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n-Hexane; CASRN 110-54-3; 00/00/0000

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/iriswebp/iris/backgr-d.htm>.

STATUS OF DATA FOR n-Hexane

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<u>Category (section)</u>	<u>Status</u>	<u>Last Revised</u>
Oral RfD Assessment (I.A.)	discussion	00/00/0000
Inhalation RfC Assessment (I.B.)	on-line	00/00/0000
Carcinogenicity Assessment (II.)	discussion	00/00/0000

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**I. CHRONIC HEALTH HAZARD ASSESSMENTS FOR NONCARCINOGENIC EFFECTS**

**I.A. REFERENCE DOSE (RfD) FOR CHRONIC ORAL EXPOSURE**

n-Hexane  
CASRN –110-54-3  
Section I.A. Last Revised -- 00/00/0000

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/iriswebp/iris/backgr-d.htm> for an elaboration of these concepts. Since RfDs

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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

No epidemiology or case report studies examining health effects in humans or any chronic laboratory studies evaluating potential health effects in animals following oral exposure to n-hexane are available. An RfD for n-hexane cannot be derived in the absence of a suitable oral study of sufficient duration that evaluates an array of endpoints. The only oral study (Krasavage et al., 1980) identified for oral exposure to n-hexane is of subchronic duration, utilized gavage exposure, and evaluated a small number (5/group) of animals. Several animals died in each dose group (two in the mid-dose and one in the high-dose groups, respectively) during the course of the study.

Krasavage et al. (1980) exposed five male COBS CD(SD) BR rats/group to doses of 0, 6.6, 13.2, and 46.2 mmol/kg (570 mg/kg) n-hexane by gavage, 5 days/week, for 90 days. The period of treatment and observation was extended to 120 days for those animals receiving 46.2 mmol/kg n-hexane to ensure that an overt neuropathological endpoint was detected. The onset of neuropathy was assessed by the initial appearance of hindlimb paralysis, at which point the animal was sacrificed and examined histopathologically. The appearance of hindlimb paralysis in animals exposed to the high dose n-hexane (3/4) was observed. Giant axonal swellings were present in the nerves of the high dose group (4/4).

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

**\_\_ I.A.2. PRINCIPAL AND SUPPORTING STUDIES**

None.

**\_\_ I.A.3. UNCERTAINTY FACTORS**

None.

**\_\_ I.A.4. ADDITIONAL STUDIES/COMMENTS**

None.

**\_\_ I.A.5. CONFIDENCE IN THE ORAL RfD**

None.

**\_\_ I.A.6. EPA DOCUMENTATION AND REVIEW OF THE ORAL RfD**

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This assessment will be peer reviewed by a group of external scientists. Comments from the peer reviewers will be evaluated carefully and considered by the Agency during the finalization of this assessment. A record of these comments will be included in Appendix A of the Toxicological Review of n-Hexane (U.S. EPA, 2005).

Agency Completion Date -- \_\_/\_\_/\_\_

**\_\_ I.A.7. EPA CONTACTS**

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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**\_\_ I.B. REFERENCE CONCENTRATION (RfC) FOR CHRONIC INHALATION EXPOSURE**

n-Hexane  
CASRN -110-54-3  
Section I.B. Last Revised -- 00/00/0000

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<sup>3</sup>) 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). Because 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<sup>3</sup> was previously entered on the IRIS data base in 1990. This value was based on a LOAEL of 204 mg/m<sup>3</sup> for neurotoxicity (electrophysical alterations) in humans (Sangi 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 NTP (1991) study (published in the literature as Dunnick et al., 1989) in which B6C3F1 mice were exposed to 0, 500, 1000, 4000, and 10000 ppm 6 hours/day, 5 days/week or 1000 ppm 22 hours/day, 5 days/ week n-hexane via inhalation for 13 weeks was used as a co-principal study. The critical effect in the subchronic study was epithelial lesions in the nasal cavity. The 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 suggests that co-exposure to acetone potentiates n-hexane metabolism and n-hexane induced neurotoxicity (Ladefoged et al., 1989; Larsen et al., 1991; Ladefoged et al., 1994; Cardona et al., 1996). Therefore, it is possible that the incidence or severity of the neurological changes observed by Sanagi et al., (1980) may have been a result of co-exposure to both solvents. Dunnick et al. (1989) was not retained as the co-principal 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 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).

