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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)

February 2005

NOTICE

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CONTENTS —TOXICOLOGICAL REVIEW OF n-HEXANE
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LIST OF TABLES AND FIGURES

FOREWORD

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

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

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

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This document and the accompanying IRIS Summary have been peer reviewed by EPA scientists and independent scientists external to EPA. Comments from all peer reviewers were evaluated carefully and considered by the Agency during the finalization of this assessment. During the finalization process, the IRIS Program Director achieved common understanding of the assessment among the Office of Research and Development; Office of Air and Radiation; Office of Prevention, Pesticides, and Toxic Substances; Office of Solid Waste and Emergency Response; Office of Water; Office of Policy, Economics, and Innovation; Office of Children's Health Protection; Office of Environmental Information, and EPA's regional offices.

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Summaries of the external peer reviewers' comments *[and public comments, if applicable]* and the disposition of their recommendations will be provided in Appendix A following external peer review.

LIST OF ABBREVIATIONS

ACGIH	American Conference of Governmental Industrial Hygienists
AIC	Akaike's Information Criterion
API	American Petroleum Institute
BEI	biological exposure index
BMD	benchmark dose
BMC	benchmark concentration
BMR	benchmark response
TWA	time weighted average
CI	confidence interval
CMAP	compound muscle action potential
CNS	central nervous system
CYP450	cytochrome P450
DL	distal latencies
EEG	electroencephalogram
ENG	electroneurography
ENM	electroneuromyographic
ERG	electroretinograms
EROD	ethoxyresorufin O-deethylase
FOB	functional observation battery
IRDC	International Research and Development Corporation
IRIS	Integrated Risk Information System
LDH	lactate dehydrogenase
MAP	motor nerve action potential
MCV	motor nerve conduction velocity
MDL	minimum detection limit
NADPH	reduced nicotinamide adenine dinucleotide phosphate
NTP	National Toxicology Program
OR	odds ratio
PBTK	physiological based toxicokinetics
PNS	peripheral nervous system
ppm	parts per million
PROD	pentoxyresorufin O-dependentylase
RfC	reference concentration
RfD	reference dose
SCV	sensory nerve conduction velocity
SDS-PAGE	sodium-dodecyl sulfate polyacrylamide gel electrophoresis
SEP	somatosensory evoked potential
SNAP	sensory nerve action potential
TSCA	Toxic Substances Control Act
TLV	threshold limit value
UPDRS	Unified Parkinson Disease Rating Scale
VEP	visual evoked potential

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. 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 µg/L drinking water or risk per $µg/m^3$ air breathed; and (3) the 95% lower bound and central estimate on the estimated concentration of the chemical substance in drinking water or air that 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, (NRC, 1983). EPA guidelines that were used in the development of this assessment may include the following: *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986a), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996b), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a), *Draft Revised Guidelines for Carcinogen Assessment* (U.S. EPA, 1999), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), (proposed) *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 1998b, 2000a), *Science Policy Council Handbook*: *Risk*

Characterization (U.S. EPA, 2000b), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000c), *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000d), 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 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. The relevant literature was reviewed through February 2004.

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

n-Hexane (CAS RN 110-54-3) is a straight-chain, fully saturated hydrocarbon with six carbon atoms also referred to as hexane and hexyl hydride. The compound 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 (ATSDR, 1999; HSDB, 2003).

Chemical Formula	C_6H_{14}
Molecular Weight	86.18
Melting Point	-95 °C
Boiling Point	69 °C
Density	0.66 g/mL (at 20 °C)
Water Solubility	9.5 mg/L (at 25 °C)
Log K_{OW}	3.29
Log K_{OC}	2.9
Vapor Pressure	150 mm Hg at 25 °C
Henry's Law Constant	1.69 atm-m^3/mol

n-Hexane 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 varying amounts of structural isomers and related compounds, such as methylpentane and methylcyclopentane.

Highly purified n-hexane is used as a reagent for chemical or chromatographic separations. It also is used as a solvent for extracting edible fats and oils in the food industry and as a cleaning agent in the textile, furniture, and printing manufacturing industries. n-Hexane is the solvent base for many commercial products, such as glues, cements, paint thinners, and degreasers (ATSDR, 1999; NSC, 2003).

The compound 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 were available. However, absorption following oral exposure has been suggested by the identification of n-hexane and its metabolites in expired air, serum, and urine (Krasavage et al., 1980; Ono et al., 1981; Baelum et al., 1998). For example, increased levels of n-hexane in exhaled air and a major metabolite of n-hexane (2,5-hexanedione) in urine was 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 compound (Krasavage et al. 1980; Ono et al., 1981).

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 ten 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 compounds 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 the compound for 4 hours and to 360 mg/m^3 (204 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 human partition coefficient values (olive oil/air, blood/air, and 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, respectively. These values are similar to those reported in some tissues from F-344 rats (2.9 for muscle, 5.2 for liver, and 159 for fat; Gargas et al., 1989). In addition, blood/air (2.13) and breast milk/air (4.66) partition coefficients for humans (8 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. (1978) monitored n-hexane in the blood of pregnant F-344 rats who were exposed via inhalation to 1000 ppm 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 (GC-MS) 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 the compound in blood and tissues are given in Table 1.

Table 1. Concentration of n-hexane in blood and tissues of pregnant F-344 rats immediately after a 6-hour exposure to 1000 ppm n-hexane

Tissues	Concentration (μg/g)
Blood	0.45 ± 0.10*
Liver	0.84 ± 0.26
Kidney	0.53 ± 0.14
Brain	0.04 ± 0.00

Fetus	0.61 ± 0.14

Source: Adapted from Bus et al. (1978)
* = units of μg/mL

Bus et al. (1979) administered a single 6-hour exposure of 1000 ppm n-hexane on GD 12 or 20 to pregnant F-344 rats and monitored the levels of n-hexane in maternal blood and tissues and in the fetuses for up to 18 hours after exposure. Levels of n-hexane in maternal blood and total fetal tissue indicate that there may be transfer of n-hexane across the placenta. Specifically, no significant differences were observed between initial maternal blood n-hexane levels (0.45 ± 0.11 μg/mL) and total fetal n-hexane levels (0.61 ± 0.14 μg/g wet weight). Initial disposition of n-hexane in maternal tissues was greatest in kidney (6.33 ± 0.75 μg/g wet weight), followed by liver (0.85 ± 0.13 μg/g wet weight), blood (0.45 ± 0.11 μg/mL), and brain (0.04 ± 0.00 μg/g wet weight).

Bus et al. (1981) administered a single dose or five daily 6-hour exposures of 0 or 1000 ppm n-hexane to male F-344 rats (3/group) and observed the appearance of the compound in blood, liver, kidney, brain, and sciatic nerve. In the single day 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 ± 10.17 μg/mL, respectively. However, n-hexane was undetected 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 F-344 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 2).

Table 2. Tissue distribution of radioactivity in male F-344 rats 72 hours after a 6-hour inhalation exposure to various concentrations of [1,2-^{14}C]-n-hexane

Tissue	Nanomole-equivalents/g wet weight or mL			
	500 ppm	**1000 ppm**	**3000 ppm**	**10,000 ppm**
Liver	63.5 ± 0.9	90.9 ± 3.0	313.8 ± 34.6	189.6 ± 10.1
Lung	58.4 ± 0.7	85.3 ± 13.1	176.9 ± 9.5	118.1 ± 2.8
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

Source: Adapted from Bus et al. (1982)
ND = Not determined

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 (DeCaprio et al., 1982; Graham et al., 1982b; Anthony et al., 1983a,b; Lapadula et al., 1986; Boekelheide et al., 1987; DeCaprio et al., 1988; Sanz et al., 1995).

Similarly, a single 6-hour exposure of n-hexane to male F-344 rats (0, 500, 1000, 3000, or 10,000 ppm), the compound was detected in blood, liver, kidney, sciatic nerve, testis, brain, and lung (Baker and Rickert, 1981; Table 3).

Table 3. Steady state concentration of n-hexane concentrations in male F-344 rats exposed via inhalation

Tissue	Nanomole-equivalents/g wet weight or mL			
	500 ppm	**1000 ppm**	**3000 ppm**	**10,000 ppm**
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

Source: Adapted from Baker and Rickert (1981)
Data are means ± SE (n=3)

3.3. METABOLISM

n-Hexane is principally metabolized in the liver. As shown in Figure 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 to 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 (Perbellini et al., 1981).

Evidence that the liver is the primary location for the initial hydroxylation step for

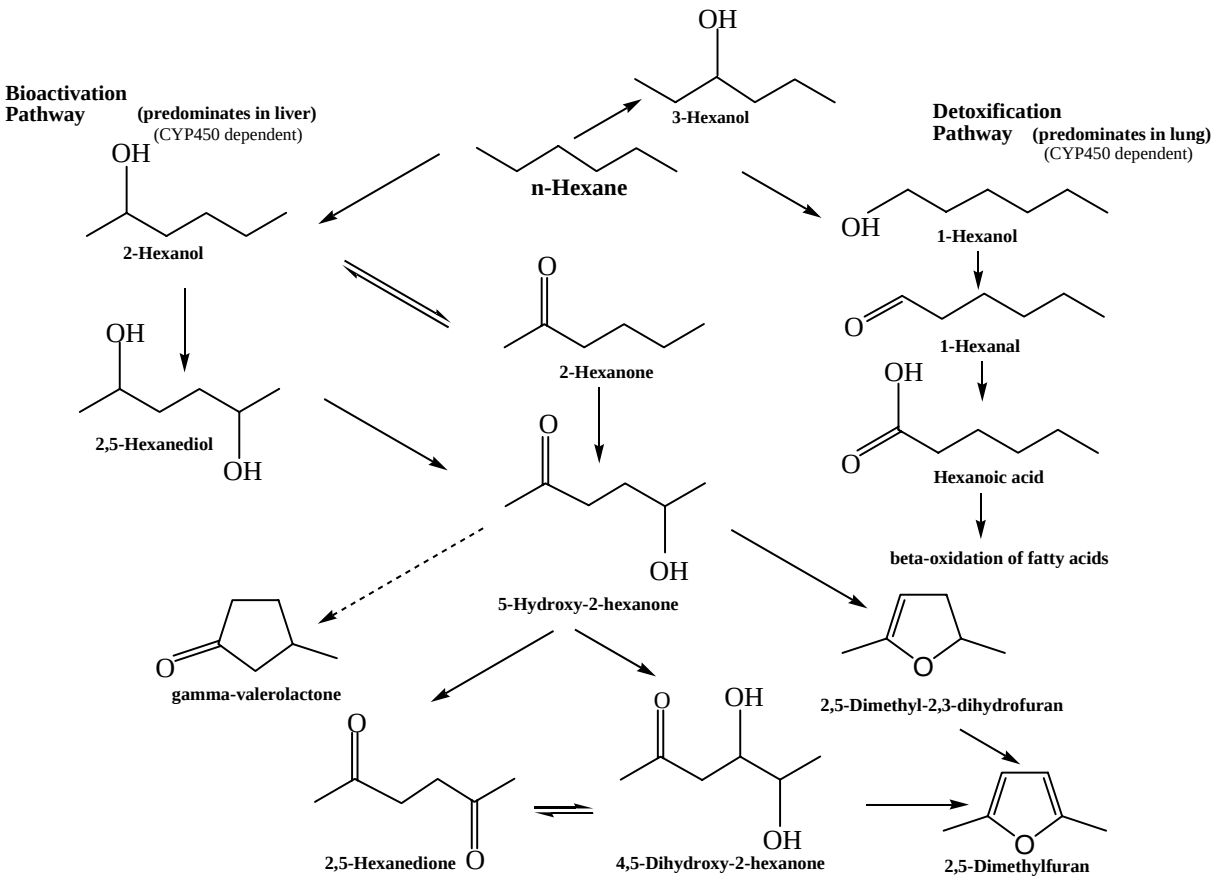

Figure 1. Biotransformation of n-hexane (Adapted from Couri and Milks, 1982 and Soriano et al., 1996)

bioactivation of n-hexane comes from the measurement of hydroxylating activity in isolated microsomes from liver, lung, brain, and the extensor digitorum longus (EDL) 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) compared to lung, brain, and soleus and EDL 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-hexanol, γ-valerolactone and 2,5-dimethylfuran 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 0.19, 5.4, 3.3, and 3.7 mg/L, respectively. n-Hexane exposure correlated well with both total metabolites (r = 0.7858) and the individual metabolites 2-hexanol (r = 0.6851) and 2,5-hexanedione (r = 0.6725). 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 hours after the start of exposure (no difference was found between morning and afternoon exposures).

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 (Gelmont et al., 1981; Kivits et al., 1981; Vaz et al., 1987). The study authors 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.

Other studies indicate that 2,5-hexanedione levels identified in the urine of humans may be an artifact of the extraction method used (Fedtke and Bolt, 1986; dos Santos et al., 2002). 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. 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).

A study in rats suggests that 2-hexanol may be the major metabolite of n-hexane following

inhalation exposure. Fedtke and Bolt (1987) exposed 3 male Wistar rats/group to mean concentrations of 0, 50 ± 3, 102 ± 6, 248 ± 6, 504 ± 20, 1003 ± 74, 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 post exposure). The formation of metabolites of n-hexane was dependent on exposure concentration (up to approximately 300 ppm). Formation of 1-, 2-, and 3-hexanol and 2-hexanone was evident during exposure but ceased by 8 hours post exposure. Levels of 2,5-hexanedione and 4,5-dihydroxy-2-hexanone were initially low and metabolism of n-hexane to these metabolites had ceased by 16 and 40 hours post exposure, 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 F-344 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 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 post exposure.

Table 4. Metabolism of n-hexane following a 6-hour exposure of pregnant F-344 rats on gestation day 20

Tissues	Time after exposure (hours)						
	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

Tissues	Time after exposure (hours)						
	0	**1**	**2**	**4**	**8**	**12**	**18**
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
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

Source: Adapted from Bus et al. (1979)
Values are μg/mL or μg/g wet weight ± SE
ND = not detected

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 avis. It is used to estimate the K_M and V_{max} for an enzyme. The estimated parameters for hexane hydroxylation in the liver and lung are presented in Table 5.

Table 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	6.0	ND
V_{MAX1} (nmoles/mg/min)	0.09	1.0	ND
K_{M2} (μM)	300	1100	290

Tissue Parameter	Product Formed		
	1-Hexanol	2-Hexanol	3-Hexanol
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

Source: Adapted from Toftgard et al. (1986)
ND = No data

The plots for 1- and 2-hexanol were similar and 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 stronger 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 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 has 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.

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 5. Based on the low K_M, and accompanying V_{max} , 1-hexanol is the favored product in the lungs. The enzyme responsible for the formation of 2-hexanol and 3-hexanol have very 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.

Nakajima et al. (1991) used phenobarbital, n-hexane, 2-hexanone, and 2,5-hexanedione to induce different CYP450s to which they also raised monoclonal antibodies. The enzyme activities, i.e., benzene aromatic hydroxylase, toluene side chain oxidation, ethoxyresorufin O-deethylase (EROD), and pentoxyresorufin O-depentylase (PROD) were measured as indirect indicators of the activity of the CYP450 species, CYP2E1, CYP2C2/6, CYP1A1/2, and CYP2B1/2, 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 probably 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 in immunoinhibition analysis 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 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 CYP2B2) and beta-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. 1-, 2-, and 3-Hexanol production was measured relative to time and expressed as nmoles metabolite/nmole enzyme protein/minute (turnover number or K_{cat}). The enriched CYP2B1 preparation produced all 3 alcohols with a turnover ratio of approximately 30:10:1 for 2-, 3-, and 1-hexanol, respectively. The CYP2B2 turnover ratio was similar to CYP2B1 (20:8:1 for the 2-, 3-, and, 1- hexanol, respectively). Turnover numbers were highest for the CYP2B1 and lowest for the CYP2A1 isozyme. The CYP2B1/2 isozymes primarily resulted in the production of 2-hexanol where the CYP2A1 isozyme primarily resulted in the production of 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 number 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 favored product in the liver.

In addition, Toftgard et al. (1986) used antibodies to these isozymes as inhibitors 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, 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 was reduced by 33% in liver microsomes and by 74% in lung microsomes. 3-Hexanol was 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 was important for n-hexane hydroxylation for detoxification in this organ. The partial reductions of 2-hexanol production in metyrapone-treated liver and lung microsomes, suggests the partial involvement of this cytochrome P450 species 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 co-exposure to methyl ethyl ketone on the toxicokinetics of n-hexane in human volunteers. Subjects (4-5 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 his/her own control by being exposed on the same day to n-hexane alone and the mixture of 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 3-fold lower after coexposure to methyl ethyl ketone. The authors suggested that an inhibition of one of the intermediate steps in the interconversion of n-hexane to 2,5-hexanedione may have been brought about by the presence of methyl ethyl ketone.

Studies in laboratory animals also demonstrate the effect of co-exposure 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 F-344 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, formed from further metabolism of 2,5-hexanedione, was also detected in increased quantities as a result of co-exposure to methyl ethyl ketone.

