# Toxicological Profile for *n*-Hexane

**Draft for Public Comment**

**May 2024**

U.S. Department of Health and Human Services
Agency for Toxic Substances and Disease Registry

CS274127-A

# DISCLAIMER

Use of trade names is for identification only and does not imply endorsement by the Agency for Toxic Substances and Disease Registry, the Public Health Service, or the U.S. Department of Health and Human Services.

This information is distributed solely for the purpose of pre dissemination public comment under applicable information quality guidelines. It has not been formally disseminated by the Agency for Toxic Substances and Disease Registry. It does not represent and should not be construed to represent any agency determination or policy.

# FOREWORD

This toxicological profile is prepared in accordance with guidelines developed by the Agency for Toxic Substances and Disease Registry (ATSDR) and the Environmental Protection Agency (EPA). The original guidelines were published in the *Federal Register* on April 17, 1987. Each profile will be revised and republished as necessary.

The ATSDR toxicological profile succinctly characterizes the toxicologic and adverse health effects information for these toxic substances described therein. Each peer-reviewed profile identifies and reviews the key literature that describes a substance's toxicologic properties. Other pertinent literature is also presented, but is described in less detail than the key studies. The profile is not intended to be an exhaustive document; however, more comprehensive sources of specialty information are referenced.

The focus of the profiles is on health and toxicologic information; therefore, each toxicological profile begins with a relevance to public health discussion which would allow a public health professional to make a real-time determination of whether the presence of a particular substance in the environment poses a potential threat to human health. The adequacy of information to determine a substance's health effects is described in a health effects summary. Data needs that are of significance to the protection of public health are identified by ATSDR.

Each profile includes the following:

(A) The examination, summary, and interpretation of available toxicologic information and epidemiologic evaluations on a toxic substance to ascertain the levels of significant human exposure for the substance and the associated acute, intermediate, and chronic health effects;

(B) A determination of whether adequate information on the health effects of each substance is available or in the process of development to determine the levels of exposure that present a significant risk to human health due to acute-, intermediate-, and chronic-duration exposures; and

(C) Where appropriate, identification of toxicologic testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans.

The principal audiences for the toxicological profiles are health professionals at the Federal, State, and local levels; interested private sector organizations and groups; and members of the public. ATSDR plans to revise these documents in response to public comments and as additional data become available. Therefore, we encourage comments that will make the toxicological profile series of the greatest use.

Electronic comments may be submitted via: www.regulations.gov. Follow the on-line instructions for submitting comments.

Written comments may also be sent to: Agency for Toxic Substances and Disease Registry
Office of Innovation and Analytics
Toxicology Section
1600 Clifton Road, N.E.
Mail Stop S106-5
Atlanta, Georgia 30329-4027

The toxicological profiles are developed under the Comprehensive Environmental Response, Compensation, and Liability Act of 1980, as amended (CERCLA or Superfund). CERCLA Section 104(i)(1) directs the Administrator of ATSDR to "…effectuate and implement the health-related authorities" of the statute. This includes the preparation of toxicological profiles for hazardous substances most commonly found at facilities on the CERCLA National Priorities List (NPL) and that pose the most significant potential threat to human health, as determined by ATSDR and the EPA. Section 104(i)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list. In addition, ATSDR has the authority to prepare toxicological profiles for substances not found at sites on the NPL, in an effort to "…establish and maintain inventory of literature, research, and studies on the health effects of toxic substances" under CERCLA Section 104(i)(1)(B), to respond to requests for consultation under Section 104(i)(4), and as otherwise necessary to support the site-specific response actions conducted by ATSDR.

This profile reflects ATSDR's assessment of all relevant toxicologic testing and information that has been peer-reviewed. Staffs of the Centers for Disease Control and Prevention and other Federal scientists have also reviewed the profile. In addition, this profile has been peer-reviewed by a nongovernmental panel and is being made available for public review. Final responsibility for the contents and views expressed in this toxicological profile resides with ATSDR.

Christopher M. Reh, Ph.D.
Associate Director
Agency for Toxic Substances and Disease Registry
Centers for Disease Control and Prevention

# VERSION HISTORY

| Date | Description |
|---|---|
| May 2024 | Draft for public comment toxicological profile released |
| July 1999 | Final toxicological profile released |

# CONTRIBUTORS & REVIEWERS

## CHEMICAL MANAGER TEAM

Obaid Faroon, D.V.M., Ph.D. (Lead)
Malcolm Williams, D.V.M., Ph.D.
Rae T. Benedict, Ph.D.
Melanie Buser, M.P.H.

Lisa Ingerman, Ph.D., D.A.B.T.
Jessica L. Myers, Ph.D.
Ramsey Hanna, Ph.D.

ATSDR, Office of Innovation and Analytics, Toxicology Section, Atlanta, GA

SRC, Inc., North Syracuse, NY

## REVIEWERS

### Interagency Minimal Risk Level Workgroup:

Includes ATSDR; National Center for Environmental Health (NCEH); National Institute for Occupational Safety and Health (NIOSH); U.S. Environmental Protection Agency (EPA); National Toxicology Program (NTP).

### Additional reviews for science and/or policy:

ATSDR, Office of Community Health Hazard Assessment; ATSDR, Office of Capacity Development and Applied Prevention Science; ATSDR, Office of Science; NCEH, Division of Laboratory Sciences; NCEH, Division of Environmental Health Science and Practice; EPA, Office of Research and Development; EPA, Office of Water.

## PEER REVIEWERS

1. Chunping Yang, Ph.D., PE (Ohio), Professor; School of Environmental Science and Engineering; Guangdong University of Petrochemical Technology; Maoming, Guangdong 525000, China and College of Environmental Science and Engineering; Hunan University; Changsha, Hunan 410082, China.

2. Marina Oktapodas Feiler, Ph.D., M.S.; Assistant Professor; Principal Investigator, Pediatric Environmental Health and Development Studies (PEDS) Laboratory; Department of Epidemiology and Environmental Health; School of Public Health and Health Professions; University at Buffalo; Buffalo, New York.

3. Hermann M. Bolt, Ph.D.; Leibnix Research Centre for Working Environment and Human Factors at TU Dortmund (IfADo), Ardeystr. 67; 44579 Dortmund, Germany.

These experts collectively have knowledge of toxicology, chemistry, and/or health effects. All reviewers were selected in conformity with Section 104(I)(13) of the Comprehensive Environmental Response, Compensation, and Liability Act, as amended.

ATSDR scientists review peer reviewers' comments and determine whether changes will be made to the profile based on comments. The peer reviewers' comments and responses to these comments are part of the administrative record for this compound.

The listing of peer reviewers should not be understood to imply their approval of the profile's final content. The responsibility for the content of this profile lies with ATSDR.

# CONTENTS

# LIST OF FIGURES

# LIST OF TABLES

# CHAPTER 1. RELEVANCE TO PUBLIC HEALTH

## 1.1 OVERVIEW AND U.S. EXPOSURES

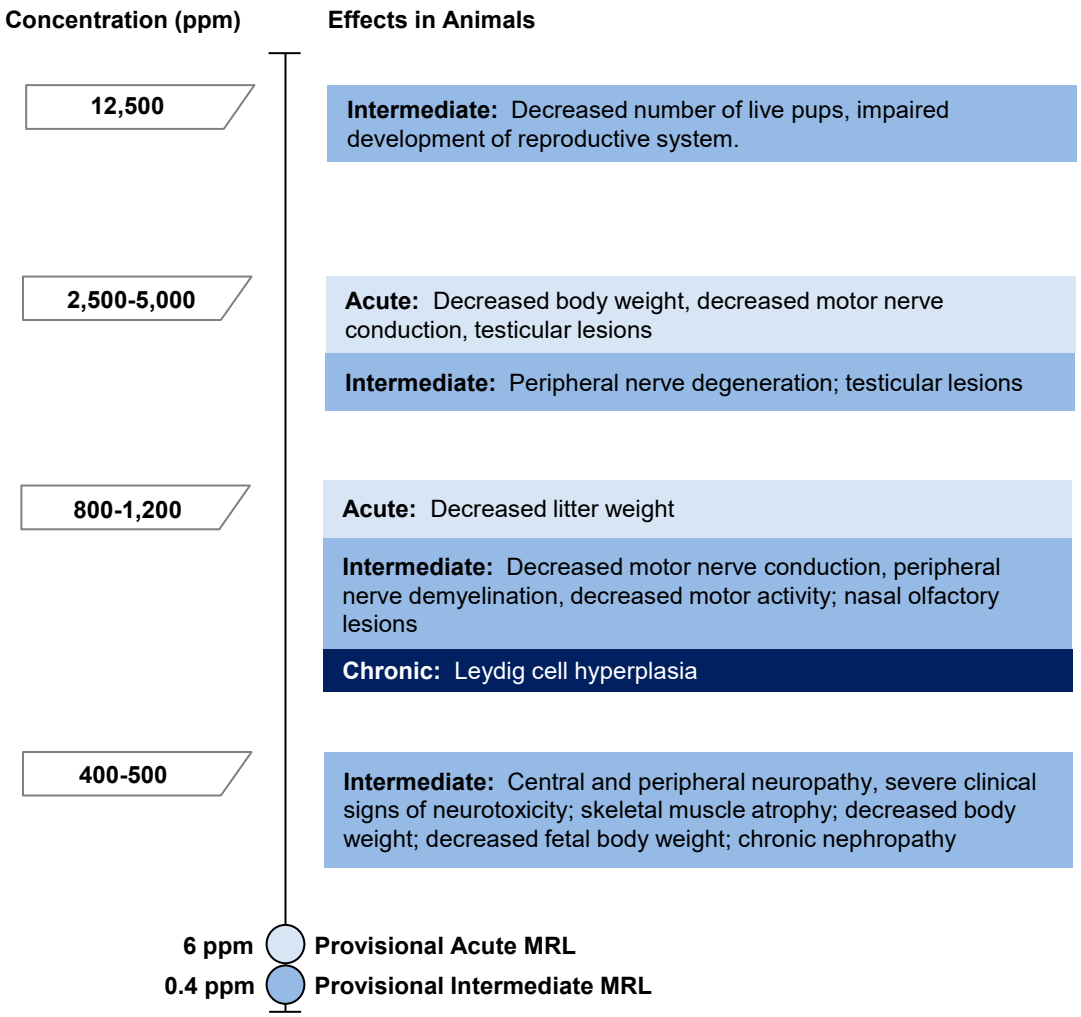
*n*-Hexane is a hydrocarbon that is refined from crude oil and is primarily used as a volatile, non-polar solvent in industry. *n*-Hexane is also used in special glues and adhesives and is present in gasoline. Pure *n*-hexane is widely used in laboratories as an extractant, while most commercial/industrial hexanes are a mixture of aliphatic hydrocarbons, including other hexane isomers. Major uses for solvents containing *n*-hexane include as cleaning agents in the printing, textile, furniture, and shoemaking industries, and for extracting vegetable oils from crops such as soybeans.

Because of the high volatility of *n*-hexane, the most likely route of human exposure is inhalation. Low-level exposures to *n*-hexane can possibly occur for much of the U.S. population, especially those who live in urban areas or those that commute in areas with heavy traffic, due to emissions of *n*-hexane associated with motor fuel use. As such, the general population will be exposed to very low levels at all times, while those living in urban centers may be exposed to slightly higher levels. Ambient air concentrations of *n*-hexane are in the parts per billion (ppb) range, with values between 0.05 and 4 ppb (EPA 2022a).

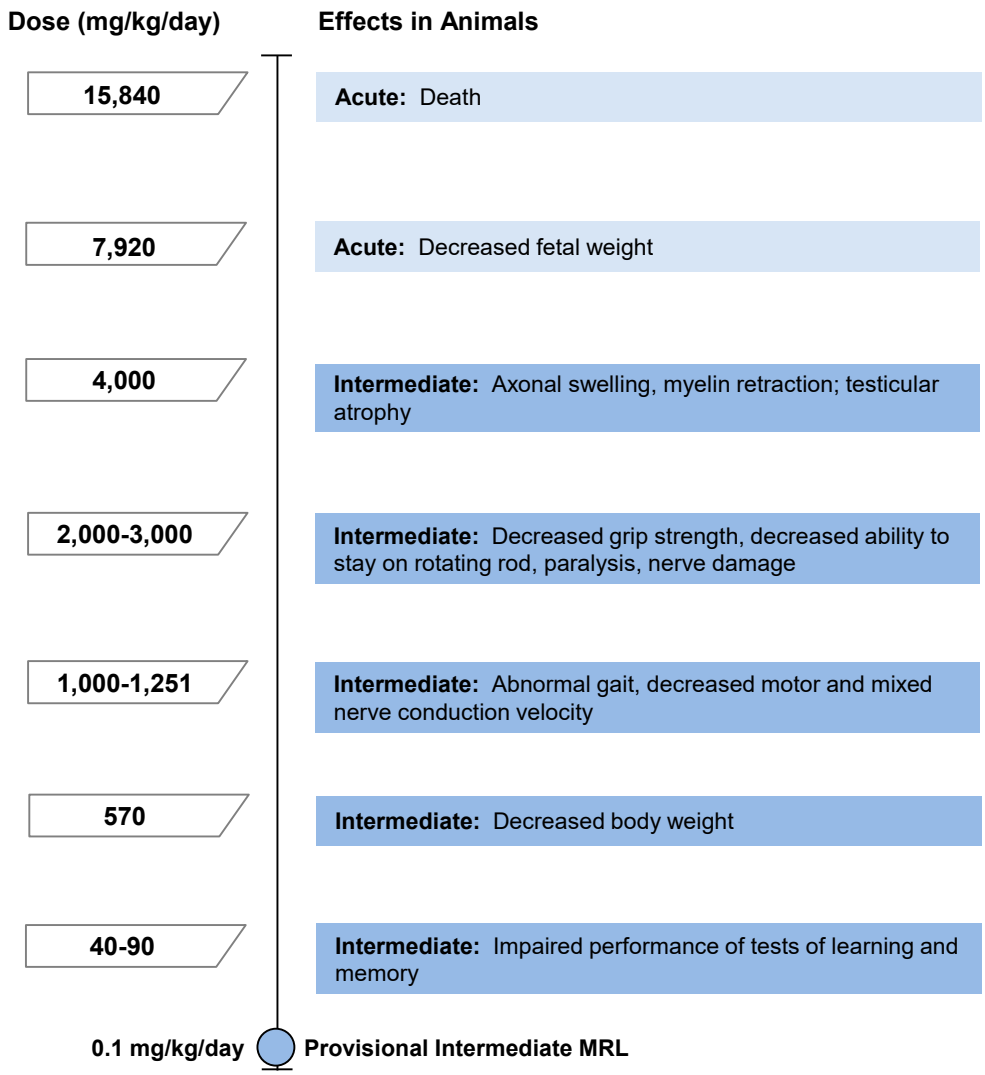
## 1.2 SUMMARY OF HEALTH EFFECTS

The health effects of *n*-hexane have been evaluated in observational occupational and population-based epidemiological studies, case reports/series, and experimental animal studies. Exposure to *n*-hexane occurs mainly through inhalation, although oral and dermal exposures may also occur. Most human studies have evaluated chronic-duration inhalation exposure, while animal studies have focused on acute- and intermediate-duration inhalation and oral exposures. Both human and animal studies were located for the majority of the endpoints evaluated in this profile. The available information suggests that adverse neurological, respiratory, developmental, and reproductive effects are the most important health concerns related to exposure to *n*-hexane (Figures 1-1 and 1-2). Muscle atrophy and decreased body weight are also common findings after exposure to *n*-hexane in experimental animals, but they are possibly secondary to the neurotoxicity that results in muscle denervation and decreased ability to move.

## Figure 1-1. Health Effects Found in Animals Following Inhalation Exposure to *n*-Hexane



## Figure 1-2. Health Effects Found in Animals Following Oral Exposure to *n*-Hexane



***Neurological Effects.*** The major public health concern regarding *n*-hexane exposure is the potential for the development of neurotoxicity. Occupational studies have documented that human exposure to *n*-hexane can result in peripheral neuropathy that in severe cases can lead to paralysis (Altenkirch et al. 1977; Wang et al. 1986; Yamamura 1969); observed clinical signs include paresthesia and leg weakness. The dose-duration relationship has not been well characterized in humans, but concentrations ≥500 ppm, and exposure for ≥2 months have been associated with human neurotoxicity. Clinical signs of peripheral neuropathy have been observed in rat studies via the inhalation and oral routes (Altenkirch et al. 1982; API 1981; De Martino et al. 1987; Huang et al. 1989; Krasavage et al. 1980; Schaumburg and Spencer 1976; Takeuchi et al. 1980). Clinical signs of neurotoxicity have not been observed in mice following intermediate-duration inhalation exposure.

***Respiratory Effects.*** Very few studies have examined the potential respiratory effects of *n*-hexane in animals and humans. *n*-Hexane was not irritating to the eyes, nose, or throat in humans at concentrations up to 500 ppm for 3–5 minutes (Nelson et al. 1943). Higher incidences of self-reported respiratory symptoms have been observed in workers exposed to *n*-hexane (Mustajbegovic et al. 2000; Nijem et al. 2001), while reduced lung function has been reported in children residing near point sources (Wichmann et al. 2009). Respiratory effects including rales, gasping, and mouth breathing were reported in rabbits throughout a 24-week inhalation exposure to 3,000 ppm *n*-hexane (Lungarella et al. 1984). Histopathological examination revealed serious effects in the lung, including centrilobular emphysema and fibrosis. Respiratory effects were also seen in mice exposed via inhalation to up to 10,000 ppm *n*-hexane for 13 weeks (NTP 1991). Mild effects were seen in the olfactory epithelium at 1,000 ppm, and in both the olfactory and respiratory tracts at 10,000 ppm. In contrast to the findings in mice, no histopathological changes were observed in the nasal cavity of male and female rats exposed up to 10,000 ppm for 13 weeks (Cavender et al. 1984) or in male rats exposed to 500 ppm for 6 months (API 1981).

***Developmental Effects.*** Associations have been reported between *n*-hexane exposure in humans and low birth weight (Gong et al. 2018) and alterations of the neonatal immune system (Lehmann et al. 2002). Rodent studies have reported decreased fetal/litter weights following inhalation (Bus et al. 1979; NIEHS 1987, 1988c; Stoltenburg-Didinger et al. 1990) or oral exposure (Marks et al. 1980) to *n*-hexane. Inhalation studies using concentrations ≥5,000 ppm have also observed more severe effects, including decreases in the number of live fetuses and increases in skeletal malformations (Li et al. 2014, 2015; NIEHS 1987, 1988c).

***Body Weight Effects.*** Data on body weight effects in humans exposed to *n*-hexane are very limited. In an offset printing factory in Hong Kong, weight loss of >5 pounds was reported in 11 out of 20 employees who developed peripheral neuropathy after exposure to solvents containing *n*-hexane, and in 5 out of 26 asymptomatic workers who were considered to have subclinical peripheral neuropathy (Chang et al. 1993). Marked decreases in body weight gain were observed at doses associated with outward clinical signs of neurotoxicity (Huang et al. 1989; API 1981; Takeuchi et al. 1980). Less severe body weight effects were observed in animal species that are less susceptible to *n*-hexane-induced neurotoxicity, such as mice (NTP 1991). In contrast, in a cross-sectional study conducted in Portugal, exposure to indoor *n*-hexane was associated with obesity in children (Paciencia et al. 2019).

***Reproductive Effects.*** Longer menstrual cycles, longer times to get pregnant, lower serum follicle-stimulating hormone (FSH) concentrations, and higher risks of spontaneous abortion and preeclampsia have been reported in human epidemiological studies evaluating exposure to *n*-hexane (Agnesi et al. 1997; Mendola et al. 2016; Nobles et al. 2019; Ruiz-García et al. 2020; Sallmen et al. 2008). Female reproductive effects have not been thoroughly examined in experimental animal studies, although several studies have reported effects in the male reproductive system. Decreased testis weights or altered testis and epididymis histopathology have been observed in rats following intermediate-duration inhalation exposure (De Martino et al. 1987; Howd et al. 1983; Nylen et al. 1989). Testicular atrophy was also noted in rats after intermediate-duration oral exposure (Krasavage et al. 1980).

***Cancer Effects.*** There is currently little information on the carcinogenic potential of *n*-hexane. A single epidemiological study identified a potential correlation between *n*-hexane exposure and intracranial tumors, but this study was extremely limited due to the small number of cases and large number of co-exposures. Papillary tumors were reported in the bronchiolar epithelium of rabbits after a 24-week exposure to 3,000 ppm *n*-hexane, but the incidence was not reported (Lungarella et al. 1984). The U.S. Environmental Protection Agency (IRIS 2005) concluded that there is inadequate information to assess the carcinogenic potential of *n*-hexane, while the Department of Health and Human Services (HHS) and the International Agency for Research on Cancer (IARC) have not assessed the carcinogenicity of *n*-hexane.

## 1.3 MINIMAL RISK LEVELS (MRLs)

***Inhalation MRLs.*** The inhalation database was considered adequate for derivation of acute- and intermediate-duration provisional MRLs for *n*-hexane. The chronic-duration data were insufficient for

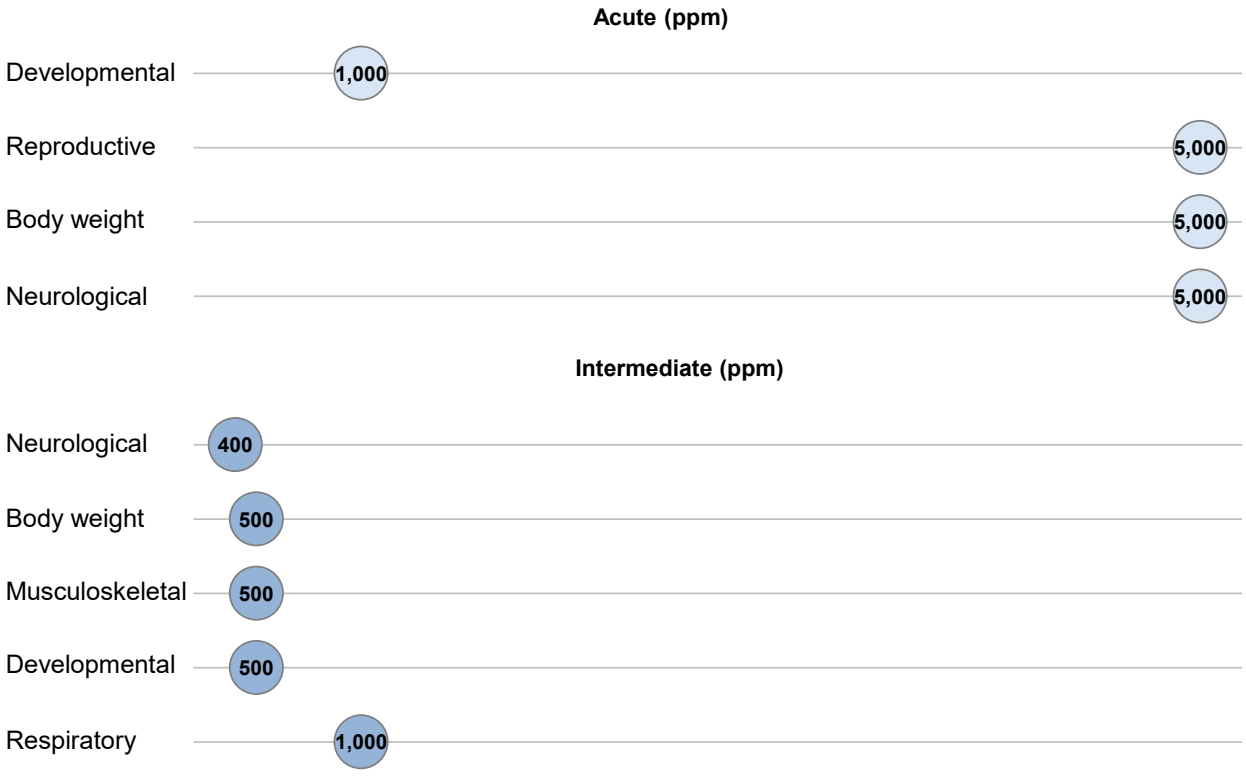
deriving a chronic-duration inhalation MRL. As illustrated in Figure 1-3, the most sensitive target of *n*-hexane toxicity following inhalation exposure is the neurological system. When air concentrations are expressed as human equivalent concentrations (HECs), the developmental and respiratory systems are the most sensitive targets. Body weight and musculoskeletal effects also have relatively low LOAEL values. The MRL values are summarized in Table 1-1 and discussed in greater detail in Appendix A.

***Oral MRLs.*** The oral database was considered adequate for derivation of a provisional intermediate-duration MRL for *n*-hexane. The acute- and chronic-duration data were insufficient for deriving provisional MRLs. As illustrated in Figure 1-4, the neurological system and body weight effects appear to be the most sensitive targets of *n*-hexane toxicity following oral exposure. The MRL values are summarized in Table 1-1 and discussed in greater detail in Appendix A.

## Figure 1-3. Summary of Sensitive Targets of *n*-Hexane – Inhalation

**The neurological and musculoskeletal systems* are the most sensitive targets of *n*-hexane inhalation exposure.**

Numbers in circles are the lowest LOAELs for all health effects in animals; no human data were identified.




*When exposure levels were expressed as human equivalent concentrations, the developmental and respiratory systems were the most sensitive targets.

## Figure 1-4. Summary of Sensitive Targets of *n*-Hexane – Oral

**Available data indicate that the nervous system and body weight changes are the most sensitive targets of *n*-hexane oral exposure.**

Numbers in circles are the lowest LOAELs for all health effects in animals.
No reliable dose response data were available for humans.

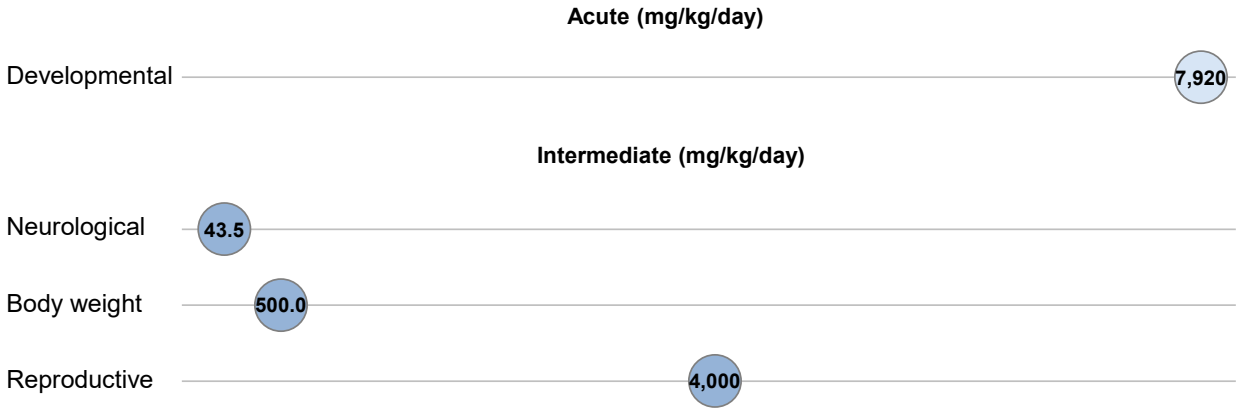


**Table 1-1. Minimal Risk Levels (MRLs) for *n*-Hexane[a]**

| Exposure route | Exposure duration | Provisional MRL | Critical effect | POD type | POD value | Uncertainty/ modifying factor | Reference |
|---|---|---|---|---|---|---|---|
| **Inhalation** | **Acute** | **6 ppm** (21 mg/m$^3$) | Decreased fetal body weight | $NOAEL_{HEC}$ | 167 ppm | UF: 30 | NIEHS 1987 |
| | **Intermediate** | **0.4 ppm** (1.4 mg/m$^3$) | Nasal cavity lesions | $LOAEL_{HEC}$ | 111 ppm | UF: 300 | NTP 1991 |
| | **Chronic** | None | – | – | – | – | – |
| **Oral** | **Acute** | None | – | – | – | – | – |
| | **Intermediate** | **0.1 mg/kg/day** | Impaired performance of a test of memory | LOAEL | 43.5 mg/kg/day | UF: 300 | Gao et al. 2019 |
| | **Chronic** | None | – | – | – | – | – |

[a]See Appendix A for additional information.

HEC = human equivalent concentration; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; UF = uncertainty factor

# CHAPTER 2. HEALTH EFFECTS

## 2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of *n*-hexane. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health. When available, mechanisms of action are discussed along with the health effects data; toxicokinetic mechanistic data are discussed in Section 3.1.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized by health effect. These data are discussed in terms of route of exposure (inhalation, oral, and dermal) and three exposure periods: acute (≤14 days), intermediate (15–364 days), and chronic (≥365 days).

As discussed in Appendix B, a literature search was conducted to identify relevant studies examining health effect endpoints. Figure 2-1 provides an overview of the database of studies in humans or experimental animals included in this chapter of the profile. These studies evaluate the potential health effects associated with inhalation, oral, or dermal exposure to *n*-hexane, but may not be inclusive of the entire body of literature. A systematic review of the scientific evidence of the health effects associated with exposure to *n*-hexane was also conducted; the results of this review are presented in Appendix C.

Animal inhalation studies are presented in Table 2-1 and Figure 2-2, animal oral studies are presented in Table 2-2 and Figure 2-3; animal dermal studies are presented in Table 2-3.

Levels of significant exposure (LSEs) for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. Effects have been classified into "less serious LOAELs" or "serious LOAELs (SLOAELs)." "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause

significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an endpoint should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these endpoints. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between “less serious” and “serious” effects. The distinction between “less serious” effects and “serious” effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

A User's Guide has been provided at the end of this profile (see Appendix D). This guide should aid in the interpretation of the tables and figures for LSEs and MRLs.

*n*-Hexane is a hydrocarbon produced from crude oil that is a component of many solvents used in industry, such as certain glues and adhesives, and it is also present in gasoline. Although pure *n*-hexane is used in laboratories, most commercial/industrial hexanes are a mixture of hexane isomers and aliphatic hydrocarbons. Major uses for solvents containing *n*-hexane include as cleaning agents in the printing, textile, furniture, and shoemaking industries, and for extracting vegetable oils from crops such as soybeans. Most of the available occupational health information comes from workers in these industries, and exposures are often to various mixtures of industrial solvents. There have been hundreds of cases of *n*-hexane neurotoxicity reported from occupational exposure throughout the world, but comparatively few in the United States. This is probably due to different use patterns; in the United States, *n*-hexane is used mainly in closed systems (e.g., for extraction of vegetable oils), while in the shoe industry cases of the 1960s and 1970s, open containers of solvents containing *n*-hexane were present in poorly ventilated workplaces.

This profile addresses the toxicological and toxicokinetics database of *n*-hexane. Animal studies utilizing pure reagent-grade *n*-hexane were evaluated, while animal studies using commercial-grade hexane were not included. Although the available occupational data is presumed to evaluate exposure to commercial-grade hexanes (and possibly other potentially neurotoxic compounds), these data provide critical information on the symptoms and progression of *n*-hexane-induced neurotoxicity.

The health effects of *n*-hexane have been evaluated in epidemiological studies, case reports, and experimental animal studies. The primary route of exposure to *n*-hexane is by inhalation due to its volatility, and it is also the most studied route in experimental animal studies. A number of case reports/ case series have extensively documented the clinical signs and altered neurological function in humans exposed to *n*-hexane, commonly referred to as peripheral neuropathy. Although useful in determining the clinical pathology of the disease, case reports and series typically lack the necessary exposure information for dose response. For the purposes of this profile, data from case reports and case series are only included to fill in gaps in outcomes with no other human data. Outcomes with sufficient information from other sources (i.e., neurological effects) do not include discussion of case reports/series. Animal inhalation data are available for each of the health effect categories and often lend support to the human data when available.

***Human studies.*** Most of the available literature on human exposure comes from occupational epidemiology studies and case reports/series examining workers with peripheral neuropathy. Human toxicity associated with *n*-hexane was first recognized in the 1960s and early 1970s in Japan and Italy. Workers in the shoe industries in these countries developed peripheral neuropathy that started with numbness in the feet and hands (sometimes referred to as glove and stocking neuropathy), followed by weakness in the lower legs and feet. In severe cases, paralysis developed. Epidemiological investigations revealed that these illnesses were linked with the use of glues and solvents containing high concentrations of *n*-hexane. In all cases, poor ventilation was a major factor in the illness. Removal from the workplace resulted in recovery for most patients over the course of several months to 2 years. Due to the volatility of *n*-hexane and its use in occupational settings, most of the available human data are from inhalation exposure and assumed to be chronic in duration.

***Animal studies.*** Information is readily available for experimental animals exposed to *n*-hexane primarily by the inhalation and oral routes and for acute and intermediate durations. The majority of these studies have evaluated neurotoxicity as the primary outcome, although data are available for all of the health categories included in this profile. Studies on the potential carcinogenicity of *n*-hexane are limited, which may be related to the lack of available chronic-duration studies and the neurotoxic effects observed at shorter durations.

***Overview of Health Effects.*** As discussed in Chapter 1, the neurological, respiratory, reproductive, and developmental systems appear to be sensitive targets of toxicity following exposure to *n*-hexane. Based on data in humans and animals, neurological effects are the primary outcome following inhalation or oral

exposure to *n*-hexane. Additional outcomes have also been reported, although some may be secondary effects resulting from overt or underlying neurological effects. The outcomes examined in human and animal studies of *n*-hexane are presented in Figure 2-1. A systematic review was conducted on the available human and animal studies for the most sensitive effects following exposure to *n*-hexane: neurological, developmental, and respiratory outcomes.

- **Neurological effects:** Neurological effects following inhalation or oral exposure are a known health effect for humans based on a high level of evidence in human studies and a high level of evidence in animal studies. Muscle weakness (particularly in the lower extremities), numbness, and decreased sensation have been reported in workers exposed to *n*-hexane, and alterations in nerve conduction and evoked potentials have been observed through further testing. Animal studies have shown similar results, with reduced motor activity, limb weakness, and paralysis being accompanied by changes in nerve conduction and nerve histopathology.

- **Respiratory effects:** Respiratory effects following inhalation exposure to *n*-hexane are a suspected health effect based on inadequate evidence in human studies and a high level of evidence in animal studies. Higher incidences of self-reported respiratory symptoms have been observed in a study of workers exposed to *n*-hexane. Animal studies have observed nasal and lung lesions following intermediate-duration inhalation exposure.

- **Developmental effects:** Developmental effects following inhalation exposure to *n*-hexane are a suspected health effect based on inadequate evidence in human studies and high evidence in animal studies. In humans, positive associations have been observed between low birth weight or alterations in the neonatal immune system and maternal exposure to *n*-hexane. Developmental effects observed in rodent inhalation and oral exposure studies include decreased fetal or litter weights and decreased number of live fetuses.

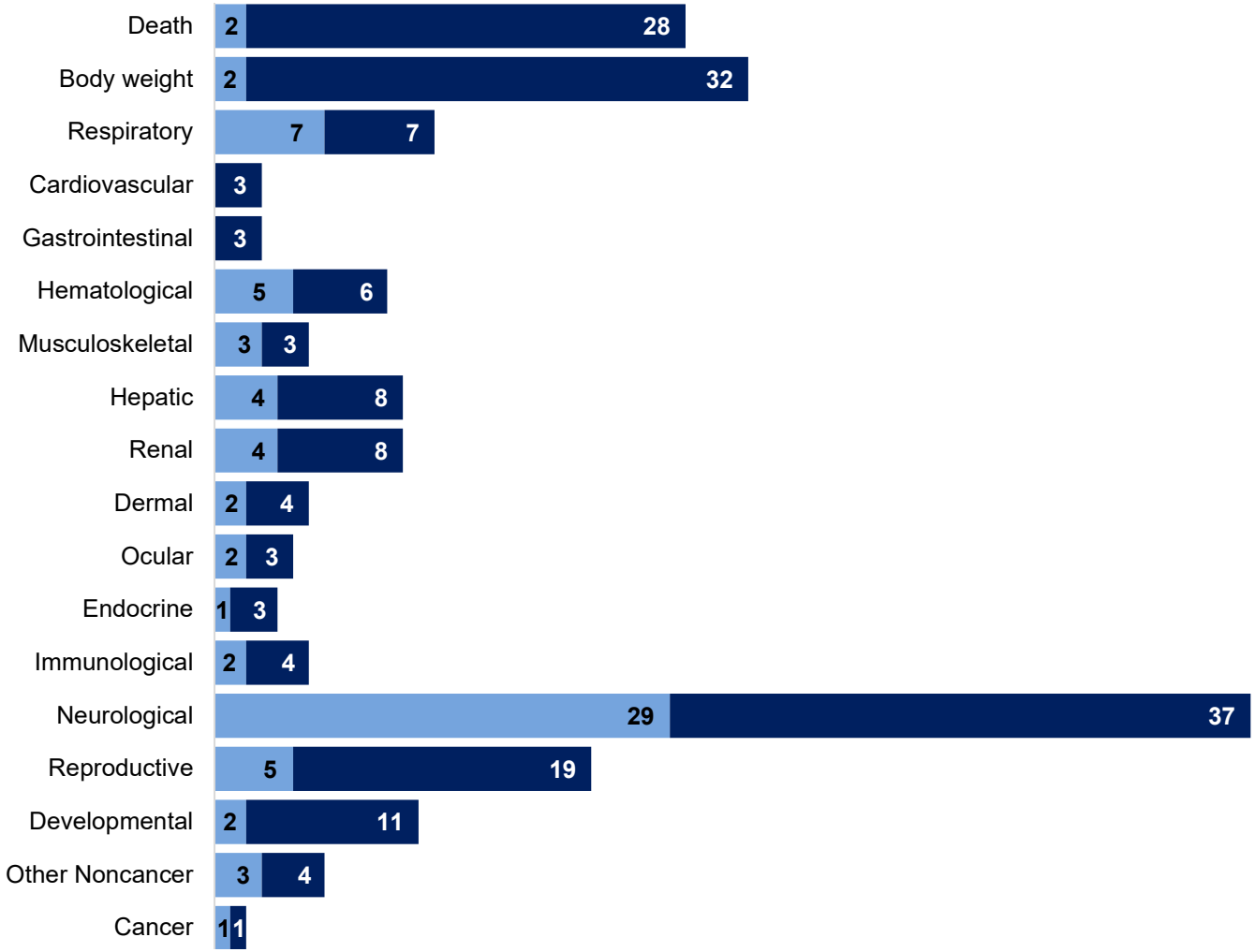
Additional sensitive effects following *n*-hexane exposure that did not undergo a full systematic review include body weight and reproductive outcomes. Although these are not the most sensitive endpoints, there is evidence to support hazard identification.

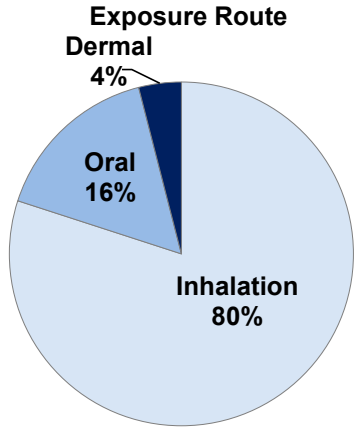
- **Body weight effects:** Although very few studies have identified weight changes in humans following exposure to *n*-hexane, decreased body weight is a common effect observed in animal studies, particularly after intermediate-duration inhalation exposure. These decreases are often accompanied by decreased food consumption and/or overt neurological effects.

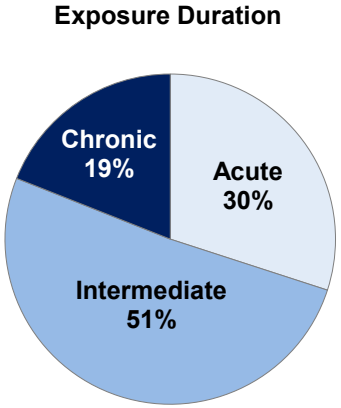
- **Reproductive effects:** Human studies have identified the female reproductive system as a potential target, with reports of longer menstrual cycles, increased risk of preeclampsia, and higher risk of spontaneous abortions in women exposed to *n*-hexane. Animal studies have observed effects in the male reproductive system, including decreased testis weight and histopathology in the testes and epididymis.

## Figure 2-1. Overview of the Number of Studies Examining *n*-Hexane Health Effects*

**Most studies examined the potential neurological, body weight, and reproductive effects of *n*-hexane**
Fewer studies evaluated health effects in **humans** than **animals** (counts represent studies examining endpoint)




*Includes studies discussed in Chapter 2. A total of 73 studies (including those finding no effect) have examined toxicity; most studies examined multiple endpoints.

## Table 2-1. Levels of Significant Exposure to *n*-Hexane – Inhalation (ppm)

| Figure key[a] | Species (strain) No./group | Exposure parameters | Doses | Parameters monitored | Endpoint | NOAEL | Less serious LOAEL | Serious LOAEL | Effects |
|---|---|---|---|---|---|---|---|---|---|
| **ACUTE EXPOSURE** | | | | | | | | | |
| **API 1979** | | | | | | | | | |
| 1 | Rat (Hybrid) 17–20 F | 10 days GDs 6–15 6 hours/day (WB) | 0, 93, 409 | BW, CS, DX, FI, GN, LE | Bd wt<br>Develop | 409<br>409 | | | |
| **Bus et al. 1979** | | | | | | | | | |
| 2 | Rat (Fischer-344) 7 F | 5 days GDs 8–12 6 hours/day (WB) | 0, 1,000 | DX | Develop | 1,000 | | | |
| **Bus et al. 1979** | | | | | | | | | |
| 3 | Rat (Fischer-344) 6–9 F | 5 days GDs 12–16 6 hours/day (WB) | 0, 1,000 | DX | Develop | 1,000 | | | |
| **Bus et al. 1979** | | | | | | | | | |
| 4 | Rat (Fischer-344) 3–8 F | 9 days GDs 8–16 6 hours/day (WB) | 0, 1,000 | DX | Develop | | 1,000 | | Decreased litter weight (13.9% at 3 weeks after birth) |
| **Chalansonnet et al. 2013** | | | | | | | | | |
| 5 | Rat (Sprague-Dawley) 8 M | 10 days 6 hours/day | 0, 1,000 | LE, CS | Neuro | 1,000 | | | |

**Table 2-1. Levels of Significant Exposure to *n*-Hexane – Inhalation (ppm)**

| Figure key[a] | Species (strain) No./group | Exposure parameters | Doses | Parameters monitored | Endpoint | NOAEL | Less serious LOAEL | Serious LOAEL | Effects |
|---|---|---|---|---|---|---|---|---|---|
| **De Martino et al. 1987** | | | | | | | | | |
| 6 | Rat (Sprague-Dawley) 8 M | 1–2 weeks 6 days/week 16 hours/day | 0, 5,000 | BW, CS, FI, LE, HP, NX | Bd wt | | | 5,000 | Decreased body weight (20–30%) |
| | | | | | Neuro | | 5,000 | | Decreased motor conduction velocity |
| | | | | | Repro | | | 5,000 | Testicular lesions (spermatocyte necrosis, exfoliation of spermatids, and Sertoli cell vacuolization) |
| **De Martino et al. 1987** | | | | | | | | | |
| 7 | Rat (Sprague-Dawley) 2–6 M | 24 hours | 0, 5,000 | CS, BW, FI, HP, RX | Repro | | | 5,000 | Testicular lesions (focal degeneration of spermatocytes and mild exfoliation of elongated spermatids) |
| **De Martino et al. 1987** | | | | | | | | | |
| 8 | Rat (Sprague-Dawley) 3 M | 2–8 days 16 hours/day | 0, 5,000 | CS, BW, FI, HP, RX | Repro | | | 5,000 | Testicular lesions (degeneration of spermatocytes, exfoliation of elongated spermatids, and Sertoli cell vacuolization) |
| **NIEHS 1987** | | | | | | | | | |
| 9 | Rat (Sprague-Dawley) 40 F | 14 days 20 hours/day GDs 6–19 (WB) | 0, 200, 1,000, 5,000 | LE, BW, DX | Bd wt | 1,000 | | 5,000 | Decreased body weight (10%), decreased extragestational body weight gain (44%) |
| | | | | | Neuro | 5,000 | | | |
| | | | | | Develop | 200[b] | | 1,000 | Decreased fetal body weight (7.5% in male offspring) |
| **NIEHS 1988a** | | | | | | | | | |
| 10 | Mouse (B6C3F1) 20 M | 5 days 20 hours/day (WB) | 0, 200, 1,000, 5,000 | LE, CS, BW, GN | Bd wt | 5,000 | | | |
| | | | | | Neuro | 5,000 | | | |
| | | | | | Repro | 5,000 | | | |

**Table 2-1. Levels of Significant Exposure to *n*-Hexane – Inhalation (ppm)**

| Figure key[a] | Species (strain) No./group | Exposure parameters | Doses | Parameters monitored | Endpoint | NOAEL | Less serious LOAEL | Serious LOAEL | Effects |
|---|---|---|---|---|---|---|---|---|---|
| **NIEHS 1988b** | | | | | | | | | |
| 11 | Mouse (Swiss CD-1) 20 M | 5 days 20 hours/day (WB) | 0, 200, 1,000, 5,000 | LE, CS, BW, RX | Bd wt | 5,000 | | | |
| | | | | | Neuro | 5,000 | | | |
| | | | | | Repro | 5,000 | | | |
| **NIEHS 1988c** | | | | | | | | | |
| 12 | Mouse (Swiss) | 12 days GDs 6–17 20 hours/day | 0, 200, 1,000, 5,000 | LE, CS, BW, DX | Bd wt | 5,000 | | | |
| | | | | | Neuro | 5,000 | | | |
| | | | | | Develop | 1,000 | | 5,000 | Decreased number of live fetuses per litter, increased incidence of late resorptions |
| **INTERMEDIATE EXPOSURE** | | | | | | | | | |
| **Altenkirch et al. 1982** | | | | | | | | | |
| 13 | Rat (Wistar) 5 M | 9 weeks 7 days/week 22 hours/day (WB) | 0, 500, 700 | BW, CS, GN, HP, LE | Neuro | | | 500 | Clinical signs (narcosis, paralysis), multifocal giant axonal swellings, primarily in the calf muscles, breakdown of axons, and myelin degradation |
| **Altenkirch et al. 1982** | | | | | | | | | |
| 14 | Rat (Wistar) 5 M | 40 weeks 7 days/week 8 hours/day (WB) | 0, 700 | BW, CS, GN, HP, LE | Bd wt | 700 | | | |
| | | | | | Neuro | | 700 | | Axonal swelling in the spinal cord |

**Table 2-1. Levels of Significant Exposure to *n*-Hexane – Inhalation (ppm)**

| Figure key[a] | Species (strain) No./group | Exposure parameters | Doses | Parameters monitored | Endpoint | NOAEL | Less serious LOAEL | Serious LOAEL | Effects |
|---|---|---|---|---|---|---|---|---|---|
| **API 1978** | | | | | | | | | |
| 15 | Rat (Sprague-Dawley) 12 M, 12 F | 26 weeks 5 days/week 6 hours/day (WB) | 0, 6, 26, 129 | BW, CS, BC, HE, LE | Bd wt | 129 | | | |
| | | | | | Hemato | 129 | | | |
| | | | | | Hepatic | 129 | | | |
| | | | | | Renal | 129 | | | |
| **API 1978** | | | | | | | | | |
| 16 | Rat (Sprague-Dawley) 12 M, 12 F | 26 weeks 7 days/week 21 hours/day (WB) | 0, 5, 27, 126 | BW, CS, BC, HE, LE | Bd wt | 126 | | | |
| | | | | | Hemato | 126 | | | |
| | | | | | Hepatic | 126 | | | |
| | | | | | Renal | 126 | | | |
| **API 1981** | | | | | | | | | |
| 17 | Rat (Sprague-Dawley) 20 M | 6 months 7 days/week 22 hours/day (WB) | 0, 500 | BW, CS, GN, HP, LE, OW | Bd wt | | | 500 | Decreased body weight (30%) |
| | | | | | Resp | 500 | | | |
| | | | | | Cardio | 500 | | | |
| | | | | | Gastro | 500 | | | |
| | | | | | Musc/skel | | | 500 | Skeletal muscle atrophy |
| | | | | | Hepatic | 500 | | | |
| | | | | | Renal | | 500 | | Increased kidney weight, chronic nephropathy |
| | | | | | Dermal | 500 | | | |
| | | | | | Ocular | 500 | | | |
| | | | | | Endocr | 500 | | | |
| | | | | | Immuno | 500 | | | |
| | | | | | Neuro | | | 500 | Abnormal gait, peripheral nerve atrophy |
| | | | | | Repro | 500 | | | |

**Table 2-1. Levels of Significant Exposure to *n*-Hexane – Inhalation (ppm)**

| Figure key[a] | Species (strain) No./group | Exposure parameters | Doses | Parameters monitored | Endpoint | NOAEL | Less serious LOAEL | Serious LOAEL | Effects |
|---|---|---|---|---|---|---|---|---|---|
| **Cavender et al. 1984** | | | | | | | | | |
| 18 | Rat (Fischer-344) 15 M, 15 F | 13 weeks 5 days/week 6 hours/day (WB) | 0, 3,000, 6,500, 10,000 | LE, CS, FI, BW, GN, HP, OP, OF, UR, HE, BC, OW | Bd wt | 10,000 F 6,500 M | 10,000 M | | Decreased body weight (11%) |
| | | | | | Resp | 10,000 | | | |
| | | | | | Hemato | 10,000 | | | |
| | | | | | Hepatic | 10,000 | | | |
| | | | | | Renal | 10,000 | | | |
| | | | | | Ocular | 10,000 | | | |
| | | | | | Immuno | 10,000 | | | |
| | | | | | Neuro | 10,000 F 3,000 M | 6,500 M | | Axonopathy in the sciatic nerve |
| | | | | | Repro | 10,000 M | | | |
| **De Martino et al. 1987** | | | | | | | | | |
| 19 | Rat (Sprague-Dawley) 3–8 M | 3–6 weeks 6 days/week 16 hours/day | 0, 5,000 | BW, CS, FI, LE, HP, NX | Bd wt | | | 5,000 | Decreased body weight (20-30%) with decreased food consumption |
| | | | | | Neuro | | | 5,000 | Decreased motor conduction velocity, peripheral neuropathy, and paralysis |
| | | | | | Repro | | | 5,000 | Testicular lesions (spermatocyte necrosis, exfoliation of spermatids, and Sertoli cell vacuolization) |
| **Frontali et al. 1981** | | | | | | | | | |
| 20 | Rat (Sprague-Dawley) 6–9 M | 7 weeks 5 days/week 9 hours/day (WB) | 0, 500, 1,500, 5,000 | BW, CS, HP | Neuro | 5,000 | | | |
| **Frontali et al. 1981** | | | | | | | | | |
| 21 | Rat (Sprague-Dawley) 6–9 M | 14 weeks 5 days/week 9 hours/day (WB) | 0, 500, 1,500, 5,000 | BW, CS, HP | Neuro | 1,500 | | 5,000 | Tibial nerve axonal degeneration |

**Table 2-1. Levels of Significant Exposure to *n*-Hexane – Inhalation (ppm)**

| Figure key[a] | Species (strain) No./group | Exposure parameters | Doses | Parameters monitored | Endpoint | NOAEL | Less serious LOAEL | Serious LOAEL | Effects |
|---|---|---|---|---|---|---|---|---|---|
| **Frontali et al. 1981** | | | | | | | | | |
| 22 | Rat (Sprague-Dawley) 6–9 M | 30 weeks 5–6 days/week 9–10 hours/day (WB) | 0, 500, 2,500 | BW, CS, HP | Neuro | 500 | | 2,500 | Tibial nerve axonal degeneration |
| **Howd et al. 1983** | | | | | | | | | |
| 23 | Rat (Fischer-344) 10 M | 11 weeks 7 days/week (4 weeks) 6 days/week (7 weeks) 24 hours/day (WB) | 0, 1,000 | LE, CS, BW, OW, NX | Bd wt | | | 1,000 | Decreased body weight (34%) |
| | | | | | Resp | 1,000 | | | |
| | | | | | Hepatic | 1,000 | | | |
| | | | | | Renal | 1,000 | | | |
| | | | | | Neuro | | | 1,000 | Decreased hindlimb and forelimb strength, ataxia, increased action potential latency and brainstem auditory-evoked response |
| | | | | | Repro | 1,000 | | | |
| Weanling rats | | | | | | | | | |
| **Howd et al. 1983** | | | | | | | | | |
| 24 | Rat (Fischer-344) 10 M | 11 weeks 7 days/week (4 weeks) 6 days/week (7 weeks) 24 hours/day (WB) | 0, 1,000 | LE, CS, BW, OW, OF, NX | Death | | | 1,000 | Increased mortality (50%) |
| | | | | | Bd wt | | | 1,000 | Decreased body weight (54%) |
| | | | | | Resp | 1,000 | | | |
| | | | | | Hepatic | 1,000 | | | |
| | | | | | Renal | 1,000 | | | |
| | | | | | Neuro | | | 1,000 | Decreased hindlimb and forelimb strength, ataxia, increased action potential latency and brainstem auditory-evoked response |
| | | | | | Repro | 1,000 | | | |
| Young adult rats | | | | | | | | | |

