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Provisional Peer-Reviewed Provisional Subchronic

Toxicity Values for *n*-Hexane
(CASRN 110-54-3)

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

COMMONLY USED ABBREVIATIONS

BMD	Benchmark Dose
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
LOAEL	lowest-observed-adverse-effect level
$LOAEL_{ADJ}$	LOAEL adjusted to continuous exposure duration
$LOAEL_{HEC}$	LOAEL adjusted for dosimetric differences across species to a human
NOAEL	no-observed-adverse-effect level
$NOAEL_{ADJ}$	NOAEL adjusted to continuous exposure duration
$NOAEL_{HEC}$	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
p-OSF	provisional oral slope factor
p-RfC	provisional inhalation reference concentration
p-RfD	provisional oral reference dose
RfC	inhalation reference concentration
RfD	oral reference dose
UF	uncertainty factor
UF_A	animal to human uncertainty factor
UF_C	composite uncertainty factor
UF_D	incomplete to complete database uncertainty factor
UF_H	interhuman uncertainty factor
UF_L	LOAEL to NOAEL uncertainty factor
UF_S	subchronic to chronic uncertainty factor

PROVISIONAL PEER-REVIEWED SUBCHRONIC TOXICITY VALUES FOR *n*-HEXANE (CASRN 110-54-3)

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (U.S. EPA) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1) U.S. EPA's Integrated Risk Information System (IRIS).
2) Provisional Peer-Reviewed Toxicity Values (PPRTVs) used in U.S. EPA's Superfund Program.
3) Other (peer-reviewed) toxicity values, including
   - Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
   - California Environmental Protection Agency (CalEPA) values, and
   - EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in U.S. EPA's IRIS. PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the U.S. EPA IRIS Program. All provisional toxicity values receive internal review by two U.S. EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multiprogram consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all U.S. EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because new information becomes available and scientific methods improve over time, PPRTVs are reviewed on a 5-year basis and updated into the active database. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV documents conclude that a PPRTV cannot be derived based on inadequate data.

Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and Resource Conservation and Recovery Act (RCRA) program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV document and understand the strengths

and limitations of the derived provisional values. PPRTVs are developed by the U.S. EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other U.S. EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the U.S. EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

n-Hexane was recently reassessed by the IRIS program and a Toxicological Review (U.S. EPA, 2005) is available. A chronic RfC and a cancer assessment are included on IRIS (U.S. EPA 2005). U.S. EPA (2005, 2008) did not derive a chronic RfD for *n*-hexane, characterizing the data as inadequate for this purpose. The Agency for Toxic Substances and Disease Registry (ATSDR, 1999) prepared a Toxicological Profile for *n*-hexane, but did not derive any oral MRL, citing inadequate data by this route. Given the limitations in the oral toxicity database for *n*-hexane, updated literature searches (2003−2007) were conducted in September 2007 to determine whether newer data (published since the IRIS Toxicological Review) were available for the derivation of oral toxicity values. An additional literature search in August 2009 revealed no recent relevant data. The following databases have been searched: MEDLINE, TOXLINE, BIOSIS, TSCATS, CCRIS, DART/ETIC, GENETOX, HSDB, and Current Contents.

The IRIS Toxicological Review (U.S. EPA, 2005) contains a comprehensive overview of the toxicology and toxicokinetics information available on *n*-hexane. This report was used to identify critical endpoints and studies for use in deriving the subchronic p-RfC for *n*-hexane. Updated literature searches did not reveal additional data beyond those that were evaluated in the previous IRIS assessment.

The derivation of subchronic toxicity values for *n*-hexane is discussed below. A brief rationale is provided for the selection of the critical study and endpoint, a summary of the critical study is presented, and the subchronic toxicity value derivation process is described. Further information on the toxicology and toxicokinetics of *n*-hexane is contained in the IRIS record (see Appendix A), IRIS Toxicological Review document (U.S. EPA, 2005), or ATSDR (1999) Toxicological Profile for *n*-hexane.

REVIEW OF PERTINENT DATA AND DERIVATION OF PROVISIONAL SUBCHRONIC TOXICITY VALUES FOR *n*-HEXANE

Subchronic p-RfD

Both of the available oral studies of *n*-hexane (Krasavage et al., 1980; Ono et al., 1981) are described in the IRIS Toxicological Review for *n*-Hexane (U.S. EPA, 2005). The IRIS Toxicological Review for *n*-Hexane concluded that a chronic RfD for *n*-hexane could not be derived due to a lack of suitable oral studies of sufficient duration that evaluated an array of endpoints. The Ono et al. (1981) study was not considered in the selection of the principal study and critical effect for the derivation of the chronic RfD in the IRIS Toxicological Review for *n*-Hexane. The Krasavage et al. (1980) study was considered, but it was determined that this study was inadequate for the development of a chronic RfD for *n*-hexane. Specifically, the Krasavage et al. (1980) study was a subchronic study, utilizing gavage exposure that evaluated a small number of animals. Additionally, several animals in each dose group died during the course of the study.

The summaries of the available oral studies described below in support of the p-RfD and RfC were taken from the text of the Toxicological Review for *n*-hexane (U.S. EPA, 2005), Additional information and details have been included where necessary for this review.

> *Krasavage et al. (1980) compared the neurotoxicity of 2-hexanone, 2-hexanol, 2,5-hexanedione, 2,5-hexanediol, 5-hydroxy-2-hexanone, n-hexane and practical grade hexane. Groups of 5 male COBS CD(SD)BR rats/group received equimolar doses of 6.6 mmol/kg of the chemicals (except practical grade hexane) by gavage (undiluted), 5 days/week for 90 days. Controls received distilled water. The mg/kg equivalent of n-hexane (99% pure) was 570 mg/kg-day. After a month of treatment, additional groups of 5 rats were administered n-hexane at 13.2 or 46.2 mmol/kg (1140 or 3980 mg/kg-day, respectively) or practical grade hexane at 46.2 mmol/kg in the same manner. The period of treatment and observation was extended to 120 days for those animals receiving 3980 mg/kg n-hexane to ensure that an overt neuropathological endpoint was detected in rats exposed to the chemical. Daily observation for condition, behavior, clinical signs, and signs of neurotoxicity (changes in posture, gait, or response to toe pinch) were made. Body weight and food consumption were recorded twice each week. The onset of neuropathy was assessed by the initial appearance of hind-limb weakness or paralysis, at which point the animal was sacrificed and examined histopathologically. In the absence of hind-limb paralysis, animals were sacrificed after 90 days (570 and 1140 mg/kg-day groups) or 120 days (3980 mg/kg-day group). After necropsy, selected target organs (tibial nerve with branches, testes, and epididymides) were subjected to microscopic examination.*
>
> *There were two rats in the 1140 mg/kg exposure group and one rat in the 3980 mg/kg n-hexane exposure group that died due to chemical pneumonitis following intubation and were not included in the determination of neurotoxic potential (Krasavage et al., 1980). The authors did not report the timing of the deaths, nor was it clear from the report whether the animals were examined for histopathology. Body weights were decreased in all rats treated with n-hexane; food consumption was reduced to 15.5 grams/day by animals exposed to the high dose of n-hexane compared with 28 grams/day in controls.*