**I.B.1. INHALATION RfC SUMMARY**

<u>Critical Effect</u>	<u>Experimental Doses*</u>	<u>UF</u>	<u>RfC</u>
Peripheral neuropathy (decreased MCV at 12 weeks)	BMC: 550 mg/m <sup>3</sup> BMCL: 430 mg/m <sup>3</sup>	1000	2E-1 mg/m <sup>3</sup>



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

Neurological deficits and respiratory lesions (mild epithelial lesions) were observed when B6C3F1 mice were exposed subchronically to 0, 500, 1000, 4000, and 10,000 ppm n-hexane, 6 hours/day, 5 days/week for 90 days or to 1,000 ppm n-hexane for 22 hours/day 6 hours/day, 5 days/week for 90 days (Dunnick et al., 1989; NTP, 1991). Dunnick et al. (1989) reported decreased locomotor activity and increased axonal swellings in the paranodal nerve in the 1000 ppm-continuous exposure group (22 hours/day) and the 10,000-ppm exposure group (6 hours/day). Histopathology of the spinal cord and tibial nerve was performed in 4 animals/sex from the 0, 1000 ppm continuous exposure, and the 10,000 ppm exposure groups only. The NOAEL (500 ppm) was based on the appearance of mild epithelial lesions in the nasal cavity. The authors suggested that this effect was more severe in the 1000-ppm continuous exposure group (22 hours/day) than the 4000-ppm exposure group (6 hour/day). They also considered these effects to be non-specific and indicative of inflammatory and regenerative changes secondary to the effects of the inhaled irritant. The authors were unclear as to whether the altered morphology was due to inflammation or direct action of n-hexane. Thus, the study authors stated that the nasal irritation was most likely secondary to the inhaled irritant. In addition, the absence of sufficient neuropathological information from the mid-concentration groups (i.e., 500, 1000, 4000 ppm for 6 hours/day) is considered to represent a significant deficiency in the interpretation of the Dunnick et al. (1989) study. Therefore, the NTP (1991)/Dunnick et al. (1989) study was not selected as the principal study for the derivation of the RfC.

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

exposure group. Samples of this brown material were subjected to infrared spectroscopy which confirmed the presence of a phthalate ester-type compound. While the observed axonal degeneration at the high dose could constitute a LOAEL, the noted contamination compromises the results. Therefore, the IRDC (1992) was not selected as the principal study for the derivation of the RfC.

Ono et al. (1982) observed subchronic effects of n-hexane on the nervous system in male Wistar rats (8/group) exposed to 0, 200, and 500 ppm n-hexane for 12 hours/day for 24 weeks. Only one animal from each group was examined histopathologically in an attempt to link any functional deficits to morphological changes that may have taken place over the duration of the experiment. The authors stated that they did not observe any definite clinical signs of neuropathy in any of the exposed groups. MCV and mixed MCVs (distal and both proximal and distal combined) were statistically significantly decreased in rats exposed to n-hexane at both 200 and 500 ppm. DL and proximal mixed MCV were statistically significantly decreased at the low dose, but not the high dose. Degeneration of the myelin sheath axons was evident in the peripheral nerves at both exposures (histopathology in one animal). While the observed decreases in MCV could constitute a LOAEL, the lack of observed clinical neuropathy and failure to evaluate nerve histopathology on a larger number of animals are limitations of this study. In addition, BMD modeling of the data was inadequate for derivation of the point of departure. Specifically, the goodness of fit p value could not be estimated from the data (Appendix B). Therefore, the Ono et al. (1982) study was not selected as the principal study for the derivation of the RfC.