Shibata et al. (1990a) monitored the appearance of 2-hexanol, 2,5-hexanedione, and 2,5-dimethylfuran in the urine after 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 for up to 48 hours after the start of exposure. Both total (free and conjugated) and free metabolite levels were decreased in urine of rats exposed to both n-hexane and methyl ethyl ketone during 48 hours from the start of the 8 hour exposure (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 co-exposed to 2000 ppm n-hexane and 2000 ppm methyl ethyl ketone, as compared to rats receiving n-hexane alone. The area under the serum concentration curve (AUC) 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) exposed five male Wistar rats/group to 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 following n-hexane alone and mixed solvent exposures. The total concentrations of metabolites decreased by approximately one-sixth following exposure to n-hexane and toluene and one-fourth following exposure to n-hexane and methyl ethyl ketone.

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 post exposure. 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 the compound in the workplace. The employees wore personal samplers that provided data on the amount of n-hexane in the air, approximately 13 – 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) observed 10 workers in a shoe factory exposed to n-hexane (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 (measured pre-shift, end of shift, and the next morning) 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. Approximately, 3 mg/g creatinine of 2,5-hexanedione corresponded 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 work week and, therefore, not consistently reflect the ambient n-hexane exposure concentration.

Several other humans 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 et al. (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 gave a urinary 2,5-hexanedione estimate of 10 ± 3 μmol/L equivalent to a 180 mg/m^3 (50 ppm) 8-hour TWA concentration of n-hexane in air.

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 urinary (total) 2,5-hexanedione concentrations. Cardona et al. (1993) reported that urinary concentrations of 2,5-hexanedione tended to increase during the work week, although a significant linear correlation was obtained between mean environmental concentration of n-hexane and the urinary metabolite. Concentrations of 2,5-hexanedione in end-of-shift urine ranged from 0.2 – 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. Some percutaneous absorption of n-hexane was thought to have occurred in some cases.

Mayan et al. (2001) analyzed urine samples in 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 – 8.5 mg/g creatinine and correlated (r = 0.85) with personal sample n-hexane concentrations ranging from 6 – 70 ppm.

A similar study by the same research group in 111 shoe workers showed a positive correlation between work station 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 means by which they were exposed to n-hexane during the course of their work. No numerical exposure data for n-hexane were provided in the report, but the 14 subjects who applied glue with a paintbrush had higher concentrations of 2,5-hexanedione in their urine than 27 individuals who used a glue handgun (for total 2,5-hexanedione, 1.5 mg/L versus 0.7 mg/L). Eleven subjects who worked under a fume hood had the lowest total 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, r = 0.91, with exposure to n-hexane. Concentrations of the metabolite ranged from 0.3–32.46 mg/L.

Studies in animals are also available suggesting the elimination of n-hexane in urine following exposure to the compound via inhalation. Bus et al. (1982) exposed F-344 rats (3/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 released in the urine. Similarly, Baker and Rickert (1981) exposed male F-344 rats exposed to a single 6-hour inhalation exposure of 500, 1000, 3000, or 10,000 ppm n-hexane. Metabolites eliminated in the urine in 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 elevated in urine samples that were acid hydrolyzed compared to untreated urine (Table 6). This change was probably caused by the hydrolysis of dimethylfuran to 2,5-hexanedione under the acidic conditions (Fedtke and Bolt, 1986).

Table 6. Metabolites excreted in urine during a 72-hour period following inhalation exposure to n-hexane in male F-344 rats

Exposure Concentration (ppm)	Metabolite	Total Metabolites Formed (μg)		
		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

Exposure Concentration (ppm)	Metabolite	Total Metabolites Formed (μg)		
		No treatment	Hydrolysis with β-glucuronidase	Hydrolysis with 3N HCl
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

Source: Adapted from Baker and Rickert (1981)
Values are means for three animals ± SE. ND = not detected

Frontali et al. (1981) exposed Sprague-Dawley rats (6-9/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 by the 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 those animals receiving n-hexane mixed with toluene (Table 7).

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

Metabolites	Treatment	
	n-Hexane (200 mg/kg)	n-Hexane (200 mg/kg) and Toluene (200 mg/kg)
2-hexanol	230.42 ± 145.83	88.74 ± 54.16*
2,5-hexanedione	138.97 ± 58.91	72.93 ± 41.06*
2,5-dimethylfuran	91.57 ± 35.83	32.50 ± 11.94**
γ-valerolactone	47.67 ± 20.71	16.70 ± 11.56**

Source: Adapted from Perbellini et al. (1982)
Data are means ± SD
Significantly different from n-hexane (* $p < 0.005$, ** $p < 0.01$), as calculated by the authors

3.5. PHYSIOLOGICALLY BASED TOXICOKINETIC MODELS

A series of reports by Perebellini and co-workers 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., 1985a, 1986, 1990). 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 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 solvent instantly establishes a balance between alveolar air and venous blood and that the solvent 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).

Perbellini and co-workers obtained much of their data on tissue volumes and blood flows from the scientific literature (Mapleson, 1973; Perbellini et al., 1985). 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 a hypothetical exposure to humans of approximately 100 ppm for 4 hours was modeled a resulting concentration of n-hexane in the venous blood of 176 μg/L was close to that obtained in the 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 :

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

with values for the constants of 0.114 (mg/L) for A, and 0.0074 $(min)^{-1}$ 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 have 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 brief interval between shifts. Similarly, the concentration of 2,5-hexanedione in

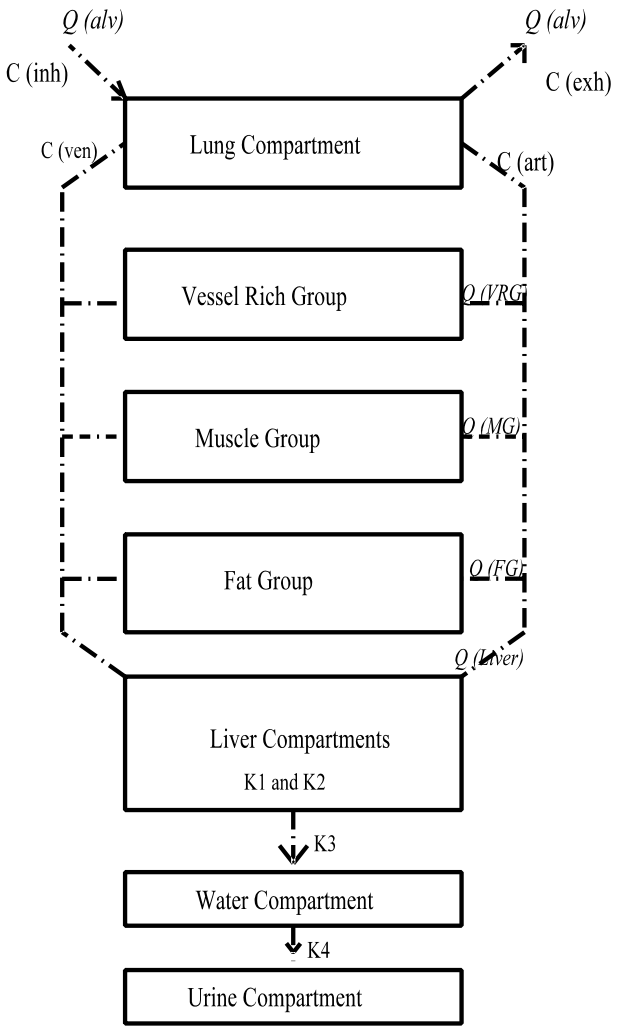

Figure 2. Physiological toxicokinetic model of the distribution of n-hexane in the body and the urinary excretion of 2,5-hexanedione
Source: Perbellini et al. (1986)

the 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 $t_{1/2}$ of 64 hours for the compound 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 work week, 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). As simulated by the model, the 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 between 3.3 to 4.3 mg/L on the morning of the other weekdays.

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 threshold limit value (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, an amount nearly two orders of magnitude lower than U.S. EPA's daily health advisory intake (Drinking Water Regulations and Health Advisory) for n-hexane of 4 mg/day (Fisher et al., 1997). The Fisher et al., (1997) model does not specifically address target tissues, 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 lactation 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 addressed the toxic effects of n-hexane in humans via the oral route.

4.1.2. Inhalation Exposure

4.1.2.1. Prechronic Exposure

No prechronic 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 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 (2 astrocytomas, 2 glioblastomas, and 2 oligodendrogliomas). Six cases had benign intracranial tumors, of which 2 were diagnosed as vestibular schwannomas (observed in 1 man and 1 woman), 2 as meningiomas (both in men), and 2 pituitary adenomas(observed in 1 man and 1 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 which 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 (OR) 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 confidence interval (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; 2 cases and 2 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.

Sanagi et al. (1980) compared peripheral nervous system function in n-hexane exposed and unexposed workers. Fourteen workers 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 5 workers who had previously been mixers in the past (exposure for 1 to 16 years with an average of 5.2 years), but who were not engaged in this job 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 life style factors that may affect the peripheral nervous system. Twenty-two breathing zone monitoring samples taken biannually over a 2-year period had 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 peripheral nervous system (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 8). The authors classified these symptoms as either persistent or persistent and transient.

Table 8. 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[§]	60	43
Heaviness in head	71	40	43
Vertigo/dizziness	50	60	38
Anosmia/dysnosia	46	20	14
Vision disturbance[**]	57	40	43
Double vision	36	20	21
Tinnitus	36	40	29
Hearing deficit[**]	71[§]	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[§]	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[§]	40	0
Writing impairment[**]	14	40	36
Unsteady gait	21	20	7

Source: Adapted from Sanagi et al. (1980)
*Symptoms classified as persistent and transient on questionnaire (unless otherwise noted)
* *Symptoms classified as persistent, no data presented as persistent and transient
[§]Statistically significant to controls ($p<0.05$)

Sanagi et al. (1980) also carried out a number of neurological tests to identify exposure-related neurological signs of n-hexane toxicity (Table 9). No objective neurological symptoms related to muscle strength by manual testing, muscle wasting, or muscle tone were reported. A statistically significant exposure-related deficit 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 compared to controls (average group values) was observed.

Table 9. Results of neurological tests in control subjects and those occupationally exposed to n-hexane in a tungsten carbide alloy factory

Test	Exposed Group	Controls
Muscle Strength		
Grip power (kg)	45.3 ± 2.9	44.9 ± 5.2
Jumping on one foot (cm)	21.3 ± 3.6*	26.0 ± 6.2
Vibration Sensation		
Radial Processes (s/16s)	13.8 ± 2.4*	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
Co-ordination 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

Source: Adapted from Sanagi et al. (1980)
Values are means ± SD
*Statistically significant versus controls ($p<0.05$)

Neurophysiological findings indicated a slowing of MCV in the posterior tibial nerve, with delayed recovery in exposed groups compared to control (Table 10). These findings are consistent with the neurological signs and subject-reported symptoms generally associated with n-hexane exposure. Consistent with the neurological signs and subject-reported symptoms, these responses may be related to n-hexane exposure. The findings of Sanagi et al. (1980) include minor neurological changes among workers exposed to n-hexane at a level close to the ACGIH recommended TLV of 50 ppm (ACGIH, 2003). However, the degree to which these changes represent impairment of neurological function is uncertain, because most neurophysiological findings and neurological signs in the n-hexane exposed group were indistinguishable from those of controls. The data suggest that exposure in the workplace to n-hexane at 58 ppm may be at or near the threshold for the onset of neurological effects.

Table 10. Nerve stimulation in control subjects and those occupationally exposed to n-hexane in a tungsten carbide alloy factory

Test (units)	Right Median and Ulna Nerves		Right Posterior Tibial Nerve	
	Exposed Group	Controls	Exposed Group	Controls
Motor nerve conduction velocity (m/s)	57.3 ± 3.4	57.5 ± 3.2	46.6 ± 2.3*	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*	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

Source: Adapted from Sanagi et al. (1980)
Values are means ± SD
* Significantly different to controls ($p<0.05$)

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 action potential (MAP) duration and decreased MCV in the median and ulnar nerves in exposed compared to unexposed workers. Exposed workers were divided into two groups (mild and high exposure) based on hydrocarbon exposure. The two groups were determined by exposure score, taking into account time in job and hygienic effect. 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 (defined as the ratio between the measured concentration of the compound and the proposed 1979 ACGIH TLV values for the compound) was 0.81 and 1.91 for the mild and highly exposed groups, respectively. The authors stated that estimation 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 time 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 to 1990 ppm), the median value for n-hexane was 448 mg/m^3 (127 ppm) and median hygienic effect was 1.24. The study began 3 months after industrial hygiene improvements were 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 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 SCV compared to controls. There was also increased latency in the somatosensory evoked potential (SEP) of exposed workers compared to 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 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).

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 which was 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) was carried out the following day. Reference values were obtained from 41 unexposed individuals. A semiquantitative rating scale of the electroneuromyographic (ENM) responses was used as a cumulative index score of the electrodiagnostic findings (Allen et al., 1975). The scale ranged from 0, where no electroneurography (ENG) abnormalities were observed, to 10, where decreases in conduction velocities and increases in distal latencies (DL) were indicative of impaired electrophysiological performance (Governa et al., 1987).

The results of the urinalysis, which measured concentrations of metabolites of cyclohexane and trichloroethylene as well as n-hexane, were only above the minimum detection limits (MDLs) in all 40 workers for two of the five metabolites of n-hexane, 2,5-hexanedione (mean 6.80 mg/L) and γ-valerolactone (mean 3.31 mg/L). The only other compound for which a significant portion of the workers had metabolite levels above the MDL was trichloroethylene. However, the trichloroethylene levels were much lower than those associated with the recommended occupational exposure limit. The urinary concentration of the n-hexane metabolite, 2-hexanol, was lower than 0.1 mg/L in 29 of 40 workers. The results of the neurological exam found 26 workers with electroneuromyographic 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 distal latencies and SCV and MCV values plus changes in MAPs in at least one muscle (ENM Score $\geq$ 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 electroneuromyographic abnormalities

(defined an 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 electroneuromyographic 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 to 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, respectively for occupationally exposed workers. Urinary concentrations of 2,5-hexanedione were 0.1-0.8; concentrations of hippuric acid were < 1.5 g/g creatinine; and concentrations of methylhippuric acid were 0 g/g creatinine, respectively for unexposed workers. No exposure concentrations of n-hexane , xylene, or toluene were 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 (ECG) 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 to 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 amounts and concentrations of metabolites in the urine may be an underestimation of the actual solvent exposure.

In a study of the same workers as those observed 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 concentration of urinary 2,5-hexanedione was 1.20 mg/L (range 0.41 to 3.06 mg/L), and the estimated mean level of 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 the period 1973-1981. 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 (EEG) 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 identified with polyneuropathy was questioned about their work experience, type of chemical material used in their 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 life style 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 the 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 direct 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 (8 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 work rooms ranged from 500 to 2500 ppm. The degree of neurological disorder was related to hygienic conditions in the work place. 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 (7 subjects), and numbness over the face (5 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 as reactions.

Paulson and Waylonis (1976) also found reduced serum cholinesterase levels among eight printing room workers who had polyneuropathy and were exposed to n-hexane in the adhesives.

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 unstated). MCVs were consistently lower among the workers exposed to n-hexane than among the 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 were found to exist between (1) frequency of polyneuropathy and n-hexane concentration in the cleaning solvents and air samples, and (2) abnormal MCV and n-hexane concentration in the cleaning solvents. 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 EMG changes also included 5 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 was seen compared to controls. Among the exposed workers with no apparent symptoms, MCVs were slower, motor DL were prolonged, and SNAP amplitudes were attenuated compared to controls. The percentage difference in these myographic 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 two years, bimonthly for the third year, and once every 3 months for the final forth 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), DL, 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 DL 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 11).

