy, such as numbness and hypoactive reflexes, Sobue et al. (1978) found evidence of cranial nerve impairment in some cases. These were indicated by blurred vision (in 13 subjects), constriction of the visual field (seven subjects), and numbness over the face (five subjects). In an earlier report on the same group of patients, Yamamura (1969) tabulated the results of semiquantitative laboratory analyses of biochemical parameters in blood and urine, most of which were unremarkable except for apparently depressed levels of serum cholinesterase activities, elevated levels of serum lactate dehydrogenase (LDH) activity, and positive urobilinogen.

Paulson and Waylonis (1976) also found reduced serum cholinesterase levels among eight printing room workers who had polyneuropathy and were exposed to n-hexane. Air levels of n-hexane taken over a 2-month period were found to be as high as 4060 mg/m^3 (1152 ppm) in the plant.

Wang et al. (1986) evaluated a group of 59 press proofing workers from 16 factories who were employed for at least 2 months. All but four of these workers had regular contact with solvents in the process of cleaning the rollers. Two exposure measures using personal air samplers were taken in 14 of the 16 factories. Samples of the bulk cleaning solvents were found to contain n-hexane at concentrations ranging from 10–65%. Referent neurological data were collected from 150 healthy individuals (50 persons from three age groups, 10–35, 36–50, and 51–80 years, sex not stated). MCVs among workers exposed to n-hexane were consistently lower than among controls. The results of the neurological examination identified 15 workers with polyneuropathy and two asymptomatic workers with abnormal MCVs. All but one of these workers were employed in factories that used solvents with n-hexane concentrations in excess of 50%. While no association was found with length of employment, statistically significant associations existed between frequency of polyneuropathy and abnormal MCV and n-hexane concentration in the cleaning solvents and between the frequency of polyneuropathy and n-hexane air concentrations. Among the workers with polyneuropathy, a high percentage

worked in factories with n-hexane air concentrations greater than 100 ppm. However, a significant reduction in the MCV was found among workers exposed to air concentrations less than 25 ppm, a result that the authors considered to be related to the prolonged exposure due to overtime work.

The 15 cases of polyneuropathy from the study by Wang et al. (1986) were included in a group of 28 color printers with polyneuropathy studied by Chang and Yip (1987). This study of electromyography (EMG) changes also included five subclinical cases, 45 workers with no apparent symptoms, and 72 normal subjects who served as the control group. Among the clinical and subclinical cases, a significant decrease in MCVs and in amplitude of MAPs and SNAPs and a significant prolongation of latencies were seen compared with controls. Among the exposed workers with no apparent symptoms, MCVs were slower, motor DLs were prolonged, and SNAP amplitudes were attenuated compared with controls. The percentage difference in these electromyographic changes from the control values increased with increasing severity of symptoms.

Chang (1990) followed 11 of the 28 polyneuropathy cases for 4 years. The authors observed the patients monthly for the first 2 years, bimonthly for the third year, and once every 3 months for the final fourth year. All 11 cases had moderate to severe polyneuropathy. There was some worsening of motor function and electrographic findings in nine of the cases even after exposure to n-hexane ceased. Delayed worsening of sensory function was not observed. Sensory disturbances usually disappeared within 4 months. All patients, including the most severely affected, who was a quadraplegic, regained full motor nerve capacity within 1–4 years. Tightness in the legs, which appeared early in the course of recovery for six of the more severe cases, was replaced by muscle cramps, which persisted up until the last clinical visit 4 years after the onset of neuropathy. Two of the six also had hyperreflexia and residual muscle atrophy in the lower extremities, and one had only residual atrophy. The inability of two of the subjects to perceive colors correctly (dyschromatopsia) persisted until the end of the study. These patients also had macular retinopathy.

In addition, Chang (1991) documented the electrophysiological performance of the 11 cases from their initial diagnosis to complete recovery of motor nerve capacity. Compound muscle action potentials (CMAPs), DLs, and nerve conduction velocities were measured in motor and sensory nerves, and pattern visual evoked potentials (VEPs) were assessed in relation to those of a group of unexposed control individuals. Full recovery was associated with a return to normal values for patterned VEPs, CMAPs, and DLs of both motor and sensory nerves. However, the nerve conduction velocity of motor nerves remained significantly lower than

normal values even when the patients had apparently made a complete recovery (Table 4-4).

Table 4-4. Motor neurographic findings in patients with n-hexane polyneuropathy

	Motor neurographic findings (mean ± SD)		
	Initial study (n = 11)	**Final study (n = 11)**	**Controls (n = 72)**
Median nerve			
CMAP amplitude (mV)	4.1 ± 2.1	10.0 ± 2.1	8.9 ± 3.0
DL (ms)	6.1 ± 0.7	3.9 ± 0.4	3.7 ± 0.4
MCV (m/s)	42.9 ± 7.1	57.3 ± 2.5[a]	61.9 ± 4.6
Ulnar nerve			
CMAP amplitude (mV)	4.2 ± 1.7	8.5 ± 1.1	7.8 ± 2.2
DL (ms)	5.0 ± 0.8	3.1 ± 0.2	3.0 ± 0.4
MCV (m/s)	40.8 ± 4.6	52.8 ± 4.1[b]	55.4 ± 4.0
Peroneal nerve			
CMAP amplitude (mV)	1.8 ± 1.0	5.6 ± 2.5	5.9 ± 1.9
DL (ms)	8.2 ± 1.1	4.8 ± 0.9	4.7 ± 1.0
MCV (m/s)	31.9 ± 3.3	46.1 ± 4.9[c]	53.1 ± 4.4
Tibial nerve			
CMAP amplitude (mV)	4.0 ± 2.4	10.7 ± 5.7	10.2 ± 3.7
DL (ms)	7.6 ± 1.3	4.5 ± 0.2	4.5 ± 0.8
MCV (m/s)	34.5 ± 4.3	46.3 ± 4.8[b]	49.6 ± 3.9

[a] ($p<0.005$); [b] ($p<0.05$); and [c] ($p<0.0005$): Significantly different from controls, as calculated by the authors.

Source: Chang, 1991.

Chang et al. (1992) further described the effects of n-hexane exposure on the neurological function of 56 offset machine workers in a printing factory. These workers, who were free of other known risk factors related to neurological function, such as alcoholism and/or diabetes mellitus, were the primary users of a cleaning solution containing 14–20% n-hexane. Subjects typically worked 12 hours/day, 6 days/week, and used a solvent-soaked cloth or sponge to manually clean the printer roller blanket surface 2 to 3 times each hour. They did not wear respirators, but all except four workers wore latex gloves when performing this cleaning operation. While other solvents were present in these cleaning solutions (toluene), n-hexane was predominant and the only one with TWA air concentrations above the TLV (50 ppm). The

workers were also exposed to lead (0.6–8.2 µg/g) and mercury (0.05–0.95µg/g) in the printing inks. The mean TWAs for n-hexane in air samples taken at the plant was 63 ppm (range 30–110 ppm) for general air and 132 ppm (range 80–210 ppm) for breathing zone air of the offset printers. The findings from the neurological examination of these workers were compared with the neurological findings of 20 age- and gender-matched unexposed controls. Among the 56 printers, 10 were asymptomatic and showed no clinical findings (classified as healthy workers); 26 were asymptomatic, but had subclinical nerve conduction deficits (classified as subclinical workers); and 20 were symptomatic for peripheral neuropathy by clinical and electrophysical findings (classified as symptomatic workers).

No relationship was observed between length of employment and the development of neuropathy. Workers that were symptomatic reported experiencing symptoms in the upper and lower extremities such as numbness (8/20 and 14/20, respectively), paraesthesia (5/20 and 13/20, respectively), pain (2/20 and 9/20, respectively), and weakness (7/20 and 15/20, respectively) of the feet and distal portion of the legs. Subjects in the symptomatic group had more severe symptoms in the lower than the upper limbs, and sensory disturbances usually appeared before motor disturbances. Four workers who did not use gloves when cleaning the roller blanket showed symptoms in the upper limbs first. No autonomic neuropathy was reported by any worker. Clinical symptoms were not reported for healthy workers or for those who were asymptomatic, but classified as having subclinical neuropathy. The authors also measured electrophysical deficits in each group of workers (Table 4-5). In healthy workers, there was a decreased amplitude of the median nerve SNAP compared with controls. Subclinical workers had more significant decrements in the SNAP amplitude and MCV. There was also a mild reduction in mean MAP amplitudes and prolongation of mean DL. The symptomatic workers were reported to have a marked reduction in mean SNAP, MAP, and MCV and prolonged DL.

Table 4-5. Nerve conduction study findings in printers with n-hexane-induced polyneuropathy

Type of nerve conduction study	Control (n = 20)[a]	Healthy worker (n = 10)[a]	Subclinical worker (n = 26)[a]	Symptomatic worker (n = 20)[a]
Amplitude of SNAP (µV)				
Median	37 (11)	27 (6)[b]	24 (8)[b]	15 (5)[b]
Ulnar	15 (4)	14 (3)	12 (5)	7 (4)[b]
Sural	24 (10)	22 (6)	18 (7)[b]	11 (8)[b]

Amplitude of MAP (mV)				
Median	7 (2)	8 (3)	6.7 (2.4)	4.6 (2.2)[b]
Ulnar	5.7 (2.1)	6.5 (1.9)	4 (2.2)[b]	3.6 (1.5)[b]
Posterior tibial	6.6 (2)	6.7 (2.3)	53 (2.5)	2.9 (1.7)[b]
Common peroneal	4.4 (1.5)	4.3 (1.4)	3.6 (1.4)	1.8 (1.4)[b]
DL of SNAP (ms)				
Median	2.3 (0.3)	2.3 (0.1)	2.6 (0.3)[b]	2.9 (0.3)[b]
Ulnar	2.1 (0.3)	2.1(0.1)	2.3 (0.3)	2.7 (0.4)[b]
Sural	3.3 (0.3)	3.1 (0.2)	3.3 (0.3)	3.7 (0.6)[b]
DL of MAP (ms)				
Median	2.9 (0.4)	3.0 (0.2)	3.6 (0.5)[b]	4.3 (1.2)[b]
Ulnar	2.2 (0.3)	2.3 (0.3)	2.6 (0.5)[b]	3.0 (0.7)[b]
Posterior tibial	4.1 (0.6)	3.9 (0.6)	4.4 (0.6)	5.6 (1.2)[b]
Common peroneal	3.9 (0.5)	3.5 (0.4)	4.2 (0.7)	5.4 (1.2)[b]
MCV (m/s)				
Median	59 (5.9)	57 (5)	55 (6.7)[b]	46 (6.5)[b]
Ulnar	61 (5.8)	59 (6)	55 (7.8)[b]	48 (7.5)[b]
Posterior tibial	50 (6.4)	46 (3.4)	45 (4.7)[b]	38 (6.5)[b]
Common peroneal	51 (4.5)	46 (3.8)	45 (5.1)[b]	37 (7.1)[b]

[a] Values presented as means with SDs in parenthesis.

[b] Significantly different from controls ($p<0.05$), as calculated by the authors.

Source: Chang et al., 1992.

Several studies (Issever et al., 2002; Seppalainen et al., 1979; Raitta et al., 1978) have investigated vision changes in relation to n-hexane exposure. Seppalainen et al. (1979) compared the VEP and electroretinograms (ERGs) of 15 workers to those of 10 healthy subjects with no occupational exposure to solvents or other neurotoxic chemicals. The highest recorded n-hexane levels in the two factories where the workers were exposed ranged from 2000 to 3250 ppm. In both factories, exposure was to technical grade hexane, which contains other aliphatic hydrocarbons with no known neurotoxic effects. Maculopathy, color discrimination deficits, flatter VEPs, and diminished peak-to-peak amplitudes of the ERGs were more common among

cases than controls.

An earlier study by the same researchers described visual defects in this same group of 15 workers, 12 of whom displayed impaired color vision (Raitta et al., 1978). The Farnsworth-Munsell (FM)-100 hue test showed 12 of the subjects to have impaired color vision, one of which was probably due to a congenital abnormality. The other cases of color vision impairment were acquired, mostly in the blue-yellow axis. In 11/15 subjects there was evidence of associated maculopathy (damage of vessels in eye that leak fluid into the center of the retina causing loss of central vision), in most cases characterized by pigment dispersion.

Issever et al. (2002) compared the color vision of 26 workers with diagnosed polyneuropathy resulting from n-hexane exposure to that of 50 nonexposed healthy controls. The workers had been exposed to n-hexane in the leather industry (exposure concentration not stated), and all complained of asthenia of upper and lower limbs, paraesthesia in hands and arms, and difficulty walking. EMG results indicated myelinic and axonal lesions of distal nerves. None had been screened for color vision during employment. All study subjects were free of any visual disorder or inherited color vision impairment. However, in the FM-100 hue test, there was significant impairment in color vision in n-hexane-exposed subjects (Table 4-6). Color vision defects did not show specificity for the blue-yellow or red-green axes but appeared to be distributed across the entire range of color vision deficits.

Table 4-6. FM-100 hue test error scores of n-hexane-exposed and nonexposed groups

Eye	Chromatic focus	Exposed group[a]	Control group[a]
Right	Blue-green	104.3 ± 37.3[b]	22.2 ± 13.5
	Red-green	64.1 ± 38.4[b]	13.8 ± 8.3
	Total	168.3 ± 70.5[b]	36.0 ± 19.8
Left	Blue-yellow	96.7 ± 55.8[b]	21.6 ± 11.0
	Red-green	81.9 ± 51.8[b]	14.0 ± 9.4
	Total	181.5 ± 103.0[b]	35.6 ± 18.2

[a] Values are presented as mean ± SD.

[b] Significantly different from controls ($p<0.001$), as calculated by the authors.

Source: Issever et al., 2002.

Huang and Chu (1989) used evoked potentials (EPs) to examine the extent to which exposure to n-hexane in the workplace might bring about subclinical effects on the central nervous system. The study involved five workers who had developed peripheral neuropathy as a result of being exposed to n-hexane in a press proofing factory. The subjects worked without protection in a poorly ventilated room and were exposed to three types of solvent mixtures, one of which contained 65% n-hexane. Two 1-hour air samples collected from the work room showed ambient air concentrations of 55 ppm for n-hexane and 9.65 ppm for benzene. Motor nerve conduction data, brainstem auditory evoked potential (BAEP), VEP, and somatosensory evoked potential (SEP) were obtained in exposed subjects and compared with those of unexposed subjects of comparable age. Four of the five exposed subjects displayed typical signs of neurological impairment, such as muscle cramps, weakness, and distal numbness. In contrast to the fifth (unaffected) subject, these four individuals had slept in the workroom between shifts. As tabulated by the authors, the four most severely affected subjects also displayed such electrophysiological deficits as lower conduction velocities and amplitudes in the median, ulnar, peroneal, and tibial nerves. Statistically significant changes also were seen in SEPs and BAEPs when the five exposed subjects were compared as a group with controls. The SEPs and BAEPs of the fifth (less severely affected) subject were higher than those of control subjects, suggesting that evoked potentials may be discriminating parameters for revealing subclinical neuropathies.

Scelsi et al. (1980) observed neuropathological symptoms and morphological changes in a small number of subjects (three women) occupationally exposed to an adhesive agent containing approximately 80% n-hexane for 2 months to 3 years. These workers experienced bodily discomfort and increasing weakness in the lower limbs. Biopsies of the sural nerves revealed axonal swelling and irregular and swollen myelin sheaths. In general, there appeared to have been a dissolution of neurotubules and an increase in the number of microfilaments. Specifically, histopathology and electron microscopy showed polymorphous changes in both myelin sheaths and axons of large diameter fibers of the sural nerve. In addition, there were irregular and swollen myelin sheaths and segmental swelling of axons with dissolution of neurotubules and increase of NFs. Polymorphous inclusion bodies were also identified in Schwann cells. The authors also observed atrophy and focal degenerative myopathic changes with lymphocytic infiltrates and phagocytosis in the soleus muscle. In one of the subjects, these changes in the muscle appeared to be associated with muscular denervation, lymphocytic infiltrations, and phagocytosis.

A follow-up study on the same subjects showed differing severities in the neuropathological responses (Scelsi et al., 1981). For example, one of the subjects suffered so

much from muscular wasting that she was unable to walk. However, neuropathy in the others was less severe, although symptoms were sufficient for a diagnosis of motor polyneuropathy. Electrophysiological measurements showed motor and sensory conduction velocities to be reduced. Light and electron microscopy of the sural nerve showed the presence of large diameter, irregularly shaped myelinated fibers, degenerated myelin, vacuoles, and abnormal organelles in two of the subjects. The third subject had scattered large fibers, with thin myelin sheaths and enlarged axons filled with packed NFs.

Yokoyama et al. (1990) examined the relationship between impaired nerve conduction velocities and morphological changes seen at biopsy in three workers exposed to n-hexane in a plant manufacturing parts for jet engines. A single air sample taken in the middle of the workroom showed the n-hexane concentration to be 195 ppm. All three workers developed progressive muscular weakness, with numbness and tingling sensations in the lower extremities. Their status progressed to the extent of work disability with difficulty walking or holding tools. The study authors measured the distribution of conduction velocities (DCVs) of sensory fibers in sural nerve at 1–2 months, 4–9 months, and 11, 23, and 36 months after exposure was ended. Values were obtained in two patients that fluctuated below the lower limit of normal as determined in 11 male subjects who had no history of exposure to chemicals. A sural nerve biopsy was obtained from one of the patients 10 weeks after cessation of exposure and showed degeneration of myelinated nerve fibers and paranodal swelling.

Electrophysiological deficits with subsequent recovery upon removal of subjects from n-hexane exposure were also demonstrated in four workers who had been exposed to n-hexane during the course of their work at a ball-manufacturing plant in Taiwan (Huang et al., 1989). The main source of exposure was solvent evaporation from a cement coating and nylon fiber winding facility. The n-hexane concentration in the poorly ventilated room averaged 108.9 ppm, the vapors resulting from a bulk solvent that contained 14.1% n-hexane by weight. The four workers displayed overt symptoms of peripheral neuropathy and showed electrophysiological deficits in motor conduction of the median, ulnar, peroneal, and tibial nerves. After cessation of exposure, there was an initial worsening in muscle strength, sensory deficit, and nerve conduction that lasted for 2–5 months, with recovery occurring over the course of the following year.

Huang et al. (1991) carried out a follow-up study of subjects potentially exposed to n-hexane at the factory described above. Forty-four workers were interviewed and classified according to their potential exposure to solvents. Five individuals were assigned to Group I because they had been involved in cement coating and the winding process; eight were assigned

to Group II because of their involvement in a gas injection process; and the remaining 31 were placed in Group III, reflective of a lower potential risk of exposure. All subjects were administered a questionnaire detailing personal history and describing subjective symptoms. The subjects also were given a physical and neurological examination. Electrophysiological determinations of nerve conduction and latencies, and electromyographic measurements were compared with those of 26 age- and sex-matched controls.

Personal air monitoring samples gave values of 109 ppm n-hexane in the cement coating section (two samples), 86.4 ppm in the nylon fiber winding section (three samples), and 75 ppm in the gas injection section (one sample). Neuropathy was evident in all subjects in Group I. Four of these individuals had severe symptoms while the other subject's symptoms were considered to be moderate. Two of eight individuals in Group II, but none in Group III, displayed mild polyneuropathy. In the EMG studies, fibrillations, positive sharp waves, and increasing polyphasic waves were found in all five Group I cases, whereas only increased polyphasic waves were noted in the eight workers in Group II. In motor nerve conduction studies, decreased nerve conduction velocity, prolonged distal latency, and reduced evoked nerve potential were observed in all four nerves studied. Workers in Group I had more severe neurological symptoms, consistent with exposure to higher n-hexane concentrations in the cement coating and nylon fiber winding sections. The factory owner installed a new ventilation system and enclosed portions of the organic solvent operations. n-Hexane concentrations in air fell to 12.9 ppm in cement coating, 14.7 ppm in nylon fiber winding, and 1.0 ppm in gas injection. There were no new cases of polyneuropathy in a 2-year follow-up at the factory, and all seven originally affected subjects recovered.

Valentino (1996) described an occupational exposure study in which 27 female and 63 male workers in the shoe industry were assessed for polyneuropathy in relation to n-hexane exposure. All of the subjects, who had been free of exposure for at least a year, were divided into groups according to whether they had been exposed to n-hexane either during the last 10 years or more than 10 years ago. In general, more recently exposed subjects had a higher incidence of neuropathological symptoms. In electrophysiological measurements, Valentino (1996) showed recovery of the motor component, while recovery of the sensory component was incomplete even 10 years after n-hexane exposure.

Bachman et al. (1993) discussed the cases of 63 workers in a metal can factory. Subjects received a neurological examination, including measurements of motor function, proprioception, sensitivity to vibrations, and vibriotactile thresholds. The workers were placed in low and high exposure groups on the basis of their duration of exposure at the subject location at which, over a

3-year period, 17 personal sampling measurements had been taken. These indicated an n-hexane concentration range of 181–2436 mg/m^3 (50–690 ppm). However, the subjects in the study showed few neurotoxic effects of n-hexane, with no overt cases of clinical neuropathy.

Smith and Albers (1997) presented a case report of an individual subacutely exposed to n-hexane vapor by sniffing glue. The authors noted that the 25-year-old male subject had a history of recreational drug use and was exposed to several other non-hexane constituents from sniffing glue. The 25-year-old male showed progressive weakness, pain, diminished sensations in the feet, and numbness in the hands. An electrophysiological examination of the subject revealed reduced SNAPs and SCVs, increased DLs, and an apparent block of conduction in the median and ulnar nerves (forearm), peroneal nerve (knee), and tibial nerve (leg). A biopsy of the sural nerve showed the classic pathological response to n-hexane exposure, including swollen axons, reduced numbers of myelinated fibers, and the appearance of abundant NFs. An initial increase in severity of the symptoms on cessation of exposure caused the subject to be confined to a wheelchair. However, a subsequent, slow improvement resulted in his strength returning to normal after a year. Reflexes were reestablished and changes to electrophysiological parameters paralleled the clinical recovery.

In a published abstract, Pezzoli et al. (1989) described a female leather goods worker who had been exposed to n-hexane for many years prior to developing symptoms of Parkinsonism. Symptoms of axonal neuropathy were evident in the patient, as would be typical in subjects chronically exposed to n-hexane. In a follow-up of the same patient, Pezzoli et al. (1996) reported that the subject's disease progressed even after she withdrew from the work environment. Pathological examination and immunohistochemical analysis of the brain showed severe and widespread dopaminergic neuronal loss, severe gliosis in the substantia nigra, and near total loss of immunostaining of tyrosine hydroxylase in the striatum. The authors stated that the patient's disease resembled a rigid akinetic form of parkinsonism with levodopa-induced dystonias. However, pathological examination of the brain revealed similarities to human 1-methyl-4-phenyl-1,2,4,6-tetrahydropyridine-induced parkinsonism. In general, interpreting these results as indicative of the etiological importance of n-hexane exposure to the onset of Parkinsonism is complicated by the fact that some symptoms are typical of the condition while others differ. The extent to which the solvent may be causative also is difficult to gauge.
The same researchers presented a case report of a 53-year-old man who had worked since the age of 15 in a leather-processing factory (Pezzoli et al., 1995). The glue used at his workstation consisted of 50% n-hexane, 17% 3-methylpentane, 15% 2-methylpentane, 13% methylcyclopentane, and 4% cyclohexane. A 24-hour urine sample from the patient contained

0.79 mg/L 2,5-hexanedione. Neurological examination of the patient showed some signs of parkinsonism (tremor, bradykinesia, and rigidity). Other symptoms and findings, when taken together, contributed to a weighted Unified Parkinson Disease Rating Scale (UPDRS) motor score of 13. UPDRS is a three part evaluation of various physical and mental characteristics (motor skills, mental status, behavior, mood, and activities of daily living) on a 0 to 5 scale. The cumulative score gives an index of severity of Parkinson's disease (on a scale of 0 to 199, with 199 being the most severe cases of Parkinson's disease). Motor and sensory nerve conduction studies showed essentially normal results, except that the medial and lateral plantar nerve sensory action potentials were bilaterally absent. Following diagnosis, the subject was removed from n-hexane exposure scenarios at work. The level of urinary 2,5-hexanedione declined to 0.25 mg/L, a value thought to be indicative of no occupational exposure. However, signs of parkinsonism worsened, and the patient achieved a weighted UPDRS motor score of 23, 30 months after the onset of the disease.

Vanacore et al. (2000) evaluated the possible association between chronic exposure to n-hexane and parkinsonism on the basis of the clinical and occupational history of a 55-year-old patient who had worked for 17 years in an environment where mixtures of aliphatic hydrocarbons (53% n-hexane) were used. Results of neurophysiological, neuroradiological, and neuropsychological tests suggested that n-hexane may affect the CNS. For example, magnetic resonance imaging showed a marked cortical cerebral atrophy, and the neuropsychological assessment revealed impaired visuomotor response, as well as loss of short- and long-term memory. The patient's apparent parkinsonism did not change much over the next 5 years.
In a further study to evaluate the effect of environmental influences on the pathogenesis of parkinsonism, Canesi et al. (2003) measured the total urinary concentration of 2,5-hexanedione and 2,5-dimethylpyrrole adducts in the urine of 108 patients with parkinsonism compared with 108 unaffected subjects. The 2,5-dimethylpyrrole adduct production is a result of the interaction of 2,5-hexanedione with NF protein lysine residues. Urinary excretion of both 2,5-hexanedione and 2,5-dimethylpyrrole were significantly reduced in patients with parkinsonism compared with controls, although there was a gradual decline in urinary metabolite levels with age in both Parkinsonism patients and controls. The data did not indicate whether the lower urinary levels were possibly the result of reduced conversion of n-hexane to its metabolites or of an increase in their further catabolism.

Karakaya et al. (1996) investigated the effects of n-hexane on the immune system. Immunological parameters in 35 workers exposed to n-hexane were compared with 23 age-matched controls with no history of n-hexane exposure. Exposure to n-hexane was measured by

TWA concentration in the breathing zone air and urinary 2,5-hexanedione (following acid hydrolysis) at the end of each shift. The mean TWA for n-hexane in air was 123 ppm (range 23–215 ppm), and the mean level of 2,5-hexanedione in urine was significantly higher in exposed workers (2.39 µg/g creatinine) compared with unexposed workers (0.41 µg/g creatinine). Whole blood samples were analyzed for immunoglobulins IgG, IgM, and IgA, and white blood cell (WBC) types. Immunoglobulin concentrations in exposed workers were significantly lower than in unexposed workers. A significant inverse correlation also was found between the immunoglobulin levels and the 2,5-hexanedione concentrations in the exposed group. No differences were detected in WBC counts between exposed and unexposed subjects.

Yucesoy et al. (1999) examined the effects of occupational coexposure to n-hexane, toluene, and methyl ethyl ketone on natural killer cell activity and some immunoregulatory cytokine levels in shoe workers. Twenty three male shoe workers were studied in comparison with 18 unexposed controls. Levels of solvents in breathing zone air were 58.07 ± 28.09 ppm (range 4.3–300 ppm) for n-hexane, 26.62 ± 10.27 ppm (range 5.37–116.2 ppm) for toluene, and 11.39 ± 4.86 ppm (range 2.43–47) for methyl ethyl ketone. Urinary levels of 2,5-hexanedione and hippuric acid were measured, and natural killer cell cytotoxic activity in peripheral lymphocytes using human erythroleukemic cells (K562) as targets was monitored. Serum levels of interleukin-2 and γ-interferon also were monitored. The authors suggested that the levels of 2,5-hexanedione in the urine of exposed subjects (3.22 ± 0.44 mg/g creatinine versus 0.98 ± 0.22 mg/g creatinine in controls) indicated that the workers had been exposed to n-hexane. Natural killer cytotoxic activity and serum levels of interleukin-2 and γ-interferon did not differ between exposed and control groups.

To protect against the onset of subclinical and clinical neuropathological symptoms of n-hexane exposure, ACGIH proposes a BEI of 0.4 mg/L as an acceptable concentration of 2,5-hexanedione in urine (non-acid hydrolyzed samples) and a BEI of 5 mg/g creatinine (acid hydrolyzed samples) at the end of shift on the last day of a workweek (ACGIH, 2003; ACGIH, 2001). The ACGIH TLV-TWA of 50 ppm is based on a review of the peer-reviewed literature indicating that commercial hexane mixtures contain approximately 50–70% n-hexane. Further, the skin notation was assigned because studies indicate that dermal exposure to n-hexane leads to peripheral neuropathy in humans (ACGIH, 2001).

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

4.2.1. Oral Exposure

4.2.1.1. *Subchronic Studies*

A few studies have addressed the toxicity of n-hexane when administered via the oral route to experimental animals. Krasavage et al. (1980) compared the neurotoxicity of 2-hexanone, 2-hexanol, 2,5-hexanedione, 2,5-hexanediol, 5-hydroxy-2-hexanone, n-hexane, and practical grade hexane. Groups of five male COBS CD(SD)BR rats/group received equimolar doses of 6.6 mmol/kg of the chemicals by gavage, 5 days/week, for 90 days. The mg/kg equivalents of the equimolar daily doses were 660 mg/kg 2-hexanone, 780 mg/kg 2,5-hexanediol, 755 mg/kg 2,5-hexanedione, 765 mg/kg 5-hydroxy-2-hexanone, 675 mg/kg 2-hexanol, 570 mg/kg n-hexane, and 4000 mg/kg practical grade hexane. After a month of treatment, additional groups of five rats were administered n-hexane at 13.2 and 46.2 mmol/kg (1140 and 3980 mg/kg, respectively). The period of treatment and observation was extended to 120 days for those animals receiving 46.2 mmol/kg n-hexane to ensure that an overt neuropathological endpoint was detected in rats exposed to the chemical. The onset of neuropathy was assessed by the initial appearance of hind-limb paralysis, at which point the animal was sacrificed and examined histopathologically.

Two rats in the 13.2 mmol/kg exposure group, one rat in the 46.2 mmol/kg n-hexane exposure group, and three rats in the practical grade hexane group died due to chemical pneumonitis following intubation and were not included in histological analyses. The authors observed the clinical manifestation of hind-limb paralysis from exposure to the following chemicals (in order of decreasing potency) 2,5-hexanedione (5/5), 5-hydroxy-2-hexanone (5/5), 2,5-hexanediol (5/5), 2-hexanone (5/5), 2-hexanol (4/5), and high dose n-hexane (3/5). Practical grade hexane and the lower doses of n-hexane did not produce hind-limb paralysis during the 90-day testing period. The relative potency of the test chemicals was compared with 2-hexanone. Specifically, the authors calculated a neurotoxic index (based on the ratio of number of days until hind-limb paralysis developed in 2-hexanone-treated animals to number of days until hind-limb paralysis developed following exposure to the test chemical). 2,5-Hexanedione, 5-hydroxy-2-hexanone, and 2,5-hexanediol had higher neurotoxic indexes than n-hexane and practical grade hexane. Neurotoxic index correlated with peak serum concentrations of 2,5-hexanedione produced and the area under the serum concentration-time curve for 2,5-hexanedione.

Decreased body weight gain and food consumption was correlated with the neurotoxic index of each chemical (statistical significance not reported by study authors). All chemicals except 570 and 1140 mg/kg n-hexane produced giant axonal swelling, adaxonal myelin infolding, and paranodal myelin retraction (incidences not reported by the study authors). The authors noted that there was an obvious gradation of histopathological response in the frequency of giant axons between chemicals with the highest neurotoxic index and those with lower neurotoxic indices.

Krasavage et al. (1980) also evaluated testicular tissue by histopathology. The authors observed various stages of atrophy of the testicular germinal epithelium following the administration of 2,5-hexanedione, 5-hydroxy-2-hexanone, 2,5-hexanediol, 2-hexanone, 2-hexanol, and the high dose of n-hexane (incidence and severity of effect not reported by the study authors). The correlation of serum concentration of 2,5-hexanedione with the onset of neuropathological symptoms suggested that a threshold serum concentration of 50 µg/mL of 2,5-hexanedione was necessary for the induction of the neuropathological effects. Only those animals receiving n-hexane at the highest dose (46.2 mmol/kg, equivalent to 3980 mg/kg-day) built up a serum concentration of 50 µg/mL 2,5-hexanedione and developed the hind-limb paralysis characteristic of hydrocarbon-induced neuropathy.

The effects of n-hexane on peripheral nerve transmission were observed by Ono et al. (1981). Male Wistar rats (5–7/group) were administered n-hexane by gavage in olive oil daily for 8 weeks. The exposure regimen consisted of administration of 0.4 mL solvent in 0.6 mL olive oil for the first 4 weeks, 0.6 mL solvent in 0.4 mL olive oil for a subsequent 2 weeks, and 1.2 mL solvent in 0.8 mL olive oil for the final 2 weeks, while a control group received olive oil alone. Body weight was measured every 2 weeks during the experimental period, resulting in dose calculations of 811 mg/kg-day (after 2 weeks), 759 mg/kg-day (2–4 weeks), 1047 mg/kg-day (4–6 weeks), and 2022 mg/kg-day (6–8 weeks). Peripheral nerve activity was measured by administering a differential pulse to electrodes inserted at different points along the tail of unanesthetized animals. Transmission of electrical charge was then detected at other points along the tail. The group mean MCV was measured at the start of the experiment and every 2 weeks until termination.

There was no change among the groups in the rates of body weight gain throughout the experiment. MCV in groups receiving n-hexane (after 4, 6, and 8 weeks exposure) was reduced by approximately 5–10% compared with controls. These changes achieved statistical significance at the 4- and 8-week time point (statistical test not stated). Distal latencies decreased as the rats grew, but there were no statistically significant differences between n-hexane-exposed and control animals. However, there were statistically significant reductions

in the proximal (approximately 6–8%) and distal (approximately 5–8%) mixed MCVs of animals receiving n-hexane compared with controls after 4, 6, and 8 weeks, respectively. In general, such changes were less severe or absent in animals exposed to isomers of n-hexane or structurally related chemicals (2- and 3-methylpentane, methylcyclopentane). In the absence of histological studies, the significance of minor MCV changes after exposure to 2-and 3-methylpentane and methylcyclopentane cannot be fully evaluated. It has been suggested that in order for an alkane to be neurotoxic it must be metabolized to a gamma diketone.

4.2.1.2. *Chronic Studies*

No chronic exposure studies were identified that involved the administration of n-hexane to experimental animals via the oral route.

4.2.2. Inhalation Exposure

4.2.2.1. *Subchronic Studies*

Ono et al. (1982) examined the subchronic effects of n-hexane on MCV and DL in eight male Wistar rats/group exposed 12 hours/day for 24 weeks to 200 and 500 ppm n-hexane. Using a modification to a previously described experimental protocol for assessing nerve conduction (MCV and mixed nerve conduction velocity) and performance (Ono et al., 1981), the authors measured MCV, mixed nerve conduction velocity, and DL before the start of the experiment and then prior to sacrifice (i.e., 0, 4, 8, 12, 16, 20, and 24 weeks exposure). The authors used the following equation to fit the data to a time-series curve of the seven observations in each rat: $Y = A - C \times e^{-Bt}$ (where Y = data for a particular endpoint over the seven observation periods; A, B, C = constant; t = weeks of exposure; and e = base of natural logarithm). The statistical differences in the average of constant A (estimated as a limiting value of the curve) were evaluated by Student's t-test. One animal from each group was examined histopathologically in an attempt to link any functional deficits to morphological changes that may have taken place over the duration of the experiment. The authors stated that they did not observe any definite clinical signs of neuropathy in any of the exposed groups. Peripheral nerve function was impaired by exposure to 500 ppm n-hexane and impaired to a lesser extent in rats exposed to 200 ppm n-hexane. Specifically, MCV and distal and total (distal plus proximal) mixed MCV were statistically significantly decreased at $\geq$200ppm (Table 4-7). DL and proximal mixed MCV were statistically significantly decreased at the low dose but not at the high dose. Degeneration of the myelinated axons was evident in the peripheral nerves of all exposed groups.

Table 4-7. Nerve conduction changes in male Wistar rats exposed 12 hours/day for 24 weeks to 200 or 500 ppm n-hexane

Dose group	MCV(m/s)	DL (ms)	Total mixed nerve conduction velocity (m/s)	Distal mixed nerve conduction velocity (m/s)	Proximal mixed nerve conduction velocity (m/s)
Control	49.6 ± 7.6	1.81 ± 0.12	59.3 ± 4.1	52.3 ± 3.3	68.6 ± 12.5
200 ppm	42.9 ± 3.5[a]	1.91 ± 0.10	53.3 ± 3.0[b]	47.6 ± 1.7[b]	58.8 ± 9.0
500 ppm	36.2 ± 1.1[b]	2.13 ± 0.20[b]	50.9 ± 1.3[b]	45.3 ± 1.6[b]	5.0 ± 3.2[a]

Curves: $Y = A - C * e^{-Bt}$; Y, data for a particular endpoint over the seven observation periods; A, B, C, constant; t, weeks of exposure; and e, base of natural logarithm.
[a] $p < 0.05$
[b] $p < 0.01$

Source: Ono et al., 1982.

Pryor et al. (1983) carried out a subchronic exposure study on the neurotoxicity potential of n-hexane by exposing male F344 rats (13–14/group) to 0 or 2000 ppm n-hexane, 14 hours/day, 7 days/week for 14 weeks. Animals were subjected to a battery of behavioral tests at intervals during the exposure period. Tests included grip strength, motor activity, startle and avoidance responses, and the acquisition of a multisensory, conditioned avoidance response. Impaired performance and n-hexane-related latencies in animals' responses to these stimuli were recorded in variance units. Exposure to 2000 ppm n-hexane (95% pure) was associated with a statistically significant reduction in undifferentiated motor activity, startle to an air-puff response, and fore-limb and hind-limb grip strength. Exposure to n-hexane also was associated with a reduction in the amplitude of the fifth component of the BAEP. Overall performance in the tests improved during a 6-week recovery period. The authors subjected n-hexane-exposed rats to some other behavioral tests in which no n-hexane-related impairment of performance was seen. These included the acquisition of a multisensory conditioned pole-climb avoidance response and a tone-intensity discrimination task. Additionally, employing only hematoxylin and eosin-stained sections of paraffin-embedded nerve, no histopathological effects on the peripheral nerves were observed at term (14 weeks).

Howd et al. (1983) compared the neuropathogenic potential of n-hexane in weanling versus young adult F344 rats, in which exposure to 1000 ppm n-hexane (95% pure) 24 hours/day, 6 days/week for 11 weeks resulted in a greater incidence of neuropathological symptoms in adults compared with weanlings. Grip strength was reduced equally in adult rats

and weanlings within 2 weeks of the beginning of exposure. However, subsequent treatments affected this parameter more in young adults than in weanlings. Older rats exhibited earlier and more severe signs of hind-limb paralysis compared with younger animals. Conversely, there was little difference in neuropathological responses between rats in the different age categories, including tail nerve conduction time and BAEP.

Cavender et al. (1984a, b) exposed F344 rats (15/sex/group) to n-hexane at 0, 3000, 6500, and 10,000 ppm for 6 hours/day, 5 days/week for 13 weeks. There were no n-hexane-related clinical signs of toxicity, effects on food consumption, ophthalmological findings, or changes in neurological function. However, there was a lowering of the urinary pH in high-dose males. There were increased organ/body weight ratios for liver, kidney, and testis in high-dose males and kidney in mid-dose males. Histopathological examination of the tibial nerves revealed paranodal axonal swelling in mid- and high-dose males (1/5 and 4/5, respectively).

Male Wistar rats (eight/group) were exposed to 0, 500, 1200, or 3000 ppm n-hexane (>99% pure) for 12 hours/day, 7 days/week for 16 weeks (Huang et al., 1989). The authors measured MCV in the tail nerve along with body weight before exposure and after 4, 8, 12, and 16 weeks of exposure to n-hexane. One animal from each group was sacrificed after 16 weeks of exposure for histopathological evaluation of the nerve fibers in the tail. In addition, Huang et al. (1989) measured the levels of neuron-specific enolase and β-S100. These nerve-specific proteins are part of a family of calcium-binding proteins that are involved in processes such as cell to cell communication, cell growth, intracellular signal transduction, and development and maintenance of the central nervous system. Some members of the S100 protein family are released into the extracellular space (depending on concentration of protein) by an unknown mechanism and modulate cell proliferation, act as chemoattractants for leukocytes, stimulate neuronal survival and/or differentiation of astrocyte proliferation, increase apoptosis of neurons, and regulate macrophage activation. Data indicates that S100 proteins may be an extracellular biomarker for natural aging or damage to the CNS or PNS (i.e., dementia associated with Alzheimer's and Parkinson's diseases) (Donato, 2001; Donato, 1999; Fano et al., 1995). Dose-dependent, statistically significant reduction in body weight gain was observed in the mid-dose (at 12 weeks) and high-dose (at 8 weeks) rats (Huang et al., 1989). Additionally, there were some neurological deficits in mid- and high-dose rats, including a reduction in grip strength and a comparative slowness of motion from week 12 of exposure. However, no hind-limb paralysis was observed by the time of termination of the experiment. Rats exposed to the mid and high doses of n-hexane showed a reduction in MCV. This reduction was statistically significant during weeks 8–16 of the exposure period compared with controls. Increased incidence of

paranodal swelling, along with some evidence of demyelination and remyelination was present in the peripheral nerves at both mid and high doses. However, these histopathologic findings were more severe in the high-dose group. Among the biochemical changes were dose-dependent reductions in nervous system-specific proteins, particularly the β-S100 protein in tail nerve fibers, which was significantly reduced by approximately 75% at all dose levels. The neurophysiologic deficits and histopathologic effects that were evident in mid- and high-dose rats suggested a NOAEL of 500 ppm.

Huang et al. (1992) exposed male Wistar rats (seven/group) to 0 or 2000 ppm (99% pure) n-hexane, for 12 hours/day, 6 days/week for 24 weeks. Body weight gain decreased at the fourth week of exposure. Effects of treatment included an overall reduction (statistically significant) in MCV after 8 weeks that gradually decreased thereafter. The authors also observed an increase in DL after 12 weeks that was even greater after 16 weeks. There was a reduction in the activity or amount of neuron-specific enolase (γ-enolase), creatine kinase-B, and the β-S100 protein with neurophysiologic deficits that were most evident in the distal segment of the sciatic nerve (64, 71, and 76%, respectively). Levels of these nerve-specific markers were positively correlated with MCV and negatively correlated with DL in the distal sciatic nerve.

The American Petroleum Institute (API) sponsored a number of toxicological studies of n-hexane in experimental animals, including a 26-week inhalation toxicity study in Sprague-Dawley rats (Biodynamics, 1978). This study, originally submitted to the EPA under the Toxic Substances Control Act, featured a complex protocol in which 12 rats/sex/group were exposed to 0, 5, 25, or 125 ppm n-hexane, 6 hours/day, 5 days/week (mean concentrations of 6, 26, and 129 ppm) for up to 34 weeks or for 21 hours/day, 7 days/week (mean concentrations of 5, 27, and 126 ppm) for up to 34 weeks. Neuropathological examinations were carried out on a subset of each group after 8, 18, 26, 31, and 34 weeks. Hematological and clinical chemistry parameters were evaluated after rats had been exposed for 13 and 26 weeks. Body weights were monitored weekly through week 12, then bimonthly until the end of the study.

The authors noted a number of fluctuations in clinical chemistry, including higher fasting glucose levels in male rats exposed to 5 ppm and 125 ppm n-hexane at 26 weeks and lower blood urea nitrogen in female rats exposed to 125 ppm n-hexane for the same duration. There were also fluctuations in hematological parameters, including reductions in hemoglobin concentration and hematocrit in females exposed at all n-hexane concentrations and durations at the 13-week interim evaluation. However, these changes showed little relationship to dose, remained within normal limits, and were not apparent in blood samples taken after 26 weeks.

Accordingly, the study authors considered the changes not to be related to treatment. An addendum to the report concluded that no animal in the study displayed signs of nervous system degeneration characteristic of n-hexane exposure.

The International Research and Development Corporation (IRDC), sponsored by Phillips Petroleum Co., continuously exposed male Sprague-Dawley rats to n-hexane and a C6-isomer mixture consisting of n-hexane, methylcyclopentane, 3-methylpentane, and 2-methylpentane as major components for 22 hours/day, 7 days/week for 6 months (IRDC, 1992a, b). This study was conducted in two phases; the exposure groups are shown in Tables 4-8 and 4-10.

Table 4-8. Experimental protocol for Phases I and II of a 6-month inhalation study of n-hexane and a mixture containing hydrocarbon isomers plus n-hexane in male Sprague-Dawley rats

Group	Treatment
I	Controls
II	125 ppm n-Hexane
III	125 n-Hexane + 125 ppm C6 isomers[a]
IV	125 n-Hexane + 375 ppm C6 isomers
V	125 ppm n-Hexane + 1375 ppm C6 isomers
VI	500 ppm n-Hexane
VII	Controls
VIII	500 ppm C6 isomers
IX	500 ppm n-Hexane + 500 ppm C6 isomers
X	500 ppm n-Hexane

[a] C6 isomers were a mixture of n-hexane-depleted C6 hydrocarbons containing methylcyclopentane, 3-methylpentane, and 2-methylpentane as major components

Source: IRDC, 1992a, b.

In both phases of this study, animals were examined daily for signs of clinical toxicity, and body weights were monitored weekly. Two controls and four rats from Group VI (see Table 4-8) were taken from their exposure groups every month for the first 5 months. These animals, plus four from all groups after 6 months of exposure, were examined histopathologically for changes to the cervical spinal cord. All surviving animals (10/group) were necropsied at term, and the weights of their major organs were recorded. Excised pieces of tissue from a variety of organs and tissues were fixed for histopathological examination, including all abnormal masses, adrenal gland, abdominal aorta, bone marrow, brain, Zymbal's gland, esophagus, epididymis,

eye and optic nerve, tongue, Harderian gland, neuroganglia, liver, kidney, lung, lymph nodes, mammary gland, pancreas, parathyroid, pituitary, prostate, salivary gland, skeletal muscle, skin, nasal turbinates, gonads, lacrimal gland, heart, thymus, thyroid, peripheral nerve, small intestine, large intestine, spinal cord, spleen, seminal vesicle, stomach, and urinary bladder.

Rats exposed to 500 ppm n-hexane (Group VI) showed an abnormal gait (10/34). These symptoms were evident in a single rat after 14 weeks but increased in incidence and severity throughout the remainder of the experiment. However, the true incidence of this response may be underestimated because of subjects withdrawn from the study before symptoms could appear.

Some changes in absolute and relative organ weights were considered to be responses to treatment, including statistically significant increases in the liver:body weight and kidney:body weight ratios (Table 4-9). Signs of liver necrosis, marked by raised discolored areas on the organ

surface and, in some cases, necrotic foci when examined histopathologically, were evident in 3/10 rats exposed to 125 ppm n-hexane (Group II) and 2/10 rats exposed to 500 ppm n-hexane (Group VI). Degenerative and regenerative changes in the kidney were observed in 4/10 rats exposed to 500 ppm n-hexane (Group VI). The authors described these kidney and liver lesions as trace to mild severity. Reevaluation of the renal pathology of the kidneys showed a solvent-induced exacerbation of the hyaline droplet nephropathy that is characteristic for male rats of this and other strains as reported by Experimental Pathology Laboratories (EPL, 1992).