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

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

Huang et al. (1989) exposed Wistar rats (8/group) via inhalation to 0, 500, 1200, or 3000 ppm (0, 1,762, 4,230, 10,574 mg/m<sup>3</sup>) n-hexane, 12 hours/day, 7 days/week for 16 weeks. Statistically significant, group-specific, dose-dependent changes in MCV were obtained in the mid- and high-concentration groups, but not in the low-concentration group. Histopathological changes to the peripheral nerves were marked by paranodal swellings and demyelination. These changes were most apparent in high-dose rats, but occurred in mid-dose animals as well. Rats exposed to mid- and high-concentrations of n-hexane in the Huang et al. (1989) study also showed some signs of behavioral deficits, including a reduction in grip strength and slowness of motion. This study was considered further for selection as the principal study for the derivation of the RfC. The data for changes in MCV were subjected to BMD modeling (Section 5.2.2 and Appendix B).

The Huang et al. (1989) study was selected as the principal study with peripheral neuropathy (decreased MCV) as the critical effect. The available human and animal n-hexane inhalation exposure data suggest that the nervous system is the primary target of n-hexane toxicity (Sections 4.1.2 and 4.2.1). Most of the reproductive and developmental studies suggest that n-hexane does not adversely affect these endpoints. For this reason and due to the uncertainty in the dose-response, the Mast (1987) study that evaluated developmental effects was considered, but not selected as the principal study for the derivation of the RfC. In addition, Huang et al. (1989) evaluated a comprehensive array of neurological endpoints, adequate number of animals and exposure groups, and was of the appropriate quality for the derivation of the RfC. The Huang et al. (1989) data set provided an adequate dose-response for BMD modeling with an estimated point of departure of a BMCL<sub>HEC</sub> of 215 mg/m<sup>3</sup> (Section 5.2.2 and Appendix B).

Several studies provide support for the selection of Huang et al. (1989) as the principal study and peripheral neuropathy as the critical effect. Specifically, studies in humans exposed to n-hexane levels in the workplace in a range of approximately 30-200 ppm (130-690 mg/m<sup>3</sup>) n-hexane show effects associated with peripheral neuropathy, such as decreased MCV (Sanagi et al., 1980; Mutti et al., 1982a; Mutti et al., 1982b; Huang and Chu, 1989; Yokoyama et al., 1990; Huang et al., 1991; Chang et al., 1992; Karakaya et al., 1996; Yucesoy et al., 1999). Studies in animals also provide support for the selection of Huang et al. (1989) as the principal study. In a follow up study, Huang et al. (1992) observed an overall reduction in MCV in rats exposed to 2000 ppm n-hexane, for 12 hours/day, 6 days/week for a total of 24 weeks, with the onset of neurophysiological deficits most evident in the distal segment of the sciatic nerve. Other sections of the central and peripheral nervous systems were comparatively unaffected. Howd et al. (1983), Pryor et al. (1983), and Ichihara et al. (1998) all used single concentrations of n-hexane in the 1000-2000 ppm range to induce compound-related neurophysiological deficits and/or behavioral changes in F-344 or Wistar rats exposed to n-hexane. Data from CIIT's 13-week toxicological study in F-344 rats exposed to n-hexane (0, 3,000, 6,500, 10,000 ppm, respectively) confirmed the neuropathological responses to the compound based on the appearance of paranodal swellings of the tibial nerves in mid- and high-dose males (Cavender et al., 1984a,b).

### I.B.3. UNCERTAINTY FACTORS

UF = 1000.

A total UF of 1000 was applied to the point of departure of 215 mg/m<sup>3</sup>: 10 for intraspecies variation (UF<sub>H</sub>: human variability); 3 for interspecies differences (UF<sub>A</sub>); 10 to extrapolate to chronic exposure from data in a less-than lifetime study (UF<sub>S</sub>); and 3 to account for database deficiencies (UF<sub>D</sub>).

An UF<sub>H</sub> of 10 was applied to account for variations in susceptibility among members of the human population (interindividual variability). In the absence of information on the variability in humans to n-hexane exposure the default of 10 was used.

An UF<sub>A</sub> 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<sub>ADJ</sub> to a BMCL<sub>HEC</sub> 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 by a certain degree by the use of the applied dosimetry method.

An UF<sub>S</sub> of 10 was applied to extrapolate from subchronic to chronic exposure. A subchronic (16 weeks) study was used for the derivation of the RfC.