Table 11. 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
Distal latency (ms)	6.1 ± 0.7	3.9 ± 0.4	3.7 ± 0.4
MCV (m/s)	42.9 ± 7.1	57.3 ± 2.5**	61.9 ± 4.6
Ulnar Nerve			
CMAP amplitude (mV)	4.2 ± 1.7	8.5 ± 1.1	7.8 ± 2.2
Distal latency (ms)	5.0 ± 0.8	3.1 ± 0.2	3.0 ± 0.4
MCV (m/s)	40.8 ± 4.6	52.8 ± 4.1*	55.4 ± 4.0
Peroneal Nerve			
CMAP amplitude (mV)	1.8 ± 1.0	5.6 ± 2.5	5.9 ± 1.9
Distal latency (ms)	8.2 ± 1.1	4.8 ± 0.9	4.7 ± 1.0
MCV (m/s)	31.9 ± 3.3	46.1 ± 4.9***	53.1 ± 4.4
Tibial Nerve			
CMAP amplitude (mV)	4.0 ± 2.4	10.7 ± 5.7	10.2 ± 3.7
Distal latency (ms)	7.6 ± 1.3	4.5 ± 0.2	4.5 ± 0.8
MCV (m/s)	34.5 ± 4.3	46.3 ± 4.8*	49.6 ± 3.9

Source: Adapted from Chang (1991)
Significance of Differences to Control, as calculated by the authors, * ($p<0.05$), ** ($p<0.005$),*** ($p<0.0005$)

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 (0.05-0.95 μg/g) in the printing inks. The mean TWA for air samples taken in the factory was 63 ppm (range 30–110 ppm) for the general air concentration and 132 ppm (range 80–210 ppm) for the breathing zone air for the offset printers. The findings from the neurological examination of these workers were compared to 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), and 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 those that were asymptomatic, but classified as having subclinical neuropathy. The authors also measured electrophysical deficits in each group of workers (Table 12). In healthy workers, there was a decreased amplitude of the median nerve SNAP compared to 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 12. Nerve conduction study findings in printers with n-hexane induced polyneuropathy

Type of nerve conduction study	control (n = 20)	healthy worker (n = 10)	subclinical worker (n = 26)	symptomatic worker (n = 20)
amplitude of SNAP (uV)				
median	37 (11)	27 (6)*	24 (8)*	15 (5)*
ulnar	15 (4)	14 (3)	12 (5)	7 (4)*
sural	24 (10)	22 (6)	18 (7)*	11 (8)*
amplitude of MAP (mV)				
median	7 (2)	8 (3)	6.7 (2.4)	4.6 (2.2)*
ulnar	5.7 (2.1)	6.5 (1.9)	4 (2.2)*	3.6 (1.5)*
posterior tibial	6.6 (2)	6.7 (2.3)	5.3 (2.5)	2.9 (1.7)*
common peroneal	4.4 (1.5)	4.3 (1.4)	3.6 (1.4)	1.8 (1.4)*
DL of SNAP (ms)				
median	2.3 (0.3)	2.3 (0.1)	2.6 (0.3)*	2.9 (0.3)*

ulnar	2.1 (0.3)	2.1(0.1)	2.3 (0.3)	2.7 (0.4)*
sural	3.3 (0.3)	3.1 (0.2)	3.3 (0.3)	3.7 (0.6)*
DL of MAP (ms)				
median	2.9 (0.4)	3.0 (0.2)	3.6 (0.5)*	4.3 (1.2)*
ulnar	2.2 (0.3)	2.3 (0.3)	2.6 (0.5)*	3.0 (0.7)*
posterior tibial	4.1 (0.6)	3.9 (0.6)	4.4 (0.6)	5.6 (1.2)*
common peroneal	3.9 (0.5)	3.5 (0.4)	4.2 (0.7)	5.4 (1.2)*
MCV (m/s)				
median	59 (5.9)	57 (5)	55 (6.7)*	46 (6.5)*
ulnar	61 (5.8)	59 (6)	55 (7.8)*	48 (7.5)*
posterior tibial	50 (6.4)	46 (3.4)	45 (4.7)*	38 (6.5)*
common peroneal	51 (4.5)	46 (3.8)	45 (5.1)*	37 (7.1)*

Source: Adapted from Chang (1992)
Values presented as mean (SD)
Significance of Differences to Control, as calculated by the authors, * ($p<0.05$)

Several studies (Seppalainen et al., 1979; Raitta et al., 1978; Issever et al., 2002) have investigated vision changes in relation to n-hexane exposure. Seppalainen et al. (1979) compared the VEP and electroretinograms (ERG) in 15 workers to those in 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 26 exposed workers were identified as having worked in the leather industry where they had been exposed to n-hexane (exposure concentration not stated) and all complained of asthenia of the upper and lower limbs, paraethesia in the hands and arm, and difficulty walking.

Electromyography (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 13). 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 13. FM-100 Hue test scores of n-hexane-exposed and non-exposed groups

Eyes	Chromatic Focus	Exposed Group	Control Group
Right	Blue-Green	104.3 ± 37.3*	22.2 ± 13.5
	Red-Green	64.1 ± 38.4*	13.8 ± 8.3
	Total	168.3 ± 70.5*	36.0 ± 19.8
Left	Blue-Yellow	96.7 ± 55.8*	21.6 ± 11.0
	Red-Green	81.9 ± 51.8*	14.0 ± 9.4
	Total	181.5 ± 103.0*	35.6 ± 18.2

Source: Adapted from Issever et al. (2002)
* Significantly different to controls ($p<0.001$), as calculated by the authors

Huang and Chu (1989) used evoked potentials 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, and brainstem auditory (BA), visual (V), and sensory somatosensory (S) evoked potentials (EPs) were obtained in exposed subjects and compared to 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 work room 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 brainstem auditory evoked potentials (BAEPs) when the five exposed subjects were compared, as a group , to 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.

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 urine was measured for 2,5-hexanedione at the end of each shift. The mean TWA for n-hexane in the air was 123 ppm (range 23–215 ppm), and the mean level of 2,5-hexanedione in the serum was significantly greater in the exposed workers (2.39 μg/g creatinine) compared to the unexposed workers (0.41 μg/g creatinine). Whole blood samples were analyzed for the immunoglobulins IgG, IgM, and IgA, and white blood cell (WBC) components. Concentrations of each of the immunoglobulins were significantly lower in exposed versus 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 co-exposure 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 to 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.

Scelsi et al. (1980) observed neuropathological symptoms and morphological changes in small numbers of subjects (3 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 swellings, 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 was irregular and swollen myelin sheaths and segmental swelling of axons with dissolution of neurotubules and increase of neurofilaments. 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 polyneuritis. 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 neurofilaments.

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 work room 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. Disability progressed to the extent of incapacity, with difficulty walking or picking up tools. Yokoyama et al. (1990) measured the distribution of conduction velocities of sensory fibers in sural nerve at 1–2 months, 4–9 months, and at 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 and subsequent recovery upon removal of subjects from the n-hexane exposure was 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 as 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 coasting and the winding process; 8 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 to 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 the more severe organic environment 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 followup 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 ten 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 measurement 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 25-year-old male showed progressive weakness, pain, diminished sensations in the feet, and numbness in the hands. An electrophysiological examination of the subjects revealed reduced sensory action potential and conduction velocities, increased conduction latencies, 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 neurofilaments. An initial increase in severity of the symptoms on cessation of exposure caused the subject to be confined to a wheel-chair. 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.

To protect against the onset of subclinical and clinical neuropathological symptoms of n-

hexane exposure, ACGIH proposes a BEI of 0.4 mg/L for 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 work week (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).

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 for 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-1,2,3,6-tetrahydropyride-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 work station 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, and mood, and activities of daily living) on a 0 to 5 scale. The cumulative score gives an index of severity of Parkinson's (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 to 108 unaffected subjects. The 2,5-dimethylpyrrole adducts production is a result of the interaction of 2,5-hexanedione with neurofilament protein lysine residues. Urinary excretion of both 2,5-hexanedione and 2,5-dimethylpyrrole were significantly reduced in patients with Parkinsonism compared to controls, although there was a gradual decline in urinary metabolite levels with age in both Parkinsonism patients and controls. The data do not discriminate between the lower urinary levels possibly being due to reduced conversion of n-hexane to its metabolites or to an increase in their further catabolism.

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

4.2.1. Oral Exposure

4.2.1.1. Prechronic 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 5 male COBS CD(SD) BR rats/group received equimolar doses of 6.6 mmol/kg of the compounds by gavage, 5 days/week, for 90 days. The mg/kg equivalents of the equimolar daily doses were 660 mg/kg 2-hexanone, 780 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 compound. The onset of neuropathy was assessed by the initial appearance of hindlimb paralysis, at which point the animal was sacrificed and examined histopathologically.

Two rats in the 13.2 mmol/kg exposure group and 1 rat in the 46.2 mmol/kg n-hexane exposure group and 3 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 hindlimb 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 hindlimb paralysis during the 90 day testing period. The relative potency of the test compounds was compared to 2-hexanone. Specifically, the authors calculated a neurotoxic index (based on days until hindlimb paralysis developed in 2-hexanone treated animals/mean days until hindlimb paralysis developed following test compound exposure). 2,5-Hexanedione, 5-hydroxy-2-hexanone, and 2,5-hexanediol were the had higher neurotoxic indexes compared to 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 compound (statistical significance not reported by study authors). All compounds except 570 and 1140 mg/kg n-hexane produced giant axonal swellings, adaxonal myelin infolding, and paranodal myelin retraction (incidence not reported by 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 testicular atrophy of the 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 study authors).

By correlating the concentration of serum 2,5-hexanedione with the onset of neuropathological symptoms, Krasavage et al. (1980) suggested that a threshold serum concentration of 50 µg/mL of 2,5-hexanedione was necessary for the induction of the neuropathological effects. For n-hexane, only those animals receiving the compound at the highest dose (46.2 mmol/kg, equivalent to 3980 mg/kg-day) resulted in a serum concentration of 50 µg/mL 2,5-hexanedione and developed the hindlimb 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 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 motor nerve conduction velocity (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 to controls. These changes achieved statistical significance at the 4- and 8-week time point (statistical test not stated). Distal latencies (DL) decreased as the rats grew according to the study authors. However, there were no statistically significant differences between n-hexane exposed and control animals. There were statistically significant reductions in the proximal (approximately 6-8%) and distal (approximately 5-8%) mixed MCVs of animals receiving n-hexane compared to controls after 4-, 6-, and 8-weeks, respectively. In general, such changes were less severe or absent in animals exposed to isomers and structurally related compounds (2- and 3-methylpentane, methylcyclopentane)of n-hexane.

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. Prechronic Studies

Altenkirch et al. (1982) exposed male Wistar rats (five/group) to 0 or 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, 22 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 distal to the axonal swellings, with an apparent intra-axonal accumulation of neurofilaments. Other morphological findings included axonal swellings 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, 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 swellings 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.

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 ppm n-hexane, 500 ppm n-hexane, and a mixed petroleum distillates fraction designated "benzine" that had been supplemented with 200 ppm n-hexane, and benzine plus 500 ppm n-hexane. Using a modification to a previously described experimental protocol for assessing nerve conduction and performance (Ono et al., 1981), the authors measured MCV and DL before the start of the experiment and then every 4 weeks prior to sacrifice. 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 and proximal) mixed MCV were statistically significantly decreased at $\geq$200ppm. DL and proximal mixed MCV were statistically significantly decreased at the low dose but not the high dose. Degeneration of the myelin sheath axons was evident in the peripheral nerves of all exposed groups.

Ichihara et al. (1998) exposed eight male Wistar rats/group to 2000 ppm n-hexane, 12 hours/day, 6 days/week for 20 weeks and showed that MCV decreased and DL increased compared to controls.

Pryor et al. (1983) carried out a subchronic exposure study on the neurotoxicity potential of n-hexane by exposing male F-344 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 compound-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- and hindlimb grip strength. Exposure to the compound also was associated with a reduction in the amplitude of the fifth component of the brainstem auditory-evoked response. 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 compound-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, 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 F-344 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 to weanlings. Grip strength was reduced equally in older rats and weanlings within 2 weeks of the beginning of the exposure period. However, subsequent effects of treatment on this parameter were greater in young adults versus weanlings. Older rats exhibited earlier and more severe signs of hindlimb paralysis compared to younger animals. Conversely, there was little difference in neuropathological responses between rats in the different age categories, including tail nerve conduction time and a brainstem auditory-evoked response.

Cavender et al. (1984a,b) exposed F-344 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 compound-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 swellings in mid- and high-dose males (1/5 and 4/5, respectively).

Male Wistar rats (8/group) were exposed to 0, 500, 1200, or 3000 ppm n-hexane (>99% pure) for 12 hours/day, 7 days/week to 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 at 16 weeks 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-S-100. These nervous specific proteins are 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. A dose-dependent, statistically significant reduction in body weight gain was observed in the mid- (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 hindlimb paralysis was observed by the termination of the experiment. Rats exposed to the mid- and high-dose of n-hexane showed a reduction in MCV. This reduction was statistically significant during weeks 8-16 of the exposure period compared to controls. Increased incidence of paranodal swellings, along with some evidence of demyelination and remyelination was present in the peripheral nerves at both mid- and high-doses. However, these histopathological findings were more severe in the high dose group. Among biochemical changes, there were dose-dependent reductions in nervous system specific proteins, particularly the β-S-100 proteins from tail nerve fibers, which were significantly reduced by approximately 75% at all dose levels. The neurophysiological deficits and histopathological effects that were evident in mid- and high-dose rats suggest a NOAEL of 500 ppm.

Huang et al. (1992) exposed male Wistar rats (7/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 and gradually decreased thereafter. The authors also observed an increase in DL after 12 weeks, which 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-S-100 protein with

neurophysiological 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 (TSCA), 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 to34 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 measurement interval. 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) continuously exposed male Sprague-Dawley rats to n-hexane and to mixtures containing n-hexane (C6 isomers were a mixture of n-hexane-depleted C6 hydrocarbons containing methylcyclopentane, 3-methylpentane, and 2-methylpentane as major components) along with other hydrocarbons for 22 hours/day, 7 days/week for 6 months (IRDC, 1992 a,b). This study was conducted in two phases, the exposure groups are shown in Tables 14 and 16.

Table 14. Experimental protocol for phase I of a 6-month inhalation study of n-hexane and mixtures containing n-hexane plus hydrocarbon isomers in male Sprague-Dawley rats

Phase I	
Group	**Treatment**
I	controls
II	125 ppm n-hexane
III	125 n-hexane + 125 ppm C6 isomers
IV	125 n-hexane + 375 ppm C6 isomers

V	125 ppm n-hexane + 1375 ppm C6 isomers
VI	500 ppm n-hexane

Source: Adapted from IRDC, 1992a, b
C6 isomers were a mixture of n-hexane-depleted C6 hydrocarbons containing methylcyclopentane, 3-methylpentane, and 2-methylpentane as major components

In both phases of this study, animals were examined daily for signs of clinical toxicity, and body weights were monitored weekly. Those animals maintained to term were necropsied, 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 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.

In phase I, rats (14-34/group) were exposed to either filtered air alone (Group I), 125 ppm n-hexane (Group II), 125 ppm n-hexane mixed with various concentrations of C6 isomers (Groups III through V), and 500 ppm n-hexane (Group VI). Two controls and four rats from Group VI were withdrawn from their exposure group 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 as described.

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 rest of the experiment. However, the true incidence of this response may be underestimated because of subjects withdrawn from the study before such 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 15). Signs of liver necrosis, marked by raised discolored areas of 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. Re-evaluation of the renal pathology of the kidneys showed a solvent-induced exacerbation of the hyaline droplet nephropathy that is characteristic of male rats in this and other strains as reported by EPL, 1992.

Table 15. Organ weight changes (relative to body weight) in male Sprague Dawley rats exposed to n-hexane 22 hours/day, 7 days/week for 6 months

Organ	Groups		
	I	II (125 ppm n-hexane)	VI (500 ppm n-hexane)
	% ± SD		
Spleen	0.18 ± 0.029	0.15 ± 0.02*	0.21 ± 0.12
Liver	2.88 ± 0.26	3.01 ± 0.27	3.31 ± 0.11**
Kidney	0.56 ± 0.047	0.55 ± 0.059	0.71 ± 0.17**
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

Source: Adapted from IRDC (1992a, b)
* statistically significant compared to controls ($p<0.05$)
** statistically significant compared to controls ($p<0.01$)

Exposure to n-hexane at 500 ppm (Group VI) was associated with axonal degeneration, myelin vacuolation, and skeletal muscular atrophy. The lesions were marked by axonal degeneration 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 also observed in the high dose group animals (9/10). No other treatment groups exhibited neuropathological/myopathic changes.

In phase II of the experiment (Table 16), rats (10/group) were exposed to either filtered air alone (Group VII), 500 ppm C6 isomers alone (Group VIII), 500 ppm n-hexane plus 500 ppm C6 isomers (Group IX), 500 ppm n-hexane (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) was observed in rats exposed to 500 ppm n-hexane. No such histopathological evidence of neuropathy was seen in controls. The authors noted a slight increased incidence and severity of chronic renal nephritis in both controls and n-hexane exposed rats (6/11 and 10/10, respectively) with a significant increase in mean absolute and/or relative kidney weight (5.32 ± 0.526 for controls and 8.44 ± 0.983 for n-hexane exposed group).

Table 16. Experimental protocol for phase II of a 6-month inhalation study of n-hexane and mixtures containing n-hexane plus hydrocarbon isomers in male Sprague-Dawley rats

Phase II	
Group	**Treatment**
VII	controls
VIII	500 ppm C6 isomers

IX	500 ppm n-hexane + 500 ppm C6 isomers
X	500 ppm n-hexane

Source: Adapted from IRDC, (1992a, b)

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 (Dunnick et al., 1989; NTP, 1991). Groups of 10 mice/sex/group were exposed to 0, 500, 1000, 4000, and 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, forelimb and hindlimb 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 to 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 the compound for 22 hours/day. There was an increased incidence of paranodal axonal swellings in high-dose or extended exposure duration mice. Table 17 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 17. 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 (extended)**	**4000**	**10,000**
Male						
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	1010 (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)
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	1/4	ND	3/4
Teased Fiber Tibial nerve	0/4	ND	ND	3/4	ND	3/4
Female						
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 (20)	10/10 (2.6)
Metaplasia	0/10	0/10	1/10 (2)	8/10 (1.9)	8/10 (2)	10/10 (2.6)

Site/Lesion	Concentration of n-Hexane (ppm)					
	0	**500**	**1000**	**1000 (extended)**	**4000**	**10,000**
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	0/4	ND	ND	0/4	ND	0/4
Spinal cord						
Tibial nerve	0/4	ND	ND	0/4	ND	3/4
Teased Fiber	0/4	ND	ND	3/4	ND	3/4
Tibial nerve						

Source: Adapted from Dunnick et al. (1989)
The number in parentheses represents the average grade of the most severe lesions observed in the eight levels of nasal cavity examined on a scale of 1-5
ND = No Data

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 further 120-day recovery period before sacrifice, at which point the cellular architecture of the lungs was examined under the light and electron microscope. 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 non-ciliated bronchial epithelial cells. However, some of these lesions were more marked in animals sacrificed immediately after the last exposure compared to 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 subjects 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 the compound in mice dosed with n-hexane observed in this portion of the study.

