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TOXICOLOGICAL REVIEW

OF

2-HEXANONE

(CAS No. 591-78-6)

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

February 2008

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CONTENTS—TOXICOLOGICAL REVIEW OF 2-HEXANONE (CAS No. 591-78-6)

LIST OF TABLES

LIST OF FIGURES

LIST OF ACRONYMS AND ABBREVIATIONS

AIC	Akaike Information Criterion
AVEP	average visual evoked potential
BMC	benchmark dose concentration
BMCL	benchmark concentration, lower 95% confidence limit
BMD	benchmark dose
BMDL	benchmark dose, lower 95% confidence limit
BMDS	benchmark dose software
BMR	benchmark response
CASRN	Chemical Abstracts Service Registry Number
CNS	central nervous system
CYP	cytochrome
CYP450	cytochrome P450
EEG	electroencephalogram
EPA	Environmental Protection Agency
EPN	O-ethyl O-4-nitrophenyl phenylphosphonothioate
EROD	ethoxyresorufin O-deethylase
GD	gestational day
HEC	human equivalent concentration
HSDB	Hazardous Substances Data Bank
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level
MAP	muscle action potential
MCV	motor (nerve) conduction velocity
MEK	methyl ethyl ketone
MiBK	methyl isobutyl ketone
NLM	National Library of Medicine
NOAEL	no-observed-adverse-effect level
NRC	National Research Council
PBTK	physiologically based toxicokinetic
PNS	peripheral nervous system
POD	point of departure
PROD	pentoxyresorufin O-depentylase
RfC	reference concentration
RfD	reference dose
s.c.	subcutaneous
TLV	threshold limit value
TSO	toluene side-chain oxidase
UF	uncertainty factor
w/w	weight/weight

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 2-hexanone. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of 2-hexanone.

The intent of Section 6, *Major Conclusions in the Characterization of Hazard and Dose Response*, is to present the major conclusions reached in the derivation of the reference dose, reference concentration, and cancer assessment, where applicable, and to characterize the overall confidence in the quantitative and qualitative aspects of hazard and dose response by addressing the quality of data and related uncertainties. The discussion is intended to convey the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's IRIS Hotline at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

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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 2-hexanone. IRIS Summaries may include oral reference dose (RfD) and inhalation reference concentration (RfC) values for chronic and less-than-lifetime exposure durations, and a carcinogenicity assessment.

The RfD and RfC, if derived, provide quantitative information for use in risk assessments for health effects known or assumed to be produced through a nonlinear (presumed threshold) mode of action. The RfD (expressed in units of mg/kg-day) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The inhalation RfC (expressed in units of mg/m^3) 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). Reference values are generally derived for chronic exposures (up to a lifetime), but may also be derived for acute (#24 hours), short-term (greater than 24 hours to 30 days), and subchronic (greater than 30 days to 10% of average lifetime) exposure durations, all of which are derived based on an assumption of continuous exposure throughout the duration specified. Unless specified otherwise, the RfD and RfC are derived for chronic exposure duration.

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 may be derived. 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 may be derived from the application of a low-dose extrapolation procedure. If derived, the oral slope factor is an upper bound on the estimate of risk per mg/kg-day of oral exposure. Similarly, a unit risk is an upper bound on the estimate of risk per $\mu g/m^3$ air breathed.

Development of these hazard identification and dose-response assessments for 2-hexanone has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidelines and technical reports that may have been used in the development of this assessment include the following: *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, 1996), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a), *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), *Supplemental Guidance for Assessing Susceptibility from Early-*

Life Exposure to Carcinogens (U.S. EPA, 2005b), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995a), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 2006, 2000a, 1998b), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000b), and *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000c).

The literature search strategy employed for this compound was based on the Chemical Abstracts Registry Service Number (CASRN) and at least one common name. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. The relevant literature was reviewed through March 2007.

2. CHEMICAL AND PHYSICAL INFORMATION

Structurally, 2-hexanone consists of a keto group flanked by a methyl group and an n-butyl group (Figure 2-1). The compound is a colorless liquid with a characteristic acetone-like odor but more pungent (NLM, 2005). Synonyms for 2-hexanone include the following: methyl butyl ketone, methyl n-butyl ketone, butyl methyl ketone, MnBK, and propylacetone.

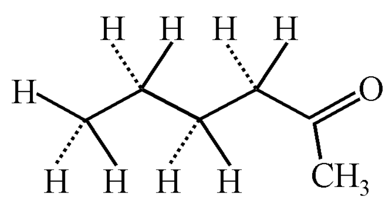

Figure 2-1. Chemical structure of 2-hexanone.

Pertinent physical and chemical properties of 2-hexanone are listed below (NLM, 2005).

Chemical formula	$C_6H_{12}O$
Molecular weight	100.16
Melting point	–55.5°C
Boiling point	127.6°C
Flash point	23°C
Density	0.8113 at 20°C
Water solubility	1.64×10^4 mg/L at 20°C
Log K_{ow}	1.38
Vapor pressure	11.6 mm Hg at 25°C
Conversion factor	1 ppm = 4.1 mg/m^3; 1 mg/m^3 = 0.244 ppm

2-Hexanone is produced commercially by the catalyzed reaction of acetic acid and ethylene under pressure followed by distillation to purify the material (NLM, 2005). The compound has been used as a solvent for lacquers, ink thinners, nitrocellulose, resins, oils, fats, and waxes. It is a medium evaporating solvent for nitrocellulose acrylates, vinyl, and alkyd coatings (polyester coating derived from an alcohol and an acid or acid anhydride).

In 1977, the combined production and import of 2-hexanone in the U.S. was between 453 and 4500 metric tons (NLM, 2005); no breakdown of these figures was provided. The only U.S. producer of 2-hexanone, the Tennessee Eastman Company division of Eastman Kodak, discontinued production of 2-hexanone in 1979 and sold its remaining reserves by 1981 (NLM, 2005). 2-Hexanone is not produced or used in the U.S., and no information on importation is available (ATSDR, 1992).

3. TOXICOKINETICS

3.1. ABSORPTION

3.1.1. Pulmonary Absorption Studies

The available data indicate that 2-hexanone is well absorbed after administration via the inhalation route. DiVincenzo et al. (1978) exposed three healthy male volunteers (ages 22 to 53 years) to 2-hexanone (>97% pure, containing methyl isobutyl ketone [MiBK] and traces of 2-hexanol) at 10 or 50 ppm for 7.5 hours or 100 ppm for 4 hours. The 7.5-hour exposures were interrupted after 4 hours for a 0.5-hour lunch period. The volunteers were sedentary during the exposure. Expired air and venous blood samples were collected before, during, and after exposure. Exposures to 10 and 50 ppm for 7.5 hours produced mean 2-hexanone breath concentrations of 1.4 and 9.3 ppm, respectively. Fifteen minutes after exposure to 10 or 50 ppm, the expired air concentrations of 2-hexanone were 0.1 and 0.5 ppm, respectively. Exposure to 100 ppm for 4 hours produced an average 2-hexanone breath concentration of 22 ppm. These results indicated that between 75 and 92% of the inhaled 2-hexanone vapor was absorbed by the lungs and respiratory tract (DiVincenzo et al., 1978). 2-Hexanone was not detected in the expired air 3 hours after cessation of exposure to 50 or 100 ppm 2-hexanone.

DiVincenzo et al. (1978) exposed four young male beagles to 2-hexanone (>97% pure, containing MiBK and traces of 2-hexanol) for 6 hours at concentrations of 50 or 100 ppm. Over the first 4 hours of the exposure period, the hexanone in exhaled air had time-weighted average concentrations of 16 and 35 ppm for the low and high exposure groups, respectively. Thirty minutes after cessation of exposure to 50 ppm 2-hexanone, the breath concentration of 2-hexanone decreased to 0.7 ppm. 2-Hexanone was below the limit of detection by 3 to 5 hours after the exposure. It was determined that about 65–68% of the inhaled vapor was absorbed by the lungs.

3.1.2. Gastrointestinal Tract Absorption Studies

2-Hexanone appears to be well absorbed after oral administration. DiVincenzo et al. (1978) administered 2 µCi of 1-[^{14}C]-hexanone dissolved in corn oil via a gelatin capsule to human volunteers; the total dose was 0.1 mg/kg. Most of the 2-hexanone-derived radioactivity was exhaled as $^{14}CO_2$, reaching a peak within 4 hours of dosing and then decreasing slowly over the next 3 to 5 days. The major portion of radioactivity excretion in urine occurred during the first 48 hours but continued at measurable levels until 8 days after dosing. The cumulative 8-day elimination of radioactivity in breath and urine averaged 39.5 and 26.3%, respectively. The overall recovery of ^{14}C was 65.8%. The authors presumed that the remainder of the radioactivity was retained in tissue or fat deposits.

Administration of 1-[^{14}C]-2-hexanone at 20 or 200 mg/kg by gavage to rats resulted in excretion of about 1.1% of the administered radioactivity in the feces, about 44% in the breath, and 38% in urine, with about 15% remaining in the carcass after 48 hours and 8% remaining after 6 days (DiVincenzo et al., 1977). The results were similar at either dose level. These findings suggest that about 98% of the administered dose was absorbed via the gastrointestinal tract.

3.1.3. Dermal Absorption Studies

2-Hexanone is also absorbed after dermal application. DiVincenzo et al. (1978) exposed six human volunteers (ages 30–53 years) to radiolabeled 1-[^{14}C]-2-hexanone (>97% purity, contaminants not stated). The labeled compound was applied to the ventral surface of the forearm that had been shaved 24 hours prior to testing. 1-[^{14}C]-2-hexanone was held in contact with the skin for 60 minutes, and precautions were taken to ensure that inhalation exposure did not occur. The surface area of the skin subjected to solvent was 55.6 cm^2. Calculated skin absorption rates were 4.8 and 8.0 µg/cm^2-minute. The quantities of 2-hexanone absorbed by two volunteers were 15.96 and 26.81 mg, respectively. The major respiratory excretion metabolite of 1-[^{14}C]-hexanone was $^{14}CO_2$. A substantial portion of the dose was also excreted in urine; however, the chemical nature of urinary radioactivity was not characterized further.

In a similar set of experiments, DiVincenzo et al. (1978) applied 1-[^{14}C]-2-hexanone (>97% purity, impurities not stated) to the clipped thorax (55.6 cm^2) of beagles. Exposures were carried out for 5 minutes to 1 hour. By 5 minutes, 11 mg of 2-hexanone had penetrated the skin, and there was no apparent change in the absorption of 2-hexanone during the next 15 minutes. However, after 20 minutes the absorption increased markedly so that, by 60 minutes, 77 mg of 2-hexanone had penetrated the skin. The 8-hour cumulative excretion of radioactivity in two dogs dosed with 1-[^{14}C]-2-hexanone was 0.5% of the dose as unchanged 2-hexanone and 9.7% as $^{14}CO_2$ in the breath; urinary radioactivity amounted to 6.5% of the dose. The 8-hour excretion of radioactivity averaged 16.8% of the dose. The fraction of the applied 2-hexanone dose that was absorbed was not calculated.

O'Donoghue and Krasavage (1981) exposed two male beagles (one of which was pretreated with 2-hexanone) to 2-hexanone by tail dipping. Both dogs were exposed to 2-hexanone on an area of 22 cm^2 on the first day of exposure, and then the exposure area was doubled on the second day (44.1 cm^2). It was found that by 8–12 minutes, both dogs had comparable serum levels of 2-hexanone. Doubling the exposed area increased serum levels of 2-hexanone 6 to 20 times. None of the blood samples contained detectable levels of the 2-hexanone metabolites 5-hydroxy-2-hexanone, 2,5-hexanedione, or 2,5-hexanediol. Similar exposures were repeated with three different dogs for 16 minutes followed by two post exposure samples 9 and 19 minutes later (25 and 35 minute samples, respectively). One animal had detectable levels of 2-hexanone in blood within 4 minutes, but the time to detectable levels was highly variable among the animals. The highest level observed was 3.2 µg/mL. Nineteen minutes post exposure serum levels of 2-hexanone were still detectable. Twenty-four hours later, no 2-hexanone was detected (O'Donoghue and Krasavage, 1981).

To examine the effects of multiple exposures, O'Donoghue and Krasavage (1981) exposed three male dogs as above to 2-hexanone on two occasions 4 hours apart. Samples obtained after the second treatment were not significantly different from the morning samples, indicating the absence of accumulation of detectable 2-hexanone and 2,5-hexanedione levels in the serum.

O'Donoghue and Krasavage (1981) performed comparison studies on percutaneous absorption of 2-hexanone between dog and rabbit skin. Significantly more 2-hexanone was absorbed through rabbit skin compared with dog skin and probably, as a consequence, the metabolite 5-hydroxy-2-hexanone was detected in the serum of rabbits. Overall, the skin studies indicated that 2-hexanone was readily absorbed through the skin; detectable serum levels were present after approximately 10 minutes of exposure to less than 1% of body skin surface; detectable serum levels persisted for approximately 20 minutes post exposure; and, in rabbits, a metabolite (5-hydroxy-2-hexanone) was rapidly formed and detectable in the serum.

3.2. DISTRIBUTION

Duguay and Plaa (1995) treated male Sprague-Dawley rats by gavage with 2-hexanone (>99%, spectrophotometric grade) at 0.5, 1, or 2 mmol/kg (50, 100, or 200 mg/kg) in corn oil (dose volume 10 mL/kg), once daily for 3 days. The animals were sacrificed 1 hour after the last gavage. Dose-dependent increases in plasma and lung 2-hexanone levels were observed, whereas the concentration in the liver increased only with the highest dose (Table 3-1). Calculations for statistically significant differences among dose groups were not performed (Duguay and Plaa 1995).

Table 3-1. Concentrations of 2-hexanone in plasma, liver, and lung of male rats following oral exposure for 3 days

Tissue concentration	Dose		
	0.5 mmol/kg	1 mmol/kg	2 mmol/kg
Plasma (µg/mL)	2.4 ± 1.2	4.7 ± 1.1	8.5 ± 2.0
Liver (µg/g)	1.7 ± 0.5	1.6 ± 0.3	3.8 ± 1.2
Lung (µg/g)	1.1 ± 0.7	4.9 ± 1.1	13.9 ± 4.9

Source: Duguay and Plaa (1995).

In a parallel series of experiments from the same study, Duguay and Plaa (1995) exposed male Sprague-Dawley rats to a total body exposure of 2-hexanone at concentrations of 75, 150, or 300 ppm (307.5, 615, or 1230 mg/m^3). Animals were exposed on 3 consecutive days for 4 hours per day. Animals were sacrificed immediately after the last exposure on the third day.

The concentration of 2-hexanone in plasma, liver, and lung increased in a dose-dependent manner (Table 3-2). It should be noted, however, that because whole body exposures were performed, some oral and dermal absorption may have taken place.

Table 3-2. Concentrations of 2-hexanone in plasma, liver, and lung of male rats following inhalation exposure for 3 days

Tissue concentration	Dose		
	75 ppm	**150 ppm**	**300 ppm**
Plasma (µg/mL)	1.2 ± 0.3	2.6 ± 0.7	9.7 ± 0.7
Liver (µg/g)	0.7 ± 0.5	1.2 ± 0.8	2.2 ± 0.4
Lung (µg/g)	0.7 ± 0.2	2.8 ± 0.5	9.3 ± 1.2

Source: Duguay and Plaa (1995).

In male CD/COBS rats administered a single gavage dose of [^{14}C]-2-hexanone at 200 mg/kg, the serum elimination for 2-hexanone was 6 hours; the 2-hexanone metabolites 5-hydroxy-2-hexanone and 2,5-hexanedione were eliminated from serum within 12 and 16 hours, respectively (DiVincenzo et al., 1977). Peak concentrations of 2-hexanone and 5-hydroxy-2-hexanone were reached at 2 hours, whereas the peak concentration of 2,5-hexanone was reached at 6 hours. Radioactivity was detected in most tissues with highest counts in liver > kidney > whole brain. The peak concentration of radiolabel in each of these tissues was observed at 4 hours and was reduced to less than 50% by 24 hours. No quantitative data were given on tissue distribution. An analysis of the subcellular distribution of the ^{14}C-label in liver, brain, and kidney tissue homogenates indicated the highest counts were associated with the protein fraction, with some recovery from DNA and little or none from RNA.

Eben et al. (1979) treated male SPF-Wistar rats with 400 mg/kg 2-hexanone (98% pure, impurities not stated) daily by stomach tube for 40 weeks. The concentrations of 2-hexanone and metabolites in the blood were determined at intervals of 4 or 5 weeks. In the case of 2-hexanone, the maximum concentration was reached 1 hour after administration throughout the study; thereafter, the concentration decreased rapidly. After 7 hours, only trace amounts could be detected. During the first few weeks of the study, 2-hexanone could not be found in the urine. Only during the third week were very small concentrations of the free compound detected in urine, suggesting that the metabolic pathways for 2-hexanone were becoming saturated. A maximum (approximately 20 µg) was reached in the 17th week (Eben et al., 1979).

Granvil et al. (1994) studied the distribution and disappearance of 2-hexanone (purity not stated) from the blood and brain. Male CD-1 mice were treated with 5 mmol/kg (500 mg/kg) 2-hexanone dissolved in corn oil by intraperitoneal (i.p.) injection at a volume of 10 mL/kg. Animals were killed by decapitation, and blood and brain samples were collected at 15, 30, 60, and 90 minutes after treatment. Blood and brain concentrations at 15 minutes were ~182 µg/mL and ~126 μg/g, respectively. By 90 minutes, the values had dropped in a uniform manner to a blood concentration of ~28 μg/mL and a brain concentration of ~25 μg/mg. The authors noted that the rapid decrease in the concentration of 2-hexanone was due to its active metabolism in these tissues (Granvil et al., 1994).

3.3. METABOLISM

2-Hexanone is hydroxylated to 5-hydroxy-2-hexanone, which is then either oxidized to 2,5-hexanedione or reduced to 2,5-hexanediol and, to a small extent, may be converted to 2,5-dimethyl-2,3-dihydrofuran. The predominant metabolite of 2-hexanone found in blood is 2,5-hexanedione. This can be reduced to 5-hydroxy-2-hexanone and further, but to a lesser extent, to 2,5-hexanediol. The formation of 2,5-hexanedione is favored over that of 5-hydroxy-2-hexanone. 5-Hydroxy-2-hexanone can be metabolized into 4,5-dihydroxy-2-hexanone (not shown in Figure 3-1) before being further converted to 2,5-dimethyl-2,3-dihydrofuran. Additionally, 4,5-dihydroxy-2-hexanone formation may be a result from 2,5-hexanedione metabolism (U.S. EPA, 2005c). Other mechanisms, such as shunting into intermediary metabolism, may accelerate metabolic clearance of 2,5-hexanedione. Reductive metabolism of 2-hexanone results in the formation of 2-hexanol, establishing an equilibrium between the two compounds. 2-Hexanol can be further metabolized to 2,5-hexanediol, 5-hydroxy-2-hexanone, and 2,5-hexanedione. The findings of Abdel-Rahman et al. (1976) that rats, guinea pigs, and rabbits exposed to 2-hexanone vapor excreted glucuronides of 2-hexanol and 2,5-hexanediol in the urine are consistent with the results by DiVincenzo et al. (1976), discussed later in this section (Abdel-Rahman et al., 1976). Although the proportions of metabolites may differ among species, ω-1-oxidation and carbonyl reduction appear to be the initial steps in the metabolism of 2-hexanone in all species tested so far (e.g., rat, cat, dog, guinea pig, and human). The metabolic pathway for 2-hexanone, as proposed by DiVincenzo et al. (1977, 1976), based on 2-hexanone metabolites identified in blood of guinea pigs, mice, and rats, is presented in Figure 3-1.

As discussed in Section 3.2, Duguay and Plaa (1995) conducted studies using male Sprague-Dawley rats exposed to 2-hexanone by gavage (0.5, 1, or 2 mmol/kg) or by inhalation (75, 150, or 300 ppm) and quantified the metabolites in the plasma, liver, and lung. The authors reported that the concentrations of metabolites, such as 2-hexanol, 5-hydroxy-2-hexanone, and 2,5-hexanedione, were readily detectable in serum. After 2-hexanone gavage or inhalation, 2-hexanol was found in low concentrations in plasma and liver (0.5–1.3 μg/mL and 0.3–1.6 μg/g, respectively). In lung, however, concentrations ranged from 2.1 to 5.1 μg/g. However, with both routes of administration, 2-hexanol concentrations did not appear to be dose dependent.

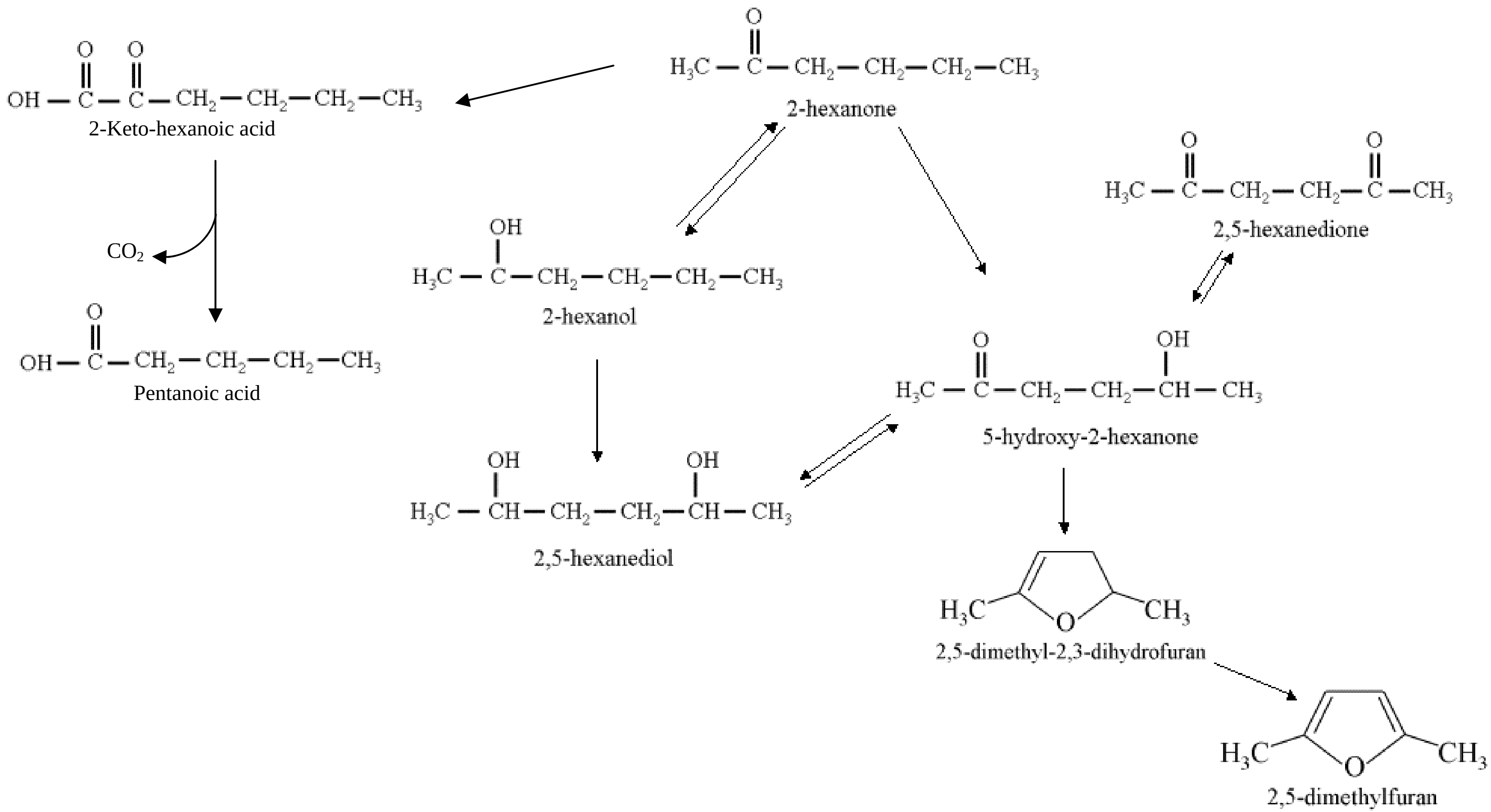

Figure 3-1. Proposed metabolic pathway for 2-hexanone.

Adapted from DiVincenzo et al. (1977, 1976).

The appearance of the 2-hexanone metabolite 2,5-hexanedione in plasma or lung, but not in liver, depended on the route of administration. The highest dose and concentration of 2-hexanone, 2 mmol/kg and 300 ppm, respectively, produced similar plasma 2-hexanone concentrations, 8.5 and 9.7 µg/mL, respectively, but the corresponding plasma 2,5-hexanedione concentrations were 7.7 µg/mL after oral and 25 µg/mL after inhalation administration. 2,5-Hexanedione was not detectable in lungs when 2-hexanone was administered orally, but significant dose-dependent amounts were found following inhalation exposure. The authors concluded that pulmonary 2-hexanone metabolism might contribute to plasma metabolite levels. 2,5-Hexanedione concentrations in liver were dose dependent but independent of the route of administration. A summary of the metabolite levels found in the plasma, liver, and lung following oral and inhalation exposures is presented in Table 3-3.

Table 3-3. 2-Hexanol and 2,5-hexanedione in the plasma, liver, and lung of male rats after oral or inhalation exposure to 2-hexanone

	Dose					
	0.5 mmol/kg (gavage)[a]	**75 ppm (inhalation)[a]**	**1 mmol/kg (gavage)[a]**	**150 ppm (inhalation)[a]**	**2 mmol/kg (gavage)[a]**	**300 ppm (inhalation)[a]**
Plasma[b]						
2-HOL[c]	0.6 ± 0.2	0.5 ± 0.1	1.2 ± 0.3	0.5 ± 0.2	1.3 ± 0.4	0.8 ± 0.3
2,5-HD[d]	5.7 ± 0.5	6.7 ± 0.8	5.8 ± 0.5	13.5 ± 2.1	7.7 ± 0.4	25 ± 3.1
Liver[e]						
2-HOL	0.4 ± 0.1	0.4 ± 0.2	1.6 ± 0.5	0.3 ± 0.1	1.2 ± 0.5	0.3 ± 0.3
2,5-HD	3.1 ± 0.2	3.1 ± 0.1	4.6 ± 0.5	4.3 ± 0.6	5.3 ± 0.5	7.3 ± 0.3
Lung[e]						
2-HOL	2.4 ± 0.5	2.1 ± 0.6	3.0 ± 1.0	2.9 ± 0.5	5.1 ± 1.5	3.7 ± 1.1
2,5-HD	ND[f]	0.9 ± 0.2	ND	4.0 ± 0.1	ND	4.8 ± 0.7

[a]Rats were sacrificed 1 hour after the last oral treatment but immediately after the last inhalation exposure. Values are mean ± SE from six animals.
[b]Plasma concentrations in µg/mL.
[c]2-HOL = 2-hexanol.
[d]2,5-HD = 2,5-hexanedione.
[e]Tissue concentrations in µg/g.
[f]ND = not detectable (<0.25 µg/mL).

Source: Duguay and Plaa (1995).

Eben et al. (1979) administered daily oral doses of 2-hexanone (400 mg/kg) over a 40-week period to male SPF-Wistar rats. The concentrations of 2-hexanone, 2-hexanol, and 2,5-hexanedione were determined in the blood at several intervals every 4 or 5 weeks. 2-Hexanone concentrations in blood peaked at 1 hour after administration then decreased rapidly, and after 7 hours only traces could be detected. The metabolite 2-hexanol was measurable in very small quantities up to 3 hours after administration (<2 µg/mL blood). In contrast, 2,5-hexanedione concentrations were relatively high as early as 1 hour after administration, and maximum values were recorded after 5 or 7 hours. 2,5-Hexanediol was not detectable in the blood at any time. A summary of the blood concentrations of 2-hexanone, 2-hexanol, and 2,5-hexanedione is presented in Table 3-4.

Table 3-4. 2-Hexanone, 2-hexanol, and 2,5-hexanedione in the blood of male rats after repeated administration of 400 mg/kg-day

	2-Hexanone (μg/mL)[a]				2-Hexanol (μg/mL)[a]				2,5-Hexanedione (μg/mL)[a]			
Week	**1 hour**	**3 hours**	**5 hours**	**7 hours**	**1 hour**	**3 hours**	**5 hours**	**7 hours**	**1 hour**	**3 hours**	**5 hours**	**7 hours**
2	26.5	15.2	5.8	–[b]	–	–	–	–	19.8	53.3	65.7	53.8
6	30.4	21.4	7.3	1.4	0.6	0.8	–	–	10.9	46.5	59.7	59.8
10	20.2	7.5	4.7	3.8	0.7	–	–	–	16.7	39.2	60.7	64
14	31.8	25.7	6.3	2.2	1.7	1.2	traces	–	10	35.1	55.2	59.1
19	32.2	22.5	3.4	0.1	–	–	–	–	16.7	50.3	62.1	55
23	35.1	19.8	6.6	0.3	–	–	–	–	10.4	46.7	68.9	63.7
27	37.8	21.3	2.9	0.7	1.3	traces	–	–	8	38.8	49.9	49.2
32	24.8	12.2	2.9	0.3	0.6	0.1	–	–	8.4	31.8	41.1	34.8
36	50.1	13.4	7.1	1	1.5	0.1	–	–	14.6	47.4	55.6	56.1
40	33.4	18.9	3.6	0.4	1.2	0.9	–	–	12.5	36.8	51.9	66.2

[a]Values represent the averages of three animals.
[b]A dash (–) indicates that the compound was below the limit of detection.

Source: Eben et al. (1979).

Granvil et al. (1994) demonstrated the rapid removal of 2-hexanone from blood and brain of male CD-1 mice following a single i.p. injection of the compound at a concentration of 5 mmol/kg. The authors observed that 2-hexanol concentrations found in whole brain at several time intervals (15, 30, and 60 minutes after dosing) were about twice as high as those found in the blood at the same time intervals and interpreted this finding as suggesting that 2-hexanol might be formed in the brain. Furthermore, the authors reported that the appearance of the reduced metabolite 2-hexanol seemed to be considerably faster than the appearance of the oxidized metabolite 2,5-hexanedione.