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

Several studies provide support for the selection of Huang et al. (1989) as the principal study and neuropathological effects as the critical effect. Occupational studies and case reports suggest that inhalation exposure to n-hexane in humans may be associated with neurotoxicity (see Section 4.1.2 of the Toxicological Review). 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 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<sup>3</sup> (69 ppm) in the mid-exposure group and 474 mg/m<sup>3</sup> (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 involving exposure to other solvents including n-hexane. These studies indicate neurological symptoms predominate including the impairment of color vision (Raitta et al., 1978; Seppalainen et al., 1979, Issever, et al., 2002, Iregren et al., 2002; Gobba and Cavalleri, 2003) and the onset of symptoms similar to Parkinson's disease (Pezzoli et al., 1989, 1995, 1996; Hageman et al., 1999, Vanacore et al., 2000; Canesi et al., 2003).

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). 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 (Ladefoged et al., 1989; Larsen et al., 1991; Ladefoged et al., 1994; Cardona et al., 1996). A study in humans showed that acetone concentrations in the workplace significantly correlated with the ratio of urinary n-hexane metabolites (specifically 2,5-hexanedione) to air n-hexane concentrations (Cardona et al., 1996). It has been suggested that induction of n-hexane metabolism by acetone may potentiate neurotoxicity by decreasing the elimination of 2,5-hexanedione. For example, studies in rodents have shown that co-exposure to acetone and 2,5-hexanedione increases the concentration of 2,5-hexanedione in the sciatic nerve compared to administration of 2,5-hexanedione alone (Ladefoged and Perbellini, 1986; Zhao et al., 1998). In addition, acetone has been shown to induce CYP2E1, one of the enzymes 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; 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). Ladefoged et al. (1989, 1994) exposed male rats to 2,5-hexanedione alone and 2,5-hexanedione plus acetone in drinking water for 6 weeks and evaluated neurological and behavioral endpoints. Rats exposed to 2,5-hexanedione alone and 2,5-hexanedione plus acetone showed decreased balance time on a rotating rod, altered behavior (ambulation, grip strength, and rearing), decreased MCV, and increased giant axonal swelling of the sciatic nerve. The authors stated that these effects were greater in severity in the rats co-exposed to 2,5-hexanedione plus acetone compared to those exposed to 2,5-hexanedione. In addition, Larsen et al. (1991) suggested that co-exposure to acetone and 2,5-hexanedione may contribute to irreversible damage to the testis and male infertility in rats. Taken together, the data suggest that acetone may alter n-hexane metabolism and potentiate n-hexane induced neurotoxicity and reproductive toxicity. Accordingly, reliable effects level cannot be identified from the available reports of occupational exposure.

The database of studies in laboratory animals exposed to n-hexane via inhalation is fairly large and numerous neurological studies are available which support selection of the principal study and critical effect (see Section 4.5.2., U.S. EPA, 2005). A review of some of these studies is presented below.

Cavender et al. (1984) exposed F-344 (15/sex/group) to n-hexane at 0, 3000, 6500, and 10,000 ppm for 6 hours/day, 5 days/week for 13 weeks, but observed no compound-related clinical signs, effects on food consumption, ophthalmological findings, or changes in neurological function. However, there were increased organ/body weight ratios for liver, kidney, and testis in high-dose males and kidney in mid-dose males. Histopathological examination of the tibial nerves revealed paranodal axonal swellings in four of five high-dose males and one of five mid-dose males.

Pryor et al. (1983) exposed F-344 rats (13-14 male/group) to 0 or 2000 ppm n-hexane (95% pure) 14 hours/day, 7 days/week for 14 weeks. Animals were subjected to a battery of

behavioral tests at intervals throughout the study including grip strength, motor activity, startle and avoidance responses, and the acquisition of a multisensory, conditioned avoidance response. Impaired performance and compound-related latencies in animals' responses to various stimuli were recorded. Exposure to 2000 ppm n-hexane was associated with a statistically significant reduction in undifferentiated motor activity, startle to an air-puff response, and fore- and hindlimb grip strength. There was also a reduction in the amplitude of the fifth component of the brainstem auditory-evoked response. No impairment of performance was seen in some behavioral tests, including the acquisition of a multisensory conditioned pole-climb avoidance response and a tone-intensity discrimination task. Additionally, no histopathological effects on the peripheral nerves were observed at term (14 weeks).