In the second portion of the study, 19–26 mice/group received cumulative doses of either 0, 1830, 2170, 7920, or 9900 mg/kg-day 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 exposed groups. Fetal birth weight was 6.35% lower in the progeny of those dams exposed to 7920 and 9920 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 compounds for spermatotoxic effects in male Sprague-Dawley rats. The experimental protocol featured the oral administration of the undiluted compound 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 heads 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 F-344 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, then 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 F-344 rats.

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

observed.

The results of a teratological study of n-hexane 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. The incidence of skeletal variations in individual live fetuses is shown in Table 18. There was a statistically significant increase in the mean percent incidence of reduced ossification of sternebrae 1-4 per litter at 5000 ppm compared to controls (38.7 ± 23.7 versus 13. ± 21.6, respectively).

Table 18. Skeletal variations in live fetuses of pregnant Sprague-Dawley rats exposed to n-hexane via inhalation

Subjects	**Concentration of n-Hexane (ppm)**			
	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: Adapted from Mast (1987)

Also, the increased mean percent incidence of reduced ossification of sternebrae 1-4 per litter was correlated (statistically significant) 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.

Mast (1988a) also described 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 to 17 (Mast, 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. The fetal weights were slightly, but not significantly reduced (means of litter means) for all groups compared to controls. However, this decrease in fetal weight did correlate significantly to 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 number of live fetuses per litter was reduced in all exposure groups compared to controls, although the observed decrease was only statistically significant at 5000 ppm. The number of live fetuses per litter in all exposure groups was also reduced compared to contemporary controls of the same strain of mice. The mean percent live implants was reduced compared to contemporary controls at 200 and 5000 ppm, but not 1000 ppm. The mean percent of intrauterine death (early and late resorptions combined) was greater for all exposed groups compared to 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 controls following exposure to 5000 ppm n-hexane relative to controls. The authors stated that trend analysis indicated an increased mean percent incidence of late resorptions was positively and significantly correlated with exposure concentration.

A number of experimental studies have 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, and (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 a n-hexane. The early meiotic prophase spermatocytes (leptotene and zygotene), 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. In these animals exposed for up to 6 weeks, subjects 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, and no increased incidence of aberrant sperm characterized by blunt-hook, banana, amorphous, or pin-head shapes. Likewise, there was no increased incidence of sperm with more than one head or tail.

Mast (1988c) carried out a further study of the effect of n-hexane in male CD-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 co-habitation 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, although Lewis (1992) reports a value of 28,710 mg/kg in rats. HSDB (2003) presents values of 24 and 45 mL/kg for juvenile and adult rats (approximately 15,840 and 29,700 mg/kg), respectively. For inhalation exposure, a 4-hour LC_{50} of 48,000 ppm has been reported (HSDB, 2003).

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 unstated), 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 19 and 20, 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) .

Table 19. Total red blood cells and nucleated cells in bronchial lavage from n-hexane-challenged New Zealand white rabbits

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

Source: Barni-Comparini et al. (1982)
* significantly different from controls (p<0.01

Table 20. Enzyme activities in lung homogenates of rabbits exposed to n-hexane

Enzyme Activities (I.U)	Exposure Groups (ppm n-hexane)	
	0	3000
Lactate dehydrogenase	75 ± 3.7	109 ± 5.1*
Glucose-6-phosphate dehydrogenase	5.10 ± 0.29	9.98 ± 1.3*
Acid phosphatase	2.31 ± 0.64	4.96 ± 0.81*
Alkaline phosphatase	1.38 ± 0.15	1.91 ± 0.19
β-glucuronidase	0.43 ± 0.09	0.95 ± 0.15*

Source: Barni-Comparini et al. (1982)
* significantly different from controls, as calculated by the authors

study. The increased levels of the lysosomal enzyme, acid phosphatase (4.96 ± 0.81 IU in exposed rabbits versus 2.31 ± 0.64 in controls), might reflect acute inflammation, while the increased activity of glucose-6-phosphate dehydrogenase (9.98 ± 1.3 IU in exposed rabbits versus 5.1 ± 0.29 IU in controls) suggests a reparative process subsequent to n-hexane-induced pulmonary disruption.

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 21).

Table 21. Concentration of biochemicals and enzyme activities in bronchial lavage extracted from male Sprague-Dawley rats inhaling 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)	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/mg protein/hour)	0.21 ± 0.03	0.27 ± 0.04	0.31 ± 0.03	0.43 ± 0.05
Alkaline phosphatase (µmol/mg protein/hour)	0.37 ± 0.05	0.41 ± 0.05	0.54 ± 0.04	0.68 ± 0.06
Lactate dehydrogenase (µmol/mg protein/min)	0.16 ± 0.03	0.19 ± 0.02	0.26 ± 0.02	0.45 ± 0.04
Glucose-6-phosphate dehydrogenase (nmol/mg protein/min)	0.90 ± 0.08	0.97 ± 0.12	1.35 ± 0.16	1.72 ± 0.16
Angiotensin-converting enzyme (nmol/mg protein/min)	0.36 ± 0.04	0.67 ± 0.08	0.86 ± 0.07	1.18 ± 0.12

Source: Adapted from Sahu et al. (1982)

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 suggest a disruptive effect of commercial hexane on one or more cell types in the lung. The three-fold 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.

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 dose groups for both n-hexane and 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 to controls. Toluene exposure (400 ppm) lead 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 level of increase in the amount of dopamine in the hippocampus was evident in animals exposed to the mixture of solvents.

Pezzoli et al. (1990) injected VCD-1(ICR)BR male mice (number unstated) intraperitoneally with 400 mg n-hexane/kg-day, 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 mid-brain (substantia nigra). This exposure was repeated again 14 days later. Endpoints evaluated were bioactive compounds of the brain such as dopamine, homovanillic acid, norepinephrine, seratonin, and 5-hydroxyindolacetic acid. Levels of dopamine and homovanillic acid were approximately 30% lower in the brains of mice treated for 3 weeks with n-hexane when compared to 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 is 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 2-fold increase was seen in the concentrations of 2,5-hexanedione (5.63 ± 0.4 μg/g versus 2–3 μg/g in controls). In contrast to the results in their earlier report (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 to control mice.

Goel et al. (1982) gave six female albino rats/group two daily intraperitoneal injections of 1mL/kg (660 mg/kg) n-hexane. Other rats received the same dose for 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 compound-related changes in clinical chemistry parameters were reported, including decreases in acetylcholinesterase, albumin, and cholesterol (in the latter, 164.72 ± 4.52 mg/dl after 2 days, 139.27 ± 4.95 mg/dl after 7 days, and 137.1 ± 5.2 mg/dl after 45 days, versus 164.8 ± 2.93 in controls). In a later study, the same researchers 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 to 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 compound-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 possibly correlated with a transient increase in plasma acetylcholinesterase activity that declined to baseline 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 to controls (Table 22). The authors speculated that the electrophysiological changes may have been related to a disruption of nerve-membrane ATPase activity.

Table 22. Changes in the 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*	0.78 ± 0.07*
	2,5-Hexanedione	ND	20.2 ± 0.46*	1.72 ± 0.11*
Sural	Control	1.04 ± 0.07	18.5 ± 0.61	0.68 ± 0.07
	n-Hexane	0.81 ± 0.30	14.7 ± 0.59*	0.92 ± 0.02*
	2,5-Hexanedione	0.90 ± 0.31	13.5 ± 0.29*	1.97 ± 0.24*

Source: Adapted from Anderson and Dunham (1984)
* statistically significant compared to controls ($p<0.05$)
ND = no data.

Khedun et al. (1996) exposed male Wistar rats for 30 days to an oral injection of 0.1 mL (66mg) n-hexane in olive oil. A Langendorff apparatus was used to measure the heart rate, coronary flow, and ventricular fibrillation threshold, the latter parameter showing a dramatic reduction compared to 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 one hour each to learn to press the level 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 post-treatment days 1 or 2 on learned behavior in a commercial operant chamber.

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 compounds of n-hexane such as 2-methylpentane (13%), 3-methylpentane (16%), methylcyclopentane (16%), 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. Prechronic Studies

Duffy et al. (1991) reported a 13-week inhalation toxicity study of commercial hexane in F-344 rats and B6C3F1 mice, in which 10 animals/sex/group were exposed to target concentrations of 0, 900, 3000, or 9000 ppm commercial hexane, 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 on 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 to controls. The kidneys of high-dose males showed mild tubular dilatation, with granular material in the lumen and signs of epithelial regeneration compared to controls. High-dose males had mild to moderate degrees of regenerative epithelium, 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 Soiefer et al., 1991). Twelve rats/sex/group were exposed to 0, 900, 3000, or 9000 ppm commercial hexane, 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 compound-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 on commercial hexane, one in F-344 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 in 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 compound-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 F-344 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 compound-related tumor formation at any tissue site in F-344 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, ophthalmoscopicoscopic effects, or hematology among the groups, and body weight changes in commercial hexane exposed mice were similar to those in controls. There was a statistically significant, dose-related increased incidence of hepatocellular neoplasms in the livers of high-dose females, compared to controls. There was also an increased incidence of pituitary hyperplasia, adenomas, and adenocarcinomas in exposed females (Table 23). Commercial hexane was associated with decreased severity and incidence of cystic endometrial hyperplasia of the uterus among high-dose females compared to controls.

Table 23. Incidence of liver and pituitary tumors in male and female B6C3F1 mice exposed to commercial hexane for 2 years

Target Organ/ Cellular Response	Target Concentrations of Commercial Hexane (ppm)			
	0	900	3000	9000
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

Target Organ/ Cellular Response	Target Concentrations of Commercial Hexane (ppm)			
	0	900	3000	9000
Carcinomas	3/50	2/50	5/49	6/50
Combined adenomas and carcinomas	7/50	8/50	9/49	16/50*T
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*	7/48**	5/49*
Adenocarcinomas	0/45	0/48	1/48	0/49
Total Neoplasms	0/45	6/48*	8/48**	5/49*

Sources: Adapted from Daughtrey et al. (1999) and Biodynamics (1993b)
* $p<0.05$ and ** $p<0.01$; Significantly different from controls, as calculated by the authors using Fisher's Exact test
T = significant dose-related trend, Cochrane Armitage test, $p<0.05$

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 (8/group) and CD-1 mice (8/group) were exposed to commercial hexane, 6 hours/day, at target concentrations of 0, 900, 3000, or 9000 ppm on GDs 6–15 (BRRC, 1989a). Termination for the female rats was on GD 21 and female mice was on GD 18. Maternal body weight gain was monitored intermittently among the dams 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 to 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 female 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, 6 hours/day on GDs 6–15 and sacrificed 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 term. As in the range finding study, number of ovarian corpora, 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 under investigation 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 compound-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 to 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 incidence of two individual skeletal variations in high-dose pups. Comparing the rate of these effects between controls and high-dose groups by litter, the incidences of these lesions 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 the 0.05 level using 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 F0 Sprague-Dawley rats/sex/group were exposed to concentrations of 0, 900, 3000, or 9000 ppm commercial hexane, 6-hours/day, 5 days/week for 10 weeks. The study has been published in the peer reviewed literature by Daughtrey et al. (1994a). Clinical signs of toxicity were observed 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 post-natal 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. Similarly, 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 the F1 high-dose males (statistically significant). The number of pups born to exposed F1 rats were not statistically different compared to controls. F2 pup body weights in the high dose group were reduced by 6 to 9% compared to 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, 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 to 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

A subset of studies have explicitly addressed the extent to which other compounds can mediate or potentiate the toxic effects of n-hexane.

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, 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 exposed to methyl ethyl ketone died at that time point. The authors suggested that this increase in mortality was due to 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 swellings 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 five male Wistar rats/group to 500 ppm n-hexane, 700 ppm n-hexane, 300 ppm n-hexane plus 200 ppm methyl ethyl ketone, 400 ppm n-hexane plus 100 ppm methyl ethyl ketone, or 500 ppm n-hexane plus 200 ppm methyl ethyl ketone. The time taken for onset of hindlimb paralysis to develop was shorter in rats exposed to 700 ppm n-hexane and to the mixture of 500 ppm n-hexane plus 200 ppm methyl ethyl ketone compared to the other single n-hexane exposure and the other n-hexane plus methyl ethyl ketone mixtures.

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 have been observed as a result of co-exposure to methyl ethyl ketone (Altenkirch et al., 1978; Shibata, 1990a,b; van Engelen et al., 1997). 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% DL was reported for those rats exposed to 2000 ppm methyl ethyl ketone and 2000 ppm n-hexane compared to 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 to the level 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 was observed to be 2-fold greater than 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, 12 hours/day for 24 weeks (Takeuchi et al., 1983). MCV and mixed MCV were similar between 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 to rats exposed singly to n-hexane- or methyl ethyl ketone-exposed. There was little change in DL among the exposure groups (both single solvent and mixtures of solvents) throughout the experiment. However, the results suggest 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 medium supplemented with various solvents for up to 56 days. Cultures 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 swellings to appear in 75% of the cultures. Veronesi et al. (1984) employed eight cultures/dose. Cultures included n-hexane in the medium at 25, 50, 80, 100, and 250 μg/mL. Concentrations of methyl ethyl ketone employed in parallel cultures were 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 (25, 50, 100, 250 μg/mL) plus methyl ethyl ketone (10, 25, 50, 100 μg/mL) were also tested and were incubated for up to 56 days (see Table 2). All cultures were evaluated for signs of neuropathological effects under the light and electron microscopes.

The authors reported the development of axonal swellings, retraction of paranodal myelin, accumulation of neurofilaments, and peripheral displacement of neurotubules and mitochondria at n-hexane concentrations of 100 and 250 μg/mL. Time-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 intra-axonal rectilinear inclusions that developed in several cultures treated with 200–400 μg/mL methyl ethyl ketone.

As shown in Table 24, the presence of methyl ethyl ketone in neurotoxic concentrations of n-hexane in this system reduced the time-to-onset as compared to equivalent incubations containing n-hexane alone. Addition of methyl ethyl ketone to nonneurotoxic concentrations of n-hexane induced an apparently neurotoxic response.

Table 24. Time-to-onset for the appearance of axonal swellings in 75% of 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)				
	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

Source: Adapted from Veronesi et al. (1984)
NC = no pathological change

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, 12 hours/day, for 16 weeks. Electrophysiological parameters such as MVC, MCV, and DL were measured at the start of the experiment, after 4, 8, 12, and 16 weeks of exposure, then for 4 weeks after exposure was discontinued. Marked reduction in conduction velocities and increased DLs in the tail nerves of rats was observed following exposure to n-hexane alone. There were no neuropathological effects of toluene exposure or exposures to the mixture of n-hexane and toluene.

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 to 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 to a mixture of 1000 ppm n-hexane and 1000 ppm toluene, 21 hours/day, 7 days/week for 28 days. The auditory and visual sensitivity of the animals was measured as their brainstem auditory evoked

potentials (BAEP) and visual evoked potentials (VEP), 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 to controls and among the groups. Changes in auditory brainstem responsiveness to a click-evoked stimulus 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 to 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 to 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, 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 to 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 post-exposure 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 to 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 brought. Exposure to the mixture caused a rapid onset of motor deficits in male F-344 rats compared to 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., 1989; Ladefoged et al., 1994). Specifically, these studies have evaluated the neurotoxic effect of acetone co-exposure with the n-hexane metabolite 2,5-hexanedione.

Cardona et al. (1996) analyzed the relationship between exposure to solvents and the various forms of 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 the place of work of each subject for periods of time between 2 and 4 hours (averaging 200 minutes) and always continued to the end of the work shift. Urine samples were collected at the end of the work shift. Alveolar concentrations (exhaled air) were also collected. The median n-hexane, toluene, and acetone concentrations 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 concentration 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 correlation with environmental n-hexane exposure ($r = 0.936$) and n-hexane in exhaled air ($r = 0.7435$). Acetone was statistically significantly correlated with total and free 2,5-hexanedione/atmospheric n-hexane concentrations ($r = 0.6459$ and 0.6965, respectively). In addition, there was significant correlation ($r = 0.96626$ 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.

Ladefoged et al., (1989) exposed male Wistar rats (6/group) to either 0.5% 2,5-hexanedione, 0.5% acetone, 5.0% ethanol, 0.5% 2,5-hexanedione plus 0.5% acetone, and 0.5% 2,5-hexanedione plus 5.0% ethanol in drinking water (w/w). Food and water consumption were measured weekly, peripheral motor nerve conduction velocity was measured weekly from the third week of exposure, and neurobehavioral toxicity (balance time in 30 second intervals on a moving rod) weekly. Body weight gain and water consumption was statistically significantly reduced after two weeks following treatment with 2,5-hexanedione, 2,5-hexanedione plus acetone, and 2,5-hexanedione plus ethanol compared to controls administered pure water. Water consumption was also reduced in the ethanol only exposure group following the first week of dosing compared to 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 to controls. Acetone only exposed rats showed a statistically significantly reduction at 6 weeks exposure. Ethanol exposure alone did not produce any significant changes in MCV, but co-exposure with 2,5-hexanedione significantly reduced MCV at 3 weeks exposure duration compared to controls. The MCV's measured following exposure to 2,5-hexanedione plus ethanol were greater than exposure to 2,5-hexanedione alone. In addition, 2,5-hexanedione plus acetone led to a greater reduction in MCV compared to 2,5-hexanedione plus ethanol (statistically significant at week 4). Table 25 presents the results of this study. Acetone and ethanol alone did not affect balance time at any duration of exposure. 2,5-Hexandione alone reduced balance time in rats exposed for 3 and 4 weeks only. Acetone plus 2,5-hexanedione reduced balance time from the second week of dosing until the end of the study (6 weeks) and this effect was significantly greater from the forth week of dosing onward. A reduction in balance time was also observed for the 2,5-hexanedione plus ethanol group, but was not significantly longer compared to rats given 2,5-hexanedione alone.