The body-weight data were presented graphically however, terminal body weights appeared to be decreased (relative to controls) by ~20%, 10%, and 30% in the low-, mid- and high-dose groups (statistical significance not reported by the study authors). The authors observed hind-limb paralysis in three rats exposed to the high dose of n-hexane. Lower doses of n-hexane did not produce hind-limb paralysis during the 90-day testing period. The relative potency of the test chemicals was compared with 2-hexanone. Specifically, the authors estimated a neurotoxic index calculated as the ratio of number of days until hind-limb paralysis developed in 2-hexanone-treated animals to the number of days in animals treated with other test materials. Based on the time taken by the rats to develop hind-limb paralysis, 2,5-hexanedione (the principal metabolite of n-hexane; U.S. EPA, 2005) had approximately 38 times the neurotoxic potency of n-hexane itself on an equimolar basis. Furthermore, the neurotoxic index correlated with peak serum concentrations of 2,5-hexanedione and the area under the serum concentration-time curve for 2,5-hexanedione. Histopathology evaluation revealed giant axonal swelling, adaxonal myelin infolding and paranodal myelin retraction (incidence of these specific findings not reported). There was histologic evidence of neuropathy in four rats in the high-dose group; no control, low- or mid-dose animals had evidence of neuropathy. In addition, atrophy of the germinal epithelium was observed in the testes of high-dose rats (incidence not reported). This study identified a LOAEL of 570 mg/kg-day 5 days/week based on body weight reductions; no NOAEL can be identified from these data.

The effects of n-hexane on peripheral nerve transmission were evaluated by Ono et al. (1981). Male Wistar rats (5−7/group) were administered n-hexane (99% pure) by gavage in olive oil daily for 8 weeks. The exposure regimen consisted of administration of 0.4 mL solvent and 0.6 mL olive oil for the first 4 weeks, 0.6 mL solvent and 0.4 mL olive oil for a subsequent 2 weeks and 1.2 mL solvent and 0.8 mL olive oil for the final 2 weeks, while a control group received olive oil alone. Body weight was measured every 2 weeks during the experimental period, resulting in dose calculations[1] of 811 mg/kg-day (after 2 weeks), 759 mg/kg-day (2−4 weeks), 1047 mg/kg-day (4−6 weeks) and 2022 mg/kg-day (6−8 weeks). Peripheral nerve activity was measured by administering a differential pulse to electrodes inserted at different points along the tail of unanaesthetized animals. Transmission of electrical charge was then detected at other points along the tail. The group mean motor nerve conduction velocity (MCV) was measured at the start of the experiment and every 2 weeks until termination. Histopathology examinations were not made.

There was no change among the groups in the rates of body-weight gain throughout the experiment (Ono et al., 1981). In groups exposed to n-hexane for at least 4 weeks, MCV was reduced by approximately 5−10% compared with controls. These changes achieved statistical significance at the 4- and 8-week time points (statistical test not stated). Distal latencies decreased as the rats grew, but there were no statistically significant differences between n-hexane-exposed and control animals. However, there were statistically significant ($p<0.05$) reductions in the distal (approximately 5−8%) mixed MCVs of animals receiving n-hexane compared with controls after 4 weeks and significant reduction in the proximal (approximately 6−8%) mixed MCVs after 6 weeks.

[1] The dose estimates are not directly proportional to the administered volumes of solvent due to increases in body weight over the course of the study.

For the purposes of the PPRTV derivation, these data were given additional quantitative consideration. There was no change among the groups in the rates of body-weight gain throughout the study (Ono et al., 1981). In groups exposed to *n*-hexane for at least 4 weeks, MCV was reduced by approximately 5−10% compared with controls. These changes were statistically significant at the 4- and 8-week time points (statistical test not reported by the study authors). Distal latencies decreased as the study progressed and the rats gained weight, but there were no statistically significant differences between *n*-hexane-exposed and control animals. However, there were statistically significant ($p < 0.05$) reductions in the distal mixed MCVs (approximately 5−8%) of animals receiving *n*-hexane compared with controls after 4 weeks and significant reduction in the proximal mixed MCVs (approximately 6−8%) after 6 weeks. The dose administered for the first 4 weeks was considered the LOAEL (for distal MCV) for this review. The dose averaged approximately 785 mg/kg-day. The dose producing this effect was actually lower than the dose at which no effect was observed (although the duration of dosing was shorter with the higher dose). This study design precludes identifying a lower dose that did not produce the response. For that reason, no NOAEL can be identified.

Both oral studies (i.e., Krasavage et al., 1980; Ono et al., 1981) were rat gavage studies of subchronic duration using small numbers (5−7/group) of animals. In the Krasavage et al. (1980) study, some animals died due to gavage errors (2/5 mid-dose and 1/5 high-dose rats). A LOAEL of 570 mg/kg-day was determined from the low-dose group in the study which was adjusted for continuous exposure (LOAEL × 5/7 days/week) to 407 mg/kg-day based on body-weight reductions of at least 10% (at study termination) in the remaining animals of the low-dose group and mid-dose group (814 mg/kg-day, adjusted). Larger body-weight reductions, along with hind-limb paralysis and histologic evidence of peripheral neuropathy and testicular effects, were observed at the high dose (2843 mg/kg-day, adjusted). In the study by Ono et al. (1981), a LOAEL of 785 mg/kg-day can be identified based on reductions in motor nerve conduction velocity (MCV) in rats exposed via daily gavage. In this study, the doses were increased over time and the LOAEL was identified using the weighted average dose estimated for the first 4 weeks (when the first evidence of MCV reductions was reported). MCV reductions are evidence of peripheral neuropathy, which is also the critical endpoint of the chronic RfC for *n*-hexane and a well-established effect of human and animal inhalation exposure to *n*-hexane (U.S. EPA, 2005). There were no effects on body weight gain in the study by Ono et al. (1981).