**Table 2-1. Levels of Significant Exposure to *n*-Hexane – Inhalation (ppm)**

| Figure key[a] | Species (strain) No./group | Exposure parameters | Doses | Parameters monitored | Endpoint | NOAEL | Less serious LOAEL | Serious LOAEL | Effects |
|---|---|---|---|---|---|---|---|---|---|
| **Huang et al. 1989** | | | | | | | | | |
| 25 | Rat (Wistar) 8 M | 16 weeks 7 days/week 12 hours/day (WB) | 0, 500, 1,200, 3,000 | BI, BW, CS, HP, LE, NX | Bd wt | 500 | 1,200 | | Decreased body weight (12%) |
| | | | | | Neuro | 500 | 1,200 | | Decreased grip strength and motor nerve conduction velocity, paranodal swelling, demyelination, and remyelination of the peripheral nerve |
| **Ichihara et al. 1998** | | | | | | | | | |
| 26 | Rat (Wistar) 8 M | 20 weeks 6 days/week 12 hours/day | 0, 2,000 | LE, CS, BW, NX | Bd wt | 2,000 | | | |
| | | | | | Neuro | | 2,000 | | Decreased motor conduction velocity |
| **Li et al. 2014, 2015** | | | | | | | | | |
| 27 | Rat (Wistar) 5 F | 20 days GDs 1–20 4 hours/day | 0, 100, 500, 2,500, 12,500 | LE, CS, DX | Neuro | 2,500 | 12,500 | | Irritability, aggression |
| | | | | | Develop | 2,500 | | 12,500 | Decreased live pups/litter, decreased percentage of secondary follicles, increased atretic follicles, alterations in estrus cycle in female offspring |
| **Nylen and Hagman 1994** | | | | | | | | | |
| 28 | Rat (Sprague-Dawley) 18–36 M | 61 days 18 hours/day (WB) | 0, 1,000 | BW, OF | Neuro | | 1,000 | | Decreased motor conduction velocity and auditory brainstem response, increased flash evoked potential latency |

**Table 2-1. Levels of Significant Exposure to *n*-Hexane – Inhalation (ppm)**

| Figure key[a] | Species (strain) No./group | Exposure parameters | Doses | Parameters monitored | Endpoint | NOAEL | Less serious LOAEL | Serious LOAEL | Effects |
|---|---|---|---|---|---|---|---|---|---|
| **Nylen et al. 1989** | | | | | | | | | |
| 29 | Rat (Sprague-Dawley) 12–18 M | 28–61 days 7 days/week 18–21 hours/day | 0, 1,000 | GN, HP | Musc/skel | | | 1,000 | Hindlimb muscular atrophy |
| | | | | | Repro | | | 1,000 | Testicular atrophy |
| **Nylen et al. 1994** | | | | | | | | | |
| 30 | Rat (Sprague-Dawley) 18 M | 28 days 21 hours/day (WB) | 0, 1,000 | BW, OF | Neuro | | 1,000 | | Decreased motor conduction velocity and flash evoked potential amplitude |
| **Pryor and Rebert 1992** | | | | | | | | | |
| 31 | Rat (Fischer-344) 12 M | 9 weeks 7 days/week 14 hours/day (WB) | 0, 4,000 | NX, OF, LE | Neuro | | 4,000 | | Decreased grip strength, decreased motor conduction velocity, decreased auditory brainstem response amplitude |
| **Pryor et al. 1983** | | | | | | | | | |
| 32 | Rat (Fischer-344) 11–12 M | 14 weeks 7 days/week 14 hours/day | 0, 2,000 | CS, BW, NX | Bd wt | | 2,000 | | Decreased body weight (19%) |
| | | | | | Neuro | | 2,000 | | Decreased limb grip strength, startle response, and motor activity; increased evoked potentials latencies |
| **Rebert and Sorenson 1983** | | | | | | | | | |
| 33 | Rat (Fischer-344) 8 M | 10-11 weeks 5 days/week 24 hours/day (WB) | 0, 500, 1,000, 1,500 | NX, BW, LE | Death | | | 1,500 | $LC_{50}$ (4/8 died during 6-week recovery period) |
| | | | | | Bd wt | 500 | | 1,000 | Decreased body weight (23%) |
| | | | | | Neuro | | 500 | 1,000 | LOAEL: Decreased fore- and hindlimb grip strength; increased latency of evoked potentials at 1,000 ppm |
| | | | | | Other noncancer | 1,500 | | | |

**Table 2-1. Levels of Significant Exposure to *n*-Hexane – Inhalation (ppm)**

| Figure key[a] | Species (strain) No./group | Exposure parameters | Doses | Parameters monitored | Endpoint | NOAEL | Less serious LOAEL | Serious LOAEL | Effects |
|---|---|---|---|---|---|---|---|---|---|
| **Schaumburg and Spencer 1976** | | | | | | | | | |
| 34 | Rat (Sprague-Dawley) 8 NS | 42–162 days 7 days/week 24 hours/day (WB) | 400–600 | GN, HP | Neuro | | | 400 | Central and peripheral neuropathy, foot-drop, waddling gait, limb weakness, swollen axons, axonal degeneration |
| **Stoltenburg-Didinger et al. 1990** | | | | | | | | | |
| 35 | Rat (Wistar) 8–20 F | 21 days GDs 1–21 7 days/week 23 hours/day | 0, 500 | OF, OW, BW, DX, CS | Neuro | 500 | | | |
| | | | | | Develop | | | 500 | Decreased fetal weight (22% at 9 days after birth) |
| **Stoltenburg-Didinger et al. 1990** | | | | | | | | | |
| 36 | Rat (Wistar) 8–20 F | 63 days GD 1–PND 42 7 days/week 23 hours/day | 0, 800 | OF, OW, BW, DX, CS | Neuro | | 800 | | Hindlimb weakness |
| **Takeuchi et al. 1980** | | | | | | | | | |
| 37 | Rat (Wistar) 7 M | 16 weeks 7 days/week 12 hours/day (WB) | 0, 3,040 | LE, CS, BW, HP, LE | Death | | | 3,040 | Increased mortality (29%) |
| | | | | | Bd wt | | | 3,040 | Decreased body weight (33%) and body weight gain (79%) |
| | | | | | Musc/skel | | | 3,040 | Muscular atrophy, denervation, irregular fibers, and disordered myofilaments |
| | | | | | Neuro | | | 3,040 | Gait disturbances, decreased motor and mixed nerve conduction velocity, axonal swelling, neurofilament accumulation, denervated neuromuscular junctions |

**Table 2-1. Levels of Significant Exposure to *n*-Hexane – Inhalation (ppm)**

| Figure key[a] | Species (strain) No./group | Exposure parameters | Doses | Parameters monitored | Endpoint | NOAEL | Less serious LOAEL | Serious LOAEL | Effects |
|---|---|---|---|---|---|---|---|---|---|
| **API 1980** | | | | | | | | | |
| 38 | Mouse (CD-1) 12 M | 8 weeks 5 days/week 6 hours/day (WB) | 0, 99, 396 | DX | Repro | 396 | | | |
| **Liu et al. 2012** | | | | | | | | | |
| 39 | Mouse (ICR) 5 F | 5 weeks 7 days/week 4 hours/day | 0, 850, 4,300, 21,500 | BW, RX | Bd wt | 4,300 | 21,500 | | 15% lower terminal body weight |
| | | | | | Repro | 4,300 | 21,500 | | Decreased duration of diestrus |
| **NTP 1991 (this study was also published as Dunnick et al. 1989)** | | | | | | | | | |
| 40 | Mouse (B6C3F1) 8 M, 8 F | 13 weeks 5 days/week 6 hours/day (WB) | 0, 580, 1,109, 4,421, 10,000 | CS, BW, NX | Neuro | 4,421 | 10,000 | | Decreased locomotor activity |
| **NTP 1991 (this study was also published as Dunnick et al. 1989)** | | | | | | | | | |
| 41 | Mouse (B6C3F1) 10 M, 10 F | 13 weeks 5 days/week 6 hours/day (WB) | 0, 580, 1,109, 4,421, 10,000 | NX, BW, CS, GN, HP, LE | Bd wt | 10,000 F 4,421 M | 10,000 M | | Decreased body weight (17%) |
| | | | | | Resp | 1,109 F 4,421 M | 4,421 F | 10,000 | LOAEL: Lesions in the nasal cavity (multifocal regeneration and metaplasia in the olfactory epithelium) SLOAEL: Lesions in the nasal cavity (multifocal erosion, regeneration, inflammation, metaplasia in the olfactory and respiratory epithelium), respiratory irritation (sneezing) |
| | | | | | Cardio | 10,000 | | | |
| | | | | | Gastro | 10,000 | | | |
| | | | | | Hemato | 10,000 F 4,421 M | 10,000 M | | Increased number of segmented neutrophils |
| | | | | | Hepatic | 10,000 | | | |
| | | | | | Renal | 10,000 | | | |

**Table 2-1. Levels of Significant Exposure to *n*-Hexane – Inhalation (ppm)**

| Figure key[a] | Species (strain) No./group | Exposure parameters | Doses | Parameters monitored | Endpoint | NOAEL | Less serious LOAEL | Serious LOAEL | Effects |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Dermal | 10,000 | | | |
| | | | | | Endocr | 10,000 | | | |
| | | | | | Immuno | 10,000 | | | |
| | | | | | Neuro | 4,421 F<br>10,000 M | 10,000 F | | Paranodal swellings in tibial nerve |
| | | | | | Repro | 10,000 | | | |
| **NTP 1991 (this study was also published as Dunnick et al. 1989)** | | | | | | | | | |
| 42 | Mouse (B6C3F1) 8 M, 8 F | 13 weeks 5 days/week 22 hours/day (WB) | 0, 1,099 | CS, BW, NX | Neuro | 1,099 M | 1,099 F | | Decreased locomotor activity |
| **NTP 1991 (this study was also published as Dunnick et al. 1989)** | | | | | | | | | |
| 43 | Mouse (B6C3F1) 10 M, 10 F | 13 weeks 5 days/week 22 hours/day (WB) | 0, 1,099 | NX, BW, CS, GN, HP, LE | Bd wt | 1,099 F | 1,099 M | | Decreased body weight (10%) |
| | | | | | Resp | | 1,099[c] | | Lesions in the nasal cavity (multifocal regeneration and metaplasia in olfactory epithelium) |
| | | | | | Cardio | 1,099 | | | |
| | | | | | Gastro | 1,099 | | | |
| | | | | | Hemato | 1,099 | | | |
| | | | | | Hepatic | 1,099 | | | |
| | | | | | Renal | 1,099 | | | |
| | | | | | Dermal | 1,099 | | | |
| | | | | | Endocr | 1,099 | | | |
| | | | | | Immuno | 1,099 | | | |
| | | | | | Neuro | | 1,099 | | Paranodal swellings in tibial nerve |

**Table 2-1. Levels of Significant Exposure to *n*-Hexane – Inhalation (ppm)**

| Figure key[a] | Species (strain) No./group | Exposure parameters | Doses | Parameters monitored | Endpoint | NOAEL | Less serious LOAEL | Serious LOAEL | Effects |
|---|---|---|---|---|---|---|---|---|---|
| **Lungarella et al. 1984** | | | | | | | | | |
| 44 | Rabbit (New Zealand) 12 M | 24 weeks 5 days/week 8 hours/day (WB) | 0, 3,000 | LE, CS, BW, HE, OP, GN, HP | Death | | | 3,000 | Increased mortality (17%) |
| | | | | | Bd wt | 3,000 | | | |
| | | | | | Resp | | | 3,000 | Upper respiratory tract irritation (nasal discharge and salivation), respiratory difficulties (gasping, lung rales, mouth breathing), histopathology (centrilobular emphysema, pulmonary fibrosis, goblet cell metaplasia, epithelial desquamation) |
| | | | | | Hemato | 3,000 | | | |
| | | | | | Ocular | | 3,000 | | Ocular irritation (lacrimation, hyperemia of the conjunctiva) |
| | | | | | Neuro | 3,000 | | | |
| | | | | | Cancer | | | 3,000 | CEL: Lung tumors (papillary tumors in the bronchiolar epithelium) |

## Table 2-1. Levels of Significant Exposure to *n*-Hexane – Inhalation (ppm)

| Figure key[a] | Species (strain) No./group | Exposure parameters | Doses | Parameters monitored | Endpoint | NOAEL | Less serious LOAEL | Serious LOAEL | Effects |
|---|---|---|---|---|---|---|---|---|---|
| **CHRONIC EXPOSURE** | | | | | | | | | |
| **Imai and Omoto 1999** | | | | | | | | | |
| 45 | Rat (F344/Jcl) 6 M | 415 days 6 days/week 4 hours/day | 0, 1,000 | LE, CS, BW, FI, OW, HP | Bd wt | 1,000 | | | |
| | | | | | Repro | | 1,000 | | Leydig cell hyperplasia |

[a]The number corresponds to entries in Figure 2-2.
[b]Used to derive a provisional acute-duration inhalation minimal risk level (MRL) of 6 ppm. The NOAEL of 200 ppm was converted to a $NOAEL_{HEC}$ of 167 ppm and then divided by a total uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustments and 10 for human variability); see Appendix A for details.
[c]Used to derive a provisional intermediate-duration inhalation MRL of 0.4 ppm. The LOAEL of 1,099 ppm was converted to a $LOAEL_{HEC}$ of 111 ppm and then divided by a total uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation from animals to humans with dosimetric adjustments and 10 for human variability); see Appendix A for details.

BC = blood chemistry; Bd wt or BW = body weight; Cardio = cardiovascular; CEL = Cancer Effect Level; CS = clinical signs; Develop = developmental; DX = developmental toxicity; Endocr = endocrine; F = female(s); FI = food intake; Gastro = gastrointestinal; GD = gestation day; GN = gross necropsy; HE = hematology; HEC = human equivalent concentration; Hemato = hematological; HP = histopathology; Immuno = immunological; $LC_{50}$ = median lethal concentration; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); Musc/skel = musculoskeletal; Neuro = neurological; NOAEL = no-observed-adverse-effect level; NS = not specified; NX = neurological function; OF = organ function; OP = ophthalmology; OW = organ weight; PND = postnatal day; Repro = reproductive; Resp = respiratory; RX = reproductive function; SLOAEL = serious lowest-observed-adverse-effect level; UR = urinalysis; (WB) = whole-body

**Figure 2-2. Levels of Significant Exposure to *n*-Hexane – Inhalation**
Acute (≤14 days)

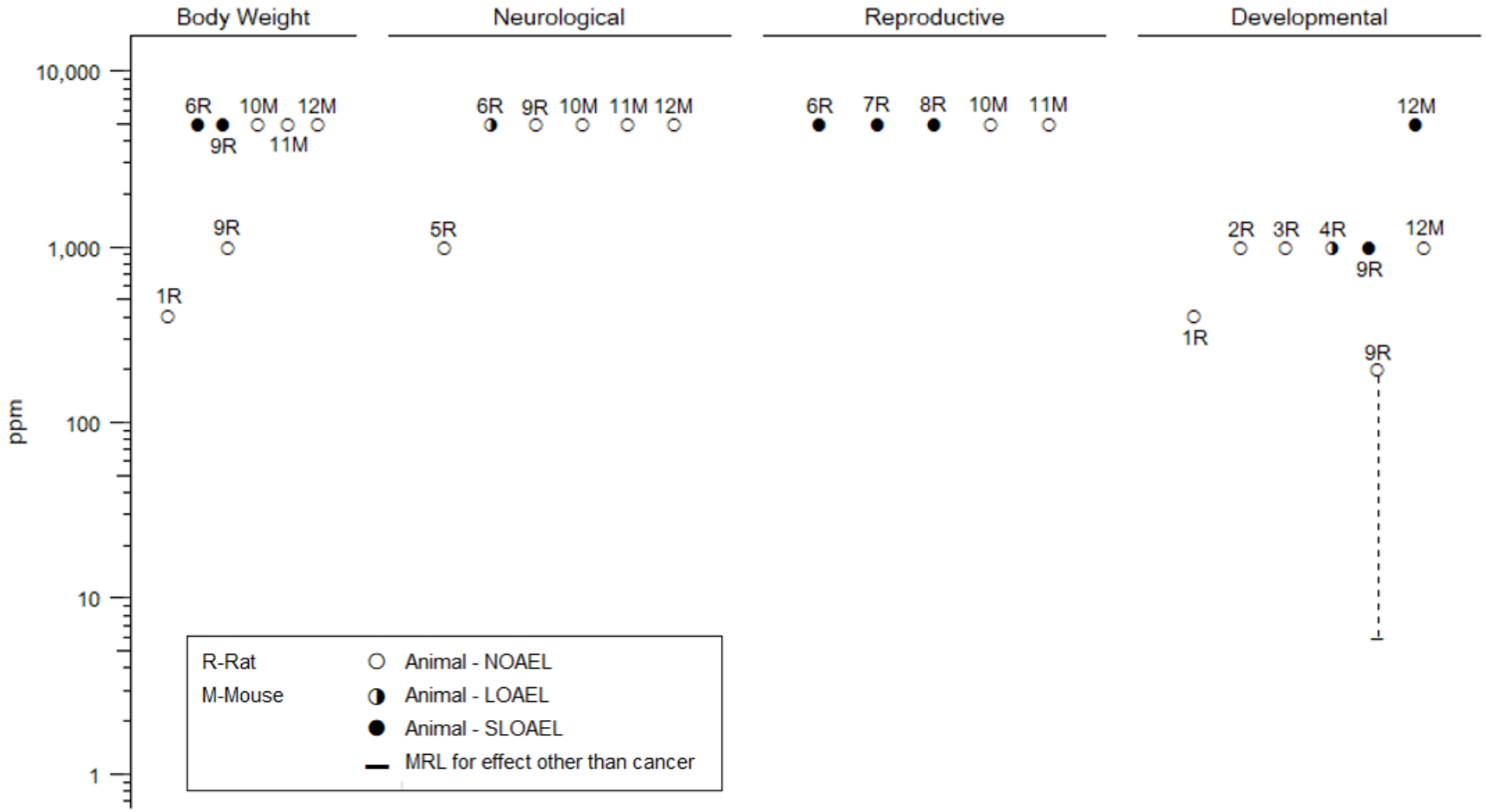


**Figure 2-2. Levels of Significant Exposure to *n*-Hexane – Inhalation**
Intermediate (15–364 days)

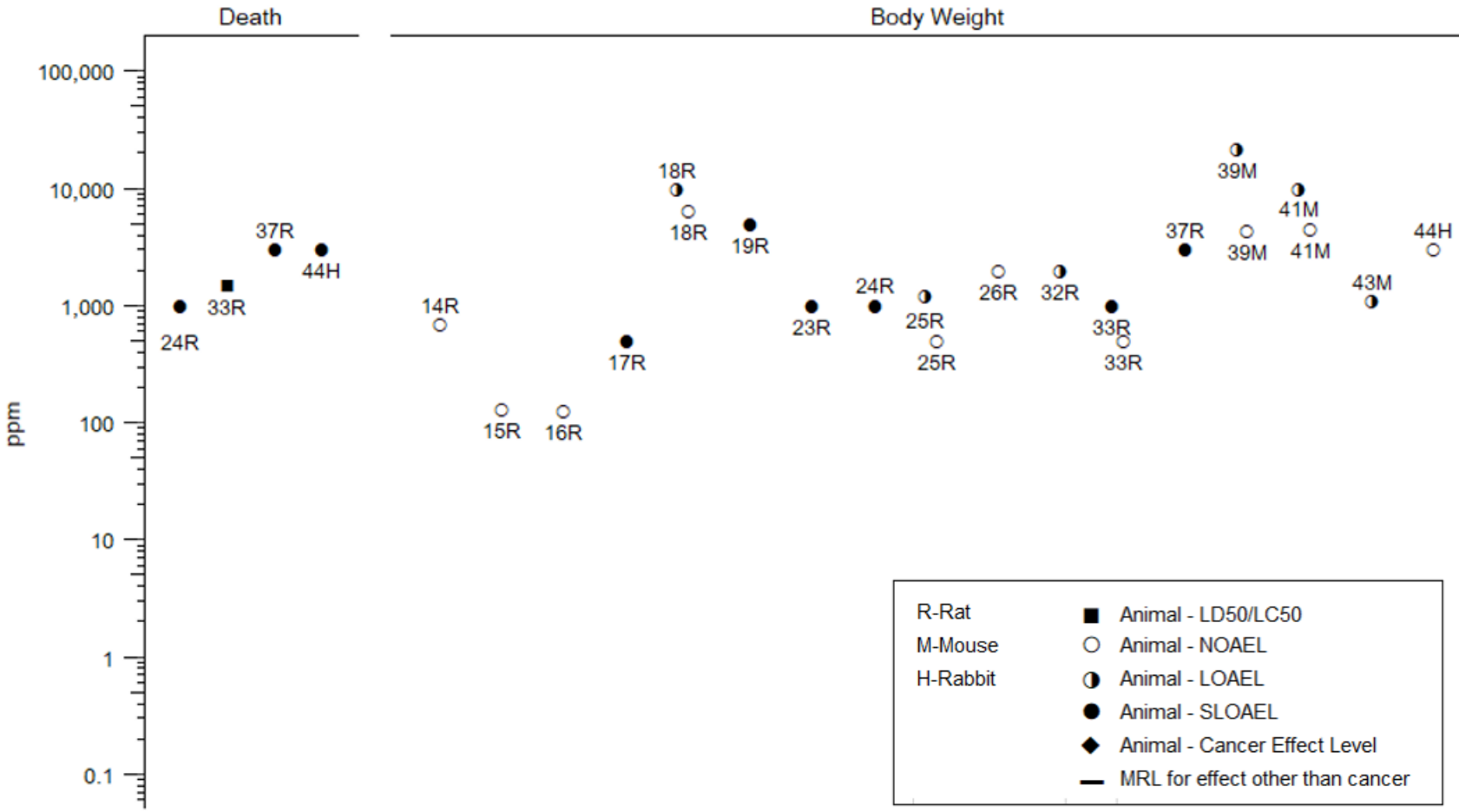


**Figure 2-2. Levels of Significant Exposure to *n*-Hexane – Inhalation**
Intermediate (15–364 days)

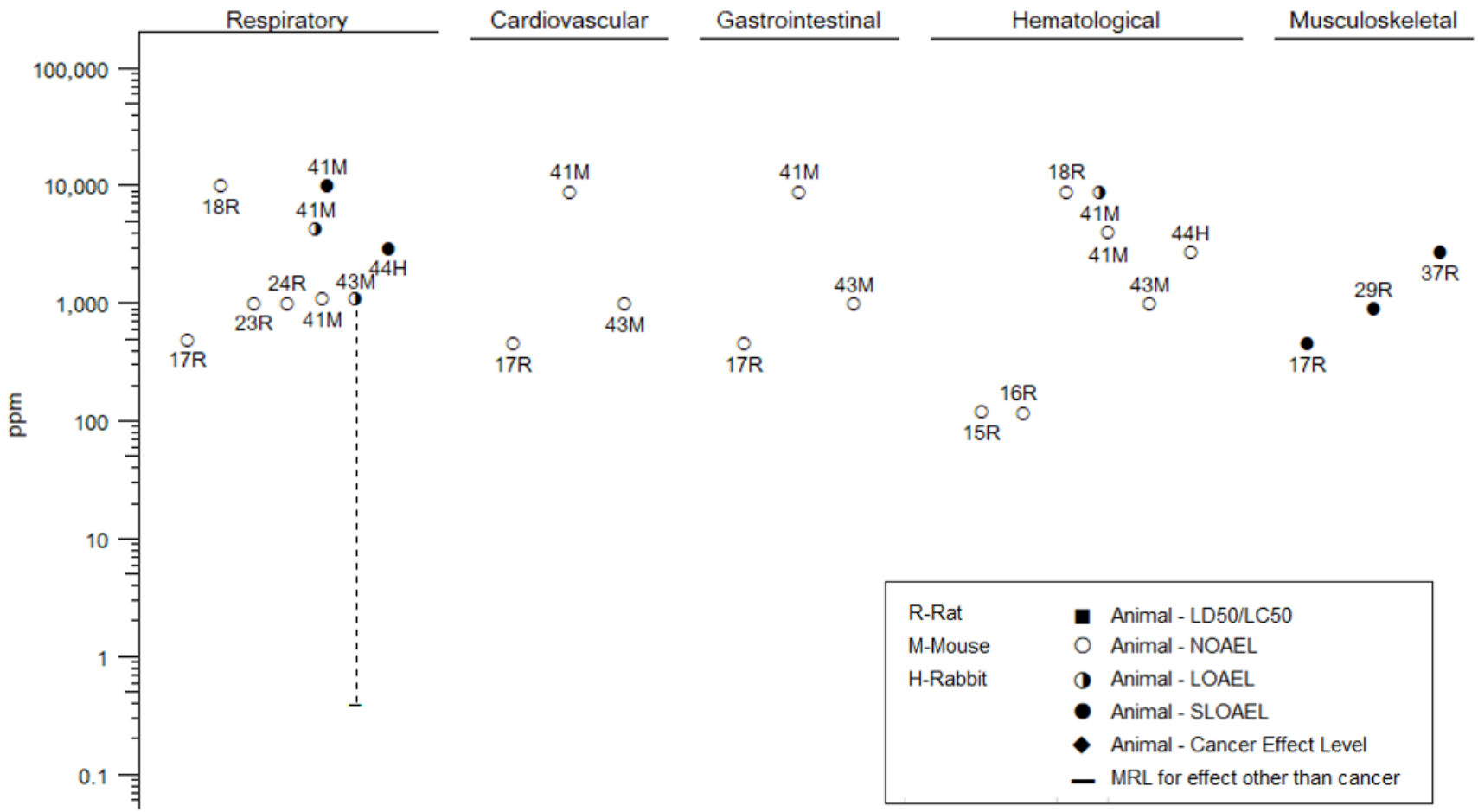


**Figure 2-2. Levels of Significant Exposure to *n*-Hexane – Inhalation**
Intermediate (15–364 days)

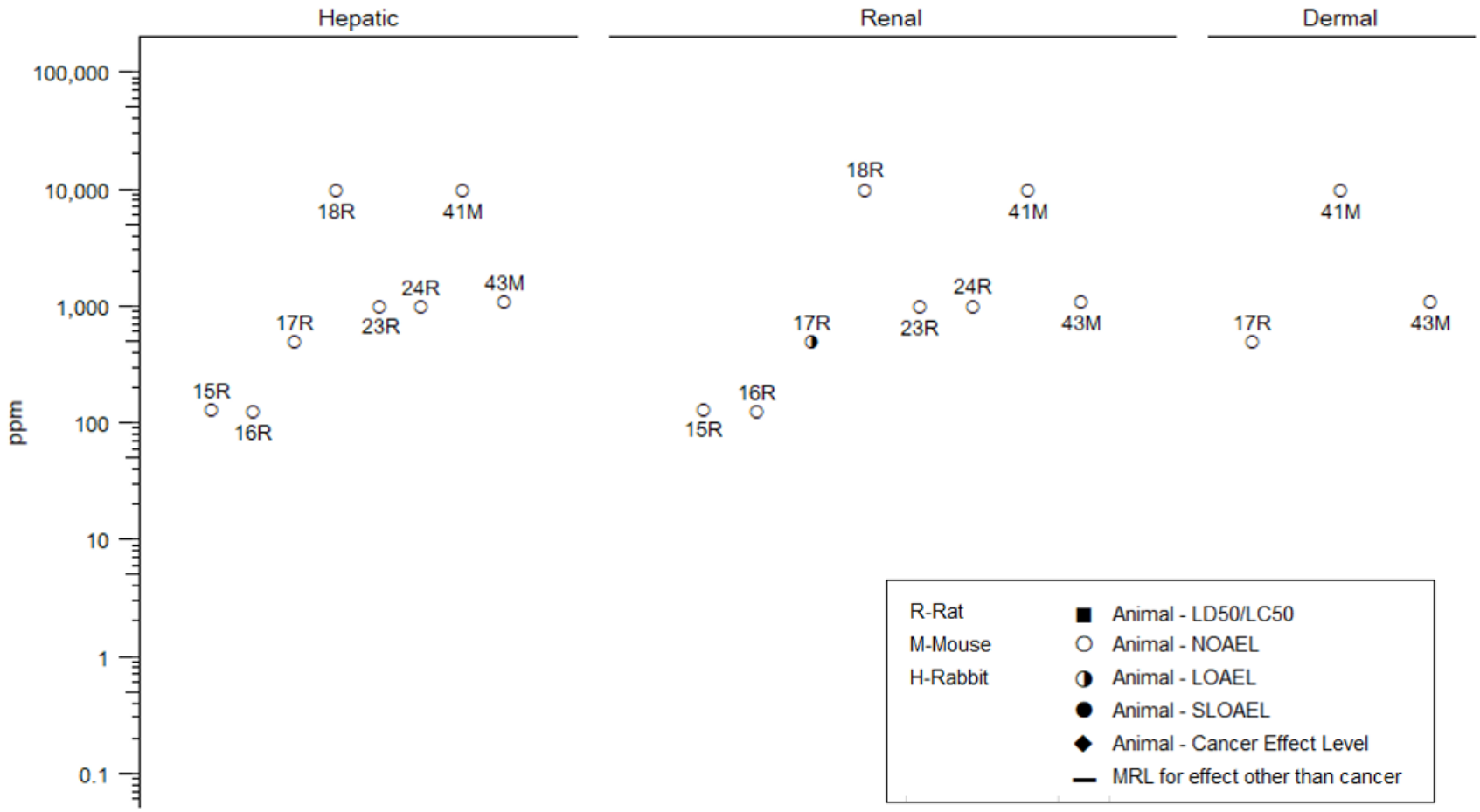


**Figure 2-2. Levels of Significant Exposure to *n*-Hexane – Inhalation**
Intermediate (15–364 days)

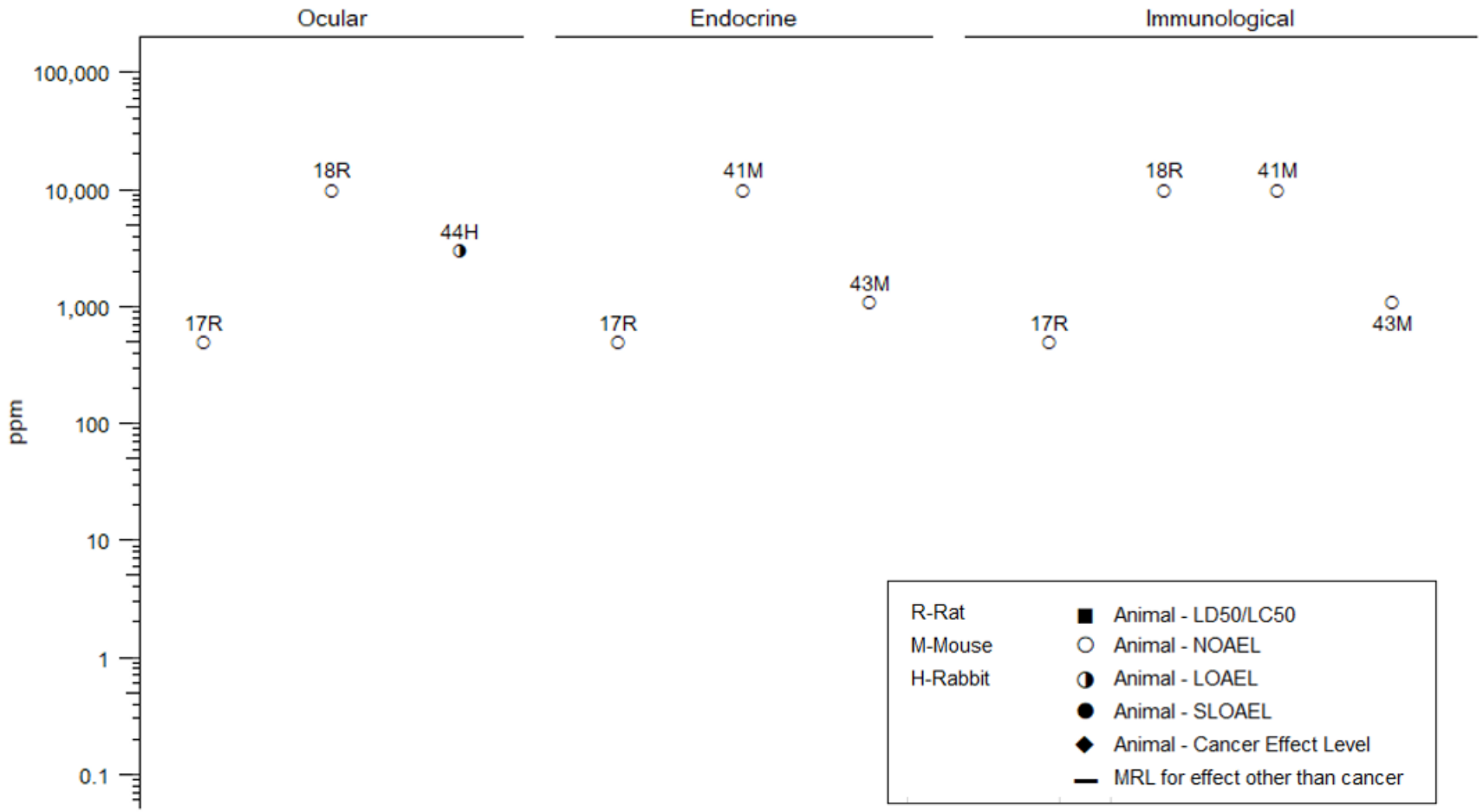


**Figure 2-2. Levels of Significant Exposure to *n*-Hexane – Inhalation**
Intermediate (15–364 days)

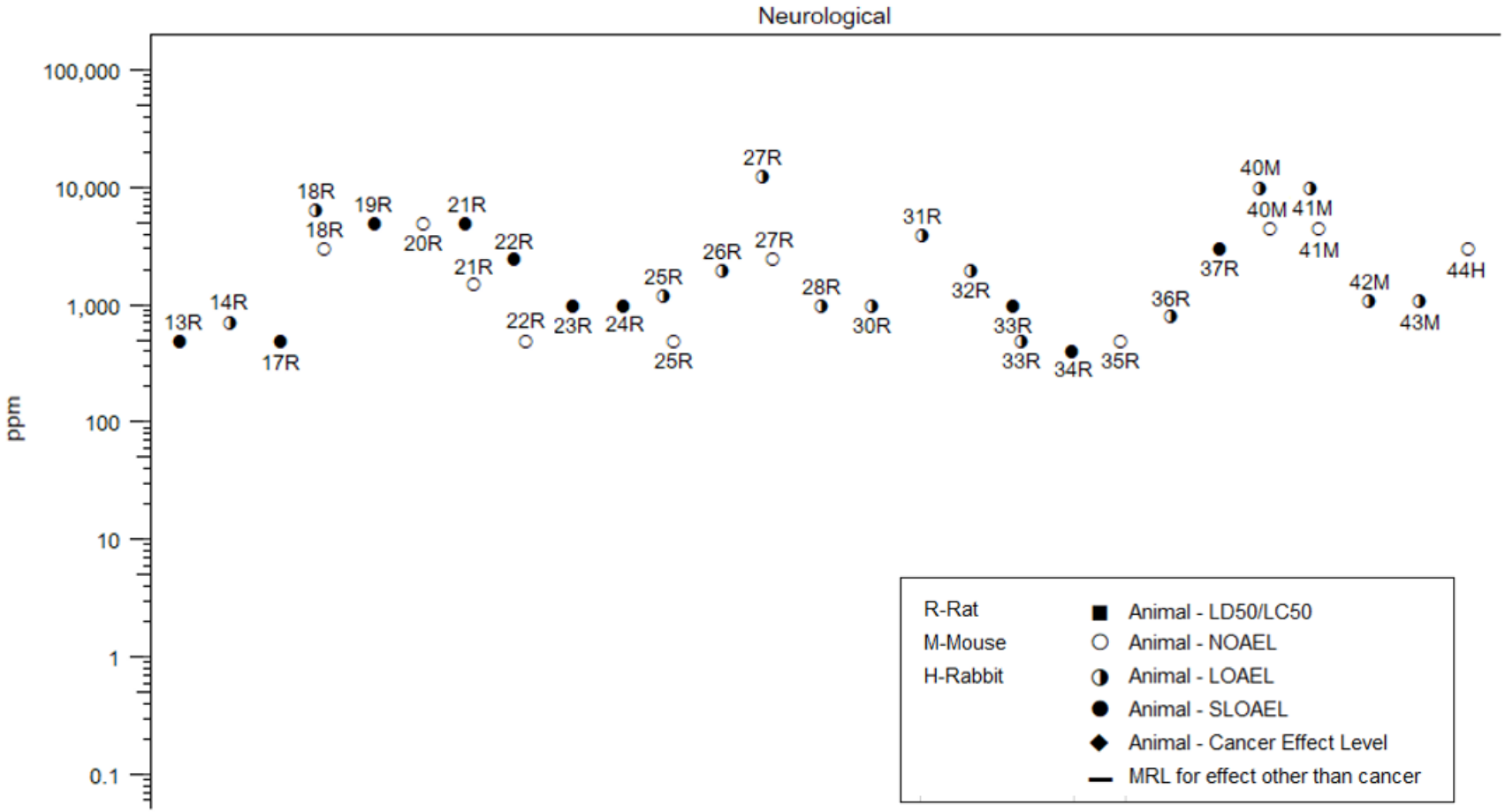


**Figure 2-2. Levels of Significant Exposure to *n*-Hexane – Inhalation**
Intermediate (15–364 days)

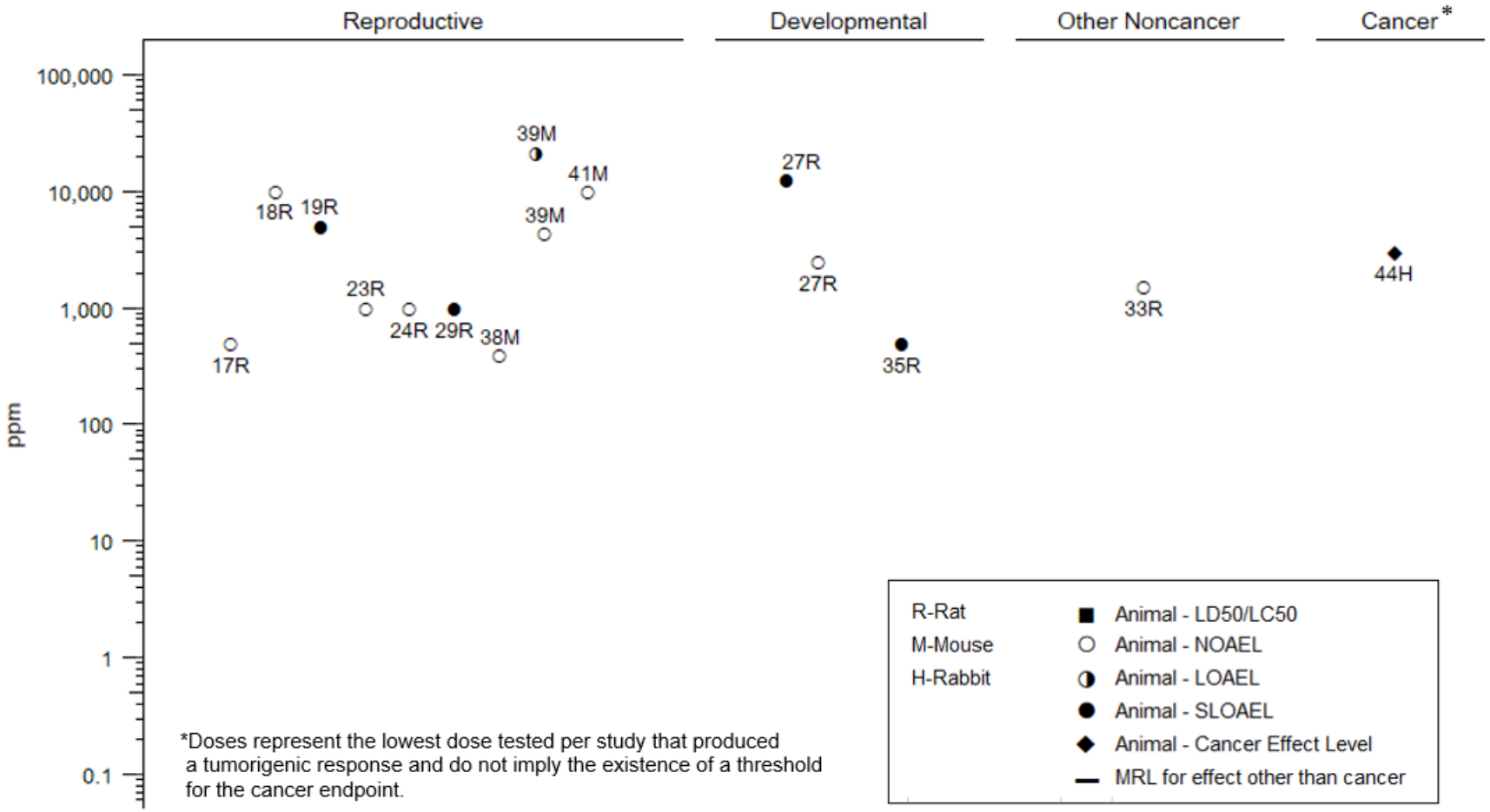


**Figure 2-2. Levels of Significant Exposure to *n*-Hexane – Inhalation**
Chronic (≥365 days)

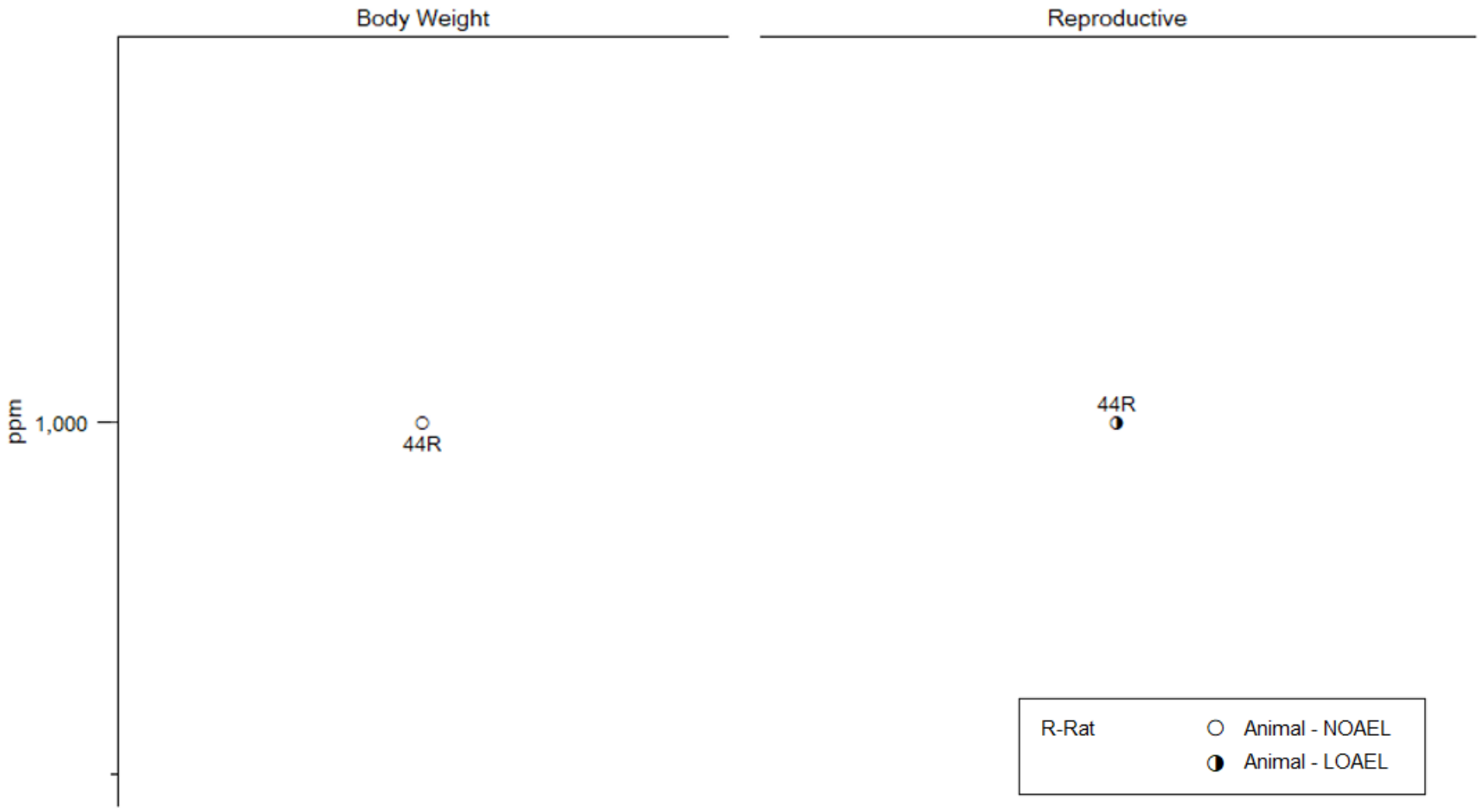


**Table 2-2. Levels of Significant Exposure to *n*-Hexane – Oral (mg/kg/day)**

| Figure key[a] | Species (strain) No./group | Exposure parameters | Doses | Parameters monitored | Endpoint | NOAEL | Less serious LOAEL | Serious LOAEL | Effects |
|---|---|---|---|---|---|---|---|---|---|
| **ACUTE EXPOSURE** | | | | | | | | | |
| **Kimura et al. 1971** | | | | | | | | | |
| 1 | Rat (Sprague-Dawley) 6–12 B | Once (G) | | LE | Death | | | 15,840 | $LD_{50}$ |
| **Linder et al. 1992** | | | | | | | | | |
| 2 | Rat (Sprague-Dawley) 6 M | 1 day 2 times/day (G) | 0, 20,000 | BW, HP, RX, OW | Repro | 20,000 | | | |
| **Linder et al. 1992** | | | | | | | | | |
| 3 | Rat (Sprague-Dawley) 6 M | 5 days 2 times/day (G) | 0, 10,000 | BW, HP, OF, OW | Repro | 10,000 | | | |
| **Marks et al. 1980** | | | | | | | | | |
| 4 | Mouse (CD-1) 24–35 F | 10 days GDs 6–15 3 times/day (GO) | 0, 2,170, 2,830, 7,920, 9,900 | CS, DX, LE | Death | | | 2,830 | Increased mortality in dams (9%, not statistically significant) |
| | | | | | Develop | 2,830 | 7,920 | | Decreased fetal weight (6%) |
| **Marks et al. 1980** | | | | | | | | | |
| 5 | Mouse (CD-1) 6–14 F | 10 days GDs 6–15 1 time/day (GO) | 0, 260, 660, 1,320, 2,200 | CS, DX, LE | Develop | 2,200 | | | |

**Table 2-2. Levels of Significant Exposure to *n*-Hexane – Oral (mg/kg/day)**

| Figure key[a] | Species (strain) No./group | Exposure parameters | Doses | Parameters monitored | Endpoint | NOAEL | Less serious LOAEL | Serious LOAEL | Effects |
|---|---|---|---|---|---|---|---|---|---|
| **INTERMEDIATE EXPOSURE** | | | | | | | | | |
| **Krasavage et al. 1980** | | | | | | | | | |
| 6 | Rat (COBS) 5 M | 90–120 days 5 days/week 1 time/day (G) | 0, 570, 1,140, 4,000 | BW, CS, HP | Bd wt | | 570 | | Decreased body weight (15%) |
| | | | | | Neuro | 1,140 | | 4,000 | Hindlimb paralysis, axonal swelling, myelin retraction |
| | | | | | Repro | 1,140 | | 4,000 | Testicular atrophy of the germinal epithelium |
| **Li et al. 2018** | | | | | | | | | |
| 7 | Rat (Wistar) 10 M | 10 weeks 6 times/week (GO) | 0, 1,000, 2,000, 3,000 | CS, BW, NX | Bd wt | 1,000 | 2,000 | 3,000 | LOAEL: Decreased body weight (19%)<br>SLOAEL: Decreased body weight (26%) |
| | | | | | Neuro | 1,000 | 2,000 | | Abnormal gait, decreased ability to stay on rotating rod |
| **Li et al. 2020a** | | | | | | | | | |
| 8 | Rat (Wistar) 10 M | 7 weeks 1 time/day (GO) | 0, 3,000 | CS, BW, HP | Bd wt | | | 3,000 | Decreased body weight (23%) |
| | | | | | Neuro | | | 3,000 | Paralysis, abnormal gait, nerve damage |
| **Li et al. 2020b** | | | | | | | | | |
| 9 | Rat (Wistar) 8 M | 10–24 weeks (GO) | 0, 500, 1,000, 2,000, 4,000 | CS, BW, NX | Bd wt | 500 | 1,000 | 4,000 | LOAEL: Decreased body weight (19%)<br>SLOAEL: Decreased body weight (28%) |
| | | | | | Neuro | 500 | 1,000 | 2,000 | LOAEL: Transient paralysis, abnormal gait, decreased rotarod latencies, decreased motor conduction velocity<br>SLOAEL: Paralysis after 14 weeks of exposure |

**Table 2-2. Levels of Significant Exposure to *n*-Hexane – Oral (mg/kg/day)**

| Figure key[a] | Species (strain) No./group | Exposure parameters | Doses | Parameters monitored | Endpoint | NOAEL | Less serious LOAEL | Serious LOAEL | Effects |
|---|---|---|---|---|---|---|---|---|---|
| **Ono et al. 1981** | | | | | | | | | |
| 10 | Rat (Wistar) 5–7 M | 8 weeks 1 time/day (GO) | 0, 1,251 | BW, CS, NX | Bd wt | 1,251 | | | |
| | | | | | Neuro | | 1,251 | | Decreased motor and mixed nerve conduction velocity |
| **Wang et al. 2017** | | | | | | | | | |
| 11 | Rat (Wistar) 12 M | 8 weeks 1 time/day (GO) | 0, 3,000 | CS | Neuro | | 3,000 | | Decreased grip strength, abnormal gait |
| **Gao et al. 2019** | | | | | | | | | |
| 12 | Mouse (Kunming) 10 M, F | 20 days (G) | 0, 43.5, 86.5, 173.0 | BW, NX | Neuro | | 43.5[b] | | Impaired performance on a test of memory |

[a]The number corresponds to entries in Figure 2-3.
[b]Used to derive a provisional intermediate-duration oral risk level (MRL) of 0.1 mg/kg/day. The LOAEL of 43.5 mg/kg/day was divided by a total uncertainty factor of 300 (3 for the use of a minimal LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability); see Appendix A for details.