Table 25. Effect of 2,5-hexanedione, acetone, ethanol, and mixtures of 2,5-hexanedione with acetone or ethanol in drinking water on average motor nerve conduction velocity in m/sec

Dose week	Control	0.5% 2,5-hexanedione	0.5% acetone	0.5% 2,5-hexanedione plus acetone	5.0% ethanol	0.5% 2,5-hexanedione plus 5.0% ethanol
3	29.6 (3.0)	26.1 (2.4)**	28.0 (2.9)	24.8 (2.9)**	29.2 (2.0)	25.1 (1.6)***
4	30.3 (2.5)	25.8 (2.3)***	28.6 (1.8)	23.5 (1.8)***§	29.6 (1.4)	26.1 (2.6)**
5	29.3 (1.1)	25.3 (2.9)**	28.8 (1.2)	22.3 (3.2)*	31.2 (0.9)*	27.1 (1.7)*
6	31.5 (2.1)	25.1 (1.8)***	29.5 (1.1)*	23.0 (2.8)***§	32.5 (1.1)	26.8 (1.7)***

(SD) standard deviation
Significantly different from control: *p < 0.05; ** p < 0.01; p< 0.001
§significantly different from group receiving 0.5% 2,5-hexanedione

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 served as controls. One a week body weight and food and water consumption were measured. In addition the following behavioral indicators were also observed weekly: ambulation (crossing in an open field for 5 minutes), rearing (number of times both forelegs were raised from the floor in 5 minutes), balance on a accelerating rotarod, and grip strength of forelimbs and hindlimbs. 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 to 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 to 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 2,5-hexanedione only treated rats, but not for rats treated with 2,5-hexanedione and acetone. During the 10 week recovery period, the reduction in grip strength was not reversible for either group.

Following 6 weeks of exposure to 2,5-hexanedione and 2,5-hexanedione plus acetone there were statistically significant increased giant axonal swellings 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), respectively and class 10 fibers was 0.2 (0.0-0.5) and 0.9 (0.0-2.1) compared to controls (class 9, 0.0 [0.0-0.5] and class 10, 0.0 [0.0-0.3]). 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 co-exposures compared to 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 co-exposure 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% 2,5-hexanedione alone or in combination (weight/volume concentrations) with 0.5% acetone for 6 weeks in drinking water. At week 5 of exposure, half of the rats were mated with females (unexposed) and the number of matings, pregnancies, and fetuses were measured. 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 observed. Water and food intake was reduced in rats receiving 2,5-hexanedione alone (dose dependent) and was slightly further decreased with co-exposures with 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 had a dose dependent decrease in body weight gain, which was greater with acetone exposure. Following the dosing period, food and water intake among all dosed groups reached control levels except in rats exposed to the high dose 2,5-hexanedione alone and high dose 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 remained in the high dose 2,5-hexanedione alone and with co-exposure to acetone. In addition, following the recovery period, testicular atrophy and reduced testis tubuli diameter were present in all dose groups except acetone alone exposure. The authors stated that acetone potentiated these 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 (Schaumburg and Spencer, 1976; Spencer and Schaumburg, 1977a). Sprague-Dawley rats (8 animals total) were exposed to 400-600 ppm n-hexane continuously for 1-23 weeks. Three additional animals received a subcutaneous injection of 650 mg/kg- 2 g/kg 5 days a week for up to 35 weeks. The animals were observed for clinical signs of neuropathy (characterized by waddling gait, hindlimb 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 neurofilaments, mitochondria, and smooth endoplasmic reticulum with increase in the number of neurofilaments. The earliest pathological indicator of peripheral nerve axonal degeneration was axonal swelling in the distal non-terminal region of the large myelinated fibers. These axonal swellings appeared first on the proximal sides of the paranodes 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 spilt and retracted leaving giant axonal swellings near the nodes of Ranvier. The study authors suggested that Schwann cells may become associated with these naked regions and remyelinate these short segments. These short 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, 5 weeks exposure. 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) after 3 weeks exposure to 2,5-hexanedione. In addition, the authors observed giant axonal swellings 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 unstated) 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 (dying back) that has been 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 compound by gavage to five male COBS, CD(SD) BR rats/group, 5 days/week for 90 days (section 4.2.1.1). As judged by the time taken for neuropathological symptoms to develop, the parent compound 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 (Krasavage et al., 1980; Couri and Milks, 1982). Abou-Donia et al. (1982) observed a similar comparative neurotoxic relationship when n-hexane, methyl b-butyl ketone, 2,5-hexanediol, and 2,5-hexanedione were administered orally or intraperitoneally to hens. Pathological examination of treated birds showed giant paranodal axonal swellings 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 compounds was, in descending order, 2,5-hexanedione, 2,5-hexanediol, methyl n-butyl ketone, 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 compounds 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 F-344 rats exposed to the compound by gavage at a dose rate of 2.2 mmol/kg-day for 90 days (Ralston et al.,1985). Compared to controls, 2,5-hexanedione-exposed animals performed progressively worse in the hindlimb grasp and hindlimb 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, no neurohistopathological lesions were observed at any other exposure than the high-dose rats after 28 days. These studies taken together suggest that 2,5-hexanedione-induced deficits in performance in a functional observational battery can precede the overt development of peripheral neuropathy, as exemplified by axonal swelling, rearrangement of neurofilaments, and regression of the myelin sheaths.

The molecular mechanisms that are involved in bringing about n-hexane induced neuropathological effects are not completely understood. Several studies have suggested that the mode of action involves the binding of the toxic metabolite, 2,5-hexanedione, to proteins forming pyrroles. For example, 2,5-hexanedione has been shown to cross-link neurofilament 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 neurofilament 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. The latter were not observed in the electrophoretic pattern of neurofilaments excised 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. This was taken as *a priori* evidence that cross-linking of neurofilamentous proteins had taken place as a result of 2,5-hexanedione exposure. As reported by the authors, a number of higher-molecular weight peptides were reactive with antibodies to all three of the neurofilament 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*. DeCaprio et al. (1982) showed non-acidic amino acids to be the most reactive species when incubated with 2,5-hexanedione and 2,4-hexanedione. Poly-lysine also was extremely reactive to both ketones. Selectively blocking the ϵ-amino group of lysine to give N-*α*-*t*butoxycarbonyl lysines provided inferential evidence that the ϵ-residue of lysine is much more reactive to 2,5-hexanedione 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 finding was considered to be consistent with a progressive loss of positive charge due to modification of lysine residues. Prolonged incubation with 2,5-hexanedione resulted in protein components that did not enter the gel, possibly indicative of the formation of higher molecular weight material due to cross-linking.

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, and 4-heptanone; 5-methyl-3-heptanone; 6-methyl-2,4-heptanedione, 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.

Evidence for pyrrole formation *in vivo* has come from Kessler et al. (1990) who detected pyrrole-like substances in the urine of a human volunteer exposed to n-hexane at a concentration of 146 ppm and in urine of male Wistar rats administered 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 neurofilament 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 enhances the compound's potential for pyrrole formation compared to that of 2,5-hexanedione. Rats given 0.25 mmols/kg 3,4-dimethyl-2,5-hexanedione every 8 hours developed severe limb paralysis within 3 days. The condition was marked with axonal swellings just proximal to the first node of Ranvier. The swellings contained masses of neurofilaments.

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 been formed during the reaction, and showed that similar entities were formed as products of reactions between other primary amines and proteins and 2,5-hexanedione and γ-diketones such as 2,5-heptandione and 3,6-octanedione. Although the sequence of critical events remains uncertain, Graham et al. (1982b) speculated that cross linking of neurofilaments as a result of n-hexane exposure likely involved some or all of the following processes: metabolism to 2,5-hexanedione, interaction of that compound with ϵ-lysyl residues of

proteins, cyclization to form a pyrrole, and 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 to that of 2,5-hexanedione. For example, in *in vivo* studies in five Sprague-Dawley rats/group (sex unstated), animals were intraperitoneally injected five times/week with either, 0 2.5, and 4 mmols/kg-day 2,5-hexanedione, or 0.0625, 0.125, and 0.25 mmols/kg-day 3,4-dimethyl-2,5-hexanedione (Anthony et al., 1983a). Dimethyl substitution led to an acceleration of peripheral neuropathy as judged by the lower dose and shorter time required for the onset of hindlimb paralysis. For example, a daily dose of 0.25 mmols/kg-day 3,4-dimethyl-2,5-hexanedione produced hindlimb paralysis after 19.6 ± 1.4 days, indicative of a cumulative toxic dose of 3.5 ± 0.29 mmols/kg. By contrast, a 16-fold higher dose of 2,5-hexanedione (4.0 mmols/kg-day) brought about hindlimb paralysis after 35.8 days, equivalent to a cumulative toxic dose of 102 ± 7.4 mmols/kg. Ultrastructural examinations of a giant axonal swelling from the anterior root of rats exposed to 3,4-dimethyl-2,5-hexanedione showed an accumulation of neurofilaments, 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. (1983b) provided further evidence for increased reactivity and pyrrole-forming capacity of 3,4-dimethyl-2,5-hexanedione compared to 2,5-hexanedione by studying their rates of reaction with 0.02M ethanolamine and ovalbumin. Reactions 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 under the action of the dimethylated diketone. Protein crosslinking was also measured in the presence of both ketones. The rates of polymer formed were 1.3–1.5 $mols^{-1}$ $hour^{-1}$ for 3,4-dimethyl-2,5-hexanedione compared to 0.034–0.037 $mols^{-1}$ $hour^{-1}$ for 2,5-hexanedione. The increase in polymer formation represents an increase in the rate of protein crosslinking.

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 capacity to form microtubules, was altered in γ-diketone-modified preparations. Specifically, the maximal velocity of assembly was consistently different between control and treated samples. Elongation phase kinetics were approximately three-fold higher in treated than in control assemblies. Gel filtration of the derivatized tubulin preparations showed that dimerization had occurred in those 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 compound 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 different amounts of pyrrole formation Table 26).

Table 26. 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	0.6 ± 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**	1.9 ± 0.4**	2.1 ± 0.5*
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**	1.7 ± 0.8	1.1 ± 0.1*

Source: Adapted from DeCaprio et al. (1988)
Significantly different to corresponding incubations with 2,5-HD (** $p<0.001$, * $p<0.01$)

DeCaprio et al. (1988) also exposed male Wistar rats via intraperitoneal injection of the separate analogues. Milder symptoms of hindlimb paralysis for the perdeuterated compound and a lower rate of adduct formation was observed. However, tissue concentrations of each diketone isomer were not markedly different (DeCaprio et al., 1988). The degree of covalent cross-linking of axonal proteins appeared to correlate with the amount of pyrrole formed. 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 hindlimb paralysis, no axonal swellings of the peripheral nerves, and aggregated neurofilaments There was a similar rate of pyrrole formation in rats receiving 3-acetyl-2,5-hexanedione compared to 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 assumed that the characteristics of the pyrrole formed by 3-acetyl-2,5-hexanedione rendered it less susceptible to oxidation. The authors suggested that the lack of an electrophilic center, would make this pyrrole derivative more stable, thus it could not take part in further protein cross-linking. This hypothesis was supported by the absence of peripheral neuropathy associated with the other γ-diketones. This evidence indicates that pyrrole oxidation and protein cross-linking are necessary steps in the pathogenesis of γ-diketone neuropathy.

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

linearly with 2,5-hexanedione. SDS-PAGE and fluorography showed prominent labeling of three neurofilament (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 have the majority of the 2,5-hexanedione binding sites, suggesting the possibility for limited and selective pyrrole adduction of neurofilament proteins. Cyanogen bromide cleavage of 2,5-hexanedione-induced pyrrole adducts of neurofilament 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).

4.4.5. Genotoxicity Studies

Data from limited short term *in vitro* tests provide minimal evidence of the genotoxicity of n-hexane (Table 27, 28, and 29). For example, NTP (1991) observed that n-hexane was negative for gene reversion in *S. typhimurium* strains T98, TA100, TA1535, or TA1537 with or without activation with S9 (Mortelmans et al., 1986; NTP, 1991). Ishidate et al. (1984) reported no increased reverse mutations in the Ames test in strains TA92, TA94, TA98, TA100, TA1535, or TA1537 due to 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 2-fold 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 *E. coli* or *B. 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 27. Summary of *in vitro* studies on the mutagenicity/genotoxicity of n-hexane

Test System	Cell/Strain	Results	Reference	Comments
Bacteria				
S. typhimurium	A98, 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
Chinese hamster fibroblasts	CHL	positive (tested - S9 only)	Ishidate et al. (1984)	Polyploidy
Human lymphocytes		borderline positive (-S9) negative (+S9)	Perocco et al. (1983)	Inhibition of DNA synthesis
Mammalian cells				
Chinese hamster ovary		negative (- S9) borderline positive (+ S9)	NTP (1991)	SCE
Chinese hamster ovary		negative (+/- S9)	NTP (1991)	CA
Chinese hamster cells	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; (positive) = borderline positive; SCE = sister chromatid exchanges; CA = chromosomal aberrations; MN = micronuclei; NCE = nonchromatic erythrocytes; PCE = polychromatic erythrocytes

Table 28. Summary of *in vivo* studies on the mutagenicity/genotoxicity of n-hexane

Species	Strain	Compound tested	Results	Reference
Mice	CD-1	n-hexane	negative (dominant lethal)	Litton Bionetics (1980)
	B6C3F1	n-hexane	negative (CA and MN)	Shelby and Witt (1995)
	ND	n-hexane	negative (SCE) negative (NCE and PCE) positive (CA)	NTP (1991)
Rats	Albino	n-hexane	positive (CA)	Hazleton Labs (1992)
	Sprague-Dawley	commercial hexane	negative (CA)	Microbiological Associates (1990)

ND = no data; (positive) = borderline positive; SCE = sister chromatid exchanges; CA = chromosomal aberrations; MN = micronuclei ; NCE = nonchromatic erythrocytes; PCE = polychromatic erythrocytes

Table 29. Summary of *in vivo* and *in vitro* studies on the mutagenicity/genotoxicity of commercial hexane mixtures

***In vitro* tests with commercial hexane mixture**				
Bacteria *S. typhimurium*	TA98, TA100, TA1535, TA1538	negative (+/- S9)	Microbiological Associates (1989)	Gene reversion (in vapor phase)
Mammalian Cells Chinese Hamster fibroblasts	CHL	negative (ND on S9 status)	Kawachi et al. (1980)	CA
***In vivo* tests with commercial hexane mixture**				
Rats	Sprague-Dawley	negative	Microbiological Associates (1990)	CA

ND = no data; (positive) = borderline positive; SCE = sister chromatid exchanges; CA = chromosomal aberrations; MN = micronuclei ; NCE = nonchromatic erythrocytes; PCE = polychromatic erythrocytes

Those *in vitro* cytogenic tests in mammalian cell lines that have included n-hexane as a test compound have been generally negative, although n-hexane induced polyploidy in Chinese fibroblast hamster cells (CHL) (Ishidate et al., 1984). DNA synthesis was inhibited in human lymphocytes in the presence of concentrations of n-hexane from 10^{-4} M to 10^{-2} M, but only in the presence of cytotoxicity (Perocco et al., 1983). NTP (1991) reported a marginally increased incidence of sister chromatid exchanges (SCE) 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 (NTP, 1991; Daughtrey et al. 1994b). 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, 1978).

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

Hazleton Laboratories (1992) recorded a slight, but significant, increase in the number of chromosomal mutations due to 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 to controls (Hazleton Laboratories 1992). n-Hexane did not increase the incidence of SCEs in an *in vivo* mouse bone marrow cells at intraperitoneal doses of 500, 1000, or 2000 mg/kg of n-hexane (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, 1989; Microbiological Associates, 1990). 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 hindlimb paralysis, a frequent symptom of n-hexane 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 to 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 to 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-hexane, 2,5-hexanediol, methyl n-butyl ketone, and 2-hexanol) by administering equimolar doses of each compound by gavage to five male COBS, CD(SD) BR rats/group, 5 days/week for 90 days. Based on the time taken by the rats to develop hindlimb 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 swellings and, in nerve fiber cross sections, a change in the distribution of fiber area size (Ladefoged et al., 1994).

Behavioral changes have also been demonstrated in female rats injected subcutaneously with 20 or 200 mg/kg 2,5-hexanedione, 7 days/week for up to 50 days (Pereira et al., 1992). The tests employed were open field, step-down inhibitory avoidance, and shuttle avoidance, with each animal receiving two sessions, the first for training and a second 24 hours later for testing. Rats exposed to 2,5-hexanedione at the high dose showed significant changes in general motor activity with a greater than 50% impairment of performance in the shuttle avoidance test.