The Ono et al. (1981) study was selected as the basis for the subchronic p-RfD for *n*-hexane. This study identified a LOAEL of 785 mg/kg-day for decreased MCV. This endpoint is supported by observed clinical signs and histological evidence of peripheral neuropathy at higher doses in the Krasavage et al. (1980) study. Krasavage et al. (1980) also observed changes in body weight at lower doses than the effects observed by Ono et al. (1981). However, taking into consideration the increased mortality observed by Krasavage et al (1980) and the observation that the nervous system is the primary target of *n*-hexane-induced toxicity in both humans and animals, this study was not selected for the determination of the subchronic p-RfD.

A composite UF of 3000 was applied to the LOAEL of 785 mg/kg-day (Ono et al., 1981) to derive a **subchronic p-RfD** as follows:

$$\begin{aligned} \textbf{Subchronic p-RfD} &= \text{LOAEL} \div \text{UF} \\ &= 785 \text{ mg/kg-day} \div 3000 \\ &= \textbf{0.3 mg/kg-day or } \mathbf{3 \times 10^{-1}} \textbf{ mg/kg-day} \end{aligned}$$

The composite UF of 3000 is composed of the following:

- An UF of 10 is applied for interspecies extrapolation to account for potential pharmacokinetic and pharmacodynamic differences between rats and humans.
- An UF of 10 for intraspecies differences is used to account for potentially susceptible individuals in the absence of information on the variability of response in humans.
- An UF of 3 ($10^{0.5}$) is applied for use of a LOAEL. Although nerve conduction velocity was reduced at the LOAEL of 785 mg/kg-day (Ono et al., 1981), there was no histopathologic evidence of damage to peripheral nerves at a similar dose, 814 mg/kg-day, or observable signs of nerve damage (e.g., limb dragging) at 1140 mg/kg-day in the supporting study (Krasavage et al., 1980). Since nerve conduction normally decreases as a function of age, this delay in nerve conduction may represent an advancing of an age-related effect. Additionally, the weighted average dose method coupled with a fixed dose and increased body weight with time precluded identifying a NOAEL that was actually lower than the weighted average LOAEL value. For these reasons, a full UF of 10 is not warranted.
- A database UF of 10 is applied. There are no two-generation reproductive studies or developmental studies. Although a large toxicological database exists for inhaled *n*-hexane, and supports peripheral nerve damage as the critical effect, the database for oral *n*-hexane exposure contains only two minimally adequate subchronic neurotoxicity studies.

Confidence in the principal study (Ono et al., 1981) and the supporting study (Krasavage et al., 1980) is low. The studies used small numbers of animals (5−7/dose) and, in the Krasavage et al. (1980) study, gavage errors resulted in the loss of three animals. Further, few endpoints were examined. However, the principal study focused on an endpoint (nerve conduction velocity) relevant to the known critical effect of inhalation exposure (U.S. EPA, 2008), and the supporting study employed several dose levels and included histopathologic examination of nervous system and testicular tissues, both critical target organs of inhalation exposure (U.S. EPA, 2005). Confidence in the database is low because the database for oral *n*-hexane exposure contains only two minimally adequate subchronic neurotoxicity studies. Thus, low confidence in the subchronic p-RfD follows.

Subchronic p-RfC

The chronic RfC for *n*-hexane (0.7 mg/m^3) on IRIS (U.S. EPA, 2005) was derived (completion date December 2005) based on peripheral neuropathy (decreased motor nerve conduction velocity ex vivo) in tail nerves from rats exposed to *n*-hexane via inhalation for 16 weeks (Huang et al., 1989). The derivation included the use of a partial UF of 3 (rather than a full UF of 10) for subchronic-to-chronic extrapolation, which was justified in the IRIS derivation because the lifetime of neurofilaments (the target of *n*-hexane toxicity) is shorter than the lifetime of an adult rat (U.S. EPA, 2008). There is no intermediate duration inhalation MRL for *n*-hexane (ATSDR, 1999). Because U.S. EPA (2005) used a subchronic study as the basis for the chronic RfC for *n*-hexane and because newer subchronic inhalation studies have not been identified, the subchronic p-RfC is based on same critical study (Huang et al., 1989), endpoint (peripheral neuropathy), and point-of-departure ($BMCL_{HEC}$) value as the chronic RfC—but without the UF for subchronic-to-chronic extrapolation.

The summary of the Huang et al. (1989) study contained herein is excerpted from the text of the Toxicological Review (U.S. EPA, 2005).

> *Male Wistar rats (8/group) were exposed to 0, 500, 1200 or 3000 ppm n-hexane (>99% pure) for 12 hours/day, 7 days/week for 16 weeks (Huang et al., 1989). The authors measured MCV in excised tail nerve preparations along with body weight before exposure and after 4, 8, 12 and 16 weeks of exposure to n-hexane. An animal from each group was sacrificed after 16 weeks of exposure for histopathological evaluation of the nerve fibers in the tail. In addition, Huang et al. (1989) measured the levels of neuron-specific enolase and ß-S100. These nerve-specific proteins are part of a family of calcium-binding proteins that are involved in processes such as cell to cell communication, cell growth, intracellular signal transduction, and development and maintenance of the CNS. Some members of the S100 protein family are released into the extracellular space (depending on concentration of protein) by an unknown mechanism and modulate cell proliferation, act as chemoattractants for leukocytes, stimulate neuronal survival and/or differentiation of astrocyte proliferation, increase apoptosis of neurons and regulate macrophage activation. Data indicates that S100 proteins may be an extracellular biomarker for natural aging or damage to the CNS or PNS (i.e., dementia associated with Alzheimer's and Parkinson's diseases) (Donato, 2001; Donato, 1999; Fano et al., 1995).*
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> *Dose-dependent, statistically significant reduction in body-weight gain was observed in the mid-dose (at 12 weeks) and high-dose (at 8 weeks) rats, but food consumption data were not reported (Huang et al., 1989). Additionally, there were some neurological deficits in mid- and high-dose rats, including a reduction in grip strength and a comparative slowness of motion from week 12 of exposure. However, no hind-limb paralysis was observed by the time of termination of the experiment. Rats exposed to the mid and high doses of n-hexane showed a reduction in MCV. This reduction was statistically significant during weeks 8−16 of the exposure period compared with controls. Increased incidence of paranodal swelling, along with some evidence of demyelination and remyelination was present in the peripheral nerves at both mid and high doses. However, these histopathologic findings were more severe in the high-dose group. Among the biochemical changes were dose-dependent reductions in nervous system-specific proteins, particularly the ß-S100 protein in tail nerve fibers, which was significantly reduced by approximately 75% at all dose levels. The neurophysiologic deficits and histopathologic effects that were evident in mid- and high-dose rats suggested a NOAEL of 500 ppm and LOAEL of 1200 ppm.*