Howd et al. (1983) exposed weanling and adult F-344 rats to 0 or 1000 ppm n-hexane (95% pure) 24 hours/day, 6 days/week for 11 weeks and found greater incidence of behavioral signs of neuropathy in adults compared to weanlings. Grip strength was reduced equally in older rats and weanlings within 2 weeks of the beginning of the exposure period. However, subsequent effects of treatment on this parameter were greater in young adults versus weanlings. Older rats exhibited earlier and more severe signs of hindlimb flaccid paralysis than did younger animals. Conversely, there was little difference in neurophysiological responses between rats in the different age categories, including tail nerve conduction time and a brainstem auditory-evoked response.

Ichihara et al. (1998) exposed male Wistar rats (8/group) to 0 or 2000 ppm n-hexane, 12 hours/day, 6 days/week for 20 weeks and showed that MCV decreased and distal latency (DL) increased compared to controls. The n-hexane metabolite, 2,5-hexanedione, was detected in the urine of exposed animals but not in that of controls.

Altenkirch et al. (1982) exposed male Wistar rats (five/group) to 0, 500 or 700 ppm n-hexane, 300 ppm n-hexane plus 200 ppm methyl ethyl ketone, 400 ppm n-hexane plus 100 ppm methyl ethyl ketone, and 500 ppm n-hexane plus 200 ppm methyl ethyl ketone, 22 hours/day, 7 days/week for up to 9 weeks. Animals were observed for clinical signs of toxicity throughout the experiment, and histopathological examinations of excised brain, spinal cord, and peripheral nerves were performed at term. All exposed rats survived to term, although some groups showed a reduction in body weight gain during the lifetime of the experiment. Clinical signs included excessive salivation and an increase in paralysis of the hind limbs. The time for this condition to develop was shorter in those rats exposed to the higher concentrations of n-hexane and to the mixtures. Histopathological examinations of the peripheral nerves showed the presence of axonal swellings, especially at the branches of the tibial and ischiatic nerves. A breakdown of axons and myelin developed distal to the axonal swellings, with an apparent intra-axonal accumulation of neurofilaments. Other morphological findings included axonal swellings of the gracile tract of the spinal cord, especially at the level of the gracile nucleus in the medulla oblongata.

A second phase of the study exposed male Wistar rats (five/group) to 0 or 700 ppm n-hexane or 500 ppm n-hexane plus 200 ppm methyl ethyl ketone, 8 hours/day, 7 days/week for 40 weeks (Altenkirch et al., 1982). The animals displayed neither the clinical signs of n-hexane-induced peripheral neuropathy nor the axonal swellings and peripheral nerve fiber degeneration that marked the histopathological responses in those animals exposed continuously for 9 weeks. After 40 weeks there was some evidence of nerve fiber destruction in all groups, including controls. These changes were thought to be age-related. Altenkirch et al. (1982) concluded that male Wistar rats exposed 8 hours/day for 40 weeks to either 700 ppm n-hexane or 500 ppm n-hexane plus 200 ppm methyl ethyl ketone developed no neuropathological or clinical signs of neuropathy. This contrasted with the rats exposed to the same concentrations for 24 hours/day in the first phase of the study. The rats in the first study developed clinical neuropathy after 4 weeks.

Huang et al. (1992) exposed male Wistar rats to 0 or 2000 ppm (99% pure) n-hexane, for 12 hours/day, 6 days/week for 24 weeks. Effects of treatment included an overall reduction in MCV after 8 weeks and an increase in DL after 12 weeks. There was a reduction in the activity or amount of neuron-specific enolase ( $\gamma$ -enolase), creatine kinase-B, and the  $\beta$ -S-100 protein with neurophysiological deficits that were most evident in the distal segment of the sciatic nerve (near the knee). Other sections of the central and peripheral nervous systems were comparatively unaffected.