B = both males and females; Bd wt or BW = body weight; CS = clinical signs; Develop = developmental; DX = developmental toxicity; F = female(s); (G) = gavage; GD = gestation day; (GO) = gavage in oil; HP = histopathology; $LD_{50}$ = median lethal dose; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); Neuro = neurological; NOAEL = no-observed-adverse-effect level; NX = neurological function; OF = organ function; OW = organ weight; Repro = reproductive; RX = reproductive function; SLOAEL = serious lowest-observed-adverse-effect level

**Figure 2-3. Levels of Significant Exposure to *n*-Hexane – Oral**
Acute (≤14 days)

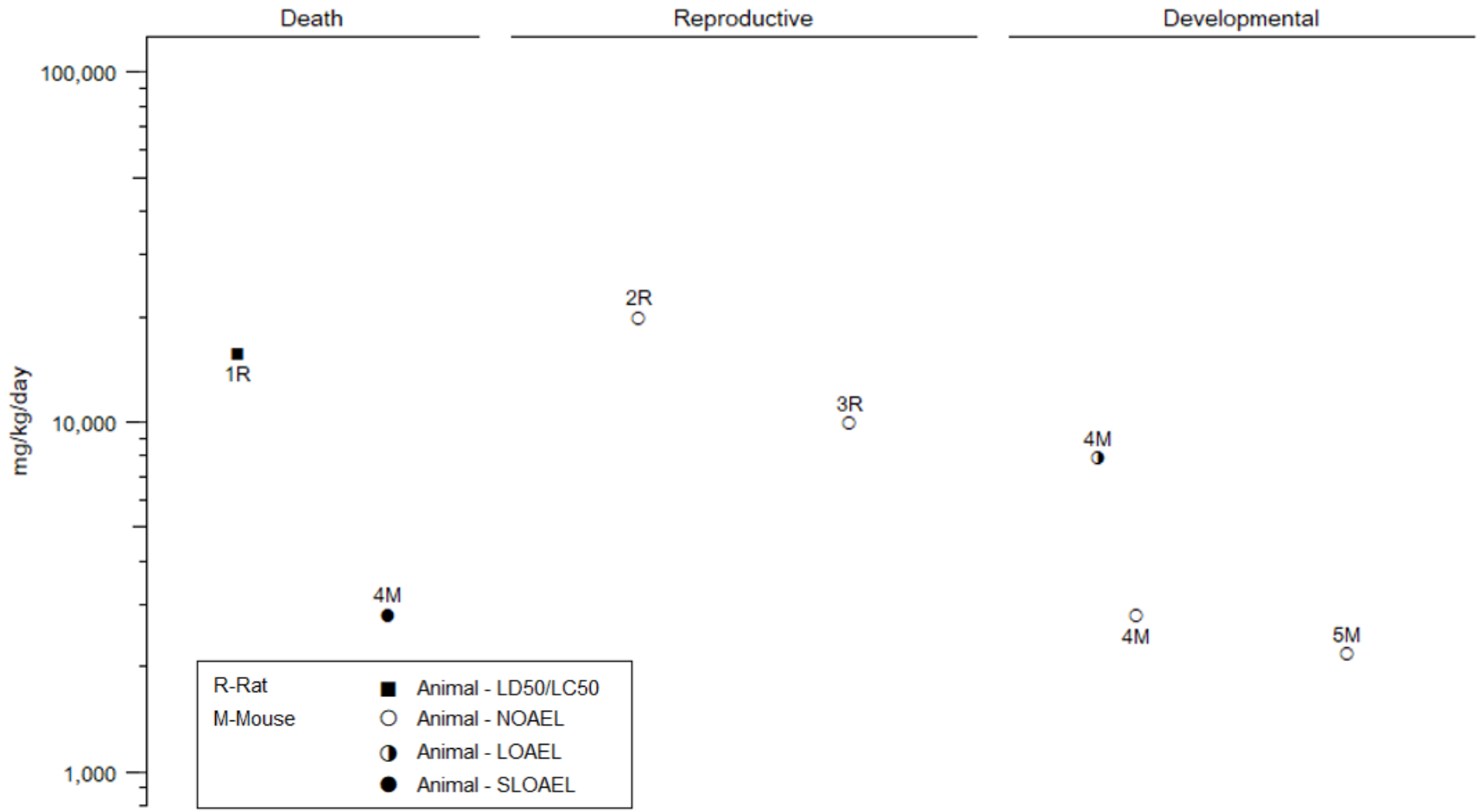
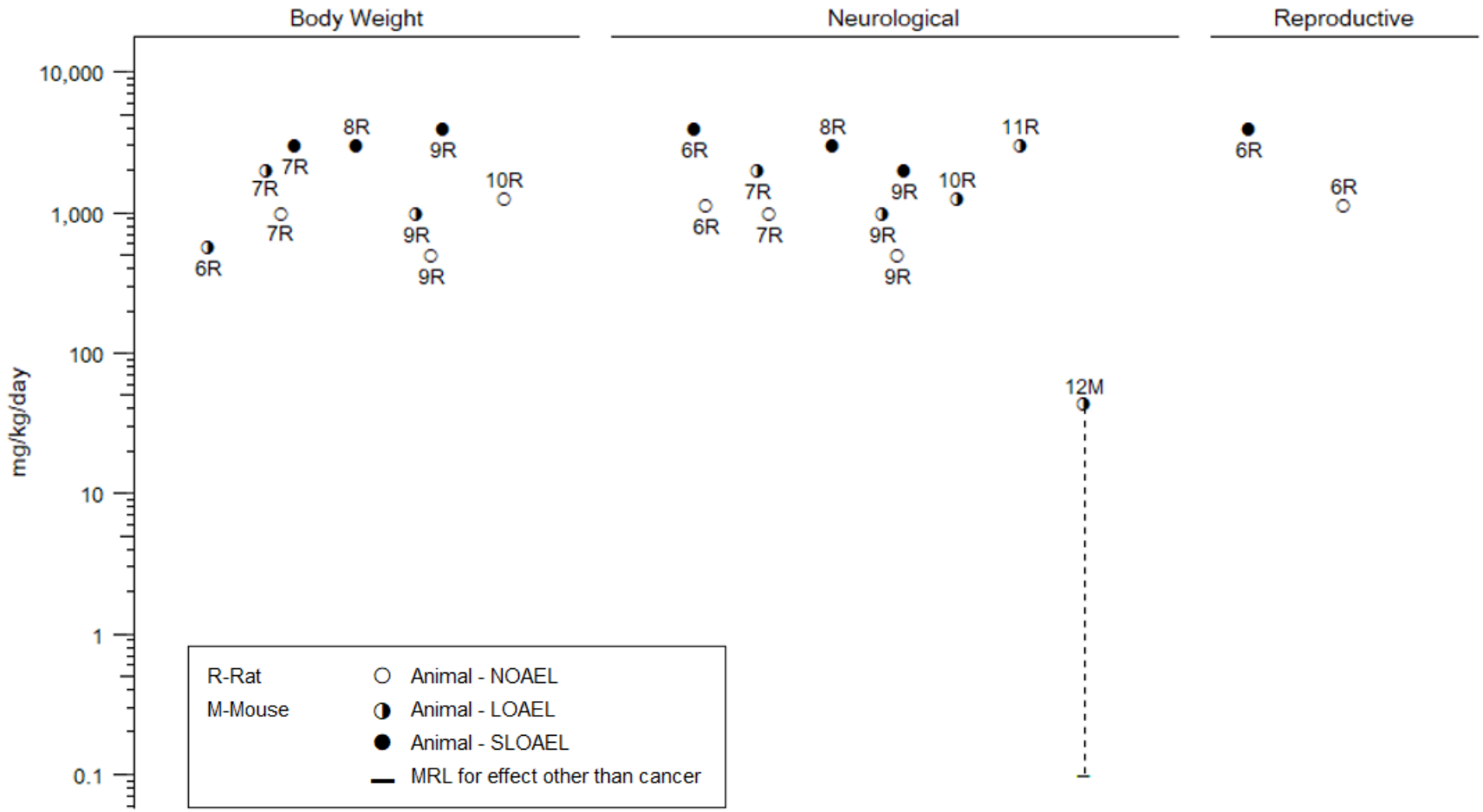


**Figure 2-3. Levels of Significant Exposure to *n*-Hexane – Oral**
Intermediate (15–364 days)



## Table 2-3. Levels of Significant Exposure to *n*-Hexane – Dermal

| Species (strain) No./group | Exposure parameters | Doses | Parameters monitored | Endpoint | NOAEL | Less serious LOAEL | Serious LOAEL | Effects |
|---|---|---|---|---|---|---|---|---|
| **ACUTE EXPOSURE** | | | | | | | | |
| **Iyadomi et al. 2000** | | | | | | | | |
| Mouse (BALB/c) 5 F | Once | 0, 80 µL | OF | Dermal | | 80 | | Increased ear thickness |
| **Wahlberg and Boman 1979** | | | | | | | | |
| Guinea pig (NS) 30 NS | Once | 2 mL | BW, CS, LE | Bd wt | 2 | | | |

Bd wt or BW = body weight; CS = clinical signs; F = female(s); LE = lethality; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; NS = not specified; OF = organ function

## 2.2 DEATH

No studies were located describing death in humans after inhalation exposure to *n*-hexane. This includes cases of occupational exposure where severe neurological effects occurred. All-cause mortality was increased in a cohort of shoe manufacturing workers compared to U.S. referent rates, but not compared to state or county reference rates (Lehman and Hein 2006). Excess deaths from lung cancer and ischemic heart disease (IHD) were the main contributors to the overall mortality elevation (compared to U.S. rates). In a population-based study from Canada, positive associations were observed between increased *n*-hexane exposure and non-accidental causes and nonmalignant respiratory mortality (Villeneuve et al. 2013). When adjusting for nitrogen dioxide ($NO_2$), only nonaccidental causes remained significant. No associations were observed between *n*-hexane and mortality from cardiovascular disease or cancer (including lung cancer).

No studies were located describing death in test animals after acute-duration inhalation exposure to *n*-hexane. No exposure-related deaths were observed in male or female mice or in female rats exposed up to 5,000 ppm for as long as 14 days (API 1979; Chalansonnet et al. 2013; NIEHS 1987, 1988a, 1988b 1988c).

Longer-duration inhalation studies have shown mixed results with regard to mortality. Increased mortality (up to 50%) was observed in several intermediate-duration inhalation studies in rats and rabbits with concentrations as low as 1,000 ppm *n*-hexane (Howd et al. 1983; Lungarella et al. 1984; Rebert and Sorenson 1983; Takeuchi et al. 1980). In contrast, no exposure-related deaths were observed in rats or mice exposed up to 10,000 ppm for as long as 40 weeks (Altenkirch et al. 1982; API 1978, 1981; Cavender et al. 1984; Howd et al. 1983; Huang et al. 1989; Ichihara et al. 1998; NTP 1991; Pryor et al. 1983) or in rats chronically exposed to 1,000 ppm (Imai and Omoto 1999).

Several oral $LD_{50}$ (lethal dose, 50% kill) values are available for *n*-hexane: 15,840 mg/kg was reported for 14-day-old rats, 32,340 mg/kg for young adult rats (80–160 g), and 28,710 mg/kg for older adult rats (300–470 g) (Kimura et al. 1971). No exposure-related deaths were observed in mice following acute-duration exposure up to 2,830 mg/kg/day or in rats exposed for up to 4,000 mg/kg/day 120 days (Bouakkaz et al. 2018; Krasavage et al. 1980; Marks et al. 1980).

Topical application of a single 2-mL dose of undiluted *n*-hexane had no effect on survival in exposed guinea pigs observed for 35 days after exposure (Wahlberg and Boman 1979).

## 2.3 BODY WEIGHT

Data on body weight effects in humans exposed to *n*-hexane are very limited. In an offset printing factory in Hong Kong, weight loss of >5 pounds was reported in employees who developed peripheral neuropathy after exposure to solvents containing *n*-hexane, and in an additional 5 out of 26 asymptomatic workers who were considered to have subclinical peripheral neuropathy (Chang et al. 1993). In a cross-sectional study conducted in Portugal, exposure to indoor *n*-hexane was associated with an increased risk of obesity in children (mean age of 9 years) (Paciencia et al. 2019).

Body weight effects were often observed in animal studies, but these effects were typically accompanied by decreased food consumption and are thought to be a secondary effect following injury to the primary neurological targets of *n*-hexane. In acute-duration inhalation studies, exposure to *n*-hexane at ≥5,000 ppm resulted in decreased body weight in female rats (NIEHS 1987), but not at concentrations ≤1,000 ppm (API 1979; NIEHS 1987), and not in male or female mice (NIEHS 1988a, 1988b, 1988c). Body weight decreases were also observed following intermediate-duration exposure to *n*-hexane. Concentrations ≥1,000 ppm resulted in 10–79% decreases body weight and/or body weight gain in rats and mice (Altenkirch et al. 1982; API 1981; Cavender et al. 1984; De Martino et al. 1987; Howd et al. 1983; Huang et al. 1989; NTP 1991; Pryor et al. 1983; Rebert and Sorenson 1983; Takeuchi et al. 1980). In contrast, several intermediate- and chronic-duration studies have not found changes in body weights in similarly exposed rats, mice, and rabbits (Altenkirch et al. 1982; API 1978; Cavender et al. 1984; Ichihara et al. 1998; Imai and Omoto 1999; Lungarella et al. 1984).

Body weight changes are also a common occurrence following oral exposure to *n*-hexane. Intermediate-duration studies have reported decreased body weights in rats with daily doses ≥570 mg/kg/day (Krasavage et al. 1980; Li et al. 2020a, 2020b). Daily oral doses of 1,251 mg/kg/day for 8 weeks of *n*-hexane had no effect on body weight in male rats (Ono et al. 1981).

Topical application of a single 2 mL dose of undiluted *n*-hexane had no effect on body weight in exposed guinea pigs followed for 35 days after exposure (Wahlberg and Boman 1979).

## 2.4 RESPIRATORY

Data on potential respiratory effects in humans are limited. In a controlled human study, vapor concentrations up to 500 ppm *n*-hexane (purity not listed) did not cause irritation to the nose or throat in 10 volunteers exposed for 3–5 minutes in an inhalation chamber (Nelson et al. 1943). Self-reported respiratory symptoms including cough, phlegm, bronchitis, and chest tightness were more frequent in solvent-exposed chemical plant workers compared to controls (Mustajbegovic et al. 2000). No association was observed between self-reported breathing difficulty and total months working with solvents in shoe workers (Nijem et al. 2000), although a second study showed that self-reported breathing difficulty was correlated with years of exposure in varnishing workers (Nijem et al. 2001).

Ambient concentrations of *n*-hexane were associated with lower forced expiratory volume ($FEV_1$) and forced vital capacity (FVC) scores in children (mean age of 8 years) living near petrochemical plants in Argentina (Wichmann et al. 2009). In a cross-sectional study, no association was observed between ambient *n*-hexane concentrations and hospital visits for children (≤16 years of age) with wheezy episodes (Buchdahl et al. 2000). Similarly, no association was observed between indoor *n*-hexane concentrations and the incidence of rhinitis in children (mean age 9 years) (Paciencia et al. 2020).

Respiratory effects have been reported in mice and rabbits following intermediate-duration inhalation studies, while rats may be less sensitive to these effects. Increased lung weights have been observed in male rats exposed to concentrations ≥1,000 ppm (Howd et al. 1983), but no histopathological changes in the lungs or nasal cavities were observed (API 1981; Cavender et al. 1984). In mice, histopathological effects in the nasal cavity (multifocal regeneration and metaplasia in the olfactory epithelium), have been observed at *n*-hexane concentrations ≥4,421 ppm for 6 hours/day, 5 days/week or 1,099 ppm for 22 hours/day, 5 days/week for 13 weeks (NTP 1991). Sneezing was observed in mice exposed to 10,000 ppm starting at 4 weeks of exposure (NTP 1991). Male rabbits exposed to 3,000 ppm for 24 weeks showed signs of respiratory tract irritation (nasal discharge) and breathing difficulties (gasping, lung rales, mouth breathing) (Lungarella et al. 1984). Additionally, histopathological examination revealed pulmonary fibrosis, centriacinar emphysema, and epithelial desquamation.

Increased lung weights were also observed in male rats administered 600 mg/kg/day *n*-hexane via gavage for 8 weeks (Bouakkaz et al. 2018). Histopathological evaluation of the lungs revealed lesions comparable to acute interstitial pneumonia, including multifocal bronchopneumonia, fibronecrotic lesions, alveoli filled with red blood cells, and inflammatory cells. The presence of erythrocytes in the

lungs was graded as a severe effect at this dose by the study authors. The nature of these lesions suggests an injury to the lungs, which could potentially occur with an error in gavage dosing.

## 2.5 CARDIOVASCULAR

No studies were located on cardiovascular effects of *n*-hexane in humans.

Histopathological examination of the heart and aorta revealed no treatment related lesions in male rats exposed up to 500 ppm *n*-hexane for 22 hours/day, 7 days/week for 6 months (API 1981). Differences in relative heart weights were reported in B6C3F1 mice exposed for 13 weeks to *n*-hexane at concentrations of 1,099 ppm for 22 hours/day, although no histopathological changes were observed (NTP 1991).

## 2.6 GASTROINTESTINAL

No studies were located on gastrointestinal effects of *n*-hexane in humans.

Histopathological examination of gastrointestinal tissues revealed no treatment-related lesions in male rats exposed to up to 500 ppm *n*-hexane for 22 hours/day, 7 days/week for 6 months (API 1981). Likewise, no gastrointestinal alterations were reported in mice exposed for 13 weeks to *n*-hexane at concentrations up to 10,000 ppm for 6 hours/day or 1,099 ppm for 22 hours/day (NTP 1991).

## 2.7 HEMATOLOGICAL

No exposure-related differences in blood parameters (e.g., complete blood counts) were reported in offset printers (Chang et al. 1993) or tungsten carbide alloy workers (Sanagi et al. 1980) occupationally exposed to *n*-hexane. White blood cell counts were unaffected by *n*-hexane exposure in 35 workers compared to 23 unexposed controls (Karakaya et al. 1996). No differences in complete blood or platelet counts were observed in *n*-hexane-exposed male shoe repairers compared to controls (Tomei et al. 1999). Decreased hematocrit (females only) and mean corpuscular volume (males only) were observed in shoe workers in Bosnia and Herzegovina compared to controls, although the study authors stated that the values still fell within the laboratory's reference range (Umicevic et al. 2022).

Hematological parameters were within normal limits in rats exposed up to 10,000 ppm for 13 weeks (Cavender et al. 1984). Similarly, no exposure-related changes were observed in male and female rats

exposed up to 129 ppm for 6 months (API 1978). In male mice exposed to 10,000 ppm for 6 hours/day for 13 weeks, increased number of segmented neutrophils was observed, which the study authors attributed to chronic active inflammation in the nasal mucosa (NTP 1991). No other biologically relevant changes were observed in female mice similarly exposed up to 10,000 ppm for 6 hours/day for 13 weeks or in male and female mice exposed to 1,099 ppm *n*-hexane for 22 hours/day for 13 weeks. No changes in hematological parameters were observed in male rabbits exposed to 3,000 ppm *n*-hexane for 24 weeks (Lungarella et al. 1984).

Increases in white blood cells were observed in male rats administered 300 mg/kg/day *n*-hexane via gavage for 8 weeks compared to vehicle controls (Bouakkaz et al. 2018). These animals presented with signs of acute pneumonia and severe erythrocytes in the lungs, which could result from gavage dosing errors, so the toxicological significance of increased inflammatory cells is questionable.

## 2.8 MUSCULOSKELETAL

Muscle wasting and atrophy have been reported in humans with severe neurotoxicity occupationally exposed to *n*-hexane (Yamamura 1969). Three women who worked as cabinet finishers and presented with signs of neuropathy had normal levels of creatine phosphokinase (data not provided), while electromyograms showed fibrillation in the proximal and distal muscles (Herskowitz et al. 1971). Additionally, histopathological evaluation of the muscle showed angulation of fibers and target fibers, both signs of denervation. Muscle weakness and denervation were also reported in offset printers occupationally exposed to *n*-hexane (Chang et al. 1993).

Hindlimb muscular atrophy characterized as “severe” was reported in male rats exposed to 986 ppm *n*-hexane for up to 61 days (Nylen et al. 1989). Skeletal muscle atrophy was observed in male rats exposed to 500 ppm *n*-hexane for 22 hours/day for 6 months (API 1981). Electron microscopy of the gastrocnemius and soleus muscles in male rats exposed to 3,040 ppm *n*-hexane for 12 hours/day for 16 weeks, revealed atrophy, denervation, irregular fibers, disordered myofilaments, zigzagging of the Z-band, and numerous invaginations of the plasma membrane (Takeuchi et al. 1980).

## 2.9 HEPATIC

There is a limited amount of information on potential hepatic effects in workers. Mean concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, and alkaline phosphatase

(AP) were higher among *n*-hexane-exposed male shoe repairers compared to controls, although no differences in gamma-glutamyl transferase (GGT), cholesterol, or triglycerides were observed (Tomei et al. 1999). Increased bilirubin, AST, and GGT were observed in male and female shoe workers in Bosnia and Herzegovina compared to controls, although the study authors stated that most values still fell within the laboratory's reference range (Umicevic et al. 2022). Serum AST and ALT activities were within normal limits in a group of shoe and leather workers (Murata et al. 1994).

Based on data from animal studies, the liver does not appear to be a primary target of *n*-hexane toxicity. Increased liver weights have been observed in mice and rats exposed to *n*-hexane concentrations ≥1,000 ppm, but histopathological changes have either not been observed or not been evaluated (API 1981; Cavender et al. 1984; Howd et al. 1983; NTP 1991). No change in liver function parameters (ALT, AST, AP) were observed in male and female rats exposed up to 10,000 ppm for 13 weeks (Cavender et al. 1984) or in male or female rats exposed up to 129 ppm for as long as 6 months (API 1978).

## 2.10 RENAL

No exposure-related differences in kidney function tests (e.g., urinalysis, blood urea nitrogen) were reported in offset printers (Chang et al. 1993), tungsten carbide alloy workers (Sanagi et al. 1980), or male shoe repairers (Tomei et al. 1999). Decreased creatinine levels were observed in female shoe workers in Bosnia and Herzegovina compared to controls, although the study authors stated that it still fell within the laboratory's reference range (Umicevic et al. 2022).

An increase in relative kidney weights along with an increased incidence and severity of chronic nephropathy were noted in male rats exposed to 500 ppm *n*-hexane for 22 hours/day for 6 months (API 1981). The study authors stated that it was unclear whether the increased incidence and severity was due to exacerbation of the process seen in the control group or if the *n*-hexane exposure caused additional tubular injury. Relative kidney weights were also increased in rats and mice exposed to *n*-hexane at ≥1,000 ppm, but no histopathological lesions were observed (Cavender et al. 1984; Howd et al. 1983; NTP 1991).

Urine pH was decreased in male rats exposed to 10,000 ppm for 13 weeks, but not in females and no other changes in urine parameters were noted in either sex (Cavender et al. 1984). Decreased blood urea nitrogen was noted in female rats, but not male rats, exposed to 126 ppm *n*-hexane for 21 hours/day, 7 days/week for 6 months (API 1978). This difference was not observed at 3 months or in female rats

exposed for 6 hours/day, 5 days/week for 6 months. The toxicological significance of these changes in urinary parameters is unknown.

## 2.11 DERMAL

Coldness, reddishness, or roughness of the skin in the distal extremities was observed in workers with peripheral neuropathy after occupational inhalation exposure to *n*-hexane (Yamamura 1969). Application of 1.5 mL analytical-grade *n*-hexane to the volar forearm of a male volunteer caused an initial increase blood flow (expressed as a relative, dimensionless value), which returned to control approximately 60 minutes after application (Wahlberg 1984). A slight transient erythema was observed after 10–20 minutes of exposure and a stinging and/or burning sensation was reported by the volunteer.

Histopathological examination of the skin after intermediate-duration inhalation exposure revealed no treatment-related lesions in rats exposed to 500 ppm (API 1981) or in mice exposed to *n*-hexane at concentrations up to 10,000 ppm for 6 hours/day for 13 weeks or 1,099 ppm for 22 hours/day for 13 weeks (NTP 1991).

Dermal application of 80 µL to the front and back of the ears of female mice resulted in increased ear thickness within 2 hours of exposure (Iyadomi et al. 2000). Peak ear thickness was observed at 6 hours and was still significantly thicker than controls 24 hours post-exposure. There were no differences in ear thickness 48 or 72 hours post-exposure, as compared to controls.

## 2.12 OCULAR

In a controlled human study, vapor concentrations up to 500 ppm *n*-hexane did not cause irritation to the eyes, nose, or throat in 10 volunteers exposed for 3–5 minutes in an inhalation chamber (Nelson et al. 1943). Maculopathy and color discrimination defects were identified in 11 out of 15 workers exposed to *n*-hexane from glues or solvents used in vegetable oil extraction, although these workers were also exposed to numerous other chemicals (Raitta et al. 1978; Seppalainen et al. 1979).

Histopathological examination of the eye and optic nerve after intermediate-duration inhalation exposure revealed no treatment-related lesions in male Sprague-Dawley rats exposed to 500 ppm *n*-hexane 22 hours/day for 6 months (API 1981). No ophthalmologic differences were reported in male and female rats exposed to up to 10,000 ppm for 13 weeks compared to controls (Cavender et al. 1984). Ocular

irritation (lacrimation, hyperemia of the conjunctiva) was observed in male rabbits exposed to 3,000 ppm *n*-hexane for 24 weeks (Lungarella et al. 1984).

## 2.13 ENDOCRINE

The data on the potential endocrine effects of *n*-hexane are very limited. Three women who worked as cabinet finishers and presented with signs of neuropathy had normal thyroxine levels (data not provided) (Herskowitz et al. 1971).

Histopathological examination of endocrine tissues after intermediate-duration inhalation exposure revealed no treatment-related lesions in male rats exposed to 500 ppm *n*-hexane daily for 22 hours/day for 6 months (API 1981). Similar results were seen in mice exposed for 13 weeks to *n*-hexane at concentrations up to 10,000 ppm for 6 hours/day or at 1,099 ppm for 22 hours/day (NTP 1991).

## 2.14 IMMUNOLOGICAL

No differences were observed in natural killer (NK) cell cytotoxic capacity or in serum interleukin-2 (IL-2) and interferon-gamma (IFN-γ) levels in two male shoe factory workers compared to controls (Yücesoy et al. 1999). Serum immunoglobulins (IgG, IgM, and IgA) were reduced in male workers compared to unexposed controls, and Ig levels were correlated with urinary 2,5-hexanedione concentrations but not with workplace *n*-hexane concentrations (Karakaya et al. 1996). The reductions also remained well within the normal ranges for immunoglobulins in blood (Jackson et al. 1997), so the toxicological significance of these findings cannot be assessed.

No changes in spleen weight or histopathology were observed in male and female rats exposed to up to 10,000 ppm for 13 weeks (Cavender et al. 1984). No treatment-related lesions were observed in the lymph nodes, thymus, bone marrow, or spleen of rats exposed to 500 ppm for 6 months (API 1981) or in mice exposed for 13 weeks to concentrations up to 10,000 ppm for 6 hours/day or 1,099 ppm for 22 hours/day (NTP 1991).

## 2.15 NEUROLOGICAL

The neurotoxicity of *n*-hexane was first observed in the shoe industries of Japan and Italy in the 1960s and 1970s (Abbritti et al. 1976; Cianchetti et al. 1976; Sobue et al. 1978; Yamamura 1969). A number of

epidemiological studies were initiated in response to outbreaks of apparent peripheral neuropathy in shoe workers. While the clinical course of the disease was well described, elucidation of a dose-duration response relationship has been difficult. In most cases, concentrations of *n*-hexane in the workplace air were either not measured at all or not until after disease developed. Also, in almost all cases, workers were concurrently exposed to other chemicals, which may have affected their response to *n*-hexane, such as acetone, methyl ethyl ketone (MEK), and toluene.

One of the first large epidemiological investigations carried out was a case series of 93 cases of peripheral neuropathy in workers exposed to *n*-hexane from glues and solvents used in sandal manufacture (Yamamura 1969). The most common physical symptoms included numbness in the distal portions of the extremities, muscle weakness, and hypoactive reflexes, while electromyography exams showed reductions in motor nerve conduction velocities and nerve biopsies revealed demyelination and axonal degeneration. Since then, numerous case reports/series have evaluated workers/patients exposed to organic solvents and exhibiting the characteristic signs of *n*-hexane-induced peripheral neuropathy (e.g., Abbritti et al. 1976; Altenkirch et al. 1977; Carelli et al. 2007; Hageman et al. 1999; Herskowitz et al. 1971; Kanavouras et al. 2011; Pastore et al. 2002; Paulson and Waylonis 1976; Pezzoli et al. 1995; Pradhan and Tandon 2015; Puri et al. 2007; Sendur et al. 2009; Smith and Albers 1997; Sun et al. 2020; Thulasirajah et al. 2020; Valentino 1996; Vanacore et al. 2000; Yokoyama et al. 1990).

Several occupational epidemiology studies are available that have evaluated a number of symptoms commonly observed with solvent-induced polyneuropathy (Table 2-4). Some of the most reported clinical signs include weakness in the extremities, absent or decreased reflexes, weight loss, muscle pain, and headaches (Chang 1987; Chang and Yip 1987; Chang et al. 1993; Huang et al. 1991; Nijem et al. 2000; Sanagi et al. 1980). Several studies have demonstrated subclinical alteration in neurological function after inhalation exposure to *n*-hexane. Abnormal nerve conduction tests (conduction velocity, distal latency, potential amplitude) have been observed in workers with and without outward clinical signs of polyneuropathy (Chang 1987; Chang and Yip 1987; Chang et al. 1993; Huang et al. 1991; Mutti et al. 1982a, 1982b; Neghab et al. 2012; Raitta et al. 1978; Seppalainen et al. 1979). Additional studies have evaluated alterations in vision (Gong et al. 2003; Issever et al. 2002; Raitta et al. 1978; Seppalainen et al. 1979; Verberk et al. 2004), hearing loss/ototoxicity (Juarez-Perez et al. 2014; Sliwinska-Kowalska

**Table 2-4. Results of Epidemiological Studies Evaluating Exposure to *n*-Hexane and Neurological Effects**

| Reference, study type, and population | Exposure/biomarker | Outcome evaluated | Result |
|---|---|---|---|
| **Bates et al. 2016, 2019**<br><br>Cross-sectional, 831 automotive workers (San Francisco Bay area, California) | Estimated from MSDS<br><br>Q1: 0 mg/m$^3$-years<br>Q2: >0–<32 mg/m$^3$ -years<br>Q3: ≥32 mg/m$^3$-years | Psychomotor speed, memory, fine motor function, mood | ↔ |
| | | Clinical signs | ↔ Ankle reflexes, touch sensation, vibration sensation |
| | | MCV | ↔ Peroneal |
| | | SSPL | ↓ (Q2 only) |
| **Beckman et al. 2016**<br><br>Cross-sectional, 689 automotive workers (San Francisco, California) | Estimated from MSDS<br><br>Q1: 0 ppm-years<br>Q2: >0–<9.6 ppm-years<br>Q3: ≥9.6 ppm-years | Color vision | ↔ |
| **Boggess et al. 2016**<br><br>Case-control, 30 children with autism spectrum disorders, 30 age-matched controls (Pennsylvania) | Serum hexane concentration<br><br>11.7 µg/g in cases versus 9.44 µg/g in controls | Autism Diagnostic Observation Schedule | ↔ |
| **Chang and Yip 1987**<br><br>Cross-sectional, 75 printing factory workers, 72 controls (Taiwan)<br><br>25 polyneuropathy (S), 5 subclinical (AS), and 45 unaffected workers (HW) | Not reported | Clinical signs | ↑ Weakness in extremities<br>↑ Absent/decreased reflexes |
| | | MCV | ↓ Median, ulnar, peroneal, tibial (S, AS, HW) |
| | | MAP amplitude | ↓ Median (S, AS, HW)<br>↓ Ulnar, peroneal, tibial (S, AS) |
| | | MAP distal latency | ↓ Median, ulnar, peroneal, tibial (HW, AS, S) |
| | | SCV | ↓ Median, ulnar, sural (S, AS) |
| | | SAP amplitude | ↓ Median, ulnar, sural (S, AS, HW) |
| | | SAP distal latency | ↓ Median, ulnar, sural (S, AS) |

**Table 2-4. Results of Epidemiological Studies Evaluating Exposure to *n*-Hexane and Neurological Effects**

| Reference, study type, and population | Exposure/biomarker | Outcome evaluated | Result |
|---|---|---|---|
| **Chang 1987**<br><br>Cross-sectional, 34 printing factory workers, 22–25 controls (Taiwan)<br><br>22 polyneuropathy (S), 5 subclinical (AS), and 7 unaffected workers (HW) | Not reported | Clinical signs | ↑ Weakness in extremities<br>↑ Absent/decreased reflexes<br>↔ Headaches, sleep disorders, mental changes |
| | | MCV | ↓ Median, ulnar, peroneal, tibial |
| | | SCV | ↓ Median, ulnar, sural |
| | | Patterned visual evoked potentials | ↑ Latency (N1, P1, N2, N1–N2)<br>↓ Amplitude (N1–P1, P1–N2) |
| | | Brainstem auditory evoked potentials | ↑ Latency (wave III, wave V, I–III, III–V, I–V)<br>↔ Latency (wave I) |
| | | Somatosensory evoked potentials | ↑ Latency (scalp median, scalp peroneal, neck median) |
| **Chang et al. 1993**<br><br>Cross-sectional, 56 printing press workers, 20 controls (Hong Kong)<br><br>10 healthy workers (HW), 26 asymptomatic (AS) and 20 symptomatic (S) neuropathic workers | Background<br>63 ppm (30–110 ppm)<br><br>Personal samplers<br>132 ppm (80–210 ppm)<br><br>2.6 years<br>(1 month–12 years)<br><br>(Work schedule<br>12 hours/day, 6 days/week) | SAP amplitude | ↓ Median (HW, AS, S)<br>↓ Ulnar (S)<br>↓ Sural (AS, S) |
| | | MAP amplitude | ↓ Median (S)<br>↓ Ulnar (AS, S)<br>↓ Posterior tibial (S)<br>↓ Common peroneal (S) |
| | | SAP distal latency | ↑ Median (AS, S)<br>↑ Ulnar (S)<br>↑ Sural (S) |
| | | MAP distal latency | ↑ Median (AS, S)<br>↑ Ulnar (AS, S)<br>↑ Posterior tibial (S)<br>↑ Common peroneal (S) |
| | | Motor conduction velocity | ↓ Median (AS, S)<br>↓ Ulnar (AS, S)<br>↓ Posterior tibial (AS, S)<br>↓ Common peroneal (AS, S) |
| | | Clinical signs | ↑ Weight loss >5 pounds (AS, S)<br>↑ CNS symptoms[a] (S) |

**Table 2-4. Results of Epidemiological Studies Evaluating Exposure to *n*-Hexane and Neurological Effects**

| Reference, study type, and population | Exposure/biomarker | Outcome evaluated | Result |
|---|---|---|---|
| **Goldman et al. 2012**<br><br>Case-control, 99 pairs of twins (World War II veterans) discordant for Parkinson's disease | Estimated from job history<br><br>Low 2.5 ppm<br>Medium 25 ppm<br>High 100 ppm | Parkinson disease | ↔ |
| **Gong et al. 2003**<br><br>Cross-sectional, 182 furniture workers, 96 unexposed controls (Japan) | Mean 0.29 ppm<br>Maximum 12.9 ppm | Color vision | ↑ Color confusion index<br>↓ Visual contrast sensitivity |
| | | Visual evoked potential | ↔ Latency, amplitude |
| **Governa et al. 1987**<br><br>Cross-sectional, 40 shoe factory workers (Italy) | Urinary 2,5-HD<br>6.8 mg/L (0.5–19 mg/L) | Electroneuromyographic abnormalities | ↑ |
| **Huang et al. 1991**<br><br>Cross-sectional, 44 ball manufacturing workers, 52 controls (Taiwan) | High 86 and 110 ppm (cement coating, nylon fiber winding)<br><br>Low 75 ppm (gas injection, outer layer production) | Clinical signs | ↑ Muscle pain, weakness |
| | | MCV | ↓ Median, ulnar, peroneal, tibial |
| | | Motor amplitude | ↓ Median, ulnar, peroneal, tibial (high only) |
| | | Motor DL | ↑ Median, ulnar, peroneal, tibial |
| | | SCV | ↓ Median, ulnar, sural (high)<br>↓ Median (low) |
| | | Sensory amplitude | ↓ Median, ulnar, peroneal, tibial (high only) |
| | | Sensory DL | ↑ Median, ulnar, peroneal, tibial |
| **Issever et al. 2002**<br><br>Case-control, 26 workers diagnosed with *n*-hexane-induced polyneuropathy, 50 unexposed controls (Turkey) | Not conducted | Color vision | ↑ Hue error test scores |

## Table 2-4. Results of Epidemiological Studies Evaluating Exposure to *n*-Hexane and Neurological Effects

| Reference, study type, and population | Exposure/biomarker | Outcome evaluated | Result |
|---|---|---|---|
| **Ithnin et al. 2011**<br><br>Cross-sectional,<br>17 locomotive depot workers,<br>17 controls (Malaysia) | 0.01–0.03 ppm | Neurobehavioral tests | ↓ Santa Ana Manual Dexterity (non-dominant hand)<br>↑ Pursuit aiming test 1<br>↔ Reaction time, digital symbol, trail making |
| **Juarez-Perez et al. 2014**<br><br>Cross-sectional, 77 paint factory workers, 84 controls (Mexico) | 0.96 ppm median<br>(0.25–19 ppm) | Hearing impairments | ↑ Hearing loss in all frequencies |
| | | Brainstem auditory-evoked potentials | ↑ Latency |
| **Murata et al. 1994**<br><br>Cross-sectional, 30 shoe and leather workers,<br>25 unexposed controls (Japan) | Urinary 2,5-HD<br>1.39 mg/L (0–3.18 mg/L) | Electrocardiographic parameters | ↓ R-R interval variability; parasympathetic activity<br>↔ Heart rate; sympathetic activity |
| | | Nerve conduction velocity | ↓ DCV, SCV (median, forearm)<br>↔ MCV (median), SCV (median, hand) |
| | | Correlation with 2,5-HD levels | ↔ |
| **Mutti et al. 1982a**<br><br>Cross-sectional, 95 shoe factory workers, 52 controls (Italy) | Mild (M) exposure group<br>49/69 ppm (median/mean)<br><br>High (H) exposure group<br>103/134 ppm (median/mean)<br><br>9.1 years<br>(1–25 years) | Clinical signs (all exposed) | ↑ Sleepiness, dizziness, weakness, paraesthesia, hypoesthesia<br>↔ Headache, muscular cramps, neurasthenic syndrome, sleep disturbances |
| | | Motor conduction velocity | ↓ Median, peroneal (M, H)<br>↔ Ulnar |
| | | MAP amplitude | ↓ Median, ulnar, peroneal (M, H) |
| | | MAP duration | ↑ Median (H)<br>↑ Ulnar (M, H)<br>↔ Peroneal (M, H), median (M) |

**Table 2-4. Results of Epidemiological Studies Evaluating Exposure to *n*-Hexane and Neurological Effects**

| Reference, study type, and population | Exposure/biomarker | Outcome evaluated | Result |
|---|---|---|---|
| **Mutti et al. 1982b**<br><br>Cross-sectional, 15 shoe factory workers, 15 controls (Italy) | 127/195 ppm (median/mean)<br><br>4.5 years (2–8 years) | Motor conduction velocity | ↓ Median, ulnar, peroneal |
| | | Distal sensory conduction velocity | ↓ Median, ulnar |
| | | Sensory nerve action potential latency | ↑ Median, ulnar |
| | | MAP duration; MAP amplitude; distal latency | ↔ Median, ulnar, peroneal |
| | | Sensory nerve action potential amplitude | ↔ Median, ulnar |
| **Neghab et al. 2012**<br><br>Cross-sectional, 27 AS shoemakers, 20 controls (Iran) | Breathing zone 33 ppm (5–85 ppm)<br><br>Urinary 2,5-HD 0.23 mg/L (0.12–0.36 mg/L)<br><br>26 years (17–44 years) | MCV, MAP, DL | ↔ Median, ulnar, posterior tibial, peroneal |
| | | SCV, DL | ↔ Median, ulnar, sural |
| | | SAP | ↓ Median, sural<br>↔ Ulnar |
| | | Correlation between SAP and 2,5-HD | ↑ |
| **Nijem et al. 2000**<br><br>Cross-sectional, 103 shoe workers (West Bank) | Not reported | Clinical signs | ↑ Headaches, mental irritability |
| | | Correlation with years of exposure | ↔ |
| **Nijem et al. 2001**<br><br>Cross-sectional, 167 shoe factory workers (West Bank) | Not reported | Correlation with years of exposure | ↔ Headaches, mental irritability<br>↑ Tingling limbs (plastic work), sore eyes (cleaning, plastic work) |
| **Park et al. 2009**<br><br>Cross-sectional, 41 solvent workers, 90 nonexposed controls (South Korea) | 0.11–1.41 ppm across 4 plants | Postural sway (eyes open) | ↑ Sway (area, length) |
| | | Postural sway (eyes closed) | ↔ Sway (area, length) |

**Table 2-4. Results of Epidemiological Studies Evaluating Exposure to *n*-Hexane and Neurological Effects**

| Reference, study type, and population | Exposure/biomarker | Outcome evaluated | Result |
|---|---|---|---|
| **Pastore et al. 1994**<br><br>Cross-sectional, 20 "healthy" workers with urinary 2,5-HD of >5 mg/L, 49–141 controls from previous studies (Spain) | Urinary 2,5-HD<br>11 mg/L (5–24 mg/L)<br><br>8 years (1.5–23 years) | Sensory nerve conduction velocity | ↔ Median, ulnar, sural |
| | | MNCV, latency | ↔ Tibial |
| | | Sensory nerve action potential amplitude | ↓ Median, sural, ulnar |
| **Raitta et al. 1978; Seppalainen et al. 1979**<br><br>Cross-sectional, 15 adhesive and oil extraction workers, 10 unexposed controls (Finland) | Highest concentrations varied from 1,500 to 3,250 ppm<br><br>12 years (5–21 years) | Visual evoked potentials | ↓ Amplitude (N1, P2, N2, P3, N3)<br>↑ Amplitude (P1)<br>↑ Latency (P1, N1)<br>↓ Latency (P2)<br>↔ Latency (N0, N2, P3, N3) |
| | | Electroretinograms | ↓ Amplitude<br>↔ Latency (a wave)<br>↓ Latency (b wave) |
| | | Visual acuity, visual fields, intraocular pressure, biomicroscopic examination findings | ↔ |
| | | Color vision defects | ↑ (12/15 examined) |
| **Sanagi et al. 1980**<br><br>Cross-sectional, 14 tungsten carbide alloy workers, 14 controls (Japan) | 58 ppm 8-hour TWA<br><br>6.2 years (1–12 years) | Clinical signs | ↑ Headache, hearing deficit, muscle weakness, dysesthesia in limbs<br>↔ Vertigo, muscle pain, numbness in limbs |
| | | Neurological tests | ↓ Muscle strength (jumping on one foot), vibration sensation on the radial processes<br>↔ Grip, position sense, coordination |
| | | Motor conduction velocity | ↔ Median<br>↓ Tibial |
| | | Residual latency | ↔ Median<br>↑ Tibial |
| | | MAP; MNCV; distal sensory conduction velocity | ↔ Median, tibial |

## Table 2-4. Results of Epidemiological Studies Evaluating Exposure to *n*-Hexane and Neurological Effects

| Reference, study type, and population | Exposure/biomarker | Outcome evaluated | Result |
|---|---|---|---|
| **Sliwinska-Kowalska et al. 2005**<br><br>Cross-sectional, 1,117 yacht, ship, paint and lacquer, plastic and shoe workers, 157 (no solvent or noise exposure) and 66 (no solvent exposure) controls (Poland) | Not reported | Hearing loss | ↑ Solvent exposed, *n*-hexane and toluene exposed |
| **Talbott et al. 2015**<br><br>Case-control, 217 children diagnosed with autism (Pennsylvania) | 0.00004 ppm ($50^{th}$ percentile) | Autism Spectrum Disorders | ↔ |
| **Tsai et al. 1997**<br><br>Cross-sectional, 298 paint factory workers (Taiwan) | 0–62.35 ppm | Neurobehavioral tests | ↑ Continuous performance test, pattern comparison test, pattern memory test latencies<br>↔ Finger tapping, associate learning, switching attention, mood scales |
| **Verberk et al. 2004**<br><br>Case-control, 30 male patients diagnosed with chronic solvent encephalopathy, 41 controls (Netherlands) | Not reported | Pattern-reversal visual evoked potentials | ↓ Low and low/high contrast (N75–P100) |
| | | Pattern-onset visual evoked potentials | ↔ |
| | | P300 | ↑ Latency<br>↓ Amplitude |
| **Wang et al. 1986**<br><br>Cross-sectional, 57 press proofing workers (Taiwan)<br><br>(54 examined) | Low 0–23 ppm<br>Mid 11–93 ppm<br>High (AS) 34–41 ppm<br>High (AS) 22–190 ppm<br><br>5.8 years (2 months–25 years) | MCV | ↓ Median (all groups)<br>↓ Ulnar, peroneal (low, mid, high symptomatic)<br>↓ Tibial (high symptomatic) |
| | | SCV | ↓ Median, ulnar, sural (high symptomatic) |
| | | Nerve histopathology | ↑ Axonal degradation, changes in the myelin sheath |

**Table 2-4. Results of Epidemiological Studies Evaluating Exposure to *n*-Hexane and Neurological Effects**

| Reference, study type, and population | Exposure/biomarker | Outcome evaluated | Result |
|---|---|---|---|
| **Yokoyama et al. 1997**<br><br>Cross-sectional, 29 shoe workers, 22 unexposed controls (Japan) | 40 ppm (13–100 ppm)<br><br>28 years (3–42 years) | Clinical signs | ↔ |
| | | Postural sway (eyes open) | ↑ Sway (2–4 Hz, anterior posterior); sway path; power of sway<br>↔ Sway (0–1, 1–2, 2–4 Hz medio-lateral; 0–1, 1–2 Hz anterior-posterior) |
| | | Postural sway (eyes closed) | ↑ Sway (0–1 Hz, anterior posterior; 0–1 Hz mediolateral); sway path; power of sway<br>↔ Sway (1–2, 2–4 Hz medio-lateral; 1–2, 2–4 Hz anterior-posterior) |

[a]Symptoms included headache, deteriorating memory, drunken feeling, and vertigo.

↑ = increase; ↔ = no change; ↓ = decrease; 2,5-HD = 2,5-hexanedione; AS = asymptomatic; CNS = central nervous system; DCV = distribution of nerve conduction velocities; DL = distal latency; HW = healthy worker; Hz = frequency; MAP = motor action potential; MCV = motor nerve conduction velocity; MNCV = mixed nerve conduction velocity; MSDS = Material Safety Data Sheet; Q = quartile; S = symptomatic; SAP = sensory action potential; SCV = sensory nerve conduction velocity; SSPL = sural sensory peak latency; TWA = time-weighted average

**Table 2-5. Results of Rodent Studies Evaluating Inhalation Exposure to *n*-Hexane and Neurological Effects**

| Reference species (strain), sex | Exposure, duration | Total hours | Outcome evaluated | Result |
|---|---|---|---|---|
| **Acute-duration exposure** | | | | |
| **Chalansonnet et al. 2013**<br>Rats (SD), M | 1,000 ppm<br>10 days, 6 hours/day | 60 | Clinical signs | ↔ |
| **NIEHS 1988a**<br>Mice (B6C3F1), M | 5,000 ppm<br>5 days, 20 hours/day | 100 | Clinical signs | ↔ |
| **NIEHS 1988b**<br>Mice (CD-1), M | 5,000 ppm<br>5 days, 20 hours/day | 100 | Clinical signs | ↔ |
| **Intermediate-duration exposure** | | | | |
| **Altenkirch et al. 1982**<br>Rats (Wistar), M | 500 ppm<br>9 weeks,<br>7 days/week,<br>22 hours/day | 1,386 | Clinical signs | ↑ Narcosis, limb weakness, paralysis |
| | | | Histopathology | ↑ Scattered multifocal giant axonal swellings, breakdown of axons, myelin degradation |
| | 700 ppm<br>40 weeks,<br>7 days/week,<br>8 hours/day | 2,240 | Clinical signs | ↔ |
| | | | Histopathology | ↑ Axonal swellings |
| **API 1981**<br>Rats (Albino), M | 500 ppm<br>6 months,<br>7 days/week,<br>22 hours/day | 3,960 | Clinical signs | ↑ Abnormal gait |
| | | | Histopathology | ↑ Peripheral nerve atrophy<br>↔ Axonal degeneration, lesions in the brain, spinal cord, or neuroganglia |

**Table 2-5. Results of Rodent Studies Evaluating Inhalation Exposure to *n*-Hexane and Neurological Effects**

| Reference species (strain), sex | Exposure, duration | Total hours | Outcome evaluated | Result |
|---|---|---|---|---|
| **Cavender et al. 1984**<br>Rats (F-344), M/F | 10,000 ppm<br>13 weeks,<br>5 days/week,<br>6 hours/day | 390 | Clinical signs | ↔ Posture, gait, tone and symmetry of facial muscles, reflexes |
| | | | Absolute brain weight | ↓ 10% (males only) |
| | | | Histopathology | ↔ Brain<br>↑ Sciatic nerve (axonopathy, paranodal axonal swelling) |
| **De Martino et al. 1987**<br>Rats (SD), M | 5,000 ppm<br>6 days, 16 hours/day | 96 | Motor conduction velocity | ↓ |
| | 5,000 ppm<br>4–6 weeks,<br>6 days/week,<br>16 hours/day | 384–576 | Clinical signs | ↑ Polyneuropathy |
| **Frontali et al. 1981**<br>Rats (SD), M | 5,000 ppm<br>14 weeks,<br>5 days/week,<br>9 hours/day | 630 | Clinical signs | ↔ |
| | | | Histopathology | ↑ Giant axonal degeneration (tibial nerve branches), paranodal and internodal swellings<br>↔ Optic nerve, medulla oblongata |
| | 2,500 ppm<br>30 weeks,<br>6 days/week,<br>10 hours/day | 1,800 | Clinical signs | ↔ |
| | | | Histopathology | ↑ Giant axonal degeneration (tibial nerve branches), paranodal and internodal swellings<br>↔ Optic nerve, medulla oblongata |

**Table 2-5. Results of Rodent Studies Evaluating Inhalation Exposure to *n*-Hexane and Neurological Effects**

| Reference species (strain), sex | Exposure, duration | Total hours | Outcome evaluated | Result |
|---|---|---|---|---|
| **Howd et al. 1983**<br><br>Rats (F-344), M<br>Weanling (21 days old) and Adults (80 days old) | 1,000 ppm<br>11 weeks, 6–7 days/week, 24 hours/day | 1,680 | Clinical signs | ↑ Ataxia, difficulty walking, flaccid hindlimbs<br>↓ Grip strength |
| | | | Compound action potential | ↓ Amplitude<br>↑ Latency |
| | | | Brainstem auditory-evoked response | ↑ Latency |
| | | | Absolute brain weight | ↓ |
| | | | Relative brain weight | ↓ (adults only) |
| | | | Histopathology | NC |
| **Huang et al. 1989**<br><br>Rats (Wistar), M | 1,200 ppm<br>16 weeks, 7 days/week, 12 hours/day | 1,344 | Clinical signs | ↓ Speed of movement<br>↔ Paralysis |
| | | | Sensory motor tests | ↓ Grip strength |
| | | | Motor nerve conduction velocity | ↓ |
| | | | Histopathology | ↑ Paranodal swellings, demyelination, remyelination |
| **Ichihara et al. 1998**<br><br>Rats (Wistar), M | 2,000 ppm<br>20 weeks, 6 days/week, 12 hours/day | 1,440 | Motor nerve conduction velocity | ↓ |
| | | | Distal latency | ↑ |
| **Li et al. 2014, 2015**<br><br>Rats (Wistar), F | 12,500 ppm<br>20 days, 4 hours/day | 80 | Clinical signs | ↑ Irritability, attack tendency |
| **Lungarella et al. 1984**<br><br>Rabbits (New Zealand), M | 3,000 ppm<br>24 weeks, 5 days/week, 8 hours/day | 600 | Clinical signs | ↔ |

**Table 2-5. Results of Rodent Studies Evaluating Inhalation Exposure to *n*-Hexane and Neurological Effects**

| Reference species (strain), sex | Exposure, duration | Total hours | Outcome evaluated | Result |
|---|---|---|---|---|
| **NIEHS 1988c**<br>Mice (CD-1), F | 5,000 ppm<br>12 days,<br>20 hours/day | 240 | Clinical signs | ↔ |
| **NIEHS 1987**<br>Rats (SD), F | 5,000 ppm<br>14 days,<br>20 hours/day | 280 | Clinical signs | ↔ |
| **NTP 1991**<br>Mice (B6C3F1), M/F | 10,000 ppm<br>13 weeks,<br>5 days/week,<br>6 hours/day | 390 | Sensory motor tests | ↓ Motor activity (females only) |
| | | | Histopathology | ↑ Paranodal swellings (tibial nerve)<br>↔ Paranodal swellings (spinal cord), axonal degeneration, demyelination, brain |
| | | | Relative brain weight | ↑ (males only) |
| | 1,099 ppm<br>13 weeks,<br>5 days/week,<br>22 hours/day | 1,430 | Sensory motor tests | ↓ Motor activity (females only) |
| | | | Histopathology | ↑ Paranodal swellings (tibial nerve)<br>↔ Paranodal swellings (spinal cord), axonal degeneration, demyelination, brain |
| | | | Relative brain weight | ↑ (males only) |
| **Nylen et al. 1994**<br>Rats (SD), M | 1,000 ppm<br>28 days<br>21 hours/day | 588 | Motor conduction velocity | ↓ |
| | | | Auditory brainstem response | ↑ Latency, amplitude |
| | | | Flash evoked potential | ↓ Amplitude |
| **Nylen and Hagman 1994**<br>Rats (SD), M | 1,000 ppm<br>61 days<br>18 hours/day | 1,098 | Motor conduction velocity | ↓ |
| | | | Auditory brainstem response | ↑ Latency |
| | | | Flash evoked potential | ↑ Latency |
| **Pryor and Rebert 1992**<br>Rats (F-344), M | 4,000 ppm<br>9 weeks<br>14 hours/day | 882 | Sensory motor tests | ↓ Grip strength |
| | | | Motor conduction velocity | ↓ |
| | | | Auditory brainstem response | ↓ Amplitude |

**Table 2-5. Results of Rodent Studies Evaluating Inhalation Exposure to *n*-Hexane and Neurological Effects**

| Reference species (strain), sex | Exposure, duration | Total hours | Outcome evaluated | Result |
|---|---|---|---|---|
| **Pryor et al. 1983**<br>Rats (F-344), M | 2,000 ppm<br>14 weeks,<br>7 days/week,<br>14 hours/day | 1,372 | Sensory motor tests | ↓ Motor activity, startle response, grip strength, conditioned avoidance response |
| | | | Auditory evoked response | ↔ Latency |
| | | | Brainstem auditory-evoked potential | ↓ Amplitude<br>↔ Latency |
| | | | Visual evoked response | ↑ Latency |
| | | | Compound action potential | ↑ Latency |
| | | | Histopathology | ↔ Tibial and sciatic nerves |
| **Rebert and Sorenson 1983**<br>Rats (F-344), M | 1,000 ppm<br>11 weeks,<br>5 days/week,<br>24 hours/day | 1,320 | Sensory motor tests | ↓ Grip strength |
| | | | Compound action potential | ↑ Latency |
| | | | Somatosensory evoked response | ↑ Latency |
| | | | Brainstem auditory-evoked response | ↑ Latency |
| | | | Auditory evoked response | ↑ Latency |
| | | | Visual evoked response | ↑ Latency |
| **Schaumburg and Spencer 1976**<br>Rats (SD), NS | 400–600 ppm<br>162 days,<br>7 days/week,<br>24 hours/day | 3,888 | Clinical signs | ↑ Unsteady, waddling gait, distal hindlimb weakness with footdrop, distal weakness of the upper extremities |
| | | | Histopathology | ↑ Axonal dilation and swellings, myelinated fiber degeneration |
| **Stoltenburg-Didinger et al. 1990**<br>Rats (Wistar), F | 500 ppm<br>21 days,<br>23 hours/day | 483 | Clinical signs | ↔ |
| | 800 ppm<br>63 days,<br>23 hours/day | 1,449 | Clinical signs | ↑ Hindlimb weakness |

**Table 2-5. Results of Rodent Studies Evaluating Inhalation Exposure to *n*-Hexane and Neurological Effects**

| Reference species (strain), sex | Exposure, duration | Total hours | Outcome evaluated | Result |
|---|---|---|---|---|
| **Takeuchi et al. 1980**<br><br>Rats (Wistar), M | 3,000 ppm<br>16 weeks,<br>7 days/week,<br>12 hours/day | 1,344 | Clinical signs | ↑ Unsteady gait, footdrop |
| | | | Nerve conduction velocity | ↓ Motor, mixed |
| | | | Distal latency | ↑ |
| | | | Histopathology | ↑ Paranodal swellings, denervated neuromuscular junctions |

↑ = increase; ↔ = no change; ↓ = decrease; F = female; M = male; NS = not specified; SD = Sprague-Dawley

et al. 2005), postural sway (Park et al. 2009; Yokoyama et al. 1997), and a variety of neurobehavioral outcomes (Ithnin et al. 2011; Tsai et al. 1997).