Given the systemic effect (peripheral neuropathy), *n*-hexane was treated as a Category 3 gas. For the chronic RfC, U.S. EPA (2005) estimated a $BMCL_{HEC}$ of 215 mg/m^3 from BMD modeling of the data on decreased motor nerve conduction velocity at 12 weeks in rats exposed via inhalation (Huang et al., 1989). Details on the modeling are available in the Toxicological Review (U.S. EPA, 2005). The $BMCL_{HEC}$ was used as the point of departure (POD). The $BMCL_{HEC}$ was divided by a total UF of 300 that included a 3-fold UF for interspecies extrapolation (dosimetric adjustments were used to extrapolate the toxicokinetic portion), a 10-fold UF for intraspecies variation, a 3-fold UF for extrapolation from subchronic to chronic exposure duration, and a 3-fold UF for database deficiencies (reflecting lack of multigeneration

reproductive and developmental studies following exposure to pure *n*-hexane and the uncertainty associated with low-dose developmental effects of exposure to *n*-hexane).

For the derivation of a subchronic p-RfC, the $BMCL_{HEC}$ of 215 mg/m^3 was divided by a total UF of 100 as follows:

Subchronic p-RfC = $BMCL_{HEC} \div UF$
= $215\ mg/m^3 \div 100$
= 2.2 or $\mathbf{2 \times 10^0\ mg/m^3}$

- An UF of 10 is applied for intraspecies differences to account for potentially susceptible individuals in the absence of information on the variability of response to humans.
- An UF of 3 is applied for interspecies extrapolation because the pharmacokinetic component of this factor is addressed by inhalation dosimetric conversion.
- An UF of 3 is applied for database deficiencies due to lack of a two-generation reproductive study and the potential for *n*-hexane-induced developmental neutrotoxicity. The database includes human occupational exposure studies, subchronic and developmental toxicity studies in rats and mice, which include evaluation of neurotoxicity following inhalation exposure to *n*-hexane. The database lacks a developmental neurotoxicity study and a multigeneration reproductive toxicity study. Prenatal exposure to pure *n*-hexane induced skeletal anomalies, decreased fetal body weight, and increased resorptions, suggesting that the fetus may be affected by *n*-hexane inhalation exposure (Mast et al., 1988a; Mast 1987; Bus et al., 1979). One of these studies indicated a developmental NOAEL of 200 ppm for reduced fetal body weight gain (Mast, 1987). However, it remains unclear whether these developmental effects occur at doses lower than those that cause neurotoxicity. Given the lack of a multigeneration reproductive toxicity study following exposure to *n*-hexane and the uncertainty associated with low-dose developmental effects of exposure to *n*-hexane, a database UF of 3 was applied.

Confidence in the principal study (Huang et al., 1989) is medium based on a relatively low but acceptable number of animals per group, investigation of endpoints relevant to the known critical target organ of this chemical, and use of an exposure range that encompassed a NOAEL and LOAEL for a duration exceeding the minimum acceptable duration for a subchronic study in rodents. Confidence in the database is low due to the lack of multigenerational reproductive studies of exposure to pure *n*-hexane. Thus, low confidence in the subchronic p-RfC follows.

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APPENDIX A. PERTINENT SECTIONS FROM IRIS SUMMARY FOR *n*-HEXANE: CHRONIC HEALTH HAZARD ASSESSMENTS FOR NONCARCINOGENIC EFFECTS

n-Hexane; CASRN 110-54-3; 12/23/2005

Human health assessment information on a chemical substance is included in IRIS only after a comprehensive review of chronic toxicity data by U.S. EPA health scientists from several program offices, regional offices, and the Office of Research and Development. Sections I (Chronic Health Hazard Assessments for Noncarcinogenic Effects) and II (Carcinogenicity Assessment for Lifetime Exposure) present the positions that were reached during the review process. Supporting information and explanations of the methods used to derive the values given in IRIS are provided in the guidance documents located on the IRIS website at http://www.epa.gov/iris/backgrd.html.

STATUS OF DATA FOR *n*-Hexane

File First On-Line 07/01/90

Category (section)	Status	Last Revised
Oral RfD Assessment (I.A.)	discussion	12/23/2005
Inhalation RfC Assessment (I.B.)	on-line	12/23/2005
Carcinogenicity Assessment (II.)	discussion	12/23/2005

_*I. Chronic Health Hazard Assessments for Noncarcinogenic Effects*

_I.A. Reference Dose for Chronic Oral Exposure (RfD)

Substance Name — n-Hexane
CASRN — 110-54-3
Section I.A. Last Revised — 12/23/2005

In general, the oral Reference Dose (RfD) is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The RfD is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis and is expressed in units of mg/kg-day. Please refer to the guidance documents at http://www.epa.gov/iris/backgrd.html for an elaboration of these concepts. Since RfDs can be derived for the noncarcinogenic health effects of substances that are also carcinogens, it is essential to refer to other sources of information concerning the carcinogenicity of this chemical substance. If the U.S. EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation will be contained in Section II of this file.

__I.A.1. Oral RfD Summary

Not available at this time.

No epidemiology or case report studies examining health effects in humans or chronic laboratory studies evaluating potential health effects in animals following oral exposure to n-hexane are available. An RfD for n-hexane cannot be derived in the absence of a suitable oral study of sufficient duration that evaluates an array of endpoints. The only study identified for oral exposure to n-hexane was of subchronic duration, utilized gavage exposure, and evaluated a small number (five/group) of animals (Krasavage et al., 1980). Several animals died in each dose group (two in the mid-dose and one in the high-dose groups, respectively) during the course of the study.