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

The authors noted a number of fluctuations in clinical chemistry, including higher fasting glucose levels in male rats exposed to 5 ppm and 125 ppm n-hexane at 26 weeks, and lower blood urea nitrogen in female rats exposed to 125 ppm n-hexane for the same duration. There were also fluctuations in hematological parameters, including reductions in hemoglobin concentration and hematocrit in females exposed at all n-hexane concentrations and durations at the 13-week measurement interval. However, these changes showed little relationship to dose, remained within normal limits, and were not apparent in blood samples taken after 26 weeks. Accordingly, the study authors considered the changes to not be related to treatment. An addendum to the report concluded that no animal in the study displayed signs of nervous system degeneration characteristic of n-hexane exposure.

#### **\_\_\_ 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. Animal studies in a second species (mice) corroborate the primacy of the neurological endpoint and confirm the validity of the critical effect for peripheral neuropathy. Confidence in the database is medium. The database lacks chronic exposure information on the pure compound via any route of exposure and a multi-generational developmental and reproductive toxicity study and a developmental neurotoxicity study. The subchronic inhalation study of Huang et al. (1989) satisfies the minimum inhalation database requirements for deriving an RfC for n-hexane. Reflecting medium confidence in the principal study and medium confidence in the database, confidence in the RfC is medium.

#### **\_\_\_ I.B.6. EPA DOCUMENTATION AND REVIEW OF THE INHALATION RfC**

Source Document -- U.S. EPA, 2005

This assessment will be peer reviewed by a group of external scientists. Comments from the peer reviewers will be evaluated carefully and considered by the Agency during the finalization of this assessment. A record of these comments will be included in Appendix A of the Toxicological Review of n-Hexane (U.S. EPA, 2005).

Agency Completion Date -- \_\_\_/\_\_\_/\_\_\_

#### **\_\_\_ 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).

## **II. CARCINOGENICITY ASSESSMENT FOR LIFETIME EXPOSURE**

n-Hexane

CASRN –110-54-3

Section II Last Revised -- 00/00/0000

Section II provides information on three aspects of the carcinogenic assessment for the substance in question: the weight-of-evidence judgment of the likelihood that the substance is a human carcinogen, and quantitative estimates of risk from oral exposure and inhalation exposure. Users are referred to Section I of this IRIS file for information on long-term toxic effects other than carcinogenicity.

The rationale and methods used to develop the carcinogenicity information in IRIS is described in the Draft Revised Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1999. Guidelines for carcinogen risk assessment. Review Draft, NCEA-F-0644, July. Risk Assessment Forum. <http://www.epa.gov/ncea/raf/cancer.htm>). The quantitative risk estimates result from application of a low-dose extrapolation procedure, and both the central estimate and upper bound estimate of risk per unit of exposure are presented. The quantitative risk estimates are presented in three ways to facilitate their use. The oral slope factor is the 95% upper bound on the estimate of risk per (mg/kg)/day of oral exposure. The unit risk is the 95% upper bound on the estimate of risk, either per µg/L drinking water or per µg/m<sup>3</sup> air breathed. The third form in which risk is presented is the 95% lower bound on the estimated concentration of the chemical in drinking water or air associated with cancer risks of 1 in 10,000, 1 in 100,000, or 1 in 1,000,000.

### **II.A. EVIDENCE FOR HUMAN CARCINOGENICITY**

#### **II.A.1. WEIGHT-OF-EVIDENCE CHARACTERIZATION**

Under EPA's Draft Revised Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1999), *data are inadequate for an assessment of the human carcinogenic potential of n-hexane* because a study (Beall et al., 2001) in humans chronically-exposed to n-hexane and other chemicals was inconclusive and there are no available animal studies examining exposure to n-hexane alone. A 2-year carcinogenicity bioassay in mice and rats exposed to a mixture containing various hydrocarbons, including n-hexane, showed an increased incidence of liver tumors in female mice (Biodynamics, 1993a; 1993b; Daughtrey et al., 1999). Daughtrey et al. (1999) observed an increased incidence of combined hepatocellular adenomas and carcinomas in female mice exposed to the highest dose of a mixture containing n-hexane (commercial hexane). In addition, the study authors identified a statistically significant trend for increased incidence of pituitary adenomas in female mice exposed to commercial hexane. Studies indicate that n-