Table 4-9. Relative organ weights of male Sprague-Dawley rats exposed to n-hexane 22 hours/day, 7 days/week for 6 months

Organ	Group [a]		
	I	II (125 ppm)	VI (500 ppm)
Spleen	0.18 ± 0.029	0.15 ± 0.02[b]	0.21 ± 0.12
Liver	2.88 ± 0.26	3.01 ± 0.27	3.31 ± 0.11[c]
Kidney	0.56 ± 0.047	0.55 ± 0.059	0.71 ± 0.17[c]
Adrenal	1.01 ± 0.23	0.91 ± 0.10	1.22 ± 0.21
Testis	0.75 ± 0.11	0.67 ± 0.08	0.62 ± 0.10
Heart	0.29 ± 0.041	0.27 ± 0.45	0.27 ± 0.021

[a] Values are percent of body weight ± SD.
[b] Statistically significant compared with controls (p<0.05).
[c] Statistically significant compared with controls (p<0.01).

Source: IRDC, 1992a, b.

Exposure to n-hexane at 500 ppm (Group VI) was associated with axonal degeneration, myelin vacuolation, and skeletal muscular atrophy. Axonal degeneration occurred in the sciatic and tibial nerves (0/10, 0/10, and 7/10) and in the spinal cord thoracic, lumbar, and sacral nerves 0/10, 0/10, and 8/10), respectively. Skeletal muscle atrophy was observed in the high-dose animals (9/10). No other treatment groups exhibited neuropathologic or myopathic changes.

In phase II of the study (Table 4-8), rats (10/group) were exposed to either filtered air (Group VII), 500 ppm C6 isomers alone (Group VIII), 500 ppm n-hexane plus 500 ppm C6 isomers (Group IX), or 500 ppm n-hexane alone (Group X). An abnormal gait developed in rats exposed to 500 ppm n-hexane (Group X) at week 16 with increasing incidence and severity over time. Atrophy of the sciatic and anterior tibial nerves (14/16) and skeletal muscle atrophy (3/10) were observed in rats exposed to 500 ppm n-hexane. No histopathologic evidence of neuropathy was seen in controls. The authors noted a slightly increased incidence and severity of chronic renal nephritis in both controls and n-hexane-exposed rats (6/11 and 10/10, respectively), and a significant increase in relative kidney weights (0.532 ± 0.053% in controls and 0.844 ± 0.098% in the n-hexane-exposed group).

The National Toxicology Program (NTP) sponsored a 13-week inhalation study of n-hexane in B6C3F1 mice, the findings of which have been reported in the peer-reviewed scientific literature (NTP, 1991; Dunnick et al., 1989). Groups of 10 mice/sex/group were exposed to 0, 500, 1000, 4000, or 10,000 ppm n-hexane (>99% pure), 6 hours/day, 5 days/week for 13 weeks, while another group of 10 mice was exposed to n-hexane at 1000 ppm for 22 hours/day, 5 days/week for 13 weeks. Separate groups of eight mice/sex/group received identical treatments but were subjected to neurobehavioral tests before the start of dosing, then again after 6 and 13 weeks of exposure. The neurobehavioral tests included undifferentiated motor activity, fore-limb and hind-limb grip strength, thermal sensitivity, startle response, and foot splay. Four males and four females were randomly selected from the 0, 1000 ppm extended duration, and 10,000 ppm exposure groups for histopathological examination of the spinal cord and tibial nerves. Animals were observed daily for signs of clinical toxicity and weighed weekly.

A full necropsy was performed at sacrifice, weights of the major organs were recorded, and histopathological evaluations (in control, high-dose, and extended duration groups) were carried out at term on a variety of excised organs and tissues, including adrenal gland, brain, bronchial lymph nodes, cecum, colon, duodenum, esophagus, gall bladder, gross lesions and tissue masses, heart, ileum, jejunum, kidney, larynx, liver, lung and mainstem bronchi, mammary

gland, mandibular and mesenteric lymph nodes, nasal cavity and turbinates, pancreas, parathyroid, pituitary, rectum, salivary glands, sciatic nerve, spinal cord, spleen, sternum including marrow, glandular and forestomach, testis with epididymis, prostate, seminal vesicles, ovary and uterus, thymus, thyroid gland, trachea, and urinary bladder. The mandibular lymph nodes, nasal cavity, and sternum with marrow were examined histopathologically in all groups. The liver was examined only in the males of all exposure groups.

All of the animals in the study survived to term, although there were some signs of nasal irritation among those animals exposed to 10,000 ppm n-hexane. However, no changes in organ weight to body weight ratio were observed in male or female mice that could be clearly attributed to exposure to n-hexane. Nonetheless, relative liver, kidney, and heart weights appeared to be increased compared with controls in exposed females. The only observed neurobehavioral deficit was a reduction in locomotor activity in females exposed to 10,000 ppm n-hexane 6 hours/day and to 1000 ppm of n-hexane for 22 hours/day. There was an increased incidence of paranodal axonal swelling in high-dose or extended exposure duration mice. Table 4-10 summarizes the incidence of neuropathological and respiratory tract lesions and gives the average grade on a scale of 1–5 for the most severe lesions observed in the eight levels of nasal cavity. The authors concluded that n-hexane caused minimal toxicity to the nervous system and/or respiratory system at 1000 ppm and above.

Table 4-10. Incidence of nasal turbinate and neuropathological lesions in B6C3F1 mice exposed to n-hexane for 13 weeks

Site/lesion	Concentration of n-hexane (ppm)					
	0	500	1000	1000 (ext.)	4000	10,000
Male[a]						
Lumen Exudate, suppurative	0/10	0/10	0/10	0/10	0/10	10/10 (2.3)
Olfactory epithelium Chronic active inflammation	0/10	0/10	0/10	0/10	0/10	10/10 (2.1)
Multifocal erosion	0/10	0/10	0/10	0/10	0/10	8/10 (3)
Multifocal regeneration	0/10	0/10	2/10 (2)	4/10 (1.5)	0/10	10/10 (2.2)
Metaplasia	0/10	0/10	2/10 (1)	4/10 (1)	1/10 (1)	10/10 (2.8)
Respiratory epithelium Chronic active inflammation	0/10	0/10	0/10	0/10	0/10	9/10 (1.9)

Site/lesion	Concentration of n-hexane (ppm)					
	0	**500**	**1000**	**1000 (ext.)**	**4000**	**10,000**
Multifocal erosion	0/10	0/10	0/10	0/10	0/10	2/10 (1.5)
Multifocal regeneration	0/10	0/10	0/10	0/10	0/10	10/10 (1.4)
Submucosa Focal fibrosis	0/10	0/10	0/10	0/10	0/10	5/10 (1.4)
Paranodal swellings Spinal cord	0/4	ND	ND	0/4	ND	0/4
Tibial nerve	0/4	ND	ND	0/4	ND	3/4
Teased fiber Tibial nerve	0/4	ND	ND	3/4	ND	3/4
Female[a]						
Lumen Exudate, suppurative	0/10	0/10	0/10	0/10	0/10	10/10 (2.3)
Olfactory epithelium Chronic active inflammation	0/10	0/10	0/10	0/10	0/10	7/10 (1.9)
Multifocal erosion	0/10	0/10	0/10	0/10	0/10	8/10 (1.7)
Multifocal regeneration	0/10	2/10 (1)	1/10 (2)	9/10 (1.7)	9/10 (2)	10/10 (2.6)
Metaplasia	0/10	0/10	1/10 (2)	8/10 (1.9)	8/10 (2)	10/10 (2.6)
Respiratory epithelium Chronic active inflammation	0/10	0/10	1/10 (2)	0/10	0/10	5/10 (1.4)
Multifocal erosion	0/10	0/10	0/10	0/10	0/10	0/10
Multifocal regeneration	0/10	0/10	0/10	0/10	1/10 (2)	6/10 (1.2)
Submucosa Focal fibrosis	0/10	0/10	0/10	0/10	1/10 (1)	9/10 (1.6)
Paranodal swellings Spinal cord	0/4	ND	ND	0/4	ND	0/4
Tibial nerve	0/4	ND	ND	0/4	ND	3/4
Teased fiber Tibial nerve	0/4	ND	ND	3/4	ND	3/4

[a] Numbers in parentheses represent the average grade (on a scale of 1-5) of the most severe lesions observed in eight levels of the nasal cavity examined.
ND = No Data.

Source: Dunnick et al., 1989.

A study by Lungarella et al. (1984) examined the effect of n-hexane in 12 male New Zealand white rabbits/group that had been exposed to either 0 or 3000 ppm n-hexane (purity not stated) for 8 hours/day, 5 days/week for 24 weeks. Some animals were afforded a 120-day recovery period before sacrifice, at which point the cellular architecture of the lungs was examined under the light and electron microscopes. Treatment-related portal-of-entry effects included an enlargement of the air spaces in respiratory bronchioles and alveolar ducts, pulmonary fibrosis, and papillary tumors of nonciliated bronchial epithelial cells. However, some of these lesions were more marked in animals sacrificed immediately after the last exposure compared with those allowed to recover for 120 days. n-Hexane exposure had no effect on body weight gain, hematological parameters, or clinical chemistry.

4.2.2.2. *Chronic Studies*

No chronic studies were identified that examined the toxicological effects of n-hexane in experimental animals by the inhalation route.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

4.3.1. Oral Studies

Marks et al. (1980) conducted a reproductive/developmental and teratological study in CD-1 mice in which dams were exposed to n-hexane (99% pure) in cottonseed oil by gavage on GDs 6–15. In the first portion of this study, 4–30 mice were dosed with vehicle or increasing concentrations of n-hexane at 260, 660, 1320, or 2200 mg/kg-day. There were no reproductive, developmental, or teratological effects of n-hexane in mice dosed with n-hexane observed in this portion of the study.

In the second portion of the study, 19–26 pregnant mice/group received cumulative doses of either 0, 1830, 2170, 7920, or 9900 mg/kg-day on GDs 6–15 in the form of three separate injections spaced throughout the day. All dams were sacrificed on GD 18, and uteri were examined for the number of implantation sites. Live fetuses were examined for external and visceral malformations and for skeletal variations. Dam mortality was increased in high-dose groups. Fetal birth weight was 6.35% lower in the progeny of those dams exposed to 7920 and 9900 mg/kg-day than in controls (0.946 g for mice dosed at 7920 and 9900 mg/kg-day versus 1.011 g in controls). However, neither these nor any other fetuses in the study showed an increased incidence of skeletal malformations or variations as a result of maternal n-hexane treatment. The authors suggested that n-hexane is not teratogenic at concentrations associated

with overt maternal toxicity.

Linder et al. (1992) included n-hexane in a survey of chemicals for spermatotoxic effects in male Sprague-Dawley rats. The experimental protocol featured the oral administration of the undiluted n-hexane either in a single dose of 20,000 mg/kg or in five consecutive daily doses of 10,000 mg/kg. The spermatotoxic tests employed included sperm head counts, sperm velocity, sperm morphology, and the histopathology of the testis and epididymis. No change was observed in any of these parameters in rats exposed to n-hexane.

4.3.2. Inhalation Studies

Groups of between three and eight pregnant F344 rats were exposed to air or 1000 ppm n-hexane, 6 hours/day on GDs 8–12, 12–16, or 8–16 (Bus et al., 1979). Dams were sacrificed on GD 22 and autopsied to evaluate reproductive parameters such as the number and position of live, dead, and resorbed fetuses, fetal weight, and the number and type of any fetal defects, malformations, or skeletal variations. Some pregnant females exposed on GDs 8–16 were allowed to deliver their progeny, and total litter body weights and mortality were monitored at weekly intervals up to 7 weeks after birth.

Exposure to n-hexane had no significant effect on the number of fetal resorptions, external anomalies, soft tissue anomalies, or skeletal variations. Pup growth from dams exposed on GDs 8–16 was 13.9% lower than controls for up to 3 weeks after birth, but had reached levels similar to controls after 7 weeks. The authors concluded that n-hexane had little effect on the reproduction and development of F344 rats.

Litton Bionetics (1979) exposed CRL:COBS CD(SD)BR rats (20 pregnant females/group) for 6 hours/day to concentrations of 0, 100, or 400 ppm n-hexane on GDs 6–15. Food consumption and body weights were monitored intermittently between GDs 0 and 20, at which point the dams were sacrificed and necropsied to facilitate the evaluation of reproductive, developmental, and teratological parameters. However, no n-hexane-related effects were observed.

The results of a teratological study of n-hexane conducted in rats on behalf of the NTP were reported in the published literature by Mast (1987). Mast (1987) exposed pregnant Sprague-Dawley rats (30/group) to 0, 200, 1000, or 5000 ppm n-hexane (>99.5% pure) for 20 hours/day on GDs 6–19. Maternal toxicity was monitored throughout the experiment. Uterine, placental, and fetal body weights were measured at sacrifice on GD 20. In addition, the number of implantation sites and live, dead, and resorbed fetuses was reported. Live fetuses were sexed and examined for gross, visceral, skeletal, or soft-tissue craniofacial defects.

There was a statistically significant reduction in maternal body weight gain in the high dose dams. There were no intrauterine deaths following exposure to n-hexane. A reduction in fetal body weight gain was apparent in the progeny of mid- and high-dose dams (3.0–7.5% at the mid dose and 14–15% at the high dose, respectively). This difference was statistically significant at both 1000 and 5000 ppm in males and at 5000 ppm in females (3.48 ± 0.37, 3.54 ± 0.36, 3.27 ± 0.32, and 2.97 ± 0.38 grams for the 0, 200, 500, and 1000 ppm exposures, respectively). The incidences of skeletal variations in individual live fetuses are shown in Table 4-11. There was a statistically significant increase in the mean percent incidence of reduced ossification of sternebrae 1–4 per litter at 5000 ppm compared with controls (38.7 ± 23.7 versus 13.8 ± 21.6, respectively). Also, this treatment effect was correlated in a statistically significant manner with exposure concentration. Mast (1987) concluded that the lowest n-hexane concentration, 200 ppm, would be a NOAEL for developmental toxicity in Sprague-Dawley rats.

Table 4-11. Skeletal variations in live fetuses of pregnant Sprague-Dawley rats exposed to n-hexane via inhalation

	Concentration of n-hexane (ppm)			
Subjects	**0**	**200**	**1000**	**5000**
Total fetuses examined	339	350	392	408
Heads examined	170	157	186	205
Skulls examined	169	193	206	203
Malformations/Variations	**Incidence (%)**			
Dilated ureters	7.4	6.9	5.1	2.9
Renal pelvic cavitation	2.4	0.0	0.8	0.5
Supernumerary ribs	1.2	1.7	3.1	3.7
Bent ribs	0.0	0.0	0.3	0.0
Reduced Ossification				
Sternebrae 1–4	12.4	15.4	26.3	38.5
Vertebral centra	8.3	4.6	4.8	8.8
Pelvis	3.2	2.0	5.4	5.1
Phalanges	1.2	0.6	0.3	1.7
Skull	5.9	3.1	5.3	5.9

Source: Mast, 1987.

Mast et al. (1988a) also reported the results of an NTP-sponsored reproductive, developmental and teratological study in mice. Pregnant CD-1 mice (30/group) were exposed to 0, 200, 1000, or 5000 ppm n-hexane (99.2% pure) for 20 hours/day on GDs 6–17 (Mast et al., 1988a). Maternal toxicity was determined at sacrifice on GD 18, and similar reproductive, developmental, and teratological effects to those described above in Sprague-Dawley rats (Mast, 1987) were examined. Among the dams, there was a statistically significant reduction in body weight gain and relative uterus weight in the high-dose group. Fetal weights were slightly, but not significantly, reduced (means of litter means) for all groups compared with controls. However, this decrease in fetal weights did correlate significantly with dose. Mean female fetal weights were significantly reduced (approximately 6%) at 5000 ppm and exhibited a significant correlation to increasing dose. There was also an increase in the mean percent incidence of supernumerary ribs that appeared to be dose related (not significant). The numbers of live fetuses per litter were reduced in all exposure groups compared with controls, although the observed decrease was statistically significant only at 5000 ppm. The numbers of live fetuses per litter in all exposure groups were also reduced compared with contemporary controls of the same strain of mice. The mean percent of live implants was reduced compared with contemporary controls at 200 and 5000 ppm but not at 1000 ppm. The mean percent of intrauterine death (early and late resorptions combined) was greater for all exposed groups compared with controls, but this difference was only statistically significant for the 200 ppm exposure group and there was no dose-related trend. An increased incidence of late resorptions was also significantly greater than in controls, following exposure to 5000 ppm n-hexane relative to controls. The author stated that trend analysis indicated that the increased mean percent incidence of late resorptions was positively and significantly correlated with exposure concentration.

A number of experimental studies examined the effect of n-hexane on the male reproductive system when administered via the inhalation route. De Martino et al. (1987) exposed male Sprague-Dawley rats (12–39/group) to 5000 ppm n-hexane (99% pure) in either (1) a single 24-hour exposure, (2) repeated 16-hour/day exposures for up to 8 days, or (3) repeated 16-hour/day exposures, 6 hours/day for up to 6 weeks. The study employed two control groups, one of which was pair-fed. Treated animals were allowed to recover for different lengths of time after the end of treatment (from 2 days to 29 weeks, depending on the original exposure duration). Rats exposed to 5000 ppm n-hexane displayed some evidence of neuropathy such as paralysis, and extreme cases were sacrificed moribund and necropsied rather than being allowed to die and undergo partial autolysis. Focal degeneration of spermatocytes and exfoliation of

elongated spermatids was observed in rats treated with n-hexane. Early meiotic prophase spermatocytes (leptotene and zygotene) and transitional spermatocytes as well as those undergoing meiotic metaphase appeared to be more susceptible to the action of n-hexane than pachytene spermatocytes. Rats receiving a single 24-hour exposure to n-hexane also showed a measure of recovery after 2–4 weeks following the termination of exposure. By contrast, rats exposed repeatedly to 5000 ppm n-hexane over a 6-week period showed complete atrophy of the seminiferous tubules. The animals exposed for up to 6 weeks displayed a reduction in food consumption and body weight gain; these effects were accompanied by signs of incipient neuropathy. There was also a wide range of testicular lesions that did not completely resolve during the recovery period even though body weights and clinical symptoms improved.

A detailed study by Mast et al. (1988b) examined the effects of n-hexane on sperm morphology in B6C3F1 mice. The experimental protocol featured the exposure of 20 male B6C3F1 mice/group to 0, 200, 1000, or 5000 ppm n-hexane, 20 hours/day for 5 consecutive days, after which the animals were examined for gross lesions of the reproductive tract and any disturbances to sperm morphology. There were no clinical signs of toxicity or body weight changes in any of the dose groups, nor was there an increased incidence of aberrant sperm characteristics such as blunt-hook, banana, amorphous, or pin-head shapes. Likewise, there was no increased incidence of sperm with more than one head or tail.

Mast et al. (1988c) carried out a further study of the effect of n-hexane in male CD-1 mice in which 20 males/group were exposed to 0, 200, 1000, or 5000 ppm n-hexane, 20 hours/day for 5 consecutive days, then mated to unexposed virgin females. Mated females were sacrificed 12 days after the last day of cohabitation, and their reproductive status and the numbers and viability of their implants were recorded to assess the capacity of n-hexane to induce male dominant lethal effects. The number of live implants was consistently greater than 10 fetuses/litter, and there was no indication of a decline in reproductive index as a result of increasing n-hexane exposure in the males. Furthermore, there was no increase in the number of dead implants or early resorptions as a result of the males being exposed to n-hexane prior to mating. The study authors concluded that short-term exposure to n-hexane vapor did not result in a male dominant lethal effect in CD-1 mice.

4.4. OTHER STUDIES

4.4.1. Acute Toxicity Data

Few data are available for a median lethal dose (LD_{50}) for n-hexane. HSDB (2005) presents values of 28,710, mg/kg, or 24 and 45 mL/kg (approximately 15,840 and 29,700 mg/kg) for juvenile and adult rats. For inhalation exposure, a 4-hour LC_{50} of 48,000 ppm has been reported for both rats and mice (HSDB, 2005).

A number of acute and subacute experimental studies have been carried out to assess pulmonary toxicity following inhalation exposure to n-hexane. For example, Lungarella et al. (1980) exposed 12 New Zealand white rabbits/group to 0 or 3000 ppm n-hexane (purity not stated), 8 hours/day on 8 consecutive days and determined the incidence and severity of pulmonary lesions by light and electron microscopy. Treated animals showed morphological signs of parenchymal changes, with lung damage being most severe in the area between the terminal bronchioles and the alveolar ducts. The lesions consisted of necrotic changes in the bronchiolar epithelium and desquamation plus an increased number of macrophages within the proximal alveoli. The architecture of alveolar type II cells also was affected.

The same research group measured the activities of LDH, β-glucuronidase, glucose-6-phosphate dehydrogenase, and acid and alkaline phosphatases in lung homogenates of New Zealand white rabbits exposed to 0 or 3000 ppm n-hexane, 8 hours/day for 8 days (Barni-Comparini et al., 1982) . As shown in Tables 4-12 and 4-13, the appearance of blood cells in bronchial lavage and the concurrent biochemical changes observed in lung homogenates were consistent with the morphological changes observed at necropsy in the Lungarella et al. (1980) study. The increased levels of the lysosomal enzyme, acid phosphatase, might reflect acute inflammation, while the increased activity of glucose-6-phosphate dehydrogenase suggests a repair process subsequent to n-hexane-induced pulmonary disruption.

Table 4-12. Total red blood cells and nucleated cells in bronchial lavage from n-hexane-challenged New Zealand white rabbits

Cell counts (× 10^7)	Exposure group (ppm n-hexane)	
	0	3000
Red blood cells	3.5 ± 0.26	8.20 ± 0.75[a]
Total nucleated cells	0.71 ± 0.06	2.06 ± 0.28[a]

[a] Significantly different from controls (p<0.01).

Source: Barni-Comparini et al., 1982.

Table 4-13. Enzyme activities in lung homogenates of rabbits exposed to n-hexane

Enzyme activities (IU)	Exposure group (ppm n-hexane)	
	0	3000
Lactate dehydrogenase	75 ± 3.7	109 ± 5.1[a]
Glucose-6-phosphate dehydrogenase	5.10 ± 0.29	9.98 ± 1.3[a]
Acid phosphatase	2.31 ± 0.64	4.96 ± 0.81[a]
Alkaline phosphatase	1.38 ± 0.15	1.91 ± 0.19
β-Glucuronidase	0.43 ± 0.09	0.95 ± 0.15[a]

[a] Significantly different from controls, as calculated by the authors.

Source: Barni-Comparini et al., 1982.

Sahu et al. (1982) exposed 20 male Sprague-Dawley rats/group to 476, 1149, or 1676 ppm n-hexane (purity not stated) for 6 hours/day, 5 days/week for 4 weeks. The authors compared the composition of a cell-free supernatant from bronchial lavage in each of the n-hexane-exposed groups to that from 10 control rats. There was a dose-dependent increase in a number of enzyme activities and functionally relevant biochemicals (Table 4-14). While no statistical treatment of the data was provided in the report (control versus test groups), the dose-dependent increases in biochemical parameters and enzyme activities suggested a disruptive effect of commercial hexane on one or more cell types in the lung. The threefold increase in LDH activity in high-dose rats versus controls is consistent with a gross disruption of the plasma membrane of the respiratory epithelial cells, leading to possible leakage of intracellular contents.

Table 4-14. Concentration of biochemicals and enzyme activities in bronchial lavage fluid from male Sprague-Dawley rats exposed to n-hexane

Biochemical parameters (units)	Concentration of n-hexane (ppm)			
	0	476	1149	1676
Protein (mg/mL)	0.13 ± 0.02	0.14 ± 0.03	0.18 ± 0.04	0.21 ± 0.04
Lipid (mg/mL)	0.81 ± 0.12	1.08 ± 0.11	1.36 ± 0.16	1.74 ± 0.11
Sialic acid (μg/mL)	0.09 ± 0.02	0.12 ± 0.02	0.16 ± 0.03	0.21 ± 0.04
Acid phosphatase (μmol/hour-mg protein)	0.21 ± 0.03	0.27 ± 0.04	0.31 ± 0.03	0.43 ± 0.05
Alkaline phosphatase (μmol/hour-mg protein)	0.37 ± 0.05	0.41 ± 0.05	0.54 ± 0.04	0.68 ± 0.06
Lactate dehydrogenase (μmol/min-mg protein)	0.16 ± 0.03	0.19 ± 0.02	0.26 ± 0.02	0.45 ± 0.04

Glucose-6-phosphate dehydrogenase (nmol/min-mg protein)	0.90 ± 0.08	0.97 ± 0.12	1.35 ± 0.16	1.72 ± 0.16
Angiotensin-converting enzyme (nmol/min-mg protein)	0.36 ± 0.04	0.67 ± 0.08	0.86 ± 0.07	1.18 ± 0.12

Source: Sahu et al., 1982.

Ikeda et al. (1986) exposed five male Wistar rats/group continuously for 30 days to either 200 or 400 ppm n-hexane, 200 ppm n-hexane with supplemental toluene at 200 ppm, or 200 and 400 ppm toluene alone. Weight gain was significantly less in the two treatment groups with n-hexane or toluene alone. When concentrations of norepinephrine and dopamine were measured in various regions of the brain, exposure at 400 ppm n-hexane resulted in significant elevations of norepinephrine levels in the thalamus (by 206%), dorsal, olfactory, and frontal cortex (by 164%, 139%, and 157%, respectively), and cerebellum (by 170%) compared with controls. Toluene exposure (400 ppm) led to a significant reduction in norepinephrine in the olfactory cortex (by 82%) and in the hypothalamus (by 81%) and elevation in the ventral cortex (by 132%). Dopamine levels were reduced in the striatum (by 91%) and elevated in the olfactory cortex (138%) at 400 ppm toluene. Equivalent changes of this magnitude were not seen as a result of exposure to mixtures of n-hexane and toluene, although the amount of norepinephrine in the olfactory cortex was increased by 129%. A similar increase in the amount of dopamine in the hippocampus was evident in animals exposed to the mixture of solvents.

Pezzoli et al. (1990) injected male VCD-1(ICR)BR mice (number not stated) intraperitoneally with 400 mg/kg-day n-hexane, 5 days/week for 3 weeks. In addition, male Sprague-Dawley rats were injected with a single 5 mg dose of n-hexane into the left midbrain (substantia nigra). This exposure was repeated again 14 days later. The concentrations of neurotransmitter-related substances of the brain, such as dopamine, homovanillic acid, norepinephrine, serotonin, and 5-hydroxyindolacetic acid, were evaluated. Levels of dopamine and homovanillic acid were approximately 30% lower in the brains of mice treated for 3 weeks with n-hexane when compared with controls. A similar result was observed in response to the single n-hexane treatment in the left brain. The authors speculated that n-hexane could possibly alter the dopaminergic pathway; however, the mechanism was unclear.

The same researchers measured the concentrations of dopamine, homovanillic acid, and 2,5-hexanedione in the cerebellum and striatum of mice injected intraperitoneally with 1000 mg/kg n-hexane (Masotto et al., 1995). A more than twofold increase was seen in the concentrations of 2,5-hexanedione (5.63 ± 0.4 versus 2–3 µg/g in controls). In contrast to the results of an earlier study (Pezzoli et al., 1990), striatal synaptosomal dopamine increased in

mice treated with n-hexane (1000 mg/kg) either 30 or 60 minutes prior to sacrifice compared with control mice.

Goel et al. (1982) gave six female albino rats/group daily intraperitoneal injections of 1mL/kg (660 mg/kg) n-hexane for 2 or 7 consecutive days or twice a week for 45 days. Animals were sacrificed after 2, 7, or 45 days, and liver homogenates were prepared. Alkaline phosphatase activity was increased in liver homogenates of all n-hexane-treated animals regardless of duration of exposure (4.15 ± 0.48 nmol/min-mg protein after 2 days, 7.8 ± 1.5 nmol/min-mg protein after 7 days, 4.67 ± 0.58 nmol/min-mg protein after 45 days, versus 1.53 ± 0.19 nmol/min-mg protein in controls), while that of fructose-1,6-diphosphate aldolase decreased. Some n-hexane-related changes in clinical chemistry parameters were reported, including decreases in acetylcholinesterase, albumin, and cholesterol (in the latter, 1647 ± 45 mg/L after 2 days, 1393 ± 5 mg/L after 7 days, and 1371 ± 5 mg/L after 45 days versus 1648 ± 29 mg/L in controls). In a later study, the same researchers (Goel et al., 1987) measured ^{59}Fe uptake in rats receiving n-hexane via the same exposure protocol and found it to be reduced in the bone marrow compared with controls (Goel et al., 1987). However, most hematological parameters were unaffected by n-hexane treatment, following either intraperitoneal injection or a 7-day oral exposure regimen. Goel et al. (1988) also used the same experimental approach to demonstrate n-hexane related reductions in hepatic total sulfhydryl content (by 15% from control values) and the activity of such mixed function oxidases as aniline hydroxylase (by 11–58%), benzo(a)pyrene hydroxylase (by 41–60%), and aminopyrine-N-demethylase (by 53–57%).

Bastone et al. (1987) exposed male Sprague-Dawley rats to 0 or 5000 ppm n-hexane, 16 hours/day, 6 days/week for 4 weeks. There was a treatment-related reduction of MCV, an effect accompanied by a transient increase in plasma acetylcholinesterase activity that declined to baseline levels 2 weeks after cessation of treatment.

Anderson and Dunham (1984) dosed five male Sprague-Dawley rats/group intraperitoneally with 540 mg/kg n-hexane or 280 mg/kg 2,5-hexanedione daily for 35 days and obtained conduction velocities in the sciatic and sural nerves that were slower compared with controls (Table 4-15). The authors speculated that the electrophysiological changes may have been related to a disruption of nerve-membrane ATPase activity.

Table 4-15. Changes in sciatic and sural nerve action potentials induced by n-hexane and 2,5-hexanedione

Nerve	Treatment	Amplitude (mV)	Peak conduction velocity (m/s)	Duration (ms)
Sciatic	Control	1.31 ± 0.16	24.2 ± 0.94	0.60 ± 0.04
	n-Hexane	0.92 ± 0.23	20.2 ± 0.92[a]	0.78 ± 0.07[a]
	2,5-Hexanedione	ND	20.2 ± 0.46[a]	1.72 ± 0.11[a]
Sural	Control	1.04 ± 0.07	18.5 ± 0.61	0.68 ± 0.07
	n-Hexane	0.81 ± 0.30	14.7 ± 0.59[a]	0.92 ± 0.02[a]
	2,5-Hexanedione	0.90 ± 0.31	13.5 ± 0.29[a]	1.97 ± 0.24[a]

[a] Statistically significant compared with controls ($p<0.05$).
ND = No data.

Source: Anderson and Dunham, 1984.

Khedun et al. (1996) exposed male Wistar rats for 30 days by gavage to daily doses of 0.1 mL (66 mg) n-hexane in olive oil. A Langendorff apparatus was used to measure heart rate, coronary flow, and ventricular fibrillation threshold, the latter parameter showing a dramatic reduction compared with controls. Concomitant reductions of magnesium and potassium were noted in the treated group. However, exogenously replacing the magnesium and potassium did not reverse the lowering of the ventricular fibrillation threshold.

Bio-Research Laboratories (1989) carried out an acute operant behavior study of inhaled commercial hexane in Sprague-Dawley rats (6/sex/group) receiving a single 6-hour nose-only exposure at either 0, 873, 2974, or 9187 ppm. Animals were tested in commercial operant chambers that were fitted with a response lever over a feeder magazine. Prior to commencement of the study, all animals were given up to seven training sessions of approximately 1 hour each to learn to press the lever to get the reward (a food pellet). On the day of treatment, rats were tested for a 30-minute session immediately following treatment. The animals in the study showed no clinical signs of toxicity, and body weight parameters and food consumption were unaffected by treatment. Analysis of the response data showed no effect of hexane treatment immediately after exposure or on posttreatment days 1 or 2 on learned behavior.

4.4.2. Studies with Mixtures Containing n-Hexane

Commercial hexane is a mixture of aliphatic hydrocarbons used as a solvent for adhesives or to clean machinery. Although the precise amount of each constituent varies, slightly more than half (about 52%) of commercial hexane consists of n-hexane. The remaining

portion is a mixture of isomers and structurally related chemicals of n-hexane, such as 3-methylpentane (16%), methylcyclopentane (16%), and 2-methylpentane (13%), as well as some minor components such as cyclohexane and 2,4-dimethylpentane.

4.4.2.1. *Oral Exposure*

No studies were identified that administered commercial hexane to experimental animals via the oral route.

4.4.2.2. *Inhalation Exposure*

4.4.2.2.1. *Subchronic studies*. Duffy et al. (1991) reported (abstract only) a 13-week inhalation toxicity study of commercial hexane in F344 rats and B6C3F1 mice, in which 10 animals/sex/group were exposed to target concentrations of 0, 900, 3000, or 9000 ppm commercial hexane for 6 hours/day, 5 days/week for 13 weeks in a published abstract of the report by Biodynamics (1989). There were no changes in body weight, food, and water consumption, no treatment-related mortality at any concentration in either species, and few, if any, clinical signs of toxicity other than lacrimation in both sexes of high-dose mice and high-dose female rats. High-dose male and female mice and high-dose male rats displayed an increase in absolute and relative liver weights. Adverse histopathological findings typical of hydrocarbon nephropathy were confined to the kidneys of high-dose male rats, as described in the experimental pathology report of the study (EPL, 1989). All male rats (controls and exposed) showed some evidence of hyaline droplet formation and related nephropathy. However, this effect was more severe in male rats exposed to commercial hexane compared with controls. The kidneys of high-dose males showed mild tubular dilatation, with granular material in the lumen and signs of epithelial regeneration compared with controls. High-dose males displayed mild to moderate degrees of epithelial regeneration, a response that was minimal in controls and in animals receiving commercial hexane at the intermediate concentrations.

Bio-Research Laboratories (1990) conducted a 13-week study of the effects of commercial hexane in Sprague-Dawley rats (also reported in an abstract by Soiefer et al., 1991). Twelve rats/sex/group were exposed to 0, 900, 3000, or 9000 ppm commercial hexane for 6 hours/day, 5 days/week for 13 weeks. The animals were evaluated in a functional observational battery (FOB) approximately 1–2 hours after the first exposure and prior to exposure on days 1, 7, 14, 35, 63, and 91. Motor activity was tested pre-study and on days 34, 62, and 90. Six animals/sex in the control and high-dose groups were assessed for histological signs of

neuropathy. No n-hexane-related effects were observed in either the FOB assessment or histologically in either sex in any treatment group.

4.4.2.2.2. ***Chronic exposure.*** API sponsored two 2-year carcinogenicity studies with commercial hexane, one in F344 rats (Biodynamics, 1993a) and the other in B6C3F1 mice (Biodynamics, 1993b). The principal features and key findings of these studies have been compiled into a single research report that was published in the peer-reviewed literature (Daughtrey et al., 1999). In each case, 50 animals/sex/group were exposed 6 hours/day, 5 days/week to a commercial hexane preparation at targeted concentrations of 0, 900, 3000, or 9000 ppm. The commercial hexane preparation used in the experiments consisted of 51.5% n-hexane, 16% methylcyclopentane, 16.1% 3-methylpentane, 12.9% 2-methylpentane, 3.3% cyclohexane, and trace amounts of other hydrocarbons.

There were no statistically significant differences in survival rates between control and exposed groups of either sex. Exposed animals showed few clinical signs of toxicity in response to exposure to commercial hexane other than lacrimation, and there were no n-hexane related necropsy findings remote from the site-of-entry. Histopathological lesions in the respiratory passages were noted, especially in the nasal turbinates and larynx. Specific findings consisted of hyperplasia of epithelial and goblet cells, chronic inflammation, and increased incidence of intracytoplasmic eosinophilic material in all groups exposed to commercial hexane. Chronic inflammation was also seen to some extent in controls. Low-, mid-, and high-dose males and females displayed squamous metaplasia/hyperplasia of the columnar epithelium. This effect was not seen in controls. No treatment-related histopathological abnormalities in sciatic nerve were observed in any group of F344 rats exposed to commercial hexane in this study. The histopathological lesions of the respiratory tract that were evident, even in low-dose rats of both sexes, suggest that a NOAEL cannot be derived from this study. There was no n-hexane-related tumor formation at any tissue site in F344 rats.

There were no statistically significant differences in survival between controls and any of the exposed mice of either sex. There were no differences in clinical signs of toxicity and ophthalmologic or hematologic effects between the groups, and body weight changes in commercial hexane-exposed mice were similar to those in controls. There was a statistically significant, dose-related increase in the incidence of hepatocellular neoplasms in the livers of high-dose females compared with controls. There was also an increased incidence of pituitary hyperplasia, adenomas, and adenocarcinomas in exposed females (Table 4-16). Commercial hexane was associated with decreased severity and incidence of cystic endometrial hyperplasia

of the uterus among high-dose females compared with controls.

Table 4-16. Incidence of liver and pituitary tumors in male and female B6C3F1 mice exposed to commercial hexane for 2 years

	Target concentration of commercial hexane (ppm)			
	0	**900**	**3000**	**9000**
Target organ / cellular response	**Liver**			
Males Adenomas	10/49	5/50	7/50	10/50
Carcinomas	7/49	11/50	10/50	3/50
Combined adenomas and carcinomas	17/49	16/50	17/50	13/50
Females Adenomas	4/50	6/50	4/49	10/50
Carcinomas	3/50	2/50	5/49	6/50
Combined adenomas and carcinomas	7/50	8/50	9/49	16/50[a,b]
	Pituitary			
Males Hyperplasia	0/46	0/11	0/6	1/46
Adenomas	1/46	0/11	0/6	0/46
Adenocarcinomas	0/46	0/11	0/6	0/46
Total neoplasms	1/46	0/11	0/6	0/46
Females Hyperplasia	2/45	4/48	4/48	6/49
Adenomas	0/45	6/48[a]	7/48[c]	5/49[a]
Adenocarcinomas	0/45	0/48	1/48	0/49
Total neoplasms	0/45	6/48[a]	8/48[c]	5/49[a]

[a] Significantly different ($p<0.05$) from controls, as calculated by the authors using Fisher's Exact test.
[b] Significant dose-related trend; Cochrane-Armitage test, $p<0.05$.
[c] Significantly different ($p<0.01$) from controls, as calculated by the authors using Fisher's Exact test.

Sources: Daughtrey et al., 1999; Biodynamics, 1993b.

4.4.2.2.3. ***Reproduction/developmental studies.*** API sponsored two reproductive studies in laboratory rats and mice exposed to commercial hexane (BRRC, 1989a, b). The first study was a range-finding study in which pregnant Sprague-Dawley rats (eight/group) and CD-1 mice

(eight/group) were exposed to commercial hexane for 6 hours/day at target concentrations of 0, 900, 3000, or 9000 ppm on GDs 6–15 (BRRC, 1989a). Pregnant rats were terminated on GD 21, pregnant mice on GD 18. Maternal body weight gain was monitored intermittently and at termination. Uterine weights, number of ovarian corpora lutea, implantation sites, and viable and nonviable implants were evaluated. All live fetuses were weighed, sexed, and examined for external and visceral malformations and skeletal variations. None of the dams of either species displayed overt maternal toxicity during the course of the experiment. There appeared to be a slight increase in body weight gain in the high-dose rats in parallel with increased food and water consumption in this group. The only sign of reproductive or developmental toxicity was a reduction in fetal weights per litter in the progeny of pregnant mice exposed to 9000 ppm commercial hexane. No treatment-related malformations or variations were observed in either the rat or mouse fetuses.

BRRC (1989b) exposed pregnant Sprague Dawley rats (30/group) to 0, 900, 3000, or 9000 ppm commercial hexane for 6 hours/day on GDs 6–15 and sacrificed the animals on GD 21. Maternal body weights and food and water consumption were recorded on GDs 0, 6, 9, 12, 15, 18, and 21, and the weights of liver, kidney, and uterus were measured at sacrifice. As in the range finding study, numbers of ovarian corpora lutea, implantation sites, resorptions, and live and dead fetuses were evaluated. Fetuses were examined for external and visceral abnormalities and for skeletal variations. There were no treatment-related effects in reproductive, developmental, or teratological parameters in any of the groups of rats in the study. Among maternal effects, body weight gain was reduced in high-dose dams and in the mid-dose group for a portion of the exposure period (GDs 9–12).

In addition, pregnant CD-1 mice (30/group) were exposed to the same regimen as that described for the Sprague-Dawley rats (BRRC, 1989b). There were no n-hexane-related effects in maternal body weight gain, no changes in food and water consumption, and no other clinical signs of toxicity among the exposed groups compared with controls. Gestational parameters, including the numbers of viable and nonviable implantations/litter and sex ratio, were unaffected by exposure to commercial hexane. However, a degree of maternal toxicity was evident when the dams were necropsied, as indicated by a dose-dependent increased incidence of discoloration of the lungs. Dark brown foci were evident in the lungs of 4/29 high-dose and 2/25 mid-dose dams. A NOAEL of 900 ppm would apply to the maternal effects of commercial hexane based on this result.

Fetal body weights were unchanged among the groups, and there were no significant changes in the incidence of individual malformations or pooled external, visceral, or skeletal

malformations. However, there were treatment-related increased incidences of two individual skeletal variations in high-dose pups. Comparing the incidences of these effects between controls and high-dose groups by litter, the numbers were 0/26 versus 6/26 for bilateral bone islands at the first lumbar arch and 20/26 versus 26/26 for all unossified intermediate phalanges (statistically significant at $p<0.05$, Fisher's Exact test as calculated by the authors). A NOAEL of 3000 ppm for these skeletal variations was identified.

BRRC (1991) carried out a two-generation reproductive/developmental toxicity study in which, prior to breeding, 25 Sprague-Dawley rats/sex/group (F0 generation) were exposed to concentrations of 0, 900, 3000, or 9000 ppm commercial hexane for 6-hours/day, 5 days/week for 10 weeks. The study was published in the peer reviewed literature by Daughtrey et al. (1994a). Clinical signs of toxicity were monitored daily, and food consumption and body weight data were recorded weekly. After 10 weeks, males and females were mated and these mating pairs were exposed to commercial hexane at the same doses for 6 hours/day, 7 days/week for 21 days. Cohabitation was maintained only long enough for pregnancy to be achieved (copulation plug present). For the dams, exposure was continued through GD 19, discontinued until postnatal day (PND) 4, then reinstituted until weaning on PND 28, at which point the F0 animals were sacrificed. On PND 4, the pups were culled to 4/sex/litter, then, on PND 28, 25 F1 rats/sex/group were randomly selected for exposure to commercial hexane for 8–11 weeks. Subjects were then mated as described for the F0 generation. All F2 rats were sacrificed on PND 28.

Among the reproductive indices evaluated were survival, mating, fertility, gestation, live births, and lactation. All subjects were necropsied, and excised pieces of liver, kidney, pituitary, upper and lower respiratory tract, and any obvious lesions were examined histopathologically. Reproductive organs and tissues taken for histopathology included the vagina, uterus, ovary, testis, epididymis, seminal vesicles, and prostate.

In the F0 generation, there were no dose-related changes in body weight gain and no clinical signs of toxicity resulting from exposure to commercial hexane at any concentration. However, hyaline droplet nephropathy was visible histopathologically in the high-dose F0 males. There were no changes in any of the mating indices, fertility, gestation, live pups/litter, or pup viability at PND 28. A treatment-related effect of commercial hexane was a reduction of mean body weight in the F1 pups of the high-dose dams, an effect that became apparent at PND 14 and beyond. The mean body weight of the F1 pups remained lower than controls throughout their pre-breeding period. The group-specific means were significantly decreased (by approximately 7%) on PND 21 (38.9 ± 4.0 g in high-dose pups versus 41.9 ± 3.95 g in control pups).

There were no overt signs of clinical toxicity and no other signs of reproductive performance deficits in the F1 generation. Similarly, no lesions in male reproductive pathology were apparent at necropsy and histopathological examination. Hyaline droplet nephropathy was observed in F1 high-dose males (statistically significant). The number of pups born to exposed F1 rats were not statistically different compared with controls. F2 pup body weights in the high-dose group were reduced by 6 to 9% compared with controls. The viability of F2 pups did not differ between the groups.

IRDC (1986) reported a single generation reproduction/developmental toxicity study in which Sprague-Dawley rats were exposed to 0, 100, 500, or 1503 ppm commercial hexane via inhalation for 6 hours/day, 7 days/week. Exposure of both sexes of rat occurred for 100 days prior to mating, through the mating period (maximum of 15 days), through GDs 1–20, and then postnatally through weaning (PND 21).

There were some statistically significant reductions in body weight gain among the groups, most notably in the F0 females exposed to 1503 ppm. Fetal weights were reduced in the F1 pups, especially in high-dose progeny on lactation day 4, where the reduction from control levels was 11–13%. The body weight of high-dose F1 pups remained 8–9% lower than controls throughout lactation. Similar body weight reductions compared with controls were also observed in the mid-dose group throughout lactation, where the reductions were 12–17% from control values at their greatest extent and achieved statistical significance on PNDs 14 and 21. There were no changes in organ weight and no teratological effects in fetuses in any of the treatment groups. Study authors considered the changes in pup weight to be of no biological significance and assigned a NOAEL of 1503 ppm to the study.

4.4.3. Potentiation and Antagonism Studies

Altenkirch et al. (1978) exposed 22 male Wistar rats/group to 0 or 10,000 ppm n-hexane, 8900 ppm n-hexane mixed with 1100 ppm methyl ethyl ketone, or 6000 ppm methyl ethyl ketone alone for 8 hours/day, 7 days/week for up to 19 weeks. The group exposed to methyl ethyl ketone alone had originally been exposed to 10,000 ppm of this solvent. However, the initial concentration had to be reduced to 6000 ppm after a few days because of severe irritation of the upper respiratory tract. All animals exposed to solvent showed immediate clinical signs of toxicity, such as excitation, ataxia, impaired gait, and drowsiness. The effects were more prominent in the group exposed to the solvent mixture. Motor deficits occurred in solvent-exposed animals, characterized by an eversion of the hind-limbs. Rats with a severe paresis could only crawl across the floor of the cage or not move at all. These deficits occurred earlier,

and their extent was more severe in rats exposed to n-hexane mixed with methyl ethyl ketone. While rats exposed to pure methyl ethyl ketone did not develop any obvious motor impairment up to week 7, all animals in this exposure group died during the eighth week. The authors suggested that the cause of this increase in mortality was bronchopneumonia.

Serial necropsies and histopathological examinations of the peripheral nerves were carried out on subsets of exposed rats throughout the course of the experiment. Changes, such as multifocal, paranodal swelling of giant axons of the tibial nerves, were detectable in rats exposed to the n-hexane/methyl ethyl ketone mixture during week 4 of exposure. However, rats exposed to n-hexane alone did not develop such manifestations of peripheral nerve damage until week 8. The authors described similar changes in the spinal cord in the long descending tracts at distal sites and in the long ascending tracts at proximal sites near the medulla oblongata. Exposure to methyl ethyl ketone alone (6000 ppm, 8 hours/day for 7 weeks) did not induce comparable histopathological changes. Neuropathological changes were more severe and occurred earlier in animals exposed to n-hexane mixed with methyl ethyl ketone than to n-hexane alone.

Altenkirch et al. (1982) exposed male Wistar rats (five/group) to 0, 500, or 700 ppm n-hexane, 300 ppm n-hexane plus 200 ppm methyl ethyl ketone, 400 ppm n-hexane plus 100 ppm methyl ethyl ketone, and 500 ppm n-hexane plus 200 ppm methyl ethyl ketone for 24 hours/day, 7 days/week for up to 9 weeks. Animals were observed for clinical signs of toxicity over the course of the experiment, and histopathological examinations of excised brain, spinal cord, and peripheral nerves were performed at term. All exposed rats survived to term, although some groups showed a reduction in body weight gain during the lifetime of the experiment. Clinical signs included excessive salivation and an increase in paralysis of the hind-limbs. This condition was thought to be indicative of peripheral neuropathy. The time for this condition to develop was shorter in those rats exposed to the higher concentrations of n-hexane and to the mixtures. Histopathological examinations of the peripheral nerves showed the presence of axonal swellings, especially at the branches of the tibial and ischiatic nerves. A breakdown of axons and myelin developed distally to the axonal swellings, with an apparent intra-axonal accumulation of NFs. Other morphological findings included axonal swelling of the gracile tract of the spinal cord, especially at the level of the gracile nucleus in the medulla oblongata.