DiVincenzo et al. (1976) administered a single dose of 2-hexanone (450 mg/kg i.p. in corn oil) to male guinea pigs (strain not stated). Blood was collected by heart puncture from four animals at 1, 2, 4, 6, 8, 12, and 16 hours after dosing. In addition to 2-hexanone, three major metabolites were identified by gas chromatography: 5-hydroxy-2-hexanone, 2,5-hexanedione, and 2-hexanol. 2,5-Dimethyl-2,3-dihydrofuran was also detected, but additional experiments revealed that this was an artifact because 5-hydroxy-2-hexanone underwent dehydration and cyclization in the gas chromatograph. The authors noted that 5-hydroxy-2-hexanone may be transformed also in vivo to 2,5-dimethyl-2,3-dihydrofuran, but the equilibrium favors the formation of 5-hydroxy-2-hexanone.

DiVincenzo et al. (1976) also conducted follow-up studies to determine the metabolic fate of 2-hexanone metabolites in guinea pigs. Each of the principal metabolites identified in the above study (5-hydroxy-2-hexanone, 2,5-hexanedione, 2,5-hexanediol, and 2-hexanol) was administered individually (450 mg/kg i.p.). 5-Hydroxy-2-hexanone was further metabolized to 2,5-hexanedione and 2,5-hexanediol. The half-life of 5-hydroxy-2-hexanone in serum was 156 minutes. The major metabolite 2,5-hexanedione was formed rapidly, and its concentration in serum was equivalent to or greater than that of the parent compound (5-hydroxy-2-hexanone) in all samples measured. Serum concentrations of 2,5-hexanediol were markedly lower than those of 2,5-hexanedione. 5-Hydroxy-2-hexanone was the only metabolite detected in serum of guinea pigs after an i.p. injection of 2,5-hexanedione. The half-life of 2,5-hexanedione was 100 minutes. Both 5-hydroxy-2-hexanone and 2,5-hexanedione were no longer detectable in serum by 16 hours. The principal metabolites in serum after i.p. injection with 2,5-hexanediol were 5-hydroxy-2-hexanone and 2,5-hexanedione. 2,5-Hexanediol was cleared within 8 hours and had a half-life of 84 minutes in serum. The following metabolites were identified after the administration of 2-hexanol: 2-hexanone, 5-hydroxy-2-hexanone, 2,5-hexanedione, and 2,5-hexanediol. The half-life and clearance time of 2-hexanol were 72 minutes and 6 hours, respectively.

The authors noted that the 2-hexanol was rapidly metabolized to 2-hexanone, which, in turn, was converted to the same metabolites identified above for animals treated with 2-hexanone. They determined that the conversion of 2-hexanol to 2,5-hexanediol seemed to be a minor pathway. The metabolites, 2,5-hexanediol and 2,5-hexanedione, were cleared in 8 and 16 hours, respectively. A summary of the half-life and clearance time of 2-hexanone and metabolites is presented in Table 3-5.

Table 3-5. Serum half-lives and clearance times of 2-hexanone and its metabolites in guinea pigs

Compound administered	Half-life (minutes)[a]	Clearance time (hours)
2-Hexanone	78	6
2-Hexanol	72	6
5-Hydroxy-2-hexanone	156	8[b]
2,5-Hexanedione	100	16
2,5-Hexanediol	84	8

[a]Half-lives were determined from the linear portion of the plasma concentration curve and extrapolated to zero time.
[b]Estimated value.

Source: DiVincenzo et al. (1976).

Bus et al. (1981) presented metabolism data for n-hexane that provide some insight on the metabolism of 2-hexanone. In the study, the authors exposed male F344 rats for 1 or 5 days, 6 hours/day, to 1000 ppm n-hexane. Animals were sacrificed immediately after exposure or at increasing time intervals for up to 24 hours after the end of exposure, and concentrations of the parent compound and two of its metabolites, 2-hexanone and 2,5-hexanedione, were measured in blood, liver, kidney, brain, and sciatic nerve. Kinetics of all three compounds were similar after 1 and 5 days of exposure, with tissue levels of the metabolites frequently exceeding those of the parent compound even immediately after the end of exposure. Tissue levels of n-hexane and 2-hexanone were always lower after 5 days of repeated exposures, compared with levels after a single exposure, consistent with self-induction of some metabolizing enzymes. On the other hand, tissue levels of 2,5-hexanedione were always slightly higher after 5 days of exposure, compared with single exposure. A compilation of the data for 2-hexanone and 2,5-hexanedione after 5 days of exposure to n-hexane is given in Table 3-6 (sciatic nerve data not included).

Table 3-6. Tissue levels of 2-hexanone and 2,5-hexanedione in male F344 rats following inhalation exposure to n-hexane for 5 days

Time (hours)	Blood[a,b]		Liver[a,c]		Brain[a,c]		Kidney[a,c]	
	2-Hx[d]	2,5-HxD[e]	2-Hx	2,5-HxD	2-Hx	2,5-HxD	2-Hx	2,5-HxD
0	0.46 ± 0.07	1.97 ± 0.38	0.12 ± 0.01	0.56 ± 0.10	0.78 ± 0.04	5.66 ± 0.17	22.9 ± 3.81	11.8 ± 1.03
1	0.23 ± 0.02	6.02 ± 0.56	0.20 ± 0.04	0.64 ± 0.13	0.18 ± 0.01	7.41 ± 0.31	9.73 ± 1.14	23.5 ± 1.85
2	0.06 ± 0.03	3.99 ± 0.37	–	0.69 ± 0.01	0.08 ± 0.01	7.17 ± 0.74	4.80 ± 0.39	26.4 ± 1.61
4	–[f]	2.12 ± 0.26	–	0.15 ± 0.02	–	2.75 ± 0.34	0.63 ± 0.23	16.8 ± 3.67
8	–	0.54 ± 0.19	–	0.03 ± 0.03	–	–	0.67 ± 0.15	9.08 ± 2.45
12	–	–	–	–	–	–	0.78 ± 0.21	0.86 ± 0.27
24	–	–	–	–	–	–	0.28 ± 0.00	0.01 ± 0.01

[a]Mean ± SE, n = 3.
[b]Values in µg/mL plasma.
[c]Values in µg/g wet weight.
[d]2-hexanone
[e]2,5-hexanedione
[f] Dash (–) = below detection limit.

Source: Bus et al. (1981).

This experiment, although conducted with n-hexane as the parent compound, provides some insight into the metabolism of 2-hexanone. The data shown in Table 3-6 indicate that the metabolism of 2-hexanone to 2,5-hexanedione (intermediates not considered) proceeds rapidly, while the further metabolism of 2,5-hexanedione and its elimination appear to proceed much more slowly. Both the resurgence of 2-hexanone levels in kidney between 8 and 12 hours and the precipitous drop of 2,5-hexanedione levels in kidney between 8 and 12 hours occurred in the same fashion with single exposure, suggesting rather complex compartmentalization and toxicokinetics that, to some extent, may be governed by the lipophilic characteristics of the compounds. The authors (Bus et al., 1981) suggested that the high levels observed in kidney for both metabolites, but not the parent compound, reflect the fact that the metabolites of n-hexane, and thus 2-hexanone, are mostly eliminated via urine.

Cytochrome P450 (CYP450) enzymes catalyze the initial steps (either detoxification or bioactivation) of 2-hexanone, but their identities have not been investigated to any great detail. Oral administration of 1-[^{14}C]-2-hexanone to humans or rats resulted in the appearance of $^{14}CO_2$ in the exhaled breath, indicating removal of the α-carbon (DiVincenzo et al., 1978, 1977). Administration of SKF525A (a mixed function oxidase inhibitor) to rats before oral administration of 2-hexanone resulted in marked decrease in the respiratory excretion of $^{14}CO_2$ for the first 4 hours after administration, followed by a marked increase at 4–8 and 12–24 hours. This suggests that this oxidative step is mediated by a microsomal mixed-function oxidase system (DiVincenzo et al., 1977).

Because inhalation exposure of humans to 1-[^{14}C]-2-hexanone resulted in the appearance of labeled carbon dioxide in expired air and 2,5-hexanedione in serum, DiVincenzo et al. (1978) hypothesized that the metabolic pathway for 2-hexanone is similar in humans and experimental animals. Metabolically, aliphatic ketones generally are in equilibrium with the corresponding secondary alcohols, which explains the presence of 2-hexanol. An alternate pathway is oxidation of the 5-methylene group to the corresponding alcohol, 5-hydroxy-2-hexanone. Another possibility in the metabolism of 2-hexanone is the cyclization of 5-hydroxy-2-hexanone to the corresponding dihydrofuran and oxidation to 2,5-dimethylfuran (DiVincenzo et al., 1977). However, the formation of these furan moieties may be an artifact resulting from thermal dehydration and cyclization during gas chromatography (DiVincenzo et al., 1977). In addition, the γ-valerolactone found in the urine was hypothesized to result from α-oxidation of 5-hydroxy-2-hexanone to 2-keto-5-hydroxyhexanoic acid, decarboxylation and oxidation to 4-hydroxypentanoic acid, and lactonization to γ-valerolactone (not shown in Figure 3-1) (DiVincenzo et al., 1977).

Although the specific isoforms of CYP450 that catalyze the metabolism of 2-hexanone have not been fully characterized, Nakajima et al. (1991) provided some insight into the effects of 2-hexanone on CYP450 induction. The authors treated male Wistar rats with 2-hexanone at 5 mmol/kg (500 mg/kg) i.p. for 4 days and demonstrated that various CYP450 isozyme activities were induced. 2-Hexanone was effective in inducing several CYP450 isoforms as indicated by the increase in activities of benzene aromatic hydroxylase (CYP2E1) and toluene side chain oxidation (CYP2C6/11) two- to threefold and pentoxyresorufin O-depentylase (PROD; CYP2B1/2) about 30-fold but barely induced ethoxyresorufin O-deethylase activity (EROD; CYP1A1/2) (Nakajima et al., 1991). Imaoka and Funae (1991) also showed that 2-hexanone induced the immunologically measured levels of several CYP450 isozymes, foremost CYPs 2B1, 2B2, 2C6, and 2E1. Minimal or equivocal induction was observed for CYPs 1A1, 1A2, 2C7, and 4A3. The levels of CYPs 2C11 and 2C13 were slightly reduced (Imaoka and Funae, 1991). However, it is not evident to what extent 2-hexanone might affect its own metabolism via enzyme induction. Similarly, the enzymes that synthesize the glucuronides of 2-hexanone metabolites, which were identified by Abdel-Rahman et al. (1976) in the urine of 2-hexanone-exposed rats, guinea pigs, and rabbits, have not been characterized further.

3.4. ELIMINATION

In humans exposed to 2-hexanone via inhalation at 10 or 50 ppm for 7.5 hours or to 100 ppm for 4 hours, unchanged 2-hexanone (but none of its metabolites) was found in expired air, and neither 2-hexanone nor any of its metabolites was found in urine during or after exposure (DiVincenzo et al., 1978). 2-Hexanone was no longer detected in expired air 3 hours after exposure to 50 or 100 ppm. In two humans who received a single oral dose of 1-[^{14}C]-2-hexanone, breath excretion of $^{14}CO_2$ reached a peak within 4 hours then decreased slowly over the next 3 to 5 days. Average overall recovery of the ^{14}C-label in 8 days was 40% in breath and 26% in urine. Feces were not analyzed (DiVincenzo et al., 1978). These results suggest slow clearance and possibly retention of 2-hexanone in humans exposed by this route.

In beagles exposed to 2-hexanone via inhalation at 50 or 100 ppm for 6 hours, 32 and 35%, respectively, of the inhaled vapor was excreted in the expired breath (DiVincenzo et al., 1978). By 3 to 5 hours after exposure, 2-hexanone was no longer detected in expired air. Excretion via other routes was not addressed.

In rats administered a single oral dose of 1-^{14}C-2-hexanone, DiVincenzo et al. (1977) observed similar results as the above findings in humans. Radioactivity in breath accounted for about 45% of the administered dose (5% was in unchanged 2-hexanone; 40% was in $^{14}CO_2$); 38% was found in urine; 1.1% was recovered in the feces; and about 15% remained in the carcass. In male rats that received daily gavage doses of 2-hexanone at 400 mg/kg-day for 40 weeks, very low concentrations of free 2-hexanone were detected in the urine from the third week on. A maximum concentration of approximately 20 μg was reached in the 17th week (Eben et al., 1979). Similarly, free 2,5-hexanediol was found in the urine after 3 weeks and peaked in the 17th week. Free and conjugated 2,5-hexanedione were present in the 7th week, whereas excretion levels of the free form were consistent throughout the study. A strong correlation was observed in this study between the onset of neuropathy and the urinary concentration of 2,5-hexanedione when 2-hexanone, 2,5-hexanedione, or 2,5-hexanediol was administered orally to rats at 400 mg/kg-day.

Radiolabeled ^{14}C from 1-^{14}C-2-hexanone applied to the forearms of two human volunteers was found in the breath and urine (DiVincenzo et al., 1978). In one subject, eliminated amounts in urine and breath were similar, while, in the other subject, the levels in breath were about three times higher than in urine. Fecal elimination was not measured.

3.5. PHYSIOLOGICALLY BASED TOXICOKINETIC MODELS

2-Hexanone was considered as a metabolite in two physiologically based toxicokinetic (PBTK) models for n-hexane that focus on its neurotoxic metabolite, 2,5-hexanedione (Hamelin et al., 2005; Perbellini et al., 1990). PBTK models that deal specifically with 2-hexanone were not identified. A blood/air partition coefficient of 127 for 2-hexanone measured using preserved human blood has been reported (Sato and Nakajima, 1979).

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS—CASE REPORTS, EPIDEMIOLOGICAL STUDIES, AND OBSERVATIONAL STUDIES

In humans, 2-hexanone vapor caused irritation of the eyes and respiratory tract during acute exposure to relatively high concentrations. Men exposed to 0.23, 0.65, or 2% 2-hexanone in air (9422, 26,600, or 81,900 mg/m^3) for 1 minute or less reported strong eye and nasal irritation (Schrenk et al., 1936). Moderate eye and nasal irritation was reported after a brief exposure to 0.1% (4100 mg/m^3). Peripheral neuropathy was reported in printers, furniture finishers, and spray painters occupationally exposed to 2-hexanone (Davenport et al., 1976; Mallov 1976; Allen et al., 1974; Billmaier et al., 1974). Several studies have described the occurrence of neurological effects after the introduction of 2-hexanone into products used in the occupational setting.

Davenport et al. (1976) reported the occurrence of symmetrical polyneuropathy in a 35-year-old male who was occupationally exposed to 2-hexanone among other compounds. The patient had worked as a furniture finisher for several years, most recently spraying lacquer compounds, sometimes without using a face mask. Initially, according to the manufacturer, MiBK was present at a concentration of 20% in the finish, 12% in the thinner, and 7% in the sealer. Toluene, xylene, n-butyl alcohol, and acetone were also present in various proportions. After repeated inquiries, the manufacturer disclosed that, for the 6-month period before the onset of the man's illness, 2-hexanone had been substituted for MiBK on a volume-for-volume basis in the formulations of lacquers and solvents because of MiBK supply limitations. The patient first noticed tingling in the soles of his feet and mild clumsiness of gait. Weakness progressed rapidly to the upper extremities, resulting in a wheelchair-bound condition. Three months after the onset of the first symptoms, routine hematology, blood chemistry, urinalysis, spinal fluid, and analysis for heavy metals and porphyrins were normal. A biopsy of the sural nerve[1] at the level of the lateral malleolus revealed diffuse fibrosis and loss of nerve fibers. Several enlarged axons, with and without myelin sheaths, with neurofibrillary tangles were evident. A clinical evaluation 3 months later indicated improved strength and ability to walk unassisted, though with some residual unsteadiness of gait. Tendon reflexes distal to the elbows and knees were still absent. The case report noted that a similar progressive distal extremity weakness developed in a 19-year-old coworker of the patient. This condition also improved following removal from contact with lacquer products.

One probable and two definite cases of 2-hexanone-induced peripheral neuropathy were found during an investigation of 26 painters who worked at Cannelton or nearby Newburgh dams

[1] A sensory nerve that innervates the skin of the back of the leg, and skin and joints on the lateral side of the heel and foot.

on the Ohio River (Mallov, 1976). Two formulations of paint were used. The older formulation contained 22% (weight/weight [w/w]) MiBK and 22% (w/w) methyl isoamyl ketone. In the newer otherwise identical formulation, these solvents were replaced by 44% (w/w) 2-hexanone. While both paint formulations were reported to contain 3.1% (w/w) of the known neurotoxicant triorthocresyl phosphate, this substance was not found in two bulk samples of the 2-hexanone paint formulation. One definite case of peripheral neuropathy was that of a 42-year-old man, a painter for 10 years, who had been painting Cannelton Dam from September 1972 until August 1973. His initial signs, including weight loss, numbness and tingling of feet, and progressive weakness in both lower extremities that progressed to his upper extremities as well, began in July 1973. Weakness progressed until he could no longer stand without assistance. Lower extremity reflexes became absent and an electromyogram was abnormal. Blood and urine lead analysis indicated slightly elevated levels but not sufficient to cause effects. The second case was that of a 35-year-old man who had been painting since he was 14 years old. He painted at Cannelton Dam from April to October 1973. He felt well until about 4 weeks prior to the termination of painting at Cannelton but eventually became unable to rise from a sitting position without help. Urine lead levels were in the lower normal range. The third painter had worked at either Cannelton or Newburgh dam from September 1970 until November 1973. He also felt well until about 4 weeks prior to termination of painting. While he experienced weakness in his extremities, he remained able to walk but reported above-normal episodes of falling and dropping things. He was not examined by a physician until 3.5 months after the onset of symptoms, at which time absent ankle reflex, foot weakness, and diminished sensation were noted. None of the three patients had a history of alcoholism or family history of neurological disease or took medications.

A cross-sectional study of peripheral neuropathy among employees at a coated fabrics plant in Ohio was started when it was noted that six workers from the print department had developed severe peripheral neuropathy (five hospitalized, one seen as outpatient) between April and August 1973 (Allen et al., 1974; Billmaier et al., 1974). The plant produced plastic-coated printed fabrics that were used mainly for wall coverings and automobile interiors. Processing steps included mixing, calendering, laminating, coating, printing, embossing, inspecting, and shipping. Starting in September 1973, all 1157 employees of the plant (including the original six cases) were screened using electromyography and nerve conduction testing. A total of 192 employees were referred for detailed neurological evaluation. On the basis of these examinations, it was concluded that 68 employees had definite signs, symptoms, and electrodiagnostic findings of peripheral neuropathy. Severity ranged from mild (electrodiagnostic findings but no physical symptoms) to moderate (distal sensory loss) to severe (distal muscle weakness and sensory loss). There were a total of nine severe cases, including the original six cases. Cases with possible causes other than a toxic chemical (e.g., diabetes) were not included in the analysis but were identified in the presentation of results. The distribution of cases within the plant is shown in Table 4-1.

Table 4-1. Prevalence of peripheral neuropathy among employees of a coated fabrics plant

Workplace	Number of cases	Number of employees examined	Prevalence (%)
Non-print departments	30[a]	984	3
Print department (total)	38[b]	173	22[c]
Operators	27	69	39[c]
Helpers	10	59	17[c]
Foreman	0	21	0
Service helper	1	16	6
Not known	0	8	0
Total	68	1157	6

[a]Includes 18 persons with diabetes or other conditions that can cause or contribute to neuropathy.
[b]Includes one person with diabetes and one person on isoniazid therapy.
[c]Significantly elevated compared with non-print departments ($p < 0.001$) using the chi-square test.

Source: Billmaier et al. (1974).

The prevalence of peripheral neuropathy was significantly higher among print department employees than among employees from other departments (22 vs. 3%, $p < 0.001$). All nine severe cases were print department workers. Within this department, prevalence was highest among printer operators (39%, $p < 0.001$ compared with non-print department employees), who spent almost 100% of their time near the printing machines. Prevalence among helpers (17%) who spent roughly 50% of their time near the printing machines was also significantly elevated compared with non-print department employees ($p < 0.001$). There was a 6% prevalence among service helpers who were in and out of the premises (one case among service helpers was a pan washer who used the solvent for cleaning). Among manufacturing departments other than the print department, the prevalence of neuropathy ranged from 0 to 6.7%. No cases of peripheral neuropathy were observed in supervisory personnel who remained at a distance from the machines or in office personnel.

In addition to job category, incidence of neuropathy was also associated with working overtime (print operators with definite neuropathy averaged 47.2 hours/week versus 42.0 hours/week for those without neuropathy [$p < 0.01$]) and with eating on the job (data not shown). Each employee generally worked on the same machine all the time. No differences in neuropathy incidence were found based on the type of printing machine or the area in which the machine was located; data were insufficient to correlate illness with individual machines. Among print department employees, there were no significant differences in neuropathy incidence related to age or tenure in the department; 90% of cases had presented within the previous year, and only 5% of the cases were known to have medical conditions that could cause or contribute to neuropathy. Among non-print department employees, cases were clustered in older (40+) employees ($p < 0.001$); only 53% had onset within the previous year, and 60% of these cases were known to have diabetes or other medical conditions that could cause or contribute to neuropathy unrelated to compound exposure.

In the search for the etiologic agent, other chemicals known to cause peripheral neuropathy were ruled out, either by clinical tests on workers or because they were not used in the plant. Based on an investigation into the relationship between the cases of peripheral neuropathy and the distribution of the roughly 275 chemicals used in the plant, the most likely agent appeared to be contained in the solvents used as ink thinners and cleaners. These had previously consisted of methyl ethyl ketone (MEK) and MiBK, but, starting in August 1972, the latter was phased out and gradually replaced by 2-hexanone, which reached maximal usage in December 1972. Substitution of 2-hexanone for MiBK was the only major change in the production process in the previous 7 years. In September 1973, the print department was closed for a month and 2-hexanone was removed from production materials. Thus, there was a 13-month window of exposure to 2-hexanone.

In addition to exposure to 2-hexanone, affected workers were also exposed to high concentrations of MEK that sometimes vastly exceeded threshold limit values (TLVs). MEK by itself does not produce this type of neuropathy in animal studies but can potentiate the effects produced by 2-hexanone (Saida et al., 1976). Thus, the presence of MEK in the coated fabrics plant study could contribute to an overestimation of the risk associated with exposure to 2-hexanone itself. Workplace levels for 2-hexanone and MEK from this study are presented in Table 4-2.

Table 4-2. Results of area atmospheric sampling for MEK and 2-hexanone in a coated fabrics plant

	Front of print machine[a]		Back of print machine[a]		Wind-up area[a]	
	MEK[b]	**2-Hexanone[c]**	**MEK**	**MEK[b]**	**2-Hexanone[c]**	**MEK**
	104	2.3	85	104.0	2.3	85.0
	109	2.6	265	3.0	44	2.0
	124	4.1	401	9.0	47	2.0
	162	5.1	440	9.8	49	2.6
Median	220	5.8	603	21.7	127	5.9
	453	9.7	608	23.9	143	6.0
	565	11.5	725	48.6	250	7.9
	570	19.8	750	49.9	289	9.8
	670	21.7	763	156.0	338	17.5

[a]Values are in ppm, listed from lowest to highest result obtained for each solvent at each work location.
[b]TLV = 200 ppm.
[c]TLV = 100 ppm.

Source: Billmaier et al. (1974).

Another confounding factor for this study is that exposure may not have been limited to the inhalation route. Poor work practices documented at the plant included washing hands in solvent, using solvent-soaked rags to clean equipment, and eating in work areas. Dermal and even oral exposure is likely to have occurred. The significance of exposure by these routes is suggested by the observations that eating on the job was associated with the development of neuropathy and that a worker whose job involved washing pans with the solvent was the only afflicted print department worker other than the print operators and their helpers. As discussed in Sections 3.1.2 and 3.1.3, 2-hexanone is absorbed readily through the skin and gut and can produce neuropathy by both routes in animals.

The researchers reported that patients removed from 2-hexanone exposure showed significant and consistent improvements. They also performed a study of workers at a comparable coated fabrics plant in California that produced the same products as the one in Ohio but without the use of 2-hexanone. Electrodiagnostic studies were conducted on 21 solvent-exposed workers at the California plant, but no peripheral neuropathy was found.

4.2. ACUTE, SUBCHRONIC, AND CHRONIC STUDIES IN ANIMALS

4.2.1. Oral Exposure

4.2.1.1. *Acute and Short-Term Oral Exposure*

Range-finding toxicity data by Smyth et al. (1954) list an oral median lethal dose (LD_{50}) of 2.59 g/kg of 2-hexanone for rats, while Tanii et al. (1986) provide an oral LD_{50} of 2.43 g/kg for mice. Details for either study are limited (Tanii et al., 1986; Smyth et al., 1954).

4.2.1.2. *Subchronic Toxicity Studies*

16-Week study: female Wistar rats

Homan et al. (1977) conducted a 120-day drinking water study with female Wistar rats. 2-Hexanone (purity not stated) was administered in drinking water at 0, 0.65, or 1.3% (0, 480, or 1010 mg/kg-day). A dose-dependent decrease in food consumption was observed in exposed animals versus controls. Water consumption in exposed animals was reduced to about half that of controls. Animals exposed to 0.65 or 1.3% 2-hexanone experienced a 45.5 and 68.8% reduction in body weight gain, respectively. A dose-dependent decrease in absolute liver weight was observed in exposed animals. Absolute kidney weights were increased, and there was a dose-dependent increase in relative kidney weights. A summary of the data for diet and water consumption, body weight gain, and organ weights is provided in Table 4-3. Neurotoxicity outcomes among the treated animals are outlined in Section 4.4.1.

Table 4-3. Gross observations in rats exposed to 2-hexanone in drinking water for 120 days

Dose (mg/kg-day)	Food intake (g/day)	Water intake (mL/day)	Body weight gain (g)	Liver weight		Kidney weight	
				Absolute (g)	Relative	Absolute (g)	Relative
0	17.99	32.29	110.2	10.30	3.10	1.97	0.60
480	16.90	17.98	60.0[a]	9.01	3.35	2.21	0.82[a]
1010	12.90	17.33	34.3[a]	7.80[a]	3.38	2.10	0.92[a]

[a]Significantly different from controls, $p < 0.01$.

Source: Homan et al. (1977).

40-Week study: male Wistar rats

Eben et al. (1979) gavaged male SPF-Wistar rats daily with 400 mg/kg 2-hexanone (98% pure) for 40 weeks. Body weight gain in treated animals was less than in controls; a decrease in body weights was observed from the 17th to the 25th weeks, followed by a slight increase until study completion. There were also symptoms of neurotoxicity in the treated animals (see Section 4.4.1).

4.2.1.3. *Chronic Toxicity Study*

13-Month study: CD/COBS(SD) rats

O'Donoghue et al. (1978)[2] conducted a 13-month study in male CD/COBS(SD) rats. The animals' drinking water contained 0, 0.25, 0.5, or 1.0% (0, 143, 266, or 560 mg/kg-day) 2-hexanone (96% pure, containing 3.2% MiBK and 0.7% unknown contaminants). 2-Hexanone produced a dose-dependent reduction in body weight at all doses tested. The effect was present by the second week in the two highest dose levels and by the third week in the low-dose group. A statistically significant increase in liver weight was found in the highest dose group compared with all groups except the 0.5% group. The 0.5% and 0.25% groups showed dose-dependent increases in relative liver weights compared with controls. A statistically significant increase in relative kidney weights was present between the 1.0% 2-hexanone group and all other groups and between the 0.5% group and all other groups. Similarly, a statistically significant increase in relative testes weight was found between the 1.0% group and all other groups. A summary of the body weight and organ weight data is present in Table 4-4 (O'Donoghue et al., 1978).

Clinical neurological deficits were noted in animals exposed to either 0.5 or 1.0% 2-hexanone. Severe deficits including decreased extension of the hind limb, hind-limb weakness, and muscular atrophy of the hind-limb musculature were noted among animals treated with 1% 2-hexanone. Deficits among animals exposed to 0.5% 2-hexanone were slight and did not result in clinical progression. Evidence of axonal swelling was noted at all dosing levels of

[2] This study is an unpublished study; accordingly, it was externally peer reviewed by EPA in December 2007.

2-hexanone. Neurological effects are discussed in further detail in Section 4.4.1.1. Other than neural effects and changes in body weight, no nonneural clinical signs related to 2-hexanone exposure were found.

Table 4-4. Pathological changes in rats exposed for 13 months to 2-hexanone

	Body weight[a]	Liver[b]		Kidney[b]		Testes[b]	
		Absolute	Relative	Absolute	Relative	Absolute	Relative
Control	710	26.71 ± 2.02	3.64 ± 0.41	4.66 ± 0.53	0.63 ± 0.87	2.99 ± 0.81	0.40 ± 0.11
2-Hexanone (0.25% or 143 mg/kg-day)	685	24.99 ± 4.33	3.97 ± 0.43	4.58 ± 0.69	0.73 ± 0.05	3.24 ± 0.38	0.52 ± 0.08
2-Hexanone (0.5% or 266 mg/kg-day)	612	25.06 ± 2.04	4.22 ± 0.43	5.33 ± 0.31	0.90 ± 0.12[c]	3.16 ± 1.04	0.54 ± 0.19
2-Hexanone (1.0% or 560 mg/kg-day)	448	20.73 ± 2.95	4.62 ± 0.32[c]	4.86 ± 0.38	1.10 ± 0.23[c]	3.29 ± 0.26	0.75 ± 0.17[c]

[a]Values are means of 10 animals.
[b]Values are mean ± SE based on four or five animals per group.
[c]Statistically different from controls, $p < 0.05$

Source: O'Donoghue et al. (1978).