Neurological outcomes are commonly evaluated in rodents following inhalation exposure to *n*-hexane (Table 2-5). No clinical signs of neurotoxicity have been observed in acute-duration inhalation studies in rats and mice following exposure up to 5,000 ppm for as long as 14 days (Chalansonnet et al. 2013; NIEHS 1987, 1988a, 1988b, 1988c), although motor conduction velocity was decreased in rats exposed to 5,000 ppm *n*-hexane for 6 days and for 2–4 weeks (De Martino et al. 1987). Signs of neurological toxicity similar to those seen in humans after inhalation exposure to *n*-hexane have been observed in many intermediate-duration studies with rats. Clinical signs of neurotoxicity (abnormal gait, hindlimb weakness), altered nerve conduction tests (decreased motor conduction velocity), and histopathology (axonal swellings, myelin degradation, denervated neuromuscular junctions) have all been reported (Altenkirch et al. 1982; API 1981; Cavender et al. 1984; Frontali et al. 1981; Howd et al. 1983; Huang et al. 1989; Ichihara et al. 1998; Li et al. 2014, 2015; NTP 1991; Pryor et al. 1983; Rebert and Sorenson 1983; Schaumburg and Spencer 1976; Takeuchi et al. 1980). No signs of peripheral neurotoxicity, such as hindlimb weakness or foot dragging, were observed in male rabbits exposed by inhalation to 3,000 ppm *n*-hexane for 24 weeks (Lungarella et al. 1984).

Exposure to *n*-hexane may also result in hearing loss (ototoxicity). Rats exposed to 1,000 ppm *n*-hexane for 21 or 61 days showed a decrease in auditory sensitivity (decreased brainstem auditory response) (Nylen and Hagman 1994; Nylen et al. 1994), although this response had disappeared 3 months after exposure (Nylen et al. 1994). Alterations in the brainstem auditory responses in rats have also been measured in several other rat studies at concentrations ≥1,000 ppm (Howd et al. 1983; Pryor and Rebert 1992; Pryor et al. 1983; Rebert and Sorenson 1983).

Similar effects have also been observed in rodents following oral exposure to *n*-hexane. Decreased motor nerve conduction velocity was reported in male rats orally exposed to 1,251 mg/kg/day for 8 weeks, although no changes in behavior or clinical signs of peripheral neurotoxicity were observed (Ono et al. 1981). Abnormal gait and decreased ability to stay on a rotating rod were observed in rats receiving gavage doses of 2,000 mg/kg/day *n*-hexane for 10 weeks, although all animals were normal in appearance (Li et al. 2018). In addition to abnormal gait, decreased grip strength was observed in male rats administered 3,000 mg/kg/day *n*-hexane for 8 weeks via gavage (Wang et al. 2017).

Clinical signs of neurotoxicity (hindlimb weakness or paralysis) were observed in male rats administered 4,000 mg/kg/day *n*-hexane via gavage for 90 days, along with tibia1 nerve alterations (multifocal axonal swellings, adaxonal myelin infolding, paranodal myelin retraction) (Krasavage et al. 1980). Abnormal gait, paralysis, decreased rotarod latency, and decreased motor nerve conduction were observed in male rats exposed to 3,000 mg/kg/day *n*-hexane for 7 weeks (Li et al. 2020a). Histopathological examination of the sciatic nerve revealed damage including loss of myelin and vacuolization. Similarly, transient paralysis, abnormal gait, decreased rotarod latency, and decreased motor nerve conduction were observed in male rats exposed to 1,000 mg/kg/day *n*-hexane for 24 weeks (Li et al. 2020b).

While nonmammalian species are not commonly used in toxicology studies, the chicken has proven to be a valuable model for human neurotoxicity caused by organophosphates, which is clinically similar to that caused by *n*-hexane (Abou-Donia and Lapadula 1990). During a continuous 90-day exposure, chickens exposed to 1,000 ppm *n*-hexane developed mild ataxia and histologic changes in the spinal cord (no changes were seen in peripheral nerves). However, chickens exposed continuously to 1,008 ppm for 30 days showed no effects (Abou-Donia et al. 1991). Chickens orally exposed to 2,000 mg/kg showed mild leg weakness followed by full recovery after 2–4 days (Abou-Donia et al. 1982). Similarly, oral exposure to 100 mg/kg/day for 90 days resulted in leg weakness, but no other serious signs of neurotoxicity (Abou-Donia et al. 1982).

The neurotoxicity of *n*-hexane is believed to ultimately result from the effects the *n*-hexane metabolite, 2,5-hexanedione, on peripheral nerves. Another potential metabolite, 2-hexanone, has also caused neurotoxicity in humans (Allen et al. 1975). The other metabolites of *n*-hexane can also produce neurotoxicity in rats via their subsequent metabolism to 2,5-hexanedione (Krasavage et al. 1980). 2,5-Hexanedione causes a peripheral neuropathy in rats virtually identical to that caused by inhalation of *n*-hexane when administered in drinking water at a concentration of 0.5% (Schaumburg and Spencer 1976; Spencer and Schaumburg 1977a, 1977b). The time to onset of peripheral neuropathy was about 12 weeks. No significant differences in histopathology of peripheral or central nerves were noted between oral exposure to 2,5-hexanedione and inhalation exposure to *n*-hexane.

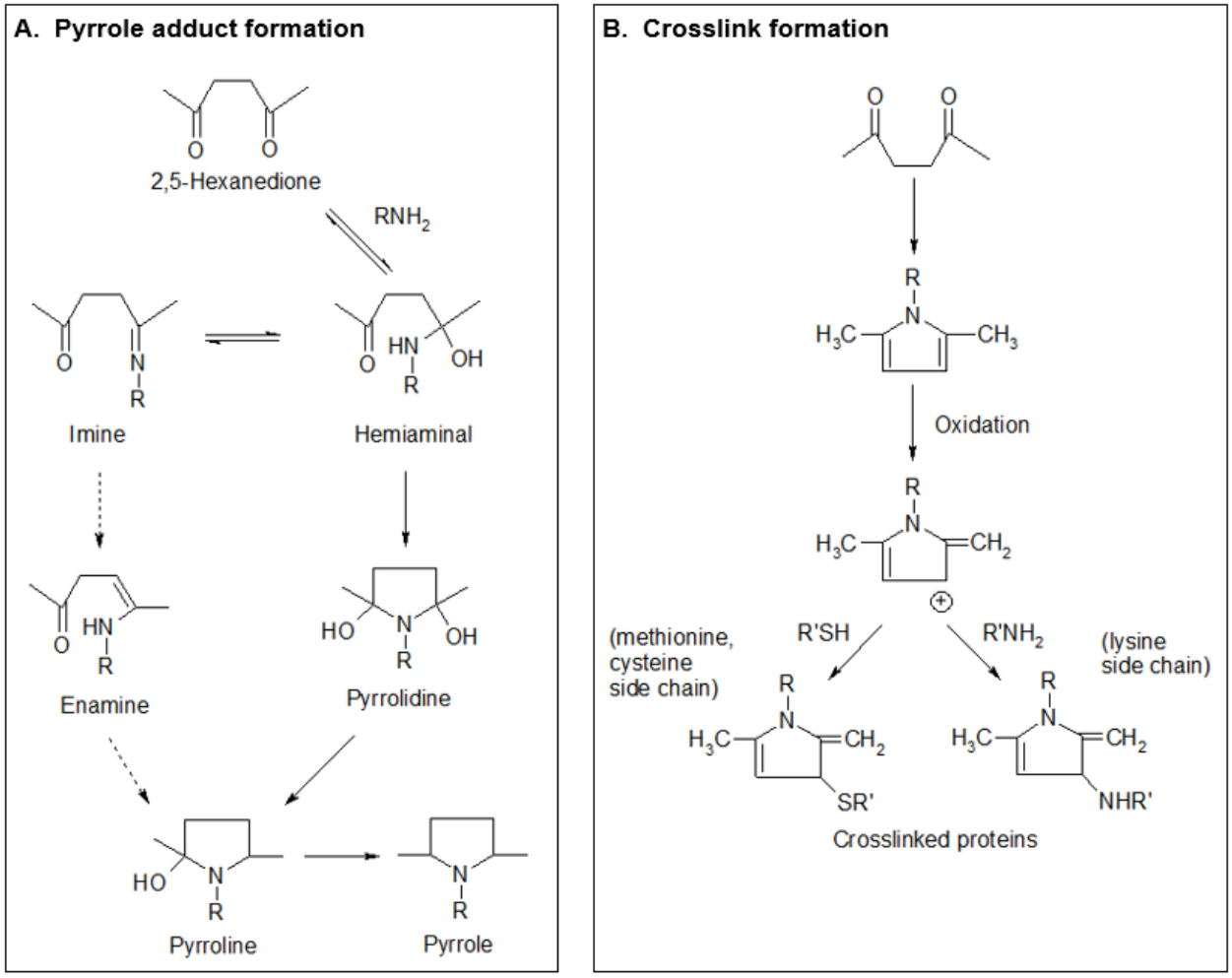
The sequence of events in *n*-hexane-induced neuropathy has been described in rats (Spencer and Schaumburg 1977a). The process appears to begin by increases in the number of 10 nm axonal neurofilaments and accumulation in swellings on the proximal sides of the nodes of Ranvier in distal regions of large, myelinated fibers. As exposure continues, there is a retrograde spread of axonal swellings up the nerve, and smaller myelinated and unmyelinated fibers become involved. The nerve

terminal is unaffected until late in the process. The enlarged axons displace the paranodal myelin sheaths, leaving denuded swellings in areas near the nodes of Ranvier. This process occurs before functional impairment is evident and can be reversed on cessation of exposure as swelling diminishes, and proliferation of Schwann cells occurs at these sites with subsequent remyelination of the axons. If exposure to *n*-hexane continues, axonal restoration and remyelination do not take place at some swellings, and the length of the nerve fiber between the swelling and the terminal undergoes breakdown, very similar to that seen when fibers are transected. Axon sprouting is often seen at the intact portion of a degenerated fiber even while intoxication continues. When intoxication ends, this regenerative process can reestablish motor and sensory function.

Peripheral neuropathy begins in the hind limbs in the rat model and eventually affects the front limbs. The nerve fibers most vulnerable to *n*-hexane exposure in rats are the branches of the tibia1 nerve serving the calf muscles, followed in order by the plantar nerve branches supplying the flexor digitorum brevis muscle, and then sensory plantar nerve branches innervating the digits. As intoxication continues, axonal degeneration ascends the plantar and tibia1 nerves (Spencer and Schaumburg 1977b). Examination of control animals indicated that the most sensitive fibers were also the largest. Effects on the central nervous system have also been observed in rats exposed to *n*-hexane or its neurotoxic metabolite, 2,5-hexanedione. Axonal swelling and degeneration were observed in the anterior vermis, spinocerebellar tract in the medulla oblongata, and gracile tracts of the spinal cord (Spencer and Schaumburg 1977b).

The chemical structure of 2,5-hexanedione suggests that it could react with lysine side-chain amino groups in proteins to form pyrroles (see Figure 2-4). *In vitro* experiments showed that this was, in fact, the case, and that the modified proteins can undergo secondary reactions to yield oxidized and polymeric products (DeCaprio et al. 1982; Graham et al. 1982). Oral administration of 2,5-hexanedione produced evidence that this process can take place *in vivo*, as demonstrated by the detection of 2,5-dimethylpyrrole adducts in serum and axonal cytoskeletal proteins (DeCaprio and O'Neill 1985). When a series of 2,5-hexanedione analogues were tested for their ability to produce neurotoxicity in rats, it was found that only those with the 2,5-gamma spacing were neurotoxic, and that potency correlated with the rate constant for pyrrole formation (Genter St. Clair et al. 1988). The role of oxidation of the pyrrole adduct in the development of neurotoxicity was demonstrated with another 2,5-hexanedione analogue that could form pyrroles but was resistant to oxidation. This analogue (3-acetyl-2,5-hexanedione) caused pyrrolidation of protein *in vivo*, but not neurotoxicity.

## Figure 2-4. Reaction of 2,5-Hexanedione with Protein




Source: Graham et al. 1995

The reaction of anti-neurofilament antibodies with high molecular weight aggregates from rat neuronal cytoskeletal proteins provided direct evidence for neurofilament cross-linking after 2,5-hexanedione administration (Lapadula et al. 1986). Immunoblotting with antibodies specific for phosphorylated forms of cytoskeletal proteins has demonstrated a reduction of phosphorylation in neurofilament proteins and microtubule-associated-protein 2 (MAP-2) after 2,5-hexanedione treatment (Abou-Donia et al. 1988).

Whether neurofilament cross-linking is related to the neurofilament accumulation, axonal swellings, and ultimate axonal degeneration observed in *n*-hexane neurotoxicity or is incidental remains to be elucidated (Graham et al. 1995). Since the maintenance of the axon depends on transport of cellular components from the neuronal cell body, the effect of 2,5-hexanedione on axonal transport has been investigated. If 2,5-hexanedione treatment slowed or stopped axonal transport, distal axonal degeneration would be an

expected consequence. Measurement of the rate of axonal transport both during and after 2,5-hexanedione intoxication showed accelerated rates of transport that persisted after treatment ended (Pyle et al. 1993). Increased rates of axonal transport may reflect a reparative response after neuronal injury (Graham et al. 1995).

## 2.16 REPRODUCTIVE

Women shoemakers reported longer menstrual cycles and longer times to get pregnant compared to controls (Ruiz-García et al. 2020). Additionally, serum FSH concentrations were inversely associated with *n*-hexane exposure and urinary 2,5-hexanedione levels. A cross-sectional study examining female shoemakers in Portugal found similar results, with increased self-reporting of longer menstrual cycles and increase difficulty of getting pregnant (Sallmen et al. 2008). A case-control study examining 108 women diagnosed with spontaneous abortion found a higher risk associated with higher solvent exposure in the shoe and leather industry (estimated by self-reported time and methods for using solvents) but not with occupation as a shoemaker (Agnesi et al. 1997).

Increased risk of preeclampsia was observed in asthmatic mothers exposed to ambient *n*-hexane during the second trimester or whole pregnancy, while mothers without asthma only showed an increased risk with the whole pregnancy average exposure (Mendola et al. 2016). Exposure to *n*-hexane was correlated with increased risk of preeclampsia during the first 20 weeks of gestation, but not when evaluated over the whole pregnancy (Nobles et al. 2019). No association was observed between ambient *n*-hexane concentrations and gestational hypertension during any window of exposure.

One study evaluated the reproductive toxicity of *n*-hexane in female mice. A 3-week exposure to 21,500 ppm *n*-hexane resulted in a decreased duration of diestrus in ICR mice (Liu et al. 2012). In super-ovulated mice, there were decreases in mature ovarian follicles and increases in primordial follicles and atretic follicles at 21,500 ppm and a decrease in the number of ovulated ova at ≥850 ppm (Liu et al. 2012).

Several acute- and intermediate-duration studies have examined the potential reproductive effects following *n*-hexane exposure in male rodents. Mice exposed up to 5,000 ppm *n*-hexane for 20 hours/day for 5 consecutive days had no changes in mouse sperm morphology or fertility compared to controls (NIEHS 1988a, 1988b). The fertility of male mice was unaffected by exposure up to 396 ppm *n*-hexane for 8 weeks (API 1980). Histopathological examination of mice exposed for 13 weeks to *n*-hexane at

concentrations up to 10,000 ppm for 6 hours/day or 1,099 ppm for 22 hours/day revealed no treatment-related lesions in any of the reproductive tissues examined (seminal vesicles, prostate, testis, epididymis, ovary, uterus) (NTP 1991).

Increased testes weights were reported in male rats exposed to 10,000 ppm *n*-hexane 13 weeks (Cavender et al. 1984), but testes weights were decreased in rats following exposure to 1,000 ppm *n*-hexane for 11 weeks (Howd et al. 1983). Histopathological lesions were either not found or not examined; therefore, the toxicological significance of this finding is uncertain. No treatment-related lesions were observed in the seminal vesicles, prostate, testis, or epididymis of male rats exposed to 500 ppm *n*-hexane 22 hours/day for 6 months (API 1981).

Bilateral testicular damage and reduced testicular size and weight was reported in male rats exposed to 1,000 ppm *n*-hexane for 18–21 hours/day for 28 or 61 days (Nylen et al. 1989). Severe testicular atrophy and loss of nerve growth factor-immunoreactive cell population were also observed. Testicular lesions were observed in male rats exposed to 5,000 ppm *n*-hexane for 24 hours or for 16 hours/day, 6 days/week for up to 6 weeks (De Martino et al. 1987). These lesions included exfoliation of spermatids and spermatocytes, degeneration of spermatocytes, and vacuolization of Sertoli cells, with some animals reaching aplasia. Morphological alterations in sperm (multinucleated round spermatids and spermatocytes) were also observed (De Martino et al. 1987). In contrast, sperm abnormalities were not observed in B6C3F1 mice exposed to up to 5,000 ppm *n*-hexane for 20 hours/day for 5 days (NIEHS 1988a). Analysis of sperm obtained 5 weeks post-exposure showed no significant effects on morphology compared to the control group. In a chronic-duration study, Leydig cell hyperplasia was observed in male rats exposed to 1,000 ppm for 60 weeks (Imai and Omoto 1999).

No changes in testicular histopathology were reported in male rats administered via gavage 20,000 mg/kg for 1 day or 10,000 mg/kg/day for 5 days (Linder et al. 1992). A decrease was noted in total sperm head counts per gram of testis following 1 day at 20,000 mg/kg, but this was not observed in rats receiving gavage doses of 10,000 mg/kg/day for 5 days, and no other changes in sperm motility or morphology were reported. Varying stages of testicular atrophy of the germinal epithelium was observed in rats administered 4,000 mg/kg/day *n*-hexane via gavage for up to 120 days (Krasavage et al. 1980).

The metabolite, 2,5-hexanedione, can also affect testicular tissue in male rats and is, in fact, used as a model for chemically induced sterility (Chapin et al. 1982; Krasavage et al. 1980). Exposure to drinking water containing 1% 2,5-hexanedione results in severe seminiferous epithelial degeneration and loss of

germ cells. In a group of rats receiving a single dose of 2,000 mg/kg 2,5-hexanedione (Linder et al. 1992), no histopathological changes were detected 2 days after treatment; however, at 14 days, testicular debris was observed in the proximal caput, sloughed epididymal cells were observed in the cauda lumen as was retention at the lumen and base of Step 19 spermatids in Stages IX–XII.

## 2.17 DEVELOPMENTAL

A positive association was observed between ambient *n*-hexane exposure (represented as a unitless exposure intensity) and low birth weight in a case-control study in Texas (Gong et al. 2018). In a study examining the effects of *n*-hexane on the neonatal immune system, the percentage of T cells producing IL-2 in cord blood was increased with increased maternal *n*-hexane exposure, although no difference was observed in T cells producing IFN-γ, tumor necrosis factor-alpha (TNF-α), or IL-4 (Lehmann et al. 2002).

Several acute- and intermediate-duration studies have examined the potential developmental effects of *n*-hexane exposure in rodents. No differences in fetal parameters (e.g., number of implantations, resorptions, fetal sex ratio, fetal malformations) were observed in rats exposed up to 409 ppm for 6 hours/day during GDs 6–15 (API 1979) or in rats exposed up to 5,000 ppm for 20 hours/day over GDs 6–19 compared to controls (NIEHS 1987). Decreased fetal body weights were observed for male offspring (7.5%) at 1,000 ppm and in both male (15%) and female (14%) offspring at 5,000 ppm (NIEHS 1987). Additionally, an increased incidence of reduced ossification in the sternebrae 1–4 was observed in the offspring of rats exposed to 5,000 ppm. Decreased litter weight (13.9% at postnatal day [PND] 21) was observed in the offspring of rats exposed to 1,000 ppm *n*-hexane for 6 hours/day on GD 8–16, although litter weights were similar to controls at week 7 and there were no developmental effects observed in the offspring of rats exposed to the same concentration on GDs 8–12 or 12–16 (Bus et al. 1979).

The number of live fetuses per litter were decreased, while the number of late resorptions per litter was increased in pregnant mice exposed to 5,000 ppm *n*-hexane for 20 hours/day during GDs 6–17 (NIEHS 1988c). Fetal weights of female offspring were decreased 6% at 5,000 ppm, but this was not observed in male offspring, and no differences were observed in the incidence of malformations in either sex. In another study, reduced pup body weight (22% at PND 9) was observed in the offspring of female rats exposed to 500 ppm *n*-hexane for 23 hours/day throughout gestation (21 days) (Stoltenburg-Didinger et al. 1990). A third study reported a decreased ratio of live pups per litter, decreased proportion of ovarian

secondary follicles, and increased proportion of atretic follicles in the female offspring of rats exposed to 12,500 ppm on GDs 1–20 (Li et al. 2014). No difference in the day of vaginal opening or ovarian pathology were observed, although slight changes in the estrus cycle were measured.

In a set of oral exposure studies, no differences were observed in total number of implants, number of resorptions, fetal deaths, sex ratio, number of stunted fetuses, live fetuses per dam, fetal weight, or incidence of malformed fetuses (visceral or skeletal) in the offspring of female mice administered doses up to 2,200 mg/kg/day via gavage on GDs 6–15 (Marks et al. 1980). Fetal weights were decreased 6% in the offspring of female mice receiving gavage doses 3 times daily at 7,920 mg/kg/day on GDs 6–15, but no other developmental effects were observed.

## 2.18 OTHER NONCANCER

No exposure-related differences in metabolic function (e.g., fasting blood glucose, electrolytes) were reported in offset printers (Chang et al. 1993), cabinet finishers (Herskowitz et al. 1971), or sandal makers (Yamamura 1969) occupationally exposed to *n*-hexane.

No effect in body temperature was observed in rats following exposure up to 1,500 ppm *n*-hexane for 11 weeks (Rebert and Sorenson 1983). No change in metabolic parameters were observed in male or female rats exposed up to 10,000 ppm for 13 weeks (Cavender et al. 1984). No exposure-related differences in mean-fasting glucose were observed in male or female rats exposed up to 129 ppm for as long as 6 months (API 1978).

## 2.19 CANCER

EPA (IRIS 2005) concluded that there is inadequate information to assess the carcinogenic potential of *n*-hexane. HHS and IARC have not assessed the carcinogenicity of *n*-hexane.

A case-control study evaluating the occurrence of intracranial tumors among employees at a petrochemical research facility found a positive association between self-reported *n*-hexane exposure and glioma cases, although no association was observed using project-based exposure estimates (Beall et al. 2001). Additionally, an analysis separating cases by duration of chemical use did show a positive association for *n*-hexane and longer duration (>48 months) of potential use. This study is limited by the

small number of cases, the number of various co-exposures, and the observed correlations with other compounds/agents, including ionizing radiation.

Papillary tumors derived from Clara cells in the bronchiolar epithelium were observed in male rabbits exposed to 3,000 ppm *n*-hexane for 24 weeks (Lungarella et al. 1984), although the incidence was not reported, making the significance of this finding difficult to determine.

## 2.20 GENOTOXICITY

Genotoxic effects have not been examined in humans after *n*-hexane exposure. The database on *n*-hexane in animals, mammalian cells, and microorganisms is limited (see Tables 2-6 and 2-7) but indicates little potential for genotoxicity. *n*-Hexane was negative in a dominant lethal test in mice by the inhalation route at up to 396 ppm *n*-hexane ppm for 6 hours/day, 5 days/week for 8 weeks (API 1980). Similar results were observed in another dominant lethal mutation study at higher concentrations in which male Swiss mice were exposed to up to 5,000 ppm *n*-hexane for 20 hours/day for 5 days (NIEHS 1988b).

**Table 2-6. Genotoxicity of *n*-Hexane *In Vitro***

| | | Results | | |
|---|---|---|---|---|
| | | Activation | | |
| Species (test system) | Endpoint | With | Without | Reference |
| Nonmammalian cells | | | | |
| *Escherichia coli* WP2, WP2 uvr A, CM611, WP67, WP100, WP110, p3478 | | – | – | McCarroll et al. 1981a |
| *Bacillus subtilis* H17, M45 | | – | – | McCarroll et al. 1981b |
| *Salmonella typhimurium* TA98, TA100, TA1535, TA1537, | Reverse mutation | – | – | Mortelmans et al. 1986 |
| *S. typhimurium* TA92, TA94, TA98, TA100, TA1535, TA1537 | Reverse mutation | – | – | Ishidate et al. 1984 |
| *S. typhimurium* TA98, TA100 | Reverse mutation | – | – | Houk et al. 1989 |
| *S. typhimurium* TA98, TA100, TA1535, TA1537 | Reverse mutation | – | – | NTP 1991[a] |
| *Saccharomyces cerevisiae* | Chromosome loss | – | – | Mayer and Goin 1994 |
| Mammalian cells | | | | |
| Human (lymphocytes) | Unscheduled DNA synthesis | – | – | Perocco et al. 1983 |
| Hamster (CHO) | Chromosomal aberration | – | – | NTP 1991[a] |

**Table 2-6. Genotoxicity of *n*-Hexane *In Vitro***

| Species (test system) | Endpoint | Results: Activation With | Results: Activation Without | Reference |
|---|---|---|---|---|
| Hamster (CHO) | Sister chromatid exchange | + | – | NTP 1991[a] |
| Hamster (CHL) | Polyploidy | ND | + | Ishidate et al. 1984 |

[a]Unpublished NTP data reported in NTP (1991).

– = negative results; CHL = Chinese hamster lung; CHO = Chinese hamster ovary; DNA = deoxyribonucleic acid; ND = not detectable; NTP = National Toxicology Program

**Table 2-7. Genotoxicity of *n*-Hexane *In Vivo***

| Species (exposure route) | Endpoint | Results | Reference |
|---|---|---|---|
| Rat | Sperm morphology | + | De Martino et al. 1987 |
| Mouse (bone marrow) | Sister chromatid exchange | – | NTP 1991[a] |
| Mouse | Dominant lethal mutation | – | API 1980 |
| Mouse | Chromosomal exchange | – | NTP 1991[a] |
| Mouse | Micronuclei formation | – | NTP 1991[a] |
| Mouse | Sperm morphology | – | NIEHS 1988a |
| Mouse | Dominant lethal mutation | – | NIEHS 1988a |

[a]Unpublished NTP data reported in NTP (1991).

– = negative result; + = positive result; NTP = National Toxicology Program

There was no increase in the incidence of micronucleated normochromatic erythrocytes or polychromatic erythrocytes in the peripheral blood of male and female mice exposed via inhalation to 1,000, 4,000, or 10,000 ppm *n*-hexane, 6 hours/day, 5 days/week for 13 weeks or in mice exposed to 1,000 ppm for 22 hours/day for 13 weeks (NTP 1991). In an *in vivo* mouse bone marrow cytogenetics assay, doses up to 2,000 mg/kg *n*-hexane dissolved in corn oil and administered by intraperitoneal injection did not increase the incidence of sister chromatid exchanges; chromosomal aberrations were slightly increased, but this increase was not significant (NTP 1991).

Results have generally been negative for *n*-hexane in bacterial tester strains such as *Escherichia coli*, *Bacillus subtilis*, and *Salmonella typhimurium* both with and without metabolic activation (Houk et al. 1989; Ishidate et al. 1984; McCarroll et al. 1981a, 1981b; Mortelmans et al. 1986). In studies conducted by the National Toxicology Program (NTP 1991), *n*-hexane was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested with a preincubation protocol at doses up to

1,000 µg/plate with or without Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9 fraction. *N*-Hexane was also negative in an *in vitro* test for induction of chromosome loss in *Saccharomyces cerevisiae* (Mayer and Goin 1994).

Negative results were also obtained in mammalian cells except for one observation of polyploidy in Chinese hamster lung cells (Ishidate et al. 1984; Perocco et al. 1983). Treatment at doses up to 5,000 µg/mL in the presence or absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 did not induce chromosomal aberrations in cultured Chinese hamster ovary (CHO) cells. Sister chromatid exchanges were induced in CHO cells, but only in the presence of S9; no dose-response was apparent (NTP 1991).

# CHAPTER 3. TOXICOKINETICS, SUSCEPTIBLE POPULATIONS, BIOMARKERS, CHEMICAL INTERACTIONS

## 3.1 TOXICOKINETICS

- Inhaled *n*-hexane is readily absorbed in the lungs, while absorption by the oral and dermal route has not been well characterized.
- Inhaled *n*-hexane distributes throughout the body; based on blood-tissue partition coefficients, preferential distribution would be in the order: body fat>>liver, brain, muscle>kidney, heart>lung, blood.
- *n*-Hexane is metabolized by cytochrome P-450 enzymes in the liver to several metabolites, including the neurotoxicant, 2,5-hexanedione.
- Approximately 10–20% of absorbed *n*-hexane is excreted unchanged in exhaled air, and 2,5-hexanedione is the major metabolite recovered in urine.

### 3.1.1 Absorption

*n*-Hexane is absorbed by passive diffusion in the lungs. Oral and dermal absorption have not been studied. Alveolar *n*-hexane reaches a steady state with the *n*-hexane in blood, as *n*-hexane is distributed and metabolized in the body more is absorbed from the alveolar air. In studies with humans, there was no evidence of saturation up to 204 ppm (Veulemans et al. 1982). During exercise in this study, the alveolar uptake rate decreased, but total intake increased slightly because of the higher ventilation rate. The absorption of inhaled *n*-hexane has been investigated in six healthy male volunteers (Veulemans et al. 1982). Three different trials were performed on each volunteer: 4-hour exposure at 102 ppm *n*-hexane, 4-hour exposure at 204 ppm, and 4-hour exposure during exercise on a stationary bicycle ergometer at 102 ppm. Each trial was done at least 2 weeks apart. Lung clearance (from alveolar air to blood) and retention were calculated from *n*-hexane concentrations in inhaled and exhaled air. After exposure, *n*-hexane in exhaled air was measured for up to 4 hours to determine respiratory elimination. Retention of *n*-hexane (calculated from lung clearance and respiratory minute volume) was approximately 20–25% of the *n*-hexane in the inhaled air. This resulted in calculated average absorption rates of 0.84 mg/minute at 102 ppm and 1.59 mg/minute at 204 ppm. Physical exercise at 102 ppm caused a significant increase in lung clearance and at peak loads (60 watts), was more than twice the value at rest, resulting in an increase in absorption rate. Pulmonary excretion of *n*-hexane after exposure ended appeared to be biphasic, with a fast drop in the first 30 minutes and a slower drop for the remainder of the 4-hour observation period.

In a workplace study, lung uptake and excretion of *n*-hexane were studied in 10 workers (sex not specified, 18–30 years old) in a shoe factory (Mutti et al. 1984). Simultaneous samples of inhaled and alveolar air (last 100 mL of the tidal volume) were collected 6 times during an 8-hour workday. Breathing-zone air was collected with personal samplers. Median time-weighted average (TWA) *n*-hexane concentrations were 243 mg/m$^3$ (69 ppm). 2-Methylpentane, 3-methylpentane, cyclohexane, and *n*-heptane were also present in the air. Alveolar excretion was monitored during a 6-hour post-exposure period. Uptake was calculated from lung ventilation, the retention coefficient (1-[Calv/Cinh]), and environmental concentrations. The total amount of exhaled *n*-hexane was calculated by integration of the decay curve for the concentration of exhaled *n*-hexane. About 25% of inhaled *n*-hexane was retained in the alveoli. Absorption into the blood in relation to total respiratory uptake was about 17%, taking into account the retention coefficient and alveolar ventilation.

No studies were located that specifically addressed absorption of *n*-hexane after oral exposure in humans or animals. Absorption of *n*-hexane by the oral route in humans can be inferred from the appearance of *n*-hexane in exhaled air and 2,5-hexanedione in urine of volunteers receiving 0.24 or 0.81 mg/kg via a gastric feeding tube (Baelum et al. 1998). Absorption of toxicologically significant amounts by this route can be inferred since neurological effects occurred in rats receiving *n*-hexane by gavage (Krasavage et al. 1980; Ono et al. 1981). Significant serum levels of the *n*-hexane metabolite, 2,5-hexanedione, were also measured in rats receiving *n*-hexane by gavage (Krasavage et al. 1980).

The permeability of human skin to *n*-hexane has been determined *in vitro* in flow-through diffusion cells (Loden 1986). Pieces of full-thickness human skin were exposed to [$^3$H]*n*-hexane in human serum, and the appearance of label in the trans compartment measured for 0.5 or 12 hours. The skin was then sectioned with a microtome into 0.25 mm slices and the quantity of label in the skin was measured. The rate of resorption (uptake of substance by the receptor fluid beneath the skin [i.e., the amount that passes through the skin]) was calculated. The rate of resorption for *n*-hexane through human skin was calculated to be 0.83 (μg·cm$^2$/hour). The permeability of *n*-hexane through human skin was much lower (approximately 100-fold) than for other chemicals tested in this study. For example, rates of resorption (in μg·cm$^2$/hour) were 99 for benzene and 118 for ethylene glycol.

No information is available on whether absorption of *n*-hexane by children differs from that of adults. Since absorption by all routes appears to be by passive diffusion, it is probable that absorption in children is similar to that of adults.

### 3.1.2 Distribution

The distribution of *n*-hexane is a function of its high lipid and very low water solubility. *n*-Hexane is transported in blood mainly by partitioning into hydrophobic regions of blood proteins (Lam et al. 1990). Transfer to tissues occurs via a similar partitioning process. *n*-Hexane can also leave the blood through the lungs via the pulmonary circulation depending on the alveolar air *n*-hexane concentration.

Partition coefficients established in human tissues indicate a distribution pattern at equilibrium of body fat>>liver, brain, muscle>kidney, heart>lung, blood (Perbellini et al. 1985). The following partition coefficients for *n*-hexane were determined: olive oil/air, 146; blood/air, 0.80; liver/air, 5.2; kidney/air, 3; brain/air, 5; fat/air, 104; muscle/air, 5; heart/air, 2.8; and lung/air, 1. Saline/air partition was not reported separately for *n*-hexane, but was very low for the range reported for the entire group of compounds (0.1–0.4). Partition coefficients for *n*-hexane in male Fischer 344 rats have been reported (blood/air, tissue/air): blood, 2.29; liver, 5.2; muscle, 2.9; and fat, 159 (Gargas et al. 1989).

In a study where blood *n*-hexane concentrations were determined in volunteers during exposure to 102 or 204 ppm for 4 hours, blood *n*-hexane reached steady state within 50 minutes and was stable until the end of exposure. Concentrations of *n*-hexane in blood at 50 minutes were 0.183 mg/L at 102 ppm and 0.3347 mg/L at 204 ppm (Veulemans et al. 1982).

In Fischer 344 rats exposed to up to 10,000 ppm *n*-hexane for 6 hours, *n*-hexane achieved an apparent steady state in all tissues within 2 hours (Baker and Rickert 1981). Steady-state concentrations were proportional to dose only in blood and liver. In brain, sciatic nerve, kidney, lung, and testes, exposure to 1,000 ppm resulted in a disproportionately greater concentration than exposure at 500 ppm. Peak blood concentrations of *n*-hexane were 1, 2, 8, and 21 μg/mL, and peak sciatic nerve concentrations were 12, 48, 130, and 430 μg/g at 500, 1,000, 3,000, and 10,000 ppm, respectively. In a study that addressed possible accumulation of *n*-hexane in tissues, *n*-hexane was not detected in any tissue besides sciatic nerve after 2 hours post-exposure in either 1- or 5-day exposures to *n*-hexane at 1,000 ppm for 6 hours/day (Bus et al. 1981). Initial concentrations after a single exposure were: sciatic nerve, 46 μg/g; kidney, 5.8 μg/g; liver, 1.2 μg/g; brain, 3 μg/g; and blood, 0.5 μg/mL. Initial concentrations after five daily exposures were similar. No studies were located regarding distribution of *n*-hexane after oral or dermal exposure in humans or animals.

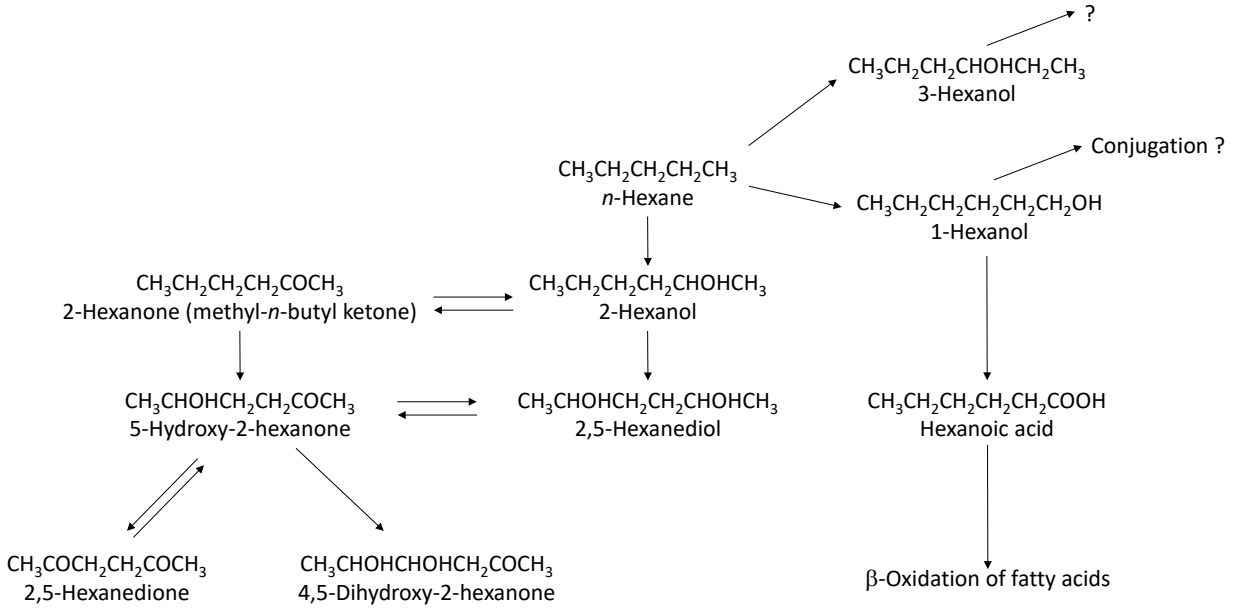
No information is available on whether distribution of *n*-hexane in children differs from that of adults. Transfer across the placenta has been demonstrated in rats for *n*-hexane and two resulting metabolites, 2-hexanone and 2,5-hexanedione (Bus et al. 1979); no preferential distribution to the fetus was observed for either *n*-hexane or the metabolites. Concentrations of *n*-hexane and its metabolites were similar between maternal tissues and the fetal tissues following a 6-hour exposure to 1,000 ppm *n*-hexane on GD 20 (Bus et al. 1979), indicating that transfer across the placenta takes place.

*n*-Hexane has not been measured in breast milk, although partition coefficients have been reported for human milk from a group of eight volunteers (Fisher et al. 1997). The milk/air coefficient was 4.66 and the blood/air coefficient was 2.13. A milk/blood partition coefficient of 2.10 was calculated from these data, indicating that there would be preferential distribution to this compartment. Due to its relatively rapid metabolism, storage of *n*-hexane in body fat does not appear to occur at air concentrations to which humans are exposed; thus, mobilization of stored *n*-hexane upon pregnancy or during lactation is unlikely. The toxic metabolite of *n*-hexane, 2,5-hexanedione, can probably be distributed to germ cells as demonstrated by the testicular effects observed in male rats after drinking water exposure to 2,5-hexanedione. High air concentrations of *n*-hexane can also produce these effects in rats, presumably via 2,5-hexanedione.

### 3.1.3 Metabolism

The metabolism of *n*-hexane takes place in the liver. The initial reaction is oxidation by cytochrome P-450 isozymes to hexanols, predominantly 2-hexanol. Further reactions convert 2-hexanol to 2-hexanone, 2,5-hexanediol, 5-hydroxy-2-hexanone, 4,5-dihydroxy-2-hexanone, and the neurotoxicant, 2,5-hexanedione. Hydroxylation at the 1- and 3- positions can be considered detoxification pathways; hydroxylation at the 2- position is a bioactivation pathway. A diagram of the proposed pathway for mammalian metabolism of *n*-hexane is presented in Figure 3-1.

**Figure 3-1. Proposed Scheme for the Metabolism of *n*-Hexane**




Adapted from Fedtke and Bolt (1987) and NTP (1991)

Approximately 10–20% of *n*-hexane absorbed by inhalation is excreted unchanged in exhaled air; the remainder is metabolized. Metabolism takes place via mixed-function oxidase reactions in the liver. In a study in which metabolites were measured in workers exposed to *n*-hexane (Perbellini et al. 1981), mean concentrations of *n*-hexane metabolites in urine were: 2,5-hexanedione, 5.4 mg/L (including 4,5-dihydroxy-2-hexanone because acid treatment of the urine converts 4,5-dihydroxy-2-hexanone to 2,5-hexadione); 2,5-dimethylfuran, 3.7 mg/L; gamma-valerolactone, 3.3 mg/L; and 2-hexanol, 0.19 mg/L (2,5-dimethylfuran and gamma-valerolactone are believed to be artifacts of sample preparation and analysis rather than true metabolites of *n*-hexane [Perbellini et al. 1981]). The first reaction that takes place is hydroxylation of *n*-hexane at the 2- position to form 2-hexanol. Further reactions result in 2,5-hexanedione, presumably through transient intermediates, including 2-hexanone, 2,5-hexanediol, and 5-hydroxy-2-hexanone. Correlations between concentrations of *n*-hexane in air and urinary metabolites were best for total *n*-hexane metabolites (r=0.7858), followed by 2-hexanol (r=0.6851) and 2,5-hexanedione (r=0.6725).

The time-course of the metabolism of inhaled *n*-hexane in a group of 19 volunteers has been estimated by determining serum 2,5-hexanedione during and after a 15.5-minute exposure to 60 ppm *n*-hexane (van Engelen et al. 1997). The time to reach the peak concentration varied from 16.2 to 19.8 minutes after the

start of exposure (i.e., 1–4 minutes following the cessation of exposure). The rate at which 2,5-hexanedione appeared in the blood ranged from 1.89 to 4.48 μM/hour.

Further studies in humans indicate that a large proportion of the 2,5-hexanedione detected in urine after *n*-hexane exposure is the result of an artifact resulting from treatment with acid to hydrolyze urinary conjugates (Fedtke and Bolt 1987). When urine from a male volunteer exposed to 217 ppm *n*-hexane for 4 hours was hydrolyzed enzymatically with β-glucuronidase, excretion of 4,5-dihydroxy-2-hexanone was approximately 4 times higher than that of 2,5-hexanedione. When the urine was hydrolyzed with acid, 4,5-dihydroxy-2-hexanone was not detected, but the amount of 2,5-hexanedione in the urine increased, indicating conversion of 4,5-dihydroxy-2-hexanone to 2,5-hexanedione by the acid treatment. The fraction of 2,5-hexanedione determined after complete acid hydrolysis minus the 2,5-hexanedione originally present was equal to the 4,5-dihydroxy-2-hexanone. Only "minor" amounts of 2-hexanol were reported.

2,5-Hexanedione has also been detected after acid hydrolysis of the urine of individuals unexposed to *n*-hexane (Fedtke and Bolt 1986; Perbellini et al. 1993). 2,5-Hexanedione was not detected without acid hydrolysis, indicating that it is formed as a result of conversion of 4,5-dihydroxy-2-hexanone. It is possible that small amounts of *n*-hexane are produced in the body by lipid peroxidation, as has been demonstrated for *n*-pentane (Filser et al. 1983). Urinary excretion of 2,5-hexanedione ranged from 0.3 to 1.2 mg in 24 hours for unexposed individuals; workers exposed to approximately 50 ppm *n*-hexane excreted 3–4 mg/24 hours (Perbellini et al. 1993).

When male Wistar rats were exposed to *n*-hexane at concentrations up to 3,074 ppm for 8 hours, analysis of urine showed that 2-hexanol was the major metabolite, accounting for about 60–70% of the total metabolites collected over the 48-hour collecting period (Fedtke and Bolt 1987). This is in contrast to humans, in which the major urinary metabolite is 2,5-hexanedione (Perbellini et al. 1981). The amounts of metabolites excreted were linearly dependent on the exposure concentration, up to an exposure of about 300 ppm. 2-Hexanol and 2-hexanone were detected in the first sample (obtained during the 8-hour exposure); excretion of 2,5-hexanedione was delayed and was not detected until 8–16 hours after exposure began. The amount of 2,5-hexanedione detected depended on sample treatment; total excreted amounts over 48 hours were approximately 350 μg/kg 2,5-hexanedione without acid treatment and 3,000 μg/kg with total acid hydrolysis, indicating conversion of 4,5-dihydroxy-2-hexanone with acid treatment.

The metabolism of *n*-hexane in rat lung and liver microsomes has been investigated (Toftgard et al. 1986). In liver microsomes, the formation of 1-, 2-, and 3-hexanol from *n*-hexane was best described kinetically by a two-enzyme system, while for lung microsomes, single-enzyme kinetics were indicated for each metabolite. For conversion to 1-hexanol, apparent K, values were 0.4 and 300 μM, and $V_{max}$ values were 0.09 and 1.2 nmol/mg protein/minute, respectively. For conversion to 2-hexanol, apparent $K_m$ values were 6 and I, 100 μM, and $V_{max}$ values were 1 and 4.6 nmol/mg protein/minute, respectively. Insufficient information was available to estimate the high-affinity activity for 3-hexanol, the low-affinity activity had an apparent $K_m$ of 290 pM and a $V_{max}$ of 0.5 nmol/mg protein/minute. In the lung, $K_m$ values were 9, 50, and 65 μM for 1-, 2-, and 3-hexanol, respectively; $V_{max}$ values were 2.2, 1.3, and 0.2 nmol/mg protein/minute, respectively. Prior induction of cytochrome P-450 enzymes with phenobarbital markedly increased the rate of formation of 2-hexanol in liver microsomes (1.8 nmol/mg/minute control versus 15 nmol/mg/minute with phenobarbital) and that of 3-hexanol (0.4 nmol/mg/minute control versus 2.8 nmol/mg/minute), while the rate of formation of 1-hexanol fell slightly (2 nmol/mg/minute control versus 0.7 nmol/mg/minute). Antibodies to cytochrome P-450 isozymes PB-B (CYP2B1-inducible by phenobarbital) and BNF-B (CYP1A1-inducible by β-naphthoflavone) were used as inhibitors to investigate the specificity of the reactions. In control liver microsomes, anti-PB-B showed no inhibitory effects while anti-BNF-B inhibited the formation of 2- and 3-hexanol by 25 and 40%, respectively, but had no effect on the formation of 1-hexanol. In microsomes from rats induced with phenobarbital, the anti-PB-B antibody reduced the formation of hexanols back to control levels. Purified cytochrome P-450 isozymes were also tested for their ability to hydroxylate *n*-hexane. The highest activity (nmol metabolite/nmol P-450/minute) was found with P-450-PB-B (CYP2B1), followed by P-450-PB-D (CYP2B2) and P-450-BNF-B (CYP1A1). Formation of 2,5-hexanediol from 2-hexanol was catalyzed by a cytochrome P-450 isozyme different from cytochrome P-450-PB-B (as judged by antibody inhibition) that was present in liver microsomes, but not in lung microsomes. This process was unaffected by prior induction of cytochrome P-450. Furthermore, alcohol dehydrogenase activity with hexanols or 2,5-hexanediol as the substrate was found exclusively in liver cytosol. These results suggest that inhaled *n*-hexane must be transported to the liver either intact or in the form of 2-hexanol before the neurotoxic metabolite, 2,5-hexanedione, can be formed. The large increase in hydroxylation of *n*-hexane upon induction (which would favor the production of 2,5-hexanedione via 2-hexanol) is a likely explanation for the potentiating effects of MEK on *n*-hexane neurotoxicity in humans and rats (Altenkirch et al. 1977, 1982) and of methyl isobutyl ketone in chickens (Abou-Donia et al. 1985).

Mortensen and Nilsen (1998) conducted an *in vitro* species comparison of *n*-hexane metabolism in the liver. Humans had the highest $K_m$ (132 μmol/L) and rats had the lowest $K_m$ (25 μmol/L), with guinea

pigs and mice having similar values as rats; however, there were no statistically significant differences between the species. $V_{max}$ values were similar in the four species, ranging from 0.19 to 0.36 µmol/g/minute.

The tissue and cytochrome P-450 isoform specificity of *n*-hexane hydroxylation to hexanols has been investigated in rat tissues and cell lines expressing specific cytochrome P-450 isoforms (Crosbie et al. 1997). The highest activity per mg protein for the production of 2-hexanol (which can be further metabolized to 2,5-hexanedione) was in liver, followed by lung (about 25% of liver activity), muscle, and brain. Activity in muscle and brain was very low compared to the liver. Membrane preparations from cells expressing human CYP2E1 had the same *n*-hexane 2-hydroxylation activity as control cells. In contrast, cells expressing human CYP2B6 had approximately 100 times the 2-hydroxylation activity of the CYP2E1 or control cells. Specific induction of the cytochrome P-450 isozyme, CYP2E1, has been reported in male Wistar rats after intraperitoneal injection of *n*-hexane (Nakajima et al. 1991). No effects on total liver microsomal protein or total cytochrome P-450 content were observed.

trans-1,2-Dichloroethylene, a specific inhibitor of CYP2E1 in rats, has also been shown to affect the metabolism of *n*-hexane (Mathews et al. 1997). Rats exhale a large number of endogenous volatile organic compounds (VOCs), including *n*-hexane. When CYP2E1 was inhibited by intraperitoneal injection of 1,2-dichloroethylene, levels of exhaled *n*-hexane increased approximately 25-fold within 4 hours and returned to pre-dose levels at approximately 24 hours, closely paralleling the inhibition and resynthesis time-course for CYP2E1. No increase in lipid peroxidation was observed, indicating that the increase in exhaled *n*-hexane was the result of inhibition of metabolism.

It is probable that many cytochrome P-450 isoforms are capable of hydroxylating *n*-hexane (both *in vivo* and under laboratory conditions); it is not possible at this time to specify which forms are definitely involved in *n*-hexane metabolism *in vivo*. The results of a study in CYP2E1 knockout and wild-type mice found more extensive metabolism of *n*-hexane to 2,5-hexanedione in wild-type mice, as compared to the knockout mice (Iba et al. 2000). The study also found that 2,5-hexanedione formation increased with duration in the wild-type mice but was unchanged in the knockout mice. The formation of 2,5-hexanedione in the knockout mouse suggests that other cytochromes P-450s can also metabolize *n*-hexane; the lack of increase in 2,5-hexanedione levels in the knockout mice suggests that these other cytochrome P-450s are not likely to be inducible by *n*-hexane.

The effect of concentration on the fate of [$^{14}$C]*n*-hexane after inhalation exposure has been studied in Fischer 344 rats (Bus et al. 1982). The disposition of radioactivity was concentration-related with the amount of the acquired body burden excreted increasing with increasing concentrations; 12, 24, 38, and 62% of the acquired body burden was excreted at exposure concentrations of 500, 1,000, 3,000, and 10,000 ppm, respectively. In contrast, 38 and 18% of the body burden of radioactivity was recovered as exhaled $CO_2$ and 35 and 18% was recovered in the urine at *n*-hexane concentrations of 500 and 10,000 ppm (exhaled air and urine were collected for 72 hours after exposure). Radioactivity remaining in the tissues and carcass 72 hours after exposure was 6.1 and 5.4% of the body burden at 500 and 10,000 ppm, respectively. The decreased total $^{14}CO_2$ and urinary $^{14}C$ excretion after exposure to 10,000 ppm, as compared to lower concentrations, was likely due to an inhibition of *n*-hexane metabolism rather than saturation of *n*-hexane excretion by the lungs or the kidneys.

In a study in which pregnant rats received a single 6-hour exposure to 1,000 ppm *n*-hexane on GD 12 or 20 (Bus et al. 1979), *n*-hexane was rapidly and extensively metabolized to methyl-*n*-butyl ketone (2-hexanone) and 2,5-hexanedione. 2-Hexanone and 2,5-hexanedione (the only metabolites measured) were detected in the maternal liver, kidney, brain, and blood. Fetal concentrations of *n*-hexane and its metabolites (entire fetus) were similar to those in maternal blood at all times after exposure. Results were similar on both GDs 12 and 20. *n*-Hexane and 2-hexanone were rapidly eliminated from maternal tissues and the fetus, with minimal or nondetectable concentrations reached 8 hours after exposure. In contrast, tissue concentrations of 2,5-hexanedione increased between 0 and 4 hours after exposure and thereafter exhibited a significantly slower elimination rate compared to *n*-hexane and 2-hexanone. 2,5-Hexanedione was not detected in the blood or tissues 24 hours after exposure. The half-life of 2,5-hexanedione in maternal blood was significantly greater than *n*-hexane and 2-hexanone (3.9 hours versus 1.24 and 0.99 hours, respectively).