A second phase of the experiment featured the exposure of male Wistar rats (five/group) to 700 ppm n-hexane or 500 ppm n-hexane plus 200 ppm methyl ethyl ketone for 8 hours/day, 7 days/week for 40 weeks. These animals displayed neither the clinical signs of n-hexane-induced peripheral neuropathy nor the axonal swelling and peripheral nerve fiber degeneration that marked the histopathological responses in those animals exposed continuously for 9 weeks.

After 40 weeks there was some evidence of nerve fiber destruction in all groups, including controls, changes that were interpreted by the authors as being age-related. Altenkirch et al. (1982) concluded that male Wistar rats exposed 8 hours/day for 40 weeks to either 700 ppm n-hexane or 500 ppm n-hexane plus 200 ppm methyl ethyl ketone developed no neuropathological or clinical signs of neuropathy. This contrasted with rats exposed to the same concentrations for 24 hours/day in the first phase of the study. The rats in the first phase of the study developed clinical neuropathy after 4 weeks.

Ichihara et al. (1998) carried out a series of studies on the toxicological interactions of n-hexane and methyl ethyl ketone that was intended to resolve the apparent contradiction between the potentiating effects of methyl ethyl ketone on n-hexane-induced neurotoxicity and the reduced urinary levels of 2,5-hexanedione that had been observed as a result of coexposure to methyl ethyl ketone (van Engelen et al., 1997; Shibata, 1990a, b; Altenkirch et al., 1978). Eight male Wistar rats/group were exposed 12 hours/day, 6 days/week for 20 weeks to either filtered air (controls), 2000 ppm n-hexane (96% purity), 2000 ppm n-hexane plus 200 ppm methyl ethyl ketone, or 2000 ppm n-hexane plus 2000 ppm methyl ethyl ketone. MCV, DL, and urinary 2,5-hexanedione were measured every 4 weeks. A several-fold decrease in MCV and an approximate 50% increase in DL were reported for those rats exposed to 2000 ppm methyl ethyl ketone and 2000 ppm n-hexane compared with those exposed to 2000 ppm n-hexane alone. These changes (decrease in MCV and increase in DL) were greater than those induced by n-hexane alone or by a mixture of 2000 ppm n-hexane and 200 ppm methyl ethyl ketone. Changes in urinary 2,5-hexanedione were biphasic. On the first day of exposure, coexposure with methyl ethyl ketone decreased urinary levels of 2,5-hexanedione compared with the levels obtained in rats exposed to 2000 ppm n-hexane. However, the urinary level of 2,5-hexanedione in rats exposed to 2000 ppm n-hexane mixed with 2000 ppm methyl ethyl ketone increased after 4 weeks and reached twice the level seen in rats exposed to 2000 ppm n-hexane alone.

Eight male Wistar rats/group were exposed to either 100 ppm n-hexane, 100 ppm n-hexane plus 200 ppm methyl ethyl ketone, or 200 ppm methyl ethyl ketone alone for 12 hours/day for 24 weeks (Takeuchi et al., 1983). MCV and mixed MCV were similar among n-hexane-exposed, methyl ethyl ketone-exposed, and controls. Small, though statistically significant, reductions in both MCV and mixed MCV were detected at various time points during the exposure period in those rats exposed to the mixture of solvents, as compared with rats exposed to n-hexane or methyl ethyl ketone alone. There was little change in DL among the exposure groups (both single solvent and mixtures of solvents) throughout the experiment. However, the results suggested that methyl ethyl ketone enhanced the subclinical

neurophysiological effects of n-hexane at comparatively low concentrations.

Veronesi et al. (1984) assessed the neurotoxicity of combinations of n-hexane and methyl ethyl ketone in a tissue culture system in which explanted fetal mouse spinal cord with attached dorsal root ganglia and striated muscle were cultured for up to 56 days in media supplemented with various solvents. Eighteen cultures/dose/solvent were examined under light and electron microscope twice a week. Veronesi et al. (1984) developed a time-to-onset metric based on the incubation time (in days) necessary for axonal swelling to appear in 75% of the cultures. Cultures (eight/dose) were exposed to n-hexane in the medium at 0, 25, 50, 80, 100, or 250 µg/mL or to methyl ethyl ketone at 0, 10, 25, 50, 200, 300, 400, or 600 µg/mL. Cultures with single solvent exposures were incubated for up to 49 days. Cultures with mixtures of n-hexane (0, 25, 50, 100, 250 µg/mL) plus methyl ethyl ketone (0, 10, 25, 50, 100 µg/mL) were incubated for up to 56 days.

The authors reported the development of axonal swelling, retraction of paranodal myelin, accumulation of NFs, and peripheral displacement of neurotubules and mitochondria at n-hexane concentrations of 100 and 250 µg/mL. Times-to-onset for n-hexane concentrations of 100 and 250 µg/mL were 43 and 28 days, respectively. Veronesi et al. (1984) described the cultures incubated in methyl ethyl ketone alone as being marked by generalized cellular breakdown at the highest dose (600 µg/mL). Other pancytotoxic responses included intraaxonal rectilinear inclusions that developed in several cultures treated with 200–400 µg/mL methyl ethyl ketone.

As shown in Table 4-17, the presence of methyl ethyl ketone in neurotoxic concentrations of n-hexane in this system reduced the time-to-onset as compared with equivalent incubations containing n-hexane alone. Addition of methyl ethyl ketone to nonneurotoxic concentrations of n-hexane-induced an apparently neurotoxic response.

Table 4-17. Time-to-onset for the appearance of axonal swelling in explanted cultures of fetal mouse spinal cord incubated with mixtures of n-hexane and methyl ethyl ketone

n-Hexane (µg/mL)	Methyl ethyl ketone (µg/mL)[a]				
	0	**10**	**25**	**50**	**100**
0	NC	Not tested	Not tested	NC	NC
25	NC	Not tested	Not tested	11	22
50	NC	14	Not tested	12	18
100	43	31	Not tested	19	25

250	28	Not tested	21	27	Not tested

[a] All values in days.
NC = No pathological change.

Source: Veronesi et al., 1984.

Takeuchi et al. (1981) exposed eight male Wistar rats/group to ambient air, 1000 ppm n-hexane, 1000 ppm toluene, and 1000 ppm n-hexane mixed with 1000 ppm toluene for 12 hours/day for 16 weeks. Electrophysiological parameters such as mixed nerve conduction velocity, MCV, and DL were measured at the start of the experiment, after 4, 8, 12, and 16 weeks of exposure, and 4 weeks after exposure was discontinued. Marked reduction in conduction velocities and increased DLs in the tail nerves of rats were observed following exposure to n-hexane alone. There was slight neuropathologic impairment following exposure to the mixture of n-hexane and toluene, while toluene exposure alone had no effect.

Ikeda et al. (1993) investigated the effects associated with exposure to either n-hexane, toluene, or a mixture of the two solvents in Wistar rats. The rats were initially taught to respond to a pre-signaled light flash in order to avoid an electric shock (by pressing a lever). Rats that had an avoidance rate of over 80% (18 rats) were selected for the study and divided into three groups of six each (toluene only, n-hexane only, and mixture of toluene and n-hexane). Controls were exposed to air. Each exposure group was first exposed to air (as an internal control) for one hour and then in sequence to 50, 100, 200, 400, or 800 ppm (in ascending order) of each solvent individually or as a 50:50 mixture. The interval between exposures for each rat was 14 days and sham exposure to air was carried out every seventh day following exposure to solvent. Shock-avoidance behavior was monitored during and after (up to an hour) exposure, and the effects of each organic solvent were evaluated by comparing the performance in individual rats during and after exposure with their own performance under sham exposure (i.e., to ambient air). The highest exposure concentration of n-hexane appeared to induce a consistent increase in the lever press rate. By contrast, the 800 ppm mixture (400 ppm n-hexane and 400 ppm toluene) decreased lever press and avoidance rates when compared with baseline behavior.

Nylen et al. (1994) and Nylen and Hagman (1994) compared the performance of the auditory and visual systems in rats after exposure to n-hexane or combined exposure to n-hexane combined with either toluene or xylene. In the study involving toluene, Nylen et al. (1994) exposed male Sprague-Dawley rats to either 1000 ppm n-hexane, 1000 ppm toluene, or a mixture of 1000 ppm n-hexane and 1000 ppm toluene for 21 hours/day, 7 days/week for 28 days. The auditory and visual sensitivity of the animals was measured as their BAEPs and VEPs,

which were recorded 2 days, 3 months, and 1 year after the completion of exposure. Conduction velocities in the nerves of the tail and compound nerve and action potentials were also compared between controls and exposed groups. Changes in auditory brainstem responsiveness to click-evoked stimuli were not observed in the rats exposed to n-hexane alone but were detected 2 days after the conclusion of the exposure regimen in toluene-exposed animals and in those inhaling the mixture of solvents. The latter group of animals showed an enhanced loss of auditory sensitivity compared with other groups 3 months following termination of exposure. Exposure of rats to n-hexane alone was associated with the lowering of one amplitude in the flash-evoked potential 2 days after exposure. This change was not observed in rats exposed to the mixture of n-hexane and toluene. There was little change in the peripheral MCV in rats 2 days and 3 months after exposure to a mixture of n-hexane and toluene, as compared with a marked decrease in MCV in rats that had been exposed to n-hexane alone (the MCVs at the 2-day time point were 9.2 ± 1.7 m/s in n-hexane receiving animals, 15.4 ± 2.0 m/s in unexposed controls, and 12.8 ± 1.5 m/s in rats exposed to mixed n-hexane and toluene).

Nylen and Hagman (1994) exposed male Sprague-Dawley rats to 1000 ppm n-hexane, 1000 ppm xylene, or a mixture of 1000 ppm n-hexane and 1000 ppm xylene for 18 hours/day, 7 days/week for 61 days. The same neurophysiological measurements as those described by Nylen et al. (1994) were carried out 2 days, 4 months, and 10 months after the conclusion of the dosing regimen. For the BAEPs, exposure to the n-hexane:xylene mixture caused a persistent loss of auditory sensitivity that, compared with controls, was statistically significant in the 7–17 dB range. For the VEPs, the latencies of the flash-evoked potentials were prolonged in the n-hexane exposure group versus controls at the 2-day postexposure time point. Exposure to n-hexane alone markedly reduced nerve conduction velocity, while the mixture of n-hexane and xylene did not have much effect on this parameter. The MCVs at the 2-day time point were 12.2 ± 3.8 m/s in n-hexane receiving animals and 17.5 ± 4.0 m/s in rats exposed to mixed n-hexane and xylene compared with 21.2 ± 2.3 m/s in unexposed controls.

Ralston et al. (1985) used an oral exposure regimen to investigate the possible potentiation of 2,5-hexanedione-induced neurotoxicity by methyl ethyl ketone. The mixture was administered via gavage at a concentration of 2.2 mmol/kg-day, 5 days/week for up to 90 days. Exposure to the mixture caused a rapid onset of motor deficits in male F344 rats compared with exposure to either chemical alone. Urinary clearance of 2,5-hexanedione was reduced, and the area under the blood concentration time course was increased in the presence of methyl ethyl ketone. This suggests that methyl ethyl ketone potentiates 2,5-hexanedione-induced neurotoxicity by increasing the persistence of 2,5-hexanedione in the circulation.

In addition to toluene, xylene, and methyl ethyl ketone, a variety of studies have shown that acetone may affect n-hexane metabolism and potentiate n-hexane induced neurotoxicity (Cardona et al., 1996; Ladefoged et al., 1994, 1989; Larsen et al., 1991). Specifically, these studies have evaluated the neurotoxic effect of acetone co-exposure with the n-hexane metabolite 2,5-hexanedione.

Ladefoged et al. (1989) exposed male Wistar rats (six/group) for up to 6 weeks to either 0.5% 2,5-hexanedione, 0.5% acetone, 5.0% ethanol, 0.5% 2,5-hexanedione plus 0.5% acetone, or 0.5% 2,5-hexanedione plus 5.0% ethanol in drinking water (w/w). Food and water consumption were measured weekly, peripheral MCV was measured weekly from the third week of exposure, and neurobehavioral toxicity (balance time in 30 second intervals on a moving rod) was measured weekly. Body weight gain and water consumption were statistically significantly reduced after two weeks following treatment with 2,5-hexanedione, 2,5-hexanedione plus acetone, and 2,5-hexanedione plus ethanol compared with controls administered pure water. Water consumption was also reduced in the ethanol-only exposure group following the first week of dosing compared with controls. Neurophysiologically, rats exposed to 2,5-hexanedione and 2,5-hexanedione plus acetone had statistically significantly reduced MCV beginning at 3 weeks exposure duration compared with controls. Acetone-only-exposed rats showed a statistically significant reduction at 6 weeks exposure. Ethanol exposure alone did not produce any significant changes in MCV, but coexposure with 2,5-hexanedione significantly reduced MCV at 3 weeks exposure duration compared with controls. MCVs measured following exposure to 2,5-hexanedione plus ethanol were greater than after exposure to 2,5-hexanedione alone. In addition, 2,5-hexanedione plus acetone led to a greater reduction in MCV compared with 2,5-hexanedione plus ethanol (statistically significant at week 4). Table 4-18 presents results of this study. Acetone and ethanol alone did not affect balance time at any duration of exposure.

Table 4-18. Effect of 2,5-hexanedione, acetone, ethanol, and mixtures of 2,5-hexanedione with acetone or ethanol in drinking water on average MCV

Dosing week	Control [a]	0.5% 2,5-Hexanedione[a]	0.5% Acetone[a]	0.5% 2,5-Hexanedione plus acetone[a]	5.0% Ethanol [a]	0.5% 2,5-Hexanedione plus 5.0% ethanol[a]
3	29.6 (3.0)	26.1 (2.4)[b]	28.0 (2.9)	24.8 (2.9)[b]	29.2 (2.0)	25.1 (1.6)[c]
4	30.3 (2.5)	25.8 (2.3)[c]	28.6 (1.8)	23.5 (1.8)[c,e]	29.6 (1.4)	26.1 (2.6)[b]
5	29.3 (1.1)	25.3 (2.9)[b]	28.8 (1.2)	22.3 (3.2)[d]	31.2 (0.9)[d]	27.1 (1.7)[d]

6	31.5 (2.1)	25.1 (1.8)[c]	29.5 (1.1)[d]	23.0 (2.8)[c,e]	32.5 (1.1)	26.8 (1.7)[c]

[a] Values are means in m/sec; numbers in parentheses are SDs.
[b] $p < 0.01$; [c] $p < 0.001$; and [d] $p < 0.05$.
[e] Significantly different from group receiving 0.5% 2,5-hexanedione.

Source: Ladefoged et al., 1989.

2,5-Hexandione alone reduced balance time in rats exposed for 3 and 4 weeks only. Acetone plus 2,5-hexanedione reduced balance time to significantly lower levels than in controls from the second week of dosing until the end of the study (6 weeks) and to significantly lower levels than in 2,5-hexanedione-only-treated animals from the fourth week of dosing onward. A reduction in balance time was also observed in the 2,5-hexanedione plus ethanol group compared with controls, but there was no significant difference compared with rats given 2,5-hexanedione alone.

Ladefoged et al. (1994) exposed male rats (20/group) to 0.5% 2,5-hexanedione, 0.5% acetone, and 0.5% 2,5-hexanedione plus 0.5% acetone in drinking water for 6 weeks. Tap water was given to controls. Once a week body weight and food and water consumption were measured. In addition, the following behavioral indicators were also monitored weekly: ambulation (crossing in an open field for 5 minutes), rearing (number of times both fore-legs were raised from the floor in 5 minutes), balance on an accelerating rotarod, and grip strength of fore-limbs and hind-limbs. After 6 weeks exposure, half of the rats were subjected to histopathological analysis of nerve fibers. The other half of the rats were allowed a 10-week recovery period followed by histological analysis of nerve fibers. Food and water consumption and body weights were decreased in both the 2,5-hexanedione alone and 2,5-hexanedione plus acetone groups during the entire 6 weeks of the study. Water consumption returned to normal in the first 4 weeks of the recovery period for these rats. Body weight decrease was most pronounced during the dosing period but remained statistically significant during the recovery period. Acetone alone had no effect on the behavioral parameters observed. The authors stated that the neurotoxicity of 2,5-hexanedione and 2,5-hexanedione plus acetone was demonstrated by statistically significant changes in the performance of the dosed rats compared with controls in the behavioral tests (data presented graphically for ambulation, rearing and balance; grip strength data were not shown). In addition, the behavioral effects were more pronounced in the animals dosed with both 2,5-hexanedione and acetone compared with rats dosed with 2,5-hexanedione alone. The reduction in ambulation following both exposures was reversed within 5 weeks of the 10-week recovery period. Effects on rearing and balance on a rotarod were reversible within 10 weeks recovery for the rats treated with only 2,5-hexanedione but not

for rats treated with 2,5-hexanedione and acetone. The reduction in grip strength was not reversible for either group during the 10-week recovery period.

Following 6 weeks of exposure to 2,5-hexanedione and 2,5-hexanedione plus acetone, there was statistically significant increased giant axonal swelling in the sciatic nerve. The median percent relative distribution of fiber area of class 9 fibers was 0.4 (0.0–0.5) and 1.6 (0.0–2.1), and that of class 10 fibers was 0.2 (0.0–0.5) and 0.9 (0.0–2.1) compared with controls (class 9, 0.0 [0.0–0.5] and class 10, 0.0 [0.0–0.3]), respectively. The same pattern was observed in the tibial nerve fibers. The authors stated that structural changes observed immediately after the 6-week exposure period were greater in rats exposed to the solvent mixture compared with those exposed to 2,5-hexanedione only. After 10 weeks recovery, the nerve tissue (sciatic and tibial) appeared normal. The neurotoxicity observed in this study was similar to that seen following exposure to n-hexane alone (Section 4.2.2.1).

Acetone coexposure has also been shown to affect male reproductive toxicity of n-hexane. Larsen et al. (1991) exposed male rats (10/group) to 0, 0.13, 0.25, or 0,5% 2,5-hexanedione alone or in combination with 0.5% acetone (weight/volume) for 6 weeks in drinking water. At week 5 of exposure, one-half of the rats were mated with unexposed females, and the numbers of matings, pregnancies, and fetuses were recorded. The other half of the rats were allowed a 10-week recovery period followed by mating and analysis of the same reproductive parameters. Testis weight and morphology were also evaluated. Water and food intake was reduced in rats receiving 2,5-hexanedione alone (dose dependent) and was slightly further decreased with coexposure to acetone. The authors calculated average intake based on water consumption to be 170, 270, and 440 mg/kg-day 2,5-hexanedione. Rats exposed to 2,5-hexanedione alone displayed a dose-dependent decrease in body weight gain, which was greater with acetone exposure. Following the dosing period, food and water intake of all dosed groups returned to control levels except in rats exposed to the high dose of 2,5-hexanedione alone or the high dose of 2,5-hexanedione plus acetone. Body weight remained significantly lower from the third week of dosing until the end of the study. Actual food and water intake and body weights were not reported. The number of matings were not affected in any of the exposure groups. A statistically significant reduction in testis weight and the number of pregnancies and fetuses was observed in rats exposed to 0.5% 2,5-hexanedione alone and 0.25 and 0.5% 2,5-hexanedione plus acetone after 6 weeks. The highest combined treatment resulted in infertility in the male rats. After the 10-week recovery period, the effects on the testis and fertility persisted in the high-dose 2,5-hexanedione-only group and in the group coexposed to acetone. In addition, following the recovery period, testicular atrophy and reduced testis tubuli

diameters were present in all dose groups except acetone alone exposure. The authors stated that acetone potentiated the effects of 2,5-hexanedione on testis.

4.4.4. Mode of Action Studies

Ultrastructural studies indicate that nervous system toxicity induced by n-hexane may be the result of a sequence of events leading to degeneration of the axons (Spencer and Schaumburg, 1977a; Schaumburg and Spencer, 1976). Sprague-Dawley rats (eight animals total) were exposed continuously to 400–600 ppm n-hexane for 1–23 weeks. Three additional animals received subcutaneous injections of 650–2000 mg/kg 5 days a week for up to 35 weeks. The animals were observed for clinical signs of neuropathy (characterized by waddling gait, hind-limb paralysis, and decreased ability to grip a rotating bar). Sciatic, tibial, and plantar nerves were subjected to light microscopy. The authors described focal condensation of NF, mitochondria, and smooth endoplasmic reticulum with increase in the number of NFs. The earliest pathological indicator of peripheral nerve axonal degeneration was axonal swelling in the distal nonterminal region of the large myelinated fibers. These axonal swellings appeared first proximal to the nodes of Ranvier and ascended the nerve with further exposure (i.e., facing paranodes and internodal loci). Paranodal swelling was accompanied by shrinkage and corrugation of the adjacent distal internode. Paranodal myelin sheaths split and retracted leaving giant axonal swellings near the nodes of Ranvier. The study authors suggested that Schwann cells may become associated with these denuded regions and remyelinate short segments. Remyelinated segments then mark the position of the axonal swellings that were resolved without fiber breakdown or total internodal demyelination.

Several studies suggest that the n-hexane metabolite, 2,5-hexanedione, is the primary toxic agent by which n-hexane brings about its neurotoxicological effects. Ladefoged et al. (1989) exposed male Wistar rats (11/group) to 0, 0.5% 2,5-hexanedione in drinking water for 6 weeks. The rats were evaluated for neurobehavioral and nervous system toxicity by the rotarod performance and measurement of MCV, respectively. MCV was significantly reduced after 3, 4, and 5 weeks of exposure (Table 4-19). Rotarod performance was significantly reduced (decreased average balance time) after 3 and 4 weeks exposure. In a follow-up study, Ladefoged et al. (1994) exposed male Wistar rats (20/group) to 0 or 0.5% 2,5-hexanedione in drinking water for 6 weeks. Statistically significant reductions in performance in neurobehavioral tests (ambulation and rearing, rotarod, and grip strength) were noted after 3 weeks exposure to 2,5-hexanedione. In addition, the authors observed giant axonal swelling in the tibial and sciatic nerve fibers after 6 weeks exposure to 2,5-hexanedione.

Schaumburg and Spencer (1978) showed the rapid onset of distal axonal degeneration in cats administered aqueous 2,5-hexanedione at low-levels (concentration and route of administration not stated) for 60–75 days. Other symptoms typical of n-hexane-induced peripheral neuropathy included progressive symmetrical weakness in all extremities, resulting in paralysis. Schaumburg and Spencer (1978) demonstrated that 2,5-hexanedione also caused widespread axonal degeneration in the mammillary body, lateral geniculate nucleus, and superior colliculus in exposed cats. These lesions were thought to be further examples of the distal axonopathy seen elsewhere in the peripheral and central nervous systems in humans and animals exposed to n-hexane.

Krasavage et al. (1980) studied the relative neurotoxicity of n-hexane and its metabolites by administering equimolar doses of each chemical by gavage to five male COBS, CD(SD) BR rats/group for 5 days/week for 90 days (section 4.2.1.1). As judged by the time taken for neuropathological symptoms to develop, the parent chemical and its metabolites could be ranked in descending order of neurotoxicity as follows: 2,5-hexanedione, 5-hydroxy-2-hexane, 2,5-hexanediol, 2-hexanone, 2-hexanol, n-hexane, and practical grade hexane. 2,5-hexanedione had approximately 38 times the neurotoxic potency of n-hexane itself on an equimolar basis (Couri and Milks, 1982; Krasavage et al., 1980). Abou-Donia et al. (1982) observed a similar comparative neurotoxic relationship when n-hexane, 2-hexanone, 2,5-hexanediol, and 2,5-hexanedione were administered orally or intraperitoneally to hens. Pathological examination of treated birds showed giant paranodal axonal swelling followed by degeneration of axons and myelin in peripheral nerves and the spinal cord. Based on the time of onset of these symptoms, the magnitude of the clinical signs of toxicity, and the severity of the histopathological lesions, the relative neurotoxicity of the subject chemicals was, in descending order, 2,5-hexanedione, 2,5-hexanediol, 2-hexanone, and n-hexane.

Nachtman and Couri (1984) carried out an electrophysiological study to evaluate the comparative neurotoxicity of 2-hexanone and 2,5-hexanedione in rats. Male Wistar rats were exposed to the chemicals at concentrations of 20 and 40 nmol/L in drinking water. Motor nerve velocities and latencies were determined at three stimulus sites, the sciatic notch, the popliteal space, and the plantaris tendon. Distal latency was significantly greater in animals exposed to 2,5-hexanedione (2 weeks at 40 nmol/L) than in those receiving 2-hexanone for the same duration. 2,5-hexanedione was also shown to induce neurobehavioral deficits in male F344 rats exposed to the chemical by gavage at a dose rate of 2.2 mmol/kg-day for 90 days (Ralston et al., 1985). Compared with controls, 2,5-hexanedione-exposed animals performed progressively worse in the hind-limb grasp and hind-limb place reflex tests and the balance beam and

accelerating rotorod functional tests. Similar deficits in performance in a functional observational battery were observed in male Long-Evans rats that were exposed to 2,5-hexanedione intraperitoneally at 0, 150, 225, and 350 mg/kg-day for 28 days (Shell et al., 1992). These became apparent at some intermediate doses and time points, but no neurohistopathological lesions were observed at any other exposure than the high dose after 28 days. Taken together, these studies suggested that 2,5-hexanedione-induced deficits in FOB performance can precede the overt development of peripheral neuropathy, as exemplified by axonal swelling, rearrangement of NFs, and regression of the myelin sheaths.

The molecular mechanisms involved in bringing about n-hexane-induced neuropathological effects have been studied extensively. Several studies have suggested that the mode of action involved the binding of the toxic metabolite, 2,5-hexanedione, to proteins forming pyrrole adducts then undergo oxidation, leading to protein cross-linking. For example, 2,5-hexanedione was shown to cross-link NF proteins of spinal cord when administered to male Sprague-Dawley rats for 180 days in drinking at a concentration of 5000 mg/L (Lapadula et al., 1986). Spinal cords were isolated after exposure and their proteins separated using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Separating the polypeptides according to molecular weight by this approach revealed a reduced content of NF triplet proteins and the additional presence of bands migrating at positions on the gel that were equivalent to molecular weights of 138,000 and 260,000 Daltons. The latter were not observed in the electrophoretic pattern of NFs obtained from unexposed animals. Lapadula et al. (1986) used immunoblotting to demonstrate that lower molecular weight bands on control gels were immunologically indistinguishable from higher molecular weight bands of gels carrying neurofilamentous proteins of exposed animals. These findings indicate that cross-linking of neurofilamentous proteins had taken place as a result of exposure to 2,5-hexanedione. As reported by the authors, a number of higher molecular weight proteins were reactive with antibodies to all three of the NF proteins under evaluation.

A substantial body of physiological and biochemical studies have explored the mechanism by which n-hexane-derived 2,5-hexanedione binds to and cross-links proteins. For example, DeCaprio et al. (1982) studied the covalent binding of 2,5-hexanedione to amino acids and polypeptides in vitro. These authors showed nonacidic amino acids to be the most reactive species when incubated with 2,5-hexanedione and 2,4-hexanedione. Polylysine also was extremely reactive to both ketones. Using lysine with selectively t-butoxycarbonyl-blocked α- or ϵ-amino groups demonstrated that the ϵ-amino group was six times more reactive than the α-amino group. Moreover, while 2,4-hexanedione and a number of other diketones reacted with

the ϵ-amino group to some extent, incubation at pH 9.5 markedly enhanced the lysyl reactivity of γ-diketones such as 2,5-hexanedione. Mass spectrometric analysis of the reaction product between the 2,5-hexanedione and the lysine ϵ-amino group suggested that a substituted pyrrole residue had been formed. Electrophoretic separation by charge of diketone-incubated bovine serum albumin showed an increased mobility within the gel of 2,4- or 2,5-hexanedione-treated protein with time. This study confirms that only diketones with γ-spacing are capable of forming pyrrole adducts, a necessary step in the neurotoxicity of alkanes.

The demonstration of pyrrole formation during 2,5-hexanedione-induced cross-linking suggests that this may be part of the mechanism by which changes in the peripheral nerve architecture are brought about. Sanz et al. (1995) carried out a series of in vitro assays to quantify pyrrole adduct formation by several non-γ- and/or γ-diketones (such as 2,5-hexanedione). The solvents assayed were 2-hexanone; 3,4-dimethylhexane; 2,5-hexanedione; 3,4-dimethyl-2,5-hexanedione; 2-hexanol and 2,5-hexanediol as derivatives of n-hexane; 5-methyl-3-heptanone; 6-methyl-2,4-heptanedione; 4-heptanone; and 4-heptanol as derivatives of n-heptane. The results showed that 3,4-dimethyl-2,5-hexanedione and 2,5-hexanedione formed pyrroles at the greatest speed and to the greatest extent. This suggests that these γ-diketones may more readily form pyrroles than their non-γ-diketone analogs. Therefore, they would be expected to have the greater capacity for inducing neuropathological effects.

In vivo evidence also supports the proposed mode of action for n-hexane-induced protein cross-linking and pyrrole formation. Kessler et al. (1990) detected pyrrole-like substances in the urine of a human volunteer exposed to n-hexane for 3 hours at a concentration of 146 ppm and in the urine of male Wistar rats administered 0, 50, 100, 250, 500, 1000, or 3000 ppm n-hexane for three 8-hour exposures. Mateus et al. (2002) detected pyrroles in the urine of male Wistar rats exposed to 200 mg/kg or 300 mg/kg 2,5-hexanedione in the diet for up to 9 weeks.

Graham et al. (1982a) hypothesized that pyrrole derivatization of lysyl residues is central to the development of NF aggregations. They used the 2,5-hexanedione analog, 3,4-dimethyl-2,5-hexanedione, as a probe. The presence of the two methyl groups of the analog enhanced the chemical's potential for pyrrole formation compared with that of 2,5-hexanedione. Rats given 0.25 mmol/kg 3,4-dimethyl-2,5-hexanedione every 8 hours developed severe limb paralysis within 3 days. The condition was marked by axonal swelling just proximal to the first node of Ranvier. The swellings contained masses of NFs.

Graham et al. (1982b) also demonstrated the in vitro interaction between 2,5-hexanedione and ethanolamine. Magnetic resonance spectroscopy characterized the product

of this reaction as 1-(2-hydroxyethyl)-2,5-dimethylpyrrole. The authors addressed the issue of the nature of an orange-colored chromophore that had formed during the reaction and showed that similar entities were formed as products of reactions between other primary amines or proteins and 2,5-hexanedione or γ-diketones such as 2,5-heptandione and 3,6-octanedione. Graham et al. (1982b) speculated that cross-linking of NFs as a result of n-hexane exposure likely involved some or all of the following processes: metabolism to 2,5-hexanedione, interaction of that chemical with ϵ-lysyl residues of proteins, cyclization to form pyrrole adducts that undergo oxidation to electrophiles, and these electrophiles then react with protein nucleophiles to result in covalent cross-linking of derivatized proteins to form higher molecular weight protein aggregates.

Two further studies by Anthony et al. (1983a, b) demonstrated the enhanced neuropathological activity of 3,4-dimethyl-2,5-hexanedione compared with that of 2,5-hexanedione. For example, in in vivo studies, five Sprague-Dawley rats/group (sex not stated) were intraperitoneally injected five times/week for at least 7 weeks with either 0, 2.5, or 4 mmol/kg-day 2,5-hexanedione or 0, 0.0625, 0.125, or 0.25 mmol/kg-day 3,4-dimethyl-2,5-hexanedione. Dimethyl substitution led to an acceleration of peripheral neuropathy as judged by the lower dose and shorter time required for the onset of hind-limb paralysis. For example, a daily dose of 0.25 mmol/kg-day 3,4-dimethyl-2,5-hexanedione produced hind-limb paralysis after 19.6 ± 1.4 days, indicative of a cumulative toxic dose of 3.5 ± 0.29 mmol/kg. By contrast, a 16-fold higher dose of 2,5-hexanedione (4.0 mmol/kg-day) brought about hind-limb paralysis after 35.8 days, equivalent to a cumulative toxic dose of 102 ± 7.4 mmol/kg. Ultrastructural examination of a giant axonal swelling from the anterior root of rats exposed to 3,4-dimethyl-2,5-hexanedione showed an accumulation of NFs but comparatively few microtubules. Light microscopy of the spinal cord showed large axonal swellings in the anterior root, white matter, and anterior horn (Anthony et al., 1983a).

Anthony et al. (1983b) provided further evidence for increased reactivity and pyrrole-forming capacity of 3,4-dimethyl-2,5-hexanedione compared with 2,5-hexanedione by studying their rates of reaction with 0.02M ethanolamine and ovalbumin. Reaction products were collected at various time points and then analyzed by electrophoresis. 3,4-Dimethyl-2,5-hexanedione displayed a greater rate of pyrrole formation with ethanolamine than did 2,5-hexanedione, and there was a greater rate of covalent cross-linking of ovalbumin with the dimethylated diketone. Protein cross-linking was also measured in the presence of both ketones. The rates of polymer formed were 1–1.5 mol^{-1} $hour^{-1}$ for 3,4-dimethyl-2,5-hexanedione compared with 0.034–0.037 mol^{-1} $hour^{-1}$ for 2,5-hexanedione. The increase in polymer

formation represents an increase in the rate of protein cross-linking.

Genter et al. (1987) separated the *d, l* from the *meso* diastereomers of 3,4-dimethyl-2,5-hexanedione, both of which form identical tetramethylpyrrol adducts in the reaction with protein amino functions. The *d,l* diastereomer both formed pyrroles more rapidly and was more neurotoxic than the *meso* diastereomer, strongly supporting the concept that pyrrole adduct formation is a necessary step in the pathogenesis of γ-diketone neurotoxicity. Rosenberg et al. (1987) showed that the axonal swelling that followed intoxication with the *d,l* diastereomer were demonstrably more proximal than those produced by the *meso* diastereomer, showing that the rate of protein cross-linking determines the proximo-distal location of the axonal swelling.

Boekelheide (1987) carried out an in vitro study of the capacity of 2,5-hexanedione and 3,4-dimethyl-2,5-hexanedione to form cross-links in the lysine-rich polypeptide, tubulin (from bovine brain and rat testis). The ability to form microtubules was altered in γ-diketone-modified preparations. Specifically, the maximal velocity of assembly was consistently different among control and treated samples. A prominent decrease in the length of the nucleation phase was observed in the presence of γ-diketone. Gel filtration of the derivatized tubulin preparations showed that dimerization had occurred in preparations exposed to 2,5-hexanedione or 3,4-dimethyl-2,5-hexanedione.

DeCaprio et al. (1988) compared the neurotoxicity and pyrrole-forming potential of 2,5-hexanedione and deuterated 2,5-hexanedione ([D_{10}]-2,5-hexanedione) in vitro and in vivo. The latter derivative was expected to form pyrroles at a slower rate than the native chemical because of a primary isotope effect on the cleavage of the C-H bond. Incubation of bovine serum albumin with 2,5-hexanedione and [D_{10}]-2,5-hexanedione resulted in lower amounts of pyrrole formation (Table 4-19).

Table 4-19. Pyrrole adduct formation in proteins from γ-diketone-treated rats

Treatment	Dose level (mg/kg-day)	Duration (days)	Pyrrole concentration (nmol/mg protein)		
			Serum	Brain stem	Spinal cord
Control	-	17	0.2 ± 0.0	0.6 ± 0.1	0.6 ± 0.2
2,5-hexanedione	3.5	17	7.5 ± 1.8	3.7 ± 0.7	4.7 ± 1.2
[D_{10}]-2,5-hexanedione	3.5	17	2.8 ± 0.8[a]	1.9 ± 0.4[a]	2.1 ± 0.5[b]
Control	-	38	0.2 ± 0.1	0.6 ± 0.4	0.3 ± 0.2
2,5-hexanedione	2.5	38	3.1 ± 1.0	1.7 ± 0.6	2.2 ± 0.7

[D_{10}]-2,5-hexanedione	2.5	38	1.2 ± 0.1[a]	1.7 ± 0.8	1.1 ± 0.1[b]

[a] $p<0.05$, significantly different from corresponding incubations with 2,5-hexanedione.
[b] $p<0.01$, significantly different from corresponding incubations with 2,5-hexanedione.

Source: DeCaprio et al., 1988.

DeCaprio et al. (1988) also exposed male Wistar rats via intraperitoneal injection of the separate analogues. Milder symptoms of hind-limb paralysis for the perdeuterated chemical and a lower rate of adduct formation were observed. However, tissue concentrations of each γ-diketone isomer were not markedly different. The degree of covalent cross-linking of axonal proteins appeared to correlate with the amount of pyrrole formed (DeCaprio et al., 1988). These findings were considered to support an absolute requirement for pyrrole formation in γ-diketone neurotoxicity.

Genter St. Clair et al. (1988) used the diketone 3-acetyl-2,5-hexanedione to show that stable pyrrole derivatives of proteins could be formed without protein cross-linking. In vitro incubations containing ovalbumin and 3-acetyl-2,5-hexanedione resulted in the formation of pyrrole derivatives, with no sign of protein cross-linking. In vivo, daily intraperitoneal injection of 3-acetyl-2,5-hexanedione in male Sprague-Dawley rats for 20 days showed no signs of hind-limb paralysis, no axonal swelling of the peripheral nerves, and no aggregated NFs. The rate of pyrrole formation in rats receiving 3-acetyl-2,5-hexanedione was similar to that in animals injected with 2,5-hexanedione and 3,4-dimethyl-2,5-hexanedione. Isolated hemoglobin from rats treated with the three γ-diketones contained equivalent amounts of pyrroles. However, when the plasma membrane protein, spectrin, was measured as an indicator of cross-linking, negative results were obtained for 3-acetyl-2,5-hexanedione in contrast to the other two γ-diketones. The authors postulated that the electron-withdrawing acetyl group on the pyrrole formed by 3-acetyl-2,5-hexanedione rendered the pyrrole ring less susceptible to oxidation. In the absence of oxidation of the pyrrole ring, cross-linking of proteins could not occur. This hypothesis was supported by the absence of peripheral neuropathy associated with the other γ-diketones. This evidence indicates that both pyrrole oxidation and protein cross-linking are necessary steps in the pathogenesis of γ-diketone neuropathy. The observation that the neurological deficits continued to worsen for several weeks after cessation of exposure of humans to n-hexane could be explained by the continuing oxidation of pyrrole rings and NF cross-linking.

DeCaprio and Fowke (1992) investigated the interaction of 2,5-hexanedione and spinal cord NFs in vitro. Isolated spinal cord proteins were incubated with [^{14}C]-2,5-hexanedione. Incorporation of radioactivity and pyrrole formation in NFs increased linearly with

2,5-hexanedione concentration. SDS-PAGE and fluorography showed prominent labeling of three NF subunits (designated H, M, and L), in addition to some high molecular weight components derived from NF-H and NF-M. Mild proteolysis permitted the isolation of the carboxyl-terminal tail domains of NF-H and NF-M. These domains appeared to contain the majority of the 2,5-hexanedione binding sites, suggesting the possibility for limited and selective pyrrole adduction of NF proteins. Cyanogen bromide cleavage of 2,5-hexanedione-induced pyrrole adducts of NF-protein "M" showed the greatest amount of 2,5-hexanedione binding in a polypeptide fragment thought to correspond to a region at the carboxyl terminus where three important lysine-containing sequences are situated (DeCaprio et al., 1997).

An observation consistently made between species, between adult and immature members of the same species, and within individual humans and animals, was that longer axons in the PNS and CNS were more vulnerable to the toxic effects of n-hexane and its metabolites than shorter axons. The axonal swellings that initially occurred proximal to nodes of Ranvier in the most distal internodes of the longest axons were filled with disorganized masses of NFs. Thus, Graham et al. (1995) postulated that during repeated exposures to n-hexane the resulting metabolism to 2,5-hexanedione resulted in progressive derivatization of protein lysyl amino groups to form pyrrolyl adducts; oxidation of the pyrrole rings to electrophiles lead to increasing levels of cross-linking of NFs during the proximo-distal transport of axoplasm. Furthermore, the observations by Cavanaugh and Bennetts (1981) suggested that the constriction of axonal diameter at nodes of Ranvier contributed to the formation of axonal swelling at these locations by presenting obstructions to the transport of the growing masses of NFs. Additionally, they observed that nonobstructing masses of NFs could be successfully transported to the synapse for proteolysis. Since the rate of NF transport is 1 mm/day (Griffin et al., 1984), axonal length can be seen to determine the period of time during which sufficient NF cross-linking must occur to produce the threshold masses necessary to occlude transport and result in axonal swellings, secondary myelin retraction and demyelination, and distal axonal degeneration.

4.4.5. Genotoxicity Studies

Data from limited short-term in vitro tests provide minimal evidence of the genotoxicity of n-hexane (Tables 4-20, 4-21, and 4-22). For example, NTP (1991) observed that n-hexane was negative for gene reversion in *Salmonella typhimurium* strains T98, TA100, TA1535, or TA1537 with or without activation with S9 (NTP, 1991; Mortelmans et al., 1986). Ishidate et al. (1984) reported no increase in reverse mutations in the Ames test in strains TA92, TA94, TA98, TA100, TA1535, or TA1537 treated with n-hexane (purity not stated). Houk et al. (1989) described a spiral salmonella assay of n-hexane in strains TA98 and TA100 in which a slightly elevated response (less than twofold over background) was reported in TA 98 without S9 activation. This finding was considered insignificant by the authors. Similarly, no DNA damage was detected in *Escherichia coli* or *Bacillus subtilis* microsuspensions (McCarroll et al., 1981a, b). n-Hexane provoked a marginal or weakly positive response in an in vitro test to induce chromosome loss in *Saccharomyces cerevisiae* D61.M (Mayer and Goin, 1994). However, the metabolite 2,5-hexanedione was clearly positive for chromosome loss in this system.

Table 4-20. Summary of in vitro assays on the mutagenicity/genotoxicity of n-hexane

Test System	Cell/Strain	Results	Reference	Comments
Bacteria				
S. typhimurium	TA98, TA100, TA1535, TA1537	Negative (+/– S9)	Mortelmans et al., 1986	Gene reversion
	TA92, TA94, TA98, TA100, TA1535, TA1537	Negative (ND on S9 status)	Ishidate et al., 1984	
	TA98, TA100	Negative (+/– S9)	Houk et al., 1989	Spiral salmonella assay
E. coli	WP2, WP2urvA, WP67, CM611, WP100 W3110polA$^+$, P3478polA$^-$	Negative (+/– S9)	McCarroll et al., 1981a	DNA damage
B. subtilis	H17, M45	Negative (+/– S9)	McCarroll et al., 1981b	
Fungi				
S. cerevisiae	D61.M	Borderline positive (tested –S9 only)	Mayer and Goin, 1994	Chromosomal loss
Mammalian cells				

Chinese hamster fibroblasts	CHL	Borderline positive (tested –S9 only)	Ishidate et al., 1984	Polyploidy
Human lymphocytes		Borderline positive (–S9) negative (+S9)	Perocco et al., 1983	Inhibition of DNA synthesis
Chinese hamster ovary		Negative (– S9) Borderline positive (+ S9)	NTP, 1991	SCE
Chinese hamster ovary		Negative (+/– S9)	NTP, 1991	CA
Chinese hamster	V79	Negative (tested –S9 only)	Lankas et al., 1978	Induction/promotion
Mouse lymphoma	L5178Y $tk^{+/-}$	Negative (+/– S9)	Hazleton Labs, 1992	Forward mutations

ND = no data.
SCE = Sister chromatid exchanges.
CA = Chromosomal aberrations.

Table 4-21. Summary of in vivo assays on the mutagenicity/genotoxicity of n-hexane

Species	Strain	Results	Comments	Reference
Mouse	CD-1	Negative	Dominant lethal	Litton Bionetics, 1980
	B6C3F1	Negative	CA and MN	Shelby and Witt, 1995
	ND	Negative Negative Positive	SCE NCE and PCE CA	NTP, 1991
Rat	Albino	Positive	CA	Hazleton Labs, 1992

CA = Chromosomal aberrations.
MN = Micronuclei.
NCE = Nonchromatic erythrocytes.
PCE = Polychromatic erythrocytes.
SCE = Sister chromatid exchanges.
ND = No data.

Table 4-22. Summary of in vivo and in vitro assays on the mutagenicity/ genotoxicity of commercial hexane mixtures

Species	Strain	Results	Comments	Reference
In vitro assays				
Bacteria *S. typhimurium*	TA98, TA100, TA1535, TA1538	Negative (+/– S9)	Gene reversion (in vapor phase)	Microbiological Associates, 1989
Mammalian Cells Chinese Hamster fibroblasts	CHL	Negative (ND on S9 status)	CA	Kawachi et al., 1980
In vivo assays				
Rat	Sprague-Dawley	Negative	CA	Microbiological Associates, 1990

CA = Chromosomal aberrations.
ND = No data.

Those in vitro cytogenic tests in mammalian cell lines that included n-hexane as a test chemical have been generally negative, although n-hexane-induced polyploidy in Chinese hamster lung fibroblast cells (CHL) (Ishidate et al., 1984). DNA synthesis was inhibited in human lymphocytes in the presence of concentrations of n-hexane from 10^{-4}–10^{-2} M but only at cytotoxic concentrations (Perocco et al., 1983). NTP (1991) reported a marginally increased incidence of sister chromatid exchanges (SCEs) in Chinese hamster ovary (CHO) cells in the presence of S9 (not dose dependent). Similarly, n-hexane was negative for chromosomal aberrations in CHO cells (Daughtrey et al., 1994b; NTP, 1991). n-Hexane was negative for forward mutations in the mouse lymphoma L5178Y $tk^{+/-}$ assay (Hazleton Laboratories, 1992). n-Hexane did not induce mutagenic activity in V79 Chinese hamster cells when a promoter, methylazoxymethanol acetate, was added to the system (Lankas et al., 1978).

Tests for the genotoxic potential of n-hexane in vivo have been predominantly negative. No dominant lethal mutations were induced following n-hexane exposure in CD-1 mice (Mast et al., 1988b; Litton Bionetics, 1980). Also, n-hexane did not induce CA and micronuclei in bone marrow cells of B6C3F1 mice injected intraperitoneally with the chemical (Shelby and Witt, 1995).

Hazleton Laboratories (1992) recorded a slight, but significant, increase in the number of chromosomal mutations induced by n-hexane in albino rat bone marrow cells. Moreover, an in vivo bone marrow cytogenetic assay found that male albino rats exposed to 150, 300, and 600 ppm of n-hexane for 5 days experienced a significant increase in CA (chromatid breaks and markers) at all treatment levels compared with controls (Hazleton Laboratories, 1992).

n-Hexane did not increase the incidence of SCEs in in vivo mouse bone marrow cells at intraperitoneal doses of 500, 1000, or 2000 mg/kg (NTP, 1991). The dosed groups displayed slight increases in CA, but this increase was not considered to be significant.

In the few studies that have addressed the genotoxicity/mutagenicity of a mixture containing approximately 50% n-hexane, no gene reversion or chromosomal aberrations in CHO cells (with or without activation) and chromosomal aberrations in CHL cells were seen in vitro (Microbiological Associates, 1990; Microbiological Associates, 1989). In addition, in vivo, no chromosomal aberrations were induced in male and female Sprague-Dawley rat bone marrow cells after nose-only inhalation exposure to commercial hexane for 6 hours/day on 5 consecutive days at concentrations of 876, 3249, and 8715 ppm (Microbiological Associates, 1990).