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 compound-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. The mechanism of n-hexane induced neurotoxicity following oral exposure is not completely understood. However, 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 observed for 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 to 14 workers who were not exposed to any solvents in the same factory (Sanagi et al., 1980). The 22 breathing zone monitoring samples taken biannually over a 2-year period had an 8-hour TWA of 58 ppm for n-hexane and 39 ppm for acetone. Compared to 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 signs relating to muscle strength, and reduced vibration sensation of the radial nerve. Neurophysiological findings suggested a delayed recovery from a slowing of motor nerve conduction in the posterior tibial nerve.

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 mean TWA for n-hexane of the 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 factor (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 compound. 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 (Ahonen et al., 1988; Saito et al., 1991; Mutti et al.,1993; Takeuchi, 1993; Cardona et al., 1993, 1996; Mayan et al., 2001; Prieto et al., 2003). 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 electrographic changes indicative of polyneuropathy and urinary excretion of metabolites indicative of exposure to n-hexane, they 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 electroneuromyographic 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 work week (ACGIH, 2003).

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

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 body weight gain and MCV in male Wistar rats (8/group) exposed to 0, 500, 1200, or 3000 ppm n-hexane (>99% pure) for 12 hours/day, 7 days/week to 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

hindlimb 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-S-100 proteins from tail nerve fibers. The neurophysiological deficits and histopathology indicate a NOAEL of 500 ppm.

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 degeneration of the myelin sheaths 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 single concentrations of n-hexane in the 1000-2000 ppm range to induce compound-related neurophysiological deficits and/or behavioral changes in animals exposed to n-hexane compared to controls. Data from CIIT's 13-week toxicological study in F-344 rats exposed to n-hexane confirmed the neuropathological responses to the compound 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 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 indicate 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-S-100 protein. An 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 F-344 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 compound-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 a compound-related reduction in body weight gain in the fetuses of mid- and high-dose dams (3.0–7.5% at the mid-dose in females and males, respectively, and 14–15% at the high-dose in females and males, respectively), 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 et al., 1987). Examination of the fetuses revealed some potentially compound-related variations, including the incidence of supernumerary ribs and reduced skeletal ossification. A companion study in CD-1 mice (Mast et al., 1988c) 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 is 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 have been observed following inhalation exposure to n-hexane (DeMartino et al., 1987; Nylen et al., 1989). 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 F-344 rats exposed to n-hexane have indicated that the kidney may be a target organ for the compound, at least in this test species (Cavender et al., 1984a,b). However, this response may be related to the well-described α2u-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 alpha 2u-globulin accumulation; the data are not to be used in the assessment of health risk (U.S. EPA, 1991b). The criteria for demonstrating this mode of action for risk assessment purposes have been clearly defined (U.S. EPA, 1991b). 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 alpha 2u-globulin; 3) additional pathological lesions associated with alpha 2u-globulin are also present. In addition, a number of mechanistic studies can be used to further support conclusions regarding the role of alpha 2u-globulin. Data suggest that kidney effects following inhalation exposure to n-hexane may be due to the accumulation of alpha 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 and fibrosis of the submucosa (NTP, 1991; Dunnick et al., 1989).

A substantial number of toxicological studies have been carried out on various formulations of a mixture (commercial hexane) containing predominantly n-hexane (approximately 50%) that provide 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).

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 (Soiefer et al., 1991).

A single toxicological study has addressed the possible impacts of commercial hexane when administered to experimental animals using a chronic dosing regimen (Biodynamics, 1993a,b; Daughtrey et al., 1999). The study exposed F-344 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 F-344 rats exposed to commercial hexane in this study. There was no compound-related incidence in tumor formation at any tissue site in F-344 rats. There was an increased incidence of hepatocellular neoplasms that were apparent in female mice only (Section 4.4.2.2.2., Table 25). There also was an increased incidence of pituitary hyperplasia, adenomas, and adenocarcinomas in females.

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, 6 hours/day on GDs 6–15 (BRRC, 1989a,b). 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 at the high-dose and at mid-dose for part of the exposure from GDs 9 to 12 in rat dams. 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 in Sprague-Dawley rats (BRRC, 1991; Daughtrey et al., 1994a). 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). Sprague-Dawley rats were exposed to 0, 100, 500, or 1503 ppm commercial hexane via inhalation, 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 weight of high-dose F1 pups remained 8–9% lower than that of controls throughout lactation. Similar body weight reductions compared to 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 toxicity of commercial hexane are provided by the histopathological lesions of high-dose male F-344 rats exposed to 0, 900, 3000, or 9000 ppm commercial hexane, 6 hours/day, 5 days/week for 13 weeks (Biodynamics, 1989; Duffy et al., 1991), 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 30.

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 this compound. 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 axonal swelling and myelin degeneration, that could possibly 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). The axonal swelling progressed and subsequent localized demyelination and remyelination produce axonal degeneration in a distal retrograde manner following further exposure to n-hexane. Shrinkage of the internode, accumulation of neurofilaments, 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 swellings accompanied by axonal and myelin degeneration in the peripheral nervous system in laboratory animals (Krasavage et al., 1980; Abou-Donia et al., 1982; Nachtman and Couri, 1984). The time to onset of these symptoms, severity of the lesions, and magnitude of the neurotoxicity indicates that 2,5-hexanedione is more toxic than any other metabolites of n-hexane or n-hexane itself. *In vivo* and *in vitro* studies indicate that the mode of action of 2,5-hexanedione may involve cross linking of the compound with proteins of spinal cord neurofilament proteins (DeCaprio et al., 1982; Lapadula et al., 1986; Kessler et al., 1990; Sanz et al., 1995; Mateus et al., 2002). Specifically, evidence suggests that 2,5-hexanedione may react with lysine residues forming pyrroles. Genter St. Clair et al. (1988) further 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 with the neurofilaments. Formation of pyrroles has been hypothesized to be responsible for the accumulation of neurofilaments observed in the distal axonal swellings of the peripheral nerves following n-hexane exposure (Graham et al., 1982a; 1982b; 1995).

Studies with structurally related chemical compounds of 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-diacetylethane) are associated with axonal swellings and neurofilament accumulation (Anthony et al., 1983a, b; Graham et al., 1995; Kim et al., 2001; Kim et al., 2002; Spencer et al., 2002). These structurally related compounds 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 the axonal swelling depends on the reactivity of the compound with the protein and the neurotoxicity associated with the compound (Anthony et al., 1983a, b; Genter St. Clair et al., 1988; Graham et al., 1995; Kim et al., 2001; Kim et al., 2002; Spencer et al., 2002).. 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 to distal locations following exposure to 2,5-hexanedione.

Table 30. Inhalation studies for n-hexane

Reference	Strain/Species	Doses (ppm)	Duration adjusted doses (mg/m^3)	Duration	Response	NOAEL (mg/m^3)	LOAEL (mg/m^3)
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
Ichihara et al. (1998)	same as above	0 2000 (12 h/d, 6 d/w)	0 3021	20 weeks	MCV↓, DL↑	none	3021
Pryor et al. (1983)	male F-344 rats	0 2000 (14 h/d)	0 4112	14 weeks	FOB effects	none	4112
Howd et al. (1983)	male F-344 rats	0 1000 (24 h/d, 6 d/w)	0 3021	11 weeks	hindlimb paralysis MCV↓	none	3021
Cavender et al. (1984a,b)	F-344 rats	0 3000 6500 10,000 (6h/d, 5 d/w)	0 1888 4091 6294	13 weeks	organ/weight↑, histopathology of peripheral NS	1888	4091
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↓, histopathology of the peripheral NS, 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, and β-S-100	none	7058

Reference	Strain/Species	Doses (ppm)	Duration adjusted doses (mg/m^3)	Duration	Response	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
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)
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)	0 315 629 2518 6294 2307	13 weeks	nasal irritation and relative liver, kidney and heart weights↑, neurobehavioral deficits and histopathology	315	6294 (and 2307)
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
Bus et al. (1979)	3 to 8 pregnant F-344 rats	0 1000 (6 h/d)	0 881	GDs 8-12 12-16 8-16	fetal weights↓, (with partial recovery)	none	881

Reference	Strain/Species	Doses (ppm)	Duration adjusted doses (mg/m^3)	Duration	Response	NOAEL (mg/m^3)	LOAEL (mg/m^3)
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
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, (1988a)	35 pregnant CD-1 mice	0 200 1000 5000 (20 h/d)	0 587.5 2937 14,686	GDs 6-17	reductions in relative uterus weight and body weight gain (HD) fetal resorptions	2937 none	14,686 587.5

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

4.6.1. Summary of Overall Weight-of-Evidence

Under EPA's Draft Revised Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1999), *data are inadequate for an assessment of the human carcinogenic potential of n-hexane* because a study (Beall et al., 2001) in humans chronically-exposed to n-hexane and other chemicals was inconclusive and there are no available animal studies examining exposure to n-hexane alone. 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 (Biodynamics, 1993a; 1993b; Daughtrey et al., 1999). 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 *Saccharomyces 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). The available studies in humans as well as laboratory animals thus far have not demonstrated a carcinogenic effect. 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 the compound and increased cancer incidence (Beall et al., 2001).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 (2 astrocytomas, 2 glioblastomas, and 2 oligodendrogliomas). Six cases had benign intracranial tumors, of which 2 were diagnosed as vestibular schwannomas (observed in 1 man and 1 woman), 2 as meningiomas (both in men), and 2 pituitary adenomas(observed in 1 man and 1 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 which 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 (OR) 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 confidence interval (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; 2 cases and 2 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 reduce the significance of this result.

In laboratory animals exposed for 2 years via inhalation to a commercial hexane mixture containing n-hexane (0, 900, 3000, 9000 ppm) there was a statistically significant increase in hepatocellular combined adenomas and carcinomas (7/50, 8/50, 9/49, 16/50, respectively) in female B6C3F1 mice (Biodynamics, 1993; Daughtrey et al., 1999). This increase was not observed in male mice or in either sex of F-344 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 the pure compound 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*, was negative for

chromosomal aberrations in Chinese hamster ovary cells and forward mutations in the mouse lymphoma L5178 $tk^{+/-}$ assay (Mortelmans et al., 1986; Ishidate et al., 1984; Houk et al., 1989; McCarroll et al., 1981a,b; NTP, 1991; Daughtrey et al., 1994; Hazleton Laboratories, 1992). n-Hexane was marginal for inducing chromosome loss in the DNA of *S. cerevisiae* D61M (Mayer and Goin, 1994). In *in vivo* tests, the compound was negative for inducing dominant lethal mutations in CD-1 mice (Litton Bionetics, 1980; Mast, 1988b). Furthermore, n-hexane was unable to induce chromosomal aberrations (CA) and micronuclei in bone marrow cells of B6C3F1 mice injected intraperitoneally with the compound (Shelby and Witt, 1995). n-Hexane did not increase the incidence of sister chromatid exchanges (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 weanling rats were more resistant to the neuropathological effects of n-hexane than adults. 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 to 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). 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 2 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 younger infants may have altered responses to the toxic effects of n-hexane exposure.

4.7.2. Possible Gender Differences

The available data do not provide unequivocal 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 (Biodynamics, 1993b; Daughtrey et al., 1999). 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 compound-related increase in the formation of adenocarcinomas of the pituitary gland in female mice compared to 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 (RfD)

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. 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 for n-hexane did not contain a derivation of an oral RfD. The only oral study (Krasavage et al., 1980) identified for oral exposure to n-hexane is of subchronic duration, utilized gavage exposure, and evaluated a small number (5/group) of animals. Several animals died in each dose group (two in the mid-dose and one in the high-dose groups, respectively) during the course of the 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 hindlimb paralysis, at which point the animal was sacrificed and examined histopathologically. The appearance of hindlimb paralysis in animals exposed to the high dose n-hexane (3/4) was observed. Giant axonal swellings were present in the nerves of the high dose group (4/4).

A route-to-route extrapolation using available inhalation data is currently not possible since limited PBTK models are available for n-hexane (Perbellini et al., 1986; Fisher et al., 1997). The Fisher et al. (1997) lactational transfer model was developed using rodent tissue solubility and allometrically-scaled metabolic rate constants available in the published literature (in abstract form only) to estimate human tissue metabolic parameters. In addition, the authors suggested that the absence of exposure and toxicokinetic data on lactation transfer of chemicals such as n-hexane to nursing infants is 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 the compound, 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 is 2-hexanol. Thus, using a PBTK model based on information from laboratory animal studies may not be appropriate.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

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

Many occupational and experimental exposure studies in humans investigate 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 co-exposure 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 Sanagi et al. (1980), which compared the neurological function 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 co-exposure to acetone was 39 ppm (biannual measurement over a two 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 was chosen as the principal study in the previous IRIS assessment (1990). The principal limitation of Sanagi et al. (1980) is that the workers were co-exposed to n-hexane and acetone, which raises the question of the role of acetone co-exposure in the observed neurological changes.

Several studies indicate that acetone may affect n-hexane metabolism, neurotoxicity, and reproductive toxicity (Ladefoged et al., 1989; Larsen et al., 1991; Ladefoged et al., 1994; Cardona et al., 1996). 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 induction of n-hexane metabolism by acetone may potentiate neurotoxicity by decreasing the elimination of 2,5-hexanedione. For example, studies in rodents have shown that co-exposure to acetone and 2,5-hexanedione increases the concentration of 2,5-hexanedione in the sciatic nerve compared to administration of 2,5-hexanedione alone (Ladefoged and Perbellini, 1986; Zhao et al., 1998). 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 (Section 3.3; Patten et al., 1986). Thus, co-exposure to acetone may induce CYP450 enzymes and increase the production of the neurotoxic metabolite, 2,5-hexanedione.

Oral co-exposure studies in rats further support acetone potentiation of n-hexane neurotoxicity (Section 4.4.3). Ladefoged et al. (1989, 1994) 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 co-exposed to 2,5-hexanedione plus acetone compared to those exposed to 2,5-hexanedione. In addition, Larsen et

al. (1991) suggested that co-exposure 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 co-exposure 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 co-exposure 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. (1982 a) 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 (mildly exposed) and 134 (highly exposed) ppm. 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 co-exposures 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 relatively low doses (Mast, 1987; Mast, 1988; Dunnick et al., 1989; Huang et al., 1989; NTP, 1991; IRDC, 1992a, b; Ono et al., 1992). These studies were considered for the selection of the principal study and are described below. 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, 6 hours/day, 5 days/week for 90 days or to 1,000 ppm n-hexane for 22 hours/day 6 hours/day, 5 days/week for 90 days (Dunnick et al., 1989; NTP, 1991). 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 performed in 4 animals/sex from the 0, 1000 ppm continuous exposure, and the 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 hour/day). They also considered these effects to be non-specific 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 amenable to 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 co-exposure 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, the IRDC (1992) 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 (8/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. DL and proximal mixed MCV were statistically significantly decreased at the low dose, but not the high dose. Degeneration of the myelin sheath 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 was inadequate for derivation of the point of departure. Specifically, the goodness of fit p value could not be estimated from the data (Appendix B). Therefore, the Ono et

al. (1982) study was not selected as the principal study for the derivation of the RfC.

Mast (1988a) exposed pregnant CD-1 mice (30/group) to 0, 200, 1000, and 5000 ppm n-hexane for 20 hours/day on GDs 6 to 17 . The authors reported a significant increased number of late resorptions in mice exposed to 5000 ppm n-hexane. The effects noted are at only the high dose. Therefore, the Mast (1988a) study was not selected as the principal study for the derivation of the RfC.

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 to 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 increased incidence of reduced skeletal ossification of sternebrae 1-4 was also observed at 5000 ppm. This study identifies a developmental NOAEL of 200 ppm from these effects, but the range between the NOAEL and the next highest dose (1000 ppm) is 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 (Bus et al., 1979; Litton Bionetics, 1979; Marks et al., 1980; De Martino et al., 1987; Mast et al., 1988b; and Mast et al., 1988c; Linder et al., 1992). In contrast to the studies by Mast (1987) and Mast (1988a), these studies do not indicate that n-hexane exposure produces adverse reproductive and 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 (8/group) via inhalation to 0, 500, 1200, or 3000 ppm (0, 1,762, 4,230, 10,574 mg/m^3) n-hexane, 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 swellings 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, adequate number of animals and exposure groups, and was of the appropriate quality for the derivation of the RfC. The Huang et

al. (1989) data set provided an adequate dose-response for BMD modeling with an estimated point of departure of a $BMCL_{HEC}$ of 215 mg/m^3 (Section 5.2.2 and Appendix B).

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 in a range of approximately 30-200 ppm (130-690 mg/m^3) n-hexane show effects associated with peripheral neuropathy, such as decreased MCV (Sanagi et al., 1980; Mutti et al., 1982a; Mutti et al., 1982b; Huang and Chu, 1989; Yokoyama et al., 1990; Huang et al., 1991; Chang et al., 1992; Karakaya et al., 1996; Yucesoy et al., 1999). 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 compound-related neurophysiological deficits and/or behavioral changes in F-344 or Wistar rats exposed to n-hexane. Data from CIIT's 13-week toxicological study in F-344 rats exposed to n-hexane (0, 3,000, 6,500, 10,000 ppm, respectively) confirmed the neuropathological responses to the compound based on the appearance of paranodal swellings of the tibial nerves in mid- and high-dose males (Cavender et al., 1984a,b).