Concentration time curves for *n*-hexane in a closed exposure system indicated that metabolism in rats was proportional to air concentration up to about 300 ppm (Filser et al. 1987). Metabolism was nonlinear above 300 ppm and appeared to be saturated at concentrations ≥3,000 ppm.

Little information is available on the metabolism of *n*-hexane after oral exposure, although it appears to be qualitatively similar to that after inhalation exposure. Peak serum concentrations of the *n*-hexane metabolite, 2,5-hexanedione, of 24, 44, and 53 μg/mL were observed in rats after a single gavage exposure to 570, 1,140, and 4,000 mg/kg *n*-hexane, respectively (Krasavage et al. 1980). Serum

2,5-hexanedione concentrations rose slowly to a peak at 12–16 hours and returned to baseline by 24 hours.

No information is available as to whether metabolism of *n*-hexane in children differs from that of adults. No studies were located comparing metabolism in young and adult animals. The toxicity of *n*-hexane results from biotransformation yielding the active metabolite, 2,5-hexanedione. The initial step is an oxidation to 2-hexanol catalyzed by a cytochrome P-450 enzyme. Some cytochrome P-450 enzymes are developmentally regulated (Leeder and Kearns 1997). As the above discussion indicates, it is not completely clear which cytochrome P-450 enzymes are involved in *n*-hexane metabolism.

### 3.1.4 Excretion

Elimination half-lives of *n*-hexane and its metabolites in the body and several tissues have been evaluated in humans and animals. In a study of workers exposed to *n*-hexane, the post-exposure alveolar excretion of *n*-hexane was about 10% of the total uptake and was in two phases: a fast phase with a half-life of 11 minutes and a slow phase with a half-life of 99 minutes (Mutti et al. 1984). Veulemans et al. (1982) estimated the elimination half-life of *n*-hexane in blood following a 4-hour inhalation exposure of humans to 102 ppm *n*-hexane. After exposure, there was a rapid fall to about 50% of the level at the end of exposure in the first 10 minutes, followed by a slower exponential time course with a half-life of 1.5–2 hours. A physiologically based pharmacokinetic (PBPK) model estimated a half-life of *n*-hexane in fat tissue of approximately 64 hours in humans (Perbellini et al. 1986). A half-life for the urinary excretion of 2,5-hexanedione was estimated by Perbellini et al. (1986) using data from workers. A study in rats exposed to 500 ppm [$^{14}$C] *n*-hexane for 6 hours estimated a urinary half-time for excretion of radioactivity of 12.7 hours (Bus et al. 1982).

Excretion of *n*-hexane after oral exposure in humans can be inferred based on elevated levels of 2,5-hexanedione in urine of volunteers receiving 0.24 or 0.81 mg/kg via a gastric feeding tube (Baelum et al. 1998). No studies were located regarding excretion of *n*-hexane or *n*-hexane metabolites following oral exposure to *n*-hexane in animals. No studies were located regarding excretion of *n*-hexane or *n*-hexane metabolites following dermal exposure to *n*-hexane.

No information is available as to whether excretion of *n*-hexane and its metabolites in children differs from that of adults. No studies were located comparing excretion in young and adult animals.

### 3.1.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

Models are simplified representations of a system with the intent of reproducing or simulating its structure, function, and behavior. PBPK models are more firmly grounded in principles of biology and biochemistry. They use mathematical descriptions of the processes determining uptake and disposition of chemical substances as a function of their physicochemical, biochemical, and physiological characteristics (Andersen and Krishnan 1994; Clewell 1995; Mumtaz et al. 2012a; Sweeney and Gearhart 2020). PBPK models have been developed for both organic and inorganic pollutants (Ruiz et al. 2011) and are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Mumtaz et al. 2012b; Ruiz et al. 2011; Sweeney and Gearhart 2020; Tan et al. 2020). PBPK models can also be used to more accurately extrapolate from animal to human, high dose to low dose, route to route, and various exposure scenarios and to study pollutant mixtures (El-Masri et al. 2004). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic endpoints (Clewell 1995).

***Summary of PBPK Models.*** The Perbellini et al. (1986, 1990b) model simulates the absorption, distribution, biotransformation, and excretion of *n*-hexane during inhalation exposure. The excretion kinetics of the neurotoxic metabolite of *n*-hexane, 2,5-hexanedione, are also simulated.

A model describing transfer of *n*-hexane via lactation from a mother to a nursing infant is also available (Fisher et al. 1997). Human milk/blood partition coefficients for 19 VOCs, including *n*-hexane, were experimentally determined using samples from volunteers. These parameters were used to estimate the amount of *n*-hexane an infant would ingest from milk if the mother was occupationally exposed to *n*-hexane at the Threshold Limit Value (TLV) throughout a workday.

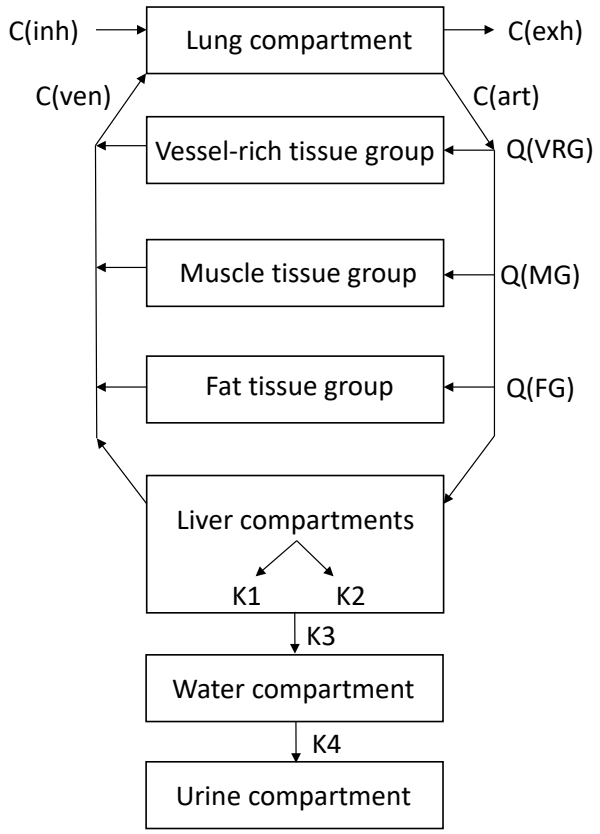
***n-Hexane PBPK Model Comparison.*** The Perbellini et al. (1986, 1990b) model is the only validated model for this chemical identified in the literature. The Fisher et al. (1997) model was intended for risk assessment to predict which of 19 VOCs may be present in milk at a high enough level after workplace exposure to raise health concerns for a nursing infant.

## The Perbellini et al. (1986, 1990b) Model

***Risk Assessment.*** The Perbellini et al. (1986, 1990b) model successfully described alveolar air and venous blood concentration of *n*-hexane following inhalation exposure in humans. Simulations indicated that exposure to 50 ppm for an 8-hour workday, 5-day workweek would result in a gradual accumulation of *n*-hexane in body fat, which is not completely cleared during the weekend.

***Description of the Model.*** The Perbellini et al. (1986, 1990b) model has eight compartments (see Figure 3-2) representing lung, liver, fat, muscle, a lumped compartment representing richly perfused tissues, urine, and a "water." The water compartment was included to simulate the transfer of the liver metabolite, 2,5-hexanedione, to urine.

### Figure 3-2. Perbellini et al. (1986, 1990b) Physiologically Based Pharmacokinetic Model




C(ven) = concentration of *n*-hexane in venous blood; C(inh) = concentration of *n*-hexane in inhaled air; C(exh) = concentration of *n*-hexane in exhaled air; C(art) = *n*-hexane in arterial blood; FG = fat tissue group; MG = muscle tissue group; VRG = vessel-rich tissue group

Source: Perbellini et al. 1986

Absorption from the lung is assumed to be flow-limited and governed by the air concentration, a blood/air partition coefficient, and blood flow to the lung. Exchanges between blood and tissues are assumed to be flow-limited and governed by the concentration gradient between blood and tissue, the tissue/blood partition coefficient, and tissue blood flow. Metabolism of *n*-hexane was attributed to the liver and was simulated as two first-order pathways ($minute^{-1}$), one of which results in production of 2,5-hexanedione; the other pathway represents all other metabolism elimination processes. The metabolite, 2,5-hexanedione, is transferred to the water compartment and, from the water compartment, to urine, with both transfers assumed to be first order ($minute^{-1}$).

Physiological parameters for volumes and blood flow of the compartments are listed in Table 3-1. Physiologic constants (compartment volume, blood flows, etc.) were taken from published values. Values for the solubility of *n*-hexane in blood and tissues (partition coefficients) are taken from human tissue (Perbellini et al. 1985). Rate constants (Table 3-1, Figure 3-2) were estimated from animal and human data and are all assumed to be first order.

**Table 3-1. Parameters Used in the Perbellini et al. (1986, 1990b) Physiologically Based Pharmacokinetic Model for *n*-Hexane**

| Parameters | Human |
|---|---|
| | Compartment volumes (L) |
| Liver | 1.7 |
| Lung | 1.0 |
| Fat | 11.5 |
| Vessel-rich compartment | 7.1 |
| Muscle compartment | 36.3 |
| | Flows (L/minute) |
| Alveolar ventilation | 6 |
| Cardiac output | 6.3 |
| | Percentage of cardiac output |
| Liver | 30 |
| Fat | 4.4 |
| Vessel-rich compartment | 50 |
| Muscle compartment | 16 |

**Table 3-1. Parameters Used in the Perbellini et al. (1986, 1990b) Physiologically Based Pharmacokinetic Model for *n*-Hexane**

| Parameters | Human |
|---|---|
| | Partition coefficients |
| Blood/air | 0.8 |
| Liver/blood | 6.5 |
| Fat/blood | 130 |
| Vessel-rich compartment | 5 |
| Muscle/blood | 6.2 |
| | Metabolic constants (minute$^{-1}$) |
| $K_1$ (catabolism of *n*-hexane to metabolites) | 0.3 |
| $K_2$ (synthesis of 2,5-hexanedione to *n*-hexane) | 0.012 |
| $K_3$ (synthesis of 2,5-hexanedione to body water) | 0.009 |
| $K_4$ (transfer of 2,5-hexanedione from body water to urine) | 0.0009 |

***Validation of the Model.*** The Perbellini et al. (1986, 1990b) model was validated using a data set for venous blood *n*-hexane values in volunteers exposed for 4 hours (Veulemans et al. 1982). The range in the study was 334–368 µg/L during exposure to 204 ppm; the model predicted values that were within 1 standard deviation of the observed means. After 4 hours of exposure to 102 ppm, the predicted value for venous blood *n*-hexane concentration was about 10% below the actual observed means. Blood *n*-hexane concentrations and air *n*-hexane concentration have shown to be strongly correlated in workers exposed to *n*-hexane (Perbellini et al. 1986). The model predicted a blood *n*-hexane level in workers exposed to 102 ppm (182 µg/L) that was similar to values predicted from the observed air-blood correlation (176 µg/L). The urinary excretion rate of 2,5-hexanedione predicted by the model was also compared to a data set from 13 workers followed for 24 hours from the beginning of a workday. The model successfully predicted the rate of 2,5-hexanedione urinary excretion.

***Target Tissues.*** Target tissues were not specifically addressed in Perbellini et al. (1986, 1990b). The target tissue for *n*-hexane is peripheral nerve (via the neurotoxic metabolite 2,5-hexanedione).

***Species Extrapolation.*** Species extrapolation was not addressed in Perbellini et al. (1986, 1990b). Results from *in vitro* studies in rat liver homogenates were used to estimate kinetic parameters for the catabolism of *n*-hexane and synthesis of 2,5-hexanedione.

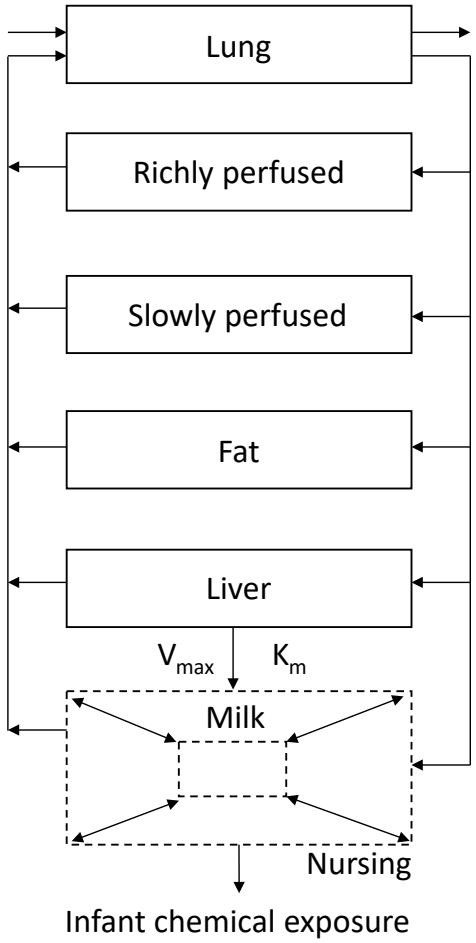
***Interroute Extrapolation.*** Interroute extrapolation was not addressed in Perbellini et al. (1986, 1990b).

## The Fisher et al. 1997 Model

***Risk Assessment.*** The purpose of this study was risk assessment. The transfer of 19 chemicals to milk was simulated to predict those that may result in exposures to infants higher than the EPA Drinking Water Health Advisory values for chronic ingestion of contaminated water by a 10-kg child. The model predicted an ingestion rate of 0.052 mg/day for *n*-hexane, which was below this value.

***Description of the Model.*** The Fisher model simulates the transfer of *n*-hexane from a mother to a nursing infant during and after occupational exposure via inhalation (Fisher et al. 1997). The model contains seven compartments: alveolar space, lung blood, fat, slowly perfused tissues, rapidly perfused tissues, liver, and milk (see Figure 3-3). Absorption from the lung is assumed to be flow-limited and governed by the air concentration, a blood/air partition coefficient, and blood flow to the lung. Exchanges between blood and tissues are assumed to be flow-limited and governed by the concentration gradient between blood and tissue, the tissue/blood partition coefficient, and tissue blood flow. Metabolism of *n*-hexane was attributed to the liver and was simulated as a capacity-limited process ($K_M$, $V_{max}$). Fate of the metabolites were not simulated in the model. Transfer of *n*-hexane to breast milk was simulated as a first-order process ($hour^{-1}$). Standard literature values were used for most parameters while blood/air and milk/air partition coefficients were determined experimentally from milk samples from nine volunteers (Table 3-2). The milk/blood partition coefficient was derived from the blood/air and milk/air coefficients. Maximum rates of hepatic metabolism ($V_{max}$) and the $K_m$ value for *n*-hexane were taken from a study in rats. The milk compartment included changes in volume in response to nursing; milk letdown from nursing is assumed to be a first-order process and milk production a zero-order process. Minimum and maximum volumes for the milk compartment were 0.010 and 0.125 L, respectively. The amount of *n*-hexane ingested by the infant was predicted using simulations run assuming an *n*-hexane air level of 50 ppm (based on the TLV) for an 8-hour working period containing two 0.5-hour and one 1-hour break periods without exposure and eight 12-minute nursing periods over 24 hours.

**Figure 3-3. Fisher et al. (1997) Physiologically Based Pharmacokinetic Model**




Milk compartment volume changes due to nursing.

Source: Fisher et al. 1997

**Table 3-2. Parameters Used in the Fisher et al. (1997) Physiologically Based Pharmacokinetic Model for *n*-Hexane**

| Parameters | Human |
|---|---|
| | % BW (BW=60 kg) |
| Liver | 1.5 |
| Richly perfused | 10 |
| Slowly perfused | 54 |
| Fat | 25 |
| Milk | 10–125 mL |
| | Flows (L/minute) |
| Alveolar ventilation | $24xBW^{0.74}$ |
| Cardiac output | $15xBW^{0.74}$ |

**Table 3-2. Parameters Used in the Fisher et al. (1997) Physiologically Based Pharmacokinetic Model for *n*-Hexane**

| Parameters | Human |
|---|---|
| | Percentage of cardiac output |
| Liver | 29 |
| Fat | 10 |
| Richly perfused | 35 |
| Slowly perfused | 19 |
| Milk | 7 |
| | Partition coefficients |
| Blood/air | 2.13 |
| Liver/blood | 2.45 |
| Fat/blood | 74.74 |
| Richly perfused blood | 2.45 |
| Slowly perfused blood | 1.36 |
| Milk/air | 4.66 |
| Milk/blood | 2.10 |
| | Metabolic constants |
| $V_{max}$ | 6.0 mg/kg/hour |
| $K_m$ | 0.3 mg/L |
| | Milk compartment |
| Nurse[a] | 20/hour |
| Prod[b] | 0.06 L/hour |

[a]Nurse is a first-order term to describe the rate of ingestion of breast milk by a nursing infant.
[b]Prod is a zero-order term to describe the rate of breast milk production at 1.3–3 months of lactation.

BW = body weight

***Validation of the Model.*** Fisher et al. (1997) did not validate that model against observations of *n*-hexane in blood, tissues, or breast milk.

***Target Tissues.*** Target tissues (peripheral nervous system) were not specifically addressed in Fisher et al. (1997).

***Species Extrapolation.*** Species extrapolation was not reported in Fisher et al. (1997).

***Interroute Extrapolation.*** Interroute extrapolation was not reported in Fisher et al. (1997).

### 3.1.6 Animal-to-Human Extrapolations

The rat is the major model system for human *n*-hexane neurotoxicity. Inhalation of *n*-hexane in this species produces clinical and histopathological effects similar to those seen in workers exposed to *n*-hexane. However, the toxicokinetics in rats are somewhat different; for example, less 2,5-hexanedione and more 2-hexanol is produced in rats as a proportion of total urinary metabolites compared to humans (Fedtke and Bolt 1987; Frontali et al. 1981). Mice do not develop clinical signs of neurotoxicity after exposure to *n*-hexane, although histopathological changes (paranodal axonal swellings) have been observed (NTP 1991). A single study in rabbits exposed to high levels of *n*-hexane (3,000 ppm) showed no evidence of neurotoxicity in this species (Lungarella et al. 1984).

## 3.2 CHILDREN AND OTHER POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Children may be more or less susceptible than adults to health effects from exposure to hazardous substances and the relationship may change with developmental age.

This section also discusses unusually susceptible populations. A susceptible population may exhibit different or enhanced responses to certain chemicals than most persons exposed to the same level of these chemicals in the environment. Factors involved with increased susceptibility may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters can reduce detoxification or excretion or compromise organ function.

Populations at greater exposure risk to unusually high exposure levels to *n*-hexane are discussed in Section 5.7, Populations with Potentially High Exposures.

***Age.*** Cases of *n*-hexane toxicity in humans have occurred as the result of workplace exposure and solvent misuse (Spencer et al. 1980). Some of these cases of peripheral neuropathy have occurred in teenagers (particularly with solvent misuse); however, none of the clinical reports indicate differences in physical signs or functional tests between this group and adults (Altenkirch et al. 1977; Yamamura 1969). While no reports of *n*-hexane toxicity in young children were located, it is probable that similar toxicity would

occur if exposure was comparable to that in affected adults. Specific information is not available on whether children are more susceptible than adults to the effects of *n*-hexane.

Animal studies provide limited further information. Only two studies were located where the responses to *n*-hexane were compared between young animals and adults. In a study in Fischer 344 rats directly comparing the effects of exposure to 1,000 ppm *n*-hexane 24 hours/day, 6 days/week for 11 weeks in weanlings (21 days old) and young adults (80 days old), peripheral neuropathy occurred in both groups, although onset was more rapid in the young adult group (Howd et al. 1983). For example, mild, slight ataxia was observed in 2/10 young adults and in 0/10 weanlings after 7 weeks of exposure; mild, slight ataxia was observed in 2/10 weanlings after 8 weeks of exposure. Age-related differences in severity were also observed. After 11 weeks of exposure, severe hindlimb ataxia, inability to stand, and flaccid hindlimbs were observed in all surviving young adults; in contrast, all of the weanling animals displayed mild, slight ataxia. No deaths were observed over the 11-week exposure period and 3-week recovery period in weanling rats. In young adults, however, 5 of 10 rats died as the result of severe neuropathy. The study authors suggested that the relative resistance of the weanling rats may have been due to shorter, smaller-diameter axons, or to a greater rate of growth and repair in their peripheral nerves compared to those of adults. In contrast, an oral $LD_{50}$ study in Sprague Dawley rats showed that 14-day-old rats were more susceptible to the lethal effects of a large dose of *n*-hexane than young adults (Kimura et al. 1971). $LD_{50}$ values for *n*-hexane were 15,840 mg/kg for 14-day-old rats and 32,340 mg/kg for the young adults. Clinical signs and time to death were not reported. Comparison of the findings in the Howd et al. (1983) neurotoxicity study and the Kimura et al. (1971) $LD_{50}$ study is limited by differences in endpoint examined, exposure routes, and rat strains.

*n*-Hexane has not caused teratogenic effects in rodent models, although some developmental effects (decreased fetal weight, decreased live fetuses per litter) have been reported in rats and mice exposed during pregnancy to ≥1,000 ppm (API 1979; Bus et al. 1979; Marks et al. 1980; NIEHS 1987, 1988c). Observation of the offspring after birth to maturity was not performed. No information is available on whether parental exposure to *n*-hexane can cause transgenerational effects in children. This appears unlikely since *n*-hexane has tested negative for genotoxicity in a number of *in vivo* and *in vitro* tests. One area of potential concern is that very high air concentrations of *n*-hexane (±1,000 ppm) administered for 21–24 hours/day resulted in signs of testicular damage in rats (De Martino et al. 1987; Nylen et al. 1989). These signs are also found in rats after large oral doses (Krasavage et al. 1980) and the administration of the *n*-hexane metabolite, 2,5-hexanedione, in drinking water (Chapin et al. 1982; Gillies et al. 1981). Severe neurotoxicity was evident in all these cases. It is not known whether or not this is a species-

specific effect since examination of sperm in a worker population with exposure to *n*-hexane and elevated 2,5-hexanedione urinary levels has not been reported.

No information is available as to whether *n*-hexane or its metabolites cross the placenta in humans. Transfer across the placenta has been demonstrated in rats for *n*-hexane and two resulting metabolites, 2-hexanone and 2,5-hexanedione (Bus et al. 1979); no preferential distribution to the fetus was observed for either *n*-hexane or the metabolites. Due to its relatively rapid metabolism, storage of *n*-hexane in body fat does not appear to occur at air concentrations to which humans are exposed; thus, mobilization of maternally stored *n*-hexane upon pregnancy or during lactation is unlikely. Data to support this assumption were not located as no studies evaluating levels of *n*-hexane in amniotic fluid, meconium, cord blood, or neonatal blood were identified. There are no studies dealing with exposure or body burden measurements in children. Given the absence of such studies targeted at children, it is unknown whether children are different in their weight-adjusted intake responses to *n*-hexane.

Hexanes ($C_6H_{14}$) have been detected in samples of human breast milk (Pellizzari et al. 1982); however, *n*-hexane was not quantified, nor was any attempt made to assess the subjects' exposure. A human milk/blood partition coefficient of 2.10 (Fisher et al. 1997) indicates that there would be preferential distribution to this compartment if significant absorption occurred; however, no pharmacokinetic experiments have been done to confirm that *n*-hexane or its metabolites are actually transferred to mammalian breast milk following confirmed exposure to *n*-hexane. No quantitative information is available on *n*-hexane metabolites in breast milk.

No information is available on the toxicokinetics of *n*-hexane in children or in young animals compared to adult animals. No information is available as to whether metabolism of *n*-hexane in children differs from that of adults; it is noted that some cytochrome P-450 enzymes are developmentally regulated (Leeder and Kearns 1997). No studies were located comparing metabolism in young and adult animals. The toxicity of *n*-hexane results from biotransformations that yields the active metabolite, 2,5-hexanedione (see Section 3.1.3 for additional information on the metabolism of *n*-hexane).

No information is available on whether biomarkers for exposure or effect of *n*-hexane validated in adults (exhaled *n*-hexane, 2,5-hexanedione in urine) also are valid for children. Interactions of *n*-hexane with other chemicals have not been reported in children but have occurred in adults (Altenkirch et al. 1977). Since interactions in adults are dependent on toxicokinetic parameters, predicting interactions in children requires greater understanding of the metabolism of *n*-hexane in children.

Children, like adults, have background exposures to *n*-hexane resulting from emissions from the combustion of motor fuels or heating oil or other uses of petroleum products. Some products used in the home, such as rubber cement, contain *n*-hexane and could pose exposure risks to children from inhalation. In addition to inhalation exposures through the normal use of such products in poorly ventilated interior areas, children may engage in "glue sniffing" substance-misuse behaviors that could pose serious inhalation exposure risks. Dermal exposures are also possible from hexane-containing household products. For very small children, accidental ingestion of hexane-containing materials is also a potential exposure risk. Other potential exposures are possible from hazardous waste sites. No studies in children have examined potential secondary exposure to *n*-hexane or take-home exposures from materials transferred from the parents' workplace on clothes, skin, hair, tools, or other. Such exposure risks are not expected to be a concern with *n*-hexane because it is highly volatile.

***Pre-existing Conditions, Diseases, and Exposure to Other Substances.*** No population has been identified that is unusually susceptible to toxic effects resulting from *n*-hexane exposure. It is possible that individuals with diminished peripheral nerve function may be more susceptible to *n*-hexane neurotoxicity than the general population. This group would include diabetics, persons with alcohol use disorder, and the aged.

***Genetic Polymorphisms.*** A case-control study examined the association between metabolic gene polymorphisms and risk of peripheral neuropathy in workers exposed to *n*-hexane (Zhang et al. 2006). The cases included 22 offset printing factory workers in China diagnosed with peripheral neuropathy, while the controls included 163 workers in the same shop but with no signs or symptoms. An association was observed between CYP2E1 Dra polymorphism and peripheral neuropathy, with 18% of cases having the CYP2E1 Dra homozygous mutation compared to 3.7% in controls. No other associations were identified.

## 3.3 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as biomarkers of exposure, biomarkers of effect, and biomarkers of susceptibility (NAS/NRC 2006).

The National Report on Human Exposure to Environmental Chemicals provides an ongoing assessment of the exposure of a generalizable sample of the U.S. population to environmental chemicals using biomonitoring (see http://www.cdc.gov/exposurereport/). If available, biomonitoring data for *n*-hexane from this report are discussed in Section 5.6, General Population Exposure.

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 2006). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. Biomarkers of exposure to *n*-hexane are discussed in Section 3.3.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that (depending on magnitude) can be recognized as an established or potential health impairment or disease (NAS/NRC 2006). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effect caused by *n*-hexane are discussed in Section 3.3.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.2, Children and Other Populations that are Unusually Susceptible.

### 3.3.1 Biomarkers of Exposure

*n*-Hexane can be measured in exhaled breath during and following exposure (Mutti et al. 1984; Raymer and Pellizzari 1996; Veulemans et al. 1982). At exposure concentrations of 100–200 ppm, *n*-hexane can be detected in exhaled air for about 12–24 hours. While this is the most direct method to identify and quantify exposure to *n*-hexane, these measurements require specialized equipment and are used mainly in research studies.

Exposure to *n*-hexane results in the production of metabolites by microsomal oxidative enzymes in the liver. In humans, the major metabolite in urine is the neurotoxic metabolite, 2,5-hexanedione. The amount of this metabolite in urine has shown a good correlation with concentrations of *n*-hexane in the workplace air (Mayan et al. 2001, 2002; Mutti et al. 1984). Urinary metabolite concentrations were lowest at the beginning of the shift, highest at the end of the shift, and still elevated the next morning. There was a strong correlation (r=0.967) between TWA *n*-hexane air concentration and end-of-shift 2,5-hexanedione in the urine; end-of-shift samples gave the best estimate of overall exposure. In this study, it was found that about 3 mg 2,5-hexanedione/g creatinine would correspond to about 50 ppm of *n*-hexane in the air.

Since *n*-hexane and its metabolites are cleared from the body within a few days, a test for 2,5-hexanedione in the urine is only a biomarker for recent exposure. Another neurotoxic solvent, 2-hexanone (methyl *n*-butyl ketone), also has 2,5-hexanedione as a metabolite; therefore, exposure to this chemical would have to be ruled out before exposure to *n*-hexane could be confirmed. 2-Hexanone is also a metabolite of *n*-hexane but is present in much smaller quantities in urine after exposure than 2,5-hexanedione (Fedtke and Bolt 1987).

2,5-Hexanedione levels in urine measure the excretion of 2,5-hexanedione (about 10% of the total) and levels of 4,5-dihydroxy-2-hexanone that are converted to 2,5-hexanedione upon acid treatment (acidification of urine samples is routinely performed in order to hydrolyze conjugates that can interfere with analysis). 2,5-Hexanedione has also been detected after acid treatment of urine from individuals not occupationally exposed to *n*-hexane (Fedtke and Bolt 1986; Perbellini et al. 1993); because the urine was acid-treated, the reported value is related to the total of 2,5-hexanedione plus 4,5-dihydroxy-2-hexanone. A reference value for 2,5-hexanedione in acid-treated urine in a non-occupationally exposed Italian population (n=123, 60 males, 63 females) has been determined (Bavazzano et al. 1998). This value, defined as the upper unilateral 95% tolerance interval at 95% confidence, was 0.795 mg 2,5-hexanedione/L in males and 0.627 mg/L for females. It is possible that small amounts of *n*-hexane are produced in the body by lipid peroxidation, as has been demonstrated for *n*-pentane (Filser et al. 1983). Urinary excretion of 2,5-hexanedione was 3–4 mg in 24 hours in workers exposed to approximately 50 ppm *n*-hexane, as compared to 0.3–1.2 mg in 24 hours in unexposed individuals (Perbellini et al. 1993).

Pyrrolidation of proteins appears to be a necessary step in *n*-hexane neurotoxicity, and the targets relevant to toxicity are thought to be neuronal axon proteins (Graham et al. 1995). However, *n*-hexane metabolites can pyrrolidate a variety of proteins at lysine residues, which upon oxidation can become crosslinked.

Pyrrolidated proteins in rat hair have been measured after intraperitoneal administration of 2,5-hexanedione (Johnson et al. 1995). Serial analysis of nose hairs taken during 2,5-hexanedione administration showed a progression with time of the region staining positively for pyrroles. Studies in rats found significant increases in serum, urine, and hair pyrrole adducts in rats exposed to *n*-hexane (Li et al. 2018, 2020b). The levels of serum and urine pyrrole adducts were dose- and exposure duration-related (Yin et al. 2014). This method may eventually be useful as a biomarker for past exposure to *n*-hexane in humans. An *in vitro* species comparison found that pyrrole formation in human serum was approximately 2 times higher than in rats (Yin et al. 2014). A more sensitive and rapid biomarker for 2,5-hexanedione exposure is the crosslinking of erythrocyte spectrin, where the altered migration of crosslinked spectrin is easily observable in polyacrylamide gels (Anthony et al. 1983). Further research is needed to determine whether exposure to *n*-hexane also results in adduct formation and/or crosslinking of spectrin via metabolism to 2,5-hexanedione.

### 3.3.2 Biomarkers of Effect

There are currently no subtle or sensitive biomarkers of effect associated specifically with exposure to *n*-hexane, although this is an active area of research. Electroneuromyographic testing may prove useful in the detection of nerve conduction abnormalities in their early stages before they are accompanied by clinical manifestations. In a study of 15 women who had been exposed to *n*-hexane in a shoe factory, all nerve conduction velocities (motor and sensory) were significantly slowed in exposed workers compared to controls (Mutti et al. 1982b); however, the effects of the *n*-hexane may have been exacerbated by co-exposure to MEK. None of these women had clinical signs of peripheral neuropathy. In a study of workers with relatively high urinary 2,5-hexanedione levels (indicating exposure), clinical exams were negative for neuropathy (Pastore et al. 1994). Sensory and motor nerve conduction velocities and distal latencies were normal in all nerves tested; however, significant decreases were found in sensory nerve action potential amplitude when compared with an unmatched control group. Neither the level of 2,5-hexanedione in urine nor the age of the workers correlated with the changes in amplitude; however, there was a significant correlation between years worked and decreased amplitude. In contrast, no correlation was found with the length of exposure in another study of asymptomatic workers where 14 of 40 showed abnormalities on electrophysiological testing. Levels of 2,5-hexanedione in the urine correlated with a numerical index for abnormalities (Governa et al. 1987).

Pyrrolidation and crosslinking of proteins can be considered biomarkers of either exposure or effect and are discussed in the previous section. As noted in Section 3.3.1, increases in serum, urine, and hair

pyrrole adducts were observed in rats administered *n*-hexane (Li et al. 2018, 2020b); the level of pyrrole adducts, particularly in hair, correlated with a gait abnormality score. The results suggest that hair pyrrole levels may be a biomarker for *n*-hexane-induced peripheral neuropathy. A 24-week study in rats found correlations between gait score and levels of serum and urine pyrrole adducts and an inverse correlation between time to paralysis and serum and urine pyrrole levels (Yin et al. 2014).

## 3.4 INTERACTIONS WITH OTHER CHEMICALS

Because many other chemicals can affect the enzymes responsible for *n*-hexane metabolism, the possibility of interactions is a significant concern. The initial step in *n*-hexane metabolism is oxidation to a hexanol by a cytochrome P-450 isozyme; other chemicals can induce these enzymes, possibly increasing the rate of metabolism to the neurotoxic 2,5-hexanedione or competing with *n*-hexane and its metabolites at enzyme active sites, reducing the rate of metabolism. Interactive effects can be concentration- and/or duration-dependent.

Co-exposure to acetone, MEK, toluene, xylenes, and phenobarbital have been shown to influence the neurotoxicity of *n*-hexane.

***Acetone.*** Evidence for an effect of co-exposure to acetone on *n*-hexane metabolism in humans has been described in a study of workers at a shoe manufacturing facility (Cardona et al. 1996). A statistically significant correlation was found between air levels of acetone and the ratios of free and total 2,5-hexanedione to air levels of *n*-hexane. Multiple regression analysis indicated that at a given level of *n*-hexane exposure, co-exposure to acetone increases the level of free 2,5-hexanedione in urine while reducing the level of 4,5-dihydroxy-2-hexanone.

Oral administration of acetone has been reported to potentiate the neurotoxicity caused by exposure to the *n*-hexane metabolite, 2,5-hexanedione, in rats (Ladefoged et al. 1989, 1994). It is possible that acetone may potentiate *n*-hexane neurotoxicity by decreasing body clearance of 2,5-hexanedione (Ladefoged and Perbellini 1986). Acetone also influences the action of many chemicals by its induction of the cytochrome P-450 isozyme, CYP2E1 (Patten et al. 1986). *n*-Hexane is metabolized by cytochrome P-450 isozymes, so induction by acetone may result in an increased production of the neurotoxic metabolite, 2,5-hexanedione.

***MEK.*** The addition of MEK to paint thinner appears to have been the cause of an outbreak of peripheral neuropathy in Berlin in the 1970s (Altenkirch et al. 1977). The *n*-hexane proportion was reduced from 31 to 16%, but the study authors hypothesized that the addition of MEK had caused a synergistic effect to occur, resulting in *n*-hexane neurotoxicity. The potentiation of *n*-hexane neurotoxicity by co-exposure to MEK may be duration-dependent, as suggested by an experiment in volunteers (van Engelen et al. 1997). Simultaneous exposure to 60 ppm *n*-hexane and either 200 or 300 ppm MEK for 15.5 minutes had no effect on exhaled *n*-hexane concentrations, and actually lowered 2,5-hexanedione serum concentrations about 3-fold. Time to peak 2,5-hexanedione concentrations was approximately doubled (18–30 minutes). These results are consistent, with MEK inhibiting the metabolism of *n*-hexane during a single acute-duration exposure.

In experiments with male Wistar rats, co-exposure to *n*-hexane and MEK for 9 weeks resulted in an earlier onset of signs of neurotoxicity than with *n*-hexane alone (Altenkirch et al. 1982). Similarly, co-exposure to *n*-hexane and MEK over 20 weeks significantly enhanced clinical and electrophysiological signs of neurotoxicity in Wistar rats compared to *n*-hexane alone (Ichihara et al. 1998). This was accompanied by an approximate doubling in urinary 2,5-hexanedione concentrations. Co-exposure to *n*-hexane and MEK also resulted in a greater decrease in motor conduction velocity and mixed nerve conduction velocity in Wistar rats exposed for 20–22 weeks, as compared to rats only exposed to *n*-hexane (Takeuchi et al. 1983). Oral exposure to MEK prior to inhalation exposure to *n*-hexane significantly increased blood levels and sciatic nerve levels of the neurotoxic metabolite, 2,5-hexanedione, and 2,5-dimethylfuran (a metabolite of 2,5-hexanedione) in Fischer 344 rats (Robertson et al. 1989). Levels of 2,5-hexanedione in blood were approximately 10-fold higher than control immediately after *n*-hexane exposure in rats and fell rapidly to approximately 2-fold after 6 hours. In sciatic nerve, increases in 2,5-hexanedione were approximately 6-fold at 2 hours and 3-fold at 4 hours. Although some studies have identified higher levels of *n*-hexane metabolites following co-exposure to MEK (Zhao et al. 1998), other studies have found that serum 2,5-hexanedione levels are decreased with increasing co-exposure to MEK (Shibata et al. 2002; van Engelen et al. 1997; Yu et al. 2002).

The mechanism of action of MEK on the neurotoxicity of *n*-hexane remains unclear.

***Toluene.*** Co-exposure of *n*-hexane and toluene resulted in a reduction in auditory sensitivity in rats compared to controls (Nylen et al. 1994). Exposure to *n*-hexane alone caused a marked decrease in peripheral nerve conduction velocities, while co-exposure with toluene prevented these effects. In a similar study where both peripheral and central nervous system effects were measured in rats co-exposed

to *n*-hexane and toluene (Pryor and Rebert 1992), toluene exposure prevented the peripheral neurotoxicity (decreased grip strength and nerve conduction velocities) caused by exposure to *n*-hexane alone. There was no reciprocal action of *n*-hexane on the motor syndrome (shortened and widened gait and widened landing foot splay) and hearing loss caused by toluene. Brainstem auditory response amplitudes were decreased by *n*-hexane, while co-exposure to toluene did not block this effect.

Co-exposure to approximately equal concentrations of toluene prevented *n*-hexane-induced testicular atrophy in rats (Nylen et al. 1989). The protective effects of toluene on peripheral neuropathy and testicular atrophy caused by *n*-hexane may result from competition for metabolism, resulting in a slowing of *n*-hexane conversion to 2,5-hexanedione. A study evaluating the toxicokinetics of toluene and *n*-hexane exposure found increased blood toluene concentrations and reduced 2,5-hexanedione urinary concentrations (Ali and Tardif 2006).

The interaction of *n*-hexane with toluene and trichloroethylene has also been examined in volunteers (Baelum et al. 1998). Exposure in these experiments was via a gastric feeding tube at controlled rates equivalent to what the study authors stated would be delivered to the liver by inhalation exposure at Danish occupational exposure limits (50 ppm *n*-hexane, 50 ppm toluene, and 30 ppm trichloroethylene). Co-exposure to toluene and trichloroethylene slightly increased the area under the curve (AUC) representing concentration versus time for end exhaled *n*-hexane air concentration, but urinary excretion of 2,5-hexanedione was unchanged. The only statistically significant interaction observed with *n*-hexane was an 18% decrease in the urinary excretion of hippuric acid, a toluene metabolite.

***Xylene.*** Co-exposure to *n*-hexane and xylene resulted in a loss of auditory sensitivity in male Sprague-Dawley rats (Nylen and Hagman 1994) as measured by the auditory brainstem response. Exposure to *n*-hexane or xylene alone caused a slight loss of auditory sensitivity when measured 2 days after the end of exposure. Simultaneous exposure to *n*-hexane and xylene caused a greater and persistent loss of auditory sensitivity that was greater than the sum of effects of exposure to *n*-hexane and xylene separately. These effects were still observed 4 and 10 months after exposure ended. In contrast, combined exposure to *n*-hexane and xylene partially reversed the decreased nerve conduction velocities and action potential amplitudes observed in the group treated with *n*-hexane alone. These effects were persistent from 2 days to 10 months after cessation of exposure. And like toluene, co-exposure to xylene also prevented *n*-hexane-induced testicular atrophy in rats (Nylen et al. 1989). Rats pretreated with xylene and then exposed to *n*-hexane by inhalation exhibited a markedly increased peak serum concentration of 2,5-hexanedione (Toftgard et al. 1983). Peak serum concentrations were approximately

4 μg/mL in control rats and 11 μg/mL in xylene-induced rats. Peaks were reached in 1–2 hours. The half-life for elimination from serum was approximately 1 hour for both pretreated and untreated rats. The high serum 2,5-hexanedione concentrations were correlated with an induction of liver microsomal cytochrome P-450 content (0.56 nmol/mg protein in control rats and 1.03 nmol/mg in xylene-induced rats).

# CHAPTER 4. CHEMICAL AND PHYSICAL INFORMATION

## 4.1 CHEMICAL IDENTITY

*n*-Hexane is a very volatile aliphatic hydrocarbon. It is a constituent in the paraffin fraction of crude oil and natural gas and is also used as an industrial chemical and laboratory solvent. Laboratory-grade *n*-hexane contains approximately 99% *n*-hexane. "Hexane" or "hexanes" is a commercial and industrial product consisting of a mixture of hydrocarbons with six carbon atoms and includes *n*-hexane and its isomers, 2-methylpentane and 3-methylpentane, as well as small amounts of other hydrocarbons (Brugnone et al. 1991). Laboratory and industrial solvents such as "hexane" and petroleum ether contain *n*-hexane from <0.1% to as much as 33% (Creaser et al. 1983). Information regarding the chemical identity of *n*-hexane is presented in Table 4-1.

**Table 4-1. Chemical Identity of *n*-Hexane**

| Characteristic | Information | Reference |
|---|---|---|
| Chemical name | *n*-Hexane | Budavari et al. 1989 |
| Synonym(s) and registered trade name(s) | Hexane<br>Hexyl hydride<br>Skellysolve B<br>Gettysolve-B | IUPAC name<br>NFPA 1991<br>NLM 2023<br>NLM 2023 |
| Chemical formula | $C_6H_{14}$ | Lide 2005 |
| SMILES | CCCCCC | NLM 2023 |
| Chemical structure | $CH_3CH_2CH_2CH_2CH_2CH_3$ | |
| CAS Registry Number | 110-54-3 | NLM 2023 |

CAS = Chemical Abstracts Service; SMILES = simplified molecular-input line-entry system

Many commercial grades of *n*-hexane contain appreciable amounts of other hydrocarbons in addition to *n*-hexane (for instance, toluene or such solvents as acetone or MEK; see below for other chemicals in such mixtures). Various types of commercial grades of *n*-hexane are available, and the constituents besides *n*-hexane are usually an intentional part of the process for preparing these commercial mixtures. Where intended for specialized oil extraction or laboratory uses, the purity of the *n*-hexane products may be in the range of 95–99% *n*-hexane. For a variety of uses where purity is not as important, commercial *n*-hexane mixtures (in the range of 20–80% of *n*-hexane) may contain small amounts of chemicals such as acetone, MEK, dichloromethane, 2- or 3-methylpentane, 2,3-dimethylbutane, cyclohexane, methyl cyclopentane, trichloroethylene, aromatics such as toluene, and other types of petroleum hydrocarbons (Jørgensen and Cohr 1981; Takeuchi et al. 1993; WHO 1991). In commercial grades of *n*-hexane, some

of the constituents are purposefully added as denaturants, often to discourage the misuse of the chemical to induce "highs" through sniffing or inhalation (Altenkirch et al. 1982).

## 4.2 PHYSICAL AND CHEMICAL PROPERTIES

The National Fire Protection Association (NFPA) has assigned *n*-hexane a health hazard identification code of 1 (slight) and flammability code of 3 (serious) (NFPA 1991). *n*-Hexane is flammable and may be ignited by heat, sparks, and flames. Flammable vapor may spread away from a spill. The vapor may be an explosion hazard. *n*-Hexane can react vigorously with oxidizing materials such as liquid chlorine, concentrated oxygen, and sodium hypochlorite. *n*-Hexane will attack some forms of plastics, rubber, and coatings. Virtually all *n*-hexane is obtained from petroleum mixtures through controlled fractional distillation and other refinery-based processes (Speight 2006). The presence of many types of hydrocarbon impurities in many commercial grades of *n*-hexane, combined with the intentional denaturing of *n*-hexane preparations to discourage substance misuse, make it difficult to establish odor thresholds for many products containing *n*-hexane. Information regarding the physical and chemical properties of hexane is presented in Table 4-2.

**Table 4-2. Physical and Chemical Properties of *n*-Hexane**

| Property | Information | Reference |
|---|---|---|
| Molecular weight | 86.18 | Lide 2005 |
| Color | Colorless | Budavari et al. 1989 |
| Physical state | Liquid | Budavari et al. 1989 |
| Melting point | -95°C | Lide 2005 |
| Boiling point | 69°C | Lide 2005 |
| Density at 20°C | 0.6606 g/cm$^3$ | Lide 2005 |
| Odor | Faint, peculiar odor | Budavari et al. 1989 |
| Odor threshold: | | |
| Water | 0.0064 mg/L | Amoore and Hautala 1983 |
| Air | 130 ppm | Amoore and Hautala 1983 |
| Taste threshold | No data | |
| Solubility: | | |
| Water | Insoluble<br>9.5 mg/L | Budavari et al. 1989<br>NLM 2023 |
| Organic solvent(s) | Miscible with alcohol, chloroform, ether | Budavari et al. 1989 |
| Partition coefficients: | | |
| Log $K_{ow}$ | 3.90, 2.90 | Coates et al. 1985;<br>Hansch et al. 1995 |

## Table 4-2. Physical and Chemical Properties of *n*-Hexane

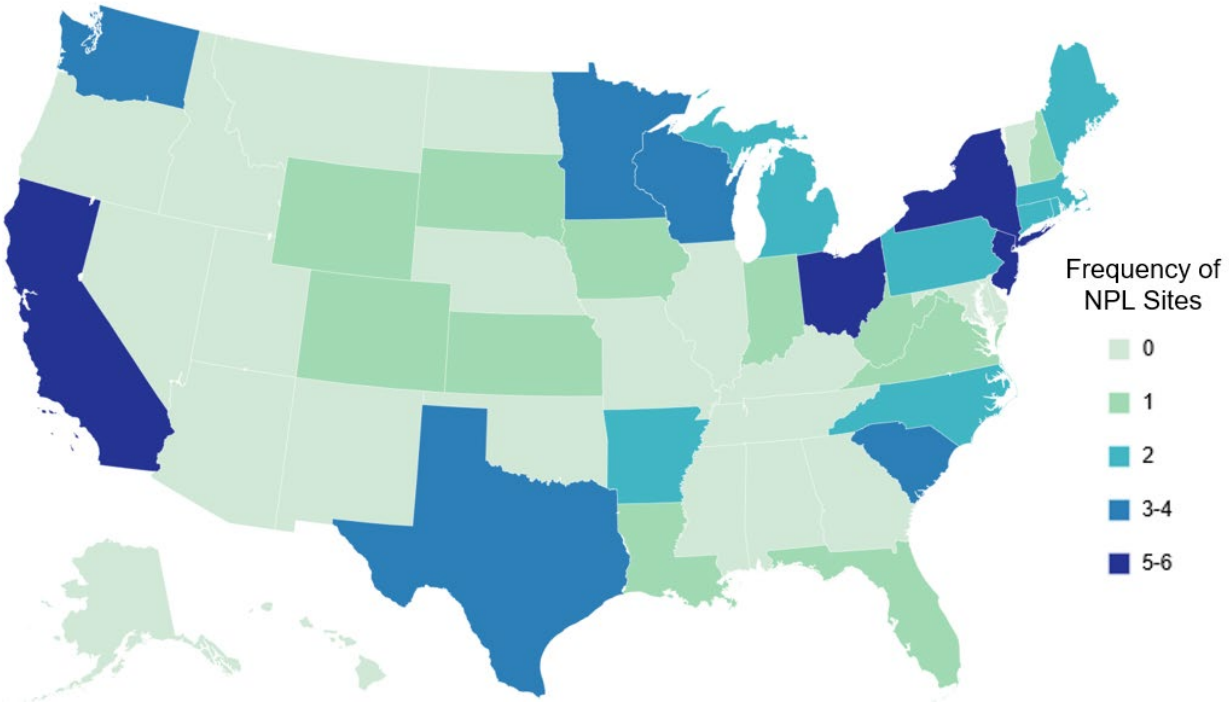
| | | |
|---|---|---|
| Vapor pressure | 138 mmHg at 24°C | Chiou et al. 1988 |
| Henry's law constant at 25°C | 1.003 atm-$m^3$/mole<br>1.3 atm-$m^3$/mole | Ashworth et al. 1988<br>Zhu et al. 2004 |
| Autoignition temperature | 225°C | NFPA 1991 |
| Flashpoint | -22°C | NFPA 1991 |
| Flammability limits | 1.1–7.5% | NFPA 1991 |
| Explosive limits | 1.1–7.5% | WHO 1991 |
| Conversion factors | 1 ppm = 3.52 mg/$m^3$<br>1 mg/$m^3$ = 0.284 ppm | WHO 1991 |

# CHAPTER 5. POTENTIAL FOR HUMAN EXPOSURE

## 5.1 OVERVIEW

*n*-Hexane has been identified in at least 67 of the 1,868 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL) (ATSDR 2022a). However, the number of sites in which *n*-hexane has been evaluated is not known. The number of sites in each state is shown in Figure 5-1.

**Figure 5-1. Number of NPL Sites with *n*-Hexane Contamination**




Source: ATSDR 2022a

- *n*-Hexane is both an anthropogenic and naturally occurring chemical. Anthropogenic hexane originates from refining crude oil and naturally occurring hexane is produced by plants, forest fires, and volcanoes.

- Vapors and emissions from refined petroleum products are the primary sources of *n*-hexane exposure to the general population.

- Since *n*-hexane has a very high vapor pressure and Henry's law volatility constant, it is expected to exist primarily in the vapor phase. In the atmosphere, the main degradation pathway will be through the reaction of free radicals such as hydroxyl radicals.

- If *n*-hexane is introduced into deeper sediments or groundwater, hexane may be persistent due to limited biodegradation under anoxic conditions.

*n*-Hexane is a highly volatile component of the paraffin (also the alkane or aliphatic) fraction of crude oil and natural gas, and it is a constituent of heating and motor fuels refined from petroleum. Exposure from contact with vapors or emissions from these refined petroleum products is the most widespread form of low-level exposure for the general population. Most *n*-hexane in these fuels is oxidized as part of the combustion process to provide heat or drive internal combustion engines. Small amounts of *n*-hexane, along with other petroleum compounds, volatilize to the atmosphere during handling, storage in fuel tanks, or through incomplete combustion. Research suggests that certain fungi may be able to produce *n*-hexane (Ahearn et al. 1996). These fungi may be common in older buildings and in some parts of the country, and they may provide exposures from previously unsuspected indoor sources. *n*-Hexane is also produced as a relatively pure product for a number of specialized end uses, primarily as a solvent especially in glues and adhesives. Especially in urban areas, *n*-hexane may be a typical component of nonpoint source runoff when rainfall washes hydrocarbons deposited on roads and other surfaces into surface waters. Spills of refined petroleum products or of commercial *n*-hexane products may introduce *n*-hexane into soils or surface waters. Around urbanized areas, spill sites, refineries, tank storage facilities, underground storage tanks (e.g., at gas stations), or waste sites, can be sources of *n*-hexane subsequently transported into sediments or groundwater.

Once introduced into deeper sediments or groundwater, *n*-hexane may be persistent since its degradation by chemical hydrolysis is negligible and opportunities for biodegradation may be limited under anoxic conditions or where nutrients such as nitrogen or phosphorus are in limited supply. In the atmosphere, the main degradation pathways involve destruction through the action of free radicals such as hydroxyl radicals.

## 5.2 PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

### 5.2.1 Production

Normal hexane (*n*-hexane) is both an anthropogenic and naturally occurring chemical. *n*-Hexane is a minor constituent of crude oil and natural gas. Its inclusion in a variety of petroleum products is a consequence of refining operations that separate hydrocarbons within specific ranges of boiling points for such uses as heating oils or automotive fuels. Virtually all *n*-hexane is obtained from petroleum mixtures

through controlled fractional distillation and other refinery-based processes (Speight 2006). *n*-Hexane may also be a metabolic byproduct from certain types of fungi (Ahearn et al. 1996).

Table 5-1 summarizes information on companies that reported the production, import, or use of *n*-hexane for the Toxics Release Inventory (TRI) in 2021 (TRI21 2023). TRI data should be used with caution since only certain types of industrial facilities are required to report. This is not an exhaustive list.