4.5. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS AND MODE OF ACTION—ORAL AND INHALATION

4.5.1. Oral Exposure

There are no studies that have examined the possible associations between oral exposure to n-hexane and noncancer health effects in humans. A few studies in animals indicate that the nervous system may be a target for the toxic effects of n-hexane following oral exposure. For example, a 90-day gavage study in male COBS CD(SD) BR rats described the characteristic appearance of axonal swellings in peripheral nerve in those subjects exposed to n-hexane at the highest dose (3980 mg/kg-day) (Krasavage et al., 1980). The histopathological lesions were accompanied by signs of hind-limb paralysis, a frequent symptom of neuropathy in experimental animals exposed to n-hexane.

Subacute exposure to n-hexane also induced deficits in nerve conduction (Ono et al., 1981). Specifically, exposures to approximately 811 mg/kg-day (after 2 weeks), 759 mg/kg-day (2–4 weeks), 1047 mg/kg-day (4–6 weeks), and 2022 mg/kg-day (6–8 weeks) resulted in statistically significant reductions in the proximal and distal MCVs of rats receiving n-hexane compared with controls.

There are data suggesting that the principal metabolite of n-hexane, 2,5-hexanedione, is responsible for the neurotoxicity associated with oral exposure to n-hexane. For example, Krasavage et al. (1980) compared the neurotoxicity of n-hexane and that of its metabolites (2,5-hexanedione, 5-hydroxy-2-hexanone, 2,5-hexanediol, 2-hexanone, and 2-hexanol) by administering equimolar doses of each chemical by gavage to five male COBS, CD(SD)BR rats/group for 5 days/week for 90 days. Based on the time taken by the rats to develop hind-limb

paralysis, 2,5-hexanedione had approximately 38 times the neurotoxic potency of n-hexane itself on an equimolar basis.

Lapadula et al. (1986) observed the formation of higher molecular weight polypeptides in excised peripheral nerve fibers in rats exposed to 2,5-hexanedione in drinking water (5000 mg/L; 180 days) than those seen in the nerves of unexposed rats. Immunoblotting techniques provided further evidence that cross-linking of neurofilamentous proteins may have occurred.

Neurophysiological and behavioral effects were observed in male Wistar rats exposed for 6 weeks to 5000 mg/L 2,5-hexanedione in drinking water (Ladefoged et al., 1989). Reduction of MCV by 17% and rotarod balance time by 50% in rats orally exposed to 2,5-hexanedione showed the capacity of the principal metabolite of n-hexane to induce subclinical symptoms of peripheral neuropathy and motor/sensory deficits. Histopathological examination of peripheral nerve fibers was also associated with the appearance of giant axonal swelling and a change in the distribution of fiber area size in nerve fiber cross sections (Ladefoged et al., 1994).

The neurotoxicity of orally administered 2,5-hexanedione in male Wistar rats was linked to the appearance of pyrrole-like substances in the urine of animals exposed to 200 or 400 mg/kg 2,5-hexanedione for 6 or 9 weeks (Mateus et al., 2002). Parallel experiments incorporated supplemental amounts of zinc acetate (300 or 500 mg/kg) in the diets. Neurobehavioral testing (rearing and ambulation in an open field) showed changes according to treatment, with those animals exposed to 2,5-hexanedione alone at the higher dose performing significantly less well than controls. The performance of animals exposed to zinc acetate plus 2,5-hexanedione was intermediate between 2,5-hexanedione-exposed animals and controls. This suggested that zinc may be protecting the animals from the neurotoxic effects of 2,5-hexanedione in some way, possibly by interfering with the n-hexane-induced cross-linking of proteins.

In summary, information on the oral toxicity of n-hexane is limited to the studies of Krasavage et al. (1980) and Ono et al. (1981). These studies provide evidence that the nervous system is the target of toxicity following oral exposure to n-hexane. Studies indicate that oral exposure to the primary metabolite of n-hexane, 2,5-hexanedione, results in many of the gross or subclinical symptoms of peripheral neuropathy also observed with n-hexane.

4.5.2. Inhalation Exposure

Several studies establish associations between inhalation exposure to n-hexane and human health effects. Specifically, occupational studies and case reports suggest that inhalation exposure to n-hexane in humans may be associated with neurotoxicity (Section 4.1). For example, Sanagi et al. (1980) monitored the neurophysiological performance of 14 workers

exposed to n-hexane in the mixing and drying jobs at a factory producing tungsten carbide alloy. The workers were examined for signs of neurological deficits compared with 14 workers who were not exposed to any solvents in the same factory (Sanagi et al., 1980). Twenty-two breathing zone air samples taken twice a year over a 2-year period indicated an 8-hour TWA of 58 ppm for n-hexane and 39 ppm for acetone. Compared with controls, exposed workers reported a significantly increased occurrence of headache, hearing deficits, dysesthesia in limbs, and muscle weakness. Exposed workers also showed an increased incidence of neurological symptoms relating to muscle strength and reduced vibration sensation of the radial nerve. Neurophysiological findings suggested that recovery from a slowing of motor nerve conduction in the posterior tibial nerve was delayed after cessation of exposure.

Mutti et al. (1982a) compared MCVs in a group of 95 shoe factory workers exposed to a mixture of hydrocarbons containing n-hexane and 52 unexposed workers from the same factory. Exposed workers were divided into two groups based on hydrocarbon exposure. The TWA for n-hexane of 108 breathing zone samples taken was 243 mg/m^3 (69 ppm) in a mildly exposed group and 474 mg/m^3 (134 ppm) in a highly exposed group. When the severity of neurological symptoms was compared, there was a gradation in response between the exposed groups, both of which displayed more severe symptoms than the controls.

The groups of workers in the Sanagi et al. (1980) and Mutti et al. (1982a) studies showed neurological symptoms as a result of n-hexane exposure. However, the subjects were also exposed to other solvents concurrently: acetone in the tungsten alloy factory (Sanagi et al., 1980) and cyclohexane, methyl ethyl ketone, and ethyl acetate in the shoe factory (Mutti et al., 1982a). Some of these components may have also contributed to the neurotoxicological effects or may have quantitatively affected the response to n-hexane to an uncertain extent. None of the occupational exposure studies or case reports of n-hexane discussed in Section 4.1 involved exposure to the single chemical. This suggests a limited utility of such data sets for dose-response modeling of n-hexane.

Industrial hygiene surveys of occupationally exposed workers have shown good correlations between the extent of occupational exposure to n-hexane and the concentration of 2,5-hexanedione in the urine (Prieto et al., 2003; Mayan et al., 2001; Cardona et al., 1996, 1993; Mutti et al., 1993; Takeuchi, 1993; Saito et al., 1991; Ahonen and Schimberg, 1988). Therefore, levels of this metabolite in the urine may be a useful, indirect means of monitoring exposure to n-hexane in the workplace. For example, when Governa et al. (1987) investigated the correlation between ENM changes indicative of polyneuropathy and urinary excretion of metabolites indicative of exposure to n-hexane, they identified a value of 7.5 mg/L 2,5-hexanedione as representing a threshold to the occurrence of abnormalities. However, some

variation from this relationship was apparent, because Governa et al. (1987) identified three workers with 2,5-hexanedione urinary concentrations of 3.0, 3.3, and 4.5 mg/L, all of whom displayed ENM changes. To protect against the onset of subclinical and clinical neuropathological symptoms of n-hexane exposure, ACGIH proposed a BEI of 0.4 mg/L as an acceptable concentration of 2,5-hexanedione in urine at the end of shift on the last day of a workweek (ACGIH, 2003).

Other responses in humans occupationally exposed to solvents containing n-hexane included the possible impairment of color vision (Gobba and Cavalleri, 2003; Iregren et al., 2002; Issever et al., 2002; Seppalainen et al., 1979; Raitta et al., 1978) and the onset of some parkinsonism-type neurological symptoms (Canesi et al., 2003; Vanacore et al., 2000; Hageman et al., 1999; Pezzoli et al., 1996, 1995, 1989).

Several studies in laboratory animals support the human data on nervous system effects following inhalation exposure to n-hexane. Huang et al. (1989) reported dose-dependent reductions in MCV in male Wistar rats (eight/group) exposed to 0, 500, 1200, or 3000 ppm n-hexane (>99% pure) for 12 hours/day, 7 days/week, for 16 weeks. Additionally, there were some behavioral deficits in high- and mid-dose rats, including a reduction in grip strength and a comparative slowness of motion indications of neurological impairment. No hind-limb paralysis was observed. Histologically there was an increased incidence of paranodal swellings, some evidence of demyelination, and remyelination was present in peripheral nerves. There were statistically significant dose-dependent reductions in nervous system specific proteins, particularly the β-S100 protein from tail nerve fibers. The neurophysiological deficits and histopathology indicate a NOAEL of 500 ppm. Effects on hind-limb grip strength typically preceded electrophysiological alterations in the progression of nerve degeneration following exposure to neurotoxic chemicals (Harry et al., 1998). For example, studies using a chemical with a similar mode of action as n-hexane, such as carbon disulfide, suggest that changes in nerve conduction velocity are usually seen in later stages of nerve degeneration following chemical exposure compared to behavioral alterations in hind-limb and fore-limb grip strength (Harry et al., 1998; Sills et al., 1998).

Other studies have also observed neurological symptoms in experimental animals exposed subchronically to n-hexane via inhalation. Ono et al. (1982) established a LOAEL of 200 ppm for histopathological effects characterized by axonal swelling and degeneration of the myelinated axons in Wistar rats subchronically exposed to 200 and 500 ppm n-hexane.

Howd et al. (1983), Pryor et al. (1983), and Ichihara et al. (1998) used n-hexane concentrations in the 1000–2000 ppm range to induce neurophysiological deficits and/or behavioral changes in laboratory animals. Data from the Chemical Industry Institute of

Toxicology's (CIIT) 13-week toxicological study in F344 rats exposed to n-hexane confirmed the neuropathological responses based on the dose-dependent appearance of paranodal swellings of the tibial nerves in high- and mid-dose males (Cavender et al., 1984a, b).

The NTP-sponsored study of n-hexane in B6C3F1 mice induced neurobehavioral deficits in high-dose mice (10,000 ppm) exposed for 6 hours/day, 5 days/week for 90 days and in another group exposed to 1000 ppm n-hexane for 22 hours/day (NTP, 1991; Dunnick et al., 1989). The authors concluded that the data in this study indicated an exposure concentration of 500 ppm n-hexane as a NOAEL.

Huang et al. (1992) exposed male Wistar rats to 2000 ppm (99% pure) n-hexane for 12 hours/day, 6 days/week for a total of 24 weeks. Effects of treatment included an overall reduction in MCV after 8 weeks and an increase in DL after 12 weeks. There was a reduction in the activity or amount of neuron-specific enolase (γ-enolase), creatine kinase-B, and the β-S100 protein. The onset of neurophysiological deficits was most evident in the distal segment of the sciatic nerve (near the knee). Other sections of the central and peripheral nervous systems were comparatively unaffected.

The reproductive/developmental toxicity of n-hexane has been investigated in a number of studies in experimental animals exposed via the inhalation route. For example, Bus et al. (1979) exposed pregnant F344 rats to 0 or 1000 ppm on GDs 8–12, 12–16, or 8–16. Progeny of exposed dams had birth weights that were approximately 14% lower than controls for up to 3 weeks after birth. Litton Bionetics (1979) did not observe any n-hexane related effects in reproductive, developmental, or teratological parameters when CRL:COBS CD(SD)BR rats were exposed for 6 hours/day to concentrations of 0, 100, and 400 ppm n-hexane on GDs 6–15.

There was an n-hexane-related reduction in body weight gain in the fetuses of mid- and high-dose dams (3.0–7.5% at the mid dose and 14–15% at the high dose in females and males), when 30 pregnant Sprague-Dawley rats/group were exposed to 0, 200, 1000, or 5000 ppm n-hexane (>99.5% pure) for 20 hours/day on GDs 6–19 (Mast, 1987). Examination of the fetuses revealed some potentially n-hexane-related variations, including the incidence of supernumerary ribs and reduced skeletal ossification. A companion study in CD-1 mice (Mast et al., 1988a) observed signs of maternal toxicity, such as reduced body weight gain and relative uterus weight in high-dose dams. Fetal resorptions were evident in all exposure groups.

There was some evidence that n-hexane can induce toxicological effects in the male reproductive system. Abnormal sperm and varying degrees of severity in the histopathology of the testis were observed following inhalation exposure to n-hexane (Nylen et al., 1989; DeMartino et al., 1987). However, exposing male B6C3F1 mice to n-hexane at concentrations of up to 5000 ppm did not result in any changes of sperm morphology or in the architecture of

the male reproductive system (Mast et al., 1988c). Similar treatments to male CD-1 mice did not induce dominant lethal effects when n-hexane-receiving animals were mated with unexposed females (Mast et al., 1988b).

There is no clear evidence of other systemic effects resulting from inhalation exposure to n-hexane. Data from CIIT's 13-week toxicological study in F344 rats exposed to n-hexane have indicated that the kidney may be a target organ of n-hexane, at least in this test species (Cavender et al., 1984a, b). However, this response may be related to the well-described α_{2u}-globulin-related hyaline droplet nephropathy that is characteristic of some strains of male rat but not of humans. When mode of action evidence convincingly demonstrates that an effect is secondary to α_{2u}-globulin accumulation, the data are not to be used in the assessment of human health risk (U.S. EPA, 1991). The criteria for demonstrating this mode of action for risk assessment purposes have been clearly defined (U.S. EPA, 1991). Three core criteria must be met: (1) increase in hyaline droplets in the renal proximal tubule cells; (2) determination that the accumulating protein in the droplets is α_{2u}-globulin; and (3) additional pathological lesions associated with α_{2u}-globulin are also present. In addition, a number of mechanistic studies can be used to further support conclusions regarding the role of α_{2u}-globulin. Data suggest that kidney effects following inhalation exposure to n-hexane may be due to the accumulation of α_{2u}-globulin. However, the evidence for this mode of action is equivocal.

There is some evidence that exposure of experimental animals to high concentrations of n-hexane via inhalation may result in portal-of-entry effects. For example, the NTP-sponsored study of inhalation exposure of B6C3F1 mice to n-hexane reported signs of irritation in the respiratory tract, such as inflammation, erosion, and regeneration of the olfactory epithelium with fibrosis of the submucosa (NTP, 1991; Dunnick et al., 1989).

A substantial number of toxicological studies were carried out on various formulations of a mixture containing approximately 50% n-hexane (commercial hexane) that provided limited support for n-hexane-induced health effects. There were no signs of nervous system degeneration when Sprague-Dawley rats were exposed subchronically to up to approximately 125 ppm commercial hexane (Biodynamics, 1978). However, 500 ppm commercial hexane was effective in inducing some signs of neuropathological degeneration in male Sprague-Dawley rats exposed in a similar exposure regimen (IRDC, 1992a, b).

Soiefer et al. (1991) reported in an abstract that Sprague-Dawley rats subchronically exposed to commercial hexane via inhalation at concentrations of 9000 ppm showed no neuropathological responses or altered performance in an FOB.

A single toxicological study has addressed the possible impacts of commercial hexane when administered to experimental animals using a chronic dosing regimen (Daughtrey et al.,

1999; Biodynamics, 1993a, b). The study exposed F344 rats and B6C3F1 mice to concentrations of up to 9000 ppm commercial hexane for 2 years. Rats displayed a variety of histopathological lesions in the respiratory epithelium at all doses in males and in mid- and high-dose females. However, no treatment-related histopathological abnormalities in sciatic nerve were observed in any group of F344 rats in this study.

Reproductive and developmental toxicological effects of commercial hexane have been studied in experimental animals. Bushy Run Research Center (1989a, b) conducted a range-finding study and a follow-up developmental toxicity study in rats and mice. BRRC (1989a, b) exposed pregnant Sprague-Dawley rats (25/group) and pregnant CD-1 mice (8–30/group) to 0, 900, 3000, or 9000 ppm commercial hexane for 6 hours/day on GDs 6–15. In the range-finding study, developmental toxicity was observed in the progeny of mice exposed to 9000 ppm commercial hexane only. Specifically, there was a reduction (per litter) in fetal weights in progeny of the high-dose dams.

In a follow-up study, body weight gain was reduced in high- and mid-dose rat dams for part of the exposure period lasting from GDs 9–12. The only developmental effects observed were an increased incidence of two individual skeletal variations in high-dose pups. The incidences of these lesions were 0/26 versus 6/26 (control versus high dose) for bilateral bone islands at the first lumbar arch and 20/26 versus 26/26 (control versus high dose) for all intermediate phalanges unossified.

Sprague-Dawley rats were exposed to commercial hexane at concentrations up to 9000 ppm in a two-generation reproductive/developmental toxicological study (Daughtrey et al., 1994a; BRRC, 1991). There was a reduction of mean body weight in the F1 pups of the high-dose dams at PND 14 and beyond. Reduced fetal weight was also observed in a single-generation reproductive/developmental study of commercial hexane in Sprague-Dawley rats (IRDC, 1986). The animals were exposed to 0, 100, 500, or 1503 ppm commercial hexane via inhalation for 6 hours/day, 7 days/week for 100 days prior to mating, through the mating period (maximum of 15 days), through GDs 1–20, and then postnatally through weaning (PND 21). Fetal weights were reduced in the F1 pups, especially in high-dose progeny on lactation day 4, where the reduction from control levels was 11–13%. The body weights of high-dose F1 pups remained 8–9% lower than those of controls throughout lactation. Similar body weight reductions compared with controls were also observed in the mid-dose group throughout lactation, where the reductions were 12–17% from control values at their greatest extent and achieved statistical significance on PNDs 14 and 21. The authors considered that the changes in pup weight were of questionable biological significance and assigned a NOAEL of 1503 ppm to the study.

Some signs of systemic kidney toxicity of commercial hexane were suggested by histopathologic lesions seen in high-dose male F344 rats exposed to 0, 900, 3000, or 9000 ppm commercial hexane for 6 hours/day, 5 days/week for 13 weeks (Duffy et al., 1991; Biodynamics, 1989), although the relevance of this finding to human toxicity is uncertain, as discussed above. A summary of toxicological studies of n-hexane in experimental animals exposed via the inhalation route is provided in Table 4-23.

Table 4-23. Toxicity findings in inhalation studies for n-hexane

Reference	Strain/species	Doses (ppm)	Duration adjusted doses (mg/m^3)	Duration	Response [a]	NOAEL (mg/m^3)	LOAEL (mg/m^3)
Biodynamics (1978)	12 Sprague-Dawley rats/sex/group	Phase I 0 6 26 129 (6 h/d, 5 d/w) Phase II 0 5 27 126 (21 h/d, 7 d/w)	 0 3.8 16.4 81.2 0 15.4 83.3 388	26 Weeks 26 Weeks	No effects No effects	81.2 388	None None
Bus et al. (1979)	3 to 8 Pregnant F344 rats	0 1000 (6 h/d)	0 881	GDs 8–12 GDs 12–16 GDs 8–16	Fetal weights ↓ (with partial recovery)	None	881
Cavender et al. (1984a, b)	F344 Rats	0 3000 6500 10,000 (6 h/d, 5 d/w)	0 1888 4091 6294	13 Weeks	Organ/weight ↑; PNS histopathology	1888	4091
Howd et al. (1983)	Male F344 rats	0 1000 (24 h/d, 6 d/w)	0 3021	11 Weeks	Hind-limb paralysis; MCV ↓	None	3021

(continued on next page)

Reference	Strain/species	Doses (ppm)	Duration adjusted doses (mg/m^3)	Duration	Response [a]	NOAEL (mg/m^3)	LOAEL (mg/m^3)
Huang et al. (1989)	8 Wistar rats/sex/group	0 500 1200 3000 (12 h/d, 7 d/w)	0 881 2115 5287	16 Weeks	Neurological FOB↓; PNS histopathology; MCV ↓	881	2115
Huang et al. (1992)	7 Male Wistar rats	0 2000 (12 h/d, 6 d/w)	0 7058	24 weeks	MCV↓ at 8 weeks DL ↑ at 12 weeks ↓ Activity of γ-enolase, creatine kinase-B, β-S100	None	7058
Ichihara et al. (1998)	7 Male Wistar rats	0 2000 (12 h/d, 6 d/w)	0 3021	20 weeks	MCV↓, DL↑	None	3021
IRDC (1992a, b)	Male Sprague-Dawley rats	0 125 500 (22 h/d, 7 d/w)	0 403 1615	6 months	Abnormal gait and peripheral nerve histopathology (HD) Liver pathology (LD)	403 None	1615 (NS) 403 (liver)
Litton Bionetics (1979)	20 Pregnant CRL:COBS CD(SD) BR rats	0 100 400 (6 h/d)	0 88.1 352.5	GDs 6–15	No effects	352.5	None
Lungarella et al. (1984)	12 Male New Zealand rabbits	0 3000 (8 h/d, 5 d/w)	0 2517	24 weeks	Lung histopathology (with partial recovery)	None	2517

(continued on next page)

Reference	Strain/species	Doses (ppm)	Duration adjusted doses (mg/m^3)	Duration	Response [a]	NOAEL (mg/m^3)	LOAEL (mg/m^3)
Mast (1987)	30 Pregnant Sprague-Dawley rats	0 200 1000 5000 (20 h/d)	0 587.5 2937 14,686	GDs 6–19	Fetal weights ↓ Some ossification changes	587.5 None	2937 587.5
Mast et al., (1988a)	35 Pregnant CD-1 mice	0 200 1000 5000 (20 h/d)	0 587.5 2937 14,686	GDs 6–17	Relative uterus weight, body weight gain ↓ (HD) Fetal resorptions	2937 None	14686
NTP (1991); Dunnick et al. (1989)	10 B6C3F1 mice/sex/group	0 500 1000 4000 10,000 (6 h/d, 5 d/w) 1000 (22 h/d, 5 d/w)	315 629 2518 6294 2307	13 Weeks	Nasal irritation; Relative liver, kidney and heart weights ↑; Neurobehavioral deficits and histopathology	315	587.5 6294 (and 2307)
Ono et al. (1982)	8 Male Wistar rats/group	0 200 500 (12 h/d, 7 d/w)	0 352 881	24 Weeks	MCV↓, DL ↑ Axon degeneration	None	352
Pryor et al. (1983)	Male F344 rats	0 2000 (14 h/d)	0 4112	14 Weeks	FOB effects	None	4112

[a] Increase (↑); decrease (↓); HD = high dose; LD = low dose.

4.5.3. Mode of Action Information

Peripheral neuropathy following inhalation exposure to n-hexane demonstrates that the nervous system is the target of toxicity for n-hexane. Further, inhalation exposure to n-hexane has been shown to decrease MCV and SCV (with increased DL) in both humans and laboratory animals. Myelin sheath thickness determines nerve conduction velocity and is proportional to the diameter of the axon and internodal length (ffrench-Constant et al., 2004; Michailov et al., 2004). Histopathological examination of the nerves of laboratory animals suggests that the mode of action of n-hexane-induced neurotoxicity may involve a sequence of events including accumulation of NF-filled axonal swellings with secondary demyelination that could lead to a decrease in MCV and SCV (Spencer and Schaumburg, 1977a). Specifically, in the nerves of rats exposed to 400–600 ppm n-hexane for up to 35 weeks, there was an increase in axonal swelling in the distal region of large myelinated fibers (Spencer and Schaumburg, 1977a). As axonal swelling progressed, subsequent localized demyelination and remyelination produced axonal degeneration in a distal retrograde manner following further exposure to n-hexane. Shrinkage of the internode, accumulation of NFs, and myelin sheath degeneration accompanied this axonal swelling.

Studies in laboratory animals suggest that the n-hexane metabolite, 2,5-hexanedione, is the primary toxic agent leading to neurological effects following exposure to n-hexane (Section 4.4.4). Administration of 2,5-hexanedione has been shown to result in axonal swelling accompanied by axonal and secondary myelin degeneration in the PNS in laboratory animals (Nachtman and Couri, 1984; Abou-Donia et al., 1982; Krasavage et al., 1980). The time to onset of these symptoms, severity of the lesions, and magnitude of the neurotoxicity indicate that 2,5-hexanedione is more toxic than n-hexane itself or any its other metabolites. In vivo and in vitro studies indicate that the mode of action of 2,5-hexanedione may involve covalent cross-linking of NF proteins in peripheral nerve and spinal cord (Mateus et al., 2002; Sanz et al., 1995; Kessler et al., 1990; Lapadula et al., 1986; DeCaprio et al., 1982). Specifically, evidence suggests that 2,5-hexanedione may react with lysine residues to form pyrrole adducts. Genter St. Clair et al. (1988) demonstrated that pyrrole derivatization is required for neuropathy to develop but that it is not sufficient alone. In addition, oxidation of the pyrrole is also necessary for cross-linking of NFs. Formation of pyrrole adducts, followed by oxidation of the pyrrole rings and cross-linking of NFs, has been hypothesized to be responsible for the accumulation of NFs observed in the distal axonal swellings of the peripheral nerves following n-hexane exposure (Graham et al., 1982a, b, 1995).

Studies with chemicals structurally related to 2,5-hexanedione provide further support for the neurotoxic mode of action of 2,5-hexanedione. Treatment with other γ-diketones (such as 3-

methyl-2,5-hexanedione, 3,4-dimethyl-2,5-hexanedione, and 1,2-diacetylbenzene) is associated with axonal swelling and NF accumulation (Kim et al., 2002, 2001; Spencer et al., 2002; Graham et al., 1995; Anthony et al., 1983a, b). These structurally related chemicals have also been utilized to provide insight into the process of axonal swelling following exposure to γ-diketones. Neurofilaments are believed to accumulate in axonal swellings due to the pyrrolization and cross-linking of their proteins as they are transported (anterograde) in the nerve. Studies indicate that the extent and location of axonal swelling depend on the reactivity of n-hexane with NF protein and the neurotoxicity associated with the n-hexane (Kim et al., 2002, 2001; Spencer et al., 2002; Graham et al., 1995; Genter St. Clair et al., 1988; Anthony et al., 1983a, b). For example, 3,4-dimethyl-2,5-hexanedione forms pyrroles faster than 2,5-hexanedione and therefore the axonal swellings occur at mid-level and proximal nerve locations compared with distal locations following exposure to 2,5-hexanedione. Further studies detailing the neurotoxicity of the *d,l* and *meso* diastereomers of 3,4-dimethyl-2,5-hexanedione (Genter et al., 1987) and of 3-acetyl-2,5-hexanedione (Genter St. Clair et al., 1988) demonstrate that both pyrrole formation and subsequent oxidation of the pyrrole rings resulting in NF cross-linking are necessary steps in the pathogenesis of n-hexane neurotoxicity.

4.6. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CHARACTERIZATION

4.6.1. Summary of Overall Weight-of-Evidence

Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), there is *inadequate information to assess the carcinogenic potential of n-hexane*. Specifically, there are no animal carcinogenicity studies available that examine exposure to n-hexane, and there is a single human study (Beall et al., 2001) where workers were chronically exposed to mixtures containing n-hexane along with other chemicals. A 2-year carcinogenicity bioassay in mice and rats exposed to a mixture containing various hydrocarbons, including n-hexane, showed an increased incidence of liver tumors in female mice (Daughtrey et al., 1999; Biodynamics, 1993a, b). Daughtrey et al. (1999) observed an increased incidence of combined hepatocellular adenomas and carcinomas in female mice exposed to the highest dose of a mixture containing n-hexane (commercial hexane). In addition, the study authors identified a statistically significant trend for increased incidence of pituitary adenomas in female mice exposed to commercial hexane. Studies indicate that n-hexane is mostly nongenotoxic in short-term testing protocols. n-Hexane showed a minimal response in *S. cerevisiae* D61.M (Mayer and Goin, 1994) and induced an increased incidence in the number of chromosomal mutations in albino rat bone marrow cells (Hazleton Laboratories, 1992). Also, the low pKa of exocyclic amino functional

groups of DNA (<5) would preclude reaction with 2,5-hexanedione to yield pyrrole adducts. Thus, these data suggest a lack of mutagenic potential of n-hexane. The available studies in humans, as well as laboratory animals, are inadequate for cancer risk assessment. The previous IRIS assessment (1990) did not contain a characterization of the carcinogenic potential of n-hexane in humans.

4.6.2. Synthesis of Human, Animal, and Other Supporting Evidence

Only one of the occupational exposure studies on n-hexane has inferred a possible association between n-hexane and increased cancer incidence. Beall et al. (2001) conducted a nested case control study evaluating the relationship between the occurrence of intracranial tumors among employees at a petrochemical plant and exposure to chemicals, including ionizing radiation, methylene chloride, acrylonitrile, vinyl chloride, formaldehyde, n-hexane, and various other chlorinated, halogenated, volative, and aromatic hydrocarbons and nitroso compounds. The workers were also exposed to organometallic and elemental metallic catalysts. The study authors selected subjects from approximately 2595 plant workers. The workers were mailed questionnaires that evaluated work history in the plant, and a total of 12 cases of intracranial tumors, which developed after hire dates at the plant, were identified from the respondents. All cases were confirmed by review of medical records and pathology specimens by four neuropathologists. Six of these cases, all of which were men, had primary brain cancers or gliomas (two astrocytomas, two glioblastomas, and two oligodendrogliomas). Six cases had benign intracranial tumors, of which two were diagnosed as vestibular schwannomas (observed in one man and one woman), two as meningiomas (both in men), and two pituitary adenomas (observed in one man and one woman). Ten healthy controls were matched to each case by age, gender, birth year, race and an initiation date for work in the building complex that was prior to the tumor diagnosis date for the case. The median length of employment at the facility was 16.8 years for cases and 10.9 years for controls.

Work histories were obtained from company records or interviews, the latter providing information about complete work history, exposures encountered, extent of hands-on work at each job, and incidence of certain other nonoccupational factors that may be related to risk of occurrence of brain cancers and intracranial tumors (exposure to diagnostic irradiation, use of anticonvulsant and ototoxic drugs, history of head trauma, seizures, meningitis, use of cellular phones and radiation badges, amateur radio operation, pesticide application, furniture refinishing, and history of hearing loss). Exposure information was obtained from company accounting records that detailed hours worked on projects during each year of employment and self-reported workplace exposure to chemicals of interest. The authors compared cases and controls with

respect to self-reported exposure to chemicals of interest, project-based work histories indicating the potential use of chemicals of interest, and self-reported exposure to any of the other nonoccupational factors that may be related to the risk of brain cancers. Conditional regression was used and maximum likelihood estimates of odds ratios with a 95% confidence interval were reported.

The authors showed that the OR for self-reported exposure to n-hexane was statistically significantly elevated (OR, infinity), with a CI of 1.4 to infinity (6 cases and 26 controls evaluated) for gliomas. The OR for potential exposure to n-hexane based on job-related exposure estimates was 2.3 (CI, 0.4 to 13.7; 4 cases and 26 controls evaluated) for gliomas. Analyses by duration indicated a statistically significantly elevated OR of 16.2 (CI, 1.1 to 227.6; two cases and two controls evaluated) for potential long-term use of n-hexane (> 48 months) for gliomas. No relationship was found between exposure to n-hexane and the occurrence of intracranial tumors. While the results of this study indicated that exposure to n-hexane may have contributed to the occurrence of brain tumors, specifically gliomas, the small number of cases, large number of chemicals to which the employees were potentially exposed, and high correlation between some of the parameters because of coexposure to several other chemicals, the results do not permit a conclusion about carcinogenicity from exposure to n-hexane alone.

In laboratory animals exposed for 2 years via inhalation to a commercial hexane mixture containing n-hexane (0, 900, 3000, or 9000 ppm), there was a statistically significant increase in hepatocellular combined adenomas and carcinomas (7/50, 8/50, 9/49, or 16/50, respectively) in female B6C3F1 mice (Daughtrey et al., 1999; Biodynamics, 1993b). This increase was not observed in male mice or in either sex of F344 rats exposed to commercial hexane under the same conditions.

Because commercial hexane is a variable mixture of hydrocarbons of which only about 52% is n-hexane, the use of commercial hexane as a toxicological surrogate for the qualitative and quantitative effects of pure n-hexane may be unjustified.

n-Hexane has shown little evidence of mutagenic activity in a number of short-term test systems. In vitro tests showed that n-hexane was not genotoxic in the salmonella (Ames) assay (with or without activation), did not cause DNA damage of *E. coli* or *B. subtilis*, and was negative for chromosomal aberrations in CHO cells and forward mutations in the mouse lymphoma L5178 $tk^{+/-}$ assay (Daughtrey et al., 1994b; Hazleton Laboratories, 1992; NTP, 1991; Houk et al., 1989; Mortelmans et al., 1986; Ishidate et al., 1984; McCarroll et al., 1981a, b). n-Hexane was marginal for inducing chromosome loss in the DNA of *S. cerevisiae* D61.M (Mayer and Goin, 1994). In in vivo tests, n-hexane was negative for inducing dominant lethal mutations in CD-1 mice (Mast et al., 1988b; Litton Bionetics, 1980). Furthermore, n-hexane did

not induce CA and micronuclei in bone marrow cells of B6C3F1 mice injected intraperitoneally (Shelby and Witt, 1995). n-Hexane did not increase the incidence of SCEs in in vivo mouse bone marrow cells (NTP, 1991). Hazleton Laboratories (1992) recorded a slight, but significant, increase in the number of chromosomal mutations due to n-hexane exposure in albino rat bone marrow cells.

4.7. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

4.7.1. Possible Childhood Susceptibility

No studies were available regarding adverse effects of n-hexane exposure in children. A single study by Howd et al. (1983) provided data indicating adult rats may be more susceptible to n-hexane-induced toxicity than weanling rats. Specifically, adult rats showed effects of greater magnitude and earlier onset compared with weanling rats. The authors suggested that this difference in neurotoxicity may be due to the decreased size of axons and greater rate of growth and repair of peripheral nerves in weanling compared with adult rats.

Metabolism may vary between children and adults due to differences in the development and maturity of phase I and phase II enzymes (Johnsrud et al., 2003; Ginsberg et al., 2002). Studies indicate that the mode of action of n-hexane toxicity may involve metabolism to more toxic metabolites. Several enzymes, such as CYP2E1, may be involved in n-hexane metabolism. Studies with human liver microsomes collected from postmortem GD 8 to PND 18 samples indicate that the amounts of CYP2E1 increased by more than two orders of magnitude with age. Because CYP2E1 has been implicated in the transformation of n-hexane to its toxic metabolite, 2,5-hexanedione, these data suggest that the lower level of CYP2E1 in children may result in altered responses (i.e., decreased susceptibility) to the toxic effects of n-hexane exposure.

4.7.2. Possible Gender Differences

The available data provide equivocal evidence suggesting gender differences in toxicity following n-hexane exposure. Some apparent sex-specific neoplastic effects occurred in B6C3F1 mice in response to inhalation exposure to commercial hexane over a 2-year period (Daughtrey et al., 1999; Biodynamics, 1993b). These include a dose-dependent appearance of hepatocellular combined adenomas and carcinomas in female mice that was significantly different to controls at the highest dose (9000 ppm) and some possibly n-hexane-related increase in the formation of adenocarcinomas of the pituitary gland in female mice compared with controls. In these cases, there are no obvious biochemical or physiological mechanisms underlying this apparent disparity of response between the sexes. Accordingly, it could be argued that the difference in response

may be accounted for by either sex differences, the influence of other hydrocarbons in the mixture, or chance. The historical background rate of hepatocellular tumor formation in this strain of mouse ranges from 11–70% with a mean of 42.1% in males and 3–54% with a mean of 25.2% in females (Daughtrey et al., 1999). Therefore, the apparent differences in incidence of hepatocellular combined adenomas and carcinomas in exposed and control females may have been due to an unusually low incidence of these lesions in the concurrent female controls.

5. DOSE RESPONSE ASSESSMENT

5.1. ORAL REFERENCE DOSE

No epidemiology or case report studies examining health effects in humans or any chronic laboratory studies evaluating potential health effects in animals following oral exposure to n-hexane are available. The only oral n-hexane exposure study (Krasavage et al., 1980) identified was of subchronic duration, utilized gavage exposure, and evaluated a small number (five/group) of animals. Several animals died in each dose group (two in the mid-dose and one in the high-dose group) during the course of the study. In this study, Krasavage et al. (1980) exposed five male COBS CD(SD) BR rats/group to doses of 0, 6.6, 13.2, and 46.2 mmol/kg (570 mg/kg) n-hexane by gavage 5 days/week for 90 days. The period of treatment and observation was extended to 120 days for those animals receiving 46.2 mmol/kg n-hexane to ensure that an overt neuropathological endpoint was detected. The onset of neuropathy was assessed by the initial appearance of hind-limb paralysis, at which point animals were sacrificed and examined histopathologically. Hind-limb paralysis was observed in 3/4 animals exposed to the high dose of n-hexane. Giant axonal swellings were present in the nerves of 4/4 animals in the high-dose group.

An RfD for n-hexane cannot be derived in the absence of a suitable oral study of sufficient duration that evaluates an array of endpoints. The previous IRIS assessment (1990) for n-hexane also did not contain a derivation of an oral RfD. A route-to-route extrapolation using available inhalation data is currently not possible since limited PBTK models are available for n-hexane (Fisher et al., 1997; Perbellini et al., 1986). The Fisher et al. (1997) lactational transfer model was developed using rodent tissue solubility and allometrically-scaled metabolic rate constants from the published literature (in abstract form only) to estimate human tissue metabolic parameters. The authors also suggested that the absence of exposure and toxicokinetic data on lactational transfer of chemicals to nursing infants was a disadvantage of this model. The PBTK model by Perbellini et al. (1986) is also inappropriate for use in route-to-route extrapolation. The dose metric for the critical effect in this model is a function of the concentration of 2,5-hexanedione in circulation. The concentration-duration-response function for 2,5-hexanedione is unknown. In addition, the oral dose of n-hexane necessary to yield the same blood-concentration-time profile for 2,5-hexanedione, taking into account gastrointestinal uptake of n-hexane, is not accounted for by Perbellini et al. (1986). Furthermore, studies indicate that the major metabolite of n-hexane in humans is 2,5-hexanedione but in laboratory animals it is 2-hexanol. Thus, using a PBTK model based on information from laboratory animal studies may not be appropriate.

5.2. INHALATION REFERENCE CONCENTRATION

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

Many occupational and experimental exposure studies in humans have investigated the health effects following inhalation exposure to n-hexane. These studies indicate that the nervous system is the target of toxicity of n-hexane (Section 4.1.2.2). Specifically, these human studies show decreased MCV following exposure to n-hexane in the range of approximately 50–2500 ppm. However, all of the human occupational n-hexane exposure studies indicate coexposure to a variety of other chemicals known to potentiate n-hexane-induced neurotoxicity (Section 4.4.3). The most well-conducted n-hexane human occupational exposure study is by Sanagi et al. (1980), which compared the neurological functions of workers in a tungsten carbide alloy factory who were exposed to n-hexane (and other solvents) to workers in the same factory who were not exposed to n-hexane. The authors determined that the breathing zone TWA concentration of n-hexane in air was 58 ppm and coexposure to acetone was 39 ppm (two measurements per year over a 2-year period). No other solvent concentrations were reported by the study authors. Exposed workers had decreased MCV and showed clinical signs of peripheral neuropathy identified by dysesthesia in limbs, muscle weakness, and reduced vibration sensation. This study had been chosen as the principal study in the 1990 IRIS assessment. The principal limitation of Sanagi et al. (1980) is that the workers were coexposed to n-hexane and acetone, which raises the question of the role of acetone coexposure in the observed neurological changes.

Several studies indicate that acetone may affect n-hexane metabolism, neurotoxicity, and reproductive toxicity (Zhao et al., 1998; Cardona et al., 1996; Ladefoged et al., 1994, 1989; Lam et al., 1991; Larsen et al., 1991; Ladefoged and Perbellini, 1986). A study in humans showed that acetone concentrations in the workplace significantly correlated with the ratio of urinary n-hexane metabolites (specifically 2,5-hexanedione) to air n-hexane concentrations (Cardona et al., 1996). It has been suggested that acetone may potentiate neurotoxicity by decreasing the elimination of 2,5-hexanedione. For example, studies in rodents and rabbits have shown that coexposure to acetone and 2,5-hexanedione decreases elimination and increases the concentration of 2,5-hexanedione in serum and sciatic nerve compared with administration of 2,5-hexanedione alone (Zhao et al., 1998; Ladefoged and Perbellini, 1986). In addition, acetone has been shown to induce CYP2E1, one of the enzymes shown to be involved in the metabolism of n-hexane to its toxic metabolite 2,5-hexanedione in rats (Patten et al., 1986) (see also Section 3.3). Thus, coexposure to acetone may induce CYP450 enzymes and increase the production of the neurotoxic metabolite 2,5-hexanedione.

Oral coexposure studies in rats further support acetone potentiation of n-hexane

neurotoxicity (Section 4.4.3). Ladefoged et al. (1994, 1989) exposed male rats to 2,5-hexanedione alone and 2,5-hexanedione plus acetone in drinking water for 6 weeks and evaluated neurological and behavioral endpoints. Rats exposed to 2,5-hexanedione alone and 2,5-hexanedione plus acetone showed decreased balance time on a rotating rod, altered behavior (ambulation, grip strength, and rearing), decreased MCV, and increased giant axonal swelling of the sciatic nerve. The authors stated that these effects were greater in severity in the rats coexposed to 2,5-hexanedione plus acetone compared with those exposed to 2,5-hexanedione alone. Lam et al. (1991) showed that coexposure of male Wistar rats to 0.5% n-hexane and 0.5% acetone resulted in inhibition of acquisition (not performance) of spatial learning skills as measured by the radial eight-arm maze. In addition, Larsen et al. (1991) suggested that coexposure to acetone and 2,5-hexanedione may contribute to irreversible damage to the testis and male infertility in rats. Taken together, the data suggest that acetone may alter n-hexane metabolism and potentiate n-hexane-induced neurotoxicity and reproductive toxicity. Thus, Sanagi et al. (1980) was not retained as the principal study for the derivation of the RfC, based on coexposure of study subjects to n-hexane and acetone.

Several additional human occupational exposure studies (see Section 4.1.2.2) support the n-hexane-induced neurotoxicity identified in humans by Sanagi et al. (1980), but all contain insufficient data on the duration or concentration of n-hexane exposure. In addition, all available human inhalation exposure studies indicate the potential for coexposure to other solvents, most of which have been shown to potentiate n-hexane-induced toxicity (Section 4.4.3). Therefore, reliable effect levels cannot be identified from the available reports of human experimental and occupational exposure. For example, Mutti et al. (1982a) showed decreased MCV in shoe factory workers exposed to mixtures containing n-hexane, cyclohexane, methyl ethyl ketone, and ethyl acetate. The authors reported that these effects occurred at TWA n-hexane concentrations in breathing zone air of 69 ppm (mildly exposed) and 134 ppm (highly exposed). Chang et al. (1992) evaluated the neurological effects of exposure to offset machine workers in a printing factory. These workers were exposed to lead and mercury in the printing inks and cleaning solutions containing mainly (14–20%) n-hexane along with a variety of other solvents (including toluene). TWA air concentrations of n-hexane were 63 ppm for the general air concentration and 134 ppm for breathing zone air for offset printing areas. The authors observed clinical symptoms of paresthesia, weakness, and numbness in the extremities and electrophysical deficits in MCV, SNAP, and MAP in exposed workers. Due to the known coexposures to other chemicals and the lack of data on duration of exposure, the available human studies were not considered further for the choice of the principal study.

As described in Section 4.2.2, the toxic effects in laboratory animals following inhalation

exposure to n-hexane support the nervous system as the primary target of toxicity. A number of studies identified a variety of effects on the nervous system, kidney, liver, and developing fetus at doses between 125 and 500 ppm (IRDC, 1992a, b; NTP, 1991; Dunnick et al., 1989; Huang et al., 1989; Mast et al., 1988a, b; Mast, 1987; Ono et al., 1982). These studies were considered for the selection of the principal study and are described below. Benchmark dose (BMD) modeling, where the data were amenable, was performed and is discussed in detail in Section 5.2.2 and Appendix B.

Neurological deficits and respiratory lesions (mild epithelial lesions) were observed when B6C3F1 mice were exposed subchronically to 0, 500, 1000, 4000, and 10,000 ppm n-hexane for 6 hours/day, 5 days/week for 90 days or to 1000 ppm n-hexane for 22 hours/day or 6 hours/day, 5 days/week for 90 days (NTP, 1991; Dunnick et al., 1989). Dunnick et al. (1989) reported decreased locomotor activity and increased axonal swellings in the paranodal nerve in the 1000 ppm continuous exposure group (22 hours/day) and the 10,000 ppm exposure group (6 hours/day). Histopathology of the spinal cord and tibial nerve was evaluated in four animals/sex from the control, 1000 ppm continuous exposure, and 10,000 ppm exposure groups only. The NOAEL (500 ppm) was based on the appearance of mild epithelial lesions in the nasal cavity. The authors suggested that this effect was more severe in the 1000 ppm continuous exposure group (22 hours/day) than the 4000 ppm exposure group (6 hours/day). They also considered these effects to be nonspecific and indicative of inflammatory and regenerative changes secondary to the effects of the inhaled irritant. The authors were unclear as to whether the altered morphology was due to inflammation or direct action of n-hexane. Thus, the study authors stated that the nasal irritation was most likely secondary to the inhaled irritant. In addition, the absence of sufficient neuropathological information from the mid-concentration groups (i.e., 500, 1000, 4000 ppm for 6 hours/day) is considered to represent a significant deficiency in the interpretation of the Dunnick et al. (1989) study. Therefore, the NTP (1991)/Dunnick et al. (1989) study was not selected as the principal study for the derivation of the RfC.

IRDC (1992a) exposed male Sprague-Dawley rats to 0, 125, and 500 ppm n-hexane subchronically for 6 months (22 hours/day, 7 days/week). n-Hexane exposure resulted in a significant decrease in mean absolute and relative liver and kidney weights at both doses. These changes in organ weights were not accompanied by any histopathological evidence of liver or kidney toxicity. In the second phase of this study, IRDC (1992b) demonstrated an increased incidence of chronic nephritis in 6/11 controls and 10/10 rats exposed to 500 ppm n-hexane. This response is considered equivocal due to the high incidence of kidney nephropathy in the control animals. Axonal degeneration and muscle atrophy were also observed but only at the

high dose. The data on axonal degeneration and muscle atrophy are not suitable for BMD modeling since each effect lacks an adequate dose-response for modeling (i.e., effects were seen at only the high dose). For example, 0/10, 0/10, and 7/10 animals showed tibial/sciatic nerve axonal degeneration and 0/10, 0/10, and 9/10 animals showed skeletal muscle atrophy at 0, 125, and 500 ppm, respectively. Finally, the results of this study are potentially compromised by possible coexposure to a phthalate ester-type compound. The authors indicated that during exposure a brown oily material collected on the glass beads of the inhalation system for each exposure group. Samples of this brown material were subjected to infrared spectroscopy, which confirmed the presence of a phthalate ester-type compound. While the observed axonal degeneration at the high dose could constitute a LOAEL, the noted contamination compromises the results. Therefore, IRDC (1992b) was not selected as the principal study for the derivation of the RfC.