5.2.2. Methods of Analysis

The RfC was derived by the benchmark dose approach (BMDS. Version 1.3.2). The benchmark response (BMR) was defined as a change of 10% for quantal endpoints such as developmental abnormalities in the fetus and a change of 1 control standard deviation (1sd) from the control mean for continuous endpoints such as fetal body weight and MCV (U.S. EPA, 2000) in the absence of any biological rationale for choosing a particular response level. Details of the model results are presented in Appendix B.

The BMCL estimates for the various studies are presented for the best fitting models in Table 31 and Appendix B. For each model, the software performed residual and overall chi-square goodness-of-fit tests, and determined the Akaike's Information Criterion (AIC). The chi-square p-value is a measure of the closeness between the observed data and the predicted data (predicted using the modeled fit). Models with chi-square 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-square p-values were identified. The BMCL estimates vary, depending on the endpoint, model selected, and parameter hypotheses and constraints. The best fitting model selection criteria were:

1. A P-value $\geq$ 0.1 for goodness of fit (hypothesis 4 for continuous models) for which a positive BMCL is estimable.
2. Least complex model (lowest AIC) which has a P-value and a positive BMCL estimate.

Table 31. 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*§	BMCL* §	$BMCL_{HEC}$**
Mast (1987)	Reduced Ossification of sternebrae 1-4	4	Nested Logistic	n $\geq$ 1	<0.0001 (Group 0.1834)#	1433	1571	943	2770
Mast (1987)	decreased fetal body weight gain	4	Hill	rho=0	0.019	-1559	1088	1034	3037
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	Quadratic	none	NE	95.98	126	71	125
Ono et al., (1982)	MNCV Total	3	Quadratic	none	NE	80.91	88	58	102
Ono et al., (1982)	MNCV Distal	3	Quadratic	none	NE	68.64	84	55	97

* units of ppm; ** $BMCL_{HEC}$ presented in mg/m^3 and conversion described in Section 5.2.2.1; § BMR = 10% for quantal endpoints and BMR = 1 standard deviation for continuous endpoints; NE Not estimable due to other computational problems

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.

Modeling results of the reduced fetal body weight gain data observed in Mast (1987) and the neurological effects (decreased MCV and MNCV) observed in Ono et al. (1992) produced inadequate P-values (Table 31). The reason that the P values were not determined is that there were too few dose groups to test hypothesis for goodness of fit. That is, with too many free parameters, the mean response data can fit for a small number of dose groups. With too few dose groups, and too many free parameters that have to be estimated from the data, there are zero or even negative degrees of freedom for the likelihood ratio test of the goodness of fit hypothesis. The P value is an upper tail probability based on the assumption that the test statistic -2 * (log of likelihood ratio) = -2 * (difference of log likelihoods of the fitted dose-response model and the model with no assumptions about means) and has a chi-squared distribution with degrees of freedom for the goodness of fit hypothesis = (number of dose groups or separate means to estimate) - (number of parameters in the dose-response model), assuming the same variance function models for both likelihoods. An attempt to fit a quadratic model (3 parameters, b0, b1, b2) to data with 3 dose groups results in degrees of freedom for the goodness of fit hypothesis = 3 - 3 = 0, and likewise for a power model (parameters intercept, slope, power) or any other 3-parameter model with no parameters specified.

No p value was obtained by using a 4-parameter model such as the cubic or the Hill, when the degrees of freedom for the goodness of fit hypothesis with 3 dose groups equals -1. Sensible specifications of some model parameters may reduce the number of parameters to be estimated from the data to less than the number of dose groups, allowing a P value to be calculated, but these specifications need to be justified. BMD modeling of the remaining endpoints from Mast (1987; 1988) and Huang et al. (1989) produced adequate P-values ($\geq$ 0.1). Huang et al. (1989) was selected as the principal study with decreased MCV at 12 weeks (lowest AIC value) n-hexane exposure in males as the critical effect (Section 5.2.1).

The data for decreased MCV were presented in Figure 1 of the study as means +/- standard error of the mean (s.e.m.) MCV. 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, converted to the scale of the measurements, and the s.e.m. was converted to standard deviation (sd). BMDS version 1.3.2 does not have a time series analysis option, thus the Huang et al. (1989) data was 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. The BMCL (1sd) of 122 ppm (430 mg/m^3) for decreased MCV in rats exposed to n-hexane for 12 weeks was chosen as the point of departure.

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 studies featuring intermittent, less-than-lifetime, exposures in experimental animals, EPA guidance (U.S. EPA, 1994) provides mechanisms for (1) adjusting experimental exposure concentrations to a value reflecting continuous exposure and (2) determining a human equivalent

concentration 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:

$$BMCL_{ADJ} = BMCL \times 12 \text{ hours/day}/24 \text{ hours/day}$$
$$= 430 \text{ mg/m}^3 \text{ x } 12 \text{ hours/day}/24 \text{ hours/day}$$
$$= 215 \text{ mg/m}^3$$

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, 1994), n-hexane is a category 3 vapor because it is largely inactive in the respiratory tract, rapidly transferred between the lungs and blood, and has toxicological effects that are extrarespiratory. Therefore, the duration adjusted $BMCL_{ADJ}$ can be factored 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 F-344 rats (Gargas et al., 1989) and an L_H in humans of 0.8 (Perbellini et al., 1985a). By convention, because a L_A/L_H greater than 1 would be derived from these values, a value of unity is adopted for this parameter by default. This allows a $BMCL_{[HEC]}$ to be derived as follows:

$$BMCL_{[HEC]} = \text{mg/m}^3 (BMCL_{ADJ}) \times 1 \text{ (interspecies conversion)}$$
$$= 215 \text{ mg/m}^3 \text{ x } 1$$
$$= \mathbf{215 \text{ mg/m}^3}$$

The $BMCL_{[HEC]}$ value of 215 mg/m^3 for reduced MCV in rats is 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 (Huang et al., 1989).

A total UF of 1000 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); 10 to extrapolate to chronic exposure from data in a less-than lifetime study (UF_S); and 3 to account for database deficiencies (UF_D).

An UF_H of 10 was applied to account for variations in susceptibility among members of

the human population (interindividual variability). Some animal data suggest that younger rats may be less sensitive to n-hexane induced neurotoxicity (Howd et al., 1983). Specifically, Howd et al. (1983) compared the neurotoxicity of n-hexane in weanling versus young adult F-344 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 hindlimb paralysis which was present earlier and was greater in severity in adults compared to weanlings. The authors suggested that weanling rats may be less sensitive to n-hexane induced neurotoxicity, due to smaller diameter and shorter axons in weanling compared to adult rats. Several studies providing support for Howd et al. (1983) indicate that 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). In the absence of information on the variability in humans to n-hexane exposure the default of 10 was used.

An 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 $BMCL_{HEC}$ already 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, 1994). The toxicodynamic uncertainty is also accounted for to a certain degree by the use of the applied dosimetry method.

A UF_S of 10 was applied to extrapolate from subchronic to chronic exposure. A subchronic (16 weeks) study was used for the derivation of the RfC.

A UF_D of 3 was applied to account for database deficiencies. The database includes many human occupational exposure studies (with co-exposure), subchronic 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 does not include a developmental neurotoxicity study or a one- or two-generation reproductive and developmental toxicity study following inhalation exposure to pure n-hexane alone. The database also lacks chronic exposure studies reporting the effects of pure n-hexane via any route of exposure. Prenatal exposure to n-hexane induced skeletal anomalies, decreased fetal body weight, and increased resorptions, suggesting that the fetus may be susceptible to n-hexane (Bus et al., 1979; Mast 1987; Mast 1988a). In addition, the nervous system has been shown to be the primary target of toxicity following n-hexane exposure in both humans and animals (see Sections 4.1 and 4.2). Given the potential increased susceptibility of the fetus to n-hexane induced neurotoxicity, a UF_D of 3 was applied.

An 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, an RfC from the Huang et al. (1989) data is calculated as follows:

$$RfC = BMCL_{[HEC]} \div UF$$
$$= 215 \text{ mg/m}^3 \div 1000$$
$$= \mathbf{2E\text{-}1 \text{ mg/m}^3}$$

5.2.4. Previous Inhalation Assessment

The previous IRIS assessment for n-hexane contained an inhalation RfC of 2E-1 mg/m^3 that was developed 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 suggests that co-exposure to acetone potentiates n-hexane metabolism and n-hexane induced neurotoxicity (Ladefoged et al., 1989; Larsen et al., 1991; Ladefoged et al., 1994; Cardona et al., 1996). 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 co-exposure to both solvents. Thus, Sanagi et al. (1980) was not retained as the principal study for the derivation of the RfC, based on co-exposure 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 10000 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 co-principal 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 the 4000 ppm exposure (6 hour/day). They also considered these effects to be non-specific 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 swellings 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, 4000 ppm for 6 hours/day). The lack of histopathology is considered to be a significant deficiency in 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) have been 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 data base for n-hexane is inadequate for an assessment of human carcinogenic potential according to the U.S. EPA's 1999 draft *Guidelines for Carcinogen Risk Assessment*. 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 (Biodynamics, 1993a,b; Daughtrey et al., 1999). However, the increased tumor incidence was of borderline statistical significance and was not present in treated male mice, nor in either sex of F-344 rats exposed to commercial hexane under the same conditions. Additionally, because commercial hexane is an 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 the pure compound would be unjustified.

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

6.1. HUMAN HAZARD POTENTIAL

n-Hexane (CAS RN 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 compounds, such as methylpentane and methylcyclopentane.

Highly purified n-hexane is used as a reagent for chemical or chromatographic separations. Other grades of the compound 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. The compound 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 this compound. 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 the compound, 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 the compound 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 2E-1 mg/m^3.

Compelling mode of action evidence has focused on the capacity of n-hexane to undergo metabolism to the γ-diketone, 2,5-hexanedione. This compound appears to have the ability to interact with specific proteins on the peripheral nerve filaments. 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 neurofilament cross-linking. The importance of γ-diketone-generated pyrrole formation also indicates the unique nature of n-hexane-generated neuropathy compared to the relative benign effects of such structural analogues as n-heptane and pentane. The inability of the latter compounds 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 is poor, because only one study has been identified in which experimental animals were exposed to the compound for an adequate duration (Krasavage et al., 1980). However, as discussed in Section 5.1.1., this study is inadequate for the development of an oral toxicity value for the compound.

Under the *Draft Revised Guidelines for Carcinogenic Risk Assessment* (U.S. EPA, 1999), *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 prechronic gavage studies, both of which were unsuitable for the calculation of an RfD.

The RfC of 2E-1 mg/m^3 arises from the neurological effects of n-hexane in Wistar rats (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 (Howd et al., 1983; Pryor et al., 1983; Cavender et al., 1984a,b; Dunnick et al., 1989; NTP, 1991; Huang et al., 1992). 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 the pure compound via any route of exposure and a multi-generational 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 *Draft Revised Guidelines for Carcinogenic Risk Assessment* (U.S. EPA, 1999), the database for n-hexane is inadequate to assess human carcinogenic potential. As such, data are unavailable to calculate quantitative cancer risk estimates.

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APPENDIX B: BENCHMARK DOSE (BMD) ANALYSIS

Benchmark dose (BMD) modeling was conducted according to draft EPA guidelines (U.S. EPA, 2000) using Benchmark Dose Software Version 1.3.2 (BMDS) available from the U.S. EPA (U.S. EPA, 2003). The BMD modeling results are summarized in Table B-1, and the model outputs are attached. A brief discussion of the modeling results is presented below.

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 (Mast, 1988a; Huang et al., 1989; Ono et al., 1992). 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 BMDS is still undergoing Beta-testing, and was not used because it was not considered 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 benchmark response (BMR) was defined as a change of 10% for quantal endpoints such as developmental abnormalities in the fetus and a change of 1 control standard deviation (1sd) from the control mean for continuous endpoints such as fetal body weight and MCV (U.S. EPA, 2000). This BMR was selected because there was no clear biological rationale for selecting an alternative response level (U.S. EPA, 2000).

The BMDS analyses shown below demonstrate a number of issues not commonly encountered in IRIS assessments. The first issue is that many dose-response functions in the Continuous, Quantal, or Nested modules allow the dose or concentration metric to be modified by raising it an exponent here called n, sometimes called "rho" in the programs. We reserve the Greek letter "rho" as used in this Appendix for the exponent used in the variance function in the continuous models, not in the dose-response function. In many cases the BMC and BMCL estimates depend on estimating n and rho from the data, or by specifying the value of these and other parameters as known constants. BMDS often assumes as a default that $n > 1$. If the optimal value of n is less than 1 (sub-linear dose-response), as happens in a number of the analyses for these data, then the BMDS program cannot allow any estimate of n to be less than 1 as long as this default is not disabled. An extensive sensitivity analyses was performed to assess the consequences of values of n less than 1 where appropriate.

A second issue arises in the analyses of teratology data using the Nested module in BMDS. This module allows analyses of quantal data such as the number of resorbed fetuses in a litter, or the number of fetal abnormalities such as the number of fetuses with reduced ossification (coded ROST) or supernumerary ribs (coded SRRR) among non-resorbed fetuses in the litter. The analyses explicitly allow for the intra-litter correlation of gender or fetal abnormalities for fetuses at adjacent prenatal sites. In the examples below the Nested analyses produce results roughly similar to quantal analyses of the number of litters with one or more

abnormalities present among pregnant females. This could also be approached by using other indicators, such as the average number of fetal abnormalities per litter as an endpoint, and calculating the mean and standard deviation of these ratios for use in the BMDS Continuous module. The Nested module allows adjustment for the possibility that the number of non-resorbed fetuses or the total litter size may also depend on the dose. It was observed that certain teratological endpoints show a clear dose-response function for the total number of fetuses with fetal abnormalities such as ROST or SRRR, but a weaker dose-response relationship for the number of litters having at least one of these abnormalities. At this time BMDS does not have a nested continuous response model for endpoints such as fetal weight, so of necessity the nested continuous responses are treated as continuous variables using the simple mean and standard deviation of the data for all fetuses across all litters within a dose group, ignoring the correlation of abnormalities or other fetal properties at adjacent sites within the litter.

The BMCL estimates for the various studies are presented for the best fitting models in Table B-1. For each model, the software performed residual and overall chi-square goodness-of-fit tests, and determined the Akaike's Information Criterion (AIC). The chi-square p-value is a measure of the closeness between the observed data and the predicted data (predicted using the modeled fit). Models with chi-square p-values $\geq$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-square p-values were identified. The BMCL estimates vary quite a bit, depending on the endpoint, model selected, and parameter hypotheses and constraints. The "best model" selection criteria were:

1. A P-value $\geq$ 0.1 for goodness of fit (Hypothesis 4 for continuous models) for which a positive BMCL is estimable.
2. Least complex model (lowest AIC) which has a P-value and a positive BMCL estimate.

BMD modeling of the reduced fetal body weight gain data (Mast, 1987) and decreased MCV and MNCV data (Ono et al.,1992) produced inadequate p values (Table B1). BMD modeling of the remaining endpoints from Mast (1987) and Huang et al. (1989) produced adequate p values ($\geq$ 0.1). Huang et al. (1989) was selected as the principal study (Section 5.2.1). Based on the criteria above, the fit of decreased MCV data (12 weeks) in male rats to the Hill model is the best fitting model. Output from the software for the Hill model run (of the male rat MCV data) follows in Output B-4.