To determine whether the concentration of MiBK, a CYP450 inducer, a contaminant in the 2-hexanone formulation used by O'Donoghue et al. (1978) may have altered the observed toxicity of 2-hexanone, other studies were evaluated that used MiBK as a test article. In a 13-week gavage study, 30 male and female Sprague-Dawley rats were treated daily with 0, 50, 250, or 1,000 mg MiBK/kg-bw (MAI, 1986). At the middle and high doses, adverse effects were observed in the liver and kidney, which progressed in severity in the high dose animals. No treatment-related effects of any kind were observed at 50 mg/kg-day. The Carnegie-Mellon Institute of Research (1977) conducted a 120-day drinking water study with 1.3% MiBK using female HLA Wistar rats. The authors estimated the dosage to be 1040 mg/kg-day. The only statistically significant finding was increased mean absolute and relative kidney weights in treated rats compared with controls. Histopathological examination revealed renal tubular cell hyperplasia in only one of five of the treated rats. No exposure-related histopathological changes were found in other organs. Based on the foregoing, it can be concluded that the dosage of MiBK received as an impurity in the study by O'Donoghue et al. (1978) did not contribute to the observed 2-hexanone related effects. O'Donoghue et al. (1978) did not observe adverse effects in the kidney or liver of treated animals, despite these organs being the target organs of toxicity in experimental studies with MiBK from both the oral and inhalation routes (U.S. EPA, 2003a).

4.2.2. Inhalation Exposures

4.2.2.1. *Acute and Short-Term Toxicity Studies*

No acute inhalation toxicity studies of 2-hexanone were identified. The National Library of Medicine's Hazardous Substances Data Bank states that a 4-hour exposure of rats to 4000 ppm 2-hexanone did not kill all animals, while exposure to 8000 ppm for 4 hours was an LD_{100} (NLM, 2005). Abdo et al. (1982) reported the death of one out of five hens exposed continuously to 200 ppm 2-hexanone (70% purity). No deaths were reported in hens exposed to 100 ppm or lower (Abdo et al., 1982).

4.2.2.2. *Subchronic Toxicity Study*

11-Week study: male rats

Groups of five male rats (Crl:COBS/CD[SD]BR) were exposed to 0 or 700 ppm (0 or 2870 mg/m^3) 2-hexanone (purity 96.1%) 72 hours/week for 11 weeks (Katz et al., 1980). The exposure schedule was as follows: two 20-hour periods and two 16-hour periods, Monday through Friday, separated by 8-hour nonexposure periods. Total white blood cell counts of treated animals were significantly ($p < 0.05$) lower than those of controls; no other differences were noted in clinical chemistry or hematological values. Gross examination of treated animals revealed marked atrophy of the hind-limb musculature, depletion of adipose tissue, and significantly decreased absolute and relative testicular weight ($p < 0.05$). Histopathological examination was performed on selected tissues, including lung and trachea (but not nasal cavities), eye, digestive tract, pancreas, thyroid, parathyroid, testes, epididymides, spleen, bone marrow, mesenteric lymph nodes, thymus, and nervous system. Atrophy of testicular germinal epithelium and grossly enlarged axons in the brain stem and cerebellum were observed in treated animals. No damage to bone marrow was evident despite the low white blood cell count. Although no discussion of findings in the lung or trachea was presented, the implication is that there were no treatment-related changes in these tissues. The treatment group developed signs of neurotoxicity (weakened hind- and forelimb grasp) by the second week of exposure, progressing to severe hind-limb weakness by 71 days, and showed decreased weight gain. Neurological effects are discussed in further detail in Section 4.4.1.2.

4.2.2.3. *Chronic Toxicity Study*

72-Week study: male Sprague-Dawley rats

Krasavage and O'Donoghue (1977) exposed groups of male Sprague-Dawley rats (18/group) to 0, 100, or 330 ppm (0, 410, or 1353 mg/m^3) 2-hexanone (purity not specified) 6 hours/day, 5 days/week for 72 weeks. Clinical signs (observed daily and examined weekly), body weight (recorded weekly), and water consumption (at 15, 22, 32, and 44 weeks of exposure) were monitored. Beginning at 4 weeks and continuing at approximately 6-week intervals for the first 52 weeks, unspecified numbers of animals were killed for microscopic examination of an extensive list of tissues, including the trachea and lung. Body weights and weight gain were comparable among groups until the 20th week. Thereafter, body weights of the high-concentration animals fell behind those of controls (data presented graphically without statistical analysis); a visual estimate of the graphic presentation suggested that the body weights of high-concentration animals were at least 10% less than those of controls. After 36 weeks of exposure, body weight gain in the low-concentration group also began to lag behind controls. Water intake was comparable among groups.

Gross postmortem findings revealed no compound-related changes. Low-concentration animals did not develop clinical signs attributed to 2-hexanone exposure or morphologic lesions of neuropathy. Histopathologic evidence for neuropathy in high-concentration rats was equivocal. Neurological effects are discussed in further detail in Section 4.4.1.2. Spontaneous lesions were present in the urogenital, cardiovascular, and endocrine systems of both treated and control animals and were therefore not attributed to 2-hexanone exposure by the study authors (Krasavage and O'Donoghue 1977).

2-Year study: cats

Groups of four domestic shorthair cats were exposed by inhalation to 0, 100, or 330 ppm (0, 410, or 1353 mg/m^3) 2-hexanone (purity not specified) for 6 hours/day, 5 days/week for 2 years (O'Donoghue and Krasavage 1979). Clinical signs and body weights were monitored. Serum was sampled after 30, 90, and 128 exposures to determine the levels of 2-hexanone and two of its metabolites, 5-hydroxy-2-hexanone and 2,5-hexanedione. Each sample set involved collection serum on a Monday prior to daily exposure, the following Tuesday prior to daily exposure, the following Friday prior to daily exposure, immediately after daily exposure and one and three quarter hours after daily exposure. Sera from high-dose and control animals were also analyzed for sodium, potassium, chloride, and calcium levels. Cats were sacrificed at the end of the treatment and were subjected to necropsy and histopathologic examinations.

No clinical neurological effects attributed to exposure to 2-hexanone were identified except that cats anesthetized with sodium pentobarbital following a 6-hour exposure had prolonged sleeping times (O'Donoghue and Krasavage, 1979). No compound-related changes of body weight or serum electrolyte values were found. Serum levels of 2-hexanone and the two metabolites, 5-hydroxy-2-hexanone and 2,5-hexanedione, were below the detection limit on Monday morning following a two-day non-exposure period. With the exception of 2,5-hexanedione in the 330 ppm group (1353 mg/m^3), serum levels on Tuesday morning following a 6-hour exposure after 30 days of exposure remained below the detection level. Of the three substances measured, 2-hexanone cleared the serum more quickly than 5-hydroxy-2-hexanone, which cleared more quickly than 2,5-hexanedione. Biopsy examinations through the first 9 months of exposure were unremarkable and did not serve as an early detection method for neuropathy. Gross postmortem findings revealed no compound-related changes. General histopathologic examinations showed no compound-related changes other than in the nervous system and musculature. Neurological effects are discussed in further detail in Section 4.4.1.2.

4.2.3. Dermal Exposure

90-Day study: hens

Abou-Donia et al. (1985b) treated leghorn laying hens (n = 5) with 2-hexanone (99% pure; topical application, 1 mmol/kg-bw). The chemical was applied daily with a micropipette over an area of 10 cm^2 on the unprotected back of the neck for 90 days. All hens developed gross ataxia. At sacrifice, no changes were observed in treated versus control animals when compared for size, shape, weight, or color. Equivocal histopathologic changes were present in the spinal cord of two hens. These histopathologic changes were characterized by swollen axons without obvious fragmentation of the axon or myelin sheath. No precautions against licking were mentioned in the study, so ingestion of 2-hexanone may have taken place.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

4.3.1. Oral Exposure

No standard two-generation studies or other studies of reproductive and developmental effects following oral administration of 2-hexanone were identified.

4.3.2. Inhalation Exposure

In a developmental study, Peters et al. (1981) exposed groups of 25 pregnant F344 rats to 0, 500, 1000, or 2000 ppm (0, 2048, 4096, or 8193 mg/m^3) 2-hexanone (purity not stated), 6 hours/day on gestational days (GDs) 1–21. A separate control group was maintained for each exposure group and the high-concentration controls were pair fed. Respective controls were exposed to ambient air in similar chambers to those of their exposed counterparts. Sexually mature female rats were impregnated and placed in exposure chambers 6 hours/day throughout gestation. Four weeks postdelivery, the dam was separated from the pups. The maternal 500 ppm group along with its control was terminated before 3 weeks because of an apparent lapse in care during which offspring were “unable to reach food and water,” resulting in reduced weight gain in this group. The pups in the control, 1000, and 2000 ppm groups were observed over a lifetime. At 4 (weaning), 8 (puberty), and 14 weeks (adult) and at 18–20 months of age (geriatric), five males and five females were taken, one per litter, for gross and histopathology studies and for measurement of organ/body weight ratios. At different periods of development (weaning, puberty, and adult), offspring underwent behavioral testing. Pentobarbital sleeping time was also measured in pubescent and geriatric animals in the high-dose and control groups.

Survival in the 2000 ppm and 1000 ppm dams was not affected by treatment. High-dose dams appeared sluggish after exposure but seemed to have recovered by the next exposure. Hair loss, lack of muscular coordination, and weakness were observed in “several” dams at the highest concentration after 20 days of exposure. Abnormal sniffing in the air was reported for dams in the 1000 ppm group. Maternal gestational body weight gain was decreased by 14 and 10% in the dams exposed to 2000 and 1000 ppm, respectively. Rats in the 2000 ppm exposure group were observed to eat less than the controls. No unusual behavior or change in maternal gestational growth was reported for the 500 ppm dams. Histopathology and neurotoxicity evaluations were not performed in the dams.

2-Hexanone exposure was found to result in statistically significant decreases (*p* value not reported) in litter size and pup weight observed at the highest exposure level (Peters et al., 1981). However, maternal toxicity, manifested as decreased maternal body weight during gestation, was also evident in high-dose dams, suggesting that maternal toxicity might have affected fetal growth. There was a significant decrease in the number and weight of live offspring of dams in the 2000 ppm exposure group. A lifelong, statistically significant, concentration-related reduction in growth was observed in male offspring. Only a slight treatment-related effect on body weight was seen in female offspring. Organ weights in weanling, pubescent, and geriatric offspring were unaffected by treatment, but brain weight in adult 1000 ppm offspring was significantly increased compared to that of control. Organ weights were not measured in high-dose adult offspring. The authors did not report any gross skeletal alterations. Beginning at 40 weeks of age, offspring of dams treated with 1000 or 2000 ppm showed a 3–5% decrease in survival relative to controls. The incidence of pathological lesions and the types of lesions contributory to death were not significantly different in treated and control groups (Table 4-5).

Table 4-5. Summary of pathological lesions in offspring of rats exposed to 2-hexanone during gestation

	Control				1000 ppm				2000 ppm			
	Male	**Female**	**Total**	**%**	**Male**	**Female**	**Total**	**%**	**Male**	**Female**	**Total**	**%**
Number of animals dead or sacrificed[a]	57	57	114	--	37	34	71	--	24	22	46	--
Pituitary tumor	1	3	4	3.5	1	1	2	3	1	0	1	2
Pituitary hemorrhage	2	0	2	2	0	0	0	0	1	2	3	6.5
Diaphragmatic hernia	1	1	1	2	0	1	1	1.4	1	2	3	6.5
Ovarian cysts	0	2	2	2	0	7	7	10	0	8	8	18
Mottled testes	26	0	26	23	16	0	16	23	1	0	1	2

[a]Animals include those dying subsequent to weaning in addition to those sacrificed at 78 ± 2 weeks of age.

Source: Peters et al. (1981)

Standard hematological tests (hemoglobin, red blood cell count, white blood cell count, lymphocytes, mean corpuscular hemoglobin, packed cell volume) showed no significant treatment effect on the processes involved in blood cell formation and function (Peters et al., 1981). Clinical chemistry findings were limited to a concentration-related decrease in creatinine phosphokinase activity in pubescent offspring, with values in the 1000 ppm and 2000 ppm groups significantly lower ($p < 0.05$) than controls. In geriatric offspring, there were significant increases ($p < 0.05$) in serum alanine aminotransferase activity in the 1000 ppm and 2000 ppm groups and sodium in the 2000 ppm group. The only lesions showing a significant concentration-response relationship ($p < 0.05$, Fisher's exact test conducted for this assessment) at the time of geriatric sacrifice were ovarian cysts that had 4% (2/57), 21% (7/34), and 36% (8/22) incidences in the control, 1000 ppm, and 2000 ppm females, respectively.

In pubescent high-dose male offspring, pentobarbital sleep time was significantly increased ($p < 0.05$) compared with controls. No significant changes in pentobarbital sleep time were noted in pubescent females or geriatric offspring of either sex. Behavioral alterations were reported in the offspring of pregnant rats exposed to 1000 ppm or 2000 ppm 2-hexanone. These effects consisted of reduced activity in the open field, increased activity in the running wheel, and deficits in avoidance conditioning. Offspring of treated dams (both dose levels) clung to an inclined screen longer than offspring of controls at all ages (newborn, weanling, puberty, and adult) except geriatric in which results were similar to those of controls. For offspring in the puberty and adult categories, pronounced sex differences were noted; females in all exposure categories (including controls) were clinging from 24–100% longer than males. However, the biological significance of this observation is unknown. There was a decreased rate of avoidance learning in puberty-aged females of treated dams and increased random movement in both puberty-aged and adult offspring of treated dams. Behavioral tests in most cases indicated that maternal exposure to 2-hexanone was associated with hyperactivity in the young and decreased activity in the geriatric stage, which the authors (Peters et al., 1981) speculated to be due to premature aging resulting from the earlier hyperactivity. It is not clear whether these effects are the result of transplacental exposure to 2-hexanone or of postnatal exposure to 2-hexanone and/or its metabolites via the milk of the exposed dams.

4.4. OTHER ENDPOINT-SPECIFIC STUDIES

4.4.1. Neurotoxicity Studies

4.4.1.1. *Oral Exposures*

90-Day study: hens

In hens that received a single gavage dose of 2-hexanone (technical grade 2-hexanone, 70% pure, containing 30% methyl isobutyl ketone) at 2000 mg/kg, mild weakness was observed on the day of administration, followed by apparent recovery in 4–5 days. Hens that received 100 mg/kg showed no signs of neurotoxicity (Abou-Donia et al., 1982). In a subchronic (90-day) phase of the same study, hens (n = 3) administered 2-hexanone at 100 mg/kg-day or higher developed severe ataxia or near paralysis. There was also evidence of histopathological changes, including swelling or degeneration of thoracic and lumbar regions of the spinal cord.

90-Day study: rats

Krasavage et al. (1980) administered 660 mg/kg 2-hexanone (96% pure) by gavage to male CD/COBS(SD) rats for up to 90 days. The authors considered severe hind-limb weakness or paralysis, as exhibited by "dragging" of at least one hind foot, to be clear indication of neuropathy. When this endpoint was reached, the treatment was terminated and the animal was processed for histological examination. There was a time- and dose-dependent depression in body weight gain and feed consumption. Treated animals consumed an average of 21 grams/day versus 28 grams/day for controls. The body weights of experimental and control animals at study completion were approximately 400 and 600 grams, respectively. Histologic examination of nerve tissue collected at termination revealed morphologic changes indicative of giant axonal neuropathy, which included multifocal axonal swellings, myelin infoldings, and paranodal myelin retraction. In this study, atrophy of the germinal epithelium of the testes was also observed, but the statistical significance of this observation was not addressed (Krasavage et al., 1980).

120-Day study: female Wistar rats

Homan et al. (1977) conducted a 120-day drinking water study with female Wistar rats (five/group). 2-Hexanone (purity not stated) was administered in drinking water at 0, 0.65, or 1.3% (0, 480, or 1010 mg/kg-day) (for further experimental details see Section 4.2.1.2). Neurological evaluations were conducted to assess balance, strength, coordination, and behavior. Performance was scored for each of the following 10 criteria: posture, gait, palpebral reflex, startle reflex, flexor reflex, extensor reflex, placing reflex, hopping reaction, righting reflex, and clinging reaction. Score ranged from 0 to 2 with 0 indicating normal and 2 being clearly deficient. The net score for each rat was calculated as the sum of the individual test scores. Scores were tabulated, ranked, and analyzed using the Kruskal-Wallis ranks sum test. The rank values (statistics generated from the Kruskal-Wallis test) for each treatment group for a given day of analysis were then averaged to generate a mean rank and standard deviation. A summary of the mean rank (mean of the values generated from the Kruskal-Wallis test) and standard deviation is provided in Table 4-6. Gross pathological evaluation revealed mild atrophy affecting skeletal muscles of the hind limbs in two of five animals in the 0.65% group and slight to severe atrophy of skeletal muscles (most pronounced in muscles of the hind limbs) affecting four of five animals in the 1.3% group (Homan et al., 1977).

Table 4-6. Time course of neuropathy scores following exposure of rats to 2-hexanone in drinking water

Treatment	Analysis after number of treatment days			
	46	57	80	110
	Mean rank value			
Control	26.1 ± 9.1	15.0 ± 0.0	22.1 ± 12.8	17.5 ± 0.0
0.65% 2-hexanone	32.0 ± 14.9	30.6 ± 12.7	30.9 ± 9.2	21.5 ± 8.0
1.3% 2-hexanone	37.5 ± 12.6	41.0 ± 5.7	40.0 ± 13.7	47.2 ± 2.8a

[a]Statistically significant versus controls, $p < 0.05$.

Source: Homan et al. (1977).

24-Week study: guinea pigs

Abdel-Rahman et al. (1978) administered 2-hexanone (purity not stated) in drinking water to guinea pigs (five/group, sex not stated) at 0, 0.1, or 0.25% (approximately 0, 97, or 243 mg/kg-day) for 24 weeks. Bibs were used to prevent dermal absorption by inadvertent contact of the animals' bodies with the solvent. The body weight of the guinea pigs was monitored each week up to the eighth week of the study. At the end of the seventh week, animals exposed to 0.25% 2-hexanone weighed an average of 600 grams versus 440 grams in controls. Similarly, animals exposed to 0.1% 2-hexanone weighed 618 grams by the eighth week compared with 490 grams in controls. Decreased locomotor activity may have contributed to increased body weights. The average motor activity counts in animals exposed to 0.25% 2-hexanone were 714 ± 130 compared to 1173 ± 201 in controls. Pupillary response to light (measured by change in pupillary diameter in response to an intense 2-second light stimulus) was abnormal in high-dose animals for the first 5 weeks of treatment as shown in Table 4-7 (data not provided for 0.1% 2-hexanone). The authors reported that by the 24th week of the study, a greatly impaired pupillary response was observed for all treatment groups (data not provided in the report) (Abdel-Rahman et al., 1978).

Table 4-7. Effect of 2-hexanone on guinea pig pupillary response of both eyes

Treatment	Week							
	1		2		3		5	
	Right[a]	Left[a]	Right	Left	Right	Left	Right	Left
Control	1.83 ± 0.00	1.66 ± 0.17	1.6 ± 0.1	1.67 ± 0.19	1.6 ± 0.06	1.7 ± 0.05	1.5 ± 0.05	1.5 ± 0.1
0.25% 2-hexanone	1.33 ± 0.19	1.33 ± 0.01[b]	1.05 ± 0.15	1.17 ± 0.01[b]	0.67 ± 0.17	0.92 ± 0.08[b]	0.59 ± 0.14[b]	0.71 ± 0.04[b]

[a]Values represent the mean ± SE of the change in pupillary diameter.
[b]Statistically significant from controls ($p < 0.001$) as calculated by study authors.

Source: Abdel-Rahman et al. (1978).

40-Week study: rats

Eben et al. (1979) gavaged male SPF-Wistar rats daily with 400 mg/kg 2-hexanone (98% pure) for 40 weeks. The authors stated that this treatment did not cause neuropathic symptoms; however, from the 17th week the authors noted that the animals exhibited weakness of the hind limbs, which continued until the 28th week. Thereafter, an improvement was observed. No further details were provided.

13-Month study: rats

As previously mentioned in Section 4.2.1.3, O'Donoghue et al. (1978) conducted a 13-month study in male CD/COBS(SD) rats. Each group of 10 rats was exposed to drinking water containing 0, 0.25, 0.5, or 1.0% (0, 143, 266, or 560 mg/kg-day) 2-hexanone (96% pure, containing 3.2% MiBK and 0.7% unknown contaminants). Body weight and neurological examinations were performed weekly. At the end of the study, a dose-dependent reduction in body weight was noted among all dose groups. All but one animal was found to have some evidence of neurotoxicity. Other than neural effects and body weight changes, no compound-related clinical signs were found.

Clinical neurological deficits were found only in animals receiving 0.5 or 1.0% 2-hexanone. Deficits were recorded as slight if there was incomplete extension of the hind limb and just detectable widening of the hind limb stance; moderate if there was obvious weakness, incomplete extension of the hind limbs, and waddling; and severe if there was dragging of at least one hind paw. In the 1.0% group, all the animals exhibited severe deficits. Gross pathological examination revealed observable muscle atrophy of hind-limb and lumbar muscles at this high-dose level. Progression of the clinical findings to a more severe state did not occur with time in the 0.5% group. In addition to the aforementioned changes, animals receiving 1% hexanone in their drinking water displayed loss of tone with grossly observable atrophy of the hind-limb musculature and axial muscles of the lumbar area. Weakness of the forelimbs with some muscle atrophy was observable in three of nine rats at the end of the study. Pain sensation, as judged by toe pinch, remained intact, but motor response such as flexor response was easily overcome. It was noted that tactile placing in the hind limbs could be elicited even in rats with severe weakness. Bowel and bladder functions remained normal. The clinical course was highly variable with improvements in the clinical symptoms being very common; thus, while all animals showed slight deficits on at least two of the weekly examinations, they showed improvements during other weeks.

Evidence of neuropathy was most common in the giant axons of animals of each dose level. In peripheral nerves from the 1.0% group, swelling of giant and other axons was common. Myelin infoldings into the axoplasm were more common than in controls. Myelin ovoids were frequently found along with degenerating axons. The second most common site of neural degeneration was in the spinal cord, particularly in the ventral and ventromedial funiculi of the thoracolumbar segments. The changes were similar to those found in peripheral nerves. In plastic embedded sections, an additional early change was noted, which consisted of clumping of axonal organelles in otherwise normal peripheral or central axons. Examination of the dorsal root ganglia did not reveal any effect on cell bodies, but in three animals single swollen axons were found in adjacent roots, indicating a very minimal effect. Axonal swelling was also very rare in the brain. No neuropathologic effects were found rostral to the pons. Small numbers of swollen axons were located in the ventromedial medulla. Rare single swollen axons were located in the ventral spino-cerebellar tracts, cerebellar peduncles, and deep cerebellar white matter.

Neurogenic skeletal muscle atrophy occurred in both proximal and distal hind-limb musculature. Myofibrillar atrophy was multifocal with foci overlapping in severe cases to produce large diffuse areas of atrophy with fatty replacement. Intramuscular nerves frequently showed an obvious loss of axons and rarely a swollen axon. No difference in the severity or frequency of atrophic foci was seen between proximal and distal muscles.

In the 0.5% group, peripheral nerve changes were identical in morphology and in the number of animals affected compared with the higher-dose animals but were reduced in severity. Swollen axons were generally few in number but were found in all animals. Myelin ovoids and frankly degenerating axons were also reduced in number. In some nerve bundles, there was obvious loss of axons. Spinal effects were reduced to a few swollen axons and rare degenerating axons in the ventromedial fasciculi of the thoracolumbar cord. Effects on the brainstem and cerebellum were minimal, consisting of only single or small numbers of swollen axons and single degenerating axons in half of the animals examined. Neurogenic skeletal muscle atrophy consisted of infrequent multifocal areas of myofibrillar atrophy that were generally regarded as minor. Two animals without myofibrillar atrophy were considered normal. Three samples from the quadriceps and two from the calf muscles, while not demonstrating myofibrillar atrophy, did have early myopathic effects consisting of foci of increased numbers of angular myofibers and increased numbers of myofibers with central or internal nuclei. In one of these animals, intramuscular axonal swelling was found.

At the 0.25% level, peripheral nerve changes were less severe than at higher doses and axonal swelling was found in 8 of 10 animals examined. In these eight rats, the number of swollen axons was very low, but additional changes, such as myelin infoldings into axons, myelin ovoids, and degenerating axons, were more common. In one animal, while no axonal swelling was observed, numerous degenerating axons were found. Another rat was indistinguishable from controls. Spinal lesions were minimal, consisting of a single or very few swollen axons. A few instances of axonal swelling were found in the medullae of two rats. Neurogenic myofibrillar atrophy was also minimal, occurring as a single or very few foci in two animals. Foci of angular myofibers were found in four additional animals but were of minimal severity. In control animals, the peripheral and central nervous system (CNS) contained a few degenerating axons and myelin ovoids, but these were minimal. A summary of animals found to have axonal swelling and the areas in which these axons or myopathic changes were found is presented in Table 4-8.

Table 4-8. Summary of neuropathologic findings in male rats administered 2-hexanone in drinking water for 13 months

Treatment	Incidence of axonal swelling				Incidence of myofibrillar atrophy	
	Brain	Spinal cord	Dorsal root ganglia	Peripheral nerve	Quadriceps muscle	Calf muscle
Control	0/10	0/5	0/5	0/10	0/10	0/10
0.25% 2-hexanone	2/10	7/10	0/7	8/10	1/10	2/10
0.5% 2-hexanone	4/10	5/5	0/5	10/10	5/10	6/10
1.0% 2-hexanone	8/10	5/5	3/5	10/10	10/10	10/10

Source: O'Donoghue et al. (1978).

4.4.1.2. *Inhalation Exposures*

6-Week study: rats

In a short communication, Duckett et al. (1974) reported the results of a study in which groups of nine rats (strain and sex not reported) were exposed to 200 ppm (819 mg/m^3) 2-hexanone (purity unspecified) 8 hours/day, 5 days/week for 6 weeks. Four rats served as controls. Animals presented with muscular weakness of all limbs that persisted for a few hours after exposure termination each day. Only the sciatic nerve was examined histologically. Axonal hypertrophy, beading, and degeneration associated with perinodal and segmental breakdown of myelin were observed in the sciatic nerve of all treated rats.

13-Week study: rats

In the same short communication discussed above, Duckett et al. (1974) discussed results of an unpublished subchronic experiment with 2-hexanone. In this experiment, groups of 20 Wistar rats of unspecified sex were exposed to 2-hexanone for 8 hours/day, 5 days/week at 40 ppm (164 mg/m^3) for 22–88 days or at 50 ppm (205 mg/m^3) for 13 weeks. Similar numbers of control rats were sham exposed. No overt signs or "pathological manifestations" of peripheral or central neuropathy were seen in exposed rats, except for demyelination of the sciatic nerve in 3 of the 20 rats exposed to 50 ppm for 13 weeks. Additional details were not provided. The results at 50 ppm for 13 weeks, when compared with the results at 50 ppm for 6 months, indicate that the incidence of neuropathy increases with increasing duration of exposure.

12-Week study: cats, rats, chickens

Mendell et al. (1974) continuously exposed groups of animals of unspecified sex (four Sprague-Dawley rats, four domestic shorthair cats, and five domestic chickens) to 2-hexanone (purity not stated) for 24 hours per day, 7 days per week for up to 12 weeks. Concentrations of 2-hexanone were initially 200 ppm (820 mg/m^3) for chickens and 600 ppm (2,460 mg/m^3) for cats and rats but were adjusted at an unspecified time to 100 and 400 ppm (410 and 1640 mg/m^3), respectively, to minimize complications from inanition and weight loss. Pair-fed controls were sacrificed when the exposed animals were sacrificed. After 5–8 weeks of exposure, the cats developed hind-limb and forelimb weakness. Focal swelling of the axon along the sciatic nerve, often associated with loss of neurotubules and denudation of myelin beginning at the nodes of Ranvier, and areas of demyelination were observed. Abnormal electromyograms were also observed in the cats exposed for 9–10 weeks; electromyograms were not measured in chickens or rats (Mendell et al., 1974).

90-Day study: hens

Abdo et al. (1982) exposed adult leghorn laying hens (*Gallus gallus domesticus*), 5 per group, to varying concentrations of 2-hexanone (10, 50, 100, 200, and 400 ppm; technical grade 2-hexanone containing 70% 2-hexanone and 30% methyl isobutyl ketone) for 90 days. Body weights were monitored weekly, and hens were examined every other day for neurological signs of 2-hexanone neurotoxicity. A 30-day observation period followed the final exposure. Clinical assessment of neurotoxicity was graded by classifying the degree of ataxia before paralysis as follows: T_1, mild ataxia, characterized by diminished leg movement and reluctance to walk, with hens tending to slide on the floor or fly; T_2, gross ataxia, characterized by a change in gait and disturbance of leg movement; T_3, severe ataxia, with severe leg weakness manifested by unsteadiness and occasional falling on the floor; T_4, ataxia, with near paralysis, marked by inability to walk (Abdo et al., 1982).

The spinal cord and the sciatic, peroneal, and tibial nerves were excised from hens that died during the experiment or were killed by heart puncture and exsanguinations. Severity of lesions was defined by the following criteria: (1) rare swollen axons without fragmentation, phagocytosis, or loss of myelin staining were designated as equivocal histological changes; (2) occasional degenerative changes of axons and myelin in peripheral nerve or within the spinal cord, which may contain nests of phagocytic cells, were termed mild to moderate degeneration; and (3) lesions were considered severe when there was almost complete destruction of axons and myelin in a given tract such as the anterior columns or within extensive areas of peripheral nerve.

Only hens exposed to one of the highest two concentrations of 2-hexanone, 400 or 200 ppm, lost significant weight at the onset of ataxia; weight loss for these two groups continued, and the hens exposed to 400 and 200 ppm 2-hexanone weighed 48.0 $\pm$ 7.4 and 63.1 $\pm$ 5.5% (mean $\pm$ SE) of the initial weights, respectively, at the onset of paralysis. Although the group exposed to 100 ppm 2-hexanone gained some weight at the onset of ataxia, they lost 24.4% of their initial weight after 69 days of exposure. This weight loss coincided with the development of severe ataxia. This treatment group, however, regained all lost weight by the end of the 30-day observation period. No appreciable change in weight was observed in hens exposed to 50 or 10 ppm 2-hexanone.