Ono et al. (1982) observed subchronic effects of n-hexane on the nervous system in male Wistar rats (eight/group) exposed to 0, 200, and 500 ppm n-hexane for 12 hours/day for 24 weeks. Only one animal from each group was examined histopathologically in an attempt to link any functional deficits to morphological changes that may have taken place over the duration of the experiment. The authors stated that they did not observe any definite clinical signs of neuropathy in any of the exposed groups. MCV and mixed MCVs (distal and both proximal and distal combined) were statistically significantly decreased in rats exposed to n-hexane at both 200 and 500 ppm. Distal latency and proximal mixed MCV were statistically significantly decreased at the low dose but not the high dose. Degeneration of the myelinated axons was evident in the peripheral nerves at both exposures (histopathology in one animal). While the observed decreases in MCV could constitute a LOAEL, the lack of observed clinical neuropathy and failure to evaluate nerve histopathology on a larger number of animals are limitations of this study. In addition, BMD modeling of the data produced poor goodness of fit values estimated from the data (Section 5.2.2). Therefore, the Ono et al. (1982) study was not selected as the principal study for the derivation of the RfC.

Mast et al. (1988a) exposed pregnant CD-1 mice (30/group) to 0, 200, 1000, and 5000 ppm n-hexane for 20 hours/day on GDs 6–17. The authors reported a significant increase in the number of late resorptions in mice exposed to 5000 ppm n-hexane. The effects noted are at only the high dose. The Mast et al. (1988a) study was not selected as the principal study for the derivation of the RfC since effects were noted only at a dose higher than doses where effects were observed in other studies.

Mast (1987) exposed pregnant Sprague-Dawley rats (30/group) to 0, 200, 1000, or 5000 ppm n-hexane for 20 hours/day on GDs 6–19. The authors observed a statistically significant

reduction in fetal body weight gain in males at 1000 and 5000 ppm n-hexane exposure. A statistically significant increase in the incidence of reduced ossification of sternebrae 1–4 was also observed at 5000 ppm. This study identified a developmental NOAEL of 200 ppm for these effects, but the range between the NOAEL and the next higher dose (1000 ppm) was considerable. This uncertainty in the dose-response makes the selection of this study as the principal study questionable. Several additional studies have evaluated the effect of n-hexane exposure on the reproductive system and the developing fetus (Linder et al., 1992; Mast et al., 1988b, c; De Martino et al., 1987; Marks et al., 1980; Bus et al., 1979; Litton Bionetics, 1979). In contrast to the studies by Mast (1987) and Mast et al. (1988a), these studies did not indicate that n-hexane exposure produced adverse reproductive or developmental effects. Nevertheless, BMD modeling was performed on the Mast (1987) data set. The results of the BMD modeling can be found in Section 5.2.2 and Appendix B.

Huang et al. (1989) exposed Wistar rats (eight/group) via inhalation to 0, 500, 1200, or 3000 ppm (0, 1762, 4230, or 10,574 mg/m^3) n-hexane for 12 hours/day, 7 days/week for 16 weeks. Statistically significant, group-specific, dose-dependent changes in MCV were obtained in the mid- and high-concentration groups but not in the low-concentration group. Histopathological changes to the peripheral nerves were marked by paranodal swelling and demyelination. These changes were most apparent in high-dose rats but occurred in mid-dose animals as well. Rats exposed to mid- and high-concentrations of n-hexane in the Huang et al. (1989) study also showed some signs of behavioral deficits, including a reduction in grip strength and slowness of motion. This study was considered further for selection as the principal study for the derivation of the RfC. The data for changes in MCV were subjected to BMD modeling (Section 5.2.2 and Appendix B).

The Huang et al. (1989) study was selected as the principal study with peripheral neuropathy (decreased MCV) as the critical effect. The available human and animal n-hexane inhalation exposure data suggest that the nervous system is the primary target of n-hexane toxicity (Sections 4.1.2 and 4.2.1). Most of the reproductive and developmental studies suggest that n-hexane does not adversely affect these endpoints. For this reason and due to the uncertainty in the dose-response, the Mast (1987) study that evaluated developmental effects was considered but not selected as the principal study for the derivation of the RfC. In addition, Huang et al. (1989) evaluated a comprehensive array of neurological endpoints, an adequate number of animals and exposure groups, and the study was of the appropriate quality for the derivation of the RfC.

Several studies provide support for the selection of Huang et al. (1989) as the principal

study and peripheral neuropathy as the critical effect. Specifically, studies in humans exposed to n-hexane levels in the workplace to a range of approximately 30–200 ppm (130–690 mg/m^3) n-hexane, showed effects associated with peripheral neuropathy, such as decreased MCV (Yucesoy et al., 1999; Karakaya et al., 1996; Chang et al., 1992; Huang et al., 1991; Yokoyama et al., 1990; Huang and Chu, 1989; Mutti et al., 1982a, b; Sanagi et al., 1980). Studies in animals also provide support for the selection of Huang et al. (1989) as the principal study. In a follow-up study, Huang et al. (1992) observed an overall reduction in MCV in rats exposed to 2000 ppm n-hexane for 12 hours/day, 6 days/week for a total of 24 weeks, with the onset of neurophysiological deficits most evident in the distal segment of the sciatic nerve. Other sections of the central and peripheral nervous systems were comparatively unaffected. Howd et al. (1983), Pryor et al. (1983), and Ichihara et al. (1998) all used single concentrations of n-hexane in the 1000–2000 ppm range to induce neurophysiological deficits and/or behavioral changes in F344 or Wistar rats exposed to n-hexane. Data from CIIT's 13-week toxicological study in F344 rats exposed to n-hexane (0, 3000, 6500, or 10,000 ppm) confirmed the neuropathological responses to n-hexane based on the appearance of paranodal swelling of the tibial nerves in mid- and high-dose males (Cavender et al., 1984a, b).

5.2.2. Methods of Analysis

As described in Section 4.2.2, the toxic effects in laboratory animals following inhalation exposure to n-hexane support the nervous system as the primary target of toxicity. A number of studies identified a variety of effects on the nervous system, kidney, liver, and developing fetus at relatively low doses (Huang et al., 1989; Mast, 1987; Ono et al., 1982). These studies were considered for the selection of the principal study and are described below.

Endpoints selected that are continuous variables (fetal body weight and MCV) were modeled with available continuous models (linear, polynomial, power, and Hill). The hybrid model software in the Benchmark dose software (BMDS) is still undergoing beta-testing and was not used because it was not considered to be sufficiently validated for use in quantitative dose-response assessment. The hybrid modeling approach defines the benchmark response (BMR) in terms of change in the mean. The BMR was defined as a change of 10% for quantal endpoints such as developmental abnormalities in the fetus and a change of one standard deviation (1SD) from the control mean for continuous endpoints such as fetal body weight and MCV (U.S. EPA, 2000c). This BMR was selected because there was no clear biological rationale for selecting an alternative BMR level (U.S. EPA, 2000c).

The 95% lower confidence limit on the benchmark concentration (BMCL) estimates for

the various studies are presented for the best fitting models in Table 5-1, and detailed discussion of the modeling for each study is presented in Appendix B. For each model, the software performed residual and overall chi-squared goodness-of-fit tests and determined the Akaike's Information Criterion (AIC). The chi-squared *p*-value is a measure of the closeness between the observed data and the data predicted by the model fit. Models with chi-squared *p*-values ≥ 0.1 were considered adequate fits. The AIC is a measure of the model fit based on the log-likelihood at the maximum likelihood estimates for the parameters. Models with lower AIC values among those with adequate chi-squared *p*-values were identified. The BMCL estimates varied quite a bit, depending on the endpoint, model selected, and parameter hypotheses and constraints. The "best model" selection criteria are presented in detail in Appendix B. Huang et al. (1989) was selected as the principal study (Section 5.2.1). Based on the criteria described in *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000c), decreased MCV data (12 weeks) in male rats provided the best fit using the Hill model. Output from the software for the Hill model run (of the male rat MCV data) follows in Appendix B.

Table 5-1. BMD results of n-hexane inhalation toxicity studies for selection of the principal study

Reference	Endpoint	Dose groups	Model	Fixed parameters	Goodness of fit *p*-value [c]	AIC	BMC[a,b] (ppm)	BMCL[b] (ppm)	$BMCL_{HEC}$ (mg/m^3)
Mast, 1987	Reduced ossification of sternebrae 1–4	4	Nested logistic	n $\geq$ 1	<0.0001 (group: 0.1834)[d]	1433	1571	943	2770
Mast, 1987	Decreased fetal body weight gain	4	Quadratic	n $\geq$ 1	0.2517	–104.4	1540	848	1494
Huang et al., 1989	MCV 8 weeks	4	Hill	rho = 0 n = 2	0.789	27.22	198	143	252
Huang et al., 1989	MCV 12 weeks	4	Hill	rho = 0 n = 1	0.313	27.35	156	122	215
Huang et al., 1989	MCV 16 weeks	4	Hill	rho = 0 n = 2	0.779	34.88	367	321	566
Ono et al., 1982	MCV	3	Power	rho = 3.5 n = 0.75	0.3745	92.84	120	85.4	150.5
Ono et al., 1982	Mixed MCV (total)	3	Power	rho = 2 n = 0.5	< 0.0001	78.39	60	33	58.1
Ono et al., 1982	Mixed MCV (distal)	3	Power	rho = 2 n = 0.5	< 0.0001	65.82	51	28	49.3

[a] BMC = Benchmark concentration.
[b] Calculated using a BMR of 10% for quantal endpoints, and a BMR of 1 standard deviation for continuous endpoints.
[c] The nested module also estimates *p*-value for goodness-of-fit by applying a chi-squared test to data grouped by the strata or levels of these covariates as well as by dose. As shown here, the chi-squared goodness-of-fit test may give different results than the usual methods used elsewhere in this table.
[d] All models were adjusted for total litter size.

5.2.2.1. ***Adjustment to a Human Equivalent Exposure Concentration***

Because the RfC is a standard applicable to continuous lifetime human exposure but derived from animal studies featuring intermittent, less-than-lifetime exposures, EPA guidance (U.S. EPA, 1994b) provides mechanisms for (1) adjusting experimental exposure concentrations to a value reflecting continuous exposure duration (ADJ) and (2) determining a human equivalent concentration (HEC) from the animal exposure data. The former employs an inverse concentration-time relationship to derive a health-protective duration adjustment to time-weight the intermittent exposures used in the principal study. The BMCL (1SD) of 122 ppm (430 mg/m^3) for decreased MCV in rats exposed to n-hexane for 12 weeks exposure (12 hours/day, 7 days/week) as reported by Huang et al. (1989) is adjusted to continuous exposure (12 hours/day to 24 hours/day) as follows:

$$\begin{aligned} BMCL_{ADJ} &= BMCL\ (mg/m^3) \times 12\ hours/day \div 24\ hours/day \\ &= 430\ mg/m^3 \times 12\ hours/day \div 24\ hours/day \\ &= 215\ mg/m^3 \end{aligned}$$

The RfC methodology provides a mechanism for deriving a human equivalent concentration from the duration-adjusted point of departure ($BMCL_{ADJ}$) determined from the animal data. The approach takes into account the extrarespiratory nature of the toxicological responses and accommodates species differences by considering blood:air partition coefficients for n-hexane in the laboratory animal (rat) and humans. According to the RfC guidelines (U.S. EPA, 1994b), n-hexane is a category 3 vapor because it is largely inactive in the respiratory tract, is rapidly transferred between the lungs and blood, and has toxicological effects that are extrarespiratory. Therefore, the duration adjusted $BMCL_{ADJ}$ is multiplied with the ratio of the animal/human blood:air partition coefficients (L_A/L_H). As set forth in Section 3.1, values reported in the literature for these parameters include an L_A of 2.29 in F344 rats (Gargas et al., 1989) and an L_H in humans of 0.8 (Perbellini et al., 1985). By default, because an L_A/L_H greater than unity would be derived from these values, a value of unity is adopted for this ratio. This allows a $BMCL_{[HEC]}$ to be derived as follows:

$$\begin{aligned} BMCL_{HEC} &= BMCL_{ADJ}\ (mg/m^3) \times 1\ (interspecies\ conversion) \\ &= 215\ mg/m^3 \times 1 \\ &= 215\ mg/m^3 \end{aligned}$$

The $BMCL_{HEC}$ value of 215 mg/m^3 for reduced MCV in rats was used to derive the RfC

for n-hexane.

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

The $BMCL_{HEC}$ of 215 mg/m^3 for reduced MCV in Wistar rats exposed to n-hexane for 12 weeks duration (12 hours/day, 7 days/week, 16 weeks total duration) was used as a point of departure for calculating the RfC for n-hexane (Huang et al., 1989).

A total UF of 300 was applied to the point of departure of 215 mg/m^3: 10 for intraspecies variation (UF_H: human variability); 3 for interspecies differences (UF_A); 3 to extrapolate from less than lifetime to chronic exposure (UF_S); and 3 to account for database deficiencies (UF_D).

A UF_H of 10 was applied to account for variations in susceptible subpopulations. One animal study suggests that weanling rats may be less susceptible to n-hexane-induced neurotoxicity than adult rats (Howd et al., 1983). Howd et al. (1983) compared the neurotoxicity of n-hexane in weanling versus young adult F344 rats, which were exposed to 0 or 1000 ppm n-hexane (95% pure) 24 hours/day, 6 days/week for 11 weeks. The authors observed significantly decreased grip strength and increased incidence of hind-limb paralysis in both weanling and adult rats. However, both endpoints appeared earlier and were of greater severity in adults compared with weanlings. The authors suggested that these differences in n-hexane-induced neurotoxicity may be due to smaller diameter and shorter axons in weanling compared with adult rats.

The CYP2E1 enzyme is responsible for metabolism of various aliphatic and aromatic hydrocarbons, solvents, and industrial monomers, including n-hexane and acetone. Polymorphism of CYP2E1 could possibly lead to interindividual differences in the toxicity of chemicals metabolized by this enzyme. n-Hexane-induced neurotoxic effects are believed to be the result of its metabolism to the toxic metabolite, 2,5-hexanedione, by CYP2E1. In addition, differences in the development and maturity of phase I and phase II metabolic enzymes (specifically CYP2E1) between adults and children have been shown in several studies (Johnsrud et al., 2003; Ginsberg et al., 2002). Taken together, these data suggest that differences in metabolism of n-hexane may exist within the human population and between adults and children.

Only one study with one dose group is available that directly observed susceptibility differences between adult and weanling animals (Howd et al., 1983). Several mode of action studies provide some evidence supporting the hypothesis that this increased susceptibility is due to differences in axonal length between adults and weanling rats. These studies did not directly observe effects of n-hexane on NFs in weanling or young animals. Given the paucity of studies

directly observing susceptibility differences between weanling and adult animals and the possibility of altered metabolic enzyme activity among individual humans and between adults and children, a UF_H of 10 was applied to account for variations in susceptible subpopulations.

A UF_A of 3 was applied to account for uncertainty in extrapolating from laboratory animals to humans. This value is adopted by convention where an adjustment from an animal-specific $BMCL_{ADJ}$ to a $BMCL_{HEC}$ has been incorporated. Application of a full uncertainty factor of 10 would depend on two areas of uncertainty (i.e., toxicokinetic and toxicodynamic uncertainties). In this assessment, the toxicokinetic component is mostly addressed by the determination of a human equivalent concentration as described in the RfC methodology (U.S. EPA, 1994b). The toxicodynamic uncertainty is also accounted for to a certain degree by the use of the applied dosimetry method.

A UF_S of 3 was applied to extrapolate from subchronic to chronic exposure. A subchronic (16 weeks) study was used for the derivation of the RfC. However, 16 weeks is half of the time required for a newly synthesized NF protein to be transported from the neuronal cell body to the axon terminal in the longest axons of the CNS and PNS of an adult rat (Griffin et al., 1984). The rate of NF transport down an adult rat axon is 1 mm/day. The longest axons extend from the lumbar spinal cord to the hind foot and measure no more than 22 cm in the adult rat. Thus, transport for the full length of the axon would take approximately 32 weeks in an adult rat. Since the lifetime of NFs (target of toxicity of n-hexane) is shorter than the lifetime of an adult rat, extrapolation from subchronic to chronic exposure is not necessary and a UF_S of 3 was applied.

A UF_D of 3 was applied to account for database deficiencies. The database includes many human occupational exposure studies (all with coexposure to other potentially neurotoxic chemicals), subchronic animal studies in rats and mice, neurotoxicity studies in both humans and laboratory animals, and developmental studies in rats and mice following inhalation exposure to pure n-hexane. The database lacks a developmental neurotoxicity study and a multigeneration reproductive and developmental toxicity study following inhalation exposure to pure n-hexane alone. Prenatal exposure to pure n-hexane-induced skeletal anomalies, decreased fetal body weight, and increased resorptions, suggesting that the fetus may be affected by n-hexane inhalation exposure (Mast et al., 1988a; Mast, 1987; Bus et al., 1979). One of these studies indicated a developmental NOAEL of 200 ppm for reduced fetal body weight gain (Mast, 1987). However, it remains unclear whether these developmental effects occur at doses lower than those that cause neurotoxicity. Studies investigating the reproductive and developmental effects of commercial hexane, a mixture containing approximately 50% n-hexane, are also available (see Section 4.4.2.2.3). These studies evaluated reproductive and developmental effects following

exposure to doses of $\geq$ 500 ppm commercial hexane and resulted in marginal decreases in pup body weights and increased skeletal variations (BRRC, 1989a, b). Given the lack of multigeneration reproductive and developmental studies following exposure to pure n-hexane and the uncertainty associated with low-dose developmental effects of exposure to n-hexane, a UF_D of 3 was applied.

A UF to account for the extrapolation from a LOAEL to a NOAEL was not applied because BMD modeling was used to determine the point of departure for derivation of the RfC. Therefore, the RfC from the Huang et al. (1989) data is calculated as follows:

$$\begin{aligned} \text{RfC} &= \text{BMCL}_{\text{HEC}} \div \text{UF} \\ &= 215 \text{ mg/m}^3 \div 300 \\ &= 0.72 \text{ mg/m}^3 \\ &= 7 \times 10^{-1} \text{ mg/m}^3 \end{aligned}$$

5.2.4. Previous RfC

The previous IRIS assessment for n-hexane contained an RfC of 2×10^{-1} mg/m^3 that was derived from the Sanagi et al. (1980) occupational exposure study in which group-specific behavioral deficits and neurophysiological changes were considered to be associated with a LOAEL of 58 ppm. However, the subjects of the study were also exposed to acetone at a mean concentration of 39 ppm. More recent data suggest that coexposure to acetone potentiates n-hexane metabolism and n-hexane-induced neurotoxicity (Cardona et al., 1996; Ladefoged et al., 1994, 1989; Larsen et al., 1991). Therefore, it is possible that the incidence or severity of the neurological changes observed by Sanagi et al. (1980) may have been a result of coexposure to both solvents. Thus, Sanagi et al. (1980) was not retained as the principal study for the derivation of the RfC, based on coexposure of study subjects to n-hexane and acetone in the current assessment.

The subchronic NTP (1991) study (published in the literature as Dunnick et al., 1989) in which B6C3F1 mice were exposed to 0, 500, 1000, 4000, and 10,000 ppm 6 hours/day, 5 days/week or 1000 ppm 22 hours/day, 5 days/ week n-hexane via inhalation for 13 weeks was used as a co-principal study. The critical effect in the subchronic study was epithelial lesions in the nasal cavity.

The chosen NOAEL (500 ppm) in the coprincipal study was based on the appearance of mild epithelial lesions in the nasal cavity. The authors suggested that this effect was more severe in the 1000 ppm continuous exposure (22 hours/day) than in the 4000 ppm exposure group (6 hours/day). They also considered these effects to be nonspecific and indicative of inflammatory and regenerative changes secondary to the effects of the inhaled irritant. The authors were unclear as to whether the altered morphology was due to inflammation or direct action of n-hexane.

Histopathology of the spinal cord and tibial nerve was reported for four males and four females from the 0, 1000 ppm continuous exposure, and the 10,000 ppm exposure groups only. Dunnick et al. (1989) reported decreased locomotor activity and increased axonal swelling in the paranodal nerve in the 1000 ppm continuous exposure (22 hours/day) and the 10,000 ppm exposure (6 hours/day). The study authors did not perform neurological histopathology at the mid concentrations (500, 1000, or 4000 ppm for 6 hours/day). The lack of histopathology is considered to be a significant deficiency in the Dunnick et al. (1989) study, since the nervous system appears to be the primary target of n-hexane-induced neurotoxicity (Section 4.5.2). Thus, Dunnick et al. (1989) was not retained as the principal study for the derivation of the RfC in the current assessment.

The available human and laboratory animal inhalation studies for n-hexane indicate that the nervous system is a target of toxicity (Sections 4.1.2.2 and 4.2.2.1). Thus, the absence of sufficient neuropathological information from among the mid-concentration groups (i.e., 500, 1000, 4000 ppm for 6 hours/day) is considered to represent a significant data gap in the Dunnick et al. (1989) study, bounding the selected NOAEL with greater than acceptable uncertainty. Therefore, Dunnick et al. (1989) and Sanagi et al. (1980) were replaced as the principal studies by that of Huang et al. (1989).

5.3. CANCER ASSESSMENT

As discussed in Section 4.6.1., the available database for n-hexane contains inadequate information to assess carcinogenic potential according to the U.S. EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). Genotoxicity evidence has been largely negative for n-hexane.

A 2-year inhalation study of commercial hexane, a mixture containing n-hexane and other structurally related hydrocarbons, showed a statistically significant increase in combined hepatocellular adenomas and carcinomas in female B6C3F1 mice (Daughtrey et al., 1999; Biodynamics, 1993a, b). However, the increased tumor incidence was of borderline statistical

significance and was not present in treated male mice nor in either sex of F344 rats exposed to commercial hexane under the same conditions. Additionally, because commercial hexane is a variable mixture of hydrocarbons of which only about 52% is n-hexane, its use as a toxicological surrogate for the qualitative and quantitative effects of pure n-hexane would be unjustified.

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

6.1. HUMAN HAZARD POTENTIAL

n-Hexane (CASRN 110-54-3) is a solvent that has many uses in the chemical and food industries, either in pure form or as a component of commercial hexane. The latter is a mixture that contains approximately 52% n-hexane; the balance is made up of structural analogs and related chemicals such as methylpentane and methylcyclopentane.

Highly purified n-hexane is used as a reagent for chemical or chromatographic separations. Other grades of n-hexane are used as solvents for extracting edible fats and oils in the food industry and as a cleaning agent in the textile, furniture, and printing manufacturing industries. Hexane is the solvent base for many commercial products, such as glues, cements, paint thinners, and degreasers. n-Hexane is a minor constituent of crude oil and natural gas and occurs in different petroleum distillates.

No data are available regarding the potential toxicity of n-hexane in humans orally exposed to n-hexane. However, as might be expected for a chemical with such wide application, the potential exists for persons to be environmentally and/or occupationally exposed to n-hexane via other routes of exposure. In fact, a considerable number of epidemiological studies (summarized in Section 4.1) have been reported on n-hexane, the majority of which have shown an association between inhalation exposure to n-hexane and neurological symptoms in occupationally exposed individuals. However, the extent of exposure to n-hexane in many, if not all, of the occupational studies is imprecise, and subjects were likely exposed concurrently to other solvents. This means that the data in these studies are inappropriate for dose-response modeling.

Animal data also indicate that the nervous system may be the primary target of toxicity following inhalation exposure. The principal study of Huang et al. (1989) identified behavioral, neurophysiological, and neuropathological effects in Wistar rats. This study has been used to derive an RfC of 7×10^{-1} mg/m^3.

Compelling mode of action evidence has focused on the capacity of n-hexane to undergo metabolism to 2,5-hexanedione. This γ-diketone appears to have the ability to interact with specific proteins on the NFs. While some of the details remain to be worked out, a preponderance of evidence suggests that pyrrole formation is critical for the induction of neurotoxicity by γ-diketones, with pyrrole oxidation a necessary further step to initiate NF cross-linking. The importance of γ-diketone-generated pyrrole formation also indicates the unique

nature of n-hexane-generated neuropathy compared with the relative benign effects of such structural analogues as n-heptane and pentane. The inability of the latter chemicals to form γ-diketones does not permit their generation of the neurotoxicological impacts manifest by n-hexane.

Data on the toxicity of n-hexane via the oral route are poor as only one study was identified in which experimental animals were exposed to n-hexane for an adequate duration (Krasavage et al., 1980). However, as discussed in Section 5.1, this study is inadequate for the development of an oral toxicity value for n-hexane.

Under the *Guidelines for Carcinogenic Risk Assessment* (U.S. EPA, 2005a), data are *inadequate for an assessment of the human carcinogenic potential* of n-hexane. This descriptor is chosen because no human or animal studies are available. Carcinogenicity data are available for the tumor-inducing capacity of commercial hexane (of which about 52% is n-hexane). However, the relevance of this study to the identification of the carcinogenic potential of n-hexane is unclear due to the unknown toxicity contribution of the other components of the mixture and uncertainty as to whether the apparent carcinogenic response in female mice was truly treatment related.

6.2. DOSE RESPONSE

6.2.1. Noncancer

The database for oral exposure to n-hexane is limited to two subchronic gavage studies, both of which were unsuitable for the calculation of an RfD.

The RfC of 7×10^{-1} mg/m^3 was derived from reduced MCV in male Wistar rats following inhalation exposure to n-hexane (Huang et al., 1989). Treatment-related changes included reduced peripheral nerve conduction, some behavioral perturbation, and histological changes indicative of peripheral neuropathy. There is sufficient evidence from other studies in experimental animals to confirm that the nervous system is the primary target for the toxicological effects of n-hexane (Huang et al., 1992; NTP, 1991; Dunnick et al., 1989; Cavender et al., 1984a, b; Howd et al., 1983; Pryor et al., 1983). Subclinical and overt symptoms of peripheral neuropathy have been described in persons exposed to n-hexane in the workplace.

The overall confidence in this RfC assessment is medium. Confidence in the principal study (Huang et al., 1989) is medium; it involves a comparatively low but acceptable number of animals per group (eight/sex) and reports behavioral deficits, neurophysiological changes, and

neuropathological effects within a dose-range in which both a NOAEL and LOAEL could be identified. Animal studies in a second species (mice) corroborate the primacy of the neurological endpoint and confirm the validity of the critical effect for peripheral neuropathy. Confidence in the database is medium. The database lacks chronic exposure information on pure n-hexane via any route of exposure and a multigenerational developmental and reproductive toxicity study and a developmental neurotoxicity study. The subchronic inhalation study of Huang et al. (1989) satisfies the minimum inhalation database requirements for deriving an RfC for n-hexane. Reflecting medium confidence in the principal study and medium confidence in the database, confidence in the RfC is medium.

6.2.2. Cancer

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), the database for n-hexane is inadequate to assess human carcinogenic potential. As such, data are unavailable to calculate quantitative cancer risk estimates.

7. REFERENCES

Abbritti, G; Siracusa, A; Cianchetti, C; et al. (1976) Shoe-makers' polyneuropathy in Italy: the aetiological problem. Br J Ind Med 33:92-99.

Abou-Donia, MB; Makkawy, HA; Graham, D. (1982) The relative neurotoxicities of n-hexane, methyl n-butyl ketone, 2,5-hexanediol, and 2,5-hexanedione following oral or intraperitoneal administration in hens. Toxicol Appl Pharmacol 62:369-389.

ACGIH (American Conference of Governmental Industrial Hygienists). (2001) Documentation of the Threshold Limit Values and Biological Exposure Indices. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

ACGIH. (2003) TLVs and BEIs based on the documentation of the Threshold Limit Values for chemical substances and physical agents and biological exposure indices. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

Ahonen, I; Schimberg, RW. (1988) 2,5-hexanedione excretion after occupational exposure to n-hexane. Br J Ind Med 45:133-136.

Allen, N; Mendell, J; Billmaier, D; et al. (1975) Toxic polyneuropathy due to methyl n-butyl ketone. An industrial outbreak. Arch Neurol 32:209-218.

Altenkirch, H; Stoltenburg, G; Wagner, H. (1978) Experimental studies on hydrocarbon neuropathies induced by methyl-ethyl-ketone (MEK). J Neurol 219:159-170.

Altenkirch, H; Wagner, H; Stoltenburg, G; et al. (1982) Nervous system responses of rats to subchronic inhalation of N-hexane and N-hexane + methyl-ethyl-ketone mixtures. J Neurol Sci 57:209-219.

Anderson, RJ; Dunham, CB. (1984) Electrophysiologic deficits in peripheral nerve as a discriminator of early hexacarbon neurotoxicity. J Toxicol Environ Health 13:835-843.

Anthony, DC; Boekelheide, K; Graham, D. (1983a) The effect of 3,4-dimethyl substitution on the neurotoxicity of 2,5-hexanedione. I. Accelerated clinical neuropathy is accompanied by more proximal axonal swellings. Toxicol Appl Pharmacol 71:362-371.

Anthony, DC; Boekelheide, K; Anderson, CW; et al. (1983b) The effect of 3,4-dimethyl substitution on the neurotoxicity of 2,5-hexanedione. II. Dimethyl substitution accelerates pyrrole formation and protein cross-linking. Toxicol Appl Pharmacol 71:372-382.

ATSDR (Agency for Toxic Substances and Disease Registry). (1993a) Toxicological profile for automotive gasoline. Available from ATSDR, Public Health Service, U.S. Department of Health and Human Services, Atlanta, GA. Available from: <http://www.atsdr.cdc.gov/toxprofiles>.

ATSDR. (1993b) Toxicological profile for jet fuels JP-4 and JP-7. Available from ATSDR, Public Health Service, U.S. Department of Health and Human Services, Atlanta, GA. Available from: <http://www.atsdr.cdc.gov/toxprofiles>.

ATSDR. (1999) Toxicological profile for n-hexane. Available from ATSDR, Public Health Service, U.S. Department of Health and Human Services, Atlanta, GA. Available from: <http://www.atsdr.cdc.gov/toxprofiles>.

Bachman, MO; De Beer, Z; Myers, JE. (1993) n-hexane neuorotoxicity in metal can manufacturing workers. Occup Med 43:149-154.

Baelum, J; Molhave, L; Hansen, SH; et al. (1998) Metabolic interaction between toluene, trichloroethylene and n-hexane in humans. Scand J Work Environ Health 24:30-37.

Baker, T; Rickert, D. (1981) Dose-dependent uptake, distribution, and elimination of inhaled n-hexane in the Fischer-344 rat. Toxicol Appl Pharmacol 61:414-422.

Barni-Comparini, I; Lungarella, G; Fonzi, L. (1982) Response of lung enzyme activities in rabbits following short-term exposure to n-hexane: correlation between morphological and biochemical changes. Agents Actions 12:737-742.

Bastone, A; Frontali, N; Mallozzi, C; et al. (1987) Cholinesterases in blood plasma and tissues of rats treated with n-hexane or with its neurotoxic metabolite 2,5-hexanedione. Arch Toxicol 61:138-144.

Beall, C; Delzell, E; Rodu, B; et al. (2001) Case-control study of intracranial tumors among employees at a petrochemical research facility. Journal of Occupational and Environmental Medicine 43:1103-1113.

Biodynamics Inc. (1978) 26-Week inhalation toxicity study of n-hexane in the rat. EPA Document No. FYI-AX-1081-0137; NTIS No. OTS0000137-0.

Biodynamics Inc. (1989) A thirteen week inhalation toxicity study of commercial hexane in rat and mouse (final report) with cover letter dated 122789. EPA Document No. FYI-AX-1189-0714; NTIS No. OTS0000714-3.

Biodynamics Inc. (1993a) Letter from American Petroleum Institute to U.S. EPA regarding an inhalation oncogenicity study of commercial hexane in rats and mice: Part 1 (rats) with attachments, dated 04/16/93. Submitted under Section 4 of TSCA. EPA Document No. 42084 L5-2; NTIS No. OTS0572989.

Biodynamics Inc. (1993b) Letter from American Petroleum Institute to U.S. EPA regarding an inhalation oncogenicity study of commercial hexane in rats and mice: Part II (mice) with attachments, dated 06/03/93. Submitted under Section 4 of TSCA. EPA Document No. 42084 L6-2; NTIS No. OTS0572994.

Bio-Research Laboratories. (1989) An acute operant behavior study of inhaled commercial hexane in the albino rat (draft) with attachments and cover letter dated 122789. EPA Document No. FYI-AX-0190-0733; NTIS No. OTS0000733.

Bio-Research Laboratories. (1990) A thirteen week inhalation toxicity study of commercial hexane on behavior and neuromorphology in rats (final report) with attached reports, appendices & letter dated 021790. Submitted under Section 4 of TSCA. EPA Document No. 40-9089428; NTIS No. OTS0524324.

Boekelheide, K. (1987) 2,5-hexanedione alters microtubule assembly. II. Enhanced polymerization of cross-linked tubulin. Toxicol Appl Pharmacol 88:383-396.

BRRC (Bushy Run Research Center). (1989a) Developmental toxicity studies of commercial hexane vapor in CD (Sprague-Dawley) rats and commercial hexane vapor in CD-1 mice (final reports) with attachments & cover letter 111789. Submitted under Section 4 of TSCA. EPA Document No. 40-8989413; NTIS No. OTS0524323.

BRRC. (1989b) Draft results of the developmental toxicity exposure range-finding studies of commercial hexane vapor in mice and rats with cover letter dated 033089. EPA Document No. FYI-AX-0489-0459; NTIS No. OTS0000459-2.

BRRC. (1991) Two-generation reproduction study of inhaled commercial hexane in CD (Sprague-Dawley) rats (final report) with attachments and cover letter dated 041791. Submitted under Section 4 of TSCA. EPA Document No. 40-9189447; NTIS No. OTS0532897.

Bus, J; White, E; Tyler, R; et al. (1979) Perinatal toxicity and metabolism of n-hexane in Fischer-344 rats after inhalation exposure during gestation. Toxicol Appl Pharmacol 51:295-302.

Bus, J; White, E; Gillies, P; et al. (1981) Tissue distribution of n-hexane, methyl n-butyl ketone, and 2,5-hexanedione in rats after single or repeated inhalation exposure to n-hexane. Drug Metab Dispos 9:386-387.

Bus, J; Demo, D; Cox, M. (1982) Dose-dependent disposition of n-hexane in F344 rats after inhalation exposure. Fundam Appl Toxicol 2:226-229.

Canesi, M; Perbellini, L; Maestros, L; et al. (2003) Poor metabolization of n-hexane in Parkinson's disease. J Neurol 250:556-560.

Cardona, A; Marhuenda, D; Marti, J; et al. (1993) Biological monitoring of occupational exposure to n-hexane by measurement of urinary 2,5-hexanedione. Int Arch Occup Environ Health 65:71-74.

Cardona, A; Marhuenda, D; Prieto, MJ; et al. (1996) Behaviour of urinary 2,5-hexanedione in occupational coexposure to n-hexane and acetone. Int Arch Occup Environ Health 68:88-93.

Cavanaugh, JB, Bennetts, BJ. (1981) On the pattern of changes in the rat nervous system produced by 2,5-hexanediol. Brain 104: 297-318.

Cavender, FL; Casey, HW; Salem, H; et al. (1984a) A 13-week vapor inhalation study of n-hexane in rats with empasis on neurotoxic effects. Fundam Appl Toxicol 4(2 Pt 1):191-201.

Cavender, FL; Casey, HW; Gralla, EJ; et al. (1984b) The subchronic inhalation toxicity of n-hexane and methyl ethyl ketone. Advances in Modern Environmental Toxicology 6:215-231.

Chang, YC. (1990) Patients with n-hexane-induced polyneuropathy: a clinical follow up. Br J Ind Med 47:485-489.

Chang, YC. (1991) An electrophysiological follow up of patients with n-hexane polyneuropathy. Br J Ind Med 48:12-17.

Chang, YC; Yip, PK. (1987) n-hexane-induced electroneurographic changes and early detection of n-hexane intoxication. J Formoson Med Assoc 86:194-200.

Chang, CM; Yu, CW; Fong, KY; et al. (1992) N-hexane neuropathy in offset printers. Journal of Neurology, Neurosurgery, and Psychiatry 56:538-542.

Cianchetti, C; Abbritti, G; Perticoni, G; et al. (1976) Toxic polyneuropathy of shoe-industry workers: a study of 122 cases. Journal of Neurology, Neurosurgery, and Psychiatry 39:1151-1161.

Couri, D; Milks, M. (1982) Toxicity and metabolism of the neurotoxic hexacarbons n-hexane, 2- hexanone, and 2,5-hexanedione. Annu Rev Pharmacol Toxicol 22:145-166.

Crosbie, SJ; Williams, FM; Blain, PG. (1994) The metabolism of n-hexane by rat skeletal muscle, liver, lung and brain microsomes. Biochem Soc Trans 22:130S.

Crosbie, SJ; Blain, PG; Williams, FM. (1997) Metabolism of n-hexane by rat liver and extrahepatic tissues and the effect of cytochrome. Human & Experimental Toxicology 16:131-137.

Daughtrey, WC; Neeper-Bradley, T; Duffy, J; et al. (1994a) Two-generation reproduction study on commercial hexane solvent. J Appl Toxicol 14:387-393.

Daughtrey, WC; Putman, DL; Duffy, J; et al. (1994b) Cytogenetic studies on commercial hexane solvent. J Appl Toxicol 14:161-165.

Daughtrey, W; Newton, P; Rhoden, R; et al. (1999) Chronic inhalation carcinogenicity study of commercial hexane solvent in F344 rats and B6C3F1 mice. Toxicol Sci 48:21-29.

DeCaprio, AP; Fowke, JH. (1992) Limited and selective adduction of carboxyl-terminal lysines in the high molecular weight neurofilament proteins by 2,5-hexanedione in vitro. Brain Res 586:219-228.

DeCaprio, AP; Olajos, EJ; Weber, P. (1982) Covalent binding of a neurotoxic n-hexane metabolite: conversion of primary amines to substituted pyrrole adducts by 2,5-hexanedione. Toxicol Appl Pharmacol 65:440-450.

DeCaprio, AP; Briggs, RG; Jackowski, SJ; et al. (1988) Comparative neurotoxicity and pyrrole-forming potential of 2,5-hexanedione and perdeuterio-2,5-hexanedione in the rat. Toxicol Appl Pharmacol 92:75-85.

DeCaprio, AP; Kinney, EA; Fowke, JH. (1997) Regioselective binding of 2,5-hexanedione to high-molecular-weight rat neurofilament proteins in vitro. Toxicol Appl Pharmacol 145:211-217.

DeMartino, C; Malorni, W; Amantini, MC; et al. (1987) Effects of respiratory treatment with N-hexane on rat testis morphology. I. A light microscopic study. Exp Mol Pathol 46:199-216.

Donato, R. (1999) Functional roles of S100 proteins, calcium-binding proteins of the EF-hand type. Biochim Biophys Acta 1450(3): 191-231.

Donato, R. (2001) S100: a multigenic family of calcium-modulated proteins of the EF-hand type with intracellular and extracellular functional roles. Int J Biochem Cell Biol 33: 637-668.

dos Santos, CR; Meyer Passarelli, MM; de Souza Nascimento, E. (2002) Evaluation of 2,5-hexanedione in urine of workers exposed to n-hexane in Brazilian shoe factories. J Chromatogr B Analyt Technol Biomed Life Sci 778:237-244.

Duffy, J; Newton, P; Cockrell, B; et al. (1991) A thirteen week inhalation toxicity study of commercial hexane in the rat and mouse. Toxicologist 11:315.

Dunnick, JK; Graham, D; Yang, RS; et al. (1989) Thirteen-week toxicity study of n-hexane in B6C3F1 mice after inhalation exposure. Toxicology 57:163-172.

EPL (Experimental Pathology Laboratories Inc.). (1989) A thirteen week inhalation toxicity study of commercial hexane in the rat and mouse (pathology report) with attachment and cover letter dated 112289. EPA Document No. FYI-AX-1189-0714; NTIS No. OTS0000714-1.

EPL. (1992) Partial study: 6-month continuous inhalation exposure of rats to hexane mixtures (re-evaluation of renal pathology). EPA Document No. FYI-0892-0901; NTIS No. OTS0000901L6.

Fano, G; Biocca, S; Fulle, S; et al. (1995) The S-100: a protein family in search of a function. Prog Neurobiol 46:71-82.

Fedtke, N; Bolt, HM. (1986) Detection of 2,5-hexanedione in the urine of persons not exposed to n-hexane. Int Arch Occup Environ Health 57:143-148.

Fedtke, N; Bolt, HM. (1987) The relevance of 4,5-dihydroxy-2-hexanone in the excretion kinetics of n-hexane. Arch Toxicol 61:131-137.

ffrench-Constant, C; Colognato, H; Franklin RJM. (2004) Neuroscience: the mysteries of myelin unwrapped. Science 304:688-689.

Fisher, J; Mahle, D; Bankston, L; et al. (1997) Lactational transfer of volatile chemicals in breast milk. Am Ind Hyg Assoc J 58:425-431.

Frontali, N; Amantini, MC; Spagnolo, A; et al. (1981) Experimental neurotoxicity and urinary metabolites of the C5-C7 aliphatic hydrocarbons used as glue solvents in shoe manufacture. Clin Toxicol 18:1357-1367.

Gargas, M; Burgess, R; Voisard, D. (1989) Partition coefficients of low-molecular-weight volatile chemicals in various liquids and tissues. Toxicol Appl Pharmacol 98:87-89.

Gelmont, D; Stein, RA; Mead, JF. (1981) Isoprene-the main hydrocarbon in human breath. Biochem Biophys Res Commun 99: 1456-1460.

Genter, MB, Szakal-Quin, G, Anderson, CW; et al. (1987) Evidence that pyrrole formation is a pathogenetic step in γ-diketone neuropathy. Toxicol Appl Pharmacol 87: 351-362.

Genter St. Clair, MB; Amarnath, V; Moody, MA; et al. (1988) Pyrrole oxidation and protein cross-linking as necessary steps in the development of gamma-diketone neuropathy. Chem Res Toxicol 1:179-185.

Ginsberg, G; Hattis, D; Sonawane, B; et al. (2002) Evaluation of child/adult pharmacokinetic differences from a database derived from the therapeutic drug literature. Toxicol Sci 66:185-200.

Gobba, F; Cavalleri, A. (2003) Color vision impairment in workers exposed to neurotoxic chemicals. Neurotoxicology 24:693-702.

Goel, SK; Rao, GS; Pandya, KP. (1982) Toxicity of n-hexane and n-heptane: some biochemical changes in liver and serum. Toxicol Lett 14:169-174.

Goel, SK; Rao, GS; Krishnamurti, CR. (1987) Toxicity of n-hexane and 2,5-hexanediol to the hemopoietic system: a preliminary report. Xenobiotica 17:217-222.

Goel, SK; Rao, GS; Pandya, KP. (1988) Hepatotoxic effects elicited by n-hexane or n-heptane. J Appl Toxicol 8:81-84.

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

Graham, D; Anthony, DC; Boekelheide, K; et al. (1982a) Studies of the molecular pathogenesis of hexane neuropathy. II. Evidence that pyrrole derivatization of lysyl residues leads to protein cross-linking. Toxicol Appl Pharmacol 64:415-422.

Graham, D; Anthony, DC; Boekelheide, K. (1982b) In vitro and in vivo studies of the molecular pathogenesis of n-hexane neuropathy. Neurobehav Toxicol Teratol 4:629-634.

Graham, D; Amarnath, V; Valentine, WM; et al. (1995) Pathogenetic studies of hexane and carbon disulfide neurotoxicity. Crit Rev Toxicol 25:91-112.

Griffin, JW, Anthony, DC, Fahnestock, KE; et al. (1984) 3,4-Dimethyl-2,5-hexanedione impairs the axonal transport of neurofilament proteings. J Neurosci 4: 1516-1526.

Hageman, G; van der, HJ; van Hout, M; et al. (1999) Parkinsonism, pyramidal signs, polyneuropathy, and cognitive

decline after long-term occupational solvent exposure. J Neurol 246:198-206.

Harry, GJ, Graham, DG, Valentine WM, et al. (1998) Carbon disulfide neurotoxicity in rats: VIII. Summary. Neurotoxicology 19: 159-161.

Hazleton Laboratories. (1992) Initial submission: in vivo and in vitro mutagenicity studies n-hexane (hexane UV) (final report) with attachments and cover letter dated 020592. Submitted under Section 8ECP of TSCA. EPA Document No. 88-920000955; NTIS No. OTS0535721.

Houk, V; Schalkowsky, S; Claxton, L. (1989) Development and validation of the spiral Salmonella assay: an automated approach to bacterial mutagenicity testing. Mutat Res 223:49-64.

Howd, R; Rebert, C; Dickinson, J; et al. (1983) Comparison of the rates of development of functional hexane neuropathy in weanling and young adult rats. Neurobehav Toxicol Teratol 5:63-68.

HSDB (Hazardous Substances Data Bank). (2005) n-Hexane. National Library of Medicine, National Institutes of Health, U.S. Department of Health and Human Services, Bethesda, MD. Available from: <http://toxnet.nlm.nih.gov>.

Huang, CC; Chu, NS. (1989) Evoked potentials in chronic n-hexane intoxication. Clin Electroencephalogr 20:162-168.

Huang, J; Kato, K; Shibata, E; et al. (1989) Effects of chronic n-hexane exposure on nervous system-specific and muscle-specific proteins. Arch Toxicol 63:381-385.

Huang, CC; Shih, TS; Cheng, SY; et al. (1991) n-hexane polyneuropathy in a ball-manufacturing factory. J Occup Med 33:139-142.

Huang, J; Shibata, E; Kato, K; et al. (1992) Chronic exposure to n-hexane induces changes in nerve-specific marker proteins in the distal peripheral nerve of the rat. Human & Experimental Toxicology 11:323-327.

Iba, MM; Fung, J; Gonzalez, FJ. (2000) Functional Cyp2e1 is required for substantial in vivo formation of 2,5-hexanedione from n-hexane in the mouse. Arch Toxicol 74:582-586.

Ichihara, G; Saito, I; Kamijima, M; et al. (1998) Urinary 2,5-hexanedione increases with potentiation of neurotoxicity in chronic coexposure to n-hexane and methyl ethyl ketone. Int Arch Occup Environ Health 71:100-104.

Ikeda, M; Koizumi, A; Kasahara, M; et al. (1986) Combined effects of n-hexane and toluene on norepinephrine and dopamine levels in rat brain tissues after long-term exposures. Bull Environ Contam Toxicol 36:510-517.

Ikeda, T; Katakura, Y; Kishi, R; et al. (1993) Acute neurobehavioral effects of coinhalation of toluene and n-hexane on schedule-controlled behavior in rats. Environ Res 63:70-81.

Imbriani, M; Ghittori, S; Pezzagno, G; et al. (1984) n-hexane urine elimination and weighted exposure concentration. Int Arch Occup Environ Health 55:33-41.

IRDC (International Research and Development Corporation). (1986) Single-generation inhalation reproductive/fertility study on a commercial hexane (Volume I-II). Submitted under Section 4 of TSCA. EPA Document No. 40-8689172; NTIS No. OTS0524106.

IRDC. (1992a) 6-Month continuous inhalation exposures of rats to hexane mixtures - Phase I. EPA Document No. FYI-0892-0901; NTIS No. OTS0000901K6.

IRDC. (1992b) 6-Month continuous inhalation exposures of rats to hexane mixtures - Phase II. EPA Document No. FYI-0892-0901; NTIS No. OTS0000901K5.

Iregren, A; Andersson, M; Nylen, P. (2002) Color vision and occupational chemical exposures: I. An overview of tests and effects. Neurotoxicology 23:719-733.

Ishidate, M; Sofuni, T; Yoshikawa, K; et al. (1984) Primary mutagenicity screening of food additives currently used in Japan. Food Chem Toxicol 22:623-636.

Issever, H; Malat, G; Sabuncu, HH; et al. (2002) Impairment of colour vision in patients with n-hexane exposure-dependent toxic polyneuropathy. Occup Med 52:183-186.