Table B-1. 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*§	BMCL*§	$BMCL_{HEC}$**
Mast (1987)	Reduced Ossification of sternebrae 1-4	4	Nested Logistic	n ≥ 1	<0.0001 (Group 0.1834)#	1433	1571	943	2770
Mast (1987)	decreased fetal body weight gain	4	Hill	rho=0	0.019	-1559	1088	1034	3037
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	Quadratic	none	NE	95.98	126	71	125
Ono et al., (1982)	MNCV Total	3	Quadratic	none	NE	80.91	88	58	102
Ono et al., (1982)	MNCV Distal	3	Quadratic	none	NE	68.64	84	55	97

* units of ppm

§ BMR = 10% for quantal endpoints and BMR = 1 standard deviation for continuous endpoints

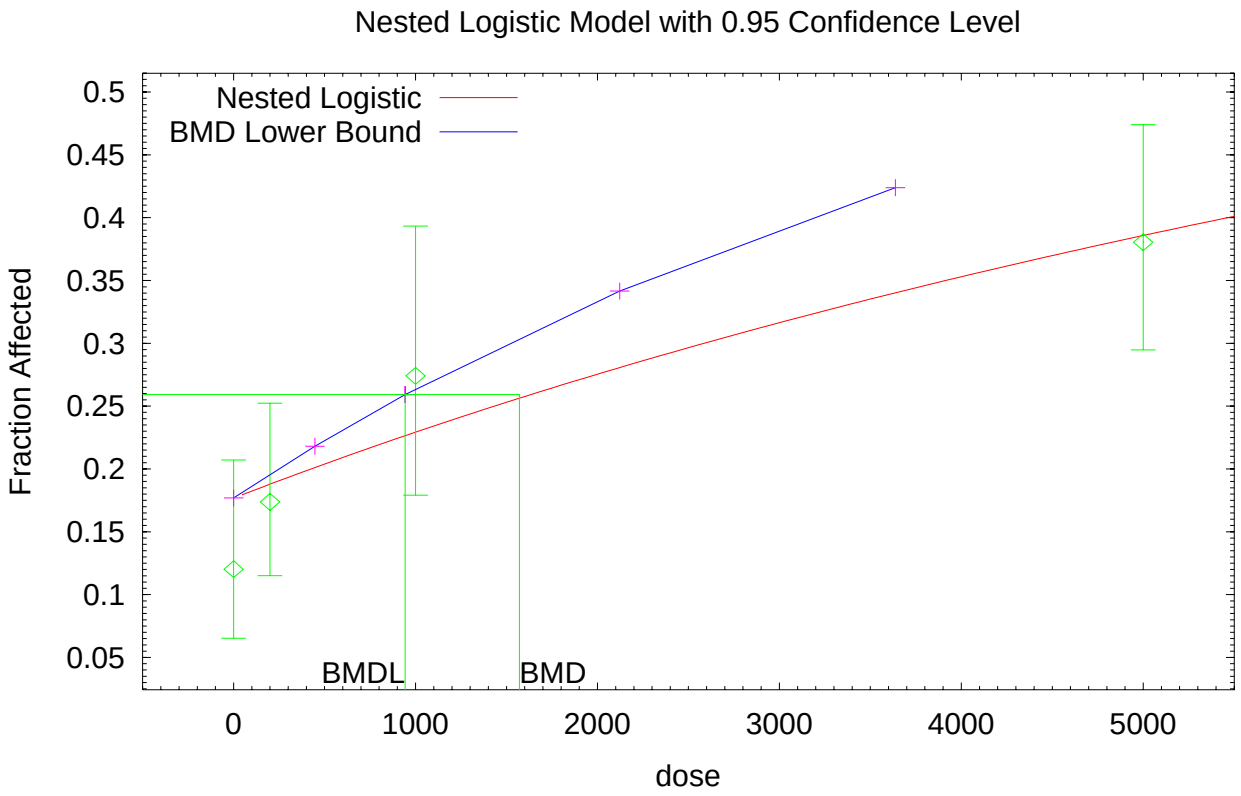
** $BMCL_{HEC}$ presented in mg/m^3 and conversion described in Section 5.2.2.1

All models have been adjusted for either total litter size

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.

NE Not estimable due to other computational problems

Output B-2: Nested, logistic model results for reduced ossification of sternebrae 1-4 in rats data from Mast et al. (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
bet	-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.	Litter Est._Prob.	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

	Mean		chi-squared		
Dose	Lit.-Spec. Cov.	Expected	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

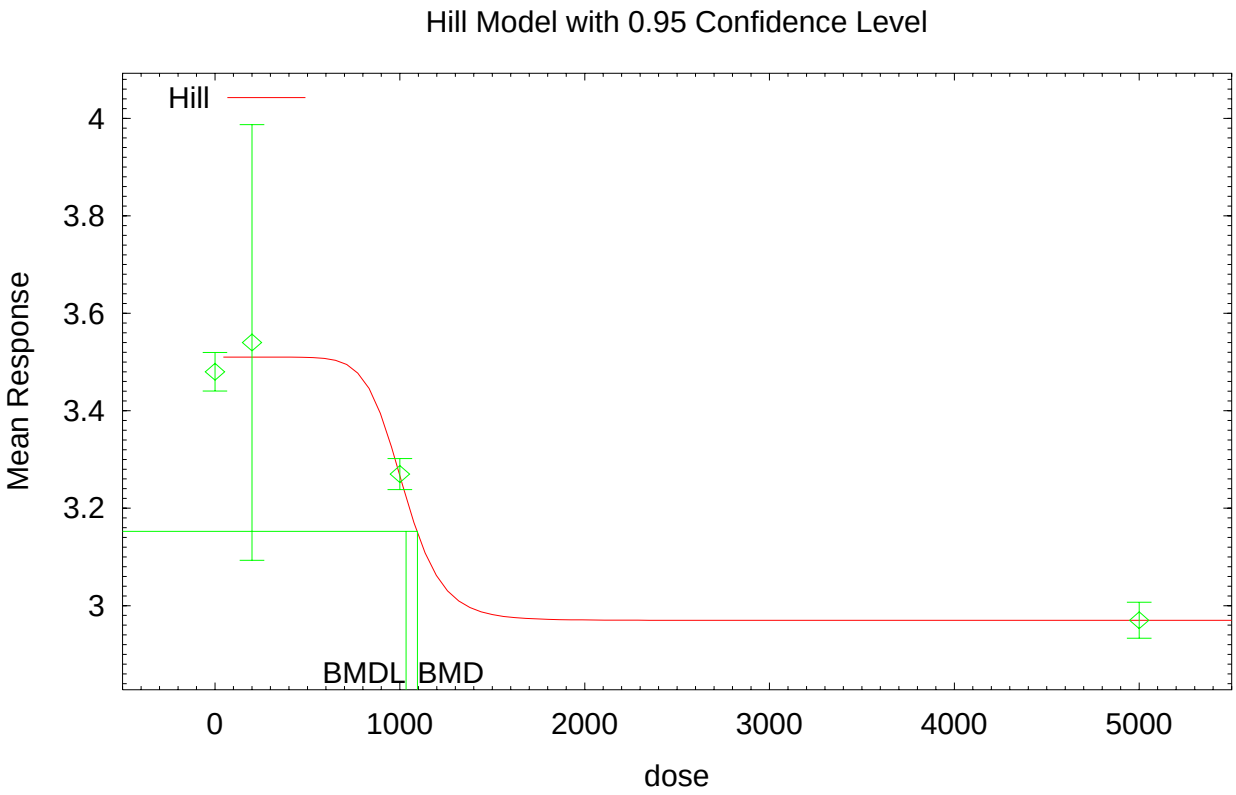
Benchmark Dose Computation
Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95

BMD = 1571.05

BMDL = 943.119

Output B-3: Continuous Hill model results for mean fetal body weight in rats from Mast et al. (1987)

Parameter constraints: Rho = 0 (constant variance)



Covariate: Number of implanted sites in the litter, not including those resorbed.
Benchmark Response: BMR = 0.10

BMDL(0.10, 95% confidence) = 1034 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 = Concs.
rho is set to 0
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 = 1250
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 = 2
k = 1000
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. Error
alpha	0.128228	1
rho	0	1
intercept	3.51048	1
v	-0.540479	1
n	9.88651	1
k	1022.62	1

Data and Estimated Values of Interest

dose	n	observed mean	observed standard deviation	estimated mean	estimated standard deviation	chi square
0	339	3.48	0.37	3.51	0.358	-0.0851
200	350	3.54	0.36	3.51	0.358	0.0824
1000	392	3.27	0.32	3.27	0.358	-1.09e-007
5000	408	2.97	0.38	2.97	0.358	-1.7e-00

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	787.082610	5	-1564.165221
A2	793.564686	8	-1571.129372
A3	787.424939	6	-1562.849879
fitted	784.661351	5	-1559.322701
R	536.633452	2	-1069.266905

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	513.862	6	<.0001
Test 2	12.9642	3	0.004715
Test 3	12.2795	2	0.002155
Test 4	5.52718	1	0.01872

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

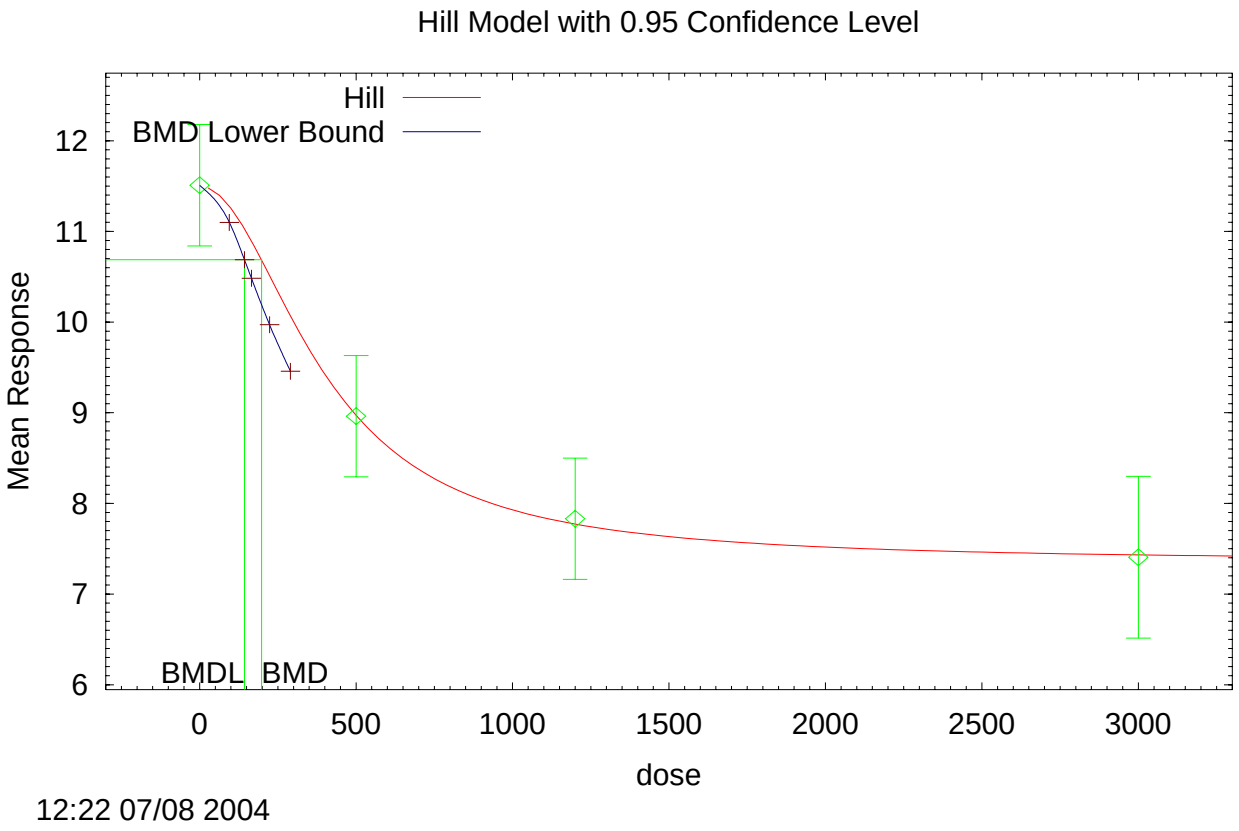
Confidence level = 0.95

BMD = 1094.84

BMDL = 1033.78

Warning: optimum may not have been found. Bad completion code in Optimization routine. BMDL computation failed for one or more point on the BMDL curve. The BMDL curve will not be plotted

Output B-4: 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 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

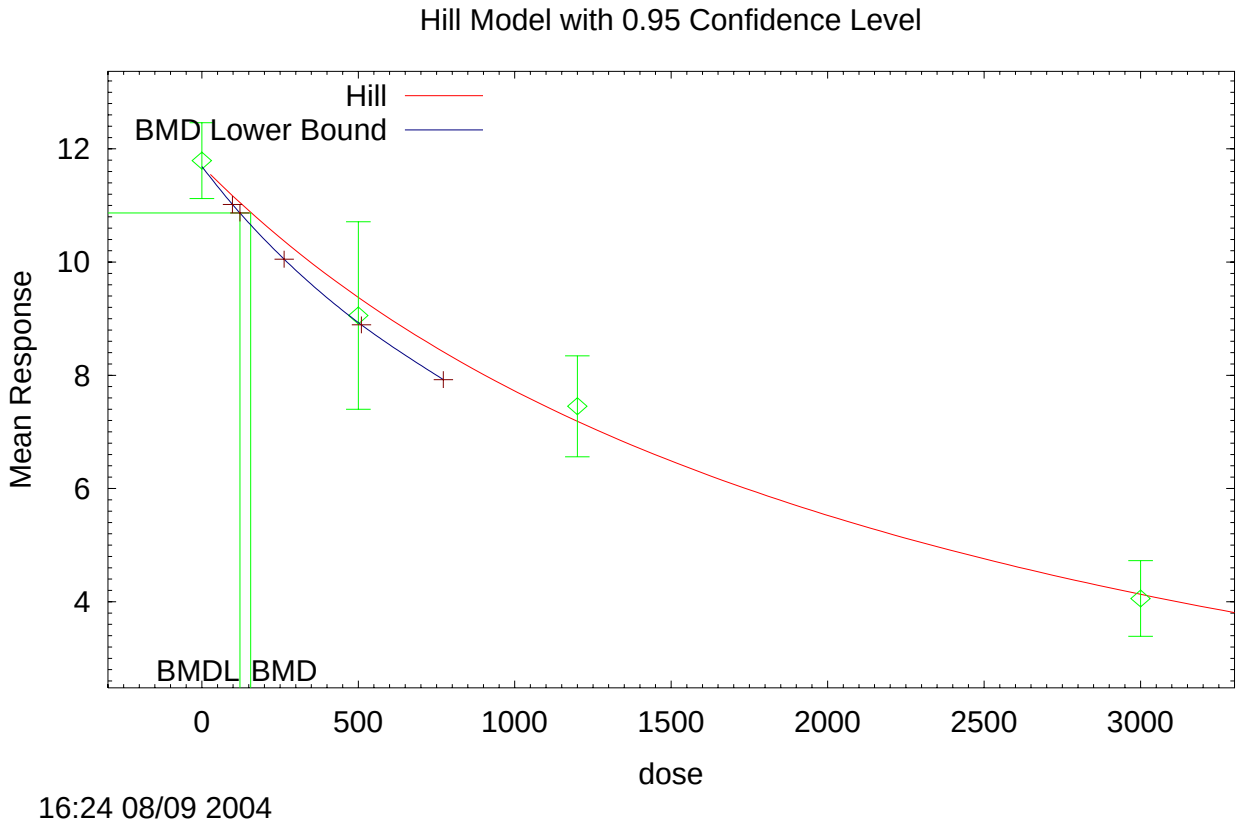
Confidence level = 0.95

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 = 2.



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

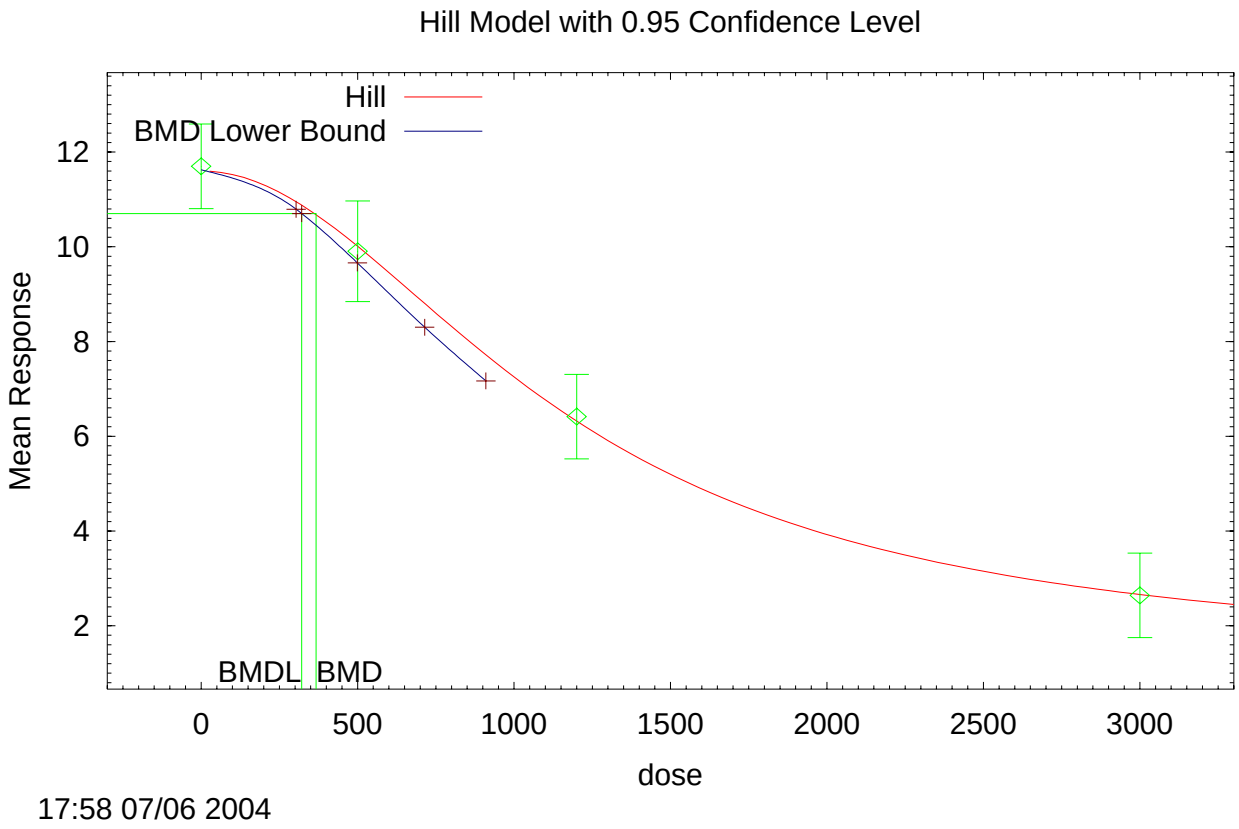
to be appropriate here

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)



Parameter constraints: Rho = 0 (constant variance) and n = 2.
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