None of the hens continually exposed to 2-hexanone vapor showed any signs of acute toxicity that are attributed to the narcotizing effects of 2-hexanone on the CNS. All hens continually exposed to 50–400 ppm 2-hexanone developed ataxia after a latent period of 6–30 days, depending on 2-hexanone concentrations. Those exposed to 400 ppm progressed to paralysis, and two died 27 days after the beginning of exposure. The remaining three chickens were in a distressed condition and were sacrificed at 31 days. The number of days of exposure to 2-hexanone vapors before the onset of ataxia was dependent on and inversely proportional to the concentration of 2-hexanone.

All hens exposed to 200 ppm 2-hexanone developed paralysis 64–72 days after the beginning of the exposure; one of these hens died at day 72 and the other four were sacrificed on day 73. Four of the hens inhaling 100 ppm 2-hexanone developed severe ataxia (T_3), while the fifth bird progressed to ataxia with near paralysis (T_4). Three hens of the group exposed to 50 ppm 2-hexanone showed severe ataxia (T_3), while the other two developed only gross ataxia (T_2). The clinical condition of all hens in this group was gross ataxia (T_2) at termination. All hens exposed to 10 ppm 2-hexanone remained normal.

Histopathological lesions in the spinal cord were dependent on concentration, duration of exposure, and duration of intoxication. Two of the hens exposed to 400 ppm did not exhibit any histopathological alterations, while another two showed equivocal changes. Hens exposed to 100 ppm 2-hexanone exhibited clinical signs of neurotoxicity for 99 $\pm$ 2 days, and all hens showed unequivocal changes in the spinal cord. Although hens exposed to 50 ppm 2-hexanone were exposed for a mean of 97 days, only four of these hens had unequivocal changes in the spinal cord. Similarly, the presence of histopathological lesions in peripheral nerves was a function of both the level of 2-hexanone inhaled and, particularly, the total dose inhaled. Although all five hens exposed to 100 ppm for 90 days survived until termination on day 120, they showed gross to severe ataxia and each had unequivocal lesions in peripheral nerves. Hens given high doses became paralyzed and thus could not be kept alive as long as those given 100 ppm 2-hexanone.

4-Month study: rats

Groups of six young adult rats (strain and sex not specified) were exposed to 1300 ppm (5325 mg/m^3) of 2-hexanone 6 hours/day, 5 days/week for up to 4 months (Spencer et al., 1975). Three rats were exposed to air only. Animals were observed for neurological signs, and histopathological examinations of several peripheral nerves, regions of the spinal cord, medulla, and cerebellum were completed. In the exposed rats, narcosis, loss of coordination, weight loss (data not presented), foot drop, and proximal hind-limb and forelimb weakness were observed. Pathological alterations included nerve fiber degeneration in the peripheral nerves, spinal cord, medulla, and cerebellum; axonal dilatation with localized fiber swelling; and secondary paranodal myelin retraction.

6-Month study: male rats

Duckett et al. (1979) exposed groups of Wistar rats (sex not specified) to 0 (n = 20) or 50 ppm (n = 40) 2-hexanone (0 or 205 mg/m^3) 8 hours/day, 5 days/week for 6 months. No overt signs of toxicity were observed during the study. Electrophysiological evaluations were performed on 5 treated and 10 control rats at the end of the experiment. The mean sciatic motor conduction velocity (MCV) in the exposed group was significantly lower ($p = 0.005$) than in the controls. No effect on the amplitude of the evoked muscle action potential (MAP) was observed. Widespread demyelination of the sciatic nerve was reported in 32 rats from the exposed group; two of the rats also had axonal hypertrophy and beading. No abnormalities were seen in the sciatic nerves of control rats. The study authors reported that the histopathology of the CNS, liver, and kidney of all rats was normal (details were not provided) (Duckett et al., 1979).

72-Week study: male rats

Krasavage and O'Donoghue (1977) exposed male Sprague-Dawley rats (18/group) to 0, 100, or 330 ppm (0, 410, or 1353 mg/m^3) 2-hexanone (purity not specified) 6 hours/day, 5 days/week for 72 weeks (for further experimental detail, see Section 4.2.2.2). Exposure to 100 ppm did not cause clinical or pathologic evidence of neurological damage. One rat exposed to the high concentration developed progressive hind-limb weakness; another three high concentration animals showed slight weakness that was not progressive. One animal in the high concentration group developed a severe polyradiculoneuritis of the nerve roots in the lumbar and sacral spinal nerves and in the sciatic and tibial nerves. The authors concluded that chronic exposure to 100 ppm 2-hexanone was not neurotoxic, while findings at 330 ppm were equivocal (Krasavage and O'Donoghue 1977).

6-Month study: male rats

Male Sprague-Dawley rats (six/group) were exposed to 0 or 100 ppm (0 or 410 mg/m^3) 2-hexanone (purity 96.66%, 2.9% MiBK) 22 hours/day, 7 days/week for 6 months (Egan et al., 1980). Two animals from each group underwent microscopic examination for neuropathologic changes following 2, 4, and 6 months of exposure. No treated or control animals displayed clinical signs of neurotoxicity during the exposure period. After four months of exposure, a typical pattern of 2-hexanone-induced neuropathology began to appear in the CNS and peripheral nervous system (PNS). At this time, PNS specimens revealed giant axonal swellings and secondary demyelination in a few large diameter fibers in the tibial nerve branches to the calf muscles. In the CNS, isolated giant axonal swellings were found in the medulla oblongata and cerebellum. By six months, more advanced degeneration was presented in teased fibers in calf muscle branches and giant axonal swelling had ascended to the level of the sciatic notch. The spinal cord revealed scattered fiber degeneration in the ventral portion of the gracile tract and the caudal portion of descending fiber tracts in the lumbar region.

10-Month study: male rats, male monkeys

Johnson et al. (1977) exposed male Sprague-Dawley rats (10/per group) and male monkeys (*Macaca fascicularis*) (8/group) to 0, 100, or 1000 ppm (0, 410, or 4100 mg/m^3) commercial grade 2-hexanone (purity not stated) for 6 hours per day, 5 days per week for up to 10 months. Rats in the 1000-ppm exposure group exhibited progressive body weight loss beginning at 16 weeks and reaching statistical significance at 20 weeks ($p < 0.01$). Monkeys in the 1000-ppm group progressively lost body weight beginning at 8 weeks. No significant effect of 2-hexanone on body weight of rats or monkeys was found in the low-dose exposure groups.

Four neurological tests were conducted on both rats and monkeys: MCV of right sciatic-tibial nerves, MCV of the right ulnar nerve, absolute refractory period of these nerves, and MAP recorded in response to both sciatic and ulnar stimulation. In addition, electroencephalograms and visual evoked potentials were recorded from monkeys. All animals were administered an anesthetic prior to neurological testing: rats received an i.p. injection of 35 mg/kg of sodium pentobarbital, and monkeys were given 15 mg/kg of ketamine hydrochloride intramuscularly.

After 25 weeks, all rats and monkeys in the high-dose exposure group were removed from further exposure because neuropathy (hind-limb drag) apparently had developed. All eight monkeys in the 100 ppm group were exposed for a total of 41 weeks. Rats in the low-dose group were removed from 2-hexanone exposures after 29 weeks. Beginning at 3 months of exposure, monkeys in the 1000 ppm group showed a progressive decrease in the MCV of the sciatic-tibial nerves. After 6 months, the mean MCV of this group was 63% of the mean of control animals. Commencing at 9 months, the MCV for the sciatic-tibial nerves in monkeys in the 100 ppm group was significantly different from control values ($p = 0.05$). At the termination of the study, the MCV of monkeys from the 100 ppm group was 12% less than in the corresponding controls ($p < 0.05$).

A similar pattern of sciatic-tibial neuropathy developed in rats exposed to the higher concentration of 2-hexanone. A significant decrease in MCV was observed at approximately 3 months (13 weeks) of exposure ($p = 0.05$). A significant difference at 8 weeks between MCVs of control and 1000 ppm rats was considered spurious. In the 100 ppm group, a significant difference in MCVs between controls and treated rats occurred at 29 weeks ($p < 0.001$).

A neuropathy similar to that observed for the sciatic-tibial nerves was noted in the ulnar nerve of both the monkeys and rats. When compared with controls, commencing at 4 months, monkeys showed a progressive decrease in the MCV of the ulnar nerve. At the end of 6 months' exposure to 1000 ppm, monkeys showed a significant decrease in ulnar MCV with values approximately 64% of those of controls ($p < 0.01$). Ulnar MCVs in the 100 ppm group showed a similar decreasing trend at about 6 months; however, these values were not statistically different from controls. In rats, ulnar MCVs were significantly decreased compared with control values ($p < 0.05$), beginning at about 17 weeks in both exposed groups.

Both monkeys and rats exposed to 1000 ppm 2-hexanone showed a continuous decrease in MAP amplitude in response to sciatic stimulation that became statistically significant in monkeys at 6 months ($p < 0.01$). This effect was not noted in the low-dose group of monkeys. Rats in the 100 ppm group had reduced MAP amplitudes for sciatic stimulation, beginning at 12 weeks. No effects of 2-hexanone on scalp-recorded electroencephalograms (EEGs) of monkeys were observed. Amplitude measures of the EEG were not affected at either exposure concentration. Visual examination of the EEG records did not reveal any abnormal patterns (e.g., spikes or abnormal waves).

Evidence of 2-hexanone-induced effects on average visual evoked potential (AVEP) was obtained in monkeys exposed to 1000 ppm. Specifically, latencies of certain AVEP components were increased beginning at 4 months. No effects on these latencies occurred as a result of the low-dose 2-hexanone exposure. The refractory time (i.e., the time that must elapse between two consecutive stimuli of a nerve in order for the second stimulus to also excite the nerve) was not affected by 2-hexanone at either level of exposure.

Only rats were examined for effects of 2-hexanone on operant behavior at 10 and 19 weeks of exposure to 100 and 1000 ppm, respectively. For operant behavior, animals were trained on a multiple fixed ratio of 5, fixed interval 3-minute (multi-FR5FI3) schedule for 20–40 days after shaping the bar press response. Once behavior was stable, animals were placed in exposure chambers and tested after exposure. A reduction in response rate in the 1000 ppm group developed by the second week of exposure; however, no effects of 2-hexanone on operant behavior were found with the 100 ppm group (Johnson et al., 1977).

2-Year study: cats

Groups of four domestic shorthair cats were exposed by inhalation to 0, 100, or 330 ppm (0, 410, or 1353 mg/m^3) 2-hexanone (purity not specified) for 6 hours/day, 5 days/week for 2 years (O'Donoghue and Krasavage, 1979). Clinical signs and body weights were monitored (for details, see Section 4.2.2.2). To follow the onset of neuropathy, biopsy specimens were collected from two randomly selected cats in each group at six intervals for the first 9 months of the exposure period. All specimens were taken from alternate hind paws and included 5–6 Pacinian corpuscles and plantar interosseous muscles. Cats were sacrificed at the end of the treatment and underwent gross and histopathologic examinations, and the nervous system was examined microscopically in detail.

No clinical neurological effects attributed to exposure to 2-hexanone were identified. Neuropathologic examination results for the control and low-dose groups were comparable. All cats in the high-dose group showed evidence of neuropathologic changes in the CNS and the PNS at and below the level of the cerebellum and pons (O'Donoghue and Krasavage, 1979). In the PNS, the highest incidences of change occurred in the tibial motor nerve branches to the musculature of the lower leg and then in the tibial nerve itself. In the branches, endoneural space was enlarged with clear fluid. Swelling of giant axons with myelin retraction was evident, and degenerating axons were found infrequently. No changes were found in the dorsal root ganglion cells. In the distal portion of the PNS in the high-dose animals, unusually large preterminal axonal processes were evident, a condition not seen in controls. Examinations of tibial nerve fibers indicated comparable percentages of the four fiber pathology categories (i.e., demyelination, remyelination, swelling, and degenerative fibers) in the control and low-dose groups, but the high-dose group had notable changes in each fiber pathology category except degenerative fibers. Demyelination, remyelination, swelling, and degeneration occurred in 12.3, 3.4, 6.3, and 0.4% of high-dose axons examined (average number of high-dose axons examined = 158), compared with 0, 0.3, 0, and 0.6% of control axons (average number of control axons examined = 84). In the CNS, swollen terminals were found in the posterior cerebellar peduncles, folial white matter, nucleus gracilis, fasciculis gracilis, spino-cerebellar tracts, medullary reticular formation, and all levels of the spinal cord.

4.4.1.3. *Other Routes of Exposure*

11-Month study: dogs (subcutaneous injections)

O'Donoghue and Krasavage (1981) administered 2-hexanone (>97% pure, with 2.9% MiBK and trace quantities of 2-hexanol) by daily subcutaneous (s.c.) injection to purebred male beagles (n = 4) for 11 months. Each dog received 300 mg/kg of the test compound or saline at first once daily and later (time not stated) divided into two equal doses 6 hours apart. All animals developed signs of neurotoxicity to varying degrees. The patellar reflex was lost unilaterally in two of the four dogs receiving 133 grams of 2-hexanone over a period of 96 days. One month later, the patellar reflex was lost bilaterally in both dogs, and clinical signs of neurotoxicity progressed with observations of muscle weakness and difficulty walking. The condition of both dogs gradually reversed during the course of the study, following an unspecified cessation of exposure. In the remaining two dogs, the clinical signs of neurotoxicity appeared later in the study or were apparent at study completion. In one dog, the patellar reflex could not be elicited after it had received 243 grams of 2-hexanone over a period of 156 days. Following cessation of exposure, the dog returned to apparent normality in approximately 56 days. In the remaining dog, no clear neuropathic abnormality was produced, but, although the patellar reflex was present, the response appeared sluggish. There was occasional evidence of hind-limb weakness.

Mean body weights of treated animals were comparable with those of controls, but individual animals showed weight loss or decreased weight gain. Hematology, clinical chemistry, and cerebrospinal fluid analysis were not affected by the treatment. Repeated biopsy examinations of distal peripheral nerves showed typical giant axonal swelling. The biopsy findings paralleled the clinical course except during a recovery phase, where the biopsy continued to be abnormal while the clinical course improved. Electromyographic examination of the treated dogs showed the persistence of abnormalities in two recovering dogs, no abnormalities in one recovering dog, and no abnormalities in the one dog that had appeared clinically normal throughout the study (O'Donoghue and Krasavage, 1981).

90-Day study: hens (intraperitoneal injections)

Abou-Donia et al. (1982) treated five groups of leghorn laying hens (*Gallus gallus domesticus*, n = 3) with daily injections of 2-hexanone (70% 2-hexanone and 30% methyl isobutyl ketone, i.p.) at 100 or 200 mg/kg for 90 days. Hens given a daily 100 mg/kg i.p. injection of 2-hexanone progressed through all successive stages of ataxia; the clinical conditions of two of them improved after treatment was stopped, while the third hen progressed to paralysis and died after 30 days of administration. Daily i.p. injection of 200 mg/kg 2-hexanone produced ataxia with near paralysis (T_4), which progressed to paralysis in one hen. The clinical condition of this hen, however, reverted to grade T_4 after cessation of administration.

Spinal cords from hens given daily i.p. 100 mg/kg injections of 2-hexanone did not exhibit any histopathologic changes. One of these hens, however, showed unequivocal histopathologic changes in the peripheral nerves. The sites of axonal degeneration were accompanied by myelin degeneration, and macrophages were observed containing debris with the staining properties of myelin. Although none of the hens given 200 mg/kg i.p. injections of 2-hexanone showed histopathologic alterations in peripheral nerves, two of these hens developed unequivocal histopathologic lesions in the spinal cord. A longitudinal section from the ventral column of the thoracic spinal cord from one of the hens showed axons with prominent swellings. These swellings have the morphologic configuration of the paranodal swelling that suddenly ends at the nodes of Ranvier. A longitudinal section of the thoracic spinal cord from the other affected hen demonstrated extensive degeneration in the ventral column and a markedly swollen axon and nests of macrophages.

4.4.2. Immunotoxicity Studies

No studies were located regarding immunological effects in humans by any route of exposure to 2-hexanone.

A reduction in total white blood cell counts to 60% of control values ($p < 0.05$), but no changes in differential white cell counts or evidence of bone marrow damage, was found in rats intermittently exposed to 700 ppm 2-hexanone after 8 weeks, during an 11-week study (Katz et al., 1980). These findings, although inconclusive, suggest that immunological effects may warrant some consideration in future assessments of the potential toxicity of exposure to 2-hexanone.

4.5. OTHER STUDIES

4.5.1. Mechanistic Studies

4.5.1.1. *2-Hexanone and Enzyme Induction*

2-Hexanone and its neurotoxic metabolite 2,5-hexanedione are both effective inducers of microsomal enzyme activities. This can affect the toxicity of other xenobiotics and also can affect the toxicity of 2-hexanone itself (or its precursor, n-hexane) by increasing or decreasing the formation of toxic metabolites.

Nakajima et al. (1991) characterized the CYP450 enzymes in the livers of male Wistar rats that are induced following exposure to 2-hexanone (5 mmol/kg-day), 2,5-hexanedione (5 mmol/kg-day), or phenobarbital (80 mg/kg-day), administered intraperitoneally for 4 days. A control group received an equivalent volume of corn oil vehicle (4 mL/kg). All three treatments caused a statistically significant increase in microsomal protein content and overall CYP450 activity (Table 4-9).

Table 4-9. Effects of 2-hexanone, 2,5-hexanedione, and phenobarbital on microsomal protein and CYP450

Treatment	Body weight (g)	Liver weight (g)	Liver/body weight ratio (%)	Microsomal protein (mg/g liver)	CYP450 (nmol/mg protein)
Control	206 ± 7	6.6 ± 0.2	3.21 ± 0.11	21.5 ± 0.8	0.92 ± 0.002
2-Hexanone	192 ± 6	7.3 ± 0.3[a]	3.80 ± 0.05[a]	25.1 ± 1.5[a]	1.49 ± 0.10[a]
2,5-Hexanedione	184 ± 7[a]	6.4 ± 0.3	3.49 ± 0.07[a]	26.2 ± 1.7[a]	1.62 ± 0.10[a]
Phenobarbital	197 ± 5	7.9 ± 0.4[a]	4.01 ± 0.13[a]	31.5 ± 3.0[a]	2.12 ± 0.19[a]

[a]Significantly different (p < 0.05) from control.

Source: Nakajima et al. (1991).

The enzyme activities (i.e., benzene aromatic hydroxylase [CYP2E1], toluene side chain oxidation [CYP2C6/11], EROD [CYP1A1/2], and PROD [CYP2B1/2]) were measured as indicators of CYP450 activity. All three treatments caused a statistically significant increase in the rate of benzene hydroxylation at low (0.2 mM) and high (6.3 mM) concentrations and toluene side chain oxidation at low (0.2 mM) and high (5.0 mM) concentrations. EROD activity was not affected by pretreatment; however, a statistically significant increase in PROD activity was observed with all three treatments. A summary of the results for the CYP450 activity measured with specific substrates is listed in Table 4-10.

Table 4-10. Effect of enzyme inducers on the activities of CYP450-related enzymes in rats exposed to 2-hexanone or 2,5-hexanedione

Treatment	Enzyme activity					
	BAH[a]		TSO[b]		EROD	PROD
	0.2 mM	6.3 mM	0.2 mM	5.0 mM		
Control	0.68 ± 0.09	0.53 ± 0.11	1.87 ± 0.15	8.34 ± 0.67	0.32 ± 0.06	0.11 ± 0.02
2-Hexanone	1.10 ± 0.19[c]	1.76 ± 0.23[c,d]	5.65 ± 0.62[c]	19.07 ± 1.64[c,e]	0.41 ± 0.30	3.68 ± 0.70[c]
2,5-Hexanedione	0.98 ± 0.16[c]	1.57 ± 0.15[c,d]	5.05 ± 0.46[c]	19.98 ± 0.78[c,e]	0.26 ± 0.44	2.92 ± 0.90[c]
Phenobarbital	0.48 ± 0.11[c]	2.80 ± 0.23[c,d]	5.59 ± 0.87[c]	25.36 ± 6.23[c,e]	0.27 ± 0.04	5.22 ± 0.70[c]

[a]BAH = benzene aromatic hydroxylase.
[b]TSO = toluene side-chain oxidase.
[c]Significantly different ($p < 0.05$) from control.
[d]Significant difference ($p < 0.05$) between 0.2 and 6.3 mM of the corresponding group.
[e]Significant difference ($p < 0.05$) between 0.2 and 5.0 mM of the corresponding group.

Source: Nakajima et al. (1991).

Using immunoblotting and immunodetection assays, Nakajima et al. (1991) did not detect CYP4501A1/2 in microsomes from treated and control animals. CYP4502B1/2 was induced by treatment with phenobarbital > 2-hexanone = 2,5-hexanedione. Only trace amounts of CYP4502E1 were detected in phenobarbital-treated rats, whereas 2-hexanone and 2,5-hexanedione both induced this isoform efficiently.

In order to explore the effects of 2-hexanone, 2,5-hexanedione, and phenobarbital on CYP4502B1/2, CYP4502E1, and CYP4502C6/11, Nakajima et al. (1991) performed immunoinhibition analyses of toluene side-chain oxidase (TSO) activity by using monoclonal antibodies directed against each of these CYP450 isoforms. Anti-CYP4502E1 inhibited TSO activity in induced microsomes as follows (values are percent of activity in the absence of anti-CYP4502E1): phenobarbital, 97 ± 2%; 2,5-hexanedione, 79 ± 3%; 2-hexanone, 75 ± 11%; and controls, 65 ± 2%. Anti-CYP4502B1/2 inhibited TSO activity in induced microsomes differently: phenobarbital, 31 ± 4%; 2-hexanone, 65 ± 3%; 2,5-hexanedione, 69 ± 5%; and controls, 99 ± 2%. Anti-CYP4502C6/11 inhibited toluene metabolism in induced microsomes as follows: phenobarbital, 75 ± 5%; 2-hexanone, 69 ± 5%; 2,5-hexanedione, 70 ± 3%; and controls, 23 ± 4%.

Similar studies were performed by Imaoka and Funae (1991). The authors treated male Sprague-Dawley rats (number of rats not provided) with 2-hexanone (purity not stated; 5 mmol/kg, i.p.; dissolved in corn oil) daily for 4 days. This dose was considered a maximum tolerated dose. Control rats were given corn oil only. Hepatic microsomes were isolated, and the activities of CYP450 enzymes were determined against specific substrates (Table 4-11).

Table 4-11. Catalytic activities of CYP450 enzyme activities in rat liver following induction by 2-hexanone

Substrate	Enzyme activity (nmol/min-mg protein)[a]	
	Uninduced control	2-Hexanone-treated
Aminopyrine	2.40 ± 0.50	4.37 ± 0.82b
Aniline	0.283 ± 0.044	0.421 ± 0.070[b]
7-Ethoxycoumarin	3.62 ± 0.13	6.01 ± 1.24b
Testosterone-2α	0.684 ± 0.114	0.431 ± 0.158[c]
Testosterone-2β	0.140 ± 0.039	0.240 ± 0.056[c]
Testosterone-6β	0.959 ± 0.176	1.45 ± 0.341[c]
Testosterone-7α	0.056 ± 0.006	0.062 ± 0.013
Testosterone-15α	0.040 ± 0.007	0.056 ± 0.017
Testosterone-16α	1.09 ± 0.203	1.07 ± 0.347
Testosterone-16β	0.058 ± 0.006	0.250 ± 0.106[c]

[a]Mean ± SD, number of rats not provided.
[b]Significantly different from control, $p < 0.01$.
[c]Significantly different from control, $p < 0.05$.

Source: Imakoa and Funae (1991).

The content of total CYP450 measured photometrically did not change much with treatment. However, the activities of aminopyrine N-demethylase, aniline hydroxylase, and 7-ethoxycoumarin O-dealkylase were increased by pretreatment with 2-hexanone. Testosterone 2β-, 6β-, and 16β-hydroxylase activities were significantly increased, whereas the 2α-hydroxylase activity was decreased by treatment with 2-hexanone. The authors also measured changes in the levels of 11 forms of CYP450 in hepatic microsomes caused by treatment with 2-hexanone (Table 4-12).

The level of CYP4502C11, a male-specific form, was decreased by treatment with 2-hexanone in parallel with a decrease in testosterone 2α-hydroxylase activity, which is catalyzed by this isozyme (Kamataki et al., 1983) (cf. Table 4-12). CYP4502A2 is a constitutive testosterone 6β-hydroxylase; the increase in the level of this isoform explained the increase in testosterone 6β-hydroxylase activity, shown in Table 4-11. CYP4502B1 and 2B2 are typical phenobarbital-inducible forms. The level of CYP4502B1 in the hepatic microsomes of control rats was very low, and CYP4502B2 was detected at a slightly higher level. Both forms were strongly induced in 2-hexanone-treated rats. These results reflected the increases in testosterone 16β-hydroxylase and aminopyrine N-demethylase activities of hepatic microsomes (cf. Table 4-11) and suggest that 2-hexanone is a phenobarbital-type inducer.

Table 4-12. Changes in CYP450 levels following treatment with 2-hexanone

CYP450 isoform	CYP450 content (pmol/mg protein)[a]	
	Uninduced control	2-Hexanone-treated
2A1	7.0 ± 1.3	7.9 ± 1.5
2A2	10.4 ± 2.3	11.7 ± 2.8
2B1	<0.5	44.3 ± 9.4c
2B2	3.8 ± 1.2	29.3 ± 6.2c
2C6	52.1 ± 17.7	93.4 ± 16.9[b]
2C7	21.9 ± 3.3	24.8 ± 5.8
2C11	457.0 ± 52.6	343.8 ± 46.3[c]
2C13	171.4 ± 35.8	159.7 ± 24.5
2E1	49.8 ± 9.6	102.6 ± 14.8[b]
4A3	17.6 ± 3.2	16.7 ± 2.8

[a]Mean ± SD, number of rats not provided.
[b]Significantly different from control, $p < 0.01$.
[c]Significantly different from control, $p < 0.05$.

Source: Imakoa and Funae (1991).

Imaoka and Funae (1991) determined that the inducibility of CYP4502B1 and 2B2 was strongly correlated with the hydrophobicity (as estimated by the octanol/water partition coefficients, log K_{ow}) of several 2-hexanone homologues: 2-hexanone (1.38) > methyl n-propyl ketone (0.91) > MEK (0.29) > acetone (−0.24). In contrast, the inducibility of P4502E1 was not dependent on hydrophobicity. Each of the aforementioned chemicals, at equimolar concentrations, induced CYP4502E1 to a similar extent, approximately twofold, while acetone, a prototypical inducer of CYP2E1, induced this isoform approximately threefold.

Based on studies of 2-hexanone and the pesticide O-ethyl O-4-nitrophenyl phenylphosphonothioate (EPN) in hens, Abou-Donia et al. (1991, 1985) speculated that the potentiation of the neurotoxic effects of 2-hexanone by EPN may be due to induction of hepatic microsomal CYP450 by EPN with increased production of 2,5-hexanedione (Abou-Donia et al., 1991, 1985a). Similarly, MEK may also potentiate the toxicity of 2-hexanone through induction of CYP450 as MEK but not 2-hexanone and has been shown to decrease hexobarbital sleep time in rats (Couri et al., 1977). While MEK has been shown to potentiate the toxicity of 2-hexanone in rats (Saida et al., 1976), Shibata et al. (2002) have demonstrated that MEK depresses the metabolism of n-hexane in human volunteer subjects. If the metabolic pathways of 2-hexanone, as detailed in Section 3.3 and Figure 3-1, are common in humans and animals and MEK depresses the metabolism of n-hexane but increases the metabolism of 2-hexanone, then the step in 2-hexanone metabolism that MEK likely affects is the ω-1-oxidation to 5-hydroxy-2-hexanone. While no specific CYP450 isoenzymes have been implicated and the mechanisms are not fully elucidated, it appears that 2-hexanone has the ability to influence its own metabolism via effects on CYP450 enzymes that need more research to be fully understood.

It should be noted that, like 2-hexanone, MiBK, a common contaminant in the formulation of the 2-hexanone, has the potential to act as a CYP450 inducer. However, the 3.2% concentration of MiBK in 96% pure formulations of 2-hexanone, as reported by O'Donoghue et al. (1978), may not have a significant impact on the toxicity of 2-hexanone. To determine whether the concentration of MiBK as a contaminant may have altered the observed toxicity of 2-hexanone, other studies were evaluated that used MiBK as a test article. In a 13-week gavage study, 30 male and female Sprague-Dawley rats were treated daily with 0, 50, 250, or 1,000 mg MiBK/kg-bw (MAI, 1986). At the middle and high doses, adverse effects were observed in the liver and kidney, which progressed in severity in the high-dose animals. No treatment-related effects of any kind were observed at 50 mg/kg-day. The Carnegie-Mellon Institute of Research (1977) conducted a 120-day drinking water study with 1.3% MiBK, using female HLA Wistar rats. The authors estimated the dosage to be 1040 mg/kg-day. The only statistically significant finding was increased mean absolute and relative kidney weights in treated rats compared with controls. Histopathological examination revealed renal tubular cell hyperplasia in only one of five of the treated rats. No exposure-related histopathological changes were found in other organs. Based on the foregoing, it can be concluded that the dosage of MiBK received as an impurity in the study by O'Donoghue et al. (1978) did not contribute to the observed 2-hexanone-related effects. O'Donoghue et al. (1978) did not observe adverse effects in the kidney or liver of treated animals, despite these organs being the target organs of toxicity in experimental studies with MiBK from both the oral and inhalation routes (U.S. EPA, 2003a).