Iwata, M; Takeuchi, Y; Hisanaga, N; et al. (1983) Changes of n-hexane metabolites in urine of rats exposed to various concentrations of n-hexane and to its mixture with toluene or MEK. Int Arch Occup Environ Health 53:1-8.

Johnsrud, EK; Koukouritaki, SB; Divakaran, K; et al. (2003) Human hepatic CYP2E1 expression during development. J Pharmacol Exp Ther 307:402-407.

Karakaya, A; Yucesoy, B; Burgaz, S; et al. (1996) Some immunological parameters in workers occupationally exposed to n-hexane. Hum Exp Toxicol 15:56-58.

Kawachi, T; Yahagi, T; Kada, T; et al. (1980) Cooperative program on short-term assays for carcinogenicity in Japan. IARC Sci Publ 217:323-330.

Kessler, W; Heilmaier, H; Kreuzer, P; et al. (1990) Spectrophotometric determination of pyrrole-like substances in urine of rat and man: an assay for the evaluation of 2,5-hexanedione formed from n-hexane. Arch Toxicol 64:242-246.

Khedun, SM; Maharaj, B; Naicker, T. (1996) Hexane cardiotoxicity–an experimental study. Israel Journal of Medical Sciences 32:123-128.

Kim, M-S; Sabri, MI; Miller, VH; et al. (2001) 1,2 -Diacetylbenzene, the neurotoxic metabolite of a chromogenic aromatic solvent, induces proximal axonopathy. Toxicol Appl Pharmacol 177: 121-131.

Kim, M-S; Hashemi, SB; Spencer PS; et al. (2002) Amino acid and protein targets of 1, 2-diacetylbenzene, a potent aromatic γ-diketone that induces proximal neurofilamentous axonopathy. Toxicol Appl Pharmacol 183: 55-65.

Kivits, GA; Ganguli-Swarttiywm MA; Christ, EJ. (1981) The composition of alkanes in exhaled air of rats as a result of lipid peroxidation in vivo. Effects of dietary fatty acids, vitamins, and selenium. Biochim Biophys Acta 665: 559-570.

Krasavage, WJ; O'Donoghue, JL; DiVincenzo, GD; et al. (1980) The relative neurotoxicity of methyl-n-butyl ketone, n-hexane and their metabolites. Toxicol Appl Pharmacol 52:433-441.

Ladefoged, O; Perbellini, L. (1986) Acetone-induced changes in the toxicokinetics of 2,5-hexanedione in rabbits. Scand J Work Environ Health 12: 627-629.

Ladefoged, O; Hass, U; Simonsen, L. (1989) Neurophysiological and behavioural effects of combined exposure to 2,5-hexanedione and acetone or ethanol in rats. Pharmacol Toxicol 65:372-375.

Ladefoged, O; Roswall, K; Larsen, JJ. (1994) Acetone potentiation and influence on the reversibility of 2,5-hexanedione-induced neurotoxicity studied with behavioural and morphometric methods in rats. Pharmacol Toxicol 74:294-299.

Lam, HR, Larsen, JJ, Ladefoged, O; et al. (1991) Effects of 2,5-hexanedione alone and in combination with acetone on radial arm maze behavior, the “brain swelling” reaction and synaptosomal functions. Neurotoxicol Teratol 13: 407-412.

Lankas, G; Baxter, C; Christian, R. (1978) Effect of alkane tumor-promoting agents on chemically induced mutagenesis in cultured V79 Chinese hamster cells. J Toxicol Environ Health 4:37-42.

Lapadula, DM; Irwin, RD; Suwita, E; et al. (1986) Cross-linking of neurofilament proteins of rat spinal cord in vivo after administration of 2,5-hexanedione. Journal of Neurochemistry 46:1843-1850.

Larsen, JJ; Lykkegaard, M; Ladefoged, O. (1991) Infertility in rats induced by 2,5-hexanedione in combination with acetone. Pharmacol Toxicol 69: 43-46.

Linder, RE; Strader, LF; Slott, VL; et al. (1992) Endpoints of spermatotoxicity in the rat after short duration exposures to fourteen reproductive toxicants. Reprod Toxicol 6:491-505.

Litton Bionetics Inc. (1979) Teratology study in rats using n-hexane (final report) with cover letter dated 011587. Submitted under Section 4 of TSCA. EPA Document No. 40-8789144; NTIS No. OTS0511634.

Litton Bionetics Inc. (1980) Mutagenicity evaluation of n-hexane in the mouse dominant lethal assay [final report]. EPA Document No. FYI-AX-1081-0137; NTIS No. OTS0000137-0.

Lungarella, G; Fonzi, L; Centini, F. (1980) Respiratory tract lesions induced in rabbits by short-term exposure to n-hexane. Res Commun Chem Pathol Pharmacol 29:129-139.

Lungarella, G; Barni-Comparini, I; Fonzi, L. (1984) Pulmonary changes induced in rabbits by long-term exposure to n-hexane. Arch Toxicol 55:224-228.

Mapleson, W. (1973) Circulation-time models of the uptake of inhaled anaesthetics and data for quantifying them. Brit J Anaesth 45:319-334.

Marks, TA; Fisher, PW; Staples, RE. (1980) Influence of n-hexane on embryo and fetal development in mice. Drug Chem Toxicol 3:393-406.

Masotto, C; Bisiani, C; Camisasca, C; et al. (1995) Effects of acute n-hexane and 2,5-hexanedione treatment on the striatal dopaminergic system in mice. J Neural Transm Suppl 45:281-285.

Mast, T. (1987) Inhalation developmental toxicology studies: Teratology study of n-hexane in rats [final report]. Public Health Service, U.S. Department of Health and Human Services; TER90082. Prepared by the Pacific Northwest Laboratory, Richland, WA, for the National Toxicology Program, National Institute for Environmental Health Services, Research Triangle Park, NC; PNL-6453.

Mast, T; Decker, J; Stoney, K; et al. (1988a) Inhalation developmental toxicology studies: Teratology study of n-hexane in mice [final report]. Public Health Service, U.S. Department of Health and Human Services; NTP TER90083. Prepared by the Pacific Northwest Laboratory, Richland, WA, for the National Toxicology Program, National Institute for Environmental Health Services, Research Triangle Park, NC; PNL-6590.

Mast, T; Hackett, P; Decker, J; et al. (1988b) Inhalation reproductive toxicology studies: sperm morphology study of n-hexane in B6C3F1 mice. Prepared by the Pacific Northwest Laboratory Richland, WA, for the National Toxicology Program, National Institute for Environmental Health Services, Research Triangle Park, NC; PNL-6672. Available from: National Technical Information Service, Springfield, VA; NTIS No. DE89000262.

Mast, T; Rommerein, R; Evanoff, J; et al. (1988c) Inhalation reproductive toxicology studies: male dominant lethal

study of n-hexane in Swiss (CD-1) mice. Prepared by the Pacific Northwest Laboratory Richland, WA, for the National Toxicology Program, National Institute for Environmental Health Services, Research Triangle Park, NC; PNL-6679. Available from: National Technical Information Service, Springfield, VA; NTIS No. DE89000271.

Mateus, ML; dos Santos Ana, PM; Batoreu, MC. (2002) Evidence of zinc protection against 2,5-hexanedione neurotoxicity: correlation of neurobehavioral testing with biomarkers of excretion. Neurotoxicology 23:747-754.

Mayan, O; Teixeira, JP; Pires, AF. (2001) Biological monitoring of n-hexane exposure in Portuguese shoe manufacturing workers. Appl Occup Environ Hyg 16:736-741.

Mayan, O; Teixeira, JP; Alves, S; et al. (2002) Urinary 2,5 hexanedione as a biomarker of n-hexane exposure. Biomarkers 7:299-305.

Mayer, V; Goin, C. (1994) Induction of chromosome loss in yeast by combined treatment with neurotoxic hexacarbons and monoketones. Mutat Res 341:83-91.

McCarroll, N; Keech, B; Piper, C. (1981a) A microsuspension adaptation of the *Bacillus subtilis* "rec" assay. Environ Mutagen 3:607-616.

McCarroll, N; Piper, C; Keech, B. (1981b) An *E. coli* microsuspension assay for the detection of DNA damage induced by direct-acting agents and promutagens. Environ Mutagen 3:429-444.

Michailov, GV; Sereda, MW; Brinkmann, BG. (2004) Axonal Neuregulin-1 regulates myelin sheath thickness. Science 304: 700-703.

Microbiological Associates. (1989) Salmonella/mammalian microsome mutagenicity assay of the vapor phase of commercial hexane using the desiccator methodology (final report) with attached appendices. Submitted under Section 4 of TSCA. EPA Document No. 40-8989391; NTIS No. OTS0524322.

Microbiological Associates. (1990) Subchronic in vivo cytogenetics assay in rats using nose-only inhalation exposure to commercial hexane (final report) with attachments and cover letter dated 061790. Submitted under Section 4 of TSCA. EPA Document No. 40-9089437; NTIS No. OTS0532896.

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

Murata, K; Araki, S; Yokoyama, K; et al. (1994) Changes in autonomic function as determined by ECG R-R interval variability in sandal, shoe and leather workers exposed to n-hexane, xylene and toluene. Neurotoxicology 15:867-875.

Mutti, A; Cavatorta, A; Lommi, G; et al. (1982a) Neurophysiological effects of long-term exposure to hydrocarbon mixtures. Arch Toxicol 5(Suppl):120-124).

Mutti, A; Ferri, F; Lommi, G; et al. (1982b) n-hexane-induced changes in nerve conduction velocities and somatosensory evoked potentials. Int Arch Occup Environ Health 51:45-54.

Mutti, A; Falzoi, M; Lucertini, S; et al. (1984) n-hexane metabolism in occupationally exposed workers. Br J Ind Med 41:533-538.

Mutti, A; Bergamaschi, E; Ghittori, S; et al. (1993) On the need of a sampling strategy in biological monitoring: the example of hexane exposure. Int Arch Occup Environ Health 65:S171-S175.

Nachtman, JP; Couri, D. (1984) An electrophysiological study of 2-hexanone and 2,5-hexanedione neurotoxicity in

rats. Toxicol Lett 23:141-145.

Nakajima, T; Elovaara, E; Park, SS; et al. (1991) Immunochemical detection of cytochrome P450 isozymes induced in rat liver by n-hexane, 2-hexanone and acetyl acetone. Arch Toxicol 65:542-547.

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

National Safety Council (NSC). (2003) n-hexane. Chemical Backgrounder. National Safety Council, Itasca, IL. Available from: <http://www.nsc.org/library/chemical/n-hexane.htm>.

National Toxicology Program (NTP). (1991) Toxicity studies of n-hexane in B6C3F1 mice (inhalation studies). Public Health Service, U.S. Department of Health and Human Services; TOX-2. Available from: National Institute for Environmental Health Services, Research Triangle Park, NC.

Nylen, P; Hagman, M. (1994) Function of the auditory and visual systems, and of peripheral nerve, in rats after long-term combined exposure to n-hexane and methylated benzene derivatives. II. Xylene. Pharmacology & Toxicology 74:124-129.

Nylen, P; Ebendal, T; Eriksdotter-Nilsson, M; et al. (1989) Testicular atrophy and loss of nerve growth factor-immunoreactive germ cell line in rats exposed to n-hexane and a protective effect of simultaneous exposure to toluene or xylene. Arch Toxicol 63:296-307.

Nylen, P; Hagman, M; Johnson, AC. (1994) Function of the auditory and visual systems, and of peripheral nerve, in rats after long-term combined exposure to n-hexane and methylated benzene derivatives. I. Toluene. Pharmacology & Toxicology 74:116-123.

Ono, Y; Takeuchi, Y; Hisanaga, N. (1981) A comparative study on the toxicity of n-hexane and its isomers on the peripheral nerve. Int Arch Occup Environ Health 48:289-294.

Ono, Y; Takeuchi, Y; Hisanaga, N; et al. (1982) Neurotoxicity of petroleum benzine compared with n-hexane. Int Arch Occup Environ Health 50:219-229.

Passero, S; Battistini, N; Cioni, R; et al. (1983) Toxic polyneuropathy of shoe workers in Italy. A clinical, neurophysiological and follow-up study. Italian Journal of Neurological Sciences 4:463-472.

Pastore, C; Marhuenda, D; Marti, J; et al. (1994) Early diagnosis of n-hexane-caused neuropathy. Muscle Nerve 17:981-986.

Patten, CJ; Ning, SM; Lu, AYH; et al. (1986) Acetone-inducible cytochrome P-450: purification, catalytic activity, and interaction with cytochrome b_5. Archives of Biochemistry and Biophysics 251:629-638.

Paulson, GW; Waylonis, GW. (1976) Polyneuropathy due to n-hexane. Arch Intern Med 136:880-882.

Perbellini, L; Brugnone, F; Faggionato, G. (1981) Urinary excretion of the metabolites of n-hexane and its isomers during occupational exposure. Brit J Indust Med 38:20-26.

Perbellini, L; Leone, R; Fracasso, ME; et al. (1982) Metabolic interaction between n-hexane and toluene in vivo and in vitro. Int Arch Occup Environ Health 50:351-358.

Perbellini, L; Brugnone, F; Caretta, D; et al. (1985) Partition coefficients of some industrial aliphatic hydrocarbons (C5-C7) in blood and human tissues. Br J Ind Med 42:162-167.

Perbellini, L; Mozzo, P; Brugnone, F; et al. (1986) Physiologicomathematical model for studying human exposure to organic solvents: kinetics of blood/tissue n-hexane concentrations and of 2,5-hexanedione in urine. Br J Ind Med 43:760-768.

Perbellini, L; Mozzo, P; Olivato, D; et al. (1990) "Dynamic" biological exposure indexes for n-hexane and 2,5-hexanedione, suggested by a physiologically based pharmacokinetic model. Am Ind Hyg Assoc J 51:356-362.

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

Pezzoli, G; Barbieri, S; Ferrante, C; et al. (1989) Parkinsonism due to n-hexane exposure. Lancet 2:874.

Pezzoli, G; Ricciardi, S; Masotto, C; et al. (1990) n-hexane induces parkinsonism in rodents. Brain Res 531:355-357.

Pezzoli, G; Antonini, A; Barbieri, S; et al. (1995) n-hexane-induced parkinsonism: pathogenetic hypotheses. Movement Disorders 10:279-282.

Pezzoli, G; Strada, O; Silani, V; et al. (1996) Clinical and pathological features in hydrocarbon-induced parkinsonism. Ann Neurol 40:922-925.

Prieto, MJ; Marhuenda, D; Roel, J; et al. (2003) Free and total 2,5-hexanedione in biological monitoring of workers exposed to n-hexane in the shoe industry. Toxicol Lett 145:249-260.

Pryor, G; Dickinson, J; Howd, R; et al. (1983) Neurobehavioral effects of subchronic exposure of weanling rats. Neurobehav Toxicol Teratol 5:47-52.

Raitta, C; Seppalainen, AN; Huuskonen, MS. (1978) N-hexane maculopathy in industrial workers. Albrecht Von Graefes Arch Klin Exp Ophthalmol 209:99-110.

Ralston, WH; Hilderbrand, RL; Uddin, DE; et al. (1985) Potentiation of 2,5-hexanedione neurotoxicity by methyl ethyl ketone. Toxicol Appl Pharmacol 81:319-327.

Ramsey, J; Andersen, ME. (1984) A physiologically based toxicokinetic description of the inhalation pharmacokinetics of styrene in rats and humans. Toxicol Appl Pharmacol 73:159-175.

Robertson, P; White, E; Bus, J. (1989) Effects of methyl ethyl ketone pretreatment on hepatic mixed-function oxidase activity and on in vivo metabolism of n-hexane. Xenobiotica 19:721-729.

Rosenberg, CK, Genter, MB, Szakal-Quin, G; et al. (1987). dl- versus meso-3,4-dimethyl-2,5-hexanedione: a morphometric study of the proximo-distal distribution of axonal swellings in the anterior root of the rat. Toxicol Appl Pharmacol. 87: 363-373.

Sahu, SC; Lowther, DK; Jones, SL. (1982) Biochemical response of rat lung to inhaled n-hexane. Toxicol Lett 12:13-17.

Saito, I; Shibata, E; Huang, J; et al. (1991) Determination of urinary 2,5-hexanedione concentration by an improved analytical method as an index of exposure to n-hexane. Br J Ind Med 48:568-574.

Sanagi, S; Seki, Y; Sugimoto, K; et al. (1980) Peripheral nervous system functions of workers exposed to n-hexane at a low level. Int Arch Occup Environ Health 47:69-79.

Sanz, P; Flores, I; Soriano, T; et al. (1995) In vitro quantitative structure-activity relationship assessment of pyrrole

adducts production by γ-diketone-forming neurotoxic solvents. Toxicol in Vitro 9:783-787.

Scelsi, R; Poggi, P; Fera, L; et al. (1980) Toxic polyneuropathy due to n-hexane. J Neurol Sci 47:7-19.

Scelsi, R; Poggi, P; Fera, L; et al. (1981) Industrial neuropathy due to n-hexane. Clinical and morphological findings in three cases. Clin Toxicol 18:1387-1393.

Schaumburg, HH; Spencer, PS. (1976) Degeneration in central and peripheral nervous systems produced by pure n-hexane: an experimental study. Brain 99: 183-192.

Schaumburg, H; Spencer, P. (1978) Environmental hydrocarbons produce degeneration in cat hypothalamus and optic tract. Science 199:199-200.

Seppalainen, A; Raitta, C; Huuskonen, MS. (1979) n-hexane-induced changes in visual evoked potentials and electroretinograms of industrial workers. Electroencephalogr Clin Neurophysiol 47:492-498.

Shelby, M; Witt, K. (1995) Comparison of results from mouse bone marrow chromosome aberration and micronucleus tests. Environ Mol Mutagen 25:302-313.

Shell, L; Rozum, M; Jortner, B; et al. (1992) Neurotoxicity of acrylamide and 2,5-hexanedione in rats evaluated using a functional observational battery and pathological examination. Neurotoxicol Teratol 14:273-283.

Shibata, E; Huang, J; Ono, Y; et al. (1990a) Changes in urinary n-hexane metabolites by coexposure to various concentrations of methyl ethyl ketone and fixed n-hexane levels. Arch Toxicol 64:165-168.

Shibata, E; Huang, J; Hisanaga, N; et al. (1990b) Effects of MEK on kinetics of n-hexane metabolites in serum. Arch Toxicol 64:247-250.

Sills, RC; Harry, GJ; Lowrey, KB; et al. (1998) Carbon disulfide neurotoxicity in rats: V. Morphology of axonal swelling in the muscular branch of the posterior tibial nerve and spinal cord. Neurotoxicology 19:117-127.

Smith, AG; Albers, JW. (1997) n-hexane neuropathy due to rubber cement sniffing. Muscle & Nerve 20:1445-1450.

Sobue, I; Iida, M; Yamamura, Y; et al. (1978) N-hexane polyneuropathy. Int J Neurol 11:317-330.

Soiefer, A; Robinson, K; Broxup, B; et al. (1991) A subchronic neurotoxicity study of commercial hexane vapor in the rat. Toxicologist 11:315.

Soriano, T; Menendez, M; Sanz, P; et al. (1996) Method for the simultaneous quantification of n-hexane metabolites: application to n-hexane metabolism determination. Human & Experimental Toxicology 15:497-503.

Spencer, PS; Schaumburg, HH. (1977a) Ultrastructural studies of the dying-back process III. The evolution of experimental peripheral giant axonal degeneration. J Neuropathol Exp Neurol 36(2):276-299.

Spencer, PS; Schaumburg, HH. (1977b) Ultrastructural studies of the dying-back process IV. Differential vulnerability of PNS and CNS fibers in experimental central-peripheral distal axonopathies. J Neuropathol Exp Neurol 36(2):300-320.

Spencer, PS; Kim, M-S; Sabri, MI. (2002) Aromatic as well as aliphatic hydrocarbon solvent axonopathy. Int J Hyg Environ Health 205:131-136.

Takeuchi, Y; Ono, Y; Hisanaga, N. (1981) An experimental study on the combined effects of n-hexane and toluene on the peripheral nerve of the rat. Br J Ind Med 38:14-19.

Takeuchi, Y; Ono, Y; Hisanaga, N; et al. (1983) An experimental study of the combined effects of n-hexane and methyl ethyl ketone. Br J Ind Med 40:199-203.

Takeuchi, Y. (1993) n-hexane polyneuropathy in Japan: a review of n-hexane poisoning and its preventive measures. Environ Res 62:76-80.

Toftgard, R; Haaparanta, T; Eng, L; et al. (1986) Rat lung and liver microsomal cytochrome P-450 isozymes involved in the hydroxylation of n-hexane. Biochemical Pharmacology 35:3733-3738.

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

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 studies. 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. Available from: National Technical Information Service, Springfield, VA; PB2000-500023, and <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (1995) Use of the benchmark dose approach in health risk assessment. U.S. Environmental Protection Agency. EPA/630/R-94/007. Available from: National Technical Information Service (NTIS) , Springfield, VA; PB95-213765, and <http://www.epa.gov/iris/backgr-d.htm>.

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. Available from: National Technical Information Service, Springfield, VA; PB98-140726, and <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA (1999) Guidelines for carcinogen risk assessment [review draft]. Risk Assessment Forum, Washington, DC; NCEA-F-0644. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (2000a) Science policy council handbook: peer review. 2nd edition. Prepared by the Office of Science Policy, Office of Research and Development, Washington, DC. EPA 100-B-00-001. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

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. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA (2000c) Benchmark dose technical guidance document [external review draft]. EPA/630/R-00/001. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S. EPA. (2002) A review of the reference dose and reference concentration processes. Risk Assessment Forum, Washington, DC; EPA/630/P-02/0002F. Available from: <http://www.epa.gov/iris/backgr-d.htm>.

U.S.EPA. (2005a) Guidelines for carcinogen risk assessment. Risk Assessment Forum, Washington, DC; EPA/630/P-03/001F. Available from: <http://cfpub.epa.gov/ncea/raf/recordisplay.cfm?deid=116283>.

U.S. EPA. (2005b) Supplementary guidance for assessing susceptibility from early-life exposure to carcinogens. Risk Assessment Forum, Washington, DC; EPA/630/R-03/003F. Available from: <http://www.epa.gov/ttn/atw/childrens_supplement_final.pdf>.

Valentino, M. (1996) Residual electroneurographic modifications in subjects with n-hexane-induced polyneuropathy: a follow-up study. La Medicina Del Lavoro 87:289-296.

van Engelen, JG; Rebel-de Haan, W; Opdam, JJ; et al. (1997) Effect of coexposure to methyl ethyl ketone (MEK) on n-hexane toxicokinetics in human volunteers. Toxicol Appl Pharmacol 144:385-395.

Vanacore, N; Gasparini, M; Brusa, L; et al. (2000) A possible association between exposure to n-hexane and Parkinsonism. Neurological Sciences 21:49-52.

Vaz, AD; Coon, MJ. (1987) Hydrocarbon formation in the reductive cleavage of hydroperoxides by cytochrome P-450. Proc Natl Acad Sci USA 84: 1172-1176.

Veronesi, B; Lington, A; Spencer, P. (1984) A tissue culture model of methyl ethyl ketone's potentiation of n-hexane neurotoxicity. Neurotoxicology 5:43-52.

Veulemans, H; Van Vlem, E; Janssens, H; et al. (1982) Experimental human exposure to n-hexane. Study of the respiratory uptake and elimination, and of n-hexane concentrations in peripheral venous blood. Int Arch Occup Environ Health 49:251-263.

Wang, JD; Chang, YC; Kao, KP; et al. (1986) An outbreak of n-hexane-induced polyneuropathy among press proofing workers in Taipei. Am J Ind Med 10:111-118.

Yamamura, Y. (1969) n-hexane polyneuropathy. Folia Psychiatr Neurol Jap 23:45-57.

Yokoyama, K; Feldman, RG; Sax, DS; et al. (1990) Relation of distribution of conduction velocities to nerve biopsy findings in n-hexane poisoning. Muscle Nerve 13:314-320.

Yokoyama, K; Araki, S; Murata, K; et al. (1997) Postural sway frequency analysis in workers exposed to n-hexane, xylene, and toluene: assessment of subclinical cerebellar dysfunction. Environ Res 74:110-115.

Yucesoy, B; Yucel, A; Erdem, O; et al. (1999) Effects of occupational chronic coexposure to n-hexane, toluene, and methyl ethyl ketone on NK cell activity and some immunoregulatory cytokine levels in shoe workers. Human & Experimental Toxicology 18:541-546.

Zhao, W; Misumi, J; Yasui, T; et al. (1998) Effects of methyl ethyl ketone, acetone, or toluene coadministration on 2,5-hexanedione in the sciatic nerve, serum, and urine of rats. Int Arch Occup Environ Health 71:236-244.

APPENDIX A

SUMMARY OF EXTERNAL PANEL PEER REVIEW AND PUBLIC COMMENTS AND DISPOSITION

The support document and IRIS summary for n-hexane have undergone both Agency review performed by scientists within EPA and a more formal external peer review performed by scientists in accordance with EPA guidance on peer review (U.S. EPA, 1998b, 2000a). Comments made by the Agency reviewers were addressed prior to submitting the documents for external peer review and are not part of this appendix. The five 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 the public and EPA's response to these comments follows.

EXTERNAL PEER REVIEW PANEL COMMENTS

The reviewers made several editorial suggestions to clarify specific portions of the text. These changes were incorporated in the document as appropriate and are not discussed further.

1) Oral reference dose (RfD) for n-hexane

> **No oral RfD has been derived. Have the rationale and justification for not deriving an RfD been transparently described in the documents? Are there additional studies that should be considered in this decision?**

COMMENT: All of the reviewers felt that the rationale and justification for not deriving an RfD had been transparently described in the assessment, and no additional studies were available for consideration in this decision.

RESPONSE: No response.

2) Inhalation reference concentration (RfC) for n-hexane

a) Have the rationale and justification for deriving an RfC been transparently described in the documents? Are there additional studies that should be considered in this decision?

COMMENT: One reviewer commented that some of the information presented in the document was repeated in multiple sections. This reviewer specifically referred to Altenkirch et al. (1982) and Ichihara et al. (1998). This reviewer, along with another reviewer, also suggested more synthesis of the literature presented in the document.

In addition, one reviewer noted that Tables 3-2 and 3-3 showed unexplained differences of n-hexane tissue levels following the same exposure scenario. This reviewer was also unclear as to why exposure units were changed from ppm to mg/m^3 in Table 4-22.

All of the remaining reviewers indicated that the rationale and justification for deriving the RfC was adequate and that no additional studies should have been considered in the derivation of the RfC.

RESPONSE: Sections of the document where studies were described multiple times were revised so that descriptions were not repeated. The literature is extensively synthesized and interpreted in Sections 4.5 and 5.2.1.

The discrepancies between Tables 3-2 and 3-3 have been clarified. Table 3-2 presents n-hexane tissue levels measured 72 hours after a 6-hour exposure and Table 3-3 presents n-hexane tissue levels measured immediately following a 6-hour exposure. Finally, Table 4-22 presents exposure concentrations in both ppm and mg/m^3.

b) The 1990 IRIS assessment for n-hexane used a human occupational exposure study by Sanagi et al. (1980) for the derivation of the RfC. The draft reassessment for n-hexane uses a subchronic rat study by Huang et al. (1989) for the derivation of the RfC. The workers evaluated in the Sanagi et al. (1980) study had coexposure to acetone and n-hexane. Data were identified that indicate n-hexane metabolism and n-hexane-induced neurotoxicity are potentiated by coexposure to acetone. Thus, this study was not selected for the derivation of the RfC in the current assessment.

The rationale supporting selection of the Huang et al. (1989) study versus the Sanagi et al. (1980) study as the principal study in the derivation of the RfC is presented in Sections 5.2.1 and 5.2.4 of the Toxicological Review. Is the Huang et al. (1989) study the most appropriate selection for the principal study (i.e., best study upon which to determine the point of departure)? Has the rationale for this choice been transparently and objectively described? Is the selection of Huang et al. (1989) as the principal study scientifically objective? Is the exclusion of Sanagi et al. (1980) as the principal study based on coexposure to acetone justified? Should the Huang et al. (1989) study and the Sanagi et al. (1980) study be considered as coprincipal studies in the derivation of the RfC?

COMMENT: Three reviewers felt that the selection of the Huang et al. (1989) study as the principal study was appropriate and that the rationale for the selection of this study as the principal study was adequately described in the document. These reviewers did agree that the human study by Sanagi et al. (1980) provided information useful to the assessment and should be discussed and integrated into the discussion of principal study selection to a greater extent.

One reviewer felt that both the animal study (Huang et al., 1989) and the human study (Sanagi et al., 1980) should be considered as coprincipal studies in the derivation of the RfC. This reviewer noted that the coexposure to acetone in the human study (Sanagi et al., 1980) did confound the results of this study. The reviewer stated that the coexposure to acetone in the human study would lead to the derivation of a more conservative RfC. Further this reviewer agreed that the 58 ppm exposure presented in the human study represents a LOAEL.

A single reviewer indicated that the selection of the human study (Sanagi et al., 1980) as the principal study for the derivation of the RfC was appropriate in this assessment. The reviewer also stated that the coexposure to acetone in the human study would lead to the derivation of a more conservative RfC. This reviewer suggested that the animal study (Huang et al., 1989) be presented as supporting data.

RESPONSE: EPA has determined that the Huang et al. (1989) animal study is the most appropriate study upon which to determine the point of departure for the derivation of the RfC for n-hexane. EPA has considered the reviewers' comments collectively and decided that the exclusion of the human study by Sanagi et al. (1980) as the principal study is scientifically

justified based on the coexposure of the study subjects to acetone and n-hexane. Specifically, more recent data indicate that acetone increases the metabolism of n-hexane to the toxic metabolite 2,5-hexanedione and decreases elimination of 2,5-hexanedione (see Section 5.2.1). In addition, acetone has been shown to exacerbate n-hexane-induced neurotoxicity and reproductive toxicity (see Sections 4.4.3 and 5.2.1). Given the uncertainty associated with the contribution of acetone to the neurological effects observed in humans by Sanagi et al., (1980), the Huang et al. (1989) animal study is most appropriate to be used in the derivation of the RfC for n-hexane. The Sanagi et al. (1980) human study is used as supporting data in the selection of the principal study and critical effect for the derivation of the RfC for n-hexane.

c) Has the most appropriate critical effect (decreased motor nerve conduction velocity in male rats following 12 weeks n-hexane exposure) been selected? Has the rationale and justification for this effect been transparently described? Is the selection of the critical effect scientifically justified?

COMMENT: All of the reviewers agreed that the selection of decreased MCV as the critical effect was the most appropriate endpoint for the derivation of the RfC. The reviewers felt that the rationale and justification were transparently presented and that the selection of this endpoint was scientifically justified. One reviewer suggested that a brief statement of the sensitivity of this endpoint compared with decreased hind-limb grip strength be added to the assessment.

RESPONSE: A statement indicating the sensitivity of measurement of motor nerve conduction velocity compared with hind-limb grip strength has been added to the document (see Section 4.5).

d) An RfC has been derived utilizing benchmark dose modeling to define the point of departure. Is benchmark dose modeling the best approach for determining the point of departure? Has the benchmark dose modeling been accurately and transparently described? In the absence of a biological rationale for choosing an appropriate effect level, a point of departure corresponding to a change in the mean equal to one control standard deviation from the control mean has been used. Is this the best approach for determining the effect level? Has the most appropriate model been utilized? Please comment on the model choice (and the values utilized for the model parameters) as well as the approach.

COMMENT: All reviewers agreed with the use of benchmark dose modeling for the derivation of the RfC for n-hexane. Two reviewers requested further explanation of the benchmark response level (one standard deviation versus 1, 5, or 10%) and suggested perhaps a lower point of departure should be used to derive the RfC to be more health protective. One of these reviewers suggested the application of an additional uncertainty factor to account for severity of effect.

One reviewer indicated that in Section 5.2.2 and/or Appendix B, the connection between the benchmark dose method and software (i.e., what input options and parameters were used) be added to the discussion and that the actual mathematical equation and an explanation for selection of the various models be included. This reviewer also requested clarification of the parameters used in modeling the Huang et al. (1989) 8- and 12-week exposure data**.**

One reviewer noted that the BMD modeling method used (fetal-based analysis) for the reduced fetal body weight data from Mast (1987) was incorrect. Specifically, this reviewer indicated that a litter-based analysis would be more appropriate. This reviewer also suggested that the data from the Mast et al. (1988a) study should be modeled.

RESPONSE: The U.S. EPA *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000c) states that in the absence of any data indicating the level of response that is considered to be biologically significant or adverse, a change in the mean equal to one control standard deviation from the control mean can be used. This gives an excess risk of approximately 10% for the proportion of individuals below the 2^{nd} percentile and above the 98^{th} percentile of controls for normally distributed effects. For the Huang et al., (1989) data set, an approximate 7% change in the mean MCV value corresponds to the point of departure. This percentage is an indication of the severity of the critical effect. The U.S. EPA does not have guidance specific to the application of an effect level extrapolation factor to take into account severity of effect. Thus, this factor is not generally applied in IRIS assessments and was not applied to the point of departure for the derivation of the RfC for n-hexane. Based on the severity of effect and the recommendations of the U.S. EPA *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000c) it was determined that a BMR of one control standard deviation from the control mean is appropriate for the derivation of the RfC for n-hexane.

Significant text has been added to Appendix B describing the input options and parameters used

in modeling of the results from the Huang et al. (1989), Ono et al. (1982), and Mast (1987) studies. Where appropriate, the mathematical equations and accompanying explanations for selection of one model over another have also been added to the text in Appendix B. Finally, clarification of parameters used in the BMD modeling of the Huang et al. (1989) data has been added to the text in Appendix B.

The reduced fetal body weight data from Mast (1987) has been modeled using a litter-based analysis rather than a fetal-based analysis to address correlation among the pups. The revised modeling results and output are presented in Section 5.2.2 and Appendix B. The Mast et al. (1988a) study showed a statistically significant decreased fetal body weight and increased incidence of late resorptions at the highest dose. In addition, the only other statistically significant finding was an increased mean percent intrauterine death (increased early and late resorptions combined) at the low dose, with no dose-related trend. Thus, this study was not considered further for the selection of the principal study.

e) Are the uncertainty factors applied to the point of departure for the derivation of the RfC scientifically justified and transparently and objectively described in the Toxicological Review?

COMMENT: Two reviewers suggested that an effect level extrapolation factor be applied to the point of departure to account for use of a BMR of 1 standard deviation.

Two reviewers felt that the uncertainty factor applied for extrapolation from a subchronic to a chronic study was inappropriate. One reviewer indicated that the length of duration of the principal study (16 weeks) is half the time required for a newly synthesized NF protein to be transported from the neuronal cell body to the axon terminal in the longest PNS and CNS axons of an adult rat. Thus, this reviewer felt that there is no justification to extrapolate to a lifetime of the rat since the lifetime (maximum 32 weeks) of the target tissue, the NF, is shorter. The second reviewer stated that "given what is known about the toxic mechanism," the full uncertainty factor should not be applied.

Two reviewers suggested that the intraspecies uncertainty factor applied to the point of departure for the derivation of the RfC for n-hexane was inappropriate. Specifically, one reviewer noted in one study younger rats were observed to be less susceptible to the effects of n-hexane-induced

neurotoxicity compared with adult rats. This reviewer suggested that this difference was due to shorter length axons in the CNS and PNS in young compared with adult rats. Thus, since the RfC was derived from data in adult rats, any variation seen between young and adult rats would be observed as a decreased sensitivity to the effects of n-hexane-induced neurotoxicity in young rats. The reviewer further commented that no data exist that report differences in absorption, distribution, metabolism, or elimination. This reviewer noted that if concerns regarding genetic polymorphisms were included, then the UF of 10 for intraspecies differences was appropriate. The second reviewer provided no specific rationale for the reduction of the intraspecies uncertainty factor.

All of the reviewers suggested that the application of a database uncertainty factor of 3 was reasonable, but several of them stated that the justification for this determination was inadequate. Specifically, concerns about increased susceptibility in the fetus are not supported by the data.

RESPONSE: The uncertainty factor for extrapolation from subchronic to chronic exposure has been reduced from 10 to 3 due to considerations of NF biology. Specifically, 16 weeks (the duration of the principal study by Huang et al., 1989) is half the time required for a newly synthesized NF protein to be transported from the neuronal cell body to the axon terminal of the longest axon in and adult rat. The longest axon of an adult rat stretches from the lumbar spinal cord to the hind foot (approximately 22 cm) and NFs are transported axonally in adult rats at a rate of 1 mm/day. Complete transport of the newly synthesized NF protein the full length of the axon takes approximately 32 weeks. Thus, since the lifetime of the target of n-hexane-induced toxicity is much less than the lifetime of the adult rat, extrapolation from subchronic to chronic exposure is not justifiable. Additional text has been added to Section 5.2.2 to justify this reduction in the uncertainty factor for extrapolation from a subchronic to a chronic exposure.

Information provided by reviewers concerning differences in susceptibility between adult versus young rat due to axonal length has also been added to the document (see Sections 4.4.4 and 5.2.3). A single study in rats with one dose group reported susceptibility differences between adult and weanling rats (adults showed earlier and more severe neurotoxic effects). Mode of action studies supporting the hypothesis that the possible increased susceptibility to n-hexane-induced neurotoxic effects in adults is due to differences in axonal length exist (Michailov et al., 2004; ffrench-Constant et al., 2004; Graham et al., 1995; Griffin et al., 1984; Cavanagh and Bennetts, 1981; Spencer and Schaumburg, 1977a, b; Schaumburg and Spencer, 1976). However,

these studies do not report data for weanling animals. Given the potential for altered metabolism of n-hexane in individuals with polymorphisms in the CYP2E1 enzyme and the uncertainties associated with susceptibility differences between weanling and adult rats, a UF of 10 for intraspecies variability was retained.

f) The database for n-hexane is lacking a developmental neurotoxicity study. Given the potential increased susceptibility of the developing fetus to n-hexane-induced toxicity and the increased neurotoxicity in humans and animals following n-hexane exposure, a UF_{DB} of 3 was applied. Has the rationale and justification for the UF_{DB} been transparently described? Is the application of this UF appropriate?

COMMENT: All of the reviewers indicated that the database uncertainty factor that was applied for the derivation of the RfC for n-hexane was appropriate and reasonable. Several reviewers asked for increased justification and clarification. Specifically, several reviewers had concerns that increased susceptibility in the fetus is not supported by the data.

RESPONSE: Clarification and justification for the database uncertainty factor have been added to the document. Specifically, information has been added to Section 5.2.3 describing in more detail the reproductive studies available with exposures to pure n-hexane and the multigeneration reproductive and developmental toxicity studies with a mixture containing n-hexane. The database for n-hexane includes human occupational exposure studies (all with coexposure to other potentially neurotoxic chemicals), subchronic animal studies, and developmental studies in animals. The database lacks a multigeneration reproductive and developmental toxicity study. Prenatal exposure to pure n-hexane was shown to induce skeletal anomalies, decreased fetal body weight, and increased resorptions (Mast et al., 1988a; Mast, 1987; Bus et al., 1979). In addition, the multigeneration reproductive and toxicity studies with the commercial hexane mixture containing n-hexane suggested that n-hexane may induce reductions in fetal body weight and increased skeletal anomalies (Daughtrey et al., 1994a; BRRC, 1991, 1989a, b; IRDC, 1986). Taken together, these studies indicate that n-hexane may have effects on the reproductive system or developing fetus. However, the data do not elucidate whether low doses of n-hexane (i.e., doses that have been shown to lead to neurotoxicity in humans and animals) cause reproductive and developmental effects. Thus, due to the lack of multigeneration reproductive and developmental toxicity studies evaluating the effects of low doses of exposure to pure n-hexane, a UF_{DB} of 3 was applied.

3) Carcinogenicity of n-hexane

> **Under EPA's *Draft Revised Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1999) (www.epa.gov/ncea), *data are inadequate for an assessment of the human carcinogenic potential* of n-hexane. Do the available data support this statement? Are there additional studies that should be considered in this decision?**
>
> **How well were the data from individual studies characterized and are the conclusions that are drawn from each study valid? How well are the data integrated into an overall conclusion and characterization of hazard as presented in Sections 4.5, 4.6, 5, and 6?**

COMMENT: All of the reviewers agreed with the characterization of n-hexane data as inadequate for the assessment of human carcinogenic potential. Two reviewers provided comments for clarification of the cancer data.

RESPONSE: Suggestions for clarification were added to the document. The reference has been changed to the 2005 U.S. EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a).

PUBLIC COMMENTS

Two public comment submissions were received during the external peer review and public comment period. These comments were distributed to the external peer review panel prior to the public panel meeting and discussion of the assessment. Both submissions had several editorial suggestions and requested clarifications to specific portions of the text. These changes were incorporated in the document as appropriate and are not discussed further.

1) Inhalation reference concentration (RfC) for n-hexane

COMMENT: A single commenter indicated that the selection of the human study (Sanagi et al., 1980) as the principal study for the derivation of the RfC for n-hexane was appropriate. The commenter noted that use of the Sanagi et al. (1980) study would provide a more conservative RfC for n-hexane due to the coexposure to acetone and possible potentiation of n-hexane-induced neurotoxic effects. In addition, this commenter suggested that the n-hexane exposure

reported in the human study by Sanagi et al. (1980) is a minimal LOAEL or a NOAEL for the following reasons: (1) the authors' conclusions regarding the significance of the reported findings; (2) differences in exposure conditions between exposed and control groups (i.e., ball milling operations workers); (3) significance of electrophysical findings; and (4) inconsistencies in the magnitude of the findings.

RESPONSE: All of the human occupational n-hexane exposure studies also contain coexposure to other chemicals, many of which have been shown to potentiate n-hexane-induced neurotoxicity and metabolism. Specifically, Sanagi et al. (1980) reported coexposure to acetone, a chemical that has been shown to exacerbate metabolism of n-hexane to its toxic metabolite, 2,5-hexanedione, and n-hexane-induced neurotoxicity and reproductive toxicity. In addition, neurological deficits such as vibration sensation loss and headache have been reported to result from exposure to high noise levels such as those present in the factory where workers observed by Sanagi et al. (1980) worked. The contribution of both acetone coexposure and noise to n-hexane-induced neurotoxicity is unknown.

The external peer review panel felt that the effects observed by Sanagi et al. (1980) did not represent a NOAEL or a minimal LOAEL but rather they agreed that the n-hexane exposure level represented a LOAEL. In addition, EPA has determined that the Huang et al. (1989) animal study is the most appropriate study upon which to determine the point of departure for the derivation of the RfC for n-hexane. EPA has considered the external peer review panel's comments collectively and decided that the exclusion of the human study by Sanagi et al. (1980) as the principal study is scientifically justified based on the coexposure of the study subjects to acetone and n-hexane (see response above).

COMMENT: A single comment was received requesting additional clarification for the basis of the selection of the BMDL for the 12-week exposure in male rats as opposed to the 8- or 16-week exposures in the Huang et al. (1989) study.

RESPONSE: The goodness of fit and AIC values for the 8- ,12-, and 16-week exposures were relatively comparable for the Huang et al. (1989) male rat MCV data set. BMCL values from these data sets were within twofold of each other, suggesting that all the modeling results are equally plausible. The lowest point of departure, based on decreased MCV at 12-weeks exposure (BMCL = 215 mg/kg-day) was selected for derivation of the RfD corresponding to the

most sensitive neurological effect. In addition, a discussion of the modeling of the 8, 12, and 16-week n-hexane exposure data has been added to Appendix B.

2) Database uncertainty factor for the derivation of the RfC for n-hexane

COMMENT: Both public comment submissions suggested that the database uncertainty factor of 3 was inappropriate given the combined databases for n-hexane and commercial hexane and the evidence for the mode of action of n-hexane-induced neurotoxicity. These commenters also felt that the concern for increased susceptibility to n-hexane-induced neurotoxicity in the developing fetus is contradictory to evidence suggesting that younger animals may be less sensitive to n-hexane-induced neurotoxicity.

RESPONSE: Clarification and justification for the database uncertainty factor have been added to the document. Specifically, information has been added to Section 5.2.3 detailing the only study reporting susceptibility differences between weanlings and adults (Howd et al., 1983) and additional multigeneration reproductive and developmental toxicity studies with a mixture containing n-hexane (Daughtrey et al., 1994a; BRRC, 1991, 1989a, b; IRDC, 1986). Due to the lack of studies investigating the susceptibility of weanling and young animals compared with adult animals and multi-generation reproductive and developmental toxicity studies evaluating the effects of pure n-hexane exposure on the fetus, a UF_{DB} of 3 was applied (see response above).

APPENDIX B: BENCHMARK DOSE (BMD) ANALYSIS

Benchmark dose modeling was performed on the Mast (1987) data sets for reduced ossification of sternebrae and reduced fetal body weight, the Ono et al. (1982) data sets for MCV and mixed MCVs, and the Huang et al. (1989) data sets for reduced MCV. The criteria for selecting models used here are consistent with recommendations in the EPA's *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000c) except as noted where model selection criteria may disagree in ranking the models.

MODELING OF DICHOTOMOUS DATA SETS

In rats, Mast (1987) reported a statistically significant increase in the incidence of litters with fetuses with reduced ossification of sternebrae 1–4 (dichotomous data). Dichotomous data from this study were analyzed by BMDS and examined for toxicological relevance. All nested models for dichotomous variables available in EPA's BMDS (version 1.3.2) were fit to the data presented in the BMDS output in this appendix. The nested logistic (NL), National Center for Toxicological Research (NCTR), and Rai and vanRyzin (RvR) models allow for the possibility that the variance among the proportions of pups affected in individual litters is greater than would be expected if the pups were responding completely independently of each other (U.S. EPA, 2000c). A 10% increase in the incidence of reduced ossification of sternebrae 1–4 was selected as the benchmark response because it was a response rate that fell within the range of experimental dose levels used in the Mast (1987) study. All of the models provided similar fits to the data, based on the summary results reported in the BMDS output and the detailed examination of graphs and goodness-of-fit statistics. Model fits were improved slightly by the incorporation of litter size (as a litter-specific covariate) and by intra-litter correlations, as determined by comparisons of AIC values.

Modeling of reduced ossification of sternebrae 1–4 litter incidence data (Mast, 1987)

A summary of the incidence data for reduced ossification of sternebrae 1–4 in individual fetuses for both the control and exposure groups is shown in the output for the BMD modeling results of the data presented in this appendix.

The best-fitting models used the total number of nonresorbed pups in the litter as the litter-specific covariate that had the smallest AIC compared with comparable models with other litter-specific covariates. The NL model with the default parameter specification n greater than or equal to one was fit to the data and compared with the NCTR and RvR models with similar specifications. The default option forced the value of the exponent to be set equal to one. While the RvR models achieved a small AIC, they were usually not able to estimate a BMCL value and so are not discussed further. The NL model in the BMDS nested module produced the highest goodness of fit *p*-values. Values were larger than 0.10 over a range of parameter values and model specifications. While the NCTR models had slightly smaller AIC statistics than comparable NL models, the differences were less than 2 AIC units, and none of the NCTR models had *p*-values greater than 0.10. We therefore selected the NL models as providing the best description of the data. The default option forced the value of the exponent to be set equal to one. The NL model had a better goodness of fit and smaller AIC. This model provided an AIC of 3.41 with a *p*-value of 0.183. The corresponding BMC and BMCL values were 1571 and 943 ppm, respectively.