Krasavage et al. (1980) exposed five male COBS CD(SD) BR rats/group to doses of 0, 6.6, 13.2, and 46.2 mmol/kg (570 mg/kg) n-hexane by gavage, 5 days/week, for 90 days. The period of treatment and observation was extended to 120 days for those animals receiving 46.2 mmol/kg n-hexane to ensure that an overt neuropathological endpoint was detected. The onset of neuropathy was assessed by the initial appearance of hindlimb paralysis, at which point the animal was sacrificed and examined histopathologically. The appearance of hindlimb paralysis and giant axonal swellings were observed in the high-dose group (3/4 and 4/4, respectively). The Krasavage et al. (1980) study provided data on neurotoxicity only; lacked data on an adequate number of animals in the various dose groups; and lacked clear dose-response preventing the use of these data to develop an oral RfD.

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

__I.A.2. Principal and Supporting Studies (Oral RfD)
Not applicable.

__I.A.3. Uncertainty and Modifying Factors (Oral RfD)
Not applicable.

__I.A.4. Additional Studies/Comments (Oral RfD)
Not applicable.

For more detail on Susceptible Populations, exit to the toxicological review, Section 4.7 (PDF).

__I.A.5. Confidence in the Oral RfD

Not applicable.

For more detail on Characterization of Hazard and Dose Response, exit to the toxicological review, Section 6 (PDF).

__I.A.6. EPA Documentation and Review of the Oral RfD

Source Document — U.S. EPA, 2005a

This assessment was peer reviewed by a group of external scientists. Comments from the peer reviewers were evaluated carefully and considered by the Agency during the finalization of this assessment. A record of these comments is included in Appendix A of the Toxicological Review of n-Hexane (U.S. EPA, 2005a). ***To review this appendix, exit to the toxicological review, Appendix A, Summary of External Panel Peer Review and Public Comments and Disposition (PDF)***

Agency Completion Date -- 12/23/2005

__I.A.7. EPA Contacts (Oral RfD)

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

_I.B. Reference Concentration for Chronic Inhalation Exposure (RfC)

Substance Name — n-Hexane
CASRN — 110-54-3
Section I.B. Last Revised — 12/23/2005

In general, the Reference Concentration (RfC) is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily inhalation exposure of the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory effects). The inhalation RfC (generally expressed in units of mg/m^3) is analogous to the oral RfD and is likewise based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis.

Inhalation RfCs are derived according to the *Interim Methods for Development of Inhalation Reference Doses* (U.S. EPA, 1989) and subsequently, according to *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994). Since RfCs can also be derived for the noncarcinogenic health effects of substances that are carcinogens, it is essential to refer to other sources of information concerning the carcinogenicity of this chemical substance. If the U.S. EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation will be contained in Section II of this file.

An RfC of 2E-1 mg/m^3 was previously entered on the IRIS database in 1990. This value was based on a LOAEL of 204 mg/m^3 for neurotoxicity (electrophysical alterations) in humans

(Sanagi et al., 1980). A total uncertainty factor of 300 was applied to the LOAEL (uncertainty factors of 10 for intraspecies variability, 10 for the use of a LOAEL, and 3 for limited reproductive and chronic respiratory toxicity data). The subchronic National Toxicology Program (NTP, 1991) study (published in the literature as Dunnick et al., 1989) in which B6C3F1 mice were exposed to 0, 500, 1000, 4000, and 10,000 ppm n-hexane 6 hours/day, 5 days/week, or 1000 ppm n-hexane 22 hours/day, 5 days/week, via inhalation for 13 weeks was used as a coprincipal study. The critical effect in the subchronic study was epithelial lesions in the nasal cavity. The change in the principal study from the previous IRIS assessment is due primarily to the identification of new literature. The Sanagi et al. (1980) occupational exposure study reported co-exposure to acetone at a mean concentration of 39 ppm. More recent data suggest that co-exposure to acetone potentiates n-hexane metabolism and n-hexane-induced neurotoxicity (Cardona et al., 1996; Ladefoged et al., 1994, 1989; Larsen et al., 1991). Therefore, it is possible that the incidence or severity of the neurological changes observed by Sanagi et al. (1980) may have been a result of co-exposure to both solvents. Dunnick et al. (1989) was not retained as the coprincipal study for the derivation of the RfC in the current assessment because the study authors did not perform neurological histopathology at the mid-concentrations (500, 1000, 4000 ppm for 6 hours/day). The lack of histopathology is considered to be a significant deficiency in the Dunnick et al. (1989) study, since the nervous system appears to be the primary target of n-hexane-induced neurotoxicity (see Section 4.5.2 of the Toxicological Review of n-Hexane [U.S. EPA, 2005a]).

__I.B.1. Inhalation RfC Summary

Critical Effect	Experimental Doses*	UF	RfC
Peripheral neuropathy (decreased MCV at 12 weeks) Rat subchronic inhalation study Huang et al., 1989	BMC: 550 mg/m^3 BMCL: 430 mg/m^3 $BMCL_{ADJ}$: 215 mg/m^3 $BMCL_{HEC}$: 215 mg/m^3	300	7E-1 mg/m^3

* Conversion Factors and Assumptions — MW = 86.18. Assuming 25°C and 760 mm Hg, 1 ppm = 86.18/24.45 = 3.52 mg/m^3. Duration adjustment of exposure concentrations was employed (12 hours/day, 7 days/week): $BMCL_{ADJ}$ = 430 mg/m^3 x 12h/24h = 215 mg/m^3. The $BMCL_{HEC}$ was calculated for an extrarespiratory effect of a category 3 gas. The blood:gas (air) partition coefficient ($H_{b/g}$) value for n-hexane in humans (H) is 0.8 (Perbellini et al., 1985) whereas a value of 2.29 has been reported in rats (A) (Gargas et al., 1989). According to the RfC methodology (U.S. EPA, 1994), where the ratio of animal to human blood:air partition coefficients $[(H_{b/g})_A/(H_{b/g})_H]$ is greater than one, a value of one is used for the ratio by default. Thus, $BMCL_{HEC} = 215 \times [(H_{b/g})_A/(H_{b/g})_H] = 215$ mg/m^3.