4.5.1.2. *2-Hexanone as a Sulfhydryl Reagent*

Both 2-hexanone and its metabolite 2,5-hexanedione can inhibit sulfhydryl-containing enzymes such as fructose-6-phosphate kinase and glyceraldehyde-3-phosphate dehydrogenase (enzymes in the pentose phosphate pathway [oxidative phase] and glycolytic pathway [nonoxidative phase], respectively) (Sabri, 1984; Sabri et al., 1979). Both of these chemicals inhibited fructose-6-phosphate kinase from rabbit muscle or rat brain homogenates; in each case, 2,5-hexanedione was the far more potent inhibitor (Sabri et al., 1979). Preincubation with dithiothreitol protected this enzyme from inhibition, which suggests that these compounds interfere with the sulfhydryl groups required for fructose-6-phosphate kinase activity. However, dithiothreitol could not restore enzyme activity after these compounds had been added. In addition, fructose-6-phosphate kinase activity was also reduced in brain homogenates of rats that had received 2,5-hexanedione at 0.5% in their drinking water for 10–12 weeks (Sabri et al., 1979). Glyceraldehyde-3-phosphate dehydrogenase from rabbit muscle (purified to crystalline state) was also inhibited in vitro by both compounds; in this case, 2-hexanone was the more potent inhibitor (Sabri, 1984). Levels of ATP were reduced in cat sciatic nerves treated with 2,5-hexanedione (Sabri, 1984), possibly an outcome of glyceraldehyde-3-phosphate dehydrogenase inhibition. 2-Hexanone was found to irreversibly inhibit rat brain and rabbit muscle creatine kinase and mouse brain adenylate kinase (Lapin et al., 1982).

4.5.1.3. *Studies Exploring the Development of Neuropathy*

Groups of 12 Sprague-Dawley rats (sex unspecified) were continuously exposed (24 hours/day) via inhalation to 0, 225, or 400 ppm (0, 922.5, or 1,640 mg/m^3) 2-hexanone (purity not stated) for 16–66 days (Saida et al., 1976). Rats exposed to 400 ppm were sacrificed at 16, 28, and 42 days, and those exposed to 225 ppm were sacrificed at 16, 25, 35, 55, and 66 days to study the sequence of morphological changes. Paralysis was observed after 66 and 42 days at the low and high concentrations, respectively. Neuropathologic changes preceded paralysis and were observed at the initial sacrifice after 16 days of exposure. Two distinct changes occurred quite early and close to the same time: the first to appear was an increase in the number of neurofilaments and the other was an in-pouching of the myelin sheath. In animals exposed to 400 ppm, the first observable change at 16 days was, in larger diameter nerve fibers, a two- to threefold increase in the number of neurofilaments. As the duration of exposure lengthened and the number of neurofilaments increased, several interrelated morphological observations were made. In teased nerve fiber preparations, swelling of the axons could be seen frequently in the paranodal area and less often at focal sites along the internodal segment. High numbers of nerve fibers with in-pouching of the myelin sheath were found per mm^2 of nerve fascicle, increasing with time after administration of the high concentration. A summary of the comparative sequential clinical and pathological observations is presented in Table 4-13.

Table 4-13. Clinical and pathological observations with time of exposure to 2-hexanone in rats

	2-Hexanone exposure						
	400 ppm			**225 ppm**			
Days exposed	**16**	**28**	**42**	**16**	**25**	**35**	**55**
Clinical findings	N[a]	N	P[b]	N	N	N	N
In-pouchings (#/mm^2)	6	142	499	23	46	92	86
Denuded fibers (#/mm^2)	0	4	11	0	0	1	2
Swollen axons >11 µm (#/300 fibers)	0	1	3	0	0	0	0

[a]N = normal.
[b]P = paralyzed.

Source: Saida et al. (1976).

The anterior horn cells, nerve roots, nerve trunks, intramuscular nerves, and motor end plates were studied sequentially to determine the site with the earliest pathological involvement. In animals exposed for 16 days to 225 ppm, no abnormalities were found in the motor end plates or intramuscular nerves of the intrinsic foot muscles. Only after prolonged exposure, 66 days, did the authors find typical signs of denervation in the motor end plates. These end plates showed atrophic axon terminals with Schwann cell processes interposed between the nerve terminal and postsynaptic membrane. There was also a loss of secondary synaptic clefts.

Anterior horn cells and dorsal root ganglion cells were also examined at various intervals of exposure. No changes were observed in these cell bodies, even after typical changes were seen in the main trunk of the sciatic nerve. Specifically, no abnormalities were seen that would suggest an increase in neurofilaments in these cell bodies, and no cells were observed undergoing chromatolysis.

4.5.2. Genotoxicity Studies

Mayer and Goin (1994) tested the ability of 2-hexanone to induce chromosome loss in strain D61.M of *Saccharomyces cerevisiae*. 2-Hexanone, alone or in combination with acetone and MEK, induced only a marginally positive chromosome loss (Mayer and Goin, 1994).

No data were identified for the mutagenicity of 2-hexanone with in vitro cytogenetic tests or in vivo tests.

4.5.3. Structure-Activity Relationships

A large body of toxicological information is available on n-hexane, a compound that is metabolized to 2-hexanone, on MiBK (a branched-chain homologue of 2-hexanone), and on MEK. These compounds have been reviewed in previous IRIS assessments, and a summary of the reference values derived for each is presented in Table 4-14. n-Hexane is the only compound of the above three that is also capable of producing the peripheral neuropathy similar to that observed in humans or animals exposed to 2-hexanone. Neither MiBK nor MEK can give rise to the neurotoxic metabolite 2,5-hexanedione.

Table 4-14. Summary of the toxicities of n-hexane, MiBK, and MEK

Chemical	Experimental dose	Critical effect	Reference value	Reference
n-Hexane (CASRN 110-54-3)	NOAEL[a]: 1762 mg/m^3	Peripheral neuropathy (decreased MCV at 12 weeks)	RfC: 7×10^{-1} mg/m^3	U.S. EPA (2005c)
Methyl isobutyl ketone (CASRN 108-10-1)	NOAEL: 1229 mg/m^3	Reduced fetal body weight, increased fetal death, and skeletal variations in mice and rats	RfC: 3 mg/m^3	U.S. EPA (2003a)
Methyl ethyl ketone (CASRN 78-93-3)	LEC[b]: 5202 mg/m^3	Developmental toxicity (skeletal variations)	RfC: 5 mg/m^3	U.S. EPA (2003b)
	NOAEL: 594 mg/kg-day (0.3% 2-butanol)	Decreased pup body weight	RfD: 0.6 mg/kg-day	

[a]NOAEL = no-observed-adverse-effect level.
[b]LEC = lowest effective concentration.

4.5.4. Potentiation and Other Interaction Studies

4.5.4.1. *Methyl Ethyl Ketone*

In a study of chemical interaction, Saida et al. (1976) exposed rats of unspecified sex (12/group) continuously, 24 hours/day, to 225 ppm (922 mg/m^3) 2-hexanone, 1125 ppm (3318 mg/m^3) MEK, or a combined exposure of 225 ppm (922 mg/m^3) 2-hexanone and 1125 ppm MEK for up to 66 days. No signs of neurotoxicity were observed in the MEK-exposed rats. Paralysis occurred earlier in the rats exposed to the mixture compared with rats exposed to 225 ppm 2-hexanone alone. In addition, an elevated severity of neuropathy, in the form of increased swollen axons, denuded fibers, and in-pouching of myelin sheaths, was observed histologically in the rats coexposed to MEK and 2-hexanone. Thus, MEK appeared to potentiate the toxicity of 2-hexanone. Yu et al. (2002) showed that the potentiating effect of MEK on n-hexane-induced neurotoxicity was due to an inhibitory effect of MEK on phase II biotransformation of 2,5-hexanedione (Yu et al., 2002). Since n-hexane is a precursor to 2-hexanone, and both compounds form the highly toxic 2,5-hexanedione, it is likely that the results of Yu et al. (2002) are applicable to coexposure studies with MEK and 2-hexanone.

As a test of in vivo enzyme induction, groups of five male Wistar rats were continuously exposed via inhalation to 225 ppm 2-hexanone, 750 ppm MEK, or the combination of 225 ppm 2-hexanone and 750 ppm MEK for 7 days (Couri et al., 1977). Subsequently, the animals were given sodium hexobarbital (100 mg/kg, i.p.), a substrate for phenobarbital-inducible CYP450 isoenzymes (Adedoyin et al., 1994; Knodell et al., 1988), and sleep time was measured. The average hexobarbital-induced sleep time of 2-hexanone-treated rats was comparable to that of controls (24.8 vs. 26.0 minutes); however, the sleep times in MEK and 2-hexanone/MEK-exposed rats were significantly ($p < 0.05$) less than in controls, 13.0 and 16.0 minutes, respectively. In a study by O'Donoghue and Krasavage (1979), sodium pentobarbital-induced sleep time was increased in 2-hexanone-treated cats.

4.5.4.2. *Chloroform*

Oral administration of 2-hexanone, followed by i.p. administration of chloroform to rats, resulted in a variety of hepatic and renal effects, including decreased hepatic glutathione levels, increased plasma levels of glutamic pyruvic transaminase and blood urea nitrogen, and degeneration and necrosis of hepatic and renal tissue (Hewitt et al., 1990, 1987; Brown and Hewitt, 1984; Branchflower and Pohl, 1981). Similarly, oral administration of both 2-hexanone and chloroform to rats resulted in altered permeability of the biliary tree (Hewitt et al., 1986). In these studies, some or no effect on the endpoints of interest was observed after administration of 2-hexanone or chloroform alone; administration of both substances resulted in statistically significant and dramatic changes in these effects. The authors speculated that 2-hexanone potentiated the hepatic toxicity of chloroform by decreasing glutathione levels and by increasing the metabolism of chloroform to the potent hepatotoxicant phosgene.

4.5.4.3. *O-Ethyl O-4-Nitrophenyl Phenylphosphonothioate*

2-Hexanone has been shown to potentiate the neurotoxic effects of EPN. In hens, dermal or inhalation exposure to 2-hexanone in combination with dermal application of the organophosphate pesticide EPN has resulted in earlier onset and far more severe clinical and histological manifestations of neurotoxic effects than with either chemical exposure alone (Abou-Donia et al., 1991, 1985a). The authors speculated that this potentiation effect may have been due to induction of hepatic microsomal CYP450 by EPN, leading to increased metabolism of 2-hexanone to its neurotoxic metabolite 2,5-hexanedione. An alternate explanation is that local trauma to the nervous tissue produced by 2-hexanone and EPN might increase vascular permeability and thus increase the entry of these compounds and their metabolites from circulation.

4.6. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS

4.6.1. Oral

There are no studies that have examined the possible association between oral exposure to 2-hexanone and noncancer health effects in humans. There are six subchronic or chronic studies in which 2-hexanone was administered orally to experimental animals. These include a 90-day gavage study in hens, 90-day and 40-week gavage studies in rats, 120-day and 13-month drinking water studies in rats, and a 24-week drinking water study in guinea pigs. These studies demonstrate that the nervous system is the target organ for 2-hexanone toxicity following oral exposure. For example, O'Donoghue et al. (1978), a 13-month drinking water study using COBS CD(SD)BR rats, described the characteristic neuropathologic evidence of giant axonal neuropathy in 80% of animals at the lowest dose tested (143 mg/kg-day).

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

In summary, the chronic and subchronic studies conducted with rats, hens, and guinea pigs provide ample evidence that the nervous system is the target of toxicity following oral exposure to 2-hexanone. A summary of the oral studies with 2-hexanone is provided in Table 4-15.

Table 4-15. A synopsis of oral toxicity studies with 2-hexanone

Species, strain	Group size (sex)	Dosage; duration; purity	Effects at LOAEL	NOAEL[a] (mg/kg-day)	LOAEL[a] (mg/kg-day)	Reference
Adult leghorn laying hens (*Gallus gallus domesticus*)	3/group (female)	100 mg/kg, gavage; 7 days/week for 90 days; technical grade containing 70% 2-hexanone and 30% MiBK	Mild ataxia at 12 ± 1 days with progression to severe ataxia by 50 ± 1 days	Not identified	100	Abou-Donia et al., 1982
Rat, COBS/ CD(SD)BR	6/group (male)	660 mg/kg, gavage; 5 days/week for 90 days; 2-hexanone containing 3.2% MiBK and 0.7% unknown contaminants	Clinical and histological findings of neuropathy at 55.8 ± 4.3 days	Not identified	660	Krasavage et al. (1980)
Rat, Wistar	5/group (female)	0, 0.65, or 1.3% (0, 480, or 1010 mg/kg-day) in drinking water; 120 days; purity not stated	Mild atrophy affecting skeletal muscles of the hind limbs in 2 of 5 animals examined	Not identified	480	Homan et al. (1977)
Guinea pig, English short hair	5/group (sex not stated)	0, 0.1, or 0.25% (0, 97, or 243 mg/kg-day) in drinking water; 24 weeks; purity not stated	Decreased pupillary response to light stimulus	Not identified	97	Abdel-Rahman et al. (1978)
Rat, Wistar	6/group (male)	400 mg/kg-day, gavage; 40 weeks; 2-hexanone 98% pure, contaminants not characterized	Hind-limb weakness from the 17th–28th week, with improvement thereafter	Not identified	400	Eben et al. (1979)
Rat, COBS/ CD(SD)BR	10/group (male)	0, 0.25, 0.5, or 1.0% (0, 143, 266, or 560 mg/kg-day) in drinking water; 13 months; 2-hexanone containing 3.2% MiBK and 0.7% unknown contaminants	Clinical neurological deficits	143	266	O'Donoghue et al. (1978)
			Neuropathologic evidence of myofibrillar atrophy of the calf muscle in 1/10 animals	143	266	
			Neuropathologic evidence of myofibrillar atrophy of the quadriceps muscle in 2/10 animals	143	266	
			Neuropathologic evidence of giant axonal neuropathy in 8/10 animals	Not identified	143	

[a]No-observed-adverse-effect levels (NOAELs) and lowest-observed-adverse-effect levels (LOAELs) determined by 2-hexanone assessment authors

4.6.2. Inhalation

Several studies have established associations between inhalation exposure to 2-hexanone and human health effects. Specifically, occupational studies and case reports suggest that inhalation exposure to 2-hexanone in humans may be associated with neurotoxicity. For example, a cross-sectional study of employees at a coated fabrics plant was conducted when it was noted that six workers from the print department had developed severe peripheral neuropathy soon after the plant began phasing in the use of 2-hexanone (Allen et al., 1974; Billmaier et al., 1974). Definite signs, symptoms and electrodiagnostic findings of peripheral neuropathy were confirmed in 68 out of 192 employees. The prevalence of peripheral neuropathy was clearly increased in jobs with evident exposure to 2-hexanone vapors and with time spent at work sites with 2-hexanone exposure.

Mallov (1976) reported one probable and two definite cases of 2-hexanone-induced peripheral neuropathy that were identified during an investigation of 26 painters. Similar to the studies reported above, (Allen et al., 1974; Billmaier et al., 1974), neuropathy was observed in the painters when the formulation of paint solvents was changed from MEK and methyl isoamyl ketone, both not neurotoxic, to 2-hexanone (Mallov, 1976). In another case of occupational exposure to 2-hexanone, symmetrical polyneuropathy was reported in a furniture finisher (Davenport et al., 1976). Six months prior to the onset of the worker's illness, 2-hexanone had been substituted for MiBK. A similar progressive distal extremity weakness developed in a coworker of the patient, which also improved following the coworker's removal from contact with lacquer products.

The toxicity of 2-hexanone via inhalation was studied extensively in experimental animals. As with oral exposures, the target organ for toxicity following inhalation exposure to 2-hexanone was the nervous system, and the most sensitive measures of intoxication were histopathological and clinical findings of peripheral neuropathy. Numerous studies are available, with duration varying from subchronic to chronic, in many different test species, including monkeys, rats, and cats. A summary of the available inhalation studies with 2-hexanone is provided in Table 4-16.

Table 4-16. Synopsis of animal inhalation toxicity studies with 2-hexanone

Species, strain	Number (sex)	Concentration; duration; purity	Effects at LOAEL	NOAEL[a] (mg/m^3)	LOAEL[a] (mg/m^3)	Reference
Developmental study						
Rat, pregnant F-344	25/group (female)	0, 1000, or 2000 ppm (0, 4100, or 8200 mg/m^3); day 0 of gestation through day 21, 6 h/day, 7 d/wk; purity not stated	Hyperactivity in behavioral testing	Not identified	4100	Peters et al. (1981)
Subchronic exposure studies						
Rat, strain not stated	9/group (sex not stated)	0 or 200 ppm (0 or 819 mg/m^3); 6 weeks, 8 h/d, 5 d/wk; purity not stated	Axonal hypertrophy, beading and degeneration of sciatic nerve	Not identified	819	Duckett et al. (1974)
Rat, Wistar	20/group (sex not stated)	40 ppm (164 mg/m^3); 22–88 days, 8h/d, 5d/wk; purity not stated	Peripheral neuropathy (demyelination of sciatic nerve) in 3/20	164	205	Duckett et al. (1979)
Rat, Sprague-Dawley	12/group (sex not stated)	0, 225, or 400 ppm (0, 922.5, or 1640 mg/m^3); 42–66 days, 24 h/d, 7 d/wk; purity not stated	Increased number of fibers with in-pouchings per mm^2 of nerve fascicle	Not identified	922.5	Saida et al. (1976)
Rat, COBS/ CD(SD) BR	5/group (male)	0 or 700 ppm (0 or 2870 mg/m^3); 81 days, 72 h/wk; 96.1% pure with 3.2% MiBK and 0.7% unidentified contaminants	Severe neuropathy consisting of difficulty extending hind limbs and a flat-footed gait with feet splayed in 5/5 at 71 ± 9 days	Not identified	2870	Katz et al. (1980)
Adult leghorn laying hens (*Gallus gallus domesticus*)	5/group	0, 10, 50, 100, 200, or 400 ppm (0, 41, 205, 410, 820, or 1640 mg/m^3); 90 days (continual exposure); technical grade containing 70% 2-hexanone and 30% MiBK	Mild ataxia (27 $\pm$ 2 days) progressing to severe ataxia/near paralysis (89 $\pm$ 1 days)	41	205	Abdo et al. (1982)
Rat, Sprague-Dawley	4/group (sex not stated)	0 or 400 ppm (0 or 1640 g/m^3) (adjusted); 12 weeks, 24 h/d, 7 d/wk; purity not stated	Dragging of hind limbs at 11–12 weeks	Not identified	1640	Mendell et al. (1974)
Domestic Chickens	5/group (not stated)	0 or 200 ppm (0 or 820 mg/m^3), adjusted to 100 ppm (410 mg/m^3)(time not stated); 12 weeks (24 h/d, 7 d/wk); purity not stated	Inability to stand on legs at 4–5 weeks	Not identified	820	Mendell et al. (1974)
Cat, domestic, strain not stated	4/group (sex not stated)	0 or 400 ppm (1640 mg/m^3) (adjusted); 12 weeks, 24 h/d, 7 d/wk; purity not stated	Dragging of hind limbs and forelimb weakness at 5–8 weeks	Not identified	1640	Mendell et al. (1974)

Table 4-16. Synopsis of animal inhalation toxicity studies with 2-hexanone

Species, strain	Number (sex)	Concentration; duration; purity	Effects at LOAEL	NOAEL[a] (mg/m^3)	LOAEL[a] (mg/m^3)	Reference
Rat, Wistar	20/group (sex not stated)	50 ppm (205 mg/m^3); 13 weeks, 8h/d, 5 d/wk; purity not stated	Peripheral neuropathy (demyelination of sciatic nerve) in 3/20	164	205	Duckett et al. (1979)
Chronic exposure studies						
Rat, strain not stated	6/group (sex not stated)	0 or 1300 ppm (5325 mg/m^3); 4 months, 6 h/d, 5d/w; purity not stated	Nerve fiber degeneration in the peripheral nerves, spinal cord, medulla, and cerebellum	Not identified	5325	Spencer et al. (1975)
Rat, Wistar	40/group (sex not stated)	50 ppm (205 mg/m^3); 6 months, 8h/d, 5 d/wk; purity not stated	Widespread demyelination of the sciatic nerve in 32/40	Not identified	205	Duckett et al. (1979)
Rat, Sprague-Dawley	6/group (male)	0 or 100 ppm (0 or 410 mg/m^3); 6 months, 22 h/d, 7 d/wk; 96.66% pure, impurities not characterized	Giant axonal swelling of peripheral nerves after 4 months	Not identified	410	Egan et al. (1980)
Rat, Sprague-Dawley	10/group (male)	0, 100, or 1000 ppm (0, 410, or 4100 mg/m^3); 10 months, 6 h/d, 5d/wk; commercial grade, impurities not stated	Decreased motor conduction velocity between treated and control animals beginning at 29 weeks	Not identified	410	Johnson et al. (1977)
Monkey, *Macaca fascicularis*	8/group (male)	0, 100, or 1000 ppm (0, 410, or 4100 mg/m^3); 10 months, 6 h/d, 5d/wk; commercial grade, impurities not stated	Decreased motor conduction velocity at 9 months (right sciatic-tibial nerve, right ulnar nerve)	Not identified	410	Johnson et al. (1977)
Rat, Sprague-Dawley	18/group (male)	0, 100, or 330 ppm (0, 410, or 1353 mg/m^3); 72 weeks, 6h/d, 5d/wk; purity not stated	Severe polyradiculoneuritis in the lumbar and sacral spinal nerves and roots and the sciatic and tibial nerves in one rat	410	1353	Krasavage and O'Donoghue (1977)
Cat, domestic shorthair	4/group (female)	0, 100, or 330 ppm (0, 410, or 1353 mg/m^3); 2 years (6h/d, 5d/wk); purity not stated	Giant axonal neuropathy of the spinal cord and peripheral nerve in 4/4	410	1353	O'Donoghue and Krasavage (1979)

[a]No-observed-adverse-effect levels (NOAELs) and lowest-observed-adverse-effect levels (LOAELs) determined by 2-hexanone assessment authors.

4.6.3. Mode-of-Action Information

Exposure to 2-hexanone in humans and experimental animals demonstrates that the nervous system is the target organ of toxicity, regardless of the route of exposure. The toxicity is attributed to the neurotoxic metabolite 2,5-hexanedione. A strong relationship has been noted between the concentration of 2,5-hexanedione in the urine and the onset of neuropathic symptoms (Eben et al., 1979). Similarly, 2,5-hexanedione has been described as eliciting severe neurotoxic symptoms following oral, dermal, or i.p. administration to hens and oral administration to rats (Abou-Donia et al., 1985a; Abdo et al., 1982; Krasavage et al., 1980).

Current research supports a mode of action for γ-diketones, such as the 2-hexanone metabolite 2,5-hexanedione, which involves the covalent cross-linking of neuronal macromolecules with proteins as the primary target. The result is axonal swelling, specifically of giant axons, that ultimately ends in retrograde degeneration of the axon. 2,5-Hexanedione is an electrophilic species that reacts with nucleophilic sites of proteins via a substitution or addition reaction, with the subsequent formation of a covalent bond (Lopachin and Decaprio, 2005). Although 2,5-hexanedione has been shown to react with sulfhydryl groups of enzymes (Section 4.5.1.2), the compound causes distal axonopathy by covalent reaction with nucleophilic lysine ε-amine groups to form 2,5-dimethylpyrrole adducts with neurofilaments and other proteins (LoPachin et al., 2005, 2004). Oxidation of the pyrrole moiety with molecular oxygen can generate a cation intermediate that can undergo further reactions with amino- or sulfhydryl groups. This results in the development of neurofilament aggregates in the distal, subterminal axon that, as they grow larger, form massive swellings, often just proximal to the nodes of Ranvier (Graham, 1999).

One of the major hypotheses related to the mechanism of neurotoxicity of 2,5-hexanedione is covalent binding with axonal components of nerve tissue. In vitro studies in which 2,5-hexanedione was incubated with proteins demonstrated that this compound binds to the lysine ε-amino group, resulting in the formation of the substituted pyrrole adduct ε-N-(2,5-dimethylpyrrole)norleucine (DeCaprio et al., 1982). Covalent binding of 2,5-hexanedione with axonal components leading to pyrrole formation and protein cross-linking was hypothesized as a possible initiation step leading to axonal degeneration and thus may account for the neurotoxic effects observed with exposure to γ-diketones in general (DeCaprio et al., 1988; DeCaprio et al., 1982). In vivo pyrrole formation was confirmed by the demonstration of ε-N-(2,5-dimethylpyrrole)norleucine in the hydrolyzed serum of a hen that had received 2,5-hexanedione at 200 mg/kg-day for two weeks (DeCaprio et al., 1982). The proposed mechanism for 2,5-hexanedione in the development of progressive sensorimotor distal axonopathy is presented in Figure 4-1.

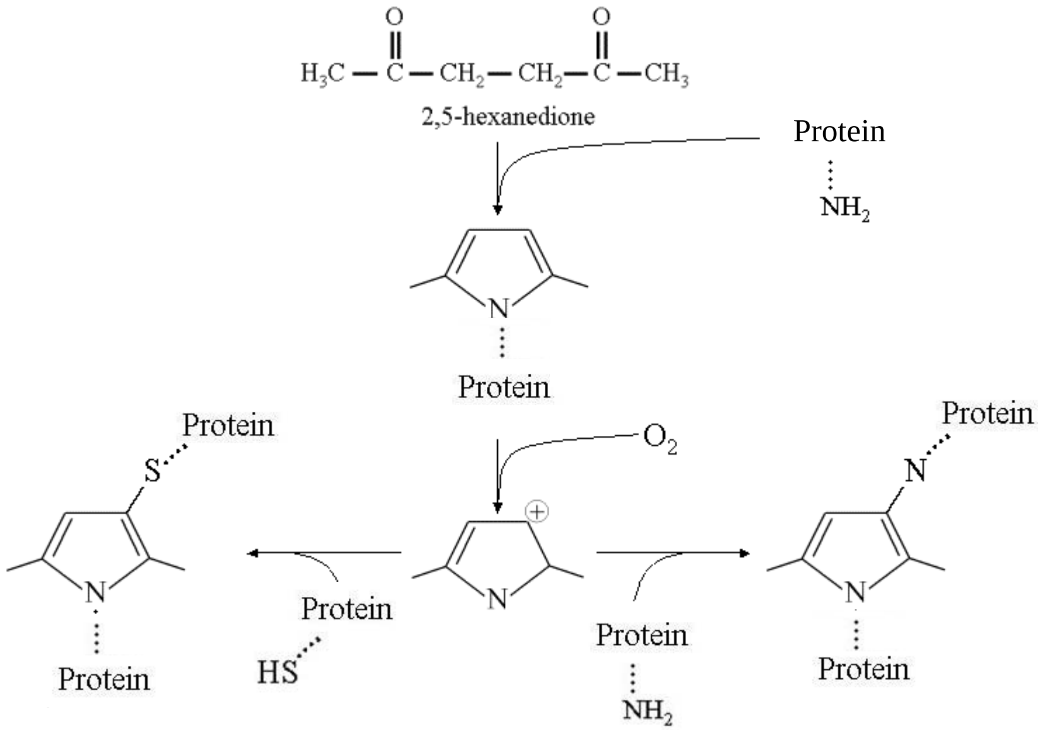

Figure 4-1. Proposed mechanism for 2,5-hexanedione-induced axonopathy.

Note: γ-Diketones, such as 2,5-hexanedione, react with amino groups in all tissues to form pyrroles. The pyrrole moiety can undergo further oxidation reactions with amino- or sulfhydryl groups. This results in the development of neurofilament aggregates (in the distal, subterminal axon), which, as they grow larger, form massive swellings of the axon.

Source: Adapted from DeCaprio et al. (1988, 1982).

4.7. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CHARACTERIZATION

4.7.1. Summary of Overall Weight of Evidence

Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), there is "inadequate information to assess the carcinogenic potential" of 2-hexanone. Specifically, there are no animal carcinogenicity studies available that examine exposure to 2-hexanone, and there are no studies available that assert a genotoxic potential of 2-hexanone. The available occupational studies do not present evidence for any carcinogenic action of 2-hexanone and are limited by frequent coexposure to other chemicals (e.g., MEK).

4.8. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

4.8.1. Possible Childhood Susceptibility

The susceptibility of the developing brain is based on the timing of neuronal development, the rapid growth that occurs in the third trimester and early infancy, and the lack of a protective barrier early in life (Costa et al., 2004). In the cerebellum, Purkinje cells develop early, weeks 5–7 in humans, whereas granule cells are generated much later, gestational weeks

24–40 in humans. The developing brain is distinguished by the absence of a blood-brain barrier. The development of this barrier is a gradual process, beginning in utero and complete at approximately 6 months of age. Because the blood-brain barrier limits the passage of substances from blood to brain, in its absence, toxic agents can freely enter the developing brain. Since Purkinje-cell degeneration has been observed with adult rats exposed to high levels of 2,5-hexanedione, infants may be at an increased risk for this type of damage at lower levels of exposures, due to the incomplete maturation of the blood-brain barrier (Hernandez-Viadel et al., 2002). However, this would depend on the capacity of infants and small children to bioactivate 2-hexanone to 2,5-hexanedione.

Metabolism of 2-hexanone 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 2-hexanone toxicity involves the metabolism to a more toxic metabolites, namely 2,5-hexanedione. Several enzymes, such as CYP2E1, CYP2B1/2, and CYP2C6/11, are inducible following administration of 2-hexanone in animal models (Imaoka and Funae 1991; Nakajima et al., 1991); however, the individual isoforms involved in its metabolism have not been fully elucidated. Toftgard et al. (1986) found that the formation of 2,5-hexanediol from 2-hexanol was catalyzed by a CYP isozyme different from CYP2B and present in liver but not in lung microsomes. The authors concluded that 2-hexanol must be transported to the liver before the neurotoxic metabolite 2,5-hexanedione can be formed (Toftgard et al., 1986). Because of this, changes in CYP protein levels and phase II enzymes during development may likely have an impact on susceptibility to 2-hexanone. As mentioned above, the possible susceptibility of 2-hexanone may be influenced by life stage, but there are few studies to confirm the impact and severity of such exposure. Thus, the evidence of possible childhood susceptibility is inconclusive.