MODELING OF CONTINUOUS DATA SETS

Continuous data models (linear, polynomial, power, or Hill where possible), either with a constant variance or with variance as a power function of the mean value (using an additional model parameter), were fit to the following continuous data sets from the Huang et al. (1989), Ono et al. (1982), and Mast (1987) studies which were selected for benchmark dose modeling: decreased MCV, decreased MCV and mixed nerve conduction velocity, and fetal body weight deficits, are the respective endpoints. The BMDS (version 1.3.2) was used to calculate potential points of departure for deriving the RfC by estimating the effective dose at a specified level of response (BMC) and its 95% lower bound (BMCL). EPA's *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000c) recommends, in the absence of some idea of the level of response to consider adverse, selecting as the BMR level for continuous data a change in the mean equal to one SD from the control mean.

The variance (square of the standard deviation) of the within-dose-group continuous endpoints is modeled in general by a two-parameter power function independently of the dose-response model for the within-dose-group mean response. This is done by fitting a model to the sample variances within each dose group, based only on the within-dose-group sample mean and not on the modeled dose-response function for the mean responses. Two situations lend themselves to a priori specification of the exponent in the variance function model, denoted rho. The first situation is to specify rho = 0, a situation often encountered in continuous models in

which the random errors appear to have a constant within-dose-group variability at all doses, as seems to be the case for the Mast (1987) fetal weight data described below. Another common situation is when the standard deviation of the within-dose-group data increases in direct proportion to the mean so that the ratio of the SD to the mean within each dose group (the within-dose-group coefficient of variation) is nearly constant, thus rho = 2. This case more accurately describes the Ono et al. (1982) MCV data. The variance model or specification of rho affects the influence (statistical weight) of observed mean values in each dose group and was considered in the analysis.

Modeling of Reduced MCV and Reduced Mixed Nerve Conduction Velocity

The Ono et al. (1982) study used three dose groups of 0, 200, and 500 ppm. The Hill model in BMDS has four adjustable parameters in the dose-response function and cannot be reliably estimated using data with only three dose groups without specifying the value of at least one parameter in either the dose-response model or the variance function model. Even when the Hill model can be fitted to the data, over-parameterizing the model may not make it possible to evaluate the goodness of fit of the model to the data or to estimate the BMCL. The Hill model was not evaluated in the Ono et al. (1982) study as there was little basis for specifying a large number of parameters in that model. The overall means and standard deviations for reduced MCV following inhalation exposure to n-hexane from the Ono et al. (1982) study are presented below in Table B-1.

Table B-1. Mean nerve conduction velocity in rats exposed to n-hexane

n-hexane exposure group	Number of rats	Mean nerve conduction velocity	Standard deviation of MCV
0	8	49.6	7.6
200	8	42.9	3.5
500	8	36.2	1.1

Source: Ono et al., 1982.

The models that were evaluated included the polynomial model of degree 1 (linear), the polynomial model of degree 2 (quadratic), and the power function model. Neither the linear nor the quadratic model provided adequate goodness of fit [all P(H4) < 0.0001] for any model specification. None of the power function models provided an adequate fit to the Ono et al. (1982) data unless one or two parameters of the model were specified. The best estimates of the shape parameter or exponent in the power function model were in the range of

0.7 to 0.8 and only provided BMCL estimates when the value of the exponent or power (rho) in the variance function model was specified as being in the range of approximately 2 to 3. Table B-2 below shows some of the BMC modeling results.

The sensitivity of the BMCL to the exponent was explored by holding γ constant at some middle values within the confidence interval for that parameter and setting rho as a constant over the range of 2 to 4. An adequate fit of the BMDS power function model can be obtained over a wide range of parameter values (Table B-1). However the BMCL estimates among acceptable models vary over a threefold range even for very small deviations in the parameter estimates, depending more on assumptions rather than what can be estimated from the data. The BMCL estimates shown in Table B-2 range from 81 to 85 ppm when rho $\geq$ 2 but could be as small as 26 to 29 ppm due to the uncertainty about the power function exponent in dose-response model (here denoted γ),

$$(\text{Mean response in dose group j}) = \text{intercept} + \text{slope} \ (\text{concentration in group j})^{\gamma}$$

The dose-response function for these data can therefore be modeled, but the estimation of the variance function leads to a high degree of uncertainty in the BMCL estimate. For example, P(H3) = 0.00147 for the bottom 4 model runs in Table B-2 and P(H3) < 0.0001 for all other models evaluated with the variance function exponent not specified. No model available in the BMDS described the sharp decrease in variance with increasing n-hexane concentration (decreasing mean MCV) unless rho > 2.5, which is not commonly encountered.

Table B-2. Parameters and modeling results for the n-hexane exposure MCV data from Ono et al. (1982)

Parameters of power model				Results					
Dose response power exponent		Variance exponent		DOF for AIC	AIC	DOF for P(H4)	P(H4)	BMC	BMCL
Estimate	Standard error	Estimate	Standard error						
0.716	0.268	2	fixed	4	98.95	1	0.0141	108	26
0.708	0.252	2.5	fixed	4	97.54	1	0.0318	107	28
0.704	0.246	2.75	fixed	4	96.85	1	0.0477	106	29
0.702	0.242	2.9	fixed	4	96.44	1	0.061	106	29
0.75	fixed	2	fixed	3	96.97	2	0.0488	116	81

0.75	fixed	2.5	fixed	3	95.57	2	0.0928	117	82
0.75	fixed	3	fixed	3	94.21	2	0.194	118	84
0.75	fixed	3.5	fixed	3	92.89	2	0.3745	120	85

DOF = Degree of freedom.

By holding one or two parameters constant, the apparent variability in the parameter estimates is constrained, and the BMCL is larger than in the unconstrained case. Specifying the two parameters decreases the uncertainty associated with the BMCL so that the BMCL values are larger than they might be if the parameter estimates were not specified or constrained (Table B-2). In summary, the data limit the confidence that can be placed in any BMCL estimated from Ono et al. (1982) data by BMDS.

The overall means and standard deviations for reduced mean total and distal mixed nerve conduction velocity following inhalation exposure to n-hexane from the Ono et al. (1982) study are presented below in Tables B-3 and B-4.

Table B-3. Mean total mixed nerve conduction velocity in rats exposed to n-hexane

Exposure group	Number of rats	Mean MCV	SD of MCV
0	8	59.3	4.1
200	8	53.3	3.0
500	8	50.9	1.3

Source: Ono et al. (1982)

Table B-4. Mean total distal nerve conduction velocity in rats exposed to n-hexane

Exposure group	Number of rats	Mean MCV	SD of MCV
0	8	52.3	3.3
200	8	47.6	1.7
500	8	45.3	1.6

Source: Ono et al., 1982.

Continuous data models (linear, polynomial, power, or Hill where possible), either with a constant variance or with variance as a power function of the mean value (using an additional model parameter), were fit to total and distal mixed nerve conduction velocity endpoints using U.S. EPA BMDS (version 1.3.2) and the same strategy as in the preceding analyses. These endpoints had the same issues as in the MCV analyses. The standard deviation and variance were much larger in the control animals than in those exposed to n-hexane and sharply decreased with increasing dose. The variance decreased much more rapidly with increasing

dose than did the mean dose-response function which required an unusually large value of the exponent rho in the variance function model.

The power function model provided the best model as indicated by the AIC criterion, but an adequate fit to either the dose-response model or the variance model was not found among the models that were evaluated unless the values of both the dose-response function power and the variance function exponent rho were specified as if they were known rather than being estimated from the data. The BMDL estimate usually was either not computable or was computable but nearly equal to zero (because the power was much less than one and the fitted power function had a very steep slope near the control exposure) unless both parameters were specified. When the constant variance option (rho = 0) was selected, either of the BMDS quadratic (polynomial) or power function models perfectly fit the mean mixed nerve conduction velocity data and fit nearly as well when the specification rho = 2 was made. The differences in fit occurred because values of rho not equal to zero in the variance function model implied different weights for fitting the mean responses at the three dose groups, but visual inspection of the results and small chi-squared residuals showed that the deviations were small and of little concern for BMDL estimation. The power function model was selected over the quadratic model because the power function model produced a decreasing estimated dose-response at all dose levels within the observed range of the data. Some quadratic models showed a small increase in mixed nerve conduction velocity at high n-hexane concentrations and were not selected as this result seemed biologically implausible.

All data sets had a large value of rho and a small value of the exponent n, usually around 0.4. The a priori plausible estimates of the variance function exponent are specified as 0 (additive error) or rho = 2, which most closely matched the data. A positive BMDL estimated concentration could be obtained unless the value of the power in the power function dose-response model was specified along with specifying rho. The results for quadratic models fit the data about as well as the power models without requiring specification of any of the parameters of the dose-response function. The advantage of the quadratic models is that they do not have an infinitely steep slope near the control concentration of 0 ppm n-hexane whereas the power function models with exponent <1 do have an infinitely steep slope near the control concentration of 0 ppm n-hexane. However, the power model has greater plausibility than the quadratic model because estimates of MCV decrease with increasing concentration.

The models evaluated included the polynomial model of degree 1 (linear), the polynomial model of degree 2 (quadratic), and the power function model. Neither the linear nor the quadratic model provided adequate goodness of fit [all P(H4) < 0.0001] for any model specification. None of the power function models provided an adequate fit to the data unless

one or two parameters of the model were specified. The best estimates of the shape parameter or exponent in the power function model were in the range of 0.36 to 0.49, and only provided BMCL estimates nontrivially greater than zero when the value of the power function exponent was specified and the power γ in the variance function model was specified as being in the range of roughly 2 to 4. The equation of the power model is the same as presented above.

Only results for specified rho of 0 and 2 are shown. Tables B-5 and B-6, below, show the results for total and distal mixed nerve conduction velocity data sets. The BMCL estimates are considered low (i.e., 13 to 33 ppm) due to the uncertainty about the power function exponent in the dose response model. The sensitivity of the BMCL to the exponent was explored by holding γ constant at middle values within the confidence interval for that parameter and setting γ as a constant over the range 2 to 4.

An adequate fit of the BMDS power function and quadratic models can be obtained over a wide range of parameter values. However, the BMCL estimates among acceptable models vary over a threefold range even for modest deviations in the parameter estimates, depending more on what one is willing to assume is known rather than what can be estimated from the data. The BMCL estimates shown above depend on the mixed MCV index and on the model. Highlighted in bold type is a set of consistent models that fit the data well as indicated by low AIC and small chi-squared residuals at concentrations around the estimated BMCL even when it is not possible to formally test the goodness of fit using the likelihood ratio test for H4, as often happens when one or more parameters are specified. The BMCL for the recommended models are shown in Table B-7.

Table B-5. Estimates of BMCL and goodness of fit statistics for total mixed nerve conduction velocity from the Ono et al. (1982) study

Exponent in power model estimate		Exponent in variance model estimate		DOF for AIC	AIC	DOF for P(H4)	P(H4)	BMC (ppm)	BMCL (ppm)
Estimate	**Standard error**	**Estimate**	**Standard error**						
0.491	0.231	14.44	7.61	5	77.31	0	NE	145	NE
0.367	0.232	0	Specified	4	81.97	0	NE	25.9	NE
0.377	0.232	2	Specified	4	80.08	0	NE	30	0+
0.4	Specified	0	Specified	3	79.99	1	0.888	33	15
0.4	Specified	2	Specified	3	78.09	1	$<10^{-4}$	35	16.8
0.5	Specified	0	Specified	3	80.27	1	0.581	56	30
0.5	Specified	2	Specified	3	78.39	1	$<10^{-4}$	60	33.2
0.382	0.205	3	Specified	4	79.25	0	NE	33	0+

0.387	0.199	4	Specified	4	78.5	0	NE	36	0+
0.4	Specified	13	6.47	4	75.5	1	$<10^{-4}$	95	NE
0.5	Specified	14.58	7.67	4	75.31	1	$<10^{-4}$	150	NE
Quadratic model									
Exponent in variance model estimate									
Estimate		**Standard error**							
3.46		4.84		5	80.89	0	NE	87	57.7
0		Specified		4	79.97	1	$<10^{-4}$	80	51.3
1.5		Specified		4	78.52	1	$<10^{-4}$	83	53.8
1.875		Specified		4	78.19	1	$<10^{-4}$	83	54.5

DOF = Degree of freedom.
NE = Could not be estimated.
0+ = any very small positive number <0.05 for BMCL in this table.

Table B-6. Estimates of power model BMCL estimates and goodness of fit statistics for distal mixed nerve conduction velocity from the Ono et al. (1982) study

Exponent in power model estimate		**Exponent in variance model estimate**		**DOF for AIC**	**AIC**	**DOF for P(H4)**	**P(H4)**	**BMC (ppm)**	**BMCL (ppm)**
Estimate	**Standard error**	**Estimate**	**Standard error**						
0.408	0.171	10.5	Specified	5	66.24	0	NE	61	NE
0.435	0.225	0	Specified	4	69.48	0	NE	34	0+
0.426	0.206	2	Specified	4	67.7	0	NE	35	0+
0.4	Specified	0	Specified	3	67.5	1	0.875	28	13
0.4	Specified	2	Specified	3	65.71	1	$<10^{-4}$	29	14.3
0.5	Specified	0	Specified	3	67.56	1	0.776	48	26.4
0.5	Specified	2	Specified	3	65.82	1	$<10^{-4}$	51	28.3
0.421	0.199	3	Specified	4	66.93	0	NE	35	0.11
0.418	0.192	4	Specified	4	66.25	0	NE	36	NE
0.4	Specified	10.5	4.82	4	64.24	1	$<10^{-4}$	59	NE
0.5	Specified	10.59	5.08	4	64.49	1	$<10^{-4}$	91	NE
Quadratic model									
Exponent in variance model estimate				**DOF for AIC**	**AIC**	**DOF for P(H4)**	**P(H4)**	**BMC (ppm)**	**BMCL (ppm)**
Estimate		**Standard error**							
4.996		0.235		5	112.3	0	NE	11850	102.3

0	Specified	4	67.48	0	NE	80	51.3
1.625	Specified	4		1	$<10^{-4}$	81	53

DOF = Degree of freedom.
NE = Could not be estimated.
0+ = any very small positive number <0.05 for BMCL in this table.

The lack of fit of the BMDS variance function to the observed variances without use of an untypically large value of rho leads to some questions about the extent to which these results can be generalized. The models selected above all have rho = 2 (constant coefficient of variation at all exposure levels) or as close as possible for the quadratic models. The power function model with power = 0.4 is probably the most typical case but will yield BMCL estimates lower than other models in Table B-4.

Table B-7. BMCL estimates for power and quadratic models with various parameters from the Ono et al. (1982) study

Model and parameters selected for BMCL estimation	Total MCV	Distal MCV
Power = 0.4, rho = 2	17	14
Power = 0.5, rho = 2	**33**	**28**
Quadratic, rho near 2	55[a]	53[a]

[a] Approximate largest value of rho for which a positive BMCL was found by halving intervals between rho = 0 and 2 among the quadratic models with specified rho.

Modeling of reduced fetal body weight data (Mast, 1987)

All four BMDS continuous endpoint models were initially considered for the Mast (1987) fetal body weight data. Specifically, continuous data models (linear, polynomial, power, or Hill where possible), either with a constant variance or with variance as a power function of the mean value (using an additional model parameter), were fit to the data using U.S. EPA BMDS (version 1.3.2). The continuous data from the Mast (1987) study for reduced mean fetal weight averaged across litters within each exposure group are shown in Table B-8.

Table B-8. Mean of litter mean pup body weight in rats exposed to n-hexane

Exposure group (ppm)	Number of litters	Mean of litter mean fetal body weight (g)	Standard deviation
0	23	3.48	0.37
200	24	3.54	0.36
1000	27	3.27	0.32
5000	28	2.97	0.38

Source: Mast, 1987.

The current version of BMDS does not provide models for continuous endpoints among the BMDS nested modules for developmental toxicology data in which individual fetal responses provided quantal response data as in Mast (1987). However, valid BMDS analyses may be carried out using each litter as the unit of analysis in the BMDS continuous model because the litters themselves correspond to different dams and are statistically independent, unlike the individual fetal weight data that exhibit intralitter correlation.

The linear, quadratic, and power dose response models among the continuous-variable models provided satisfactory goodness of fit *p*-values [P(H4) > 0.10] using a constant variance model (rho = 0) or a variance model with rho estimated from these data (BMDS version 1.3.2) and provided an adequate fit to the data (with a goodness of fit *p*-value $\geq$0.1). The model with the lowest AIC was used to establish the BMC (see BMDS output). The continuous Hill model with an unspecified exponent could not be fitted to the data due to lack of degrees of freedom (DOFs) since the number of dose groups in the modeled data set were equal to or less than the number of parameters estimated in the Hill dose response model. The results are shown in Table B-9. Even the Hill model gave a satisfactory fit to the data by specifying an exponent of 1 or 2, either of which is a plausible value. The lowest AIC occurred when the data were fit by a quadratic (polynomial of degree 2) model with constant variance, but the linear model with constant variance had an AIC value close to that of the quadratic and also provided an adequate fit to the data. The quadratic model with constant variance was therefore selected and provided a BMCL estimate of 848 ppm n-hexane.

Visual inspection of a plot of the predicted and observed means also indicated a reasonable fit of these models to the data overall, especially in the range nearest the estimated BMCL to be used as a point of departure (see BMDS outputs). The model-predicted BMC value associated with an approximate decrease in mean pup body weight of 10.6 % was 1540 ppm. The corresponding BMCL value was 848 ppm.

The estimated BMCL of 848 ppm for a quadratic model with constant variance (Table

B-9) is reasonably robust to model specification or selection among models that fit the data well: quadratic with nonconstant variance, BMCL = 838 ppm; Hill with exponent = 1 and constant variance, 835 ppm; and Hill with exponent 2 and constant variance, 873 ppm. The power function models did not fit the mean fetal body weight reduction data as well but produced modestly larger (23% and 26%, respectively) BMCL values with either constant or nonconstant variance. The linear model with constant variance has nearly as low an AIC value as the quadratic, does not fit as well as the quadratic, power, or Hill models, and has an estimated BMCL about three times larger.

Table B-9. Results from fitting BMDS models to mean fetal weight data from Mast et al. (1987)

Dose-response model	Variance model	DOF for AIC	AIC	P(H4) DOF	P(H4)	BMC (ppm)	BMCL (ppm)
Linear	Power	4	–100.12	2	0.1635	3361	2507
	Constant	3	–102.02	2	0.1603	3435	2655
Quadratic	Power	5	–102.4	1	0.2466	1535	838
	Constant	**4**	**–104.37**	**1**	**0.2517**	**1540**	**848**
Power	Power	5	–99.28	1	0.1164	2467	1094
	Constant	4	–101.22	1	0.1166	2506	1120
Hill exponent = 1	Power	5	–100.17	1	0.2097	1839	824
	Constant	4	–102.13	1	0.2130	1858	835
Hill exponent = 2	Power	5	–101.17	1	0.4476	1451	862
	Constant	4	–103.13	1	0.4326	1464	873

Modeling of reduced MCV following 8-, 12-, and 16-week n-hexane inhalation exposure data

The overall observed means and standard deviations for reduced MCV following 8-, 12-, and 16-week exposure to n-hexane from the Huang et al. (1989) study are presented in Table B-10. The data for decreased MCV were presented graphically in the Huang et al. (1989) study as mean MCV ± SEM . The study authors were contacted to obtain the raw data for decreased MCV. Dr. Huang was unable to provide these data due to length of time since the study was completed and his relocation to several other institutions. Thus, these values were taken directly from the graph in this figure by physical measurement AND converted to the scale of the measurements, and the SEM was converted to SD. BMDS version 1.3.2 does not have a time series analysis option, thus the Huang et al. (1989) data were analyzed separately for each of the successive four-week observations, rather than as a time series in which each individual measurement (4, 8, 12, and 16 weeks) may be correlated with those at another week.

Table B-10. Mean reduced MCV in rats exposed to n-hexane for 8, 12, and 16 weeks

Exposure (ppm)	Number of animals	Observed mean MCV[a]	Observed SD
8 weeks exposure			
0	8	11.5	0.8
500	8	8.96	0.8
1200	8	7.83	0.8
3000	8	7.41	1.07
12 weeks exposure			
0	8	11.8	0.8
500	8	8	0.667
1200	8	7.45	1.07
3000	8	4.06	0.8
16 weeks exposure			
0	8	11.7	1.07
500	8	9.91	0.667
1200	8	6.42	1.07
3000	8	2.64	1.07

Source: Huang et al., 1989.

[a]The data for decreased MCV displayed in Table B-10 were presented graphically in Figure 2 of the Huang et al. (1989) study as mean MCV ± SEM . As stated above, these values were taken directly from the graph in this figure by physical measurement and the SEM was converted to SD. The data presented in Table B-10 are the values that were modeled with EPA's BMDS version 1.3.2 and used to determine the POD for the derivation of the RfC for n-hexane in Section 5.2.3. EPA has ascertained that the scale intercept was inadvertently not added back in after estimating MCVs from Figure 2 of the Huang et al. (1989) data. The corrected mean MCV values were run in the most current version of EPA's BMDS, version 1.4.1.C, and the BMD modeling results have been included in this appendix (Outputs B-9, 10, and 11).

The Hill model provided the best fit to the data for 8-, 12-, and 16-week exposure to n-hexane, but the parameters for the 12-week exposure duration were substantially different from those for the 8-week and 16-week exposure duration data (Table B-11). The best fitting model for 8- and 16-week exposure was the constant-variance Hill dose-response function with the exponent specified as equal to 2 (Table B-11). The Hill model for 12 weeks exposure had P(H4) = 0.3132 and an AIC = 27.35, the second best fitting modeling results. However, when the Hill exponent was estimated from the 12-week exposure data and a constant-variance model was assumed, the AIC was only slightly larger at 27.36 and the value of P(H4) = 0.5655 was much larger. The goodness of fit statistics P(H4) for the models with the Hill exponent = 2 were < 0.002 and considered inadequate. The AIC values for these models were about 10 units higher than for the smaller values of the shape parameter (specified as 1 or estimated as 0.6), so the value of 2 was rejected. We cannot offer any explanation for this difference, but note that the best empirical estimate for the shape parameter was about 0.6, even further from the value of 2 used to model the other two exposure durations. The variance function P(H3) = 0.3946 fit the variances well and the chi-squared residuals were much smaller for exponents fixed at 1 or estimated at 0.6, than when the shape parameter was set equal to 2.

For the 12-week exposure data, a BMCL of 121.6 ppm n-hexane was estimated from a

constant-variance model in which exponent or shape parameter in the Hill dose-response function was specified as 1, but estimating the shape parameter from the data as about 0.604 yielded a BMCL of 34.7 ppm, greater than threefold lower. The numerical stability of the estimates from the model with the shape parameter estimated from the data is doubtful, as all four of the Hill model parameters were estimated from only four dose groups and the standard error of the shape parameter could not be estimated. The BMCL = 121.6 ppm appears to be a more reliable estimate.

Table B-11. Results of fitting the Hill function dose-response model to rat MCV data for 8, 12, and 16-week exposures to n-hexane (Huang et al., 1989)

Hill model exponent		Variance model exponent		DOF for AIC	AIC	DOF for P(H4)	P(H4)	BMC (ppm)	BMCL (ppm)	$BMCL_{HEC}$ (mg/m^3)
Fixed	Estimated	Fixed	Estimated							
2	NA	NA	0.525	5	39.97	1	0	448	217	382.4
2	NA	0	NA	4	38.3	2	0.0013	396	310	546.3
1	NA	NA	–0.127	5	29.32	1	0.1301	150.5	111.4	196.3
1	NA	0	NA	4	27.35	2	0.3132	156.3	121.6	214.3
NA	0.607	NA	–0.134	6	29.34	0	NE	60.5	NE	NE
NA	0.604	0	NA	5	27.36	1	0.5655	69.2	34.7	61.2

NA = Not applicable.
NE = Cannot be estimated.

The BMCL (1SD) of 121.6 ppm (430 mg/m^3) for decreased MCV in rats exposed to n-hexane for 12 weeks was chosen as the point of departure based on the sensitivity of this neurological effect following n-hexane exposure and the confidence in the modeling results from this study at low doses. For the Huang et al. (1989) data set, the excess risk is equivalent to an approximately 6.8% change in response. A summary of the BMD modeling results of the Huang et al. (1989), Ono et al. (1982), and Mast (1987) data is presented in Table B-12.

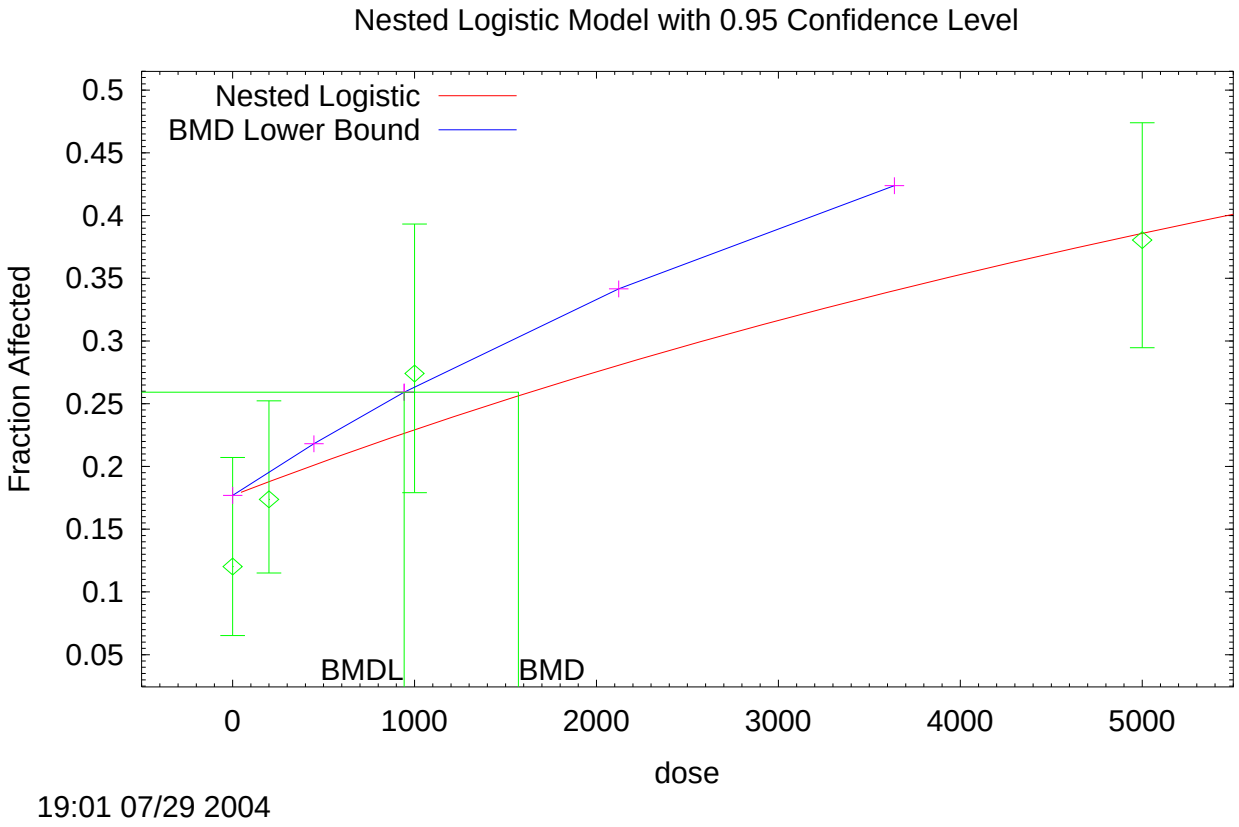
Table B-12. Benchmark dose modeling results of n-hexane inhalation toxicity studies for selection of the principal study

Reference	Endpoint	Dose groups	Model	Fixed parameters	Goodness of fit *p*-value	AIC	BMC[a] (ppm)	BMCL[a] (ppm)	$BMCL_{HEC}$ (mg/m^3)

Mast, 1987	Reduced ossification of sternebrae 1–4	4	Nested logistic	n ≥ 1	<0.0001 (group: 0.1834)[c]	1433	1571	943	2770
Mast, 1987	Decreased fetal body weight gain	4	Quadratic	n ≥ 1	0.2517	-104.37	1540	848	1494
Huang et al., 1989[d]	MCV 8 weeks	4	Hill	rho = 0 n = 2	0.789	27.22	198	143	252
Huang et al., 1989[d]	MCV 12 weeks	4	Hill	rho = 0 n = 1	0.313	27.35	156	122	215
Huang et al., 1989[d]	MCV 16 weeks	4	Hill	rho = 0 n = 2	0.779	34.88	367	321	566
Ono et al., 1982	MCV	3	Power	rho = 3.5 n = 0.75	0.3745	92.84	120	85.4	150.5
Ono et al., 1982	Mixed MCV (total)	3	Power	rho = 2 n = 0.5	< 0.0001	78.39	60	33	58.1
Ono et al., 1982	Mixed MCV (distal)	3	Power	rho = 2 n = 0.5	< 0.0001	65.82	51	28	49.3

[a] BMR = 10% for quantitative endpoints and BMR = 1 SD for continuous endpoints.
[b] $BMCL_{HEC}$ presented in mg/m^3 and conversion described in Section 5.2.2.1.
[c] All models have been adjusted for either total litter size (late resorptions) or number of viable fetuses for other endpoints. The nested module estimates *p*-value for goodness of fit by applying a chi-squared test to data grouped by the strata or levels of these covariates as well as by dose. The chi-squared goodness of fit test may give different results than the standard methods used elsewhere in this table.
[d] The corrected mean MCV values from Huang et al. (1989) data were run in the most current version of EPA's BMDS version 1.4.1.C. The BMD modeling results for these corrected data have been added to this appendix in Outputs B-9, 10, and 11 for informational purposes. These BMD modeling results provided similar BMCLs to those presented in Table B-12 above. Thus, no revision to the POD and derivation of the RfC for n-hexane has been included.

Output B-1: Nested, logistic model results for reduced ossification of sternebrae 1-4 in rats data from Mast (1987)



Parameter constraints: Exponent n $\geq$ 1.
Covariate: Number of implanted sites in the litter, including those resorbed.
Benchmark Response: BMR = 0.10 (nested quantal endpoint).

BMDL(0.10, 95% confidence) = 943 ppm n-hexane.

The probability function is:
Prob. = alpha + theta1*Rij + [1 - alpha - theta1*Rij]/ [1+exp(-beta-theta2*Rij-rho*log(Dose))],
where Rij is the litter specific covariate.
Restrict Power rho >= 1.

Total number of observations = 102
Total number of records with missing values = 0
Total number of parameters in model = 9
Total number of specified parameters = 0

Maximum number of iterations = 1250
Relative Function Convergence has been set to: 1e-008

Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
alpha = 0.174419
beta = -9.52447
theta1 = 0
theta2 = 0
rho = 1
phi1 = 0.29301
phi2 = 0.130921
phi3 = 0.279439
phi4 = 0.178497

Parameter Estimates

variable	estimate	standard error
alpha	0.416634	0.140121
beta	-10.3618	2.39704
theta	-0.0161654	0.0172964
theta 2	0.0543009	0.151612
rho	1	bounded
phi 1	0.274038	0.13398
phi 2	0.122715	0.0625457
phi 3	0.272467	0.0889558
phi 4	0.178054	0.0654785

Analysis of Deviance

Model	Log(likelihood)	Deviance	Test	DF	P-value
Full model	-582.697				
Fitted model	-708.705	252.015		94	2.2259458e-016
Reduced model	-832.35	499.306		101	<0.0001

AIC: 1433.41

Litter Data

Dose	Lit.-Spec. Cov.	Est._Prob.	Litter Size	chi-squared Expected	Observed	Residual
0.0000	9.0000	0.271	9	2.440	9	2.7529
0.0000	10.0000	0.255	10	2.550	3	0.1754
0.0000	11.0000	0.239	11	2.627	1	-0.5949
0.0000	12.0000	0.223	12	2.672	0	-0.9253
0.0000	12.0000	0.223	12	2.672	0	-0.9253
0.0000	12.0000	0.223	12	2.672	2	-0.2327
0.0000	13.0000	0.206	13	2.684	1	-0.5573

0.0000	13.0000	0.206	13	2.684	0	-0.8881
0.0000	14.0000	0.190	14	2.664	0	-0.8493
0.0000	15.0000	0.174	15	2.612	0	-0.8087
0.0000	15.0000	0.174	15	2.612	0	-0.8087
0.0000	16.0000	0.158	16	2.528	0	-0.7664
0.0000	16.0000	0.158	16	2.528	2	-0.1600
0.0000	16.0000	0.158	16	2.528	5	0.7496
0.0000	16.0000	0.158	16	2.528	0	-0.7664
0.0000	16.0000	0.158	16	2.528	3	0.1432
0.0000	17.0000	0.142	17	2.411	6	1.0753
0.0000	17.0000	0.142	17	2.411	2	-0.1231
0.0000	17.0000	0.142	17	2.411	1	-0.4227
0.0000	18.0000	0.126	18	2.262	0	-0.6761
0.0000	18.0000	0.126	18	2.262	2	-0.0783
0.0000	19.0000	0.109	19	2.080	1	-0.3259
0.0000	19.0000	0.109	19	2.080	3	0.2774
200.0000	9.0000	0.279	9	2.507	3	0.2603
200.0000	10.0000	0.263	10	2.630	2	-0.3119
200.0000	12.0000	0.232	12	2.784	3	0.0965
200.0000	13.0000	0.217	13	2.815	0	-1.2054
200.0000	13.0000	0.217	13	2.815	3	0.0793
200.0000	13.0000	0.217	13	2.815	0	-1.2054
200.0000	13.0000	0.217	13	2.815	1	-0.7772
200.0000	13.0000	0.217	13	2.815	3	0.0793
200.0000	13.0000	0.217	13	2.815	8	2.2205
200.0000	14.0000	0.201	14	2.816	8	2.1457
200.0000	15.0000	0.186	15	2.787	2	-0.3168
200.0000	15.0000	0.186	15	2.787	2	-0.3168
200.0000	15.0000	0.186	15	2.787	2	-0.3168
200.0000	15.0000	0.186	15	2.787	0	-1.1221
200.0000	15.0000	0.186	15	2.787	2	-0.3168
200.0000	16.0000	0.170	16	2.728	4	0.5018
200.0000	16.0000	0.170	16	2.728	3	0.1073
200.0000	17.0000	0.155	17	2.640	0	-1.0268
200.0000	17.0000	0.155	17	2.640	0	-1.0268
200.0000	17.0000	0.155	17	2.640	5	0.9183
200.0000	17.0000	0.155	17	2.640	2	-0.2488
200.0000	17.0000	0.155	17	2.640	0	-1.0268
200.0000	18.0000	0.140	18	2.522	6	1.3444
200.0000	18.0000	0.140	18	2.522	2	-0.2017
1000.0000	2.0000	0.405	2	0.811	2	1.5187
1000.0000	9.0000	0.307	9	2.762	3	0.0965
1000.0000	9.0000	0.307	9	2.762	0	-1.1194
1000.0000	13.0000	0.254	13	3.305	7	1.1389
1000.0000	13.0000	0.254	13	3.305	9	1.7554
1000.0000	13.0000	0.254	13	3.305	1	-0.7106
1000.0000	14.0000	0.242	14	3.382	2	-0.4050
1000.0000	14.0000	0.242	14	3.382	9	1.6457
1000.0000	15.0000	0.229	15	3.438	12	2.3972
1000.0000	15.0000	0.229	15	3.438	1	-0.6825
1000.0000	15.0000	0.229	15	3.438	13	2.6771
1000.0000	15.0000	0.229	15	3.438	2	-0.4025

1000.0000	15.0000	0.229	15	3.438	2	-0.4025
1000.0000	15.0000	0.229	15	3.438	1	-0.6825
1000.0000	16.0000	0.217	16	3.472	1	-0.6648
1000.0000	16.0000	0.217	16	3.472	4	0.1419
1000.0000	16.0000	0.217	16	3.472	11	2.0243
1000.0000	16.0000	0.217	16	3.472	3	-0.1270
1000.0000	16.0000	0.217	16	3.472	0	-0.9337
1000.0000	16.0000	0.217	16	3.472	0	-0.9337
1000.0000	16.0000	0.217	16	3.472	6	0.6797
1000.0000	17.0000	0.205	17	3.486	6	0.6522
1000.0000	17.0000	0.205	17	3.486	0	-0.9046
1000.0000	17.0000	0.205	17	3.486	0	-0.9046
1000.0000	18.0000	0.193	18	3.482	5	0.3818
1000.0000	18.0000	0.193	18	3.482	6	0.6332
1000.0000	18.0000	0.193	18	3.482	2	-0.3726
5000.0000	6.0000	0.442	6	2.651	3	0.2086
5000.0000	8.0000	0.427	8	3.417	1	-1.1527
5000.0000	10.0000	0.414	10	4.143	4	-0.0571
5000.0000	12.0000	0.404	12	4.843	11	2.1063
5000.0000	14.0000	0.395	14	5.529	7	0.4418
5000.0000	14.0000	0.395	14	5.529	8	0.7421
5000.0000	14.0000	0.395	14	5.529	6	0.1415
5000.0000	14.0000	0.395	14	5.529	0	-1.6603
5000.0000	14.0000	0.395	14	5.529	8	0.7421
5000.0000	14.0000	0.395	14	5.529	3	-0.7594
5000.0000	15.0000	0.391	15	5.871	4	-0.5296
5000.0000	15.0000	0.391	15	5.871	0	-1.6619
5000.0000	15.0000	0.391	15	5.871	2	-1.0958
5000.0000	15.0000	0.391	15	5.871	4	-0.5296
5000.0000	15.0000	0.391	15	5.871	2	-1.0958
5000.0000	15.0000	0.391	15	5.871	8	0.6026
5000.0000	15.0000	0.391	15	5.871	1	-1.3789
5000.0000	16.0000	0.388	16	6.216	9	0.7454
5000.0000	16.0000	0.388	16	6.216	4	-0.5931
5000.0000	16.0000	0.388	16	6.216	10	1.0132
5000.0000	16.0000	0.388	16	6.216	11	1.2809
5000.0000	16.0000	0.388	16	6.216	8	0.4777
5000.0000	16.0000	0.388	16	6.216	12	1.5486
5000.0000	17.0000	0.386	17	6.564	4	-0.6510
5000.0000	17.0000	0.386	17	6.564	9	0.6186
5000.0000	18.0000	0.384	18	6.918	9	0.5028
5000.0000	18.0000	0.384	18	6.918	3	-0.9460
5000.0000	19.0000	0.383	19	7.279	5	-0.5245

Combine litters with adjacent levels of the litter-specific covariate within dose groups until the expected count exceeds 3.0, to help improve the fit of the X^2 statistic to chi-squared.

Grouped Data

Dose	Mean Lit.-Spec. Cov.	Expected	chi-squared Observed	Residual
0.0000	9.5000	4.990	12	2.0018

0.0000	11.5000	5.299	1	-1.0809
0.0000	12.0000	5.344	2	-0.8188
0.0000	13.0000	5.369	1	-1.0221
0.0000	14.5000	5.277	0	-1.1718
0.0000	15.5000	5.140	0	-1.1134
0.0000	16.0000	10.111	10	-0.0168
0.0000	17.0000	7.233	9	0.3057
0.0000	18.0000	4.524	2	-0.5334
0.0000	19.0000	4.161	4	-0.0343
200.0000	9.5000	5.137	5	-0.0496
200.0000	12.5000	5.598	3	-0.8028
200.0000	13.0000	14.074	15	0.1774
200.0000	14.5000	5.602	10	1.2692
200.0000	15.0000	11.147	6	-1.0362
200.0000	16.0000	5.456	7	0.4307
200.0000	17.0000	13.198	7	-1.0783
200.0000	18.0000	5.044	8	0.8080
1000.0000	5.5000	3.572	5	0.5515
1000.0000	11.0000	6.067	7	0.2289
1000.0000	13.0000	6.610	10	0.7388
1000.0000	14.0000	6.765	11	0.8773
1000.0000	15.0000	20.627	31	1.1856
1000.0000	16.0000	24.305	25	0.0706
1000.0000	17.0000	10.459	6	-0.6680
1000.0000	18.0000	10.445	13	0.3709
5000.0000	7.0000	6.068	4	-0.7711
5000.0000	10.0000	4.143	4	-0.0571
5000.0000	12.0000	4.843	11	2.1063
5000.0000	14.0000	33.172	32	-0.1437
5000.0000	15.0000	41.098	21	-2.1502
5000.0000	16.0000	37.293	54	1.8260
5000.0000	17.0000	13.128	13	-0.0229
5000.0000	18.0000	13.836	12	-0.3134
5000.0000	19.0000	7.279	5	-0.5245

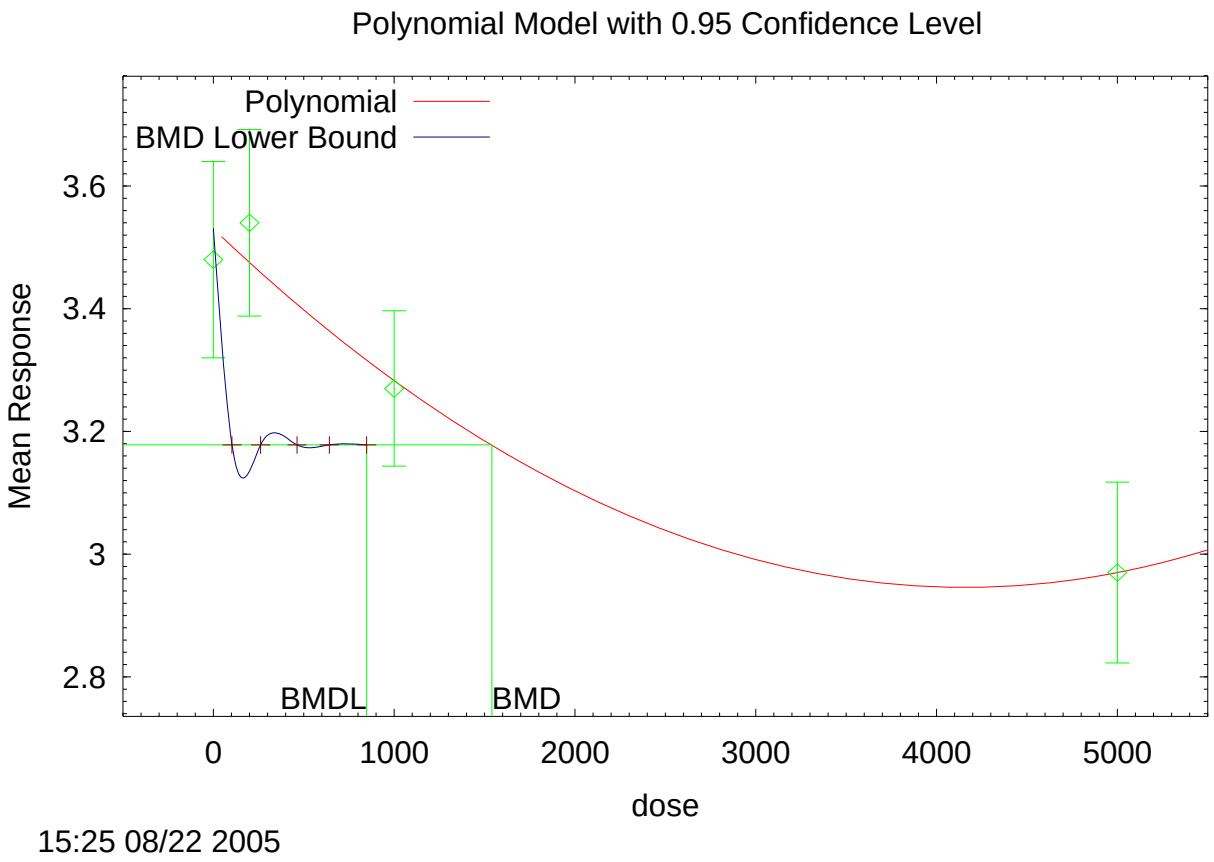
Chi-square = 32.30 DF = 26 P-value = 0.1834

To calculate the BMD and BMDL, the litter specific covariate is fixed at the mean litter specific covariate of control group: 14.826087

Benchmark Dose Computation Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95