**Table 5-1. Facilities that Produce, Process, or Use *n*-Hexane**

| State[a] | Number of facilities | Minimum amount on site in pounds[b] | Maximum amount on site in pounds[b] | Activities and uses[c] |
|---|---|---|---|---|
| AK | 23 | 100 | 49,999,999 | 1, 3, 4, 5, 7, 8, 9, 12, 14 |
| AL | 13 | 0 | 9,999,999 | 1, 2, 3, 4, 5, 7, 8, 9, 10, 12, 13, 14 |
| AR | 11 | 0 | 9,999,999 | 1, 2, 3, 5, 6, 9, 10, 12, 13, 14 |
| AZ | 17 | 100 | 9,999,999 | 1, 5, 9, 12 |
| CA | 63 | 0 | 49,999,999 | 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 |
| CO | 16 | 1,000 | 499,999,999 | 1, 2, 5, 7, 9, 10, 12, 13, 14 |
| CT | 6 | 1,000 | 9,999,999 | 2, 3, 4, 7, 9, 10, 12 |
| DE | 4 | 0 | 999,999 | 1, 2, 3, 5, 9, 10, 11, 12 |
| FL | 24 | 1,000 | 49,999,999 | 1, 5, 7, 9, 10, 11, 12, 14 |
| GA | 14 | 100 | 9,999,999 | 1, 5, 7, 8, 9, 10, 12, 14 |
| GU | 5 | 1,000 | 999,999 | 1, 5, 7, 9, 12 |
| HI | 8 | 100 | 9,999,999 | 1, 2, 3, 4, 5, 6, 9, 12, 13, 14 |
| IA | 59 | 0 | 9,999,999 | 1, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 |
| ID | 4 | 1,000 | 999,999 | 9 |
| IL | 53 | 0 | 99,999,999 | 1, 2, 3, 4, 5, 7, 8, 9, 10, 11, 12, 13, 14 |
| IN | 49 | 0 | 49,999,999 | 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 |
| KS | 29 | 0 | 999,999,999 | 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 |
| KY | 17 | 1,000 | 49,999,999 | 1, 3, 5, 6, 7, 8, 9, 10, 11, 13, 14 |
| LA | 56 | 0 | 10,000,000,000 | 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 |
| MA | 14 | 1,000 | 9,999,999 | 2, 4, 7, 9, 10, 11, 12 |
| MD | 5 | 100,000 | 9,999,999 | 9, 10, 12 |
| ME | 8 | 0 | 9,999,999 | 1, 2, 3, 4, 5, 7, 9, 12 |
| MI | 42 | 0 | 499,999,999 | 1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 |
| MN | 30 | 100 | 49,999,999 | 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 |
| MO | 29 | 0 | 9,999,999 | 1, 5, 7, 8, 9, 10, 11, 12, 13, 14 |
| MP | 2 | 100,000 | 999,999 | 1, 5, 7, 9 |
| MS | 15 | 0 | 99,999,999 | 1, 2, 3, 4, 5, 6, 8, 9, 10, 13, 14 |
| MT | 8 | 100,000 | 49,999,999 | 1, 2, 3, 4, 5, 6, 9, 12, 13, 14 |
| NC | 24 | 0 | 9,999,999 | 1, 5, 7, 9, 10, 12 |

**Table 5-1. Facilities that Produce, Process, or Use *n*-Hexane**

| State[a] | Number of facilities | Minimum amount on site in pounds[b] | Maximum amount on site in pounds[b] | Activities and uses[c] |
|---|---|---|---|---|
| ND | 19 | 0 | 49,999,999 | 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14 |
| NE | 28 | 100 | 9,999,999 | 1, 2, 3, 5, 7, 8, 9, 10, 12, 13, 14 |
| NH | 6 | 10,000 | 9,999,999 | 7, 9, 12 |
| NJ | 21 | 1,000 | 49,999,999 | 1, 2, 3, 4, 5, 6, 7, 9, 10, 12, 14 |
| NM | 7 | 10,000 | 9,999,999 | 1, 3, 5, 6, 9, 12 |
| NV | 10 | 100 | 9,999,999 | 1, 5, 7, 8, 9, 12 |
| NY | 23 | 0 | 49,999,999 | 1, 2, 4, 5, 7, 8, 9, 10, 12 |
| OH | 57 | 0 | 49,999,999 | 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 |
| OK | 11 | 10,000 | 49,999,999 | 1, 2, 3, 4, 5, 6, 7, 9, 12, 13, 14 |
| OR | 3 | 100,000 | 999,999 | 1, 5, 7, 9, 14 |
| PA | 32 | 0 | 49,999,999 | 1, 2, 5, 6, 7, 8, 9, 10, 12, 13, 14 |
| PR | 11 | 0 | 49,999,999 | 1, 2, 3, 4, 5, 7, 8, 9, 12, 13, 14 |
| RI | 4 | 10,000 | 9,999,999 | 1, 5, 7, 9, 12 |
| SC | 9 | 100 | 999,999 | 1, 5, 7, 10, 12, 13, 14 |
| SD | 19 | 1,000 | 999,999 | 1, 5, 7, 8, 9, 10, 12, 13, 14 |
| TN | 22 | 1,000 | 9,999,999 | 1, 2, 3, 5, 7, 8, 9, 10, 11, 12, 13 |
| TX | 178 | 0 | 10,000,000,000 | 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 |
| UT | 14 | 10,000 | 49,999,999 | 1, 2, 3, 4, 5, 6, 7, 8, 9, 12, 14 |
| VA | 35 | 0 | 9,999,999 | 1, 5, 7, 9, 10, 11, 12 |
| VI | 4 | 10,000 | 49,999,999 | 1, 5, 7, 9, 12, 14 |
| WA | 14 | 10,000 | 10,000,000,000 | 1, 2, 3, 4, 5, 6, 7, 9, 12, 13, 14 |
| WI | 22 | 0 | 9,999,999 | 1, 5, 7, 8, 9, 10, 11, 12, 13, 14 |
| WV | 9 | 1,000 | 49,999,999 | 1, 3, 5, 6, 7, 8, 9, 11, 12, 13 |
| WY | 6 | 10,000 | 9,999,999 | 1, 2, 3, 4, 5, 6, 8, 9, 12, 13, 14 |

[a]Post office state abbreviations used.
[b]Amounts on site reported by facilities in each state.
[c]Activities/uses:

1. Produce
2. Import
3. Used Processing
4. Sale/Distribution
5. Byproduct
6. Reactant
7. Formulation Component
8. Article Component
9. Repackaging
10. Chemical Processing Aid
11. Manufacture Aid
12. Ancillary
13. Manufacture Impurity
14. Process Impurity

Source: TRI21 2023 (Data are from 2021)

### 5.2.2 Import/Export

No current information concerning the import or export of *n*-hexane in the United States was located in the literature.

### 5.2.3 Use

A number of *n*-hexane uses have been identified. Its primary use is as an edible oil extractant for seed crops. Other uses include special-purpose solvent and cleaning agent and as a component of adhesives. Pure *n*-hexane is also used as a laboratory extractant and for instrument calibration.

*n*-Hexane is used mainly as an edible-oil extractant for a variety of seed crops such as soybeans, cottonseed, rape seed (canola), flax (linseed), mustard seed, peanuts, safflower seed, and corn germ, which are then processed into foods for humans or livestock (Bhagya and Srinivas 1992; Conkerton et al. 1995; Domínguez et al. 1995; Kim and Yoon 1990; Lawson 1995; Srinivas et al. 1992; Wanasundara and Shahidi 1994). While other petroleum-derived solvents (e.g., pentane) or other organic solvents (e.g., chloroform, methanol, ethanol, or ammonia-alcohol mixtures) are currently being studied or are used for certain processes, *n*-hexane has been widely used since the early part of this century, especially with soybeans, cottonseed, and linseed (Conkerton et al. 1995). Part of *n*-hexane's appeal relates to aesthetic properties such as preserving the colors of the original plant materials. Different extractant mixtures can also have significant effects on the levels of materials that can cause bitter tastes (e.g., tannins) and on the degree to which certain flatulence-causing sugars are removed. While other solvents could be used in the initial oil extraction phases, several decades of experience in combining the oil-extraction steps with other procedures to preserve desirable colors and eliminate unwanted tastes or other undesirable food properties have worked to maintain a heavy reliance on *n*-hexane for edible-oil extraction (Lawson 1995). After extraction, the *n*-hexane is distilled off and little to none of *n*-hexane remains in the final extracted oil.

*n*-Hexane has other major uses as a special-purpose solvent and cleaning agent (degreaser) in such industries as textile manufacture, shoe and leather making, and furniture manufacturing (Jørgensen and Cohr 1981). It is used in the printing industry as a cleaner and as a component of some inks (EPA 1996; Wadden et al. 1995). Facilities that use rotogravure printers (facilities that produce catalogues, magazines, "glossy" newspaper inserts, or telephone directories) or similar rotogravure or flexographic technologies (for labels, gift wrap, metal foils, flexible packaging materials, and some floor coverings) also use *n*-hexane (EPA 1996).

While not used in most glues or epoxy cements (Rastogi 1993), *n*-hexane is the solvent used in "rubber" cement (also known as gum adhesive) widely used in schools and libraries and by artists (McCann 1992). Various glues, adhesives, and leather-dressing preparations, especially those used in assembling shoes,

may contain *n*-hexane (Cardona et al. 1993; Periago et al. 1993; Takeuchi et al. 1993). In bookbinding and leather working, *n*-hexane, often mixed with other hydrocarbon solvents, is used as a carrier for cedar oil, beeswax, or lanolin dressings (Jørgensen and Cohr 1981; Roberts and Etherington 1996).

Adhesives, cleaners, or lacquers containing *n*-hexane are also used to prepare the veneers used in making many types of furniture or ornamental boxes (Graham et al. 1995). Additional uses for adhesives containing *n*-hexane include holding the ends of tin cans during the sealing process (Bachmann et al. 1993) and holding strings or yarns together to create the cores of balls used in several sports (Huang et al. 1991). Certain types of tapes, bandages, and dressings used in hospitals also use adhesives containing *n*-hexane (Jørgensen and Cohr 1981).

In the petrochemical industry, lighter alkane fractions, including *n*-hexane, may be used as feedstocks in the manufacture of polyethylene or polypropylene (Jørgensen and Cohr 1981). In the manufacture of truck and automobile tires, *n*-hexane is a solvent in mixtures (called “thinners”) used to adjust the viscosity of the rubber while it is being polymerized and formed into tires (Jørgensen and Cohr 1981; Van Ert et al. 1980).

Furthermore, *n*-hexane may be used as a carrier or aerosol (propellant) agent in some perfumes (Bouhamra 1995; Jørgensen and Cohr 1981). It is used in the pharmaceutical industry to help shape pills and tablets, which are then dried to vent off the *n*-hexane before packaging (Jørgensen and Cohr 1981). *n*-Hexane is also used in some typeover correction (“white-out”) fluids (Ong et al. 1993). It has been used in many types of non-mercury thermometers, especially for thermometers used in low temperature ranges (EPA 2000). New roofing materials using rubber or plastic films and membranes held together by adhesives, sealants, or hardening agents may contain *n*-hexane (Herbert et al. 1995).

Pure *n*-hexane is widely used in laboratories as an extractant for nonpolar compounds and in calibrating instruments for analyses of VOCs or total petroleum hydrocarbons (TPH) (Kanatharana et al. 1993). Since such analyses may require very high levels of purity, laboratories sometimes carry out their own fractional distillation or other pretreatment-purification procedures to remove petroleum hydrocarbon impurities found in commercially available grades of *n*-hexane (Kanatharana et al. 1993).

Finally, *n*-hexane may be a component of many types of commercial preparations or in mixtures produced in small batches onsite such as paint thinners, general-purpose solvents, degreasing agents, or cleaners. For instance, until the 1970s, naphtha, a mixture with a high *n*-hexane content, was widely used as a dry-

cleaning agent. Since the early 1900s, construction workers, metal workers, janitors, furniture workers, motor-vehicle mechanics, and print-shop workers have used these general-purpose mixtures. Such mixtures have also been used extensively for home repair and hobby projects. These mixtures have wide variations in their compositions but often contain up to 20% *n*-hexane even when the main components are other petroleum alkane fractions (e.g., kerosene), aromatic hydrocarbons (e.g., toluene), chlorinated hydrocarbon solvents, or other organic liquids (Farmer 1996; Veulemans et al. 1987).

### 5.2.4 Disposal

Limited information was located in the literature concerning the disposal of *n*-hexane. Since it is highly flammable, *n*-hexane, or mixtures with significant amounts of *n*-hexane, are regulated under the Resource Conservation and Recovery Act (RCRA) disposal procedures covering D001 wastes for ignitable wastes and petroleum solvents. For printing operations, it could also be considered under the K086 ink sludges designation (EPA 1996). *n*-Hexane is listed as a toxic substance under Section 313 of the Emergency Planning and Community Right to Know Act (EPCRA) under Title III of the Superfund Amendments and Reauthorization Act (SARA) (EPA 1993). It is also listed as a Hazardous Air Pollutant (HAP) in the Clean Air Act Amendments of 1990 (EPA 2000). Disposal of wastes containing *n*-hexane is controlled by a number of federal regulations.

## 5.3 RELEASES TO THE ENVIRONMENT

The Toxics Release Inventory (TRI) data should be used with caution because only certain types of facilities are required to report (EPA 2022b). This is not an exhaustive list. Manufacturing and processing facilities are required to report information to the TRI only if they employ ≥10 full-time employees; if their facility's North American Industry Classification System (NAICS) codes is covered under EPCRA Section 313 or is a federal facility; and if their facility manufactures (defined to include importing) or processes any TRI chemical in excess of 25,000 pounds, or otherwise uses any TRI chemical in excess of 10,000 pounds, in a calendar year (EPA 2022b).

Since *n*-hexane is a component of refined petroleum products, there is considerable potential for releases to environmental media through the use of heating and motor fuels. Table 5-2 summarizes the uses of petroleum products according to major demand categories (e.g., "transportation") and displays estimated use levels in barrels (and liter equivalents) and by percentages for the various end-use demands for specific fuel types (e.g., kerosene or fuel oil). While *n*-hexane can be a minor constituent (<1% by

weight) of several of these petroleum products, its physical properties as a light alkane make it most suitable for use in gasoline. Approximately 98% of the demand for gasoline involves transportation, mainly cars and trucks. The composition of gasolines has changed over the years, mainly in an effort to maintain the so-called octane ratings of the fuels. Since the 1980s, the growing use of nonleaded gasolines has led to a growing percentage of high-octane benzene and toluene in gasoline blends. For modern gasoline mixtures, the total percentage by weight of the *n*-hexane component is approximately 3% (Brugnone et al. 1991; Heath et al. 1993; Stelljes and Watkin 1993). Of the 2,608 million barrels of motor gasoline consumed for transportation in 1992 (designated "transportation" in Table 5-2), about 27,300 million pounds (12,409 million kg) are from the *n*-hexane fraction (PennWell 1994; Stevens 1988). This figure is about 76 times the 358 million pounds (143 million kg) of commercial *n*-hexane produced annually in the 1970s (Marks et al. 1980). Most gasoline, along with its *n*-hexane fraction, is consumed during its combustion in motor cars and other engines. However, gasoline use results in a variety of emission losses from refueling, evaporation while gasoline is stored in fuel tanks or ignition systems, and exhaust releases when there is incomplete combustion of fuels (EPA 1994a). EPA only tracks trends in total hydrocarbon or total VOC emissions, so that quantitative estimates for the *n*-hexane released from automobiles and trucks are not available. Assuming that only 1% of the *n*-hexane of motor fuels is released to environmental media, such releases could be on the same order of magnitude as the total amount of relatively pure *n*-hexane associated with the major end-uses described in Section 5.2.3.

In addition to emissions to the atmosphere, releases from heating and motor fuel uses to other environmental media are possible. For example, soil and water may contain *n*-hexane as a result of leaks and spills at refineries, pipelines, large tank batteries (or tank "farms"), above- and below-ground storage tanks, tanker trucks, and railroad tanker cars. Additionally, minor environmental releases could occur at garages or around homes and workplaces. Crude oil spills also result in the release of *n*-hexane to the air or other environmental media.

**Table 5-2. Demand Patterns for Major Petroleum Products (1992)[a]**

| Product | Residential | Commercial | Industrial | Transportation | Electric utilities | Total |
|---|---|---|---|---|---|---|
| Motor gasoline[b] | 0 (0.0)<br>0 | 15 (<1.0)<br>2,385 | 37 (1.4)<br>5,883 | 2,608 (98.0)<br>414,672 | 0 (0.0)<br>0 | 2,660 |
| Kerosene | 11 (73.3)<br>1,749 | 2 (13.3)<br>318 | 2 (13.3)<br>318 | 0 (0.0)<br>0 | 0 (0.0)<br>0 | 15 |
| Distillate fuel oil | 148 (13.6)<br>23,532 | 80 (7.3<br>12,720 | 196 (18.0)<br>31,164 | 654 (60.0)<br>103,986 | 12 (1.1)<br>1,908 | 1,090 |
| Residual fuel oil | 0 (0.0)<br>0 | 30 (7.5)<br>4,770 | 62 (15.5)<br>9,858 | 172 (42.9)<br>27,348 | 136 (33.9)<br>21,942 | 400 |

**Table 5-2. Demand Patterns for Major Petroleum Products (1992)[a]**

| Product | Residential | Commercial | Industrial | Transportation | Electric utilities | Total |
|---|---|---|---|---|---|---|
| Liquid petroleum gas and ethanes | 106 (16.5) | 19 (3.0) | 513 (79.9) | 5 (0.8) | 0 (0.0) | 642 |

[a]Expressed in millions of barrels (percent of total sectoral demand; 1 barrel = 42 U.S. gallons = 159 L.
[b]Typically contains >1% *n*-hexane.

Source: PennWell 1994

In addition to releases associated with the ordinary use of refined petroleum products as a fuel, ongoing research (Ahearn et al. 1996) suggests that a variety of fungi found in ducts and insulation materials in homes or office buildings are capable of releasing gases that include *n*-hexane. There is also evidence that marine phytoplankton produce a variety of non-methane hydrocarbons, including small amounts of *n*-hexane, from the metabolism of polyunsaturated lipids in dissolved organic materials (McKay et al. 1996). Very small amounts of *n*-hexane may also be among the biogenic emissions from different types of terrestrial vegetation (Isidorov et al. 1985; Winer et al. 1992).

When buildings have poor ventilation properties, commonly referred to as “sick-building syndrome” (Sundell 1996), the indoor air releases of *n*-hexane may sometimes be sufficient to pose public health concerns. Lastly, *n*-hexane is also among the various off-gassing constituents encountered at sanitary landfills (Brosseau and Heitz 1994; O’Leary and Walsh 1995).

### 5.3.1 Air

Most releases of *n*-hexane to environmental media are to air; as an example, see Table 5-3. Based on its Henry’s law constant, *n*-hexane discharged to water will volatilize rapidly; however, the amount volatilized will vary depending on several factors including the temperature, turbulence, and depth of the receiving water. *n*-Hexane spilled onto surface soils will also volatilize to the air. In addition to releases from commercial applications as edible oil extraction, the other major sources of atmospheric releases would be from emissions related to the *n*-hexane contained in heating and motor fuels.

Estimated releases of 37,567,136 pounds (~17,040 metric tons) of *n*-hexane to the atmosphere from 1,189 domestic manufacturing and processing facilities in 2021, accounted for about 99% of the estimated total environmental releases from facilities required to report to the TRI (TRI21 2023). These releases are summarized in Table 5-3.

**Table 5-3. Releases to the Environment from Facilities that Produce, Process, or Use *n*-Hexane[a]**

| | | Reported amounts released in pounds per year[b] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Total release | | |
| State[c] | RF[d] | Air[e] | Water[f] | UI[g] | Land[h] | Other[i] | On-site[j] | Off-site[k] | On- and off-site |
| AL | 13 | 1,146,286 | 397 | 0 | 0 | 93 | 1,146,286 | 489 | 1,146,775 |
| AK | 23 | 23,819 | 0 | 0 | 40 | 1,205 | 23,835 | 1,229 | 25,064 |
| AZ | 17 | 4,054 | 0 | 0 | 5 | 4 | 4,059 | 4 | 4,063 |
| AR | 11 | 654,496 | 394 | 0 | 21 | 730 | 654,505 | 1,136 | 655,641 |
| CA | 58 | 126,281 | 174 | 0 | 7,401 | 1,196 | 126,446 | 8,605 | 135,051 |
| CO | 16 | 55,327 | 0 | 0 | 0 | 688 | 55,327 | 688 | 56,015 |
| CT | 6 | 4,200 | 3 | 0 | 0 | 0 | 4,203 | 0 | 4,203 |
| DE | 4 | 20,146 | 5 | 0 | 0 | 114 | 20,151 | 114 | 20,265 |
| FL | 24 | 70,104 | 1 | 0 | 1 | 1 | 70,105 | 2 | 70,107 |
| GA | 14 | 1,205,395 | 6,513 | 0 | 0 | 62 | 1,205,396 | 6,574 | 1,211,970 |
| HI | 8 | 52,385 | 0 | 0 | 0 | 0 | 52,385 | 0 | 52,385 |
| ID | 4 | 1,000 | 0 | 0 | 1 | 0 | 1,000 | 1 | 1,001 |
| IL | 50 | 4,667,231 | 4,555 | 0 | 24 | 1,744 | 4,667,324 | 6,230 | 4,673,555 |
| IN | 48 | 3,216,909 | 1,089 | 0 | 3,726 | 322 | 3,217,501 | 4,545 | 3,222,046 |
| IA | 59 | 3,918,059 | 22,772 | 0 | 0 | 250 | 3,918,059 | 23,022 | 3,941,081 |
| KS | 29 | 885,785 | 131 | 13 | 2,211 | 5 | 887,280 | 865 | 888,145 |
| KY | 17 | 523,555 | 16 | 0 | 188 | 0 | 523,565 | 194 | 523,759 |
| LA | 51 | 2,668,642 | 584 | 251 | 33,932 | 14 | 2,670,119 | 33,304 | 2,703,423 |
| ME | 8 | 3,044 | 40 | 0 | 541 | 70 | 3,044 | 651 | 3,695 |
| MD | 5 | 350,355 | 69 | 0 | 9 | 0 | 350,431 | 1 | 350,432 |
| MA | 14 | 27,724 | 2 | 0 | 6 | 33,821 | 27,726 | 33,827 | 61,553 |
| MI | 40 | 425,002 | 24 | 0 | 0 | 1 | 425,002 | 25 | 425,027 |
| MN | 30 | 2,491,776 | 514 | 0 | 0 | 0 | 2,491,781 | 509 | 2,492,291 |
| MS | 15 | 1,410,951 | 29 | 0 | 0 | 0 | 1,410,980 | 0 | 1,410,980 |
| MO | 29 | 1,490,095 | 244 | 0 | 0 | 19,928 | 1,490,095 | 20,172 | 1,510,267 |
| MT | 8 | 74,255 | 9 | 0 | 81 | 10 | 74,344 | 11 | 74,355 |
| NE | 27 | 1,929,854 | 312 | 0 | 619 | 0 | 1,929,854 | 931 | 1,930,785 |
| NV | 10 | 4,080 | 0 | 0 | 0 | 8 | 4,080 | 8 | 4,088 |
| NH | 6 | 330 | 2 | 0 | 0 | 0 | 332 | 0 | 332 |
| NJ | 21 | 30,326 | 38 | 0 | 0 | 109 | 30,364 | 109 | 30,473 |
| NM | 7 | 48,911 | 0 | 0 | 0 | 0 | 48,911 | 0 | 48,911 |
| NY | 23 | 18,191 | 7 | 0 | 12 | 66 | 18,199 | 77 | 18,276 |
| NC | 23 | 946,661 | 2,254 | 0 | 2 | 1,337 | 946,684 | 3,571 | 950,255 |
| ND | 18 | 831,216 | 1,576 | 0 | 1 | 0 | 831,217 | 1,576 | 832,793 |

**Table 5-3. Releases to the Environment from Facilities that Produce, Process, or Use *n*-Hexane[a]**

| | | Reported amounts released in pounds per year[b] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Total release | | |
| State[c] | RF[d] | Air[e] | Water[f] | UI[g] | Land[h] | Other[i] | On-site[j] | Off-site[k] | On- and off-site |
| OH | 56 | 1,650,522 | 6,466 | 0 | 10,939 | 21,082 | 1,650,570 | 38,440 | 1,689,010 |
| OK | 10 | 169,965 | 10 | 0 | 250 | 0 | 169,975 | 250 | 170,225 |
| OR | 3 | 2,235 | 4 | 0 | 0 | 0 | 2,236 | 3 | 2,239 |
| PA | 32 | 250,188 | 332 | 0 | 10 | 250 | 250,436 | 344 | 250,780 |
| RI | 4 | 2,225 | 4 | 0 | 0 | 877 | 2,229 | 877 | 3,106 |
| SC | 9 | 220,466 | 19 | 0 | 224 | 2,993 | 220,683 | 3,019 | 223,702 |
| SD | 19 | 1,268,648 | 1,469 | 0 | 1 | 0 | 1,268,649 | 1,469 | 1,270,119 |
| TN | 22 | 496,442 | 434 | 0 | 0 | 250 | 496,443 | 683 | 497,126 |
| TX | 178 | 3,294,681 | 1,076 | 217,460 | 47,806 | 15,559 | 3,336,814 | 239,768 | 3,576,583 |
| UT | 13 | 107,807 | 422 | 0 | 31 | 85 | 107,838 | 507 | 108,344 |
| VA | 34 | 480,205 | 52 | 0 | 0 | 368 | 480,205 | 420 | 480,624 |
| WA | 14 | 66,188 | 600 | 0 | 38 | 0 | 66,306 | 521 | 66,827 |
| WV | 9 | 5,683 | 0 | 0 | 33 | 1 | 5,716 | 1 | 5,716 |
| WI | 22 | 35,844 | 0 | 0 | 0 | 0 | 35,844 | 0 | 35,844 |
| WY | 6 | 17,271 | 0 | 0 | 622 | 0 | 17,888 | 5 | 17,893 |
| GU | 5 | 3,268 | 0 | 0 | 0 | 0 | 3,268 | 0 | 3,268 |
| MP | 2 | 546 | 0 | 0 | 0 | 0 | 546 | 0 | 546 |
| PR | 11 | 120,627 | 0 | 0 | 0 | 0 | 120,627 | 0 | 120,627 |
| VI | 4 | 47,878 | 173 | 0 | 42 | 0 | 48,093 | 0 | 48,093 |
| Total | 1,189 | 37,567,136 | 52,816 | 217,724 | 108,817 | 103,242 | 37,614,958 | 434,777 | 38,049,735 |

[a]The TRI data should be used with caution since only certain types of facilities are required to report. This is not an exhaustive list. Data are rounded to nearest whole number.
[b]Data in TRI are maximum amounts released by each facility.
[c]Post office state abbreviations are used.
[d]Number of reporting facilities.
[e]The sum of fugitive and point source releases are included in releases to air by a given facility.
[f]Surface water discharges, wastewater treatment (metals only), and publicly owned treatment works (POTWs) (metal and metal compounds).
[g]Class I wells, Class II-V wells, and underground injection.
[h]Resource Conservation and Recovery Act (RCRA) subtitle C landfills; other onsite landfills, land treatment, surface impoundments, other land disposal, other landfills.
[i]Storage only, solidification/stabilization (metals only), other off-site management, transfers to waste broker for disposal, unknown.
[j]The sum of all releases of the chemical to air, land, water, and underground injection wells.
[k]Total amount of chemical transferred off-site, including to POTWs.

RF = reporting facilities; UI = underground injection

Source: TRI21 2023 (Data are from 2021)

EPA's National Emission Inventory (NEI) database contains information regarding sources that emit criteria air pollutants (CAPs) and their precursors, and hazardous air pollutants (HAPs) for the 50 United States, Washington DC, Puerto Rico, and the U.S. Virgin Islands. Emissions are estimated from multiple sources, including state and local environmental agencies; the TRI database; computer models for on- and off-road emissions; and databases related to EPA's Maximum Achievable Control Technology (MACT) programs to reduce emissions of HAPs. Hexane emissions estimated from the 2017 inventory are summarized in Table 5-4.

**Table 5-4. Estimated Hexane Emitted in Pounds to the Environment According to EPA's National Emission Inventory**

| Emission sector | Pounds of hexane emitted |
|---|---|
| Mobile; on-road gasoline light duty vehicles | 72,224,392 |
| Mobile; non-road equipment; gasoline | 29,212,385 |
| Industrial processes; not elsewhere classified | 25,263,505 |
| Gas stations | 16,912,830 |
| Solvent; industrial surface coating and solvent use | 11,771,007 |
| Fires; wildfires | 8,423,741 |
| Industrial processes; oil and gas production | 7,122,755 |
| Industrial processes; chemical manufacturing | 6,875,081 |
| Miscellaneous non-industrial; not elsewhere classified | 6,754,985 |
| Industrial processes; storage and transfer | 6,749,765 |
| Bulk gasoline terminals | 4,377,265 |
| Fuel combustion; industrial boilers, internal combustion engines; natural gas | 3,290,331 |
| Fires; prescribed fires | 3,154,563 |
| Industrial processes; petroleum refineries | 1,513,562 |
| Mobile; on-road gasoline heavy duty vehicles | 1,495,321 |
| Fuel combustion; electric generation; natural gas | 1,189,607 |
| Solvent; non-industrial surface coating | 1,045,477 |
| Agriculture; livestock waste | 913,252 |
| Fuel combustion; residential; natural gas | 822,820 |
| Fuel combustion; commercial/institutional; natural gas | 808,868 |
| Fires; agricultural field burning | 796,004 |
| Solvent; degreasing | 589,792 |
| Mobile; on-road diesel heavy duty vehicles | 539,283 |
| Waste disposal | 311,943 |
| Fuel combustion; industrial boilers, internal combustion engines; coal | 256,527 |
| Mobile; commercial marine vessels | 241,278 |
| Mobile; non-road equipment; diesel | 198,891 |

**Table 5-4. Estimated Hexane Emitted in Pounds to the Environment According to EPA's National Emission Inventory**

| Emission sector | Pounds of hexane emitted |
|---|---|
| Solvent; consumer and commercial solvent use | 196,243 |
| Mobile; on-road diesel light duty vehicles | 171,854 |
| Mobile; locomotives | 171,448 |
| Fuel combustion; industrial boilers, internal combustion engines; other | 160,511 |
| Industrial processes; ferrous metals | 103,250 |
| Industrial processes; pulp and paper | 96,948 |
| Industrial processes; non-ferrous metals | 80,220 |
| Fuel combustion; industrial boilers, internal combustion engines; biomass | 67,690 |
| Fuel combustion; electric generation; oil | 67,417 |
| Mobile; aircraft | 38,759 |
| Solvent; graphic arts | 38,418 |
| Industrial processes; cement manufacture | 32,879 |
| Commercial cooking | 29,751 |
| Fuel combustion; industrial boilers, internal combustion engines; oil | 28,157 |
| Fuel combustion; electric generation; coal | 21,712 |
| Fuel combustion; electric generation; other | 11,953 |
| Industrial processes; mining | 6,832 |
| Fuel combustion; comm/institutional; other | 3,884 |
| Fuel combustion; comm/institutional; coal | 2,697 |
| Fuel combustion; comm/institutional; oil | 2,388 |
| Fuel combustion; comm/institutional; biomass | 1,529 |
| Fuel combustion; electric generation; biomass | 749 |
| Fuel combustion; residential; oil | 499 |
| Solvent; dry cleaning | 47 |
| Dust; construction dust | 4 |
| Total | 112,754,292 |

Source: EPA 2022a

### 5.3.2 Water

Estimated releases of 52,816 pounds (~23.96 metric tons) of *n*-hexane to surface water from 1,189 domestic manufacturing and processing facilities in 2021, accounted for <1% of the estimated total environmental releases from facilities required to report to the TRI (TRI21 2023). This estimate includes releases to waste water treatment and publicly owned treatment works (POTWs) (TRI21 2023). These releases are summarized in Table 5-3.

*n*-Hexane is probably released to water from a number of sources including industrial discharges, effluents from municipal waste-treatment plants, and nonpoint-source runoff from roads and other surfaces. Insufficient information is available to quantify the releases from all sources in a comprehensive fashion.

### 5.3.3 Soil

Estimated releases of 108,817 pounds (~49.36 metric tons) of *n*-hexane to soil from 1,189 domestic manufacturing and processing facilities in 2021, accounted for about <1% of the estimated total environmental releases from facilities required to report to the TRI (TRI21 2023). An additional 217,724 pounds (~98.76 metric tons), constituting about <1% of the total environmental emissions, were released via underground injection (TRI21 2023). These releases are summarized in Table 5-3.

*n*-Hexane is probably released to soil or sediments from spills and during the landfilling of sludges and other wastes generated from industrial processes and municipal sewage treatment; however, no specific quantitative information concerning levels for *n*-hexane released from wastes was identified in the literature.

## 5.4 ENVIRONMENTAL FATE

### 5.4.1 Transport and Partitioning

The physical properties of *n*-hexane (see Table 4-2) that affect its transport and partitioning in the environment are: water solubility of 9.5 mg/L; log $K_{ow}$ (octanol/water partition coefficient), estimated as 2.90 or 3.90; Henry's law constant, 1.003 or 1.3 atm-$m^3$ mol; and vapor pressure, 138 mmHg at 25°C. As with many alkanes, experimental methods for the estimation of the $K_{oc}$ parameter are lacking, so that estimates must be made based on theoretical considerations (Montgomery 1991).

**Air.** Organics with a vapor pressure of $>10^{-4}$ mmHg should exist almost entirely in the vapor phase in the atmosphere (Eisenreich et al. 1981). Hexane, which has a vapor pressure of 138 mmHg at 25°C, is not expected to partition from the vapor phase to particulates in the atmosphere.

**Water.** The dominant transport process from water is volatilization. Based on mathematical models developed by the EPA, the half-life for *n*-hexane in bodies of water with any degree of turbulent mixing (e.g., rivers) would be <3 hours. For standing bodies of water (e.g., small ponds), a half-life no longer than 1 week (6.8 days) is estimated using the Exposure Analysis Modeling System (EXAMS) (EPA 2004). Based on the log $K_{ow}$ and the estimated log $K_{oc}$ (see Table 4-2), *n*-hexane is not expected to become concentrated in biota (Swann et al. 1983). An estimated bioconcentration factor (BCF) of 174 and an estimated bioaccumulation factor (BAF) of 307 suggests a low potential for *n*-hexane to bioconcentrate or bioaccumulate in trophic food chains (EPA 2012).

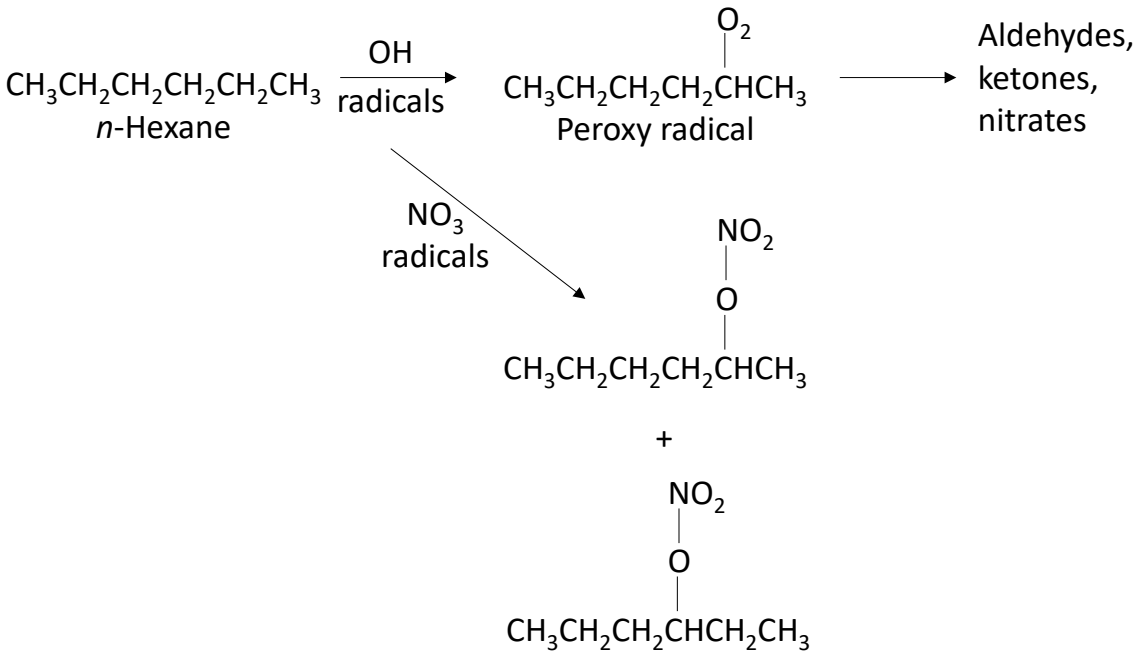
**Sediment and Soil.** In soil, the dominant transport mechanism for *n*-hexane present near the surface probably is volatilization (based on its Henry's law constant, water solubility, vapor pressure, and $K_{oc}$), but no experimental information focusing directly on *n*-hexane was found to confirm this assumption. While its estimated $K_{oc}$ values suggest a moderate ability to sorb to soil particles, *n*-hexane has a density (0.6606 g/mL at 20°C) well below that of water and a very low water solubility of 9.5 mg/L. *n*-Hexane would, therefore, be viewed as a light nonaqueous phase liquid (LNAPL), which would suggest a low potential for leaching into the lower soil depths since the *n*-hexane would tend to float on the top of the saturated zone of the water table (Feenstra et al. 1991; Hunt et al. 1988). Unless present in deeper soil layers (which can sometimes happen at waste sites or with underground storage tank leaks), *n*-hexane would generally stay near the soil surface and, if not appreciably sorbed into the soil matrix, would likely volatilize to the atmosphere. Exceptions would involve locations with shallow groundwater tables where there were large spills of hexane products. In such cases, the *n*-hexane could spread out to contaminate a large volume of soil materials.

### 5.4.2 Transformation and Degradation

**Air.** *n*-Hexane does not absorb ultraviolet (UV) light at 290 nm and is thus not expected to undergo direct photolysis reactions. The dominant tropospheric removal mechanism for *n*-hexane is generally regarded to be decomposition by hydroxyl radicals (Atkinson and Carter 1984; Atkinson et al. 1982). Calculations assuming typical hydroxyl radical concentrations suggest a half-life of approximately 1.96 days (EPA 2012). While *n*-hexane can react with nitrogen oxides to produce ozone precursors under controlled laboratory conditions (Montgomery 1991), the smog-producing potential of *n*-hexane is very low compared to that of other alkanes or chlorinated VOCs (Kopczynski et al. 1972). Hydroxyl ion reactions in the upper troposphere, therefore, are probably the primary mechanisms for *n*-hexane

degradation in the atmosphere. As with most alkanes, *n*-hexane is resistant to hydrolysis (Lyman et al. 1982). The proposed decomposition of *n*-hexane in air is shown in Figure 5-2.

**Figure 5-2. Degradation of *n*-Hexane in Air by Free Radicals**



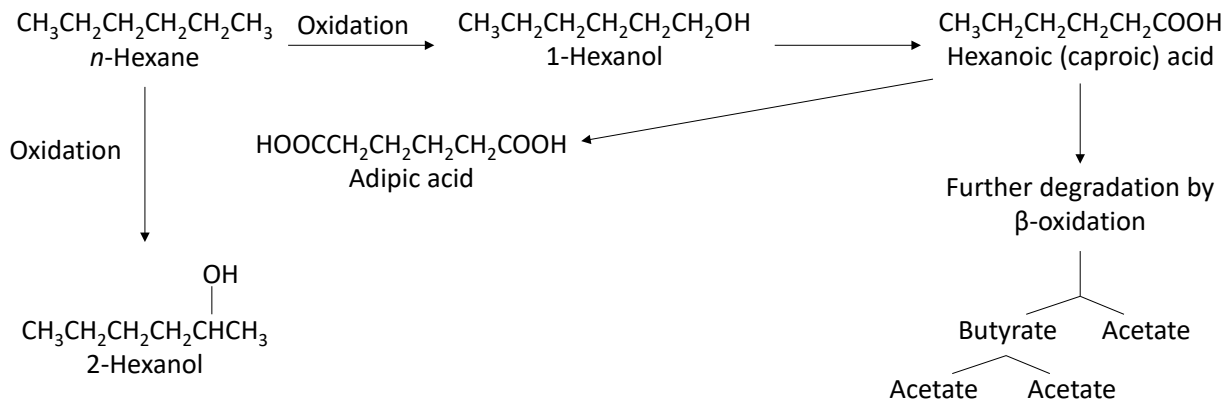

Source: Atkinson 1985

**Water.** Although few data are available dealing explicitly with the biodegradation of *n*-hexane in water, neither hydrolysis nor biodegradation in surface waters appears to be rapid compared with volatilization. In surface waters, as in the atmosphere, alkanes such as *n*-hexane would be resistant to hydrolysis (Lyman et al. 1982). Biodegradation is the most significant degradation mechanism in groundwater. One study was identified (McClay et al. 1995) that documented the ability of *Pseudomonas mendocina* bacteria to metabolize *n*-hexane in laboratory microcosms simulating groundwater conditions. Mixed bacterial cultures as well as pure cultures are documented as capable of metabolizing *n*-hexane under aerobic conditions (Heringa et al. 1961; Rosenberg et al. 1992). A study of a biofiltration system to remove VOCs from air used a sludge-like composting biofiltering system that was effective in causing the biodegradation of *n*-hexane (Morgenroth et al. 1996); this study involved a special composting system to allow the introduction of nitrogen fertilizers to overcome a nutrient limitation. Most of the available literature deals with petroleum mixtures containing several types of alkanes. In general, linear alkanes (such as *n*-hexane) are viewed as the most readily biodegradable fractions in petroleum (Leahy and Colwell 1990), particularly when oxygen is present in solution.

Since *n*-hexane is highly volatile, it is often excluded from the list of constituents included in studies on biodegradation or bioremediation of petroleum wastes or in studies of surface waters receiving pollutant loads from runoff or discharges. Attention is generally focused on complex mixtures of hydrocarbons, starting with fractions heavier or less volatile (usually C10 or longer chain alkanes, aromatics such as benzene or toluene, and polycyclic aromatic hydrocarbons (PAHs) than the lighter constituents of gasoline (Crawford et al. 1995; Latimer et al. 1990; Rosenberg et al. 1992; Sauer et al. 1993; Shaw et al. 1986). Once introduced into groundwater, *n*-hexane may be fairly persistent since its degradation by chemical hydrolysis is slow and opportunities for biodegradation may be limited under anoxic conditions or where nutrients such as nitrogen or phosphorus are in limited supply.

**Sediment and Soil.** The findings presented on bioremediation in groundwater are relevant for many soil and sediment systems. Figure 5-3 outlines the probable biodegradation of *n*-hexane based on metabolites isolated from a pure culture of *Pseudomonas* (Heringa et al. 1961). The most important biodegradation processes involve the conversion of the *n*-hexane to primary alcohols, aldehydes, and, ultimately, into fatty acids. Similar processes are encountered with other light hydrocarbons such as heptane. In general, unless the *n*-hexane is buried at some depth within a soil or sediment, volatilization is generally assumed to occur at a much more rapid rate than chemical or biochemical degradation processes. Once introduced into deeper sediments, *n*-hexane may be fairly persistent since its degradation by chemical hydrolysis is slow and opportunities for biodegradation may be limited under anoxic conditions or where nutrients such as nitrogen or phosphorus are in limited supply.

**Figure 5-3. Aerobic Biodegradation of *n*-Hexane in Sediment and Soil**




Source: Heringa et al. 1961

## 5.5 LEVELS IN THE ENVIRONMENT

Reliable evaluation of the potential for human exposure to *n*-hexane depends, in part, on the reliability of supporting analytical data from environmental samples and biological specimens. Concentrations of *n*-hexane in unpolluted atmospheres and in pristine surface waters are often so low as to be near the limits of current analytical methods. In reviewing data on *n*-hexane levels monitored or estimated in the environment, it should also be noted that the amount of chemical identified analytically is not necessarily equivalent to the amount that is bioavailable.

The widespread use of *n*-hexane as an extractant in the laboratory creates problems in interpreting concentration readings at low levels. Even with good quality control, it may often be impossible to determine whether to attribute a measured value to the actual levels in a sample or to contamination from *n*-hexane in the laboratory environment (Otson et al. 1994). For the most part, *n*-hexane is not a common target analyte from water or soil samples. While data based on ambient air samples or sampling in the air of various workplace or residential environments are more numerous, most EPA regulatory programs rely on bulk measurements of total hydrocarbons or total volatile compounds rather than on measurements of specific compounds such as *n*-hexane (Bishop et al. 1994; DeLuchi 1993).

Table 5-5 shows the lowest limit of detections that are achieved by analytical analysis in environmental media. An overview summary of the range of concentrations detected in environmental media is presented in Table 5-6.

**Table 5-5. Lowest Limit of Detection Based on Standards[a]**

| Media | Detection limit | Reference |
|---|---|---|
| Air (ppm) | 0.093 | OSHA 2010 |
| Drinking water | No data | |
| Surface water and groundwater | No data | |
| Soil | No data | |
| Sediment | No data | |
| Whole blood | No data | |

[a]Detection limits based on using appropriate preparation and analytics. These limits may not be possible in all situations.

**Table 5-6. Summary of Environmental Levels of *n*-Hexane**

| Media | Low | High | For more information |
|---|---|---|---|
| Outdoor air (ppbv) | 0.01 | 82 | Section 5.5.1 |
| Indoor air (ppbv) | 0.01 | 2,500 (ppmv) | Section 5.5.1 |
| Surface water (ppb) | 1.5 | 7.8 | Section 5.5.2 |

The available data on the levels of *n*-hexane in air, water, or soil at NPL sites are listed in Table 5-7 (ATSDR 2022a).

**Table 5-7. *n*-Hexane Levels in Water, Soil, and Air of National Priorities List (NPL) Sites**

| Medium | Median[a] | Geometric mean[a] | Geometric standard deviation[a] | Number of quantitative measurements | NPL sites |
|---|---|---|---|---|---|
| Water (ppb) | 91 | 91 | 1 | 2 | 1 |
| Soil (ppb) | | | No data | | |
| Air (ppbv) | 2.25 | 13.7 | 185 | 14 | 12 |

[a]Concentrations found in ATSDR site documents from 1981 to 2022 for 1,868 NPL sites (ATSDR 2022a). Maximum concentrations were abstracted for types of environmental media for which exposure is likely. Pathways do not necessarily involve exposure or levels of concern.

### 5.5.1 Air

*n*-Hexane is found at low levels in both rural and urban ambient air, with concentrations generally well below 55 ppbv for ambient air. In remote sites, readings of <0.5 ppbv are typical. A study of four rural sites in southern Canada showed median ambient air concentrations of *n*-hexane in the range of 0.01–0.12 ppbv (Bottenheim and Shepherd 1995). Higher levels can be encountered in urban areas, largely due to emissions from automobile exhaust. In the polluted atmosphere of the Los Angeles central business district, ambient air concentrations as high as 27 ppbv were documented in the 1960s (Neligan 1962); these levels are very similar to the concentrations of *n*-hexane measured in automobile exhaust collected during the same time period. Samples from Los Angeles in 1968 showed *n*-hexane levels of 82 ppbC (14 ppbv) (Kopczynski et al. 1972). With progressive improvements in emission controls, the levels of many air pollutants in urbanized areas today are generally far lower. A study of average VOC concentrations in the ambient air of several large cities showed the following results for *n*-hexane: Vienna 2.2 ppbv; Hamburg 3.8 ppbv; Sydney 2.1 ppbv; Chicago 2.0 ppbv; Osaka 5.5 ppbv; and Athens 1.6 ppbv (Moschonas and Glavas 1996). Air samples from Kuwaiti houses after the Gulf War (which introduced

large amounts of air pollutants from burning oil) showed average *n*-hexane levels of only 4.4 ppbv (Bouhamra 1995).

*n*-Hexane may be expected to comprise around 2% of the VOCs in urban air polluted with hydrocarbons from automobile emissions or other combustion byproducts (Barrefors and Petersson 1993). Close proximity to the exhaust systems of cars or other gasoline-powered vehicles can lead to exposures to increased concentrations of *n*-hexane. Under rush-hour conditions, the concentrations in the interior air of buses will tend to be lower (55 μg/m$^3$ or 19.8 ppbv) than the interior levels in cars (69 μg/m$^3$ or 24.9 ppbv) or the air around persons riding motorcycles (106 μg/m$^3$ or 38.1 ppbv) (Chan et al. 1994). Transportation tunnels may contain hydrocarbon concentrations around 6 times the levels encountered with ordinary open-air vehicular traffic; this is probably associated with similarly elevated levels of *n*-hexane (Barrefors and Petersson 1993). Measurements of hydrocarbons from vehicular exhaust at the Fort McHenry Tunnel in Baltimore, Maryland have shown *n*-hexane levels just under 60 ppbv (Zielinska et al. 1996).

*n*-Hexane does not seem to be present in tobacco smoke, although such smoke can lead to elevations in the concentrations of other hydrocarbons in the air of interior rooms (Barrefors and Petersson 1993). A complication in such testing is that hydrocarbons in the smoke may have been introduced from sources such as polluted urban air or *n*-hexane from cigarette lighters.

The air in well-ventilated office buildings in urban areas of California contained *n*-hexane levels of approximately 0.55 μg/m$^3$ (1.5 ppbv) (Daisey et al. 1994). Other studies of heavily polluted urban areas have suggested that the air in offices will have *n*-hexane levels at least an order of magnitude lower than the peak levels in rush-hour traffic in cars or other vehicles (Chan et al. 1994); the same studies showing that the median concentrations averaged over an entire commuting trip are about the same as for the time-averaged median concentrations of *n*-hexane in office buildings (<9 μg/m$^3$ or <3.2 ppbv).

Research suggests that gases released by various fungi in ductwork, inner walls, and crawl spaces contain a variety of VOCs, including *n*-hexane (Ahearn et al. 1996). Of the total levels of VOCs measured *in situ* from fungal-colonized insulation materials in a 2-year-old office building, *n*-hexane comprised about 2.69% of the total measured VOCs; in air samples collected under laboratory conditions using cultures prepared from fungal isolates, the *n*-hexane contribution to the total measured VOCs was 4.85% (Ahearn et al. 1996). Measurements of the interior air of an office building with fungi in air ducts and fiberglass

insulation located in the Houston area showed total VOC concentrations >100 ppbv. The VOCs levels included detectable amounts of *n*-hexane as well as toluene and benzene (Ahearn et al. 1996).

*n*-Hexane was detected in various media (indoor air, groundwater, soil gas, or outdoor air) at 17 vapor intrusion sites evaluated between 2002 and 2009 (Burk and Zarus 2013). Ten of the sites had indoor air measurements, with one site in Hartford, Illinois (IDPH 2002) exceeding the acute- and intermediate-duration indoor air MRLs with a peak indoor air concentration of 12,218 ppb (approximately 43 mg/m$^3$). The Hartford site experienced explosions and fires in homes from vapor intrusion following release of millions of gallons of petroleum to a light nonaqueous layer on groundwater. The groundwater was about 10 feet deep and homes experienced petroleum vapor intrusion following heavy rain events. Streamlined cleanup efforts are still underway at the Hartford site (EPA 2023).

Concentrations in some workplace settings may be higher than typical ambient air levels. Samples collected in tire factories during the 1970s showed median *n*-hexane levels of 25.9 ppmv around the work area where the rubber curing took place (Van Ert et al. 1980). When workers have assembled items in poorly ventilated rooms, *n*-hexane levels ranging from 500 to 2,500 ppmv have been documented (Iida 1982). Similar workplace findings, usually in countries other than the United States, have been documented, with *n*-hexane concentrations in workspace air in excess of 500 ppmv (Graham et al. 1995). Elevated levels in air are also found in substance misuse cases, where pure *n*-hexane or mixtures containing significant amounts of *n*-hexane are used to produce a “high” (Altenkirch et al. 1977; Graham et al. 1995).

*n*-Hexane is a common trace component in landfill gases at many waste sites (Brosseau and Heitz 1994; O’Leary and Walsh 1995). The *n*-hexane concentrations of these emissions have been documented to range from 3 to 10 mg/m$^3$ (0.85–2.8 ppm). While these levels would be expected to decrease rapidly as the landfill gases were dispersed into the ambient air, areas near the ground or pockets of air in trenches or excavations could reach levels significantly above the concentrations normally encountered in ambient air. For instance, data averaged over 15-minute intervals during site remediation work at a reclaimed oil refinery site showed levels as high as 121.51 mg/m$^3$ (34.51 ppmv) in the air around a backhoe digging trench in the petroleum-contaminated soils (Verma et al. 1992). Samples from the same study averaged over a typical 8-hour workshift for the area around the backhoe showed average levels of 3.06 mg/m$^3$ (0.87 ppmv). Even higher levels (perhaps in excess of 10,000 ppmv) are possible around large spills of *n*-hexane; at such elevated concentrations, as with many components of gasoline-type hydrocarbons, there could be considerable danger from explosions, which are possible when the *n*-hexane levels exceed

approximately 1.2% of the volume of air (Budavari et al. 1989). Since 0.1% by volume is equivalent to 1,000 ppmv, this flash-point level for *n*-hexane would be at a level of ≥12,000 ppmv.

*n*-Hexane is a pollutant monitored in the national Air Quality System (AQS) database, which contains ambient air pollution data collected by EPA, state, local, and tribal air pollution control agencies from monitors throughout the country. Table 5-8 shows the yearly mean 24-hour percentile distributions of *n*-hexane at monitoring stations across the United States.