4.8.2. Possible Gender Differences

Evaluations of human occupational exposures have not provided evidence that 2-hexanone acts in a gender-specific way. Most animal studies also have not brought forth strong evidence of a sex-specific action of 2-hexanone. However, it should be mentioned that in a few rat studies 2-hexanone appeared to affect the male reproductive system (Katz et al., 1980; Krasavage et al., 1980; O'Donoghue et al., 1978).

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

The RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. It can be derived from a no-observed-adverse-effect level (NOAEL), lowest-observed-adverse-effect level (LOAEL), or benchmark dose (BMD), with uncertainty factors generally applied to reflect limitations of the data used.

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

The 13-month drinking water study (10 animals/dose/sex) conducted by O'Donoghue et al. (1978) is the most suitable study for deriving a 2-hexanone RfD assessment Five other subchronic studies are available and are considered as supporting studies. Of these five studies, Krasavage et al. (1980) and Eben et al. (1979) both observed neurotoxicity after administration of single doses of 2-hexanone via gavage. These two studies were not considered as principal studies because only single relatively high doses were administered and gavage administration is less relevant to human exposure than administration in drinking water. Abdo et al. (1982) observed mild ataxia, which progressed to severe ataxia, in hens gavaged daily with 100 mg/kg 2-hexanone. This study was not chosen as the principal study because the hen's digestive system is anatomically distinct from humans and thus a poor model for assessing the effects of human oral exposure.[3] Finally, two subchronic drinking water studies that utilized multiple doses of 2-hexanone and identified neurotoxicological outcomes were considered. The first study, conducted by Homan et al. (1977), utilized doses that were higher than those used by O'Donoghue et al. (1978), and the purity of 2-hexanone was not stated. The second study, by Abdel-Rahman et al. (1978), utilized lower doses than the chronic study by O'Donoghue et al. (1978); however, the authors did not include complete data sets; that is, only data from the first 4 weeks of the study were presented. Further, the purity of the compound used was not stated.

O'Donoghue et al. (1978) conducted a 13-month study in male COBS/CD(SD) rats. The animals' drinking water contained 0, 0.25, 0.5, or 1.0% (0, 143, 266, or 560 mg/kg-day) 2-hexanone (96% pure, containing 3.2% MiBK and 0.7% unknown contaminants). In this study, 2-hexanone produced a dose-dependent reduction in body weight at all doses tested. The critical endpoints evaluated from this study were the incidences of myofibrillar atrophy of the quadriceps muscle and the calf muscle in male rats. These endpoints were chosen over the other neuropathologic endpoints in Table 5-1 because they occur due to axonal atrophy, an endpoint

[3] The lower portion of a hen's esophagus forms a pouch called the crop, which serves as a temporary storage site for food prior to passage to the stomach. The two-part structure of the hen's stomach, which consists of the proventriculus and the gizzard, further alters the absorption and distribution of chemicals.

identified as the best correlate of nerve dysfunction, regardless of route of exposure (Lehning et al., 2000). Though axonal swelling was observed with high incidence in the peripheral nerve and spinal cord at the lowest dose tested, axonal swelling poorly correlates with nerve dysfunction and can occur without progression to nerve dysfunction (LoPachin et al., 2004, 2003; Lehning et al., 2000, 1995). Because myofibrillar atrophy of the quadriceps and calf muscles displayed a clear dose-dependent response, these data were evaluated further by BMD modeling.

5.1.2. Method of Analysis: Benchmark Dose Modeling

The animal data evaluated for derivation of an RfD for 2-hexanone are displayed in Table 5-1. These data are from a chronic toxicity study in rats in which 10 animals per dose group were administered 2-hexanone in drinking water at four different concentrations (i.e., 0, 0.25, 0.5, and 1.0%) for 13 months (O'Donoghue et al., 1978). The critical endpoints evaluated from this study were the incidences of myofibrillar atrophy of the quadriceps muscle and the calf muscle in male rats, which displayed a clear dose-dependent response.

Table 5-1. Summary of neuropathologic findings in male rats administered 2-hexanone in drinking water for 13 months

Treatment (dose)	Incidence of axonal swelling				Incidence of myofibrillar atrophy	
	Brain	Spinal cord	Dorsal root ganglia	Peripheral nerve	Quadriceps muscle[a]	Calf muscle[a]
Control	0/10	0/5	0/5	0/10	**0/10**	**0/10**
0.25% 2-hexanone (143 mg/kg-day)	2/10	7/10	0/7	8/10	**1/10**	**2/10**
0.5% 2-hexanone (266 mg/kg-day)	4/10	5/5	0/5	10/10	**5/10**	**6/10**
1.0% 2-hexanone (560 mg/kg-day)	8/10	5/5	3/5	10/10	**10/10**	**10/10**

[a]The data in bold were further evaluated for RfD derivation.

Source: O'Donoghue et al. (1978).

The BMD software (BMDS, version 1.3.2) (U.S. EPA, 1999) was used to estimate a point of departure (POD) for deriving an RfD for 2-hexanone from data on myofibrillar atrophy of the quadriceps and calf muscles. This POD, called the BMDL, is defined as the 95 percent lower bound on the benchmark dose (BMD) associated with the benchmark response (BMR). For this study, a BMR of 10% extra risk (ER) was selected because it represents a response at the lower end of the observable range of the data, and provides a consistent basis of comparison across assessments. Table 5-2 presents the "best–fit" model results based on data on the incidence of myofibrillar atrophy of the quadriceps and calf muscles in rats exposed to 2-hexanone in drinking water. A more detailed presentation of the BMD modeling results is contained in Appendix B-1. In the absence of any compelling biological reason to choose one of these endpoints over the other for RfD derivation, myofibrillar atrophy of the quadriceps muscle was used because this endpoint yielded a slightly lower BMDL than myofibrillar atrophy of the calf muscle.

Table 5.2. Best fit BMD modeling results for data on myofibrillar atrophy of the quadriceps muscle and calf muscle

Endpoint (myofibrillar atrophy)	Model	AIC[a]	*p* Value	BMD (mg/kg-day)	BMDL (mg/kg-day)	BMD/BMDL
Quadriceps muscle	Multistage	22.3952	0.9995	141.4	49.9	2.8
Calf muscle	Quantal quadratic	25.8664	0.9701	88.7	69.2	1.3

[a]AIC = Akaike Information Criterion.

5.1.3. Derivation of Human Equivalent Doses

For 2-hexanone, no PBTK model is currently available. Therefore, the first step required for the final chronic RfD derivation is to determine whether intermittent doses were employed in the animal study and, if so, to adjust these doses to reflect continuous exposures, based on the assumption that the product of dose and time is constant (U.S. EPA, 2002). In the principal study (O'Donoghue et al., 1978), animals were administered 2-hexanone in drinking water 24 hours/day, 7 days/week for 13 months. Therefore, in this case, a duration adjustment is not required (i.e., the POD [adjusted BMDL or $BMDL_{ADJ}$] for 2-hexanone equals the study BMDL) as follows:

$$BMDL_{ADJ} = BMDL \times (\text{\# of hours per day exposed/24 hours}) \times (\text{\# of days per week exposed/7 days})$$

$$BMDL_{ADJ} = 50 \text{ mg/kg-day} \times (24 \text{ hours/24 hours}) \times (7 \text{ days/7 days}) = 50 \text{ mg/kg-day}$$

EPA currently does not provide a specific procedure for calculating a human equivalent dose for oral (or dermal) exposure scenarios that parallel calculation of the inhalation human equivalent concentration (HEC). Hence, the $BMDL_{ADJ}$ is used as the point of departure from which to apply uncertainty factors.

5.1.4. Calculation of the RfD—Application of Uncertainty Factors

The RfD for myofibrillar atrophy of the quadriceps muscle as the critical effect is calculated from the $BMDL_{ADJ}$ by application of uncertainty factors (UFs) as follows:

$$RfD = BMDL_{ADJ} \div UF$$

$$RfD = 50 \text{ mg/kg-day} \div 300 = 0.166 \text{ mg/kg-day} = 2 \times 10^{-1} \text{ mg/kg-day}$$

The composite UF of 300 was derived as follows:

- An intraspecies uncertainty factor (UF_H) of 10 was applied to adjust for potentially sensitive human subpopulations. A default value is warranted because insufficient information is currently available to assess human-to-human variability in 2-hexanone toxicokinetics or toxicodynamics.
- A default interspecies uncertainty factor (UF_A) of 10 was applied for extrapolation from animals to humans. No suitable data on the toxicity of 2-hexanone to humans exposed by the oral route only were identified. Insufficient information is currently available to assess rat-to-human differences in 2-hexanone toxicokinetics or toxicodynamics.
- A UF of 3 was applied to account for database deficiencies (UF_D). The database includes subchronic animal studies in rats and hens and a chronic study in rats but does not include multigenerational reproductive and developmental studies. Though no 2-hexanone-specific developmental studies are available, supporting developmental studies with n-hexane, a precursor of 2-hexanone, and 2,5-hexanedione, the ultimate toxic metabolite of n-hexane and 2-hexanone, have been primarily negative. Mouse reproductive/developmental and teratological studies with n-hexane have been negative with doses administered on GDs 6–15 by gavage of up to 2200 mg/kg-day or by daily injection up to 9900 mg/kg-day (Marks et al., 1980), and rat developmental neurotoxicity studies with 2,5-hexanedione have found minimal effects (e.g., aggregated and fused axons, identified with electron microscopy) from daily s.c. injections on GDs 12–20 with 340 mg/kg 2,5-hexanedione (Ogawa et al., 1993). It should be noted that the available studies with 2-hexanone following inhalation exposure suggest the possibility of immunotoxicity and reproductive toxicity as areas of potential concern with human exposure. For example, a reduction in total white blood cell count to ~60% of control values was reported in rats intermittently exposed to 700 ppm 2-hexanone via inhalation for 8 weeks (Katz et al., 1980). In addition, behavioral alterations observed in offspring of pregnant rats exposed to 1000 ppm 2-hexanone (Peters et al., 1981) and atrophy of testicular germinal epithelium observed in male rats exposed to 700 ppm 2-hexanone (Katz et al., 1980) suggest there may be cause for concern. However, there are no studies that evaluate immunotoxicity following oral exposure to 2-hexanone. Because of the absence of studies evaluating the possible immunotoxicity or reproductive toxicity of 2-hexanone following exposure via the oral route, a UF_D of 3 is warranted.
- An uncertainty factor for LOAEL-to-NOAEL extrapolation was not used because the current approach is to address this factor as one of the considerations in selecting a BMR for benchmark dose modeling. In this case, a BMR of a 10% extra risk of myofibrillar atrophy of the quadriceps muscle was selected under an assumption that it represents a minimal biologically significant change.

- A subchronic-to-chronic UF (UF_S) was not applied because the principal study involved a chronic exposure.

5.1.5. RfD Comparison Information

Figure 5-1 presents PODs, applied UFs, and derived RfD for the endpoints considered for 2-hexanone. As stated previously, of the available chronic and subchronic studies, the 13-month drinking water study conducted by O'Donoghue et al. (1978) was considered the most suitable study to derive an RfD. Within this study, two potential endpoints, myofibrillar atrophy of either the quadriceps muscle or the calf muscle were considered. The points of departure based on the best fit models from BMD models from Table 5.2 are presented in Figure 5-1. Axonal swelling in the brain, spinal cord and dorsal root ganglia were endpoints noted in O'Donoghue et al. (1978). These endpoints are also illustrated in Figure 5-1, but as previously mentioned, axonal swelling poorly correlates with nerve dysfunction and can occur without progression to nerve dysfunction and thus is deemed less relevant endpoints. The supporting studies outlined in Table 4-15 were deemed less relevant to human exposure as they either involved single relatively high doses via gavage in rodents (Krasavage et al., 1980; Eben et al., 1979), used a test species such as hens (which might be a poor model for assessing human exposure) (Abdo et al., 1982), were subchronic in design with higher doses administered than the chronic study by O'Donoghue et al. (1978) (Homan et al, 1977), or did not include complete data (Abdel-Rahman et al., 1978).

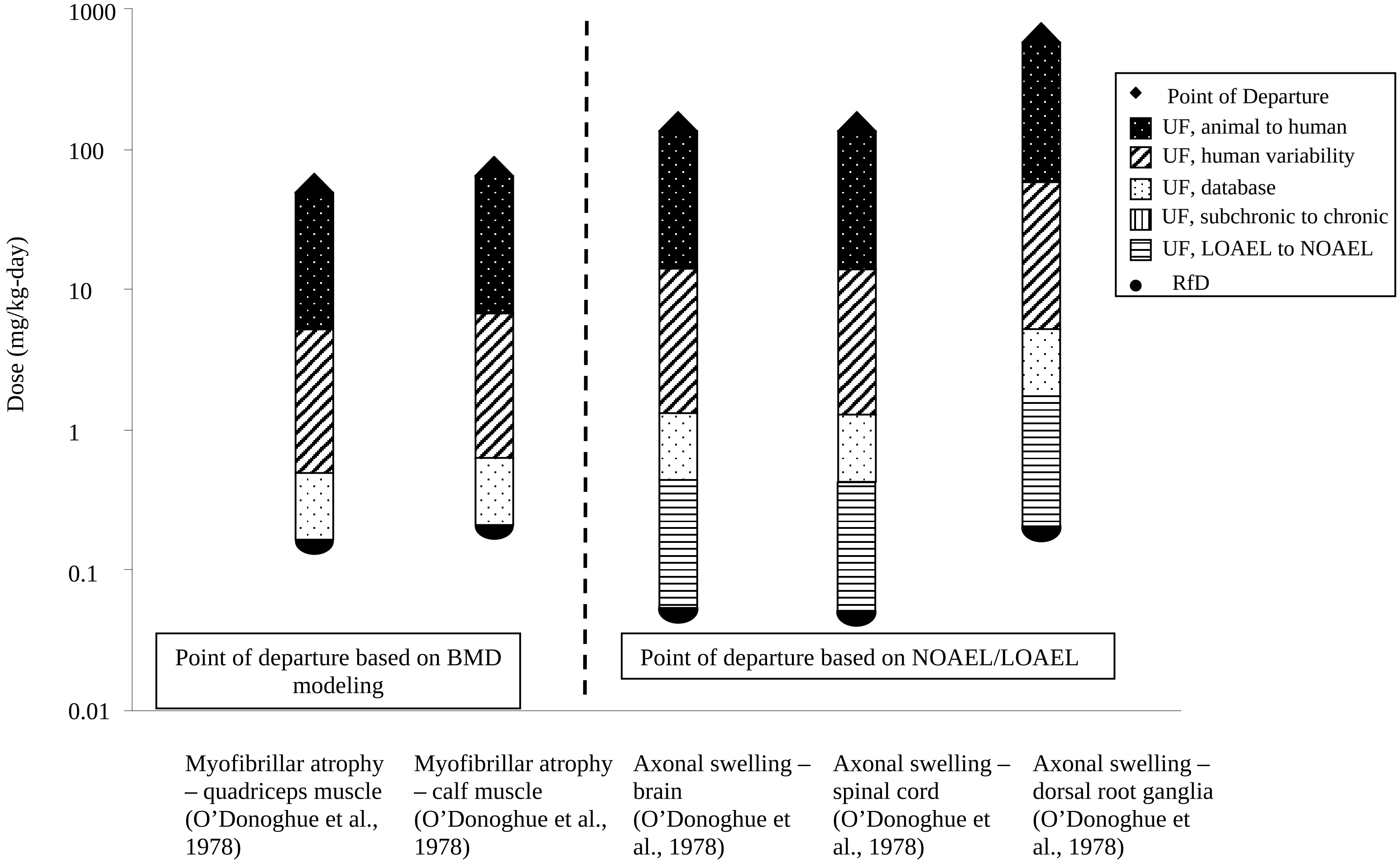

Figure 5-1. Points of departure for endpoints from O'Donoghue et al. (1978) with corresponding applied uncertainty factors and derived RfD.

5.1.6. Previous Oral Assessment

There was no previous RfD assessment for 2-hexanone with which to compare and contrast the RfD developed in this assessment.

5.2. INHALATION REFERENCE CONCENTRATION

The inhalation RfC is an estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human general population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects over a lifetime. It can be derived from a NOAEL, a LOAEL, or a benchmark concentration (BMC), with UFs generally applied to reflect uncertainties and/or limitations in the data used.

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

An inhalation study that exposed monkeys and rats to 0, 100, or 1000 ppm (0, 410, or 4100 mg/m^3) commercial grade 2-hexanone for 6 hours per day, 5 days per week for up to 10 months was used as the principal study in the derivation of the RfC (Johnson et al., 1977).

As discussed in Chapter 4, human and animal data indicate that neurological effects are a characteristic and sensitive endpoint of inhalation exposure to 2-hexanone. Neuropathy has been observed in humans following inadvertent occupational exposure (Allen et al., 1975; Billmaier et al, 1974; Gilchrist et al., 1974) and has been demonstrated repeatedly in laboratory animals (Katz et al., 1980; Egan et al., 1980; O'Donoghue and Krasavage, 1979; Johnson et al., 1979, 1977; Duckett et al., 1979, 1974; O'Donoghue et al., 1978; Krasavage and O'Donoghue, 1977; Spencer et al., 1975; Mendell et al., 1974).

Several studies of workers in a coated fabrics plant (Allen et al., 1975; Billmaier et al., 1974; Gilchrist et al., 1974) provide evidence in humans of a concentration-dependent neurotoxic response to 2-hexanone exposure. Although personal air samples were not collected in these studies, the available measures of exposure were sufficient to produce quantitative estimates of 2-hexanone inhalation exposure for two groups of workers (i.e., print operators and print helpers), both of whom exhibited peripheral neuropathy. In these workers, exposure to 2-hexanone also occurred via oral and dermal routes, as the study authors noted that individuals frequently ate at the work site and were accustomed to washing their hands with 2-hexanone. Because the magnitude of exposure to 2-hexanone from these two other exposure routes (i.e., oral and dermal), which could have been considerable, was not quantified by the study authors and the workers were coexposed with MEK, which can potentiate the toxicity of 2-hexanone, this study was deemed unsuitable for use in RfC derivation.

Of the available animal studies on 2-hexanone, the subchronic studies by Abdo et al. (1982), Duckett et al. (1979, 1974), Krasavage and O'Donoghue (1977), Saida et al. (1976), Mendell et al. (1974) and the chronic study by O'Donoghue and Krasavage (1979) were not selected for use in deriving the RfC. Duckett et al. (1979, 1974) did not report the sex of the animals or the purity of 2-hexanone used. Further, the authors used only one exposure concentration per series of experiments. Krasavage and O'Donoghue (1977) utilized two exposure concentrations (e.g., 100 and 330 ppm); however, the purity of 2-hexanone was not stated and limited data were provided. As mentioned previously, MiBK, a potential inducer of CYP450, is a common contaminant in the formulations of 2-hexanone. Without the more information on the purity of the 2-hexanone administered, it is difficult to ascertain if MiBK impacted the toxicity of 2-hexanone in Krasavage and O'Donoghue (1977). Saida et al. (1976) used two exposure concentrations (e.g., 225 ppm and 400 ppm) but did not state the sex of the animals or the purity of 2-hexanone used. Finally, Mendell et al. (1974) and Abdo et al. (1982) reported findings using hens. Although the exposure-concentration regimen used by Abdo et al. (1982) included five exposure concentrations (i.e., 10, 50, 100, 200, and 400 ppm), hens are not a suitable model for extrapolating experimental results to humans.[4] The remaining studies by Johnson et al. (1977), Katz et al. (1980), and Egan et al. (1980) were all considered further as possible principal studies.

The study by Johnson et al. (1977) was performed in two different animal species, monkeys and rats, with 8 and 10 animals per dose group, respectively. Two concentrations of commercial grade 2-hexanone were employed (100 and 1000 ppm in air) with exposures occurring 6 hours per day, 5 days per week for a duration of 10 months. Concurrent control groups were used in both species. As part of this study, Johnson et al. (1977) conducted four neurological tests in each species (usually once per month) to identify adverse effects in treated versus control animals. These four tests were MCV of the right sciatic-tibial nerve, MCV of the right ulnar nerve, absolute refractory period of these two nerves, and muscle action potentials in response to both sciatic and ulnar nerve stimulation.

The animal studies by Katz et al. (1980) and Egan et al. (1980) consisted of exposure to 2-hexanone (purity > 96%) at a single concentration for a period of 6 months or less, using only one strain and sex of rats. Both Katz et al. (1980) and Egan et al. (1980) utilized clinical chemistry and histopathologic changes to identify treatment-related effects of 2-hexanone. Despite the use of commercial grade 2-hexanone, the study by Johnson et al. (1977) was chosen as the most suitable study on which to base the RfC because Johnson et al. (1977) used two different animal species, including nonhuman primates, and two 2-hexanone exposure concentrations, while also employing larger treatment groups and longer exposure durations than either Katz et al. (1980) or Egan et al. (1980). Although duration of the study by Krasavage and O'Donoghue (1979) was longer than the study by Johnson et al. (1977), the latter utilized monkeys, a more biologically relevant species than rats, when assessing inhalation exposure. Also, Krasavage and O'Donoghue (1979) provide limited information to serve as the basis for a reference concentration.

[4] Birds have an intricate respiratory system that is exclusive to birds and includes a system of air sacs that surround the internal organs and provide reserve air space to increase lung capacity.

As previously discussed, the toxic effects seen in humans and experimental animals following exposure to 2-hexanone via inhalation provide evidence that the nervous system is the primary target of 2-hexanone toxicity. Data from Johnson et al. (1977) on both sciatic-tibial and ulnar nerve MCVs in 2-hexanone-exposed monkeys and rats were considered for use in deriving the RfC, but, ultimately, the monkey sciatic-tibial nerve MCV data were selected for the following reasons. Both monkeys and rats exhibited significant decrements in sciatic-tibial nerve MCVs at the lowest administered concentration of 2-hexanone, beginning at 9 and 7 months on study, respectively. A neuropathy similar to that observed for the sciatic-tibial nerves occurred in the ulnar nerves of both monkeys and rats. Although monkeys in the low exposure group exhibited statistically significant decreases in ulnar nerve MCVs relative to control values at 1 and 3 months, beginning at 6 months on study, this decline was not statistically significant. As monkeys have a similar respiratory tract and breathing patterns to humans, and the 2,5-hexanedione, the metabolite of 2-hexanone, typically affects long axons such as the sciatic-tibial nerve prior to other nerves, the sciatic-tibial nerve motor conduction velocity in monkeys is used to derive the RfC, though both sciatic-tibial MCV and ulnar MCV for both monkeys and rats were modeled.

5.2.2. Methods of Analysis: Benchmark Concentration Modeling

Table 5-3 displays monthly mean MCV values (in m/second) for both the sciatic-tibial and ulnar nerves of monkeys exposed to three different concentrations of 2-hexanone in air (i.e., 0, 100, or 1000 ppm) for durations ranging from 1 to 10 months. These data were extracted (via digitization [5]) from Figure 1 (for the sciatic-tibial nerve) and Figure 3 (for the ulnar nerve) of Johnson et al. (1977). Similarly, Table 5-4 displays monthly mean MCV values (in m/second) for both the sciatic-tibial and ulnar nerves of rats exposed to three different concentrations of 2-hexanone in air (i.e., 0, 100, or 1,000 ppm) for durations ranging from 2 to 29 weeks. These data were extracted (via digitization) from Figure 2 (for the sciatic-tibial nerve) and Figure 4 (for the ulnar nerve) of Johnson et al. (1977).

Because MCV values are continuous (as opposed to dichotomous), the data in Tables 5-3 and 5-4 were subjected to BMD modeling employing the available continuous models in EPA's

[5] Values from Johnson et al. (1977) were digitized using the line tool on Microsoft Office Word 2003, followed by measuring the values with the distance tool function on Adobe® Acrobat® 6.0 Professional (version 6.0.0, 5/19/2003). To accomplish this task, the figures from Johnson et al. (1977) were inserted into a Word document using the snapshot tool from Adobe® Acrobat® 6.0 Professional. Then, horizontal lines were applied over the data points, the measurement markers on the y-axis, and extended through the y-axis. Lines from the data points to the x-coordinates were not traced over, since Johnson et al. (1977) provided the absolute values in the text. Once all of the lines were traced from the data points through the y-coordinates, a vertical line was traced over the y-axis. Then the Word document was saved in portable document format (pdf) and opened using Adobe® Acrobat® 6.0 Professional. The y-axis was viewed at 300% magnification, and the distance tool was used to measure from the origin to each y-coordinate for each horizontal line, including data points and measurement markers. The distance tool allows measurements to be made down to one hundredth of a millimeter, and repeated measures placed the reproducibility of this technique at greater than 99%.

BMDS, version 1.3.2 (i.e., linear, polynomial, power, and Hill models). The BMR was defined as a 10% relative change in nerve conduction velocity from the control mean. Changes in nerve conduction velocity are thought to represent a clinically significant effect.

A difficulty encountered in conducting a BMD analysis on these data was that no information was provided regarding the standard errors or confidence limits for the mean nerve conduction velocities shown in Figures 1 through 4 in Johnson et al. (1977) nor was any of the raw data on which these means were based presented in the paper. Attempts to obtain the raw data from the investigators were unsuccessful. In BMDS, estimates of the standard deviation of the response in each dose group are needed to calculate BMDs and their corresponding BMDLs. Therefore, an indirect method for estimating this missing information on response variability was devised.

Information regarding the variability in MCV measurements in Johnson et al. (1977) can be derived from the results of statistical tests that are reported in the paper. In this study, two different statistical procedures were employed. An ANOVA was used to test for statistically significant differences in mean MCVs at specific test periods (usually monthly) whenever data across the three exposure groups (i.e., 0, 100, or 1000 ppm) were compared. After approximately 6 months on study, however, animals (both monkeys and rats) in the highest exposure group (1000 ppm) were removed from further 2-hexanone exposure. Consequently, with termination of this 1000 ppm exposure group, only two dose groups remained for each species. Thus, subsequently, the Student's *t*-test was used to test for statistically significant changes in mean MCVs across these two groups (i.e., 0 and 100 ppm) for the remaining test periods.

In an ANOVA, an F statistic is used to test for a significant difference among the means of g groups. An F statistic is defined as, $F(g\text{-}1, N\text{-}g)$ = between-group variance/within-group variance, where g-1 represents the numerator degrees of freedom and N-g represents the denominator degrees of freedom (g is the number of groups and N is the sample size within each group). In the specific case where only two group means are being compared, the F statistic reduces to a t statistic (i.e., $F(1, N\text{-}g) = t(N\text{-}g)^2$), where t has a Student's t-distribution. In order to fit a continuous dose-response model in BMDS, an estimate of the within-group variance or s^2 is needed from which the estimated standard deviation can be obtained simply by taking the square root of this variance estimate.

The estimated within-group variance can be derived using the following procedure. If the within-group means and the numbers of observations on which each of these means are based are known, the between-group variance can be calculated. Once the between-group variance has been determined and the corresponding value of the F or t statistic is known, an estimate of the within-group variance or s^2 can be derived from the following equation: s^2 = (between-group variance)/F(g − 1, N – g) or s^2 = (between-group variance)/$t(N\text{-}g)^2$. In Johnson et al. (1977), for monkeys, F statistics were reported for mean MCVs at both 4 and 6 months, while t statistics were reported for mean MCVs at both 9 and 10 months. These data yielded four estimates of the within-group variance or standard deviation. The arithmetic average of these four estimates was then used in BMD modeling as the estimated standard deviation for MCVs in each dose group, assuming a constant variance across dose groups. For rats, *F* statistics were reported in Johnson et al. (1977) for mean MCVs at both 13 and 17 weeks, while a *t* statistic was reported for mean MCVs at 29 weeks. These data yielded three estimates of the within-group variance or standard deviation. The arithmetic average of these three estimates was then used in BMD modeling as the estimated standard deviation for MCVs in each dose group, assuming a constant variance across dose groups.

Table 5-3. Effect of 2-hexanone inhalation exposure on the MCV of the sciatic-tibial and ulnar nerves in monkeys

Exposure duration (months)	2-Hexanone concentration (ppm in air)	Mean MCV: sciatic-tibial nerve (m/s)[a]	Mean MCV: ulnar nerve (m/s)[b]
1	0	42	54
	100	42	**46**[c]
	1000	40	**47**[c]
2	0	51	61
	100	46	63
	1000	44	**49**[c]
3	0	54	53
	100	48	**47**[c]
	1000	46	**45**[c]
4	0	56	63
	100	50	58
	1000	**41**[c]	**49**[c]
5	0	53	61
	100	48	63
	1000	**36**[c]	**43**[c]
6	0	50	58
	100	47	56
	1000	**33**[c]	**41**[c]
7	0	51	65
	100	48	62
8	0	50	58
	100	46	58
9	0	53	63
	100	**49**[c]	60
10	0	53	58
	100	**48**[c]	57

[a]Values extracted from Figure 1 in Johnson et al. (1977).
[b]Values extracted from Figure 3 in Johnson et al. (1977).
[c]Statistically significantly different compared with corresponding controls ($p < 0.05$), as determined by the study authors.

Table 5-4. Effect of 2-hexanone inhalation exposure on the MCV of the sciatic-tibial and ulnar nerves in rats

Exposure duration (weeks)	2-Hexanone concentration (ppm in air)	Mean MCV: sciatic-tibial nerve (m/s)[a]	Mean MCV: ulnar nerve (m/s)[b]
13	0	34	
	100	37	
	1000	**40**[c]	
17	0		42
	100		**36**[c]
	1000		**38**[c]
25	0	42	40
	100	41	37
	1000	**27**[c]	**31**[c]
29	0	39	45
	100	**25**[c]	**30**[c]

[a]Values extracted from Figure 2 in Johnson et al. (1977).
[b]Values extracted from Figure 4 in Johnson et al. (1977).
[c]Statistically significantly different compared with corresponding controls ($p < 0.05$), as determined by the study authors.