hexane is mostly nongenotoxic in short-term testing protocols. n-Hexane showed a minimal response in *Saccharomyces cerevisiae* D61.M (Mayer and Goin, 1994) and induced an increased incidence in the number of chromosomal mutations in albino rat bone marrow cells (Hazleton Laboratories, 1992). The available studies in humans as well as laboratory animals thus far have not demonstrated a carcinogenic effect. The previous IRIS assessment (1990) did not contain a characterization of the carcinogenic potential of n-hexane in humans.

## **II.A.2. HUMAN CARCINOGENICITY DATA**

Only one of the occupational exposure studies on n-hexane has inferred a possible association between the compound and increased cancer incidence (Beall et al., 2001). Beall et al. (2001) conducted a nested case control study evaluating the relationship between the occurrence of intracranial tumors among employees at a petrochemical plant and exposure to chemicals including ionizing radiation, methylene chloride, acrylonitrile, vinyl chloride, formaldehyde, n-hexane, and various other chlorinated, halogenated, volatile, and aromatic hydrocarbons and nitroso compounds. The workers were also exposed to organometallic and elemental metallic catalysts. The study authors selected subjects from approximately 2595 plant workers. The workers were mailed questionnaires that evaluated work history in the plant and a total of 12 cases of intracranial tumors, which developed after hire dates at the plant, were identified from the respondents. All cases were confirmed by review of medical records and pathology specimens by four neuropathologists. Six of these cases, all of which were men, had primary brain cancers or gliomas (2 astrocytomas, 2 glioblastomas, and 2 oligodendrogliomas). Six cases had benign intracranial tumors, of which 2 were diagnosed as vestibular schwannomas (observed in 1 man and 1 woman), 2 as meningiomas (both in men), and 2 pituitary adenomas (observed in 1 man and 1 woman). Ten healthy controls were matched to each case by age, gender, birth year, race and an initiation date for work in the building complex that was prior to the tumor diagnosis date for the case. The median length of employment at the facility was 16.8 years for cases and 10.9 years for controls.

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

confidence interval were reported.

The authors showed that the OR for self-reported exposure to n-hexane was statistically significantly elevated (OR, infinity), with a confidence interval (CI) of 1.4 to infinity (6 cases and 26 controls evaluated) for gliomas. The OR for potential exposure to n-hexane based on job-related exposure estimates was 2.3 (CI, 0.4 to 13.7; 4 cases and 26 controls evaluated) for gliomas. Analyses by duration indicated a statistically significantly elevated OR of 16.2 (CI, 1.1 to 227.6; 2 cases and 2 controls evaluated) for potential long-term use of n-hexane (> 48 months) for gliomas. No relationship was found between exposure to n-hexane and the occurrence of intracranial tumors. While the results of this study indicated that exposure to n-hexane may have contributed to the occurrence of brain tumors, specifically gliomas, the small number of cases, large number of chemicals to which the employees were potentially exposed, and high correlation between some of the parameters reduce the significance of this result.

### **\_\_\_II.A.3. ANIMAL CARCINOGENICITY DATA**

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

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

### **\_\_\_II.A.4. SUPPORTING DATA FOR CARCINOGENICITY**

n-Hexane has shown little evidence of mutagenic activity in a number of short-term test systems. *In vitro* tests showed that n-hexane was not genotoxic in the Salmonella (Ames) assay (with or without activation), did not cause DNA damage of *E. coli* or *B. subtilis*, was negative for chromosomal aberrations in Chinese hamster ovary cells and forward mutations in the mouse lymphoma L5178 tk<sup>+/−</sup> assay (Mortelmans et al., 1986; Ishidate et al., 1984; Houk et al., 1989; McCarroll et al., 1981a,b; NTP, 1991; Daughtrey et al., 1994; Hazleton Laboratories, 1992). n-Hexane was marginal for inducing chromosome loss in the DNA of *S. cerevisiae* D61M (Mayer and Goin, 1994). In *in vivo* tests, the compound was negative for inducing dominant lethal mutations in CD-1 mice (Litton Bionetics, 1980; Mast, 1988b). Furthermore, n-hexane was unable to induce chromosomal aberrations (CA) and micronuclei in bone marrow cells of B6C3F1 mice injected intraperitoneally with the compound (Shelby and Witt, 1995). n-Hexane