__I.B.2. Principal and Supporting Studies (Inhalation RfC)

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

Male Wistar rats (eight/group) were exposed to 0, 500, 1200, or 3000 ppm (0, 1762, 4230, 10,574 mg/m^3) n-hexane (>99% pure) for 12 hours/day, 7 days/week for 16 weeks (Huang et al., 1989). The authors measured motor nerve conduction velocity (MCV) in the tail nerve along

with body weight before exposure and after 4, 8, 12, and 16 weeks of exposure to n-hexane. One animal from each group was sacrificed at 16 weeks exposure for histopathological evaluation of the nerve fibers in the tail. In addition, Huang et al. (1989) measured the levels of neuron-specific enolase and beta-S-100. These nervous system-specific proteins are a family of calcium binding proteins that are involved in processes such as cell-to-cell communication, cell growth, intracellular signal transduction, and development and maintenance of the central nervous system. A dose-dependent, statistically significant reduction in body weight gain was observed in the mid- (at 12 weeks) and high-dose (at 8 weeks) rats. Additionally, there were some neurological deficits in mid- and high-dose rats, including a reduction in grip strength and a comparative slowness of motion from week 12 of exposure. However, no hindlimb paralysis was observed by the termination of the experiment. Rats exposed to the mid and high doses of n-hexane showed a reduction in MCV. This reduction was statistically significant during weeks 8-16 of the exposure period compared with controls. Increased incidence of paranodal swellings, along with some evidence of demyelination and remyelination, was present in the peripheral nerves at both mid and high doses. However, these histopathological findings were more severe in the high dose group. Among biochemical changes, there were dose-dependent reductions in nervous system specific proteins, particularly the beta-S-100 proteins from tail nerve fibers, which were significantly reduced by approximately 75% at all dose levels. The neurophysiological deficits and histopathological effects that were evident in mid- and high-dose rats indicate a NOAEL of 500 ppm.

The Huang et al. (1989) study was selected as the principal study with peripheral neuropathy (decreased MCV at 12 weeks) in male rats as the critical effect. The available human and animal n-hexane inhalation exposure data suggest that the nervous system is the primary target of n-hexane toxicity (Sections 4.1.2 and 4.2.1 of the Toxicological Review of n-Hexane [U.S. EPA, 2005a]). In addition, Huang et al. (1989) evaluated a comprehensive array of neurological endpoints and an adequate number of animals and exposure groups and was of the appropriate quality for the derivation of the RfC. The Huang et al. (1989) data set provided an adequate dose response for BMD modeling with an estimated point of departure of a $BMCL_{HEC}$ of 215 mg/m^3 (Section 5.2.2 and Appendix B of the Toxicological Review of n-Hexane [U.S. EPA, 2005a]).

As described in Section 4.2.2 of the Toxicological Review of n-Hexane (U.S. EPA, 2005a), the toxic effects in laboratory animals following inhalation exposure to n-hexane support the nervous system as the primary target of toxicity. A number of studies identified a variety of effects on the nervous system, kidney, liver, and developing fetus at doses between 125-500 ppm (IRDC, 1992a, b; NTP, 1991; Dunnick et al., 1989; Huang et al., 1989; Mast et al., 1988a; Mast, 1987; Ono et al., 1982). These studies were considered for the selection of the principal study and are described below. Benchmark dose (BMD) modeling, where the data were amenable, was performed and is discussed in detail in Section 5.2.2 and Appendix B of the Toxicological Review of n-Hexane (U.S. EPA, 2005a).

Neurological deficits and respiratory lesions (mild epithelial lesions) were observed when B6C3F1 mice were exposed subchronically to 0, 500, 1000, 4000, and 10,000 ppm n-hexane, 6 hours/day, 5 days/week for 90 days or to 1000 ppm n-hexane for 22 hours/day, 5 days/week for 90 days (NTP, 1991; Dunnick et al., 1989). Dunnick et al. (1989) reported decreased locomotor activity and increased axonal swellings in the paranodal nerve in the 1000 ppm continuous exposure group (22 hours/day) and the 10,000-ppm exposure group (6 hours/day).

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

The International Research and Development Corporation (IRDC, 1992a) exposed male Sprague Dawley rats to 0, 125, and 500 ppm n-hexane subchronically for 6 months (22 hours/day, 7 days/week). n-Hexane exposure resulted in a significant decrease in mean absolute and relative liver and kidney weights at both doses. These changes in organ weights were not accompanied by any histopathological evidence of liver or kidney toxicity. In the second phase of this study, IRDC (1992b) demonstrated an increased incidence of chronic nephritis in 6/11 controls and 10/10 rats exposed to 500 ppm n-hexane. This response is considered equivocal due to the high incidence of kidney nephropathy in the control animals. Axonal degeneration and muscle atrophy were also observed but only at the high dose. The data on axonal degeneration and muscle atrophy are not amenable to BMD modeling because each effect lacks an adequate dose response for modeling (i.e., effects were seen at only the high dose). For example, 0/10, 0/10, and 7/10 animals showed tibial/sciatic nerve axonal degeneration and 0/10, 0/10, and 9/10 animals showed skeletal muscle atrophy at 0, 125, and 500 ppm, respectively. Finally, the results of this study are potentially compromised by possible co-exposure to a phthalate ester-type compound. The authors indicated that during exposure a brown oily material collected on the glass beads of the inhalation system for each exposure group. Samples of this brown material were subjected to infrared spectroscopy, which confirmed the presence of a phthalate ester-type compound. While the observed axonal degeneration at the high dose could constitute a LOAEL, the noted contamination compromises the results. Therefore, the IRDC (1992a, b) study was not selected as the principal study for the derivation of the RfC.

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

study. In addition, BMD modeling of the data produced poor goodness of fit values estimated from the data (Appendix B of the Toxicological Review for n-Hexane [U.S. EPA, 2005a]). Therefore, the Ono et al. (1982) study was not selected as the principal study for the derivation of the RfC.