The "best-fit" model from BMDS was selected by examining the results of the chi-squared goodness-of-fit test and comparing the magnitudes of the Akaike's Information Criterion (AIC). All models with chi-squared p values ≥0.1 were considered to exhibit an adequate fit to the data. Of the models exhibiting adequate fit, the model with the lowest AIC was selected as the best-fit model. These criteria for model selection are consistent with those described in the *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000c). For the MCV data in both monkeys and rats, the 1st-degree polynomial model provided the best fit for both sciatic-tibial and ulnar nerve MCVs.

The 95% lower confidence limits on the benchmark concentration estimates (BMCLs) derived from the best-fit models for sciatic-tibial and ulnar nerve MCV values in monkeys and rats are presented in Table 5-4. Detailed BMDS outputs from the BMD of the monkey and rat MCV data are contained in Appendix B-1.

5.2.3. Exposure Duration Adjustments and Conversion to Human Equivalent Concentrations

Because the RfC is a metric that assumes continuous human exposure for a lifetime, adjustments need to be made to animal (or human) data obtained from intermittent and/or less-than-lifetime exposures, as outlined in the *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994). The first step in this process is adjusting intermittent inhalation exposures to continuous inhalation exposures, based on the assumption that the product of exposure concentration and time is constant (U.S. EPA, 2002). In Johnson et al (1977), animals were exposed to 2-hexanone for 6 hours/day, 5 days/week. Therefore, the $BMCL_{ADJ}$, reflecting continuous inhalation exposure to 2-hexanone, is derived as follows:

$BMCL_{ADJ}$ = BMCL × hours exposed per day/24 hours × Days exposed per week/7 days.
$BMCL_{ADJ}$ = 243 × 6/24 × 5/7 = 43 ppm, based on monkey sciatic-tibial nerve MCV
= 278 × 6/24 × 5/7 = 50 ppm, based on monkey ulnar nerve MCV
= 232 × 6/24 × 5/7 = 41 ppm, based on rat sciatic-tibial nerve MCV
= 352 × 6/24 × 5/7 = 63 ppm, based on rat ulnar MCV

Furthermore, because RfCs are typically expressed in units of mg/m^3, the above ppm values need to be converted to mg/m^3 using the conversion factor specific to 2-hexanone of 1 ppm = 4.1 mg/m^3. Thus, the final $BMCL_{ADJ}$ values are as follows:

$BMCL_{ADJ}$ = 43 × 4.1 = 176.3 mg/m^3, monkey sciatic-tibial nerve MCV
= 50 × 4.1 = 205 mg/m^3, based on monkey ulnar nerve MCV
= 41 × 4.1 = 168.1 mg/m^3, based on rat sciatic-tibial nerve MCV
= 63 × 4.1 = 258.3 mg/m^3, based on rat ulnar nerve MCV

Finally, this $BMCL_{ADJ}$ value must be converted to an HEC. The HEC that elicits decreased MCV, which is not a respiratory (or portal-of-entry) effect, but a systemic effect, is derived based on the following. For systemic effects, 2-hexanone is classified as a category 3 gas under EPA guidelines (U.S. EPA, 1994). According to this guidance, in order to convert the concentration effective in animals to human equivalents, a multiplicative factor based on the ratio of blood:gas partition coefficients is employed as follows:

$$HEC = BMCL_{ADJ} \times [(H_{b/g})_A/(H_{b/g})_H]$$

Where,

$(H_{b/g})_A$ = blood:gas partition coefficient for 2-hexanone in animals
$(H_{b/g})_H$ = blood:gas partition coefficient for 2-hexanone in humans.

The blood:gas partition coefficient $(H_{b/g})_H$ for 2-hexanone in humans is 127 (Sato and Nakajima, 1979); however, no value has been reported for monkeys or rats. In the absence of a measured blood:gas partition coefficient in the test species, the ratio $[(H_{b/g})_A/(H_{b/g})_H]$ defaults to unity, and the conversion to a HEC becomes the following:

$BMCL_{HEC}$ = $BMCL_{ADJ} \times [(H_{b/g})_A/(H_{b/g})_H]$ = 176.3 × 1 = 176.3 mg/m^3 based on monkey sciatic-tibial nerve MCV

$= 205 \times 1 = 205$ mg/m^3 based on monkey ulnar nerve MCV

$= 168.1 \times 1 = 168.1$ mg/m^3 based on rat sciatic-tibial nerve MCV

$= 258.3 \times 1 = 258.3$ mg/m^3 based on rat ulnar nerve MCV

These HEC values are presented in the last column of Table 5-5.

Table 5-5. Summary of BMCLs and HECs for 2-hexanone

Study reference	Study duration and type	2-Hexanone exposure (ppm)	Species/sex	Toxicological endpoint	BMDS "best fit" continuous model	BMC (ppm)	BMCL or POD[a] (ppm)	Adjusted BMCL ($BMCL_{ADJ}$)[b]	HEC[c]
Johnson et al. (1977)	10-month inhalation (subchronic)	0, 100, 1000	Male monkeys (n = 8 per dose group)	Sciatic-tibial nerve motor conduction velocity (at 6 months)	1st degree polynomial	293	243	176	176
				Ulnar nerve motor conduction velocity (at 6 months)	1st degree polynomial	335	278	205	205
Johnson et al. (1977)	29-week inhalation (subchronic)	0, 100, 1000	Male rats (n = 10 per dose group)	Sciatic-tibial nerve motor conduction velocity (at 25 weeks)	1st degree polynomial	271	232	168	168
				Ulnar motor nerve conduction velocity (at 25 weeks)	1st degree polynomial	471	352	258	258

[a]BMCLs or PODs were estimated at a BMR of 0.1 or 10% relative change from controls.

[b]Conversion factors and assumptions: molecular weight (2-hexanone) = 100.16 and 1 ppm = 100.16/24.45 = 4.1 mg/m^3 (at 25°C and 760 mm Hg). Duration adjustment of exposure concentrations and conversion to mg/m^3 was accomplished as follows: $BMCL_{ADJ}$ = 243 ppm × 6h/24h × 5 d/7d = 43 ppm × 4.1 = 176 mg/m^3.

[c]The $BMCL_{HEC}$ was calculated for an extrarespiratory effect of a category 3 gas. The blood:gas partition coefficient (Hb/g) value for 2-hexanone in humans is 127 (Sato and Nakajima, 1979); however, no value has been reported for monkeys or rats. According to EPA's RfC methodology (U.S. EPA, 1994), when the ratio of animal to human blood:gas partition coefficients [(Hb/g)A/(Hb/g)H] is greater than one or the values are unknown, a value of one is used for the ratio by default. Thus, $BMCL_{HEC}$ = 176 × [(Hb/g)A/(Hb/g)H] = 176 mg/m^3.

5.2.4. Calculation of the RfC: Application of Uncertainty Factors

As monkeys have a similar respiratory tract and breathing patterns to humans, and the 2,5-hexanedione, the metabolite of 2-hexanone, typically affects long axons such as the sciatic-tibial nerve prior to other nerves, the $BMCL_{HEC}$ based on sciatic-tibial nerve motor conduction velocity in monkeys (Table 5-4) is used to derive the RfC. It should be noted that ulnar nerve motor conduction velocity in monkeys and sciatic-tibial nerve motor conduction velocity in rats were found to have similar $BMCL_{HEC}$ as the endpoint selected above and would not result in significantly different RfCs if those alternatives were utilized.

The RfC for 2-hexanone based on peripheral neuropathy as the critical effect is derived from the $BMCL_{HEC}$ by application of UFs as follows:

$$\text{RfC} = BMCL_{HEC} \div UF$$

$$\text{RfC} = 176 \div 1000 = 0.168 \text{ mg/m}^3 \approx 2 \times 10^{-1} \text{ mg/m}^3$$

This composite UF of 1000 is composed of the following:

- A default intraspecies uncertainty factor (UF_H) of 10 was applied to adjust for potentially sensitive human subpopulations (intraspecies variability).
- A default subchronic-to-chronic uncertainty factor (UF_S) of 10 was applied to account for the less-than-lifetime exposure (10 months) in the principal study.
- A factor of 3 was selected to account for uncertainties in extrapolating from rats to humans. This value is adopted by convention where an adjustment from an animal-specific $NOAEL_{ADJ}$ to a $NOAEL_{HEC}$ has been incorporated. Application of a full uncertainty factor of 10 would depend on two areas of uncertainty (i.e., toxicokinetic and toxicodynamic uncertainties). In this assessment, the toxicokinetic component is mostly addressed by the determination of a human equivalent concentration as described in the RfC methodology (U.S. EPA, 1994b). The toxicodynamic uncertainty is also accounted for to a certain degree by the use of the applied dosimetry method and an UF of 3 is retained to fully address this component. An uncertainty factor for LOAEL-to-NOAEL extrapolation was not used because the current approach is to address this factor as one of the considerations in selecting a BMR for benchmark dose modeling. In this case, a BMR of a 10% change in nerve conduction velocity from the control mean was selected under an assumption that it represents a minimal biologically significant change. An uncertainty factor of 3 was applied to account for database deficiencies (UF_D). The database includes a human occupational exposure study (with coexposure to MEK), subchronic animal studies in rats and hens, and a chronic study in cats. One postnatal development and behavior study on 2-hexanone (Peters et al., 1981) exists, identifying a LOAEL of 1000 ppm (no NOAEL

reported), but no other developmental or teratology studies on 2-hexanone exist. However, support for applying a UF_D of 3 in the absence of other 2-hexanone-specific studies is based on the availability of developmental and teratology studies with n-hexane, a precursor of 2-hexanone, and 2,5-hexanedione, a metabolite of n-hexane and 2-hexanone. The rationale for the UF_D of 3 is based on the following: (1) developmental studies with n-hexane concentrations of 100 (GDs 6–15), 400 (GDs 6–15), or 1000 ppm (GDs 8–16) have been negative (Bus et al., 1979; Litton Bionetics, 1979); (2) a teratology study conducted on behalf of the National Toxicology Program, in which dams were exposed on GDs 6–19 to 200, 1000, or 5000 ppm n-hexane, identified a NOAEL of 200 ppm (Mast, 1987), a concentration nearly double the highest NOAEL identified from inhalation studies with 2-hexanone (See Table 4-16); and (3) rat developmental neurotoxicity studies with 2,5-hexanedione, the ultimate toxic metabolite of n-hexane and 2-hexanone, have found minimal effects (e.g., aggregated and fused axons, identified with electron microscopy) from daily s.c. injections on GDs 12–20 with 340 mg/kg 2,5-hexanedione (Ogawa et al., 1993).

5.2.5. RfC Comparison Information

Figure 5-2 presents PODs, applied UFs, and derived RfCs for several studies and endpoints considered for 2-hexanone. Of the chronic and subchronic studies available on inhalation exposure to 2-hexanone, Johnson et al. (1977) was deemed the most suitable to derive an RfC. The endpoints considered from Johnson et al. (1977) include MCV for both sciatic-tibial and ulnar nerves of both rats and monkeys. The PODs based on the best fit models from BMD models from Table 5-4 are presented in Figure 5-2. Subchronic rodent studies by Katz et al. (1980) and Egan et al. (1980) were also considered; however, both studies evaluated exposure to a single concentration of 2-hexanone for a period of less than 6 months, using clinical chemistry or histopathologic changes to identify treatment-related effects. The unpublished study by Krasavage and O'Donoghue (1977) was longer in exposure duration than the study by Johnson et al. (1977) and utilized two exposure concentrations, though purity of 2-hexanone was not specified. The Johnson et al. (1977) study is preferred because the study involved nonhuman primates that are more relevant to assessing human exposure than obligatory nose-breathing species such as rats. Figure 5-2 provides LOAEL and NOAEL PODs from Katz et al. (1980), Egan et al. (1980), and Krasavage and O'Donoghue (1977) as a comparison to the four BMCL endpoints from the Johnson et al. (1977) study.

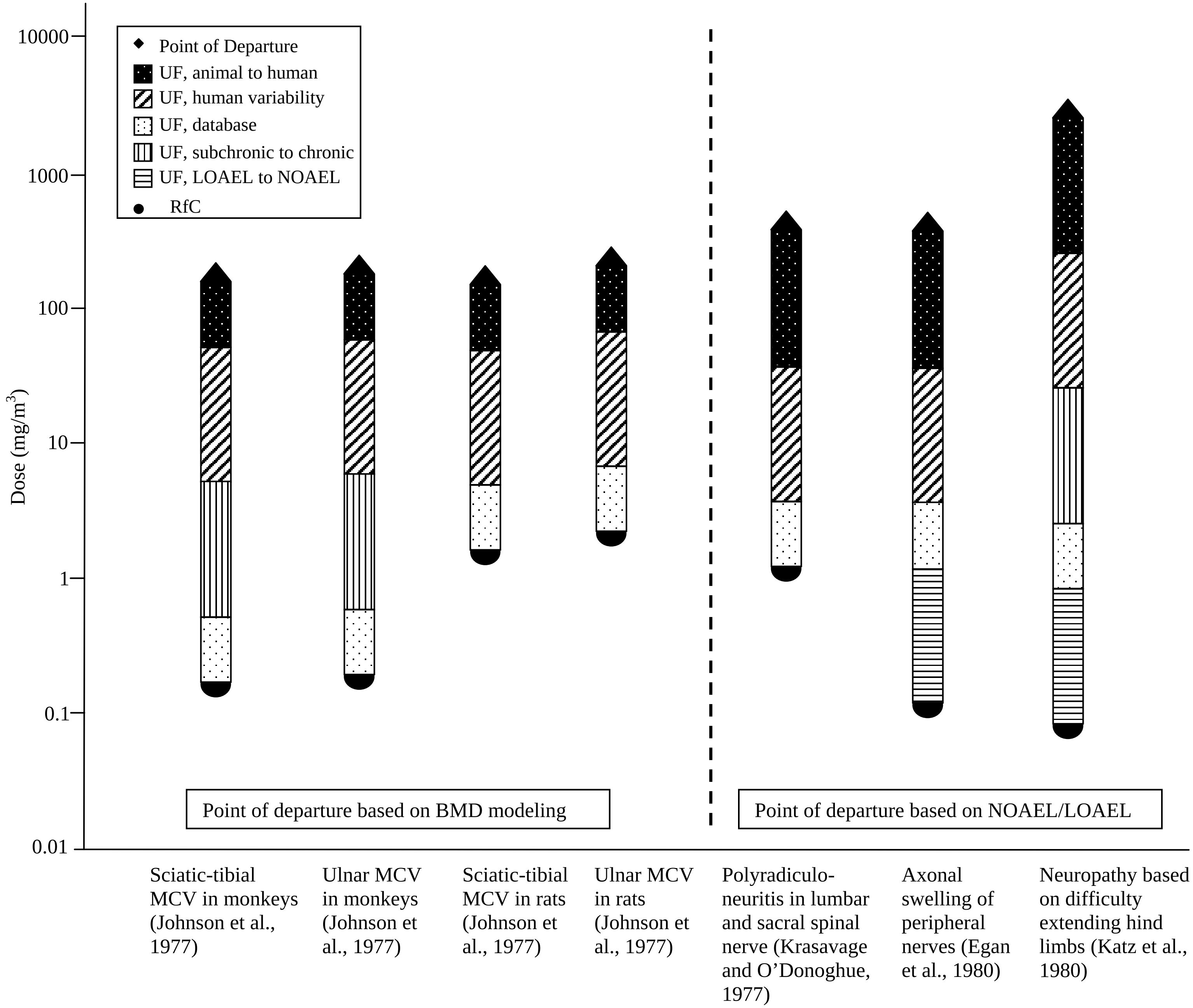

Figure 5-2. PODs for endpoints from select studies from Table 4-16, with corresponding applied UFs and derived RfCs.

5.2.6. Previous Inhalation Assessment

No previous RfC assessment for 2-hexanone exists on IRIS.

5.3. CANCER ASSESSMENT

As discussed in Section 4.6.1, the available database for 2-hexanone contains inadequate information to assess the carcinogenic potential according to *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a).

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

6.1. HUMAN HAZARD POTENTIAL

2-Hexanone (methyl butyl ketone, CASRN 591-78-6) has the chemical formula $C_6H_{12}O$ and a molecular weight of 100.16. It is a clear, volatile, flammable fluid with a pungent, acetone-like odor. 2-Hexanone is most commonly used as a paint or printing ink thinner, as a solvent for oils, waxes, and resins, or as a cleaning agent. It is currently not produced commercially in the U.S., and no information on importation is available (ATSDR, 1992).

2-Hexanone is well absorbed by the inhalation route and in the gastrointestinal tract. Animal studies suggest that 2-hexanone does not penetrate skin very efficiently, but there is evidence that it penetrates human skin easily (Bos et al., 1991). The distribution of 2-hexanone has not been studied thoroughly. In a rat study, it appeared in the plasma and the lung at higher concentrations than in the liver after both oral and inhalation administration (Duguay and Plaa, 1995) but did not show an affinity for a lipid rich tissue such as the brain (Granvil et al., 1994). In guinea pigs, 2-hexanone was eliminated quite rapidly, with a half-life of a little more than 1 hour for the parent compound and values not exceeding 2½ hours for the major metabolites (DiVincenzo et al., 1976). In rats, on the other hand, 2-hexanone was eliminated more slowly (Bus et al., 1981). The biological half-life of 2-hexanone in humans is not known. A PBTK model has not been proposed.

Metabolites of 2-hexanone include 2-hexanol, 2,5-hexanediol, 5-hydroxy-2-hexanone, 2,5-hexanedione, and some cyclic furan derivatives. The enzymes that metabolize 2-hexanone have not been characterized well. Among the metabolites of 2-hexanone, 2,5-hexanedione is the most important because it is a well-known neurotoxicant. It causes neuropathy specifically of the peripheral giant axons that involves neurofilament cross-linking and axonal swelling and proceeds to retrograde axonal degeneration. 2-Hexanone-induced neuropathy has been observed clinically in occupationally exposed humans (Davenport et al., 1976; Mallov, 1976; Allen et al., 1974; Billmaier et al., 1974), but the findings are frequently obscured by coexposure to other solvents, most frequently MEK, which is known to potentiate the toxicity of 2-hexanone.

A significant number of studies have been conducted in which laboratory animals were exposed orally or via inhalation for up to 2 years. Oral exposure studies used rats (Krasavage et al., 1980) and guinea pigs (Abdel-Rahman et al., 1978), with doses ranging up to 600 mg/kg-day by gavage or up to 1.3% in drinking water (amounting to 1010 mg/kg-day). The 13-month study in rats by O'Donoghue et al. (1978) that gave a detailed report of neuropathy incidences was used in the RfD assessment for 2-hexanone. Inhalation studies employed rats (Egan et al., 1980; Katz et al., 1980; Duckett et al., 1979, 1974; Johnson et al., 1977; Krasavage and O'Donoghue, 1977; Saida et al., 1976; Spencer et al., 1975), cats (O'Donoghue and Krasavage, 1979), and monkeys (Johnson et al., 1977), with exposures ranging from 10–1300 ppm (41–5325 mg/m^3). The hallmark symptom observed in any of these studies was neuropathy. The 2-hexanone-induced neuropathy also has been characterized mechanistically in animal studies (DeCaprio et al., 1988, 1982). Also a study in beagles that received 2-hexanone via the subcutaneous route reported neuropathy (O'Donoghue and Krasavage, 1981).

It is not clear whether 2-hexanone causes any other significant illness in humans. The available animal studies do not provide sufficient information to assess carcinogenicity of 2-hexanone. Currently, there is no evidence that this chemical causes cancer in humans or animals. According to *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), there is "inadequate information to assess the carcinogenic potential" of 2-hexanone.

Estimates of risks from other organizations

Estimates of risk for 2-hexanone derived by other organizations are compiled by the National Library of Medicine and can be found on the TOXNET Web page at http://toxnet.nlm.nih.gov/.

6.2. DOSE RESPONSE

6.2.1. Noncancer/Oral

There are no chronic or subchronic data for oral exposure of humans to 2-hexanone. There is no standard 2-year bioassay for 2-hexanone in any animal species; the only study with a chronic exposure time, the 13-month study in rats by O'Donoghue et al. (1978), was adequately conducted and reported critical, chemical-related effects with sufficient detail to be eligible as the principal study. Myofibrillar atrophy of the quadriceps muscle and the calf muscle in male rats is the critical endpoint evaluated. This endpoint was chosen over other neuropathic endpoints because it occurs due to axonal atrophy, an endpoint that correlates best to nerve dysfunction regardless of route of exposure. Another endpoint described in O'Donoghue et al. (1978) was peripheral nerve axonopathy. This endpoint was not well characterized, with incidences jumping from 0% (0/10 animals) in controls to 80% (8/10 animals) at the lowest dose (143 mg/kg-day) and 100% (10/10 animals) at both higher doses (266 and 560 mg/kg-day). Though peripheral nerve axonopathy may have exhibited the most sensitive response to 2-hexanone exposure, axonal swelling may not be an appropriate proximal marker of neuropathy because this endpoint poorly correlates with nerve dysfunction.

The RfD of 2×10^{-1} mg/kg-day was derived from myofibrillar atrophy of the quadriceps muscle in male COBS/CD(SD)BR rats following 13 months of oral exposure to 2-hexanone (O'Donoghue et al., 1978). 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 2-hexanone (Abdo et al., 1982; Krasavage et al., 1980; Eben et al., 1979; Abdel-Rahman et al., 1978; Homan et al., 1977). Because there are no compelling biological reasons to choose myofibrillar atrophy of the quadriceps muscle over myofibrillar atrophy of the calf muscle, the slightly lower BMDL was chosen. A graphical comparison of the RfDs from these two endpoints is illustrated in Figure 5-1.

A composite UF of 300 was applied; 10 for intraspecies (interindividual) variability, 10 for interspecies variability, and 3 for database uncertainty. Information was unavailable to quantitatively assess toxicokinetic or toxicodynamic differences between animals and humans and the potential variability in human susceptibility; thus, the interspecies and intraspecies UFs of 10 were applied. A threefold database deficiency UF was applied to reflect that, though chronic and subchronic information on 2-hexanone was available, there are no 2-hexanone-specific multigenerational reproductive and developmental studies. Developmental studies on n-hexane, a precursor of 2-hexanone and 2,5-hexanedione, have shown low risk of toxicity, but there is still a level of concern because available studies on 2-hexanone via inhalation exposure have suggested the possibility of immunotoxicity and reproductive toxicity. Rat developmental neurotoxicity studies with 2,5-hexanedione have found minimal effects (e.g., aggregated and fused axons, identified with electron microscopy) from daily s.c. injections on GDs 12–20 with 340 mg/kg 2,5-hexanedione. Thus, due to the absence of studies specifically evaluating immunotoxicity or reproductive toxicity of 2-hexanone via an oral route of exposure, a UF of 3 was applied to account for database deficiency.

The overall confidence in this RfD assessment is medium. Confidence in the principal study (O'Donoghue et al., 1978) is medium. The study involves a comparatively low but acceptable number of animals per group (10) and reports clinical neurological deficits and neuropathologic effects within a dose range in which LOAEL could be identified for the critical effect. Animal studies in two additional species (guinea pigs and hens) corroborate the primacy of the neurological endpoint and confirm the validity of peripheral neuropathy as the critical effect. Confidence in the database is medium. The database lacks chronic exposure information on pure 2-hexanone via any route of exposure, as well as a multigenerational developmental and reproductive toxicity study and a developmental neurotoxicity study. The chronic drinking water study of O'Donoghue et al. (1978) satisfies the minimum oral database requirements for deriving an RfD for 2-hexanone. Reflecting medium confidence in the principal study and medium confidence in the database, confidence in the RfD is medium.

6.2.2. Noncancer/Inhalation

Dose-dependent development of 2-hexanone-induced neuropathy was confirmed in numerous subchronic studies in rats (Egan et al., 1980; Katz et al., 1980; Duckett et al., 1979, 1974; Johnson et al., 1977; Krasavage and O'Donoghue, 1977; Saida et al., 1976; Spencer et al., 1975) and one chronic study in cats (O'Donoghue and Krasavage, 1979). One 10-month study was Johnson et al. (1977), using two different species (monkeys and rats; n = 8 and n = 10 per group, respectively) with two concentrations of 2-hexanone (commercial grade). Johnson et al. (1977) utilized four sensitive neurological tests to identify subtle changes in treated versus control animals. The study by Johnson et al. (1977) was chosen as the most suitable study for RfC development. Both sciatic-tibial MCV and ulnar MCV in 2-hexanone-exposed monkeys and rats were considered in deriving the RfC. A graphical comparison of the potential RfCs from these endpoints, as well as other endpoints and studies considered, are illustrated in Figure 5-2. Because monkeys have a similar respiratory tract and breathing patterns to humans and 2,5-hexanedione, the metabolite of 2-hexanone, typically affects long axons such as the sciatic-tibial nerve prior to other nerves, sciatic-tibial nerve MCV in monkeys was used to derive the RfC. It should be noted that ulnar nerve MCV in monkeys and sciatic-tibial nerve MCV in rats were found to have similar $BMCL_{HEC}$ as the endpoint selected above and would not result in significantly different RfCs if those alternatives were utilized.

The RfC of 2×10^{-1} mg/m^3 was derived from the decrease in sciatic-tibial MCV in monkeys exposed to 2-hexanone for 10 months (Johnson et al., 1977). A composite UF of 1000 was applied in the derivation of the RfC: a default of 10 for intraspecies (interindividual) variability, a default of 10 for subchronic-to-chronic uncertainty, 3 for interspecies variability, and 3 for database uncertainty. Information was unavailable to predict potential variability in susceptibility among the population; thus, the intraspecies variability UF of 10 was applied. A subchronic-to-chronic UF of 10 was applied to account for the less-than-lifetime exposure in the principal study, because the data utilized for calculating the RfC were based on values obtained at 25 weeks. An interspecies UF of 3 (rather than 10) was applied because a dosimetric adjustment was made. A UF of 3 was applied to account for database deficiencies. Although a developmental study exists that did not identify a NOAEL (a LOAEL of 1000 ppm was identified), the available developmental studies with n-hexane, a precursor of 2-hexanone, provide support for applying a UF_D of 3. Namely, developmental studies with n-hexane concentrations of 100 (GDs 6–15), 400 (GDs 6–15), or 1000 ppm (GDs 8–16) have been negative; a teratology study in which dams were exposed to n-hexane identified nearly double the highest NOAEL identified from inhalation studies with 2-hexanone. Also, rat developmental neurotoxicity studies with 2,5-hexanedione, the ultimate toxic metabolite of n-hexane and 2-hexanone, have found minimal effects from daily s.c. injections on GDs 12–20 with 340 mg/kg 2,5-hexanedione.

The overall confidence in this RfC assessment is medium. Confidence in the principal study is medium; it involves exposures in two species via the inhalation route and sensitive diagnostic tests for determining treatment-related neurotoxicity. In addition, animal studies in four different species (monkeys, rats, cats, and hens) and occupational exposures corroborate the primacy of the neurological endpoint and confirm the relevance of the critical effect for decreased MCV values.

6.2.3. Cancer

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), there is inadequate information to assess the carcinogenic potential of 2-hexanone. As such, data are unavailable to calculate quantitative cancer risk estimates.

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APPENDIX B-1. DOSE-RESPONSE MODELING FOR DERIVATION OF AN RfD FOR 2-HEXANONE

B-1.1. METHODS

The models in U.S. EPA's benchmark dose (BMD) software (BMDS) (version 1.3.2) were fit to data sets for myofibrillar atrophy of the quadriceps and calf muscle in a 13-month drinking water study with exposure to 2-hexanone (O'Donoghue, 1978). The dose levels used were those reported in the study. A BMR of a 10% extra risk of myofibrillar atrophy of the quadriceps muscle or calf muscle was selected under an assumption that it represents a minimal biologically significant change. Models were run using the default restrictions on parameters built into the BMDS.

B-1.2. RESULTS

The BMD modeling results for myofibrillar atrophy of the quadriceps and calf muscles are summarized in Table B-1.1 and Table B-1.2, respectively. The tables show the BMDs and the 95% lower bounds on the doses (BMDLs) derived from each endpoint modeled. The remainder of this section shows detailed summaries of the best-fit models for myofibrillar atrophy of the quadriceps and calf muscles, presented sequentially.

Table B-1.1. BMD modeling results for animals with myofibrillar atrophy of the quadriceps muscle

Model	AIC[a]	*p* Value	BMD	BMDL	BMD/BMDL
Gamma multi-hit	24.6565	0.9034	150.665	86.2822	1.746189
Log logistic	25.1541	0.7602	156.315	96.3847	1.621782
Logistic	24.5648	0.9392	155.905	94.5301	1.649263
Multistage	**22.3952**	**0.9995**	**141.38**	**49.9434**	2.830804
Log probit	24.9892	0.7974	152.728	98.9646	1.543259
Probit	24.4241	0.9822	148.53	86.9997	1.707247
Quantal linear	29.9156	0.1628	34.9514	22.8722	1.528117
Quantal quadratic	23.7952	0.8118	100.711	78.3857	1.284813
Weibull	24.3816	0.9945	145.517	78.2565	1.859488

[a]AIC = Akaike Information Criterion.

Table B-1.2. BMD modeling results for animals with myofibrillar atrophy of the calf muscle

Model	AIC[a]	*p* Value	BMD	BMDL	BMD/BMDL
Gamma multi-hit	27.7837	0.8972	117.834	48.9374	2.407852
Log logistic	28.36	0.7402	122.843	63.2262	1.942913
Logistic	27.9769	0.8457	120.47	70.8106	1.701299
Multistage	27.4841	0.9956	95.8576	30.1238	3.182122
Log probit	28.0906	0.8019	123.737	67.2193	1.840796
Probit	27.7116	0.9227	114.43	65.7944	1.739206
Quantal linear	30.2036	0.3498	28.7546	19.0312	1.510919
Quantal quadratic	**25.8664**	**0.9701**	**88.7125**	**69.2097**	1.281793
Weibull	27.5386	0.9756	109.348	45.8927	2.382688

[a]AIC = Akaike Information Criterion.