BMD = 1571.05 BMDL = 943.119

Output B-2: Continuous Hill model results for mean fetal body weight in rats from Mast (1987)




```
==========================================================================
  Polynomial Model.  Revision: 2.2  Date: 9/12/2002
 Input Data File: U:\IRIS\N-hexanE\MAST\MASTRATFW082205.(d)
 Gnuplot Plotting File:  U:\IRIS\N-hexanE\MAST\MASTRATFW082205.plt
                          Mon Aug 22 15:25:53 2005
==========================================================================

Mast Rat Fetal Wt by Litter Quadratic model rho==0
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

  The form of the response function is:

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

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

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

User Inputs Initial Parameter Values

alpha =	1	
rho =	1	Specified
beta_0 =	3	
beta_1 =	-0.001	
beta_2 =	0	

Parameter Estimates

			95.0% Wald Confidence Interval	
Variable	Estimate	Std. Err.	Lower Conf. Limit	Upper Conf. Limit
alpha	0.124672	0.0174576	0.090456	0.158889
beta_0	3.53112	0.0584714	3.41652	3.64572
beta_1	-0.000281312	0.000116308	-0.000509272	-5.33529e-005
beta_2	3.38002e-008	2.19346e-008	-9.19091e-009	7.67912e-008

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	beta_0	beta_1	beta_2
alpha	1	1.8e-007	-2.6e-007	2.6e-007
beta_0	1.8e-007	1	-0.7	0.64
beta_1	-2.6e-007	-0.7	1	-0.99
beta_2	2.6e-007	0.64	-0.99	1

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	23	3.48	0.37	3.53	0.353	-0.694
200	24	3.54	0.36	3.48	0.353	0.885
1000	27	3.27	0.32	3.28	0.353	-0.2
5000	28	2.97	0.38	2.97	0.353	0.00656

Model Descriptions for likelihoods calculated

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

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

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

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	55.842420	5	-101.684839
A2	56.285063	8	-96.570125
fitted	55.185409	3 4	-104.370819 -102.370819
R	37.662042	2	-71.324083

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

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	37.246	6	<.0001
Test 2	0.885286	3	0.829
Test 3	1.31402	1	0.2517

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

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

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

Benchmark Dose Computation
Specified effect = 1

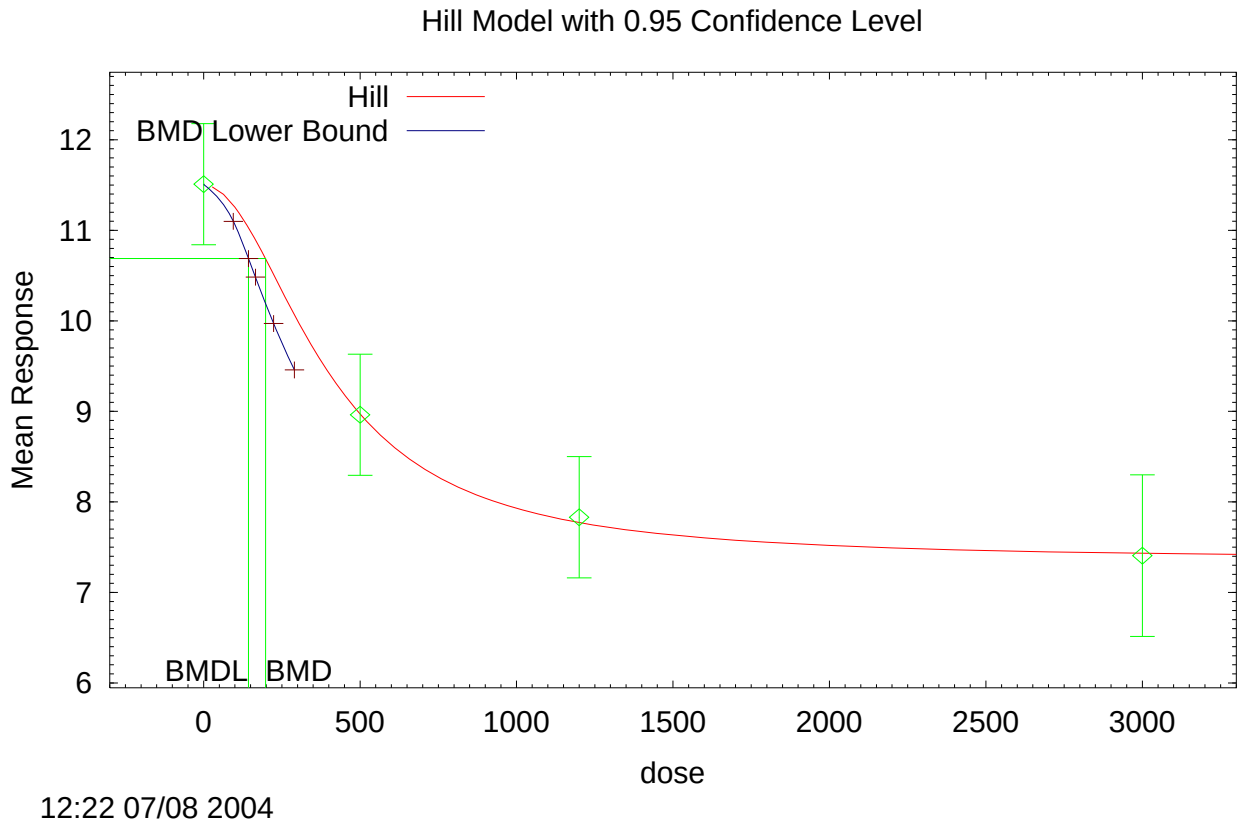
Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 1540.16

BMDL = 848.016

Output B-3: Continuous, Hill model results for decreased MCV in rats following 8 weeks exposure to n-hexane from Huang et al. (1989)



Parameter constraints: Rho = 0 (constant variance) and n = 2.
Benchmark Response: BMR = 1 standard deviation of control group.

BMDL(1 std. Dev., 95% confidence) = 143 ppm n-hexane.

The form of the response function is:
Y[dose] = intercept + v*dose^n/(k^n + dose^n)
Dependent variable = MEAN
Independent variable = HEXANE_CONC
rho is set to 0
n is set to 2
Power parameter is not restricted
The variance is to be modeled as Var(i) = alpha * mean(i) ^ rho

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

User Inputs Initial Parameter Values
alpha = 0.1
rho =1 Specified
intercept = 12
v = -3
n = 1 Specified
k = 100

Asymptotic Correlation Matrix of Parameter Estimates

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

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	0.67071	1
rho	0	1
intercept	11.5075	1
v	-4.13841	1
n	2	1
k	398.844	1

Data and Estimated Values of Interest

dose	n	observed mean	observed standard deviation	estimated mean	estimated standard deviation	chi square
0	8	11.5	0.8	11.5	0.819	0.00239
500	8	8.96	0.8	8.98	0.819	-0.0197
1200	8	7.83	0.8	7.78	0.819	0.0604
3000	8	7.41	1.07	7.44	0.819	-0.0431

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

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

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = alpha*(Mu(i))^rho

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

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-9.585726	5	29.171453
A2	-9.044284	8	34.088568
A3	-9.371958	6	30.743917
fitted	-9.609309	4	27.218617
R	-35.201386	2	74.402771

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test	df	p-value
Test 1	52.3142		6	<.0001
Test 2	1.08288		3	0.7812
Test 3	0.655348		2	0.7206
Test 4	0.474701	2	0.7887	

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

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

The p-value for Test 3 is greater than .05. The modeled variance appears
to be appropriate here

The p-value for Test 4 is greater than .05. The model chosen seems
to adequately describe the data

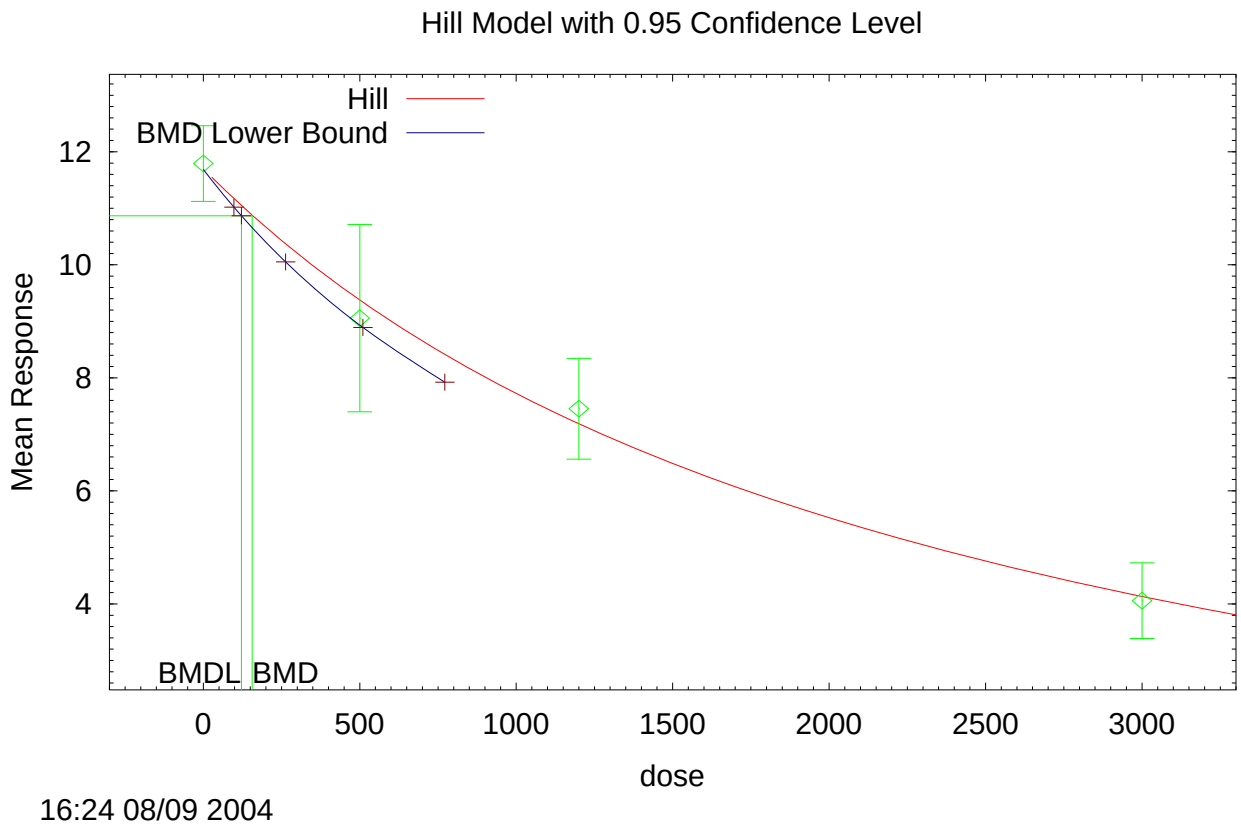
Benchmark Dose Computation
Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 198.109
BMDL = 143.112

Output B-4: Continuous, Hill model results for decreased MCV in rats following 12 weeks exposure to n-hexane from Huang et al. (1989)



Parameter constraints: Rho = 0 (constant variance) and n = 1.
Benchmark Response: BMR = 1 standard deviation of control group.

BMDL(1 std. Dev., 95% confidence) = 122 ppm n-hexane.

The form of the response function is:
Y[dose] = intercept + v*dose^n/(k^n + dose^n)

Dependent variable = MEAN
Independent variable = HEXANE_CONC
rho is set to 0
n is set to 1
Power parameter restricted to be greater than 1
The variance is to be modeled as Var(i) = alpha * mean(i) ^ rho

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

User Inputs Initial Parameter Values
alpha = 0.0001
rho = 1 Specified
intercept = 0
v = -1
n = 1 Specified
k = 1000

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

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

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	0.673532	1
rho	0	1
intercept	11.6883	1
v	-13.7905	1
k	2469.9	1

Data and Estimated Values of Interest

dose	n	observed mean	observed standard deviation	estimated mean	estimated standard deviation	chi square
0	8	11.8	0.8	11.7	0.821	0.127
500	8	9.06	0.667	9.37	0.821	-0.378
1200	8	7.45	1.07	7.18	0.821	0.334
3000	8	4.06	0.8	4.12	0.821	-0.083

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

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

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = alpha*(Mu(i))^rho

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

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-8.528285	5	27.056569
A2	-7.585712	8	31.171423
A3	-8.515700	6	29.031399
fitted	-9.676481	3	25.352961
R	-50.666148	2	105.332296

Explanation of Tests
Test 1: Does response and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test	df	p-value	
Test 1	86.1609		6	<.0001	
Test 2	1.88515		3	0.5966	
Test 3	1.85998		2	0.3946	
Test 4	2.32156		2		0.3132

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

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

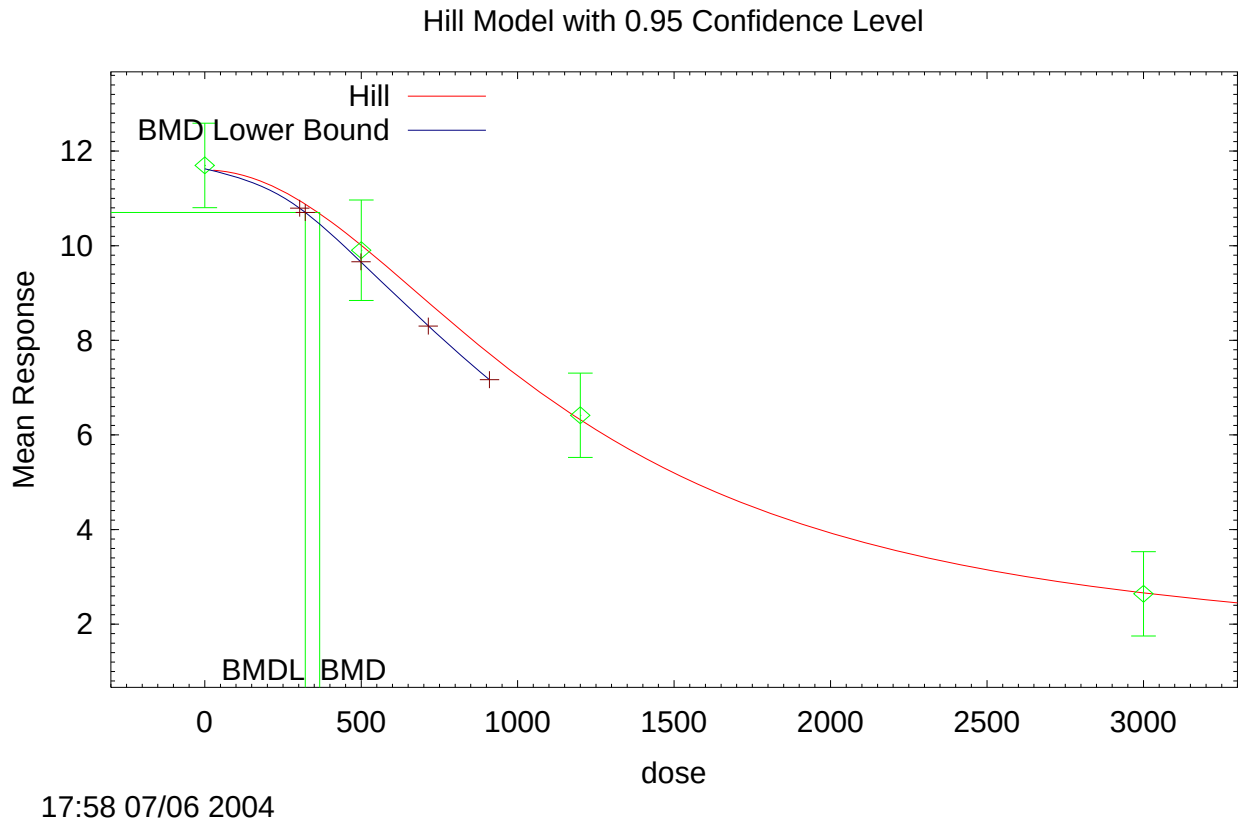
The p-value for Test 3 is greater than .05. The modeled variance appears
to be appropriate here

The p-value for Test 4 is greater than .05. The model chosen seems
to adequately describe the data

Benchmark Dose Computation
Specified effect = 1

Risk Type = Estimated standard deviations from the control mean
Confidence level = 0.95
BMD = 156.287
BMDL = 121.613

Output B-5: Continuous, Hill model results for decreased MCV in rats following 16 weeks exposure to n-hexane from Huang et al. (1989)



Benchmark Response: BMR = 1 standard deviation of control group.

BMDL(1 std. Dev., 95% confidence) = 321 ppm n-hexane.

The form of the response function is:
Y[dose] = intercept + v*dose^n/(k^n + dose^n)

Dependent variable = MEAN
Independent variable = HEXANE_CONC
rho is set to 0
n is set to 2
Power parameter is not restricted not restricted
The variance is to be modeled as Var(i) = alpha * mean(i) ^ rho
Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

User Inputs Initial Parameter Values

alpha = 0.1
rho = 1 Specified
intercept = 20
v = -10
n = 1 Specified
k = 1000

Asymptotic Correlation Matrix of Parameter Estimates

Parameter Estimates

Variable	Estimate	Std. Err.	
alpha	0.852015		1
rho	0	1	
intercept	11.6245		1
v	-10.3269	1	
n	2		1
k	1171.69	1	

Data and Estimated Values of Interest

dose	n	observed mean	observed standard deviation	estimated mean	estimated standard deviation	chi square
0	8	11.7	1.07	11.6	0.923	0.0797
500	8	9.91	0.667	10	0.923	-0.139
1200	8	6.42	1.07	6.34	0.923	0.0837
3000	8	2.64	1.07	2.66	0.923	-0.0248

Model Descriptions for likelihoods calculated

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

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

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = alpha*(Mu(i))^rho

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

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-13.304172	5	36.608345
A2	-12.188625	8	40.377250
A3	-13.188105	6	38.376209
fitted	-13.437576	4	34.875151
R	-57.381404	2	118.762807

Explanation of Tests
Test 1: Does response and/or variances differ among Dose levels?

(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio) Test	df	p-value
Test 1	90.3856	6	<.0001
Test 2	2.2311	3	0.5258
Test 3	1.99896	2	0.3681
Test 4	0.498942	2	0.7792

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

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

The p-value for Test 3 is greater than .05. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .05. The model chosen seems to adequately describe the data

Benchmark Dose Computation
Specified effect = 1

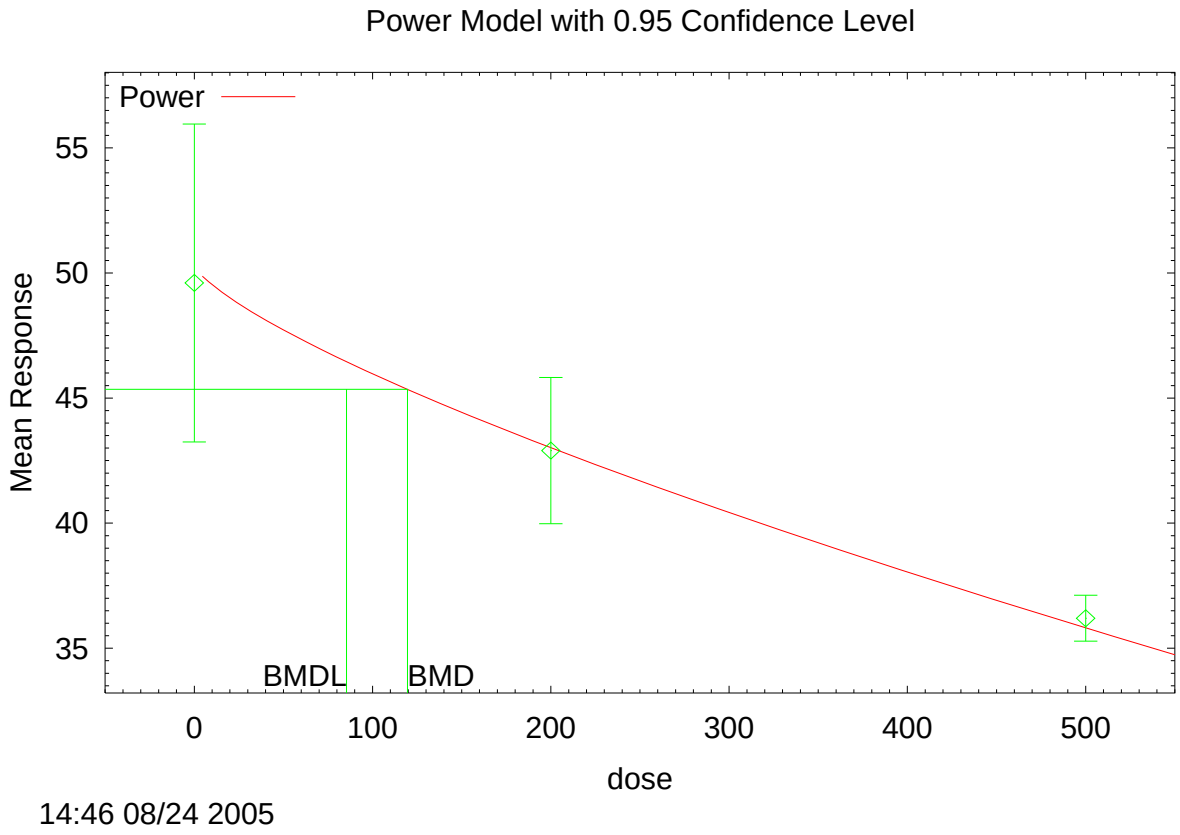
Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 367.09

BMDL = 321.332

Output B-6: Power model results for decreased MCV in rats following exposure to n-hexane from Ono et al. (1982)




```
==========================================================================
     Power Model.  $Revision: 2.1 $ $Date: 2000/10/11 20:57:36 $
    Input Data File: U:\IRIS\N-hexanE\ONO\ONO_RAT.(d)
    Gnuplot Plotting File:  U:\IRIS\N-hexanE\ONO\ONO_RAT.plt
                                Wed Aug 24 14:46:47 2005
==========================================================================

 Ono Rat MCV Power Model rho==3.5  n==0.75
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

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


   Dependent variable = MEAN
```

Independent variable = DOSE
rho is set to 3.5
power is set to 0.75
The variance is to be modeled as Var(i) = alpha*mean(i)^rho

Total number of dose groups = 3
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

User Inputs Initial Parameter Values

alpha =	0.001	
rho =	1	Specified
control =	59	
slope =	-0.0001	
power =	1	Specified

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	rho	control	slope	power
alpha	1	-1	-0.37	0.27	0.26
rho	-1	1	0.36	-0.27	-0.25
control	-0.37	0.36	1	-0.64	-0.59
slope	0.27	-0.27	-0.64	1	1
power	0.26	-0.25	-0.59	1	1

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	2.71502e-005	0.000344776
rho	3.5	3.28236

control	50.2999	1.91213
slope	-0.136897	0.23451
power	0.75	0.2655

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	8	49.6	7.6	50.3	4.95	-0.141
200	8	42.9	3.5	43	3.77	-0.0317
500	8	36.2	1.1	35.8	2.73	0.137

Model Descriptions for likelihoods calculated

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

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

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = alpha*(Mu(i))^rho

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

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-48.403560	4	104.807120
A2	-37.407394	6	86.814789
A3	-42.464475	5	94.928950
fitted	-43.446606	3	92.893212
R	-59.621629	2	123.243258

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	d.f	p-value
Test 1	44.4285	4	<.00001
Test 2	21.9923	2	1.677e-005
Test 3	10.1142	1	0.001471
Test 4	1.96426	2	0.3745

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

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

The p-value for Test 3 is less than .05. You may want to consider a different variance model

The p-value for Test 4 is greater than .05. The model chosen seems to adequately describe the data

Benchmark Dose Computation
Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

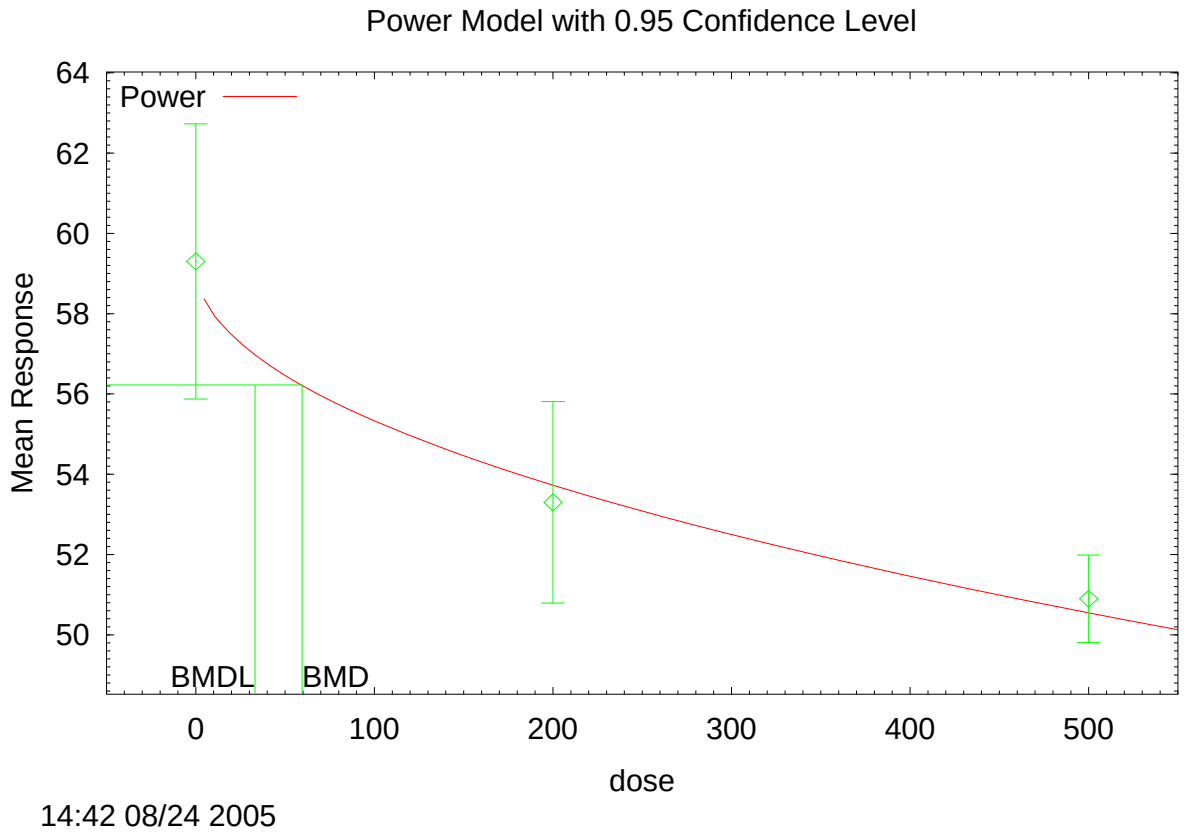
Confidence level = 0.95

BMD = 119.577

BMDL = 85.3538

Output B-7: Power model results for decreased total mixed nerve conduction velocity in rats following exposure to n-hexane from Ono et al. (1982)

==========



Power Model. $Revision: 2.1 $ $Date: 2000/10/11 20:57:36 $
Input Data File: U:\IRIS\N-hexanE\ONO\ONO_RAT.(d)
Gnuplot Plotting File: U:\IRIS\N-hexanE\ONO\ONO_RAT.plt
Wed Aug 24 14:41:58 2005

==========

Ono Rat (Total) MNCV Power Model rho==2 n==0.5

~~~~~~

The form of the response function is:

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

Dependent variable = MEAN
Independent variable = DOSE
~~~~~~

rho is set to 2
power is set to 0.5
The variance is to be modeled as Var(i) = alpha*mean(i)^rho

Total number of dose groups = 3
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

User Inputs Initial Parameter Values

alpha =	0.001	
rho =	1	Specified
control =	59	
slope =	-0.0001	
power =	1	Specified

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	rho	control	slope	power
alpha	1	-1	-0.17	0.1	0.085
rho	-1	1	0.17	-0.098	-0.083
control	-0.17	0.17	1	-0.49	-0.42
slope	0.1	-0.098	-0.49	1	1
power	0.085	-0.083	-0.42	1	1

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	0.00253828	0.0536687
rho	2	5.24144
control	59.2077	1.10404

slope	-0.38656	0.621848
power	0.5	0.256451

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	8	59.3	4.1	59.2	2.98	0.0309
200	8	53.3	3	53.7	2.71	-0.163
500	8	50.9	1.3	50.6	2.55	0.132

Model Descriptions for likelihoods calculated

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

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

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = alpha*(Mu(i))^rho

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

Warning: Likelihood for fitted model larger than the Likelihood for model A3.

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-36.984508	4	81.969016
A2	-32.573332	6	77.146663
A3	-68.919854	5	147.839707
fitted	-36.192522	3	78.385045
R	-48.756302	2	101.512604

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	d.f	p-value
Test 1	32.3659	4	<.00001
Test 2	8.82235	2	0.01214
Test 3	72.693	1	<.00001
Test 4	-65.4547	2	<.00001

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

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

The p-value for Test 3 is less than .05. You may want to consider a different variance model

The p-value for Test 4 is less than .05. You may want to try a different model

Benchmark Dose Computation
Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

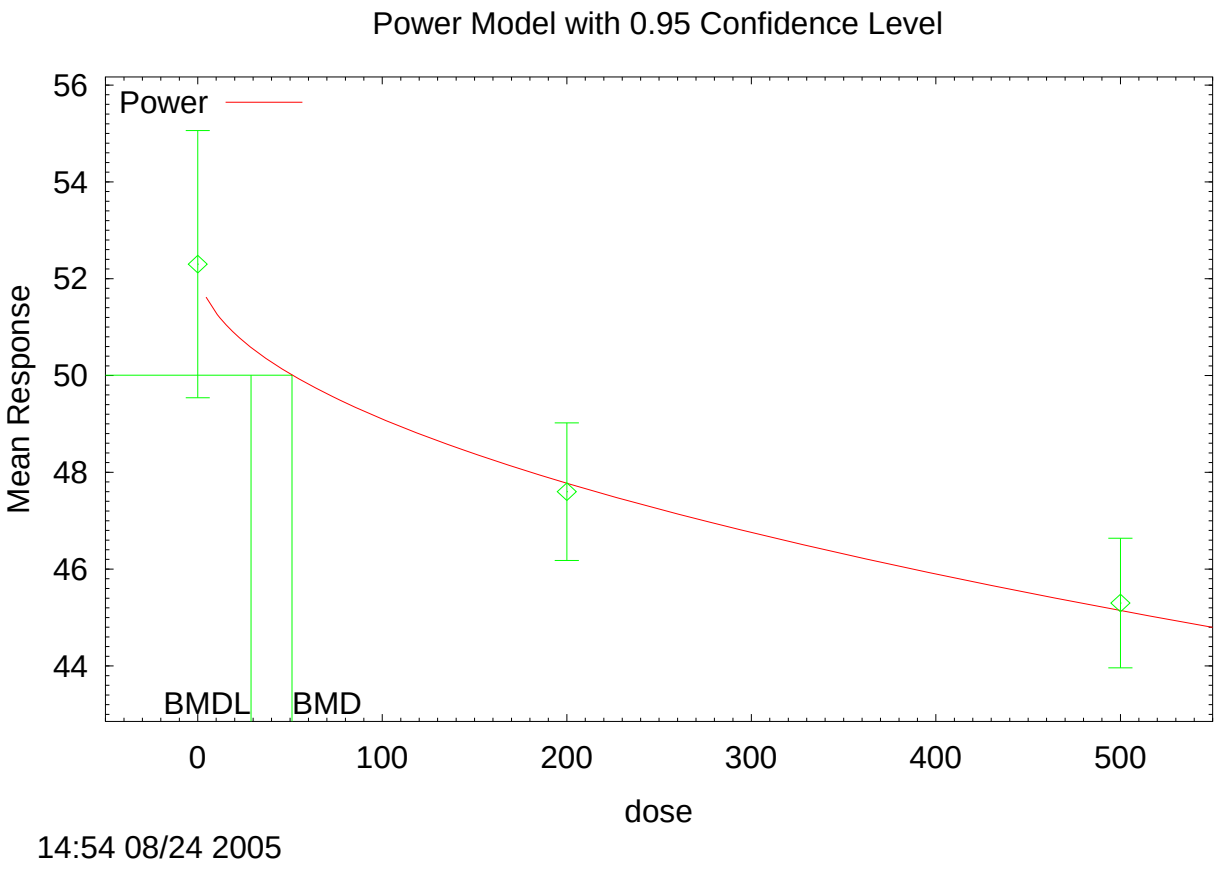
Confidence level = 0.95

BMD = 59.5474

BMDL = 33.1628

Output B-8: Power model results for decreased total distal nerve conduction velocity in rats following exposure to n-hexane from Ono et al. (1982)

==========



Power Model. $Revision: 2.1 $ $Date: 2000/10/11 20:57:36 $
Input Data File: U:\IRIS\N-hexanE\ONO\ONO_RAT.(d)
Gnuplot Plotting File: U:\IRIS\N-hexanE\ONO\ONO_RAT.plt
Wed Aug 24 14:54:16 2005

==========

Ono Rat Distal MNCV Power Model rho==2 n==0.5

~~~~~~

The form of the response function is:

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

Dependent variable = MEAN
~~~~~~

Independent variable = DOSE
rho is set to 2
power is set to 0.5
The variance is to be modeled as Var(i) = alpha*mean(i)^rho

Total number of dose groups = 3
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

User Inputs Initial Parameter Values

alpha =	0.001	
rho =	1	Specified
control =	59	
slope =	-0.0001	
power =	1	Specified

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	rho	control	slope	power
alpha	1	-1	-0.21	0.23	0.22
rho	-1	1	0.21	-0.23	-0.22
control	-0.21	0.21	1	-0.48	-0.4
slope	0.23	-0.23	-0.48	1	0.99
power	0.22	-0.22	-0.4	0.99	1

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	0.00190554	0.0368726
rho	2	4.94577

control	52.2904	0.838647
slope	-0.319513	0.468668
power	0.5	0.234407

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	8	52.3	3.3	52.3	2.28	0.0042
200	8	47.6	1.7	47.8	2.09	-0.0824
500	8	45.3	1.6	45.1	1.97	0.0782

Model Descriptions for likelihoods calculated

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

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

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = alpha*(Mu(i))^rho

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

Warning: Likelihood for fitted model larger than the Likelihood for model A3.

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-30.737669	4	69.475338
A2	-27.954058	6	67.908116
A3	-44.665496	5	99.330992
fitted	-29.909322	3	65.818644

R -43.520950 2 91.041900

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	d.f	p-value
Test 1	31.1338	4	<.00001
Test 2	5.56722	2	0.06181
Test 3	33.4229	1	<.00001
Test 4	-29.5123	2	<.00001

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

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

The p-value for Test 3 is less than .05. You may want to consider a different variance model

The p-value for Test 4 is less than .05. You may want to try a different model

Benchmark Dose Computation
Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

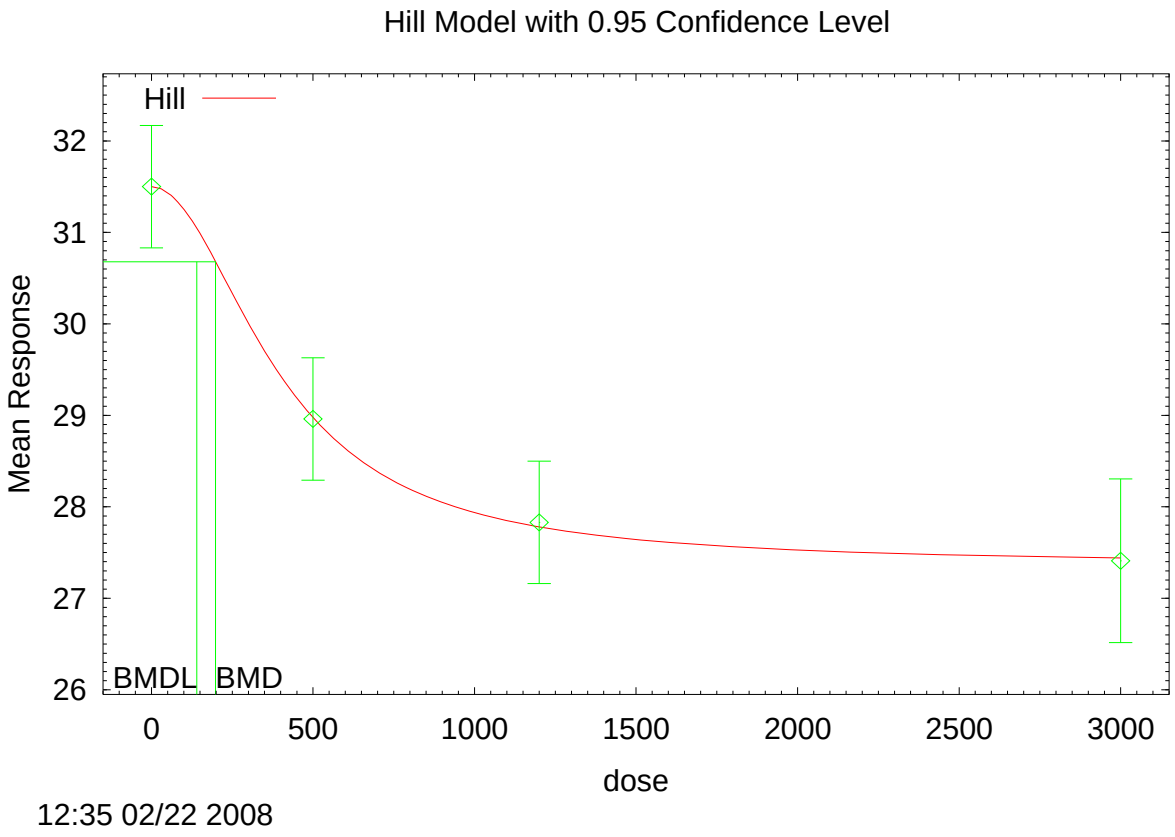
Confidence level = 0.95

BMD = 51.0371

BMDL = 28.8322

EPA ascertained that the scale intercept was inadvertently not added back in after estimating MCVs from Figure 2 of the Huang et al. (1989) data at the time of derivation of the RfC for n-hexane presented in this assessment. The corrected mean MCV values from the Huang et al. (1989) data were run in the current (2008) version of EPA's BMDS version 1.4.1.C. The BMD modeling results for these corrected data are presented below in Outputs B-9, 10, and 11 for informational purposes. These BMD modeling results provided similar BMCLs to those presented in Table B-12. Thus, no revision to the POD and derivation of the RfC for n-hexane has been included.

Output B-9: Continuous, Hill model results using EPA BMDS version 1.4.1C for decreased MCV in rats following 8 weeks exposure to n-hexane from Huang et al. (1989)



The form of the response function is: Y[dose] = intercept + v*dose^n/(k^n + dose^n)

Dependent variable = MEAN
Independent variable = HEXANECONC
rho is set to 0
n is set to 2
Power parameter restricted to be greater than 1
A constant variance model is fit

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

Default Initial Parameter Values
alpha = 0.740162
rho = 0 Specified
intercept = 31.5
v = -4.09
n = 4.26317 Specified
k = 402.559

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	intercept	v	k
alpha	1	-6.1E-007	5E-007	-9.4E-007
intercept	-6.1E-007	1	-0.73	-0.31
v	5E-007	-0.73	1	-0.2
k	-9.4E-007	-0.31	-0.2	1

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	0.671364	5.95802
intercept	31.4981	0
v	-4.12568	6.90392
k	398.583	5.0623

Data and Estimated Values of Interest

dose	n	observed mean	observed SD	estimated mean	estimated SD	chi square
0	8	31.5	0.8	31.5	0.819	0.00649
500	8	29.0	0.8	29.0	0.819	-0.0535
1200	8	27.8	0.8	27.8	0.819	0.164
3000	8	27.4	1.07	27.4	0.819	-0.117

Model Descriptions for likelihoods calculated:

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

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

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = alpha*(Mu(i))^rho

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

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-9.603027	5	29.206054
A2	-9.049322	8	34.098643
A3	-9.603027	5	29.206054
Fitted	-9.624893	4	27.249786
R	-35.128248	2	74.256497

Explanation of Tests
Test 1: Do responses and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value

Test 1	52.1579	6	<.0001
Test 2	1.10741	3	0.7753
Test 3	1.10741	3	0.7753
Test 4	0.0437323	1	0.8344

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

The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

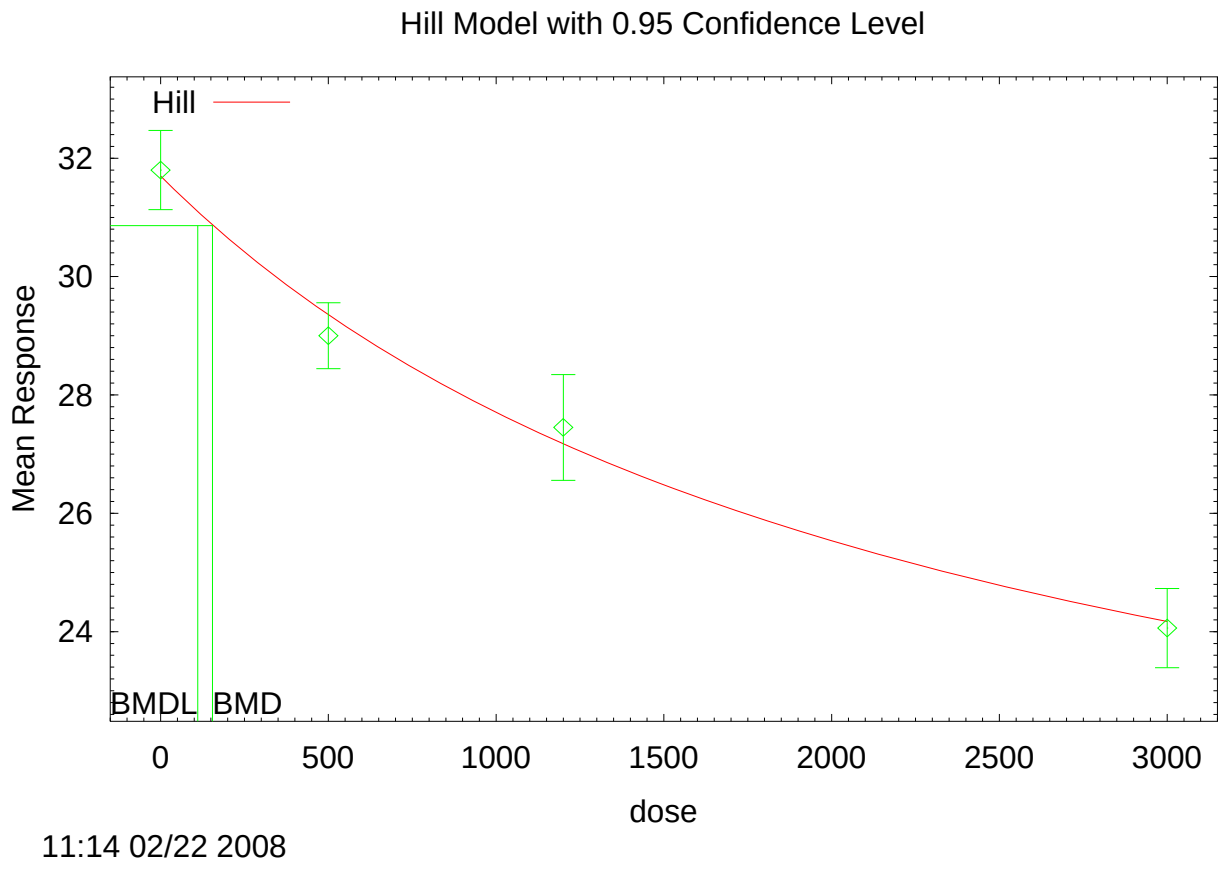
Benchmark Dose Computation

Specified effect = 1
Risk Type = Estimated standard deviations from the control mean
Confidence level = 0.95

BMC = 198.42

BMCL = 140.194

Output B-10: Continuous, Hill model results using EPA BMDS version 1.4.1C for decreased MCV in rats following 12 weeks exposure to n-hexane from Huang et al. (1989)



The form of the response function is: Y[dose] = intercept + v*dose^n/(k^n + dose^n)

Dependent variable = MEAN
Independent variable = HEXANECONC
rho is set to 0
n is set to 1
Power parameter is not restricted
A constant variance model is fit

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

Default Initial Parameter Values
alpha = 0.717447
rho = 0 Specified
intercept = 31.8
v = -7.74
n = 2.09107 Specified
k = 983.226

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	intercept	v	k
alpha	1	7.6E-008	1.4E-007	-1.5E-007
intercept	7.6E-008	1	0.3	-0.56
v	-1.4E-007	0.3	1	-0.94
k	-1.5E-007	-0.56	-0.94	1

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	0.683441	0.17086
intercept	31.6881	0.28884
v	-13.5438	20.336
k	2380.29	7658.052

Data and Estimated Values of Interest

dose	n	observed mean	observed SD	estimated mean	estimated SD	chi square
0	8	31.7	0.8	31.7	0.827	0.383
500	8	29.3	0.667	29.3	0.827	-1.15
1200	8	27.4	1.07	27.1	0.827	1.03
3000	8	24.1	0.8	24.1	0.827	-0.261

Model Descriptions for likelihoods calculated:

Model A1: $Y_{ij} = \mu_i + e_{ij}$
$Var\{e_{ij}\} = \sigma^2$

Model A2: $Y_{ij} = \mu_i + e_{ij}$
$Var\{e_{ij}\} = \sigma_i^2$

Model A3: $Y_{ij} = \mu_i + e_{ij}$

Var{e(ij)} = Sigma^2

Model A3 uses any fixed variance parameters that were specified by the user

Model R: Yi = Mu + e(i)

Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-8.550604	5	27.101208
A2	-7.594748	8	31.189496
A3	-8.550604	5	27.101208
fitted	-9.910165	4	27.820330
R	-50.124466	2	104.248931

Explanation of Tests:

Test 1: Do responses and/or variances differ among Dose levels? (A2 vs. R)

Test 2: Are Variances Homogeneous? (A1 vs A2)

Test 3: Are variances adequately modeled? (A2 vs. A3)

Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)

(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	85.0594	6	<.0001
Test 2	1.91171	3	0.5909
Test 3	1.91171	3	0.5909
Test 4	2.71912	1	0.09915

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

The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

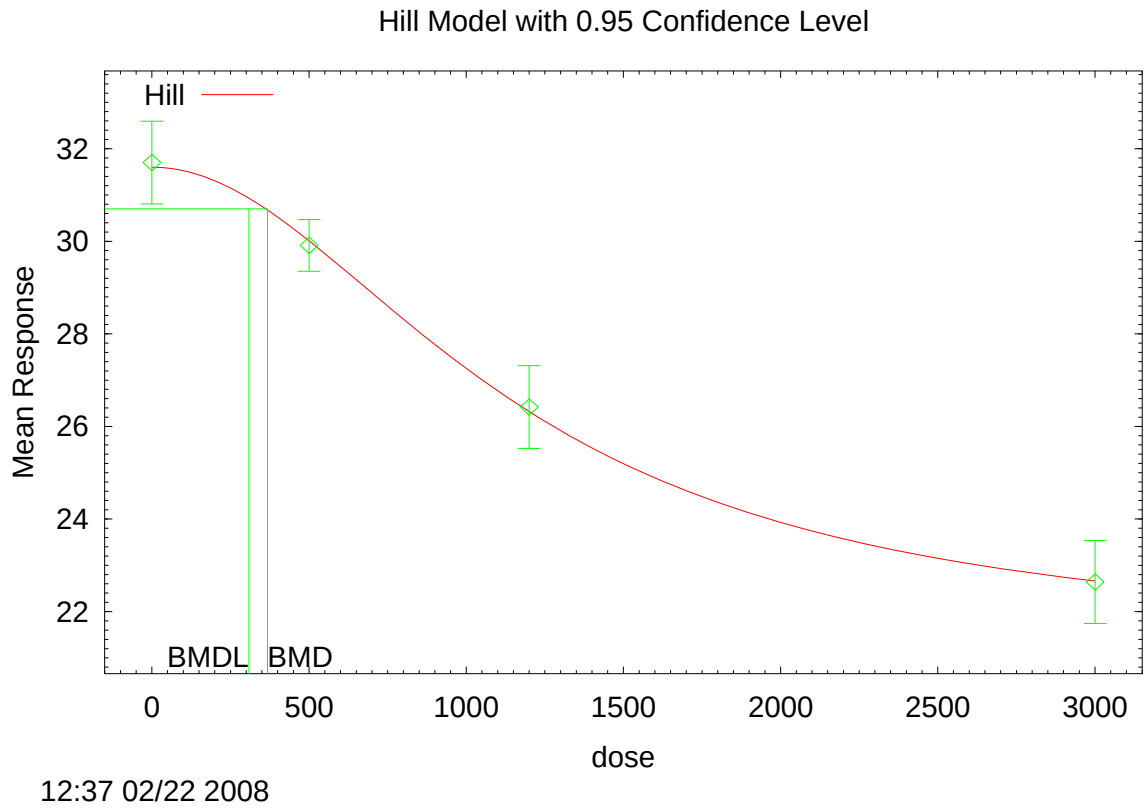
The p-value for Test 4 is less than .1. You may want to try a different model

Benchmark Dose Computation
Specified effect = 1
Risk Type = Estimated standard deviations from the control mean
Confidence level = 0.95

BMC = 154.736

BMCL = 110.399

Output B-11: Continuous, Hill model results using EPA BMDS version 1.4.1C for decreased MCV in rats following 16 weeks exposure to n-hexane from Huang et al. (1989)



The form of the response function is: Y[dose] = intercept + v*dose^n/(k^n + dose^n)

Dependent variable = MEAN
Independent variable = HEXANECONC
rho is set to 0
n is set to 2
Power parameter restricted to be greater than 1
A constant variance model is fit

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

Default Initial Parameter Values

alpha = 0.903938
rho = 0 Specified
intercept = 31.7
v = -9.06
n = 4.38522 Specified
k = 1049.57

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	intercept	v	k
alpha	1	-1.8E-007	-6.2E-007	1.8E-007
intercept	-1.8E-007	1	-0.28	-0.54
v	-6.2E-007	-0.28	1	-0.5
k	1.8E-007	-0.54	-0.5	1

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	0.855733	4.67436
intercept	31.6263	0
v	-10.3347	6.11513
k	1173.33	3.11398

Data and Estimated Values of Interest

dose	n	observed mean	observed SD	estimated mean	estimated SD	chi square
0	8	31.7	1.07	31.7	0.925	10.0173
500	8	29.9	0.667	29.9	0.925	31.6263
1200	8	26.4	1.07	26.4	0.925	1.65078
3000	8	22.6	1.07	22.6	0.925	1179.43

Model Descriptions for likelihoods calculated

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

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

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = alpha*(Mu(i))^rho

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

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-13.374455	5	36.748911
A2	-12.247583	8	40.495167
A3	-13.374455	5	36.748911
fitted	-13.507244	4	35.014489
R	-57.397708	2	118.795417

Explanation of Tests
Test 1: Do responses and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)
Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	90.3003	6	<.0001
Test 2	2.25374	3	0.5214
Test 3	2.25374	3	0.5214
Test 4	0.265578	1	0.6063

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

The p-value for Test 2 is greater than .1. A homogeneous variance
model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears
to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems
to adequately describe the data

Benchmark Dose Computation

Specified effect = 1
Risk Type = Estimated standard deviations from the control mean
Confidence level = 0.95

BMC = 367.89

BMCL = 308.931