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

Mast (1987) exposed pregnant Sprague-Dawley rats (30/group) to 0, 200, 1000, or 5000 ppm n-hexane for 20 hours/day on GDs 6-19. The authors observed a statistically significant reduction in fetal body weight gain in males at 1000 and 5000 ppm n-hexane exposure. A statistically significant increased incidence of reduced skeletal ossification of sternebrae 1-4 was also observed at 5000 ppm. This study identifies a developmental NOAEL of 200 ppm from these effects, but the range between the NOAEL and the next highest dose (1000 ppm) is considerable. This uncertainty in the dose response makes the selection of this study as the principal study questionable. Several additional studies have evaluated the effect of n-hexane exposure on the reproductive system and the developing fetus (Linder et al., 1992; Mast et al., 1988a, b; De Martino et al., 1987; Marks et al., 1980; Bus et al., 1979; Litton Bionetics Inc., 1979). In contrast to the studies by Mast (1987) and Mast et al. (1988a), these studies do not indicate that n-hexane exposure produces adverse reproductive and developmental effects. Nevertheless, BMD modeling was performed on the Mast (1987) data set. The results of the BMD modeling can be found in Section 5.2.2 and Appendix B of the Toxicological Review of n-Hexane (U.S. EPA, 2005a).

__I.B.3. Uncertainty and Modifying Factors (Inhalation RfC)

UF = 300.

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

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

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

Only one study with one dose group is available that directly observed susceptibility differences between adult and weanling animals (Howd et al., 1983). Several mode of action studies provide some evidence supporting the hypothesis that this increased susceptibility is because of differences in axonal length between adults and weanling rats. These studies did not directly observe effects of n-hexane on neurofilaments in weanling or young animals. Given the paucity of studies directly observing susceptibility differences between weanling and adult animals and the possibility of altered metabolic enzyme activity among individual humans and between adults and children, an UF_H of 10 was applied to account for variations in susceptible subpopulations.

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

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

A UF_D of 3 was applied to account for database deficiencies. The database includes many human occupational exposure studies (all with co-exposure to other potentially neurotoxic chemicals), subchronic animal studies in rats and mice, neurotoxicity studies in both humans and laboratory animals, and developmental studies in rats and mice following inhalation exposure to pure n-hexane. The database lacks a developmental neurotoxicity study and a multigeneration reproductive and developmental toxicity study following inhalation exposure to pure n-hexane

alone. Prenatal exposure to pure n-hexane induced skeletal anomalies, decreased fetal body weight, and increased resorptions, suggesting that the fetus may be affected by n-hexane inhalation exposure (Mast et al., 1988a; Mast 1987; Bus et al., 1979). One of these studies indicated a developmental NOAEL of 200 ppm for reduced fetal body weight gain (Mast, 1987). However, it remains unclear whether these developmental effects occur at doses lower than those that cause neurotoxicity. Studies investigating the reproductive and developmental effects of commercial hexane, a mixture containing approximately 50% n-hexane, are also available (see Section 4.4.2.2.3 of the Toxicological Review for n-Hexane [U.S. EPA, 2005a]). The studies with commercial hexane mixtures evaluated reproductive and developmental effects following exposure to doses of >= 500 ppm commercial hexane and resulted in marginal decreases in pup body weights and increased skeletal variations (BRRC, 1989a, b). Given the lack of a multigeneration reproductive and developmental studies following exposure to pure n-hexane and the uncertainty associated with low-dose developmental effects of exposure to n-hexane, an UF_D of 3 was applied.

An UF to account for the extrapolation from a LOAEL to a NOAEL was not applied because BMD modeling was used to determine the point of departure for derivation of the RfC.

__I.B.4. Additional Studies/Comments (Inhalation RfC)

Several studies provide support for the selection of Huang et al. (1989) as the principal study and peripheral neuropathy as the critical effect. Specifically, studies in humans exposed to n-hexane levels in the workplace in a range of approximately 30-200 ppm (130-690 mg/m^3) n-hexane show effects associated with peripheral neuropathy, such as decreased MCV (Yucesoy et al., 1999; Karakaya et al., 1996; Chang et al., 1992; Huang et al., 1991; Yokoyama et al., 1990; Huang and Chu, 1989; Mutti et al., 1982a, b; Sanagi et al., 1980). No human studies are available where exposure was to n-hexane alone.

Sanagi et al. (1980) monitored the neurophysiological performance of 14 workers exposed to n-hexane and other solvents in the mixing and drying jobs at a factory producing tungsten carbide alloy. The workers were examined for signs of neurological deficits compared to 14 workers who were not exposed to any solvents in the same factory (Sanagi et al., 1980). The 22 breathing zone monitoring samples taken biannually over a 2-year period had an 8-hour time weighted average (TWA) of 58 ppm for n-hexane and 39 ppm for acetone. No other solvent concentrations were reported by the study authors. Compared to controls, exposed workers reported a significantly increased occurrence of headache, hearing deficits, dysesthesia in limbs, and muscle weakness. Exposed workers also showed an increased incidence of neurological signs relating to muscle strength and reduced vibration sensation of the radial nerve. Neurophysiological findings suggested a delayed recovery from a slowing of motor nerve conduction in the posterior tibial nerve.

Mutti et al. (1982a) compared MCVs in a group of 95 shoe factory workers exposed to a mixture of hydrocarbons containing n-hexane and 52 unexposed workers from the same factory. Exposed workers were divided into two groups based on hydrocarbon exposure. The mean TWA for n-hexane of the 108 breathing zone samples taken was 243 mg/m^3 (69 ppm) in the mid-exposure group and 474 mg/m^3 (134 ppm) in the high-exposure group. When the severity of neurological

symptoms was compared, there was a gradation in response between the exposed groups, both of which displayed more severe symptoms than the controls.

Numerous additional occupational exposure studies involved exposure to other solvents including n-hexane. These studies indicate neurological symptoms predominate, including the impairment of color vision (Gobba and Cavalleri, 2003; Iregren et al., 2002; Issever et al., 2002; Seppalainen et al., 1979; Raitta et al., 1978) and the onset of symptoms similar to Parkinson's disease (Canesi et al., 2003; Vanacore et al., 2000; Hageman et al., 1999; Pezzoli et al., 1996, 1995, 1989).