==
Multistage Model
MYOFIBRILLAR ATROPHY OF THE QUADRICEPS MUSCLE
==

BMDS MODEL RUN
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The form of the probability function is:

P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1-beta2*dose^2-beta3*dose^3)]

The parameter betas are restricted to be positive

Dependent variable = Incidence
Independent variable = Dose

Total number of observations = 4
Total number of records with missing values = 0
Total number of parameters in model = 4
Total number of specified parameters = 0
Degree of polynomial = 3

Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 0
Beta(1) = 0
Beta(2) = 0
Beta(3) = 5.88262e+011

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Background -Beta(1) -Beta(2)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Beta(3)
Beta(3)	1

Parameter Estimates

Variable	Estimate	Std. Err.
Background	0	NA
Beta(1)	0	NA
Beta(2)	0	NA
Beta(3)	3.72829e-008	2.10431e-008
~~~~~~

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

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-10.1823			
Fitted model	-10.1976	0.0306013	3	0.9986
Reduced model	-26.9205	33.4763	3	<.0001

AIC: 22.3952

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Chi^2 Res.
i: 1					
0.0000	0.0000	0.000	0	10	0.000
i: 2					
143.0000	0.1033	1.033	1	10	-0.036
i: 3					
266.0000	0.5043	5.043	5	10	-0.017
i: 4					
560.0000	0.9986	9.986	10	10	1.001

Chi-square = 0.02 DF = 3 P-value = 0.9995

Benchmark Dose Computation

Specified effect = 0.1

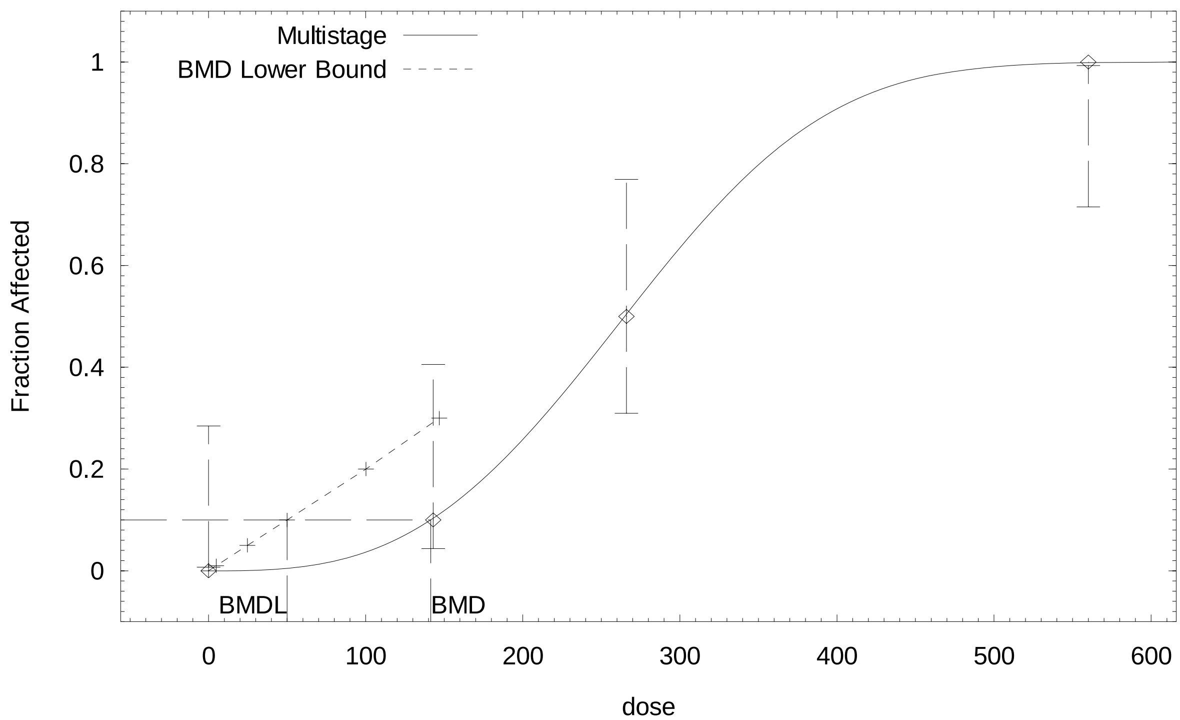
Risk Type = Extra risk

Confidence level = 0.95

BMD = 141.38

BMDL = 49.9434

Multistage Model with 0.95 Confidence Level



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==

Quantal Quadratic Model

MYOFIBRILLAR ATROPHY OF THE CALF MUSCLE

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BMDS MODEL RUN

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The form of the probability function is:

P[response] = background + (1-background)*[1-EXP(-slope*dose^2)]

Dependent variable = Incidence
Independent variable = Dose

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

Default Initial (and Specified) Parameter Values
Background = 0.0454545
Slope = 9.7083e-006
Power = 2 Specified
~~~~~~

Asymptotic Correlation Matrix of Parameter Estimates

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

	Slope
Slope	1

Parameter Estimates

Variable	Estimate	Std. Err.
Background	0	NA
Slope	1.33878e-005	4.17409e-006

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

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-11.7341			
Fitted model	-11.9332	0.398099	3	0.9406
Reduced model	-27.5256	31.5828	3	<.0001

AIC: 25.8664

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0	10	0
143.0000	0.2395	2.395	2	10	-0.2926
266.0000	0.6122	6.122	6	10	-0.07918
560.0000	0.9850	9.850	10	10	0.3905

Chi-square = 0.24 DF = 3 P-value = 0.9701

Benchmark Dose Computation

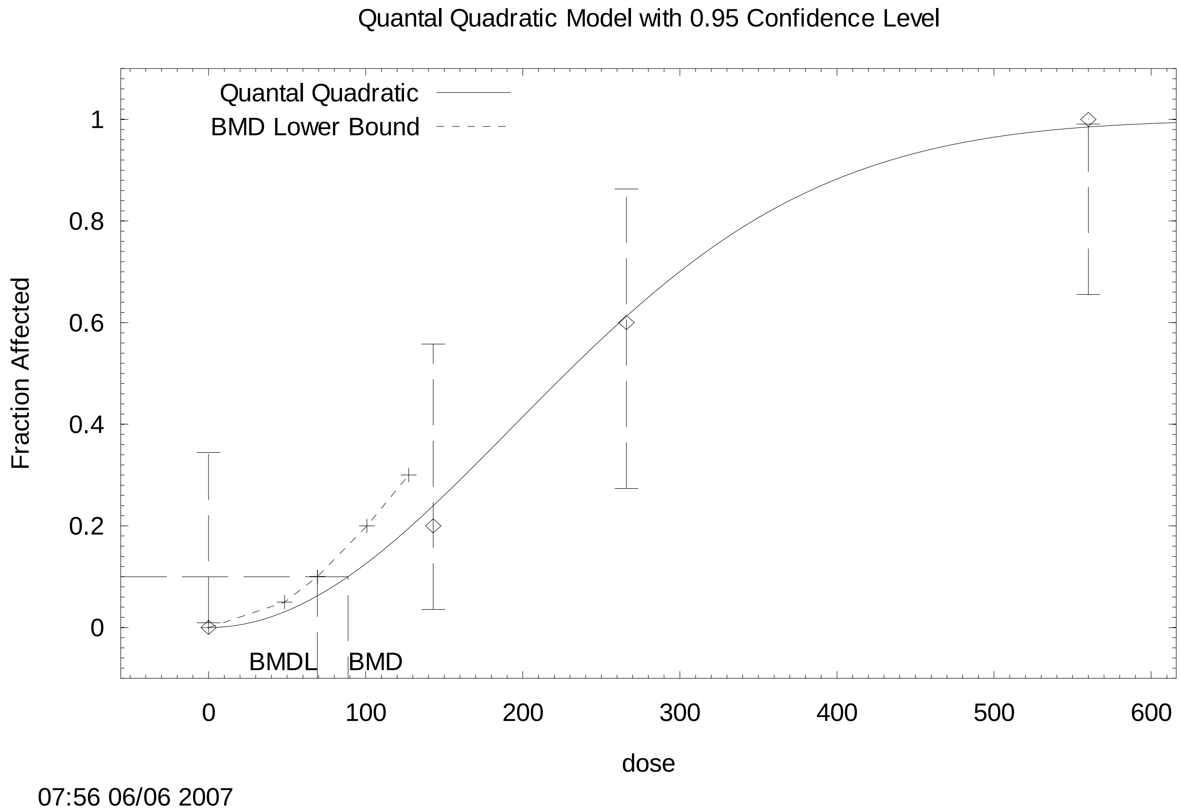
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 88.7125

BMDL = 69.2097


Quantal Quadratic Model with 0.95 Confidence Level
Quantal Quadratic
BMD Lower Bound
Fraction Affected
1
0.8
0.6
0.4
0.2
0
BMDL
BMD
0
100
200
300
400
500
600
dose
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APPENDIX B-2. EXPOSURE-RESPONSE MODELING FOR DERIVATION OF AN RfC FOR 2-HEXANONE

B-2. 1. METHODS

The models in U.S. EPA's benchmark dose (BMD) software (BMDS) (version 1.3.2) were fit to multiple data sets presented in an inhalation study with exposure to monkeys and rats (Johnson et al., 1977). Motor conduction velocity (MCV) was determined to be the most relevant endpoint in both species and was modeled for the sciatic and tibial nerves. The exposure concentrations used were those reported in the study. The U.S. EPA (2000c) BMD methodology suggests that in the absence of any other idea of what level of response to consider adverse, a change in the mean equal to one control standard deviation from the control should be used as the benchmark response (BMR). A BMR of a 10% change in nerve conduction velocity from the control mean was selected under an assumption that it represents a minimal biologically significant change. Thus, a 10% BMR was utilized in the derivation of the reference concentration (RfC).

B-2.2. RESULTS

The BMD modeling results are summarized in Table B-2.1. This table shows the BMDs and 95% lower bounds on doses (BMDLs) derived from each endpoint modeled in monkeys and rats. The remainder of this section shows detailed summaries of the modeling results for monkey sciatic and ulnar nerves for both monkeys and rats (all 1st degree polynomial), presented sequentially.

Table B-2.1. Summary of BMDS modeling results for 2-hexanone

Animal/endpoint	Model[a]	*p* Value	AIC[b]	BMD	BMDL
Monkey sciatic-tibial nerve (MCV at 6 months)	**1st degree polynomial**	**0.59**	**105.59**	**293.184**	**243.262**
	2nd degree polynomial	--	107.29	169.254	68.8063
	Power	--	105.58	293.184	243.262
	Hill	--	--	--	--
Monkey ulnar nerve (MCV at 6 months)	**1st degree polynomial**	**0.90**	**105.31**	**334.691**	**278.471**
	2nd degree polynomial	--	107.29	293.8	94.1174
	Power	--	109.31	334.691	278.471
	Hill	--	--	--	--
Rat sciatic-tibial nerve (MCV at 25 weeks)	**1st degree polynomial**	**0.79**	**121.55**	**270.959**	**232.105**
	2nd degree polynomial	--	123.48	355.94	100.045
	Power	--	125.48	329.704	232.56
	Hill	--	--	--	--
Rat ulnar nerve (MCV at 25 weeks)	**1st degree polynomial**	**0..26**	**122.77**	**470.964**	**352.274**
	2nd degree polynomial	--	606.18	133.181	87.3758
	Power	--	125.48	177.555	14.1388
	Hill	--	--	--	--

[a]Bolded values were the models used for further evaluation and RfC derivation.
[b]AIC = Akaike Information Criterion.

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1st Degree Polynomial Model
MONKEYS MCV SCIATIC TIBIAL
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BMDS MODEL RUN
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The form of the response function is:

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

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

Total number of dose groups = 3
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
alpha = 28.6225
rho = 0 Specified
beta_0 = 49.3606
beta_1 = -0.0168361

Parameter Estimates

95.0% Wald Confidence Interval

Variable	Estimate	Std. Err.	Lower Conf. Limit	Upper Conf. Limit
alpha	25.351	7.31821	11.0076	39.6944
beta_0	49.3606	1.3264	46.761	51.9603
beta_1	-0.0168361	0.0023421	-0.0214265	-0.0122456

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	beta_0	beta_1
alpha	1	4.8e-015	-5.1e-015
beta_0	4.8e-015	1	-0.63
beta_1	-5.1e-015	-0.63	1

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
------	---	--------	-----------	--------	-----------	----------
0	8	50	5.35	49.4	5.03	0.359
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98	8	47	5.35	47.7	5.03	-0.399
976	8	33	5.35	32.9	5.03	0.0401

Model Descriptions for likelihoods calculated

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

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

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

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

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-50.647941	4	109.295882
A2	-50.647941	6	113.295882
fitted	-50.793824	2	105.587648
R	-65.085067	2	134.170135

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

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	28.8743	4	<.0001
Test 2	-1.42109e-014	2	<.0001
Test 3	0.291767	1	0.5891

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

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

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

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Relative risk

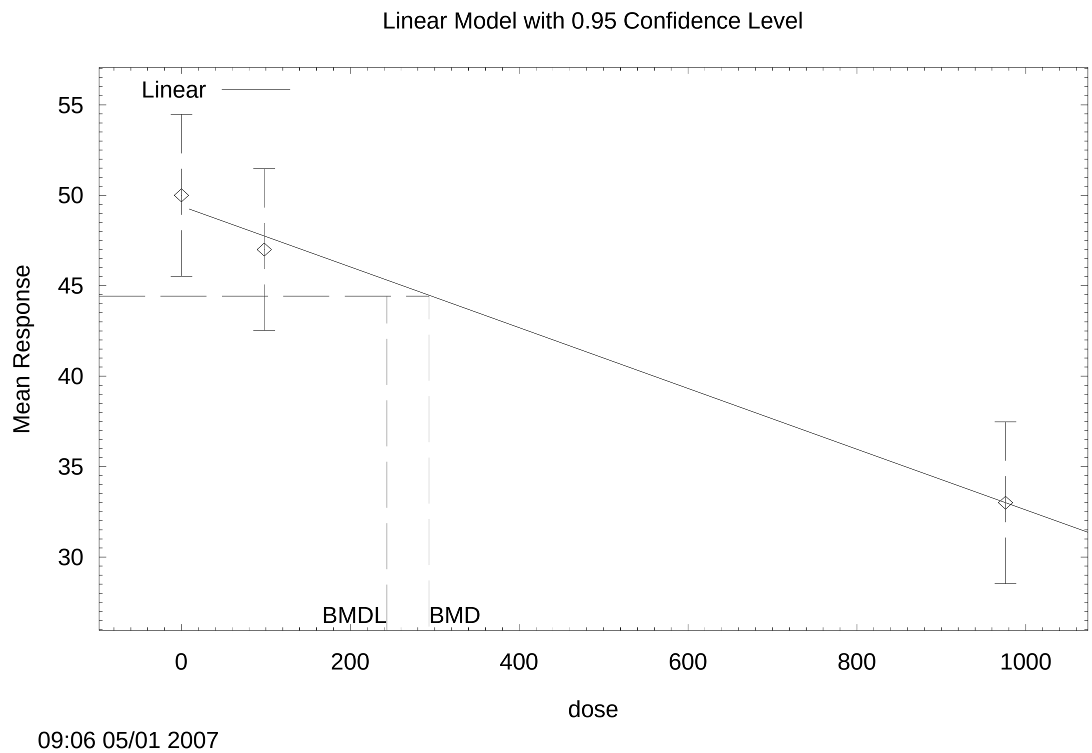
Confidence level = 0.95

BMD = 293.184

BMDL = 243.262

BMDL computation failed for one or more points on the BMDL curve.

The BMDL curve will not be plotted



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1st Degree Polynomial Model.

MONKEYS MCV ULNAR

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BMDS MODEL RUN

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The form of the response function is:

Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...
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Dependent variable = MEAN
Independent variable = Dose
rho is set to 0
Signs of the polynomial coefficients are not restricted
A constant variance model is fit

Total number of dose groups = 3
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

alpha =	28.6225	
rho =	0	Specified
beta_0 =	57.8551	
beta_1 =	-0.0172861	

Parameter Estimates

			95.0% Wald Confidence Interval	
Variable	Estimate	Std. Err.	Lower Conf. Limit	Upper Conf. Limit
alpha	25.0604	7.23432	10.8814	39.2394
beta_0	57.8551	1.31877	55.2704	60.4399
beta_1	-0.0172861	0.00232864	-0.0218502	-0.0127221

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	beta_0	beta_1
alpha	1	-4.3e-012	8.7e-012
beta_0	-4.3e-012	1	-0.63
beta_1	8.7e-012	-0.63	1

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
------	---	--------	-----------	--------	-----------	----------
0	8	58	5.35	57.9	5.01	0.0819
98	8	56	5.35	56.2	5.01	-0.091
976	8	41	5.35	41	5.01	0.00914

Model Descriptions for likelihoods calculated

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

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

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

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

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-50.647941	4	109.295882
A2	-50.647941	6	113.295882
fitted	-50.655476	2	105.310953
R	-65.478867	2	134.957734

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

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	29.6619	4	<.0001
Test 2	-1.42109e-014	2	<.0001
Test 3	0.0150715	1	0.9023

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

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

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

Benchmark Dose Computation
Specified effect = 0.1

Risk Type = Relative risk

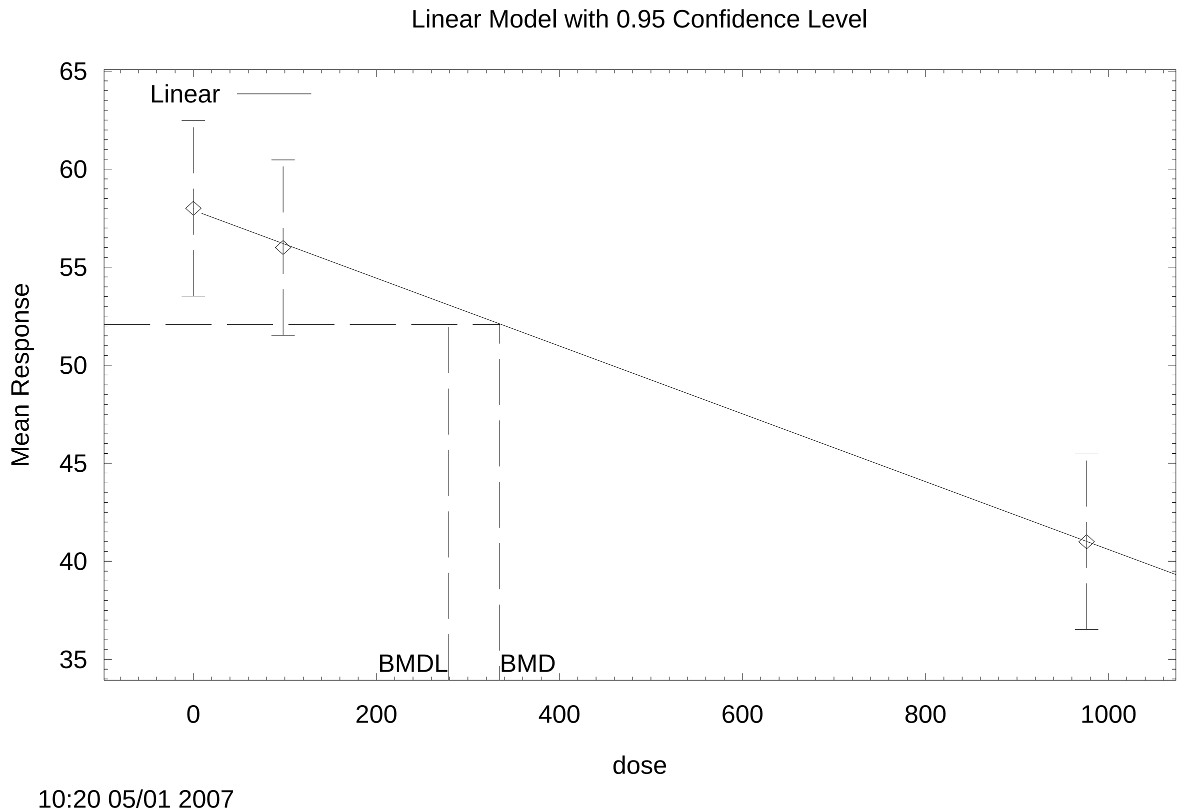
Confidence level = 0.95

BMD = 334.691

BMDL = 278.471

BMDL computation failed for one or more points on the BMDL curve.

The BMDL curve will not be plotted



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1st Degree Polynomial Model.

RATS MCV SCIATIC TIBIAL

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BMDS MODEL RUN

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The form of the response function is:

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

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

Total number of dose groups = 3
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008
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Default Initial Parameter Values
alpha = 20.5209
rho = 0 Specified
beta_0 = 42.2427
beta_1 = -0.0155901

Parameter Estimates

			95.0% Wald Confidence Interval	
Variable	Estimate	Std. Err.	Lower Conf. Limit	Upper Conf. Limit
alpha	18.5129	4.78001	9.14425	27.8815
beta_0	42.2427	1.01325	40.2568	44.2287
beta_1	-0.0155901	0.00178935	-0.0190972	-0.012083

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	beta_0	beta_1
alpha	1	-4.2e-008	6.6e-008
beta_0	-4.2e-008	1	-0.63
beta_1	6.6e-008	-0.63	1

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	10	42	4.53	42.2	4.3	-0.178
97	10	41	4.53	40.7	4.3	0.198
976	10	27	4.53	27	4.3	-0.0197

Model Descriptions for likelihoods calculated

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

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

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

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-58.741250	4	125.482501
A2	-58.741250	6	129.482501
fitted	-58.777017	2	121.554034

R	-78.206652	2	160.413304

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

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	38.9308	4	<.0001
Test 2	0	2	1
Test 3	0.0715333	1	0.7891

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

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

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

Benchmark Dose Computation
Specified effect = 0.1

Risk Type = Relative risk

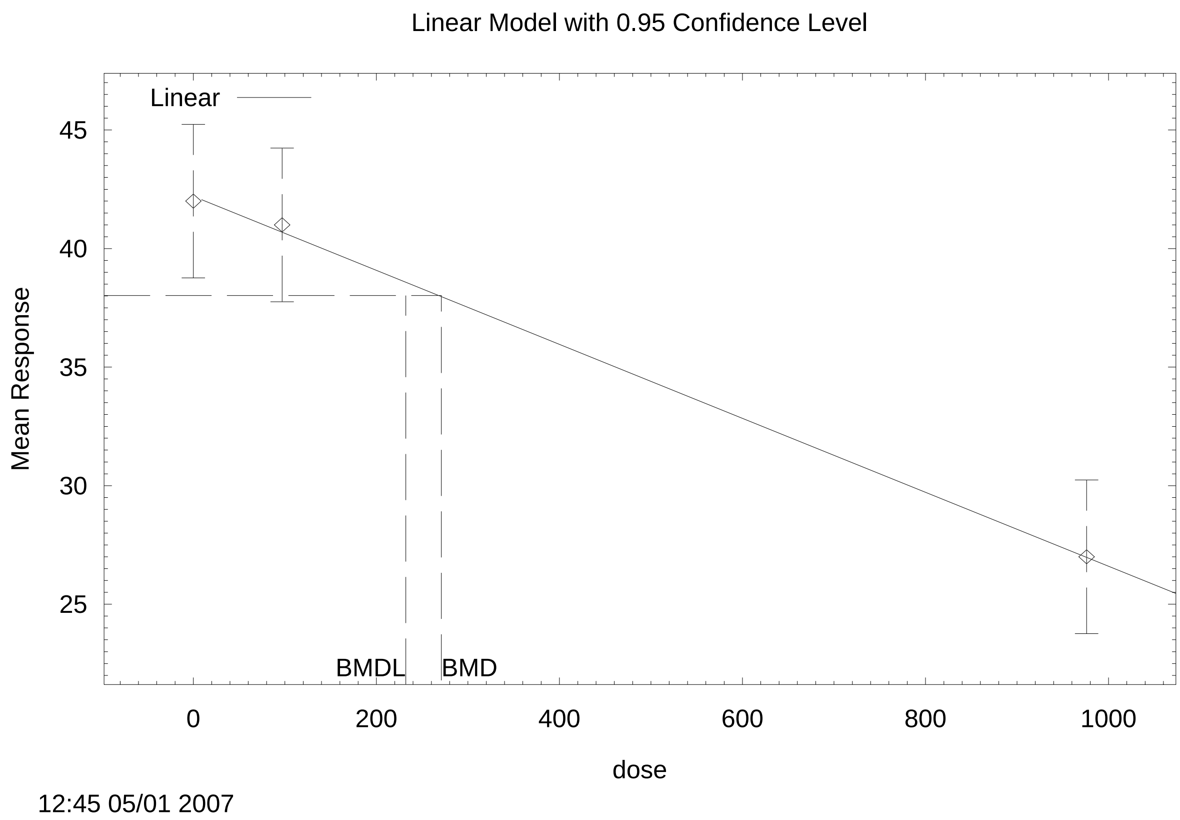
Confidence level = 0.95

BMD = 270.959

BMDL = 232.105

BMDL computation failed for one or more points on the BMDL curve.

The BMDL curve will not be plotted



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1st Degree Polynomial Model.
RATS MCV ULNAR
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BMDS MODEL RUN
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The form of the response function is:

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

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

Total number of dose groups = 3
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008
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Default Initial Parameter Values
alpha = 20.5209
rho = 0 Specified
beta_0 = 38.9587
beta_1 = -0.0082721

Parameter Estimates

			95.0% Wald Confidence Interval	
Variable	Estimate	Std. Err.	Lower Conf. Limit	Upper Conf. Limit
alpha	19.2803	4.97816	9.52331	29.0373
beta_0	38.9587	1.03404	36.932	40.9853
beta_1	-0.0082721	0.00182606	-0.0118511	-0.00469309

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	beta_0	beta_1
alpha	1	7.6e-015	3.4e-015
beta_0	7.6e-015	1	-0.63
beta_1	3.4e-015	-0.63	1

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
------	---	--------	-----------	--------	-----------	----------
0	10	40	4.53	39	4.39	0.75
97	10	37	4.53	38.2	4.39	-0.833
976	10	31	4.53	30.9	4.39	0.0828

Model Descriptions for likelihoods calculated

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

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

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

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-58.741250	4	125.482501
A2	-58.741250	6	129.482501
fitted	-59.386277	2	122.772554
R	-67.712722	2	139.425445

Test 1: Does response and/or variances differ among dose

levels (A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	17.9429	4	0.000127
Test 2	0	2	1
Test 3	1.29005	1	0.256

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

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

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

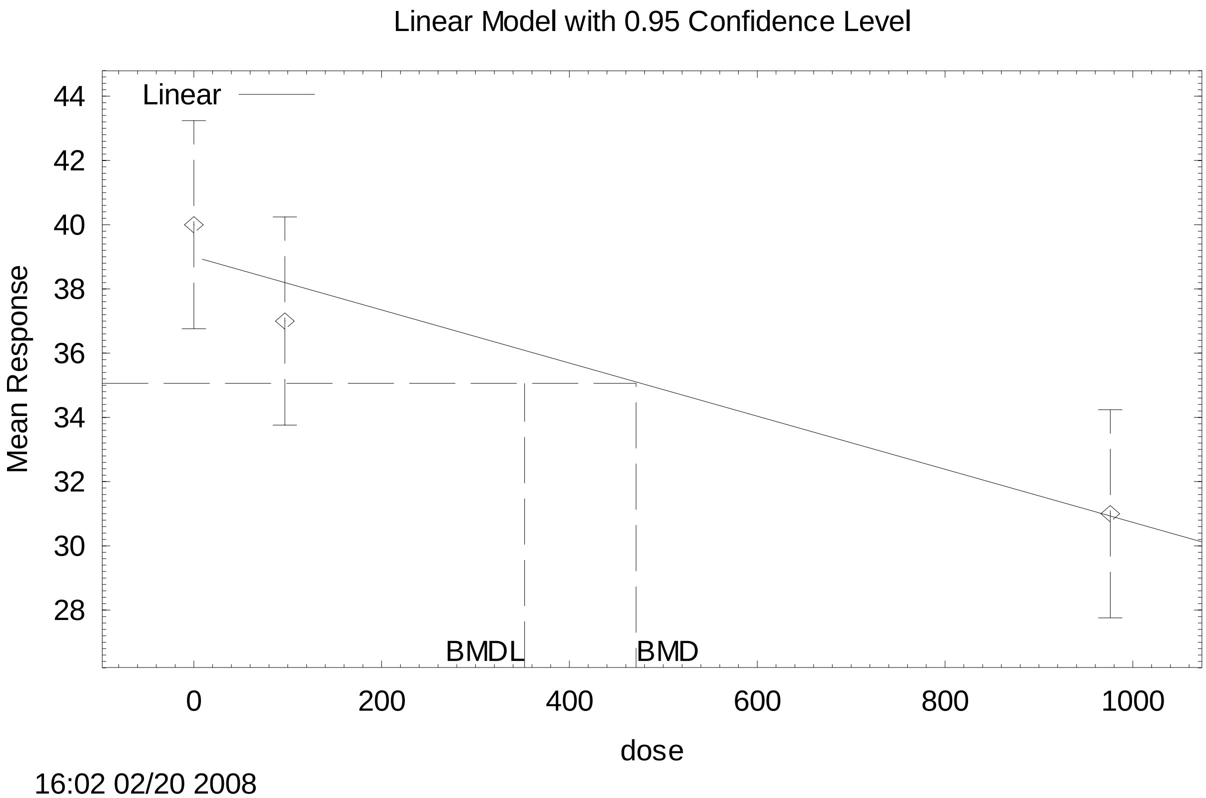
Benchmark Dose Computation
Specified effect = 0.1

Risk Type = Relative risk

Confidence level = 0.95

BMD = 470.964

BMDL = 352.274


Linear Model with 0.95 Confidence Level
Linear
44
42
40
38
36
34
32
30
28
Mean Response
BMDL
BMD
0
200
400
600
800
1000
dose
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