did not increase the incidence of sister chromatid exchanges (SCEs) in *in vivo* mouse bone marrow cells (NTP, 1991). Hazleton Laboratories (1992) recorded a slight, but significant, increase in the number of chromosomal mutations due to n-hexane exposure in albino rat bone marrow cells.

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**\_\_II.B. QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FROM ORAL EXPOSURE**

Not applicable since data are inadequate for derivation of an oral slope factor for n-hexane.

**\_\_II.B.1. SUMMARY OF RISK ESTIMATES**

Not applicable.

**\_\_II.B.2. DOSE-RESPONSE DATA**

Not applicable.

**\_\_II.B.3. ADDITIONAL COMMENTS**

Not applicable.

**\_\_II.B.4. DISCUSSION OF CONFIDENCE**

Not applicable.

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**\_\_II.C. QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FROM INHALATION EXPOSURE**

Not applicable since data are inadequate for derivation of inhalation unit risk for n-hexane.

**\_\_II.C.1. SUMMARY OF RISK ESTIMATES**

Not applicable.

**\_\_II.C.2. DOSE-RESPONSE DATA**

Not applicable.

**\_\_II.C.3. ADDITIONAL COMMENTS**

Not applicable.

**\_\_II.C.4. DISCUSSION OF CONFIDENCE**

Not applicable.

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**\_\_II.D. EPA DOCUMENTATION, REVIEW, AND CONTACTS (CARCINOGENICITY ASSESSMENT)**

**\_\_II.D.1. EPA DOCUMENTATION**

Source Document -- U.S. EPA, 2005

This assessment will be peer reviewed by a group of external scientists. Comments from the peer reviewers will be evaluated carefully and considered by the Agency during the finalization of this assessment. A record of these comments will be included in Appendix A of the Toxicological Review of n-Hexane (U.S. EPA, 2005).

**\_\_II.D.2. EPA REVIEW**

Agency Completion Date -- \_\_/\_\_/\_\_

**\_\_II.D.3. EPA CONTACTS**

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).

\_III. [reserved]

\_IV. [reserved]

\_V. [reserved]

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**\_VI. BIBLIOGRAPHY**

n-hexane

CASRN –110-54-3

Section VI. Last Revised –00/00/0000

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## **\_VII. REVISION HISTORY**

n-hexane

CASRN -- 110-54-3

File First On-Line 07/01/1990

<u>Date</u>	<u>Section</u>	<u>Description</u>
07/01/1990	I.B.	Inhalation RfC summary on-line
07/01/1990	VI.	Bibliography on-line
02/01/1991	I.B.	Text edited
09/01/1991	II.	Carcinogenicity assessment now under review
07/01/1993	I.B.1.	LOAEL(HEC) added
08/01/1995	II.	EPA's RfD/RfC and CRAVE workgroups were discontinued in May, 1995. Chemical substance reviews that were not completed by September 1995 were taken out of IRIS review. The IRIS Pilot Program replaced the workgroup functions beginning in September, 1995.
04/01/1997	III., IV., V.	Drinking Water Health Advisories, EPA Regulatory Actions, and Supplementary Data were removed from IRIS on or before April 1997. IRIS users were directed to the appropriate EPA Program Offices for this information.
12/03/2002	I.B.6.	Screening-Level Literature Review Findings message has been added. 07/30/2003 I., II. This chemical is being reassessed under the IRIS Program.
00/00/0000	I., II, VI	New RfD, RfC and cancer assessment.

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## **\_VIII. SYNONYMS**

n-hexane

110-54-3

hexyl hydride

Skellysolve B

NCI-C60571

Section VIII Last Revised -- 00/00/0000

2/8/05

DRAFT-DO NOT CITE OR QUOTE