Despite the large number of human inhalation exposure studies for n-hexane, these studies are considered inappropriate for dose-response assessment. The available occupational exposure studies and case reports contain insufficient data on the duration or concentration of n-hexane exposure and are confounded by co-exposure to other solvents including solvents that may potentiate n-hexane-induced toxicity. A variety of solvents such as toluene, methyl ethyl ketone, acetone, and xylene have been shown to potentiate n-hexane-induced neurotoxicity (see Section 4.4.3 of the Toxicological Review of n-Hexane [U.S. EPA, 2005a]). For example, it is possible that the incidence or severity of the neurological changes observed by Sanagi et al. (1980) may have been a result of co-exposure to both n-hexane and acetone. Supporting evidence for such an association comes from studies indicating that acetone may affect n-hexane metabolism, neurotoxicity, and reproductive toxicity following exposure to 2,5-hexanedione (Cardona et al., 1996; Ladefoged et al., 1994; Larsen et al., 1991; Ladefoged et al., 1989). A study in humans showed that acetone concentrations in the workplace significantly correlated with the ratio of urinary n-hexane metabolites (specifically 2,5-hexanedione) to n-hexane air concentrations (Cardona et al., 1996). It has been suggested that induction of n-hexane metabolism by acetone may potentiate neurotoxicity by decreasing the elimination of 2,5-hexanedione. For example, studies in rodents have shown that co-exposure to acetone and 2,5-hexanedione increases the concentration of 2,5-hexanedione in the sciatic nerve compared to administration of 2,5-hexanedione alone (Zhao et al., 1998; Ladefoged and Perbellini, 1986). In addition, acetone has been shown to induce CYP2E1, one of the enzymes showed to be involved in the metabolism of n-hexane to its toxic metabolite 2,5-hexanedione in rats (see Section 3.3 of the Toxicological Review of n-Hexane [U.S. EPA, 2005a]; Patten et al., 1986). Thus, co-exposure to acetone may induce CYP450 enzymes and increase the production of the neurotoxic metabolite, 2,5-hexanedione.

Oral co-exposure studies in rats further support acetone potentiation of n-hexane neurotoxicity (see Section 4.4.3 of the Toxicological Review of n-Hexane [U.S. EPA, 2005a]). Ladefoged et al. (1994, 1989) exposed male rats to 2,5-hexanedione alone and 2,5-hexanedione plus acetone in drinking water for 6 weeks and evaluated neurological and behavioral endpoints. Rats exposed to 2,5-hexanedione alone and 2,5-hexanedione plus acetone showed decreased balance time on a rotating rod, altered behavior (ambulation, grip strength, and rearing), decreased MCV, and increased giant axonal swelling of the sciatic nerve. The authors stated that these effects were greater in severity in the rats co-exposed to 2,5-hexanedione plus acetone compared with those exposed to 2,5-hexanedione. In addition, Larsen et al. (1991) suggested that co-exposure to acetone and 2,5-hexanedione may contribute to irreversible damage to the testis and male infertility in rats. Taken together, the data suggest that acetone may alter n-hexane metabolism

and potentiate n-hexane-induced neurotoxicity and reproductive toxicity. Accordingly, a reliable effects level cannot be identified from the available reports of occupational exposure.

Studies in animals also provide support for the selection of Huang et al. (1989) as the principal study. In a follow-up study, Huang et al. (1992) observed an overall reduction in MCV in rats exposed to 2000 ppm n-hexane, 12 hours/day, 6 days/week for a total of 24 weeks, with the onset of neurophysiological deficits most evident in the distal segment of the sciatic nerve. Other sections of the central and peripheral nervous systems were comparatively unaffected.

Altenkirch et al. (1982) exposed male Wistar rats (five/group) to 0, 500, or 700 ppm n-hexane for up to 9 weeks. Clinical signs included excessive salivation and an increase in paralysis of the hind limbs. The time for this condition to develop was shorter in those rats exposed to the higher concentrations of n-hexane and to the mixtures. Histopathological examinations of the peripheral nerves showed the presence of axonal swellings, especially at the branches of the tibial and ischiatic nerves. A breakdown of axons and myelin developed distal to the axonal swellings, with an apparent intra-axonal accumulation of neurofilaments. Other morphological findings included axonal swellings of the gracile tract of the spinal cord, especially at the level of the gracile nucleus in the medulla oblongata.

Howd et al. (1983), Pryor et al. (1983), and Ichihara et al. (1998) all used single concentrations of n-hexane in the 1000-2000 ppm range to induce neurophysiological deficits and/or behavioral changes in F344 or Wistar rats exposed to n-hexane. Data from the Chemical Industry Institute of Toxicology's 13-week toxicological study in F344 rats exposed to n-hexane (0, 3000, 6500, 10,000 ppm, respectively) confirmed the neuropathological responses to the n-hexane based on the appearance of paranodal swellings of the tibial nerves in mid- and high-dose males (Cavender et al., 1984a, b).

For more detail on Susceptible Populations, exit to the toxicological review, Section 4.7 (PDF).

__I.B.5. Confidence in the Inhalation RfC

Study — Medium
Data Base -- Medium
RfC — Medium

The overall confidence in this RfC assessment is medium. Confidence in the principal study (Huang et al., 1989) is medium; it involves a comparatively low but acceptable number of animals per group (eight/sex) and reports behavioral deficits, neurophysiological changes, and neuropathological effects within a dose range in which both a NOAEL and LOAEL could be identified. Numerous studies both in humans and laboratory animals support the selection of the nervous system as the target of n-hexane-induced toxicity. Confidence in the database is medium. The database lacks chronic exposure information on the pure compound via any route of exposure, a multigenerational developmental and reproductive toxicity study, and a developmental neurotoxicity study. The subchronic inhalation study of Huang et al. (1989) satisfies the minimum inhalation database requirements for deriving an RfC for n-hexane.

Reflecting medium confidence in the principal study and medium confidence in the database, confidence in the RfC is medium.

For more detail on Characterization of Hazard and Dose Response, exit to the toxicological review, Section 6 (PDF)

__I.B.6. EPA Documentation and Review of the Inhalation RfC

Source Document — U.S. EPA, 2005a

This assessment was peer reviewed by a group of external scientists. Comments from the peer reviewers were evaluated carefully and considered by the Agency during the finalization of this assessment. A record of these comments is included in Appendix A of the Toxicological Review of n-Hexane (U.S. EPA, 2005a). ***To review this appendix, exit to the toxicological review, Appendix A, Summary of External Panel Peer Review and Public Comments and Disposition (PDF)***

Agency Completion Date -- 12/23/2005

__I.B.7. EPA Contacts (Inhalation RfC)

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

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_VI.B. Inhalation RfC References

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