**Table 5-8. Summary of Annual Concentration of *n*-Hexane (ppbv) Measured in Ambient Air at Locations Across the United States[a,b]**

| Year | Number of monitors | Number of samples | Average of the arithmetic mean at all locations | Maximum concentration |
|---|---|---|---|---|
| 2018 | 105 | 5,776 | 0.18 | 49.2 |
| 2019 | 71 | 4,038 | 0.21 | 33 |
| 2020 | 85 | 4,928 | 0.49 | 141 |
| 2021 | 81 | 6,326 | 0.77 | 955 |
| 2022 | 64 | 1,597 | 0.25 | 50 |
| 2023 | 55 | 1,110 | 0.99 | 278 |

[a]Values were originally reported in parts per billion carbon (ppbC) and converted to ppbv by dividing by the number of carbons in *n*-hexane.
[b]24-hour sampling period.

Source: EPA 2024

### 5.5.2 Water

In general, data on levels in water or groundwater are very limited, with only a little bit of information available in the literature. *n*-Hexane was identified as a contaminant in well water from the Upper Potomac aquifer (DeWalle and Chian 1981). *n*-Hexane was identified in all eight sites with values between 0.6 and 4.7 µg/L, with a median value of 1.4 µg/L (determined using ATSDR's SHOWER model, discussed in Section 5.6). In another study on surface water in the Gulf of Mexico (Sauer et al. 1978), *n*-hexane was identified in five of eight sites with values between 1.5 and 7.8 ng/L.

*n*-Hexane is highly volatile and typical treatment techniques for drinking water supplies in larger towns and cities would be expected to volatilize the *n*-hexane before it could enter the distribution system. It is likely that some *n*-hexane would be found in groundwater contaminated by gasoline leaks from underground storage tanks (UST). This could be a matter of concern for some domestic groundwater

wells used for drinking water supplies. Since the emphasis in UST programs is usually on the more soluble aromatic fractions (e.g., benzene) or on bulk measurements of TPHs (Potter 1993), no information could be identified in the literature dealing explicitly with *n*-hexane.

### 5.5.3 Sediment and Soil

Very little information could be identified dealing with *n*-hexane levels in sediments and soils. *n*-Hexane has been identified among the contaminants in an offsite oilfield-disposal pit in New Mexico (Eiceman et al. 1986). Since *n*-hexane is a trace constituent of crude oil and natural gas, as well as a component of refined petroleum products, soil or sediment contamination with *n*-hexane can be expected near oilfield production sites, large soil spills, slush pits and other areas around refineries, and in waste sites where petroleum products or other *n*-hexane-containing wastes had been disposed. Detections would also be likely near many tank storage facilities, pipelines, truck or rail transfer sites, car repair facilities, automobile assembly or storage facilities, and auto and truck fueling facilities (DeLuchi 1993).

At many waste sites, *n*-hexane has been detected in the landfill gases vented from the soils at the disposal sites (Brosseau and Heitz 1994; O'Leary and Walsh 1995). While information in the literature is extremely limited, trace levels of *n*-hexane are probably found in the soils or the soil gases at many waste disposal sites.

### 5.5.4 Other Media

Testing for alkanes is often directed at compounds less volatile (e.g., C10 or higher) than *n*-hexane (Hernandez et al. 1995). There is, therefore, limited information in the literature on the levels of *n*-hexane encountered in foodstuffs. Analyses carried out in the 1960s and 1970s would have sometimes involved analytical methods not considered accurate by contemporary standards. Caution is also needed in interpreting published results to make sure the testing did not involve materials that had not yet gone through the complete cycle of solvent recovery, heating, and final vacuum treatment to recover the *n*-hexane solvent and remove as much as possible of this hydrocarbon from the final product intended for human consumption. Before these recovery processes, the crude oil or meal products can be expected to show appreciably high levels of *n*-hexane. In studies of fully processed edible oil products carried out in the 1960s, it was determined that *n*-hexane residues were generally at levels <10 ppm (Watts and Holswade 1967). Investigations using more precise modern analysis techniques (Hautfenne et al. 1987) concluded that residual *n*-hexane residues for refined food products would be <2 ppm. If the standard

assumption of 80 g of fat consumed per 70-kg person per day is made, such residual levels would be the equivalent of no more than 2.29 µg/kg/day of *n*-hexane, which is a toxicologically insignificant amount.

*n*-Hexane is present in a variety of products commonly used in household settings. Given its volatility, this creates possibilities for exposures from inhalation as well as by dermal contact and ingestion. In a study of >1,000 common household products, *n*-hexane was detected in 101 products. *n*-Hexane was detected in >10% of the items sampled in the following product categories: automotive products; oils, greases, and lubricants; and adhesive-related products (Sack et al. 1992).

## 5.6 GENERAL POPULATION EXPOSURE

Low-level exposures to *n*-hexane may occur for much of the U.S. population, especially those who live in urban areas or those that commute in areas with heavy traffic, due to emissions of *n*-hexane associated with motor fuel use. As such, the general population will be exposed to very low levels at all times, while those living in urban centers may be exposed to slightly higher levels. *n*-Hexane blood levels have been measured in National Health and Nutrition Examination Survey (NHANES) samples beginning in 2009–2010; however, the levels were below the detection limit of 0.122 ng/mL.

ATSDR's three-compartment Shower and Household-Use Exposure (SHOWER) model predicts air concentrations in the shower stall, bathroom, and main house throughout the day by estimating the contribution from showering or bathing and the contribution from other water sources in the house, such as the dishwasher, clothes washer, and faucets. This information along with human activity patterns are used to calculate a daily time-weighted average exposure concentration via inhalation exposure and from dermal uptake from skin contact. ATSDR's SHOWER model is available by sending a request to showermodel@cdc.gov. Using median treated water levels as discussed in Section 5.5.2 (1.4 ng/L, based on DeWalle and Chian 1981) and representative outdoor air levels discussed in Section 5.5.1 (EPA 2024) reasonable maximum exposure (RME) levels for *n*-hexane were calculated for different exposure groups (Table 5-9) (ATSDR 2022b).

**Table 5-9. Reasonable Maximum Exposure Daily Inhalation Dose in µg/kg/day and Administered Dermal Dose of *n*-Hexane for the Target Person**

| Exposure group | Inhalation | Dermal |
|---|---|---|
| Birth–<1 year | 0.22 | 0.018 |
| 1–<2 years | 0.24 | 0.016 |

**Table 5-9. Reasonable Maximum Exposure Daily Inhalation Dose in µg/kg/day and Administered Dermal Dose of *n*-Hexane for the Target Person**

| Exposure group | Inhalation | Dermal |
|---|---|---|
| 2–<6 years | 0.15 | 0.014 |
| 6–<11 years | 0.085 | 0.011 |
| 11–<16 years | 0.057 | 0.0093 |
| 16–<21 years | 0.044 | 0.0085 |
| Adult | 0.039 | 0.0083 |
| Pregnant and breastfeeding women | 0.056 | 0.0084 |

Source: ATSDR 2022b

## 5.7 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

In addition to individuals who are occupationally exposed to *n*-hexane, there are several groups within the general population that have potentially high exposures (higher than background levels) to *n*-hexane. These populations include individuals living near *n*-hexane production or disposal sites. Individuals who subject themselves to substance misuse by inhaling *n*-hexane or vapors from products containing significant levels of *n*-hexane would also experience potentially high exposure levels (Altenkirch et al. 1982; Graham et al. 1995).

Work situations where *n*-hexane is used as a solvent or in adhesives and where there are very poor ventilation conditions could also have elevated exposure risks. Workers in poorly ventilated confined areas (e.g., warehouses, garages, tunnels) or trenches where *n*-hexane levels could build up from engine exhaust or from off-gassing, as in some landfill sites, might also experience higher exposures. Likewise, workers in tire-manufacturing facilities may have a heightened potential for health hazards since the rubber vulcanization process can involve exposures to *n*-hexane (Graham et al. 1995). Vehicle repair technicians that use an aerosol solvent product containing *n*-hexane, acetone, and toluene may also be exposed to elevated levels. A study of such workers reported exposure "pulses" with an average breathing zone VOC level of 394 mg/m$^3$ from the solvent formulation (Wilson et al. 2007).

# CHAPTER 6. ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of *n*-hexane is available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research designed to determine the adverse health effects (and techniques for developing methods to determine such health effects) of *n*-hexane.

Data needs are defined as substance-specific informational needs that, if met, would reduce the uncertainties of human health risk assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

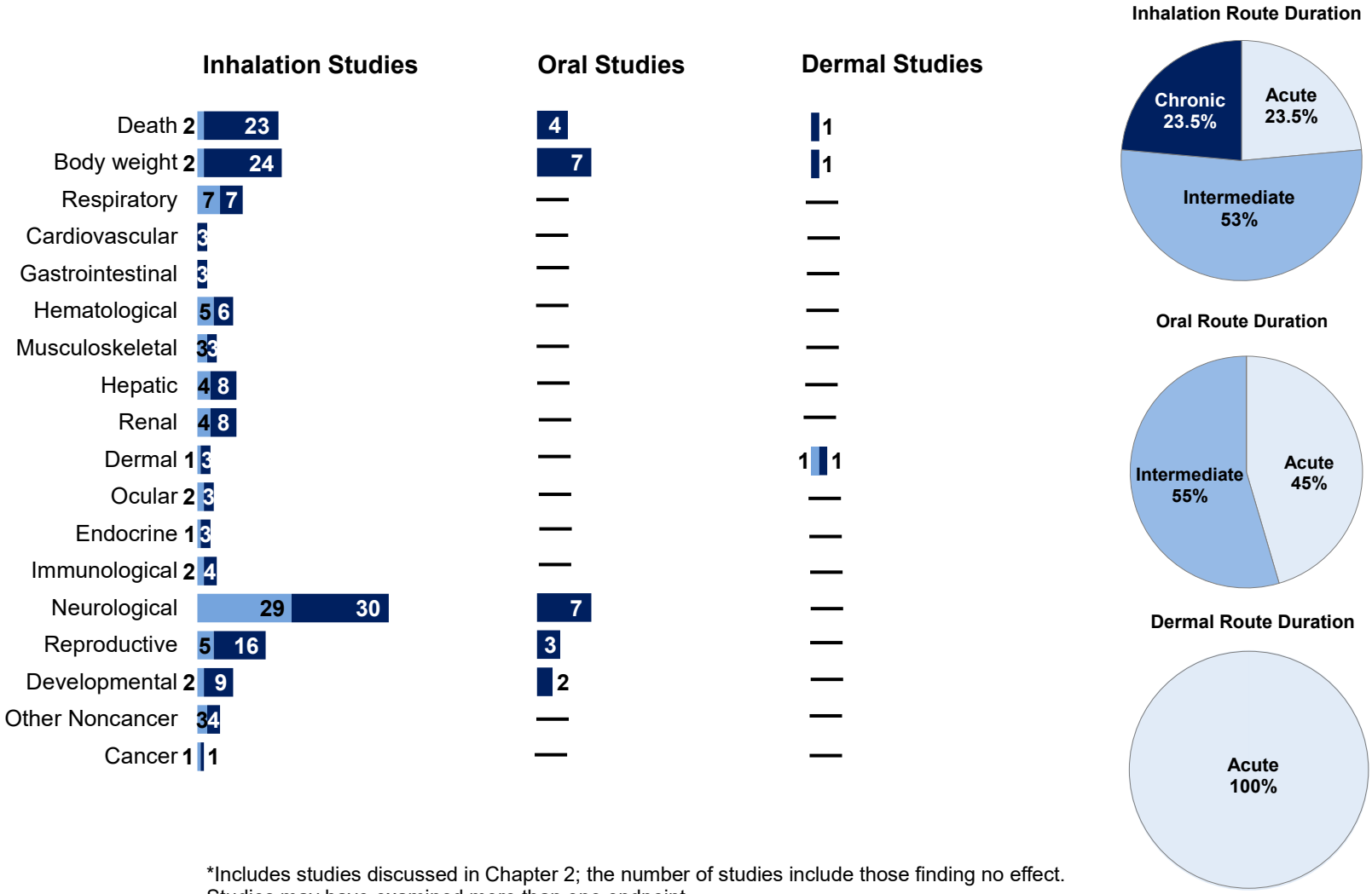
## 6.1 EXISTING INFORMATION ON HEALTH EFFECTS

Studies evaluating the health effects of inhalation, oral, and dermal exposure of humans and animals to *n*-hexane that are discussed in Chapter 2 are summarized in Figure 6-1. The purpose of this figure is to illustrate the information concerning the health effects of *n*-hexane. The number of human and animal studies examining each endpoint is indicated regardless of whether an effect was found and the quality of the study or studies.

As illustrated in Figure 6-1, most of the data on the toxicity of *n*-hexane comes from inhalation exposure studies in humans and animals. Most of the epidemiological studies have evaluated occupational exposure, which are presumed to be chronic-duration inhalation exposures. For animal studies, intermediate-duration inhalation studies are the most common. The primary health outcomes evaluated in epidemiological studies include neurological and respiratory, while animal studies have evaluated neurological, reproductive, and developmental effects. No oral exposure studies were identified in humans, and very few dermal studies were located for both humans and animals.

## Figure 6-1. Summary of Existing Health Effects Studies on *n*-Hexane by Route and Endpoint*

**Potential neurological, body weight, and reproductive effects were the most studied endpoints**
The majority of the studies examined inhalation exposure in **animals** (versus **humans**)




*Includes studies discussed in Chapter 2; the number of studies include those finding no effect. Studies may have examined more than one endpoint

## 6.2 IDENTIFICATION OF DATA NEEDS

Missing information in Figure 6-1 should not be interpreted as a "data need." A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (ATSDR 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

**Acute-Duration MRLs.** The inhalation dataset is adequate to derive an acute-duration inhalation MRL. The oral database is inadequate to derive an acute-duration oral MRL. Available oral data are limited to three acute-duration gavage studies, which were limited to examination of reproductive or developmental toxicity endpoints. Additional acute-duration oral studies examining a wide range of potential effects, particularly neurotoxicity, are needed to identify the most sensitive targets of toxicity and establish dose-response relationships. However, since the predominant route expected for human exposure is via inhalation, oral data may be less relevant to ongoing exposure scenarios in humans.

**Intermediate-Duration MRLs.** The inhalation database is adequate to derive intermediate-duration inhalation and oral MRLs. However, additional intermediate-duration oral studies examining a wide range of potential effects are needed to identify the most sensitive targets of toxicity and establish dose-response relationships. Since the predominant route expected for human exposure is via inhalation, oral data may be less relevant to ongoing exposure scenarios in humans.

**Chronic-Duration MRLs.** The inhalation and oral databases are inadequate to derive chronic-duration MRLs. Although there are many human epidemiological studies available, the lack of exposure data and concurrent exposure to neurotoxicants makes these data unsuitable. Only a single chronic-duration study in experimental animals was identified, and no chronic-duration oral studies were located. Additional low-concentration studies in animals could support the derivation of a chronic-duration inhalation MRL. Chronic-duration oral studies examining a wide range of potential effects are needed to identify the most sensitive targets of toxicity and establish dose-response relationships. However, since the predominant route expected for human exposure is via inhalation, oral data may be less relevant to ongoing exposure scenarios in humans.

**Health Effects.** Since *n*-hexane is highly volatile, the primary concern regarding toxicity relates to exposure via inhalation. Very few studies have evaluated oral or dermal exposure to *n*-hexane.

Therefore, a data need for all endpoints includes information on health effects resulting from oral and dermal exposure.

***Neurotoxicity.*** The major public health concern regarding *n*-hexane exposure is the potential for the development of neurotoxicity. Occupational studies have documented that human exposure to *n*-hexane can result in a peripheral neuropathy that in severe cases can lead to paralysis. The dose-duration relationship has not been well characterized in humans, but concentrations ≥500 ppm and exposure for ≥6 months have been associated with human neurotoxicity. Clinical neurotoxicity can be reproduced in rats via the inhalation and oral routes. Mice exhibited histopathological lesions and decreased locomotor activity; overt clinical signs such as gait disturbances or paralysis seen in rats have not been reported in mice. Other data needs are the determination of threshold levels for neurotoxicity for acute-, intermediate-, and chronic-duration inhalation exposure in the rat model, and the effect of age on susceptibility to *n*-hexane. There are no chronic-duration neurotoxicity studies in animals; such an inhalation study should evaluate both peripheral and central targets. Oral exposure is an unlikely route for human exposure due to the volatility of *n*-hexane, so oral neurological studies are not needed as critically. However, with deeply buried waste or leaking underground storage tanks, private drinking well water (municipal treatment is likely to volatilize all *n*-hexane at the plant) could become contaminated with *n*-hexane, so oral drinking water studies might be appropriate. Because of the volatility of *n*-hexane, exposure by the dermal route is unlikely and neurological toxicity studies are not needed as critically.

The molecular mechanism responsible for the axonal swelling, demyelination, and axonal degeneration seen in human *n*-hexane neurotoxicity has not been completely proven, although it is believed to be related to the pyrrolidation of neuronal proteins by the neurotoxic metabolite, 2,5-hexanedione. Whether neurofilament cross-linking is key to the neurofilament accumulation, axonal swellings, and ultimate axonal degeneration observed in *n*-hexane neurotoxicity or is incidental remains to be elucidated (Graham et al. 1995). Additional research is needed replicate the finding that the active *n*-hexane metabolite, 2,5-hexanedione, speeds rather than slows axonal transport (Pyle et al. 1993). Further studies in the rat model to answer this important question would be helpful in human risk assessment.

***Respiratory.*** Very few studies have examined the potential respiratory effects of *n*-hexane in animals and humans. *n*-Hexane is not irritating to the eyes, nose, or throat at concentrations up to

500 ppm for 3–5 minutes (Nelson et al. 1943). Higher incidences of self-reported respiratory symptoms have been observed in workers exposed to *n*-hexane (Mustajbegovic et al. 2000; Nijem et al. 2001), while reduced lung function has been reported in a study of children residing near point sources (Wichmann et al. 2009). Respiratory effects including rales, gasping, and mouth breathing were reported in rabbits throughout a 24-week inhalation exposure to 3,000 ppm *n*-hexane (Lungarella et al. 1984). Histopathological examination revealed serious effects in the lung, including centrilobular emphysema and fibrosis. Respiratory effects were also seen in mice exposed via inhalation to up to 10,000 ppm *n*-hexane for 13 weeks (NTP 1991); olfactory epithelial lesions were observed in mice exposed 1,099 ppm 22 hours/day, 5 days/week or 4,421 ppm 6 hours/day, 5 days/week, and in both the olfactory and respiratory epithelium at 10,000 ppm 6 hours/day, 5 days/week. In contrast, no histopathological changes were observed in the nasal cavity of male and female rats exposed up to 10,000 ppm 6 hours/day, 5 days/week for 13 weeks (Cavender et al. 1984), or in male rats exposed to 500 ppm 22 hours/day, 7 days/week for 6 months (API 1981). Data needs include additional acute- and intermediate-duration inhalation rodent studies to further elucidate the mechanism and species differences of the respiratory toxicity observed following *n*-hexane exposure.

***Developmental.*** Associations have been reported between *n*-hexane exposure in humans and low birth weight (Gong et al. 2018) and alterations of the neonatal immune system (Lehmann et al. 2002). Rodent studies have reported decreased fetal/litter weights following inhalation (Bus et al. 1979; NIEHS 1987, 1988c; Stoltenburg-Didinger et al. 1990) or oral exposure (Marks et al. 1980) to *n*-hexane. Inhalation studies using concentrations ≥5,000 ppm have also observed more severe effects, including decreases in the number of live fetuses or increases in skeletal malformations (Li et al. 2014, 2015; NIEHS 1987, 1988c). Developmental studies via the inhalation route in a species other than rats (e.g., mice) may be useful to assess the potential developmental toxicity of *n*-hexane exposure in humans since rats and mice have shown differences in susceptibility with other outcomes. There is also a need for developmental studies in animal models where assessment of neurological, reproductive, and possibly other endpoints continues up to sexual maturity after exposure to *n*-hexane *in utero* and during maturation. These studies would provide valuable information to assess possible differences in the toxicity between exposure to developing animals and mature animals.

***Reproductive.*** Longer menstrual cycles, longer times to get pregnant, lower serum FSH concentrations, and higher risks of spontaneous abortion and preeclampsia have been reported in

human epidemiological studies (Agnesi et al. 1997; Mendola et al. 2016; Nobles et al. 2019; Ruiz-García et al. 2020; Sallmen et al. 2008). Female reproductive effects have not been thoroughly examined in experimental animal studies, although several studies have reported effects in the male reproductive system. Decreased testis weights and/or testis and epididymis histopathology have been observed in rats following intermediate-duration inhalation exposure (De Martino et al. 1987; Howd et al. 1983; Nylen et al. 1989). Testicular atrophy was also noted in rats after intermediate-duration oral exposure (Krasavage et al. 1980). A study of endpoints of testicular function should be done in an occupationally exposed group of humans to determine if the effects seen in animals also occur in humans. Animal inhalation studies to determine the dose-response and threshold levels more accurately for testicular effects should also be conducted.

**Epidemiology and Human Dosimetry Studies.** Epidemiological information is available for the effects caused by occupational exposure to *n*-hexane. A complicating factor in these studies is that workers are almost always exposed to many other chemicals besides *n*-hexane. Epidemiological studies that followed populations exposed to *n*-hexane either in the workplace or near hazardous waste sites would be useful in assessing adverse effects in humans. Additionally, studies are needed of community populations. Of particular importance are respiratory effects, reproductive effects in males, and whether any relationship exists between *n*-hexane exposure and chronic degenerative neurological diseases. Human dosimetry studies would be useful in associating *n*-hexane levels with the reported effects.

**Biomarkers of Exposure and Effect.** The presence of the *n*-hexane metabolite, 2,5-hexanedione, in the urine is a reasonably reliable marker for exposure to *n*-hexane and has been correlated with air concentrations in the workplace. This is not a specific marker since 2-hexanone is also metabolized to 2,5-hexanedione. The levels of this metabolite in the urine associated with neurotoxicity are not known. A more sensitive marker for exposure may be the presence of pyrrolidated proteins in the blood or hair, a result of the reaction of 2,5-hexanedione with the side-chain amino group of lysine (Graham et al. 1995; Johnson et al. 1995). These methods have only been tested after oral exposure to 2,5-hexanedione in the rat model (Li et al. 2020b). It would be very useful to know if measurement of pyrrole adducts or cross-linked proteins is also feasible after inhalation exposure to *n*-hexane in the rat model. Further development and validation of this method in an occupationally exposed population may then be useful.

There are no subtle or sensitive biomarkers of effects associated specifically with exposure to *n*-hexane. Electroneurographic testing may prove useful in the detection of nerve conduction abnormalities in their

early stages before they are accompanied by clinical manifestations. In a study of 15 women who had been exposed to *n*-hexane in a shoe factory, all nerve conduction velocities (motor and sensory) were significantly slowed in exposed workers compared to controls (Mutti et al. 1982b). None of these women had clinical signs of peripheral neuropathy. Two studies suggest that the most sensitive electrophysiological biomarker of effect in *n*-hexane exposed workers may be the amplitude of the sensory nerve action potential, while amplitude of the motor nerve action potential, nerve conduction velocities, and distal latencies are less sensitive (Chang et al. 1993; Pastore et al. 1994). Further studies correlating electrophysiological studies with biomarkers of *n*-hexane exposure would be useful.

**Absorption, Distribution, Metabolism, and Excretion.** Toxicokinetic information is available for the inhalation route in humans and animals but is almost totally lacking for the oral and dermal routes. Inhaled *n*-hexane is readily absorbed in the lungs. In humans, approximately 20–30% of inhaled *n*-hexane is absorbed systemically. Absorption takes place by passive diffusion through epithelial cell membranes. Inhaled *n*-hexane distributes throughout the body; based on blood-tissue partition coefficients, preferential distribution would be in the order: body fat>>liver, brain, muscle>kidney, heart, lung>blood. *n*-Hexane is metabolized by mixed function oxidases in the liver to several metabolites including the neurotoxicant, 2,5-hexanedione. Approximately 10–20% of absorbed *n*-hexane is excreted unchanged in exhaled air, and 2,5-hexanedione is the major metabolite recovered in urine. *n*-Hexane metabolites in the urine and *n*-hexane in exhaled air do not account for total intake, suggesting that some of the metabolites of *n*-hexane enter intermediary metabolism. Saturation of metabolism occurs in rats at ≥3,000 ppm, far above any plausible human exposure. Further studies in animals via the oral and dermal routes are necessary to assess whether significant toxicity is likely to occur in humans exposed by these routes. A PBPK model exists for *n*-hexane that successfully predicts blood levels of *n*-hexane and urinary excretion of 2,5-hexanedione (Perbellini et al. 1986, 1990a) in exposed humans; however, it lacks the ability to extrapolate across species.

**Comparative Toxicokinetics.** The toxicokinetic studies available indicate that the rat is a good model for human neurotoxicity observed after occupational exposure to *n*-hexane. Mild signs can be produced in chickens and mice, but these do not progress to the serious neurotoxicity observed in humans and rats. Toxicokinetic data from other species (absorption, distribution, metabolism, excretion) could provide insight on the molecular mechanism(s) of the species specificity of *n*-hexane toxicity and would be valuable for predicting toxic effects in humans.

**Children's Susceptibility.** Several studies have examined potential respiratory effects in children. Altered lung function was observed in children living near petrochemical plants (Wichmann et al. 2009). Two general population studies did not find associations between *n*-hexane levels and respiratory effects (Buchdahl et al. 2000; Paciencia et al. 2020). Peripheral neuropathy has been reported in several teenagers after *n*-hexane exposure by solvent misuse (Altenkirch et al. 1977) and in the workplace (Yamamura 1969). These reports did not indicate any difference in susceptibility or clinical signs between teenagers and adults. Due to the limited number of reports of *n*-hexane toxicity in children, there are no data available as to whether children differ in their susceptibility to *n*-hexane toxicity compared to adults. Animal studies provide limited further information; only two studies were located where the responses to *n*-hexane were compared between young animals and adults (Howd et al. 1983; Kimura et al. 1971). An oral $LD_{50}$ study showed 14-day-old rats were more susceptible to the acute effects of a large dose of *n*-hexane than young adults (Kimura et al. 1971), while weanling rats (21 days old) were more resistant to the development of *n*-hexane peripheral neuropathy than young adults (80 days old) during an exposure to 1,000 ppm *n*-hexane (Howd et al. 1983). If cases of clinical *n*-hexane neurotoxicity occur in the future in adults in a setting where children are likely to have been exposed (e.g., home use of *n*-hexane containing products), thorough neurological and electrophysiological examinations should be performed on the children. Additionally, both immediate and long-term health effects caused by *n*-hexane in neonatal and juvenile animals could be investigated, possibly in some of the same studies examining postnatal exposures and developmental effects that are discussed in a previous data needs section.

There is no experimental evidence available to assess whether the toxicokinetics of *n*-hexane differ between children and adults. Experiments in the rat model comparing kinetic parameters in weanling and mature animals after exposure to *n*-hexane would be useful. These experiments should be designed to determine the concentration-time dependence (area under the curve) for blood levels of the neurotoxic *n*-hexane metabolite, 2,5-hexanedione. *n*-Hexane and its metabolites cross the placenta in the rat (Bus et al. 1979); however, no preferential distribution to the fetus was observed. *n*-Hexane has been detected, but not quantified, in human breast milk (Pellizzari et al. 1982), and a milk/blood partition coefficient of 2.10 has been determined experimentally in humans (Fisher et al. 1997). However, no pharmacokinetic experiments are available to confirm that *n*-hexane or its metabolites are transferred to breast milk. Based on studies in humans, it appears unlikely that significant amounts of *n*-hexane would be stored in human tissues at likely levels of exposure, so it is unlikely that maternal stores would be released upon pregnancy or lactation. A PBPK model is available for the transfer of *n*-hexane from milk to a nursing infant (Fisher et al. 1997); the model predicted that *n*-hexane intake by a nursing infant whose mother was exposed to

50 ppm at work would be well below the EPA advisory level for a 10-kg infant. However, this model cannot be validated without data on *n*-hexane content in milk under known exposure conditions.

There is no experimental evidence adequate to evaluate whether metabolism of *n*-hexane is different in children. Similarly, there is no information available from animal experiments. The initial step in *n*-hexane metabolism in animals is a hydroxylation step catalyzed by a cytochrome P-450 enzyme. Since some of these enzymes are developmentally regulated, it would be of interest to know: (1) if there are specific cytochrome P-450 isozymes involved in *n*-hexane hydroxylation and (2) if so, whether these isozymes are known to be developmentally regulated.

**Physical and Chemical Properties.** Data on physical and chemical properties are essential for estimating the partitioning of a chemical in the environment. The data on known physical and chemical properties form the basis of many of the input requirements for environmental models that predict the behavior of a chemical under specific conditions including those in hazardous waste landfills. Most of the necessary data on physical and chemical properties are available for *n*-hexane.

**Production, Import/Export, Use, Release, and Disposal.** Production methods for *n*-hexane are described in the literature, and there does not appear to be a need for further information. Uses of *n*-hexane are documented, although a detailed description of all uses is not available. Quantitative estimates of production levels for the more highly purified forms of *n*-hexane are available. The amounts of *n*-hexane associated with many types of motor and heating fuels can only be roughly estimated. Information on import and export levels is lacking. This information would be useful for estimating the potential for environmental releases from manufacturing and use industries as well as the potential environmental burden. However, it is difficult to obtain this information in the detail desired since it is generally considered to be confidential business information for those industries that manufacture *n*-hexane. Information on disposal practices is limited.

**Environmental Fate.** *n*-Hexane is a highly volatile hydrocarbon and will partition to the atmosphere if released into surface waters or onto land surfaces. The fate of *n*-hexane in air is reasonably well-described, with free radical degradation from hydroxyl radicals being of major importance. In water, biodegradation studies in surface water and groundwater are very limited, with most studies involving various petroleum fractions. Few studies were identified dealing explicitly with the fate of *n*-hexane in soils. Available studies (Heringa et al. 1961, Leahy and Colwell 1990, Rosenberg et al. 1992) indicate that *n*-hexane, along with other linear alkanes, is readily biodegraded under aerobic conditions. In soils

near the surface, *n*-hexane's high volatility will usually result in its rapid transfer to the atmosphere. Given the volatility of *n*-hexane and its ready biodegradation under aerobic conditions, the most important data need would involve degradation processes in groundwater, especially under anoxic conditions. Further research is needed to identify the rates of any relevant abiotic decay and transformation mechanisms (e.g., hydrolysis). These kinds of studies are important because they provide information about the movement or fundamental mechanisms of destruction of *n*-hexane in the environment and aid in understanding the behavior of *n*-hexane at hazardous waste sites.

**Bioavailability from Environmental Media.** Inhalation studies of humans indicate that *n*-hexane is bioavailable from the atmosphere. Although *n*-hexane in water or soil is likely to undergo transport to the air because of its volatility (although this would not necessarily be the case with *n*-hexane in groundwater), pharmacokinetic absorption studies using the oral and dermal routes of exposure would help clarify the bioavailability of *n*-hexane from water, soil, plant material, and other environmental media.

**Food Chain Bioaccumulation.** The physical constants for *n*-hexane (high volatility) and a low estimated BCF and BAF values (EPA 2012) suggest that *n*-hexane will not concentrate significantly in aquatic organisms. No empirical information is available concerning BCFs for a particular species or concerning the bioaccumulation or biomagnification of *n*-hexane in environmental media other than water. Information concerning the accumulation of *n*-hexane in several trophic levels would be useful in estimating human dietary intake; however, little intake is expected.

**Exposure Levels in Environmental Media.** Some environmental monitoring data are available for *n*-hexane in air, while very limited data are available for drinking water, surface water, groundwater, and foodstuffs. Available data for air provide a very uneven coverage for background ambient settings, and recent investigations for contexts associated with commuter traffic or workplace settings are very sparse. The data for water are not sufficient to accurately characterize the concentrations present in drinking water, surface water, and groundwater. Virtually no data are available for soils. These data would be helpful in determining the environmental concentrations of *n*-hexane so that exposure of the general population as well as of terrestrial and aquatic organisms could be estimated.

Reliable monitoring data for the levels of *n*-hexane in contaminated media at hazardous waste sites are needed so that the information obtained on levels of *n*-hexane in the environment can be used in

combination with the known body burdens of *n*-hexane to assess the potential risk of adverse health effects in populations living in the vicinity of hazardous waste sites.

**Exposure Levels in Humans.** The database for *n*-hexane exposure levels in humans is limited to a few older detections of *n*-hexane in breast milk and determinations of levels in body fluids and alveolar air collected in foreign countries. A more current and complete database would be helpful in determining the current exposure levels, thereby permitting the estimation of the average daily dose associated with various scenarios (e.g., living near a hazardous waste site). Since *n*-hexane is rapidly metabolized within the human body, further studies correlating levels in the environment with the levels of metabolites and biomarkers in humans would be helpful. This information is necessary for assessing the need to conduct health studies on these populations.

**Exposures of Children.** Better documentation of the types of household products that still contain *n*-hexane would be extremely valuable since inhalation of vapors from such products in poorly ventilated interior rooms could pose exposure risks to children. Additional studies on *n*-hexane concentrations in breast milk are important to validate the findings from PBPK modeling discussed in Chapter 3.

## 6.3 ONGOING STUDIES

No ongoing studies were identified in the National Institute of Health (NIH) RePORTER (2023) database.

# CHAPTER 7. REGULATIONS AND GUIDELINES

Pertinent international and national regulations, advisories, and guidelines regarding *n*-hexane in air, water, and other media are summarized in Table 7-1. This table is not an exhaustive list, and current regulations should be verified by the appropriate regulatory agency.

ATSDR develops MRLs, which are substance-specific guidelines intended to serve as screening levels by ATSDR health assessors and other responders to identify contaminants and potential health effects that may be of concern at hazardous waste sites. See Section 1.3 and Appendix A for detailed information on the provisional MRLs for *n*-hexane.

**Table 7-1. Regulations and Guidelines Applicable to *n*-Hexane**

| Agency | Description | Information | Reference |
|---|---|---|---|
| | **Air** | | |
| EPA | RfC | 0.7 mg/m$^3$ (0.2 ppm) | IRIS 2005 |
| | Provisional peer reviewed toxicity values | | |
| | Provisional subchronic RfC | 2 mg/m$^3$ (0.6 ppm) | EPA 2009a |
| WHO | Air quality guidelines | No data | WHO 2010 |
| | **Water & Food** | | |
| EPA | Drinking water standards and health advisories | | EPA 2018a |
| | 1-Day health advisory (10-kg child) | 10 mg/L | |
| | 10-Day health advisory (10-kg child) | 4 mg/L | |
| | Lifetime health advisory | No data | |
| | National primary drinking water regulations | Not listed | EPA 2009b |
| | RfD | Not assessed | IRIS 2005 |
| | Provisional peer reviewed toxicity values | | EPA 2009a |
| | Provisional subchronic RfD | 0.3 mg/kg/day | |
| WHO | Drinking water quality guidelines | No data | WHO 2022 |
| FDA | Substances added to food (formerly EAFUS) | Allowed as extractant in the preparation of several food/color additives, with restrictions on residue levels; allowed in some indirect food additives (particular coatings used in food packaging) | FDA 2023 |
| | **Cancer** | | |
| HHS | Carcinogenicity classification | Not evaluated | NTP 2021 |
| EPA | Carcinogenicity classification | Inadequate information to assess carcinogenic potential | IRIS 2005 |

## Table 7-1. Regulations and Guidelines Applicable to *n*-Hexane

| Agency | Description | Information | Reference |
|---|---|---|---|
| IARC | Carcinogenicity classification | Not evaluated | IARC 2023 |
| | **Occupational** | | |
| OSHA | PEL (8-hour TWA) for general industry, shipyards, and construction | 500 ppm (1800 mg/m³) | OSHA 2021a, 2021b, 2021c |
| NIOSH | REL (up to 10-hour TWA) | 50 ppm (180 mg/m³) | NIOSH 2019 |
| | IDLH | 1,100 ppm[a] | |
| | **Emergency Criteria** | | |
| EPA | AEGLs-air | | EPA 2018b |
| | AEGL 1[b] | | |
| | 10-minute, 30-minute, 60-minute, 4-hour, 8-hour | No recommendation due to insufficient data | |
| | AEGL 2[b] | | |
| | 10-minute | 4,000 ppm[c] | |
| | 30-minute | 2,900 ppm[c] | |
| | 60-minute | 2,900 ppm[c] | |
| | 4-hour | 2,900 ppm[c] | |
| | 8-hour | 2,900 ppm[c] | |
| | AEGL 3[b] | | |
| | 10-minute | 12,000 ppm[d] | |
| | 30-minute | 8,600 ppm[e] | |
| | 60-minute | 8,600 ppm[e] | |
| | 4-hour | 8,600 ppm[e] | |
| | 8-hour | 8,600 ppm[e] | |
| DOE | PACs-air | | DOE 2018a |
| | PAC-1[f] | 260 ppm | |
| | PAC-2[f] | 2,900 ppm[c] | |
| | PAC-3[f] | 8,600 ppm[e] | |

[a]Based strictly on safety considerations; IDLH is 10% of LEL of *n*-hexane in air (11,000 ppm).
[b]Definitions of AEGL terminology are available from EPA (2018c).
[c]Value is greater than 10% of the LEL; safety considerations against explosion hazard must be taken into account.
[d]Value is greater than the LEL; extreme safety considerations against explosion hazard must be taken into account.
[e]Value is greater than 50% of the LEL; extreme safety considerations against explosion hazard must be taken into account.
[f]Definitions of PAC terminology are available from DOE (2018b).

AEGL = acute exposure guideline levels; DOE = Department of Energy; EAFUS = Everything Added to Food in the United States; EPA = Environmental Protection Agency; FDA = Food and Drug Administration; HHS = Department of Health and Human Services; IARC = International Agency for Research on Cancer; IDLH = immediately dangerous to life or health; IRIS = Integrated Risk Information System; LEL = lower explosive limit; NIOSH = National Institute for Occupational Safety and Health; NTP = National Toxicology Program; OSHA = Occupational Safety and Health Administration; PAC = protective action criteria; PEL = permissible exposure limit; REL = recommended exposure limit; RfC = inhalation reference concentration; RfD = oral reference dose; TWA = time-weighted average; WHO = World Health Organization

# CHAPTER 8. REFERENCES

Abbritti G, Siracusa A, Cianchetti C, et al. 1976. Shoe-makers' polyneuropathy in Italy: the aetiological problem. Br J Ind Med 33(2):92-99. http://doi.org/10.1136/oem.33.2.92.

Abou-Donia MB, Lapadula DM. 1990. Mechanisms of organophosphorus ester-induced delayed neurotoxicity: type I and type II. Annu Rev Pharmacol Toxicol 30:405-440. http://doi.org/10.1146/annurev.pa.30.040190.002201.

Abou-Donia MB, Makkawy HA, Graham DG. 1982. The relative neurotoxicities of n-hexane, methyl n-butyl ketone, 2,5-hexanediol, and 2,5-hexanedione following oral or intraperitoneal administration in hens. Toxicol Appl Pharmacol 62(3):369-389. http://doi.org/10.1016/0041-008x(82)90139-9.

Abou-Donia MB, Lapadula DM, Campbell G, et al. 1985. The synergism of n-hexane-induced neurotoxicity by methyl isobutyl ketone following subchronic (90 days) inhalation in hens: induction of hepatic microsomal cytochrome P-450. Toxicol Appl Pharmacol 81(1):1-16. http://doi.org/10.1016/0041-008x(85)90114-0.

Abou-Donia MB, Lapadula DM, Suwita E. 1988. Cytoskeletal proteins as targets for organophosphorus compound and aliphatic hexacarbon-induced neurotoxicity. Toxicology 49(2-3):469-477. http://doi.org/10.1016/0300-483x(88)90033-9.

Abou-Donia MB, Hu ZH, Lapadula DM, et al. 1991. Mechanisms of joint neurotoxicity of n-hexane, methyl isobutyl ketone and O-ethyl O-4-nitrophenyl phenylphosphonothioate in hens. J Pharmacol Exp Ther 257(1):282-289.

Agnesi R, Valentini F, Mastrangelo G. 1997. Risk of spontaneous abortion and maternal exposure to organic solvents in the shoe industry. Int Arch Occup Environ Health 69(5):311-316. http://doi.org/10.1007/s004200050153.

Ahearn DG, Crow SA, Simmons RB, et al. 1996. Fungal colonization of fiberglass insulation in the air distribution system of a multi-story office building: VOC production and possible relationship to a sick building syndrome. J Ind Microbiol 16(5):280-285. http://doi.org/10.1007/BF01570035.

Ali N, Tardif R. 2006. Toxicokinetic modeling of the combined exposure to toluene and n-hexane in rats and humans. J Occup Health 41(2):95-103. http://doi.org/10.1539/joh.41.95.

Allen N, Mendell JR, Billmaier DJ, et al. 1975. Toxic polyneuropathy due to methyl n-butyl ketone. Arch Neurol 32:209-218. http://doi.org/10.1001/archneur.1975.00490460025001.

Altenkirch H, Mager J, Stoltenburg G, et al. 1977. Toxic polyneuropathies after sniffing a glue thinner. J Neurol 214(2):137-152. http://doi.org/10.1007/BF02430351.

Altenkirch H, Wagner HM, Stoltenburg G, et al. 1982. Nervous system responses of rats to subchronic inhalation of N-hexane and N-hexane + methyl-ethyl-ketone mixtures. J Neurol Sci 57(2-3):209-219. http://doi.org/10.1016/0022-510x(82)90028-4.

Amoore JE, Hautala E. 1983. Odor as an aid to chemical safety: odor thresholds compared with threshold limit values and volatilities for 214 industrial chemicals in air and water dilution. J Appl Toxicol 3(6):272-290. http://doi.org/10.1002/jat.2550030603.

Andersen ME, Krishnan K. 1994. Relating in vitro to in vivo exposures with physiologically based tissue dosimetry and tissue response models. In: Salem H, ed. Animal test alternatives: Refinement, reduction, replacement. New York, NY: Marcel Dekker, Inc, 9-25.

Anthony DC, Boekelheide K, Anderson CW, et al. 1983. The effect of 3,4-dimethyl substitution on the neurotoxicity of 2,5-hexanedione. II. Dimethyl substitution accelerates pyrrole formation and protein crosslinking. Toxicol Appl Pharmacol 71(3):372-382. http://doi.org/10.1016/0041-008x(83)90024-8.

API. 1978. 26 Week inhalation toxicity study of n-hexane in the rat. American Petroleum Institute. Submitted to the U.S. Environmental Protection Agency under TSCA Section FYI. OTS0000137-0. FYI-AX-1081-0137. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/OTS00001370.xhtml. February 23, 2023.

API. 1979. Teratology study in rats: n-hexane. Submitted to the U.S. Environmental Protection Agency under TSCA section FYI. OTS0000231-0. FYIAX01830231. LBI Project No. 20698-9. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/OTS00002310.xhtml. February 16, 2023.

API. 1980. Mutagenicity evaluation of n-hexane in the mouse dominant lethal assay final report. American Petroleum Institute. Submitted to the U.S. Environmental Protection Agency under TSCA Section FYI. OTS0000137-0. FYI-AX-1081-0137. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/OTS00001370.xhtml. February 23, 2023.

API. 1981. Six-month continuous inhalation exposure of rats to hexane mixtures-Phase II. American Petroleum Institute. Submitted to the U.S. Environmental Protection Agency under TSCA section FYI. OTS00001660. FYI-AX-0282-0166. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/OTS00001660.xhtml. February 23, 2023.

Ashworth RA, Howe GB, Mullins ME, et al. 1988. Air-water partitioning coefficients of organics in dilute aqueous solutions. J Hazard Mater 18:25-33. http://doi.org/10.1016/0304-3894(88)85057-X.

Atkinson R, Carter WPL. 1984. Kinetics and mechanisms of the gas phase reactions of ozone with organic compounds under atmospheric conditions. Chem Rev 84:437-470. http://doi.org/10.1021/cr00063a002.

Atkinson R, Aschmann SM, Carter WPL, et al. 1982. Kinetics of the reactions of OH radicals with n-alkanes at 299 ± 2 K. Int J Chem Kinet 14(7):781-788. http://doi.org/10.1002/kin.550140706.

ATSDR. 1989. Decision guide for identifying substance-specific data needs related to toxicological profiles. Agency for Toxic Substances and Disease Registry. Fed Regist 54(174):37618-37634. https://archives.federalregister.gov/issue_slice/1989/9/11/37613-37634.pdf#page=6. February 23, 2023.

ATSDR. 2022a. n-Hexane. Full SPL data. Substance priority list (SPL) resource page. Agency for Toxic Substances and Disease Registry. https://www.atsdr.cdc.gov/SPL/resources/index.html. June 8, 2023.

ATSDR. 2022b. Sanders2015 HLCs used in SHOWER Model v3 and PHAST. Atlanta, GA: Agency for Toxic Substances and Disease Registry. https://www.atsdr.cdc.gov/pha-guidance/toolbox/ATSDR_SHOWER_Model_v3_0_0.zip. May 3, 2023.

Bachmann MO, De Beer Z, Myers JE. 1993. n-Hexane neurotoxicity in metal can manufacturing workers. Occup Med (Lond) 43(3):149-154. http://doi.org/10.1093/occmed/43.3.149.

Baelum J, Mølhave L, Hansen SH, et al. 1998. Metabolic interaction between toluene, trichloroethylene and n-hexane in humans. Scand J Work Environ Health 24(1):30-37. http://doi.org/10.5271/sjweh.275.

Baker TS, Rickert DE. 1981. Dose-dependent uptake, distribution, and elimination of inhaled n-hexane in the Fischer-344 rat. Toxicol Appl Pharmacol 61(3):414-422. http://doi.org/10.1016/0041-008x(81)90364-1.

Barnes DG, Dourson M. 1988. Reference dose (RfD): description and use in health risk assessments. Regul Toxicol Pharmacol 8(4):471-486. http://doi.org/10.1016/0273-2300(88)90047-5.

Barrefors G, Petersson G. 1993. Assessment of ambient volatile hydrocarbons from tobacco smoke and from vehicle emissions. J Chromatogr 643(1-2):71-76. http://doi.org/10.1016/0021-9673(93)80542-g.

Bates MN, Reed BR, Liu S, et al. 2016. Solvent exposure and cognitive function in automotive technicians. Neurotoxicology 57:22-30. http://doi.org/10.1016/j.neuro.2016.08.009.

Bates MN, Pope K, So YT, et al. 2019. Hexane exposure and persistent peripheral neuropathy in automotive technicians. Neurotoxicology 75:24-29. http://doi.org/10.1016/j.neuro.2019.08.008.

Bavazzano P, Apostoli P, Balducci C, et al. 1998. Determination of urinary 2,5-hexanedione in the general Italian population. Int Arch Occup Environ Health 71(4):284-288. http://doi.org/10.1007/s004200050282.

Beall C, Delzell E, Rodu B, et al. 2001. Case-control study of intracranial tumors among employees at a petrochemical research facility. J Occup Environ Med 43(12):1103-1113. http://doi.org/10.1097/00043764-200112000-00012.

Beckman S, Eisen EA, Bates MN, et al. 2016. Acquired color vision defects and hexane exposure: a study of San Francisco Bay area automotive mechanics. Am J Epidemiol 183(11):969-976. http://doi.org/10.1093/aje/kwv328.

Bhagya S, Srinivas H. 1992. Extraction of soybean (Glycine max.) with hexane-acetic acid: Effect on oil quality. Food Chem 44(2):123-125. http://doi.org/10.1016/0308-8146(92)90323-t.

Bishop GA, Zhang Y, McLaren SE, et al. 1994. Enhancement of remote sensing for vehicle emissions in tunnels. Air Waste 44:169-175. http://doi.org/10.1080/1073161X.1994.10467246.

Boggess A, Faber S, Kern J, et al. 2016. Mean serum-level of common organic pollutants is predictive of behavioral severity in children with autism spectrum disorders. Sci Rep 6:26185. http://doi.org/10.1038/srep26185.

Bottenheim JW, Shepherd MF. 1995. C2-C6 Hydrocarbon measurements at four rural locations across Canada. Atmos Environ 29(6):647-664. http://doi.org/10.1016/1352-2310(94)00318-F.

Bouakkaz I, Khelili K, Rebai T, et al. 2018. Pulmonary toxicity induced by n-hexane in Wistar male rats after oral subchronic exposure. Dose Response 16(4):1559325818799560. http://doi.org/10.1177/1559325818799560.

Bouhamra WS. 1995. Estimation of volatile organic compounds in Kuwaiti houses after the Gulf war. Environ Pollut 90(1):121-125. http://doi.org/10.1016/0269-7491(94)00086-s.

Brosseau J, Heitz M. 1994. Trace gas compound emissions from municipal landfill sanitary sites. Atmos Environ 28(2):285-293. http://doi.org/10.1016/1352-2310(94)90103-1.

Brugnone F, Maranelli G, Romeo L, et al. 1991. Ubiquitous pollution by n-hexane and reference biological levels in the general population. Int Arch Occup Environ Health 63(3):157-160. http://doi.org/10.1007/BF00381562.

Buchdahl R, Willems CD, Vander M, et al. 2000. Associations between ambient ozone, hydrocarbons, and childhood wheezy episodes: a prospective observational study in south east London. Occup Environ Med 57(2):86-93. http://doi.org/10.1136/oem.57.2.86.

Budavari S, O'Neill MJ, Smith A, et al. 1989. n-Hexane. In: Merck index: An encyclopedia of chemicals, drugs, and biologicals. 11th ed. Rahway NJ: Merck & Co., Inc, 741.

Burk T, Zarus G. 2013. Community exposures to chemicals through vapor intrusion: a review of past ATSDR public health evaluations. J Environ Health 75(9):36-41.

Bus JS, Deyo D, Cox M. 1982. Dose-dependent disposition of n-hexane in F-344 rats after inhalation exposure. Fundam Appl Toxicol 2(5):226-229. http://doi.org/10.1016/s0272-0590(82)80031-6.

Bus JS, White EL, Tyl RW, et al. 1979. Perinatal toxicity and metabolism of n-hexane in Fischer-344 rats after inhalation exposure during gestation. Toxicol Appl Pharmacol 51(2):295-302. http://doi.org/10.1016/0041-008x(79)90472-1.

Bus JS, White EL, Gillies PJ, et al. 1981. Tissue distribution of n-hexane, methyl n-butyl ketone, and 2,5-hexanedione in rats after single or repeated inhalation exposure to n-hexane. Drug Metab Dispos 9(4):386-387.

Cardona A, Marhuenda D, Marti J, et al. 1993. Biological monitoring of occupational exposure to n-hexane by measurement of urinary 2,5-hexanedione. Int Arch Occup Environ Health 65(1):71-74. http://doi.org/10.1007/BF00586062.

Cardona A, Marhuenda D, Prieto MJ, et al. 1996. Behaviour of urinary 2,5-hexanedione in occupational co-exposure to n-hexane and acetone. Int Arch Occup Environ Health 68(2):88-93. http://doi.org/10.1007/BF00381240.

Carelli V, Franceschini F, Venturi S, et al. 2007. Grand rounds: could occupational exposure to n-hexane and other solvents precipitate visual failure in leber hereditary optic neuropathy? Environ Health Perspect 115(1):113-115. http://doi.org/10.1289/ehp.9245.

Cavender FL, Casey HW, Salem H, et al. 1984. A 13-week vapor inhalation study of n-hexane in rats with emphasis on neurotoxic effects. Fundam Appl Toxicol 4(2 Pt 1):191-201. http://doi.org/10.1016/0272-0590(84)90119-2.

Chalansonnet M, Carabin N, Boucard S, et al. 2013. Study of the potential oxidative stress induced by six solvents in the rat brain. Neurotoxicology 35:71-83. http://doi.org/10.1016/j.neuro.2012.12.002.

Chan C, Liu S, Her G. 1994. Office workers exposure to volatile organic compounds while commuting and working in Taipei City. Atmos Environ 28(14):2351-2359. http://doi.org/10.1016/1352-2310(94)90489-8.

Chang YC. 1987. Neurotoxic effects of n-hexane on the human central nervous system: evoked potential abnormalities in n-hexane polyneuropathy. J Neurol Neurosurg Psychiatry 50(3):269-274. http://doi.org/10.1136/jnnp.50.3.269.

Chang YC, Yip PK. 1987. N-hexane-induced electroneurographic changes and early detection of N-hexane intoxication. Taiwan Yi Xue Hui Za Zhi 86(2):194-200.

Chang CM, Yu CW, Fong KY, et al. 1993. N-hexane neuropathy in offset printers. J Neurol Neurosurg Psychiatry 56(5):538-542. http://doi.org/10.1136/jnnp.56.5.538.

Chapin RE, Norton RM, Popp JA, et al. 1982. The effects of 2,5-hexanedione on reproductive hormones and testicular enzyme activities in the F-344 rat. Toxicol Appl Pharmacol 62(2):262-272. http://doi.org/10.1016/0041-008x(82)90124-7.

Chiou CT, Kile DE, Malcolm RL. 1988. Sorption of vapors of some organic liquids on soil humic acid and its relation to partitioning of organic compounds in soil organic matter. Environ Sci Technol 22(3):298-303. http://doi.org/10.1021/es00168a010.

Cianchetti C, Abbritti G, Perticoni G, et al. 1976. Toxic polyneuropathy of shoe-industry workers. A study of 122 cases. J Neurol Neurosurg Psychiatry 39(12):1151-1161. http://doi.org/10.1136/jnnp.39.12.1151.

Clewell HJ. 1995. The application of physiologically based pharmacokinetic modeling in human health risk assessment of hazardous substances. Toxicol Lett 79(1-3):207-217. https://doi.org/10.1016/0378-4274(95)03372-r.

Clewell HJ, Andersen ME. 1985. Risk assessment extrapolations and physiological modeling. Toxicol Ind Health 1(4):111-131. http://doi.org/10.1177/074823378500100408.

Coates M, Connell DW, Barron DM. 1985. Aqueous solubility and octan-1-ol-water partition coefficients of aliphatic hydrocarbons. Environ Sci Technol 19(7):628-632. http://doi.org/10.1021/es00137a008.

Conkerton EJ, Wan PJ, Richard OA. 1995. Hexane and heptane as extraction solvents for cottonseed: A laboratory-scale study. J Am Oil Chem Soc 72(8):963-965. http://doi.org/10.1007/bf02542075.

Crawford DW, Bonnevie NL, Wenning RJ. 1995. Sources of pollution and sediment contamination in Newark Bay, New Jersey. Ecotoxicol Environ Saf 30(1):85-100. http://doi.org/10.1006/eesa.1995.1010.

Creaser C, Welch J, Bishop K. 1983. Letters to the editor: n-Hexane in petroleum ether fractions. Ann Occup Hyg 27(2):229-230. http://doi.org/10.1093/annhyg/27.2.229.

Crosbie SJ, Blain PG, Williams FM. 1997. Metabolism of n-hexane by rat liver and extrahepatic tissues and the effect of cytochrome P-450 inducers. Hum Exp Toxicol 16(3):131-137. http://doi.org/10.1177/096032719701600301.

Daisey JM, Hodgson AT, Fisk WJ, et al. 1994. Volatile organic compounds in twelve California office buildings: Classes, concentrations and sources. Atmos Environ 28(22):3557-3562. http://doi.org/10.1016/1352-2310(94)00200-5.

De Martino C, Malorni W, Amantini MC, et al. 1987. Effects of respiratory treatment with n-hexane on rat testis morphology. I. A light microscopic study. Exp Mol Pathol 46(2):199-216. http://doi.org/10.1016/0014-4800(87)90066-9.

DeCaprio AP, O'Neill EA. 1985. Alterations in rat axonal cytoskeletal proteins induced by in vitro and in vivo 2,5-hexanedione exposure. Toxicol Appl Pharmacol 78(2):235-247. http://doi.org/10.1016/0041-008x(85)90287-x.

DeCaprio AP, Olajos EJ, Weber P. 1982. Covalent binding of a neurotoxic n-hexane metabolite: conversion of primary amines to substituted pyrrole adducts by 2,5-hexanedione. Toxicol Appl Pharmacol 65(3):440-450. http://doi.org/10.1016/0041-008x(82)90389-1.

DeLuchi MA. 1993. Emissions from the production, storage, and transport of crude oil and gasoline. Air Waste 43(11):1486-1495. http://doi.org/10.1080/1073161X.1993.10467222.

DeWalle FB, Chian ESK. 1981. Detection of trace organics in well water near a solid waste landfill. J Am Water Works Assoc 73(4):206-211. http://doi.org/10.1002/j.1551-8833.1981.tb04681.x.

DOE. 2018a. Table 3: Protective action criteria (PAC) rev. 29a based on applicable 60-minute AEGLs, ERPGs, or TEELs. The chemicals are listed by CASRN. June 2018. U.S. Department of Energy. https://edms3.energy.gov/pac/docs/Revision_29A_Table3.pdf. July 6, 2022.

DOE. 2018b. Protective action criteria (PAC) with AEGLs, ERPGs, & TEELs: Rev. 29A, June 2018. U.S. Department of Energy. https://edms3.energy.gov/pac/. July 6, 2022.

Domínguez H, Núñez MJ, Lema JM. 1995. Enzyme-assisted hexane extraction of soya bean oil. Food Chem 54(2):223-231. http://doi.org/10.1016/0308-8146(95)00018-e.

Dunnick JK, Graham DG, Yang RS, et al. 1989. Thirteen-week toxicity study of n-hexane in B6C3F1 mice after inhalation exposure. Toxicology 57(2):163-172. https://doi.org/10.1016/0300-483x(89)90162-5.

Eiceman GA, McConnon JT, Zaman M, et al. 1986. Hydrocarbons and aromatic hydrocarbons in groundwater surrounding an earthen waste disposal pit for produced water in the Duncan oil field of New Mexico. Int J Environ Anal Chem 24(2):143-162. http://doi.org/10.1080/03067318608076465.

Eisenreich SJ, Looney BB, Thornton JD. 1981. Airborne organic contaminants in the Great Lakes ecosystem. Environ Sci Technol 15(1):30-38. http://doi.org/10.1021/es00083a002.

El-Masri HA, Mumtaz MM, Yushak ML. 2004. Application of physiologically-based pharmacokinetic modeling to investigate the toxicological interaction between chlorpyrifos and parathion in the rat. Environ Toxicol Pharmacol 16(1-2):57-71. https://doi.org/10.1016/j.etap.2003.10.002.

EPA. 1993. Determination of reportable quantities for hazardous substances. U.S. Environmental Protection Agency. Fed Regist 58(203):54840-54862. https://www.govinfo.gov/app/details/FR-1993-10-22. May 2, 2023.

EPA. 1994a. Automobile emissions: an overview. Ann Arbor, MI: U.S. Environmental Protection Agency. EPA400F92007. https://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=P10001KF.txt. February 21, 2023.

EPA. 1994b. Methods for derivation inhalation reference concentrations and application of inhalation dosimetry. Washington, DC: U.S. Environmental Protection Agency. PB2000100284. EPA600890066F. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/PB2000100284.xhtml. February 17, 2023.

EPA. 1996. National emission standards for hazardous air pollutants; final standards for hazardous air pollutant emissions from the printing and publishing industry. U.S. Environmental Protection Agency. Fed Regist 61(105):27132-27159. https://www.govinfo.gov/content/pkg/FR-1996-05-30/pdf/96-13084.pdf. May 4, 2023.

EPA. 2000. Hexane: 110-54-3. Health effects notebook for hazardous air pollutants. U.S. Environmental Protection Agency. https://www.epa.gov/sites/default/files/2016-09/documents/hexane.pdf. February 20, 2023.

EPA. 2004. Exposure analysis modeling system (EXAMS). Athens, GA: U.S. Environmental Protection Agency. EPA600R00081. https://www.epa.gov/ceam/exposure-analysis-modeling-system-exams-user-manual-and-system-documentation. June 4, 2023.

EPA. 2009a. Provisional peer-reviewed toxicity values for 1,2-dichloroethane (CASRN 107-06-2). Cincinnati, OH: U.S. Environmental Protection Agency. EPA690R09025F. https://cfpub.epa.gov/ncea/pprtv/documents/HexaneN.pdf. January 30, 2023.

EPA. 2009b. National primary drinking water regulations. Washington, DC: U.S. Environmental Protection Agency. EPA816F09004. https://www.epa.gov/sites/production/files/2016-06/documents/npwdr_complete_table.pdf. August 2, 2019.

EPA. 2012. n-Hexane. Estimation Programs Interface Suite™ for Microsoft® Windows, v 4.11. Washington, DC: U.S. Environmental Protection Agency. https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface.

EPA. 2018a. 2018 Edition of the drinking water standards and health advisories. Washington, DC: U.S. Environmental Protection Agency. EPA822F18001. https://www.epa.gov/system/files/documents/2022-01/dwtable2018.pdf. June 15, 2022.

EPA. 2018b. Compiled AEGL values. U.S. Environmental Protection Agency. https://www.epa.gov/sites/production/files/2018-08/documents/compiled_aegls_update_27jul2018.pdf. April 12, 2020.

EPA. 2018c. About acute exposure guideline levels (AEGLs). U.S. Environmental Protection Agency. https://www.epa.gov/aegl/about-acute-exposure-guideline-levels-aegls. July 26, 2018.

EPA. 2022a. 2017 NEI data. U.S. Environmental Protection Agency. https://www.epa.gov/air-emissions-inventories/2017-national-emissions-inventory-nei-data. May 3, 2023.

EPA. 2022b. Toxic chemical release inventory reporting forms and instructions: Revised 2021 version. U.S. Environmental Protection Agency. EPA740B22002. https://ordspub.epa.gov/ords/guideme_ext/guideme_ext/guideme/file/ry_2021_rfi.pdf. August 22, 2023.

EPA. 2023. Innovations in site characterization: Streamlining cleanup at vapor intrusion and product removal sites using the triad approach: Hartford Plume Site, Hartford, Illinois. Washington, DC: U.S. Environmental Protection Agency. EPA542R10006. https://www.epa.gov/remedytech/innovations-site-characterization-streamlining-cleanup-vapor-intrusion-and-product. June 9, 2023.

EPA. 2024. Annual summary data: n-hexane. Air quality system: Concentration by monitor. U.S. Environmental Protection Agency. https://aqs.epa.gov/aqsweb/airdata/download_files.html. January 18, 2024.

Farmer TH. 1996. Occupational hygiene limits for hydrocarbon solvents. Ann Occup Hyg 40(2):237-242. http://doi.org/10.1093/annhyg/40.2.237.

FDA. 2023. Hexane. Substances added to food. U.S. Food and Drug Administration. https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=FoodSubstances&id=HEXANE. January 29, 2023.

Fedtke N, Bolt HM. 1986. Detection of 2,5-hexanedione in the urine of persons not exposed to n-hexane. Int Arch Occup Environ Health 57(2):143-148. http://doi.org/10.1007/BF00381382.

Fedtke N, Bolt HM. 1987. The relevance of 4,5-dihydroxy-2-hexanone in the excretion kinetics of n-hexane metabolites in rat and man. Arch Toxicol 61:131-137. http://doi.org/10.1007/BF00661371.

Feenstra S, Mackay DM, Cherry JA. 1991. A method for assessing residual NAPL based on organic chemical concentrations in soil samples. Ground Water Monit Remediat 11(2):128-136. http://doi.org/10.1111/j.1745-6592.1991.tb00374.x.

Filser JG, Bolt HM, Muliawan H, et al. 1983. Quantitative evaluation of ethane and n-pentane as indicators of lipid peroxidation in vivo. Arch Toxicol 52:135-147. http://doi.org/10.1007/BF00354773.

Filser JG, Peter H, Bolt HM, et al. 1987. Pharmacokinetics of the neurotoxin n-hexane in rat and man. Arch Toxicol 60(1-3):77-80. http://doi.org/10.1007/BF00296952.

Fisher J, Mahle D, Bankston L, et al. 1997. Lactational transfer of volatile chemicals in breast milk. Am Ind Hyg Assoc J 58(6):425-443. http://doi.org/10.1080/15428119791012667.

Frontali N, Amantini MC, Spagnolo A, et al. 1981. Experimental neurotoxicity and urinary metabolites of the C5-C7 aliphatic hydrocarbons used as glue solvents in shoe manufacture. Clin Toxicol 18(12):1357-1367. http://doi.org/10.3109/15563658108990344.

Gargas ML, Burgess RJ, Voisard DE, et al. 1989. Partition coefficients of low-molecular-weight volatile chemicals in various liquids and tissues. Toxicol Appl Pharmacol 98(1):87-99. http://doi.org/10.1016/0041-008x(89)90137-3.

Gao JF, Zhang XY, Lin Q, et al. 2019. [Changes to learning and memory ability and the expression of NGF/NGF-R mRNA in the brain tissue in mice exposed to n-hexane.] Clin J Ind Hyg Occup Dis 37(3):217-221. https://doi.org/10.3760/cma.j.issn.1001-9391.2019.03.013. (Chinese)

Genter St Clair MB, Amarnath V, Moody MA, et al. 1988. Pyrrole oxidation and protein cross-linking as necessary steps in the development of gamma-diketone neuropathy. Chem Res Toxicol 1(3):179-185. http://doi.org/10.1021/tx00003a009.

Gillies PJ, Norton RM, Baker TS, et al. 1981. Altered lipid metabolism in 2,5-hexanedione-induced testicular atrophy and peripheral neuropathy in the rat. Toxicol Appl Pharmacol 59(2):293-299. http://doi.org/10.1016/0041-008x(81)90201-5.

Goldman SM, Quinlan PJ, Ross GW, et al. 2012. Solvent exposures and Parkinson disease risk in twins. Ann Neurol 71(6):776-784. http://doi.org/10.1002/ana.22629.

Gong Y, Kishi R, Kasai S, et al. 2003. Visual dysfunction in workers exposed to a mixture of organic solvents. Neurotoxicology 24(4-5):703-710. http://doi.org/10.1016/S0161-813X(03)00034-2.

Gong X, Lin Y, Bell ML, et al. 2018. Associations between maternal residential proximity to air emissions from industrial facilities and low birth weight in Texas, USA. Environ Int 120:181-198. http://doi.org/10.1016/j.envint.2018.07.045.

Governa M, Calisti R, Coppa G, et al. 1987. Urinary excretion of 2,5-hexanedione and peripheral polyneuropathies workers exposed to hexane. J Toxicol Environ Health 20(3):219-228. http://doi.org/10.1080/15287398709530976.

Graham DG, Anthony DC, Boekelheide K, et al. 1982. Studies of the molecular pathogenesis of hexane neuropathy. II. Evidence that pyrrole derivatization of lysyl residues leads to protein crosslinking. Toxicol Appl Pharmacol 64(3):415-422. http://doi.org/10.1016/0041-008x(82)90237-x.

Graham DG, Amarnath V, Valentine WM, et al. 1995. Pathogenetic studies of hexane and carbon disulfide neurotoxicity. Crit Rev Toxicol 25(2):91-112. http://doi.org/10.3109/10408449509021609.

Hageman G, van der Hoek J, van Hout M, et al. 1999. Parkinsonism, pyramidal signs, polyneuropathy, and cognitive decline after long-term occupational solvent exposure. J Neurol 246(3):198-206. http://doi.org/10.1007/s004150050334.

Hansch C, Leo A, Hoekman D. 1995. n-Hexane. In: Exploring QSAR: Hydrophobic, electronic, and steric constants. Vol. 2. Washington, DC: American Chemical Society, 24.

Hautfenne A, Pocklington WD, Wolfe JP. 1987. Determination of hexane residues in oils: Results of a collaborative study and the standardised method. Pure Appl Chem 59(11):1561-1570. http://doi.org/10.1351/pac198759111561.

Heath JS, Koblis K, Sager SL, et al. 1993. Risk assessment for total petroleum hydrocarbons. In: Kostecki PT, Calabrese EJ, eds. Hydrocarbon contaminated soils and groundwater. Vol. 3. Chelsea, MI: Lewis Publisher, 267-301.

Herbert R, Gerr F, Luo J, et al. 1995. Peripheral neurological abnormalities among roofing workers: Sentinel case and clinical screening. Arch Environ Health 50(5):349-354. http://doi.org/10.1080/00039896.1995.9935966.

Heringa JW, Huybregtse R, van der Linden AC. 1961. n-Alkane oxidation by a Pseudomonas formation and B-oxidation of intermediate fatty acids. Antonie Van Leeuwenhoef 27:51-58. http://doi.org/10.1007/BF02538422.

Hernandez JE, Machado LT, Corbella R, et al. 1995. n-Alkanes and polynuclear aromatic hydrocarbons in fresh-frozen and precooked-frozen mussels. Bull Environ Contam Toxicol 55(3):461-468. http://doi.org/10.1007/BF00206687.

Herskowitz A, Ishii N, Schaumburg H. 1971. n-Hexane neuropathy: A syndrome occurring as a result of industrial exposure. N Engl J Med 285:82-85. http://doi.org/10.1056/NEJM197107082850204.

Houk VS, Schalkowsky S, Claxton LD. 1989. Development and validation of the spiral Salmonella assay: an automated approach to bacterial mutagenicity testing. Mutat Res 223(1):49-64. http://doi.org/10.1016/0165-1218(89)90062-1.

Howd RA, Rebert CS, Dickinson J, et al. 1983. A comparison of the rates of development of functional hexane neuropathy in weanling and young adult rats. Neurobehav Toxicol Teratol 5(1):63-68.

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(5):381-385. http://doi.org/10.1007/BF00303127.

Huang CC, Shih TS, Cheng SY, et al. 1991. n-Hexane polyneuropathy in a ball-manufacturing factory. J Occup Med 33(2):139-142.

Hunt JR, Sitar N, Udell KS. 1988. Nonaqueous phase liquid transport and cleanup: 1. Analysis of mechanisms. Water Resour Res 24(8):1247-1258. http://doi.org/10.1029/WR024i008p01247.

IARC. 2023. Agents classified by the IARC Monographs, volumes 1–132. International Agency for Research on Cancer. https://monographs.iarc.fr/list-of-classifications. January 29, 2023.

Iba MM, Fung J, Gonzalez FJ. 2000. Functional Cyp2e1 is required for substantial in vivo formation of 2,5-hexanedione from n-hexane in the mouse. Arch Toxicol 74(10):582-586. https://doi.org/10.1007/s002040000182.

Ichihara G, Saito I, Kamijima M, et al. 1998. Urinary 2,5-hexanedione increases with potentiation of neurotoxicity in chronic coexposure to n-hexane and methyl ethyl ketone. Int Arch Occup Environ Health 71(2):100-104. http://doi.org/10.1007/s004200050255.

IPDH. 2002. Public health assessment: Response to vapors in Hartford Homes, IL. Illinois Department of Public Health. http://www.idph.state.il.us/envhealth/pdf/Hartford%20PHA%20PC%20R.pdf. June 8, 2023.

Iida M. 1982. Neurophysiological studies of n-hexane polyneuropathy in the sandal factory. Electroencephalogr Clin Neurophysiol Suppl 36:671-681.

Imai T, Omoto M. 1999. A preliminary report on the tumorigenic effect of long-term exposure to n-hexane in the rat testis. J Occup Health 41(4):261-262. http://doi.org/10.1539/joh.41.261.

IRIS. 2005. n-Hexane; CASRN 110-54-3. Integrated risk information system: Chemical assessment summary. Washington, DC: U.S. Environmental Protection Agency. https://iris.epa.gov/static/pdfs/0486_summary.pdf. January 30, 2023.

Ishidate M, Sofuni T, Yoshikawa K, et al. 1984. Primary mutagenicity screening of food additives currently used in Japan. Food Chem Toxicol 22(8):623-636. http://doi.org/10.1016/0278-6915(84)90271-0.

Isidorov VA, Zenkevich IG, Ioffe BV. 1985. Volatile organic compounds in the atmosphere of forest. Atmos Environ 19(1):1-8. http://doi.org/10.1016/0004-6981(85)90131-3.

Issever H, Malat G, Sabuncu HH, et al. 2002. Impairment of colour vision in patients with n-hexane exposure-dependent toxic polyneuropathy. Occup Med (Lond) 52(4):183-186. http://doi.org/10.1093/occmed/52.4.183.

Ithnin A, Shaari RAH, Sahani M, et al. 2011. Effects of pollutant diesel fuels on neurobehavioral performance among workers in locomotive depot. Am J Environ Sci 7(3):248-253. http://doi.org/10.3844/ajessp.2011.248.253.

Iyadomi M, Ichiba M, Zhang J, et al. 2000. Evaluation of skin irritants caused by organic solvents by means of the mouse ear thickness measurement method. J Occup Health 42(1):44-46. http://doi.org/10.1539/joh.42.44.

Jackson JR, deKechoue G, Karakaya A. 1997. Letter to the editor [Changes in immunoglobulin concentrations and urinary 2,5-hexanedione concentrations]. Hum Exp Toxicol 16(5):284-285. http://doi.org/10.1177/096032719701600511.

Johnson DJ, Lack L, Ibrahim S, et al. 1995. Protein-bound pyrroles in rat hair following subchronic intraperitoneal injections of 2,5-hexanedione. J Toxicol Environ Health 45(3):313-324. http://doi.org/10.1080/15287399509531998.

Jørgensen NK, Cohr KH. 1981. n-Hexane and its toxicologic effects: a review. Scand J Work Environ Health 7(3):157-168. http://doi.org/10.5271/sjweh.3107.

Juarez-Perez CA, Torres-Valenzuela A, Haro-Garcia LC, et al. 2014. Ototoxicity effects of low exposure to solvent mixture among paint manufacturing workers. Int J Audiol 53(6):370-376. http://doi.org/10.3109/14992027.2014.888597.

Kanatharana P, Chindarasamee C, Kaewnarong B. 1993. Extracting solvents for pesticide residues. J Environ Sci Health 28(10):2323-2332. http://doi.org/10.1080/10934529309376012.

Kanavouras K, Tzatzarakis MN, Mastorodemos V, et al. 2011. A case report of motor neuron disease in a patient showing significant level of DDTs, HCHs and organophosphate metabolites in hair as well as levels of hexane and toluene in blood. Toxicol Appl Pharmacol 256(3):399-404. http://doi.org/10.1016/j.taap.2011.07.022.

Karakaya A, Yucesoy B, Burgaz S, et al. 1996. Some immunological parameters in workers occupationally exposed to n-hexane. Hum Exp Toxicol 15(1):56-58. http://doi.org/10.1177/096032719601500110.

Kim IH, Yoon SH. 1990. Effect of extraction solvents on oxidative stability of crude soybean oil. J Am Oil Chem Sot 67(3):165-167. http://doi.org/10.1007/BF02539618.

Kimura ET, Ebert DM, Dodge PW. 1971. Acute toxicity and limits of solvent residue for sixteen organic solvents. Toxicol Appl Pharmacol 19(4):699-704. http://doi.org/10.1016/0041-008x(71)90301-2.

Kopczynski SL, Lonneman WA, Sutterfield FD, et al. 1972. Photochemistry of atmospheric samples in Los Angeles. Environ Sci Technol 6(4):342-347. http://doi.org/10.1021/es60063a005.

Krasavage WJ, O'Donoghue JL, DiVincenzo GD, et al. 1980. The relative neurotoxicity of methyl-n-butyl ketone, n-hexane and their metabolites. Toxicol Appl Pharmacol 52(3):433-441. http://doi.org/10.1016/0041-008x(80)90338-5.

Krishnan K, Andersen ME, Clewell HJ, et al. 1994. Physiologically-based pharmacokinetic modeling of chemical mixtures. In: Yang RSA, ed. Toxicology of chemical mixtures. New York, NY: Academic Press, 399-437.

Krotoszynski BK, Bruneau GM, Oweill HJ. 1979. Measurement of chemical inhalation exposure in urban population in the presence of endogenous effluents. J Anal Toxicol 3(6):225-234. http://doi.org/10.1093/jat/3.6.225.

Ladefoged O, Perbellini L. 1986. Acetone-induced changes in the toxicokinetics of 2,5-hexanedione in rabbits. Scand J Work Environ Health 12(6):627-629. http://doi.org/10.5271/sjweh.2092.

Ladefoged O, Hass U, Simonsen L. 1989. Neurophysiological and behavioural effects of combined exposure to 2,5-hexanedione and acetone or ethanol in rats. Pharmacol Toxicol 65(5):372-375. http://doi.org/10.1111/j.1600-0773.1989.tb01191.x.

Ladefoged O, Roswall K, Larsen JJ. 1994. Acetone potentiation and influence on the reversibility of 2,5-hexanedione-induced neurotoxicity studied with behavioural and morphometric methods in rats. Pharmacol Toxicol 74(4-5):294-299. http://doi.org/10.1111/j.1600-0773.1994.tb01114.x.

Lam CW, Galen TJ, Boyd JF, et al. 1990. Mechanism of transport and distribution of organic solvents in blood. Toxicol Appl Pharmacol 104(1):117-129. http://doi.org/10.1016/0041-008x(90)90287-5.

Lapadula DM, Irwin RD, Suwita E, et al. 1986. Cross-linking of neurofilament proteins of rat spinal cord in vivo after administration of 2,5-hexanedione. J Neurochem 46(6):1843-1850. http://doi.org/10.1111/j.1471-4159.1986.tb08503.x.

Latimer JS, Hoffman EJ, Hoffman G, et al. 1990. Sources of petroleum hydrocarbons in urban runoff. Water Air Soil Pollut 52:1-21. http://doi.org/10.1007/BF00283111.

Lawson H. 1995. Processing technology. In: Food oils and fats: Technology, utilization, and nutrition. New York, NY: Springer, 49-65. http://doi.org/10.1007/978-1-4757-2351-9.

Leahy JG, Colwell RR. 1990. Microbial degradation of hydrocarbons in the environment. Microbiol Rev 54(3):305-315. http://doi.org/10.1128/mr.54.3.305-315.1990.

Leeder JS, Kearns GL. 1997. Pharmacogenetics in pediatrics. Implications for practice. Pediatr Clin North Am 44(1):55-77. http://doi.org/10.1016/s0031-3955(05)70463-6.

Lehman EJ, Hein MJ. 2006. Mortality of workers employed in shoe manufacturing: an update. Am J Ind Med 49(7):535-546. http://doi.org/10.1002/ajim.20322.

Lehmann I, Thoelke A, Rehwagen M, et al. 2002. The influence of maternal exposure to volatile organic compounds on the cytokine secretion profile of neonatal T cells. Environ Toxicol 17(3):203-210. http://doi.org/10.1002/tox.10055.

Li H, Liu J, Sun Y, et al. 2014. N-hexane inhalation during pregnancy alters DNA promoter methylation in the ovarian granulosa cells of rat offspring. J Appl Toxicol 34(8):841-856. http://doi.org/10.1002/jat.2893.

Li H, Zhang C, Ni F, et al. 2015. Gestational n-hexane inhalation alters the expression of genes related to ovarian hormone production and DNA methylation states in adult female F1 rat offspring. Toxicol Lett 239(3):141-151. http://doi.org/10.1016/j.toxlet.2015.09.018.

Li X, Wang Q, Li M, et al. 2018. Hair pyrrole adducts serve as biomarkers for peripheral nerve impairment induced by 2,5-hexanedione and n-hexane in rats. pLoS ONE 13(12):e0209939. http://doi.org/10.1371/journal.pone.0209939.

Li X, Yu T, Wang S, et al. 2020a. Diallyl sulfide-induced attenuation of n-hexane-induced peripheral nerve impairment is associated with metabolic inhibition of n-hexane. Food Chem Toxicol 137:111167. http://doi.org/10.1016/j.fct.2020.111167.

Li X, Jiang L, Yu T, et al. 2020b. No-observed-adverse-effect level of hair pyrrole adducts in chronic n-hexane intoxication in rats. Neurotoxicology 78:11-20. http://doi.org/10.1016/j.neuro.2020.02.002.

Liu J, Huang HL, Pang F, et al. 2012. The effect of n-hexane on the gonad toxicity of female mice. Biomed Environ Sci 25(2):189-196. https://doi.org/10.3967/0895-3988.2012.02.010.

Lide DR. 2005. Hexane. In: CRC handbook of chemistry and physics. 74th ed. Boca Raton, FL: CRC Press Inc., 3-308.

Linder RE, Strader LF, Slott VL, et al. 1992. Endpoints of spermatotoxicity in the rat after short duration exposures to fourteen reproductive toxicants. Reprod Toxicol 6(6):491-505. http://doi.org/10.1016/0890-6238(92)90034-q.

Loden M. 1986. The in vitro permeability of human skin to benzene, ethylene glycol, formaldehyde, and n-hexane. Acta Pharmacol Toxicol (Copenh) 58(5):382-389. http://doi.org/10.1111/j.1600-0773.1986.tb00126.x.

Lungarella G, Barni-Comparini I, Fonzi L. 1984. Pulmonary changes induced in rabbits by long-term exposure to n-hexane. Arch Toxicol 55(4):224-228. http://doi.org/10.1007/BF00341015.

Lyman WJ, Reehl WF, Rosenblatt DH. 1982. Rate of hydrolysis. In: Handbook of chemical property estimation methods: Environmental behavior of organic compound. New York, NY: McGraw-Hill Book Company, 7-1 to 7-48.

Marks TA, Fisher PW, Staples RE. 1980. Influence of n-hexane on embryo and fetal development in mice. Drug Chem Toxicol 3(4):393-406. http://doi.org/10.3109/01480548009030128.

Mathews JM, Raymer JH, Etheridge AS, et al. 1997. Do endogenous volatile organic chemicals measured in breath reflect and maintain CYP2E1 levels in vivo? Toxicol Appl Pharmacol 146(2):255-260. http://doi.org/10.1006/taap.1997.8257.

Mayan O, Teixeira JP, Pires AF. 2001. Biological monitoring of n-hexane exposure in Portuguese shoe manufacturing workers. Appl Occup Environ Hyg 16(7):736-741. https://doi.org/10.1080/10473220116711.

Mayan O, Teixeira JP, Alves S, et al. 2002. Urinary 2,5 hexanedione as a biomarker of n-hexane exposure. Biomarkers 7(4):299-305. https://doi.org/10.1080/13547500210136796.

Mayer VW, Goin CJ. 1994. Induction of chromosome loss in yeast by combined treatment with neurotoxic hexacarbons and monoketones. Mutat Res 341(2):83-91. http://doi.org/10.1016/0165-1218(94)90090-6.

McCann MF. 1992. Occupational and environmental hazards in art. Environ Res 59(1):139-144. http://doi.org/10.1016/s0013-9351(05)80233-6.

McCarroll NE, Keech BH, Piper CE. 1981a. A microsuspension adaptation of the Bacillus subtilis "ret" assay. Environ Mutagen 3(6):607-616. http://doi.org/10.1002/em.2860030603.

McCarroll NE, Piper CE, Keech BH. 1981b. An E coli microsuspension assay for the detection of DNA damage induced by direct-acting agents and promutagens. Environ Mutagen 3(4):429-444. http://doi.org/10.1002/em.2860030404.

McClay K, Streger SH, Steffan RJ. 1995. Induction of toluene oxidation activity in Pseudomonas mendocina KRl and Pseudomonas sp. Strain ENVPCS by chlorinated solvents and alkanes. Appl Environ Microbial 61(9):3479-3481. http://doi.org/10.1128/aem.61.9.3479-3481.1995.

McKay WA, Turner MF, Jones BMR, et al. 1996. Emission of hydrocarbons from marine phytoplankton - Some results from controlled laboratory experiments. Atmos Environ 30(14):2583-2593. http://doi.org/10.1016/1352-2310(95)00433-5.

Mendola P, Wallace M, Liu D, et al. 2016. Air pollution exposure and preeclampsia among US women with and without asthma. Environ Res 148:248-255. http://doi.org/10.1016/j.envres.2016.04.004.

Montgomery JH. 1991. n-Hexane. In: Groundwater chemicals desk reference. Vol. 2. Ann Arbor: Lewis Publisher, 570-572.

Morgenroth E, Schroeder ED, Chang DP, et al. 1996. Nutrient limitation in a compost biofilter degrading hexane. J Air Waste Manag Assoc 46(4):300-308. http://doi.org/10.1080/10473289.1996.10467464.

Mortelmans K, Haworth S, Lawlor T, et al. 1986. Salmonella mutagenicity tests: II. Results from the testing of 270 chemicals. Environ Mutagen 8(Suppl 7):1-119. http://doi.org/10.1002/em.2860080703.

Mortensen B, Nilsen OG. 1998. Allometric species comparison of toluene and n-hexane metabolism: prediction of hepatic clearance in man from experiments with rodent liver S9 in a head space vial equilibration system. Pharmacol Toxicol 82(4):183-188. https://doi.org/10.1111/j.1600-0773.1998.tb01422.x.

Moschonas N, Glavas S. 1996. C3-C10 Hydrocarbons in the atmosphere of Athens, Greece. Atmos Environ 30(15):2769-7272. http://doi.org/10.1016/1352-2310(95)00488-2.

Mumtaz MM, Ray M, Crowell SR, et al. 2012a. Translational research to develop a human PBPK models tool kit-volatile organic compounds (VOCs). J Toxicol Environ Health A 75(1):6-24. https://doi.org/10.1080/15287394.2012.625546.

Mumtaz M, Fisher J, Blount B, et al. 2012b. Application of physiologically based pharmacokinetic models in chemical risk assessment. J Toxicol 2012:904603. https://doi.org/10.1155/2012/904603.

Murata K, Araki S, Yokoyama K, et al. 1994. Changes in autonomic function as determined by ECG R-R interval variability in sandal, shoe and leather workers exposed to n-hexane, xylene and toluene. Neurotoxicology 15(4):867-875.

Mustajbegovic J, Zuskin E, Schachter EN, et al. 2000. Respiratory findings in chemical workers exposed to low concentrations of organic and inorganic air pollutants. Am J Ind Med 38(4):431-440. http://doi.org/10.1002/1097-0274(200010)38:4<431::aid-ajim9>3.0.co;2-q.

Mutti A, Cavatorta A, Lommi G, et al. 1982a. Neurophysiological effects of long-term exposure to hydrocarbon mixtures. Arch Toxicol Suppl 5:120-124. http://doi.org/10.1007/978-3-642-68511-8_22.

Mutti A, Ferri F, Lommi G, et al. 1982b. n-Hexane-induced changes in nerve conduction velocities and somatosensory evoked potentials. Int Arch Occup Environ Health 51(1):45-54. http://doi.org/10.1007/BF00378409.

Mutti A, Falzoi M, Lucertini S, et al. 1984. n-Hexane metabolism in occupationally exposed workers. Br J Ind Med 41(4):533-538. http://doi.org/10.1136/oem.41.4.533.

Nakajima T, Elovaara E, Park SS, et al. 1991. Immunochemical detection of cytochrome P450 isozymes induced in rat liver by n-hexane, 2-hexanone and acetyl acetone. Arch Toxicol 65:542-547. http://doi.org/10.1007/BF01973714.

NAS/NRC. 2006. Human biomonitoring for environmental chemicals. Washington, DC: National Academy of Sciences/National Research Council. National Academy Press. https://doi.org/10.17226/11700.

Neghab M, Soleimani E, Khamoushian K. 2012. Electrophysiological studies of shoemakers exposed to sub-TLV levels of n-hexane. J Occup Health 54(5):376-382. http://doi.org/10.1539/joh.12-0029-fs.

Neligan RE. 1962. Hydrocarbons in the Los Angeles atmosphere. A comparison between the hydrocarbons in automobile exhaust and those found in the Los Angeles atmosphere. Arch Environ Health 5:581-591. http://doi.org/10.1080/00039896.1962.10663334.

Nelson KW, Ege JF, Ross M, et al. 1943. Sensory response to certain industrial solvent vapors. J Ind Hyg Toxicol 25:282-285.

NFPA. 1991. Hexane. Fire protection guide to hazardous materials. Quincy, MA: National Fire Protection Association, 325M-58.

NIEHS. 1987. Inhalation developmental toxicology studies: Teratology study of n-hexane in rats: Final report. Washington, DC: National Institute of Environmental Health Sciences. DE88006812. PNL-645. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/DE88006812.xhtml. October 20, 2022.

NIEHS. 1988a. Inhalation reproductive toxicology studies: Sperm morphology study of n-hexane in B6C3F1 mice. Washington, DC: National Institute of Environmental Health Sciences. DE89000262. PNL-667. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/DE89000262.xhtml. October 20, 2022.

NIEHS. 1988b. Inhalation reproductive toxicology studies: Male dominant lethal study of n-hexane in Swiss (CD-1) mice. Washington, DC: National Institute of Environmental Health Sciences. DE89000271. PNL-667. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/DE89000271.xhtml. October 20, 2022.

NIEHS. 1988c. Inhalation developmental toxicology studies: Teratology study of n-hexane in mice. Washington, DC: National Institute of Environmental Health Sciences. PB2003107682. PNL-6590. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/PB2003107682.xhtml. October 20, 2022.

Nijem K, Kristensen P, al-Khatib A, et al. 2000. Prevalence of self-reported health complaints among shoe workers of small workshop exposed to organic solvents in Hebron City, West Bank: A cross-sectional survey. Med Lav 91(3):206-216.

Nijem K, Kristensen P, Al-Khatib A, et al. 2001. Prevalence of neuropsychiatric and mucous membrane irritation complaints among Palestinian shoe factory workers exposed to organic solvents and plastic compounds. Am J Ind Med 40(2):192-198. http://doi.org/10.1002/ajim.1087.

NIOSH. 1995. Report to Congress on workers' home contamination study conducted under the Workers' Family Protection Act (29 U.S.C 671a). Cincinnati, OH: National Institute for Occupational Safety and Health. Publication No. 95-123. https://www.cdc.gov/niosh/docs/95-123/pdfs/95-123.pdf. February 16, 2023.

NIOSH. 1996. Method 2549. Volatile organics compounds (screening). NIOSH manual for analytical methods. National Institute for Occupational Safety and Health. https://www.cdc.gov/niosh/docs/2003-154/pdfs/2549.pdf. February 20, 2023.

NIOSH. 2019. n-Hexane. NIOSH pocket guide to chemical hazards. Atlanta, GA: National Institute for Occupational Safety and Health. RTECS No. MN9275000. https://www.cdc.gov/niosh/npg/npgd0322.html. January 30, 2023.

NLM. 2023. n-Hexane. PubChem. U.S. National Library of Medicine. https://pubchem.ncbi.nlm.nih.gov/compound/8058. February 20, 2023.

Nobles CJ, Williams A, Ouidir M, et al. 2019. Differential effect of ambient air pollution exposure on risk of gestational hypertension and preeclampsia. Hypertension 74(2):384-390. http://doi.org/10.1161/HYPERTENSIONAHA.119.12731.

NTP. 1991. Toxicity studies of n-hexane in B6C3F1 (inhalation studies). Research Triangle Park, NC: National Toxicology Program. PB91185322. NIH Publication No. 91-3121 https://ntp.niehs.nih.gov/ntp/htdocs/st_rpts/tox002.pdf. July 25, 2022.

NTP. 2013. Draft OHAT approach for systematic review and evidence integration for literature-based health assessments - February 2013. National Toxicology Program. https://ntp.niehs.nih.gov/ntp/ohat/evaluationprocess/draftohatapproach_february2013.pdf. February 20, 2023.

NTP. 2015. OHAT risk of bias rating tool for human and animal studies. National Toxicology Program. https://ntp.niehs.nih.gov/ntp/ohat/pubs/riskofbiastool_508.pdf. March 19, 2019.

NTP. 2021. CASRN index. Report on carcinogens. 15th ed. National Toxicology Program. https://ntp.niehs.nih.gov/pubhealth/roc/index-1.html#P. January 10, 2022.

Nylen P, Hagman M. 1994. Function of the auditory and visual systems, and of peripheral nerve, in rats after long-term combined exposure to n-hexane and methylated benzene derivatives. II. Xylene. Pharmacol Toxicol 74(2):124-129. http://doi.org/10.1111/j.1600-0773.1994.tb01086.x.

Nylen P, Ebendal T, Eriksdotter-Nilsson M, et al. 1989. Testicular atrophy and loss of nerve growth factor-immunoreactive germ cell line in rats exposed to n-hexane and a protective effect of simultaneous exposure to toluene or xylene. Arch Toxicol 63(4):296-307. http://doi.org/10.1007/BF00278643.

Nylen P, Hagman M, Johnson AC. 1994. Function of the auditory and visual systems, and of peripheral nerve, in rats after long-term combined exposure to n-hexane and methylated benzene derivatives. I. Toluene. Pharmacol Toxicol 74(2):116-123. http://doi.org/10.1111/j.1600-0773.1994.tb01085.x.

O'Leary P, Walsh P. 1995. Landfill gas: Movement, control, and uses. Waste Age 17(4):114-123.

Ong CN, Koh D, Foo SC, et al. 1993. Volatile organic solvents in correction fluids: identification and potential hazards. Bull Environ Contam Toxicol 50(6):787-793. http://doi.org/10.1007/BF00209939.

Ono Y, Takeuchi Y, Hisanaga N. 1981. A comparative study on the toxicity of n-hexane and its isomers on the peripheral nerve. Int Arch Occup Environ Health 48(3):289-294. http://doi.org/10.1007/BF00405616.

OSHA. 2010. Organic vapor sampling group 1 (OVSG-1): Carbon disulfide-extracted analytes collected on coconut charcoal sorbent tubes. Occupational Safety and Health Administration. OSHA Method 5000. https://www.osha.gov/sites/default/files/methods/5000.pdf. November 28, 2023.

OSHA. 2021a. Occupational safety and health standards. Subpart Z - Toxic and hazardous substances. Air contaminants. Table Z-1: Limits for air contaminants. Occupational Safety and Health Administration. Code of Federal Regulations. 29 CFR 1910.1000. https://www.govinfo.gov/content/pkg/CFR-2021-title29-vol6/pdf/CFR-2021-title29-vol6-sec1910-1000.pdf. August 28, 2022.

OSHA. 2021b. Occupational safety and health standards for shipyard employment. Subpart Z - Toxic and hazardous substances. Air contaminants. Occupational Safety and Health Administration. Code of Federal Regulations. 29 CFR 1915.1000. https://www.govinfo.gov/content/pkg/CFR-2021-title29-vol7/pdf/CFR-2021-title29-vol7-sec1915-1000.pdf. August 28, 2022.

OSHA. 2021c. Safety and health regulations for construction. Subpart D - Occupational health and environment controls. Gases, vapors, fumes, dusts, and mists. Occupational Safety and Health Administration. Code of Federal Regulations. 29 CFR 1926.55. https://www.govinfo.gov/content/pkg/CFR-2021-title29-vol8/pdf/CFR-2021-title29-vol8-sec1926-55.pdf. August 28, 2022.

Otson R, Fellin P, Tran Q. 1994. VOCs in representative Canadian residences. Atmos Environ 28(22):3563-3569. http://doi.org/10.1016/1352-2310(94)00201-U.

Paciencia I, Cavaleiro Rufo J, Silva D, et al. 2019. Exposure to indoor endocrine disrupting chemicals and childhood asthma and obesity. Allergy 74(7):1277-1291. http://doi.org/10.1111/all.13740.

Paciencia I, Cavaleiro Rufo J, Silva D, et al. 2020. Effects of indoor endocrine-disrupting chemicals on childhood rhinitis. J Investig Allergol Clin Immunol 30(3):195-197. http://doi.org/10.18176/jiaci.0471.

Park JB, Lee KJ, Lee KW, et al. 2009. Neurotoxic effect of occupational exposure to mixed organic solvents in Korea: posturographic study. Am J Ind Med 52(5):429-437. http://doi.org/10.1002/ajim.20679.

Pastore C, Marhuenda D, Marti J, et al. 1994. Early diagnosis of n-hexane-caused neuropathy. Muscle Nerve 17(9):981-986. http://doi.org/10.1002/mus.880170904.

Pastore C, Izura V, Marhuenda D, et al. 2002. Partial conduction blocks in n-hexane neuropathy. Muscle Nerve 26(1):132-135. http://doi.org/10.1002/mus.10183.

Patten CJ, Ning SM, Lu AY, et al. 1986. Acetone-inducible cytochrome P-450: purification, catalytic activity, and interaction with cytochrome b5. Arch Biochem Biophys 251(2):629-638. http://doi.org/10.1016/0003-9861(86)90373-5.

Paulson GW, Waylonis GW. 1976. Polyneuropathy due to n-hexane. Arch Intern Med 136(8):880-882. http://doi.org/10.1001/archinte.1976.03630080022008.

Pellizzari ED, Hartwell TD, Harris BS, et al. 1982. Purgeable organic compounds in mother's milk. Bull Environ Contam Toxicol 28(3):322-328. http://doi.org/10.1007/BF01608515.

PennWell. 1994. Motor gasoline produced from n-hexane fraction. In: Energy statistics sourcebook. 9th ed. Tulsa, OK: PennWell Publishing Company.

Perbellini L, Brugnone F, Faggionato G. 1981. Urinary excretion of the metabolites of n-hexane and its isomers during occupational exposure. Br J Ind Med 38(1):20-26. http://doi.org/10.1136/oem.38.1.20.

Perbellini L, Brugnone F, Caretta D, et al. 1985. Partition coefficients of some industrial aliphatic hydrocarbons (C5-C7) in blood and human tissues. Br J Ind Med 42(3):162-167. http://doi.org/10.1136/oem.42.3.162.

Perbellini L, Mozzo P, Brugnone F, et al. 1986. Physiologicomathematical model for studying human exposure to organic solvents: kinetics of blood/tissue n-hexane concentrations and of 2,5-hexanedione in urine. Br J Ind Med 43(11):760-768. http://doi.org/10.1136/oem.43.11.760.

Perbellini L, Marhuenda Amoros DM, Cardona Llorens AC, et al. 1990a. An improved method of analysing 2,5-hexanedione in urine. Br J Ind Med 47(6):421-424. http://doi.org/10.1136/oem.47.6.421.

Perbellini L, Mozzo P, Olivato D, et al. 1990b. "Dynamic" biological exposure indexes for n-hexane and 2,5-hexanedione, suggested by a physiologically based pharmacokinetic model. Am Ind Hyg Assoc J 51(7):356-362. http://doi.org/10.1080/15298669091369781.

Perbellini L, Pezzoli G, Brugnone F, et al. 1993. Biochemical and physiological aspects of 2,5-hexanedione: endogenous or exogenous product? Int Arch Occup Environ Health 65(1):49-52. http://doi.org/10.1007/BF00586058.

Periago JF, Cardona A, Marhuenda D, et al. 1993. Biological monitoring of occupational exposure to n-hexane by exhaled air analysis and urinalysis. Int Arch Occup Environ Health 65(4):275-278. http://doi.org/10.1007/BF00381203.

Perocco P, Bolognesi S, Alberghini W. 1983. Toxic activity of seventeen industrial solvents and halogenated compounds on human lymphocytes cultured in vitro. Toxicol Lett 16(1-2):69-75. http://doi.org/10.1016/0378-4274(83)90012-7.

Pezzoli G, Antonini A, Barbieri S, et al. 1995. n-Hexane-induced parkinsonism: pathogenetic hypotheses. Mov Disord 10(3):279-282. http://doi.org/10.1002/mds.870100308.

Potter TL. 1993. Analysis of petroleum contaminated soil and water: An overview. In: Calabrese EJ, Kostecki PT, eds. Principles and practices for petroleum contaminated soils. Ann Arbor, MI: Lewis Publishers, 1-14.

Pradhan S, Tandon R. 2015. N-hexane neuropathy with vertigo and cold allodynia in a silk screen printer: A case study. Int J Occup Med Environ Health 28(5):915-919. http://doi.org/10.13075/ijomeh.1896.00327.

Pryor GT, Rebert CS. 1992. Interactive effects of toluene and hexane on behavior and neurophysiologic responses in Fischer-344 rats. Neurotoxicology 13(1):225-234.

Pryor GT, Dickinson J, Howd RA, et al. 1983. Neurobehavioral effects of subchronic exposure of weanling rats to toluene or hexane. Neurobehav Toxicol Teratol 5(1):47-52.

Puri V, Chaudhry N, Tatke M. 2007. N-hexane neuropathy in screen printers. Electromyogr Clin Neurophysiol 47(3):145-152.

Pyle SJ, Amarnath V, Graham DG, et al. 1993. Decreased levels of the high molecular weight subunit of neurofilaments and accelerated neurofilament transport during the recovery phase of 2,5-hexanedione exposure. Cell Motil Cytoskeleton 26(2):133-143. http://doi.org/10.1002/cm.970260205.

Raitta C, Seppalainen AN, Huuskonen MS. 1978. N-hexane maculopathy in industrial workers. Albrecht Von Graefes Arch Klin Exp Ophthalmol 209(2):99-110. http://doi.org/10.1007/BF00407843.

Rastogi SC. 1993. Organic solvent levels in model and hobby glues. Bull Environ Contam Toxicol 51(4):501-507. http://doi.org/10.1007/BF00192164.

Raymer JH, Pellizzari ED. 1996. Estimation of recent exposures to volatile organic chemicals using alveolar air measurements. Toxicol Ind Health 12(2):201-210. http://doi.org/10.1177/074823379601200208.

Rebert C, Sorenson SS. 1983. Concentration-related effects of n-hexane on evoked responses from brain and peripheral nerve of the rat. Neurobehav Toxicol Teratol 5(1):69-76.

RePORTER. 2023. n-Hexane. Research Portfolio Online Reporting Tools. National Institutes of Health. https://reporter.nih.gov/. February 23, 2023.

Roberts MT, Etherington D. 1996. Hexane. In: Bookbinding and the conservation of books: A dictionary of descriptive terminology. Menlo Park, CA: Stanford University, 131.

Robertson P, White EL, Bus JS. 1989. Effects of methyl ethyl ketone pretreatment on hepatic mixed-function oxidase activity and on in vivo metabolism of n-hexane. Xenobiotica 19(7):721-729. http://doi.org/10.3109/00498258909042310.

Rooney AA, Boyles AL, Wolfe MS, et al. 2014. Systematic review and evidence integration for literature-based environmental health science assessments. Environ Health Perspect 122(7):711-718. http://doi.org/10.1289/ehp.1307972.

Rosenberg E, Legmann R, Kushmaro A, et al. 1992. Petroleum bioremediation- a multiphase problem. Biodegradation 3(2-3):337-350. http://doi.org/10.1007/bf00129092.

Ruiz P, Ray M, Fisher J, et al. 2011. Development of a human Physiologically Based Pharmacokinetic (PBPK) Toolkit for environmental pollutants. Int J Mol Sci 12(11):7469-7480. https://doi.org/10.3390/ijms12117469.

Ruiz-García L, Figueroa-Vega N, Malacara JM, et al. 2020. Possible role of n-hexane as an endocrine disruptor in occupationally exposed women at reproductive age. Toxicol Lett 330:73-79. http://doi.org/10.1016/j.toxlet.2020.04.022.

Sack TM, Steele DH, Hammerstrom K, et al. 1992. A survey of household products for volatile organic compounds. Atmos Environ 26A:1063-1070. http://doi.org/10.1016/0960-1686(92)90038-M.

Sallmen M, Neto M, Mayan ON. 2008. Reduced fertility among shoe manufacturing workers. Occup Environ Med 65(8):518-524. http://doi.org/10.1136/oem.2007.032839.

Sanagi S, Seki Y, Sugimoto K, et al. 1980. Peripheral nervous system functions of workers exposed to n-hexane at a low level. Int Arch Occup Environ Health 47(1):69-79. http://doi.org/10.1007/BF00378330.

Sauer TC, Sackett WM, Jeffrey LM. 1978. Volatile liquid hydrocarbons in the surface coastal waters of the Gulf of Mexico. Mar Chem 7(1):1-16. http://doi.org/10.1016/0304-4203(78)90039-7.

Sauer TC, Brown JS, Boehm PD, et al. 1993. Hydrocarbon source identification and weathering characterization of intertidal and subtidal sediments along the Saudi Arabian coast after the Gulf War Oil Spill. Mar Pollut Bull 27:117-134. http://doi.org/10.1016/0025-326X(93)90016-D.

Schaumburg HH, Spencer PS. 1976. Degeneration in central and peripheral nervous systems produced by pure n-hexane: an experimental study. Brain 99(2):183-192. http://doi.org/10.1093/brain/99.2.183.

Sendur OF, Turan Y, Bal S, et al. 2009. Toxic neuropathy due to n-hexane: report of three cases. Inhal Toxicol 21(3):210-214. http://doi.org/10.1080/08958370802311169.

Seppalainen AM, Raitta C, Huuskonen MS. 1979. n-Hexane-induced changes in visual evoked potentials and electroretinograms of industrial workers. Electroencephalogr Clin Neurophysiol 47(4):492-498. http://doi.org/10.1016/0013-4694(79)90165-2.

Shaw DG, Hogan TE, McIntosh DJ. 1986. Hydrocarbons in the sediments of Port Valdez, Alaska: Consequences of five years' permitted discharge. Estuarine Coastal Shelf Sci 21:131-144. http://doi.org/10.1016/0272-7714(85)90093-9.

Shibata E, Johanson G, Löf A, et al. 2002. Changes in n-hexane toxicokinetics in short-term single exposure due to co-exposure to methyl ethyl ketone in volunteers. Int Arch Occup Environ Health 75(6):399-405. http://doi.org/10.1007/s00420-002-0325-5.

Sliwinska-Kowalska M, Zamyslowska-Szmytke E, Szymczak W, et al. 2005. Exacerbation of noise-induced hearing loss by co-exposure to workplace chemicals. Environ Toxicol Pharmacol 19(3):547-553. http://doi.org/10.1016/j.etap.2004.12.018.

Smith AG, Albers JW. 1997. n-Hexane neuropathy due to rubber cement sniffing. Muscle Nerve 20(11):1445-1450. http://doi.org/10.1002/(sici)1097-4598(199711)20:11<1445::aid-mus13>3.0.co;2-0.

Sobue T, Iida M, Yamamura Y, et al. 1978. n-Hexane polyneuropathy. Int J Neurol 11:317-330.

Speight JG. 2006. Introduction to refining processes. In: The chemistry and technology of petroleum. 2nd ed. New York, NY: M. Dekker, 430-431.

Spencer PS, Schaumburg HH. 1977a. Ultrastructural studies of the dying-back process. III. The evolution of experimental peripheral giant axonal degeneration. J Neuropathol Exp Neurol 36(2):276-299. http://doi.org/10.1097/00005072-197703000-00005.

Spencer PS, Schaumburg HH. 1977b. Ultrastructural studies of the dying-back process. IV. Differential vulnerability of PNS and CNS fibers in experimental central-peripheral distal axonopathies. J Neuropathol Exp Neurol 36(2):300-320. http://doi.org/10.1097/00005072-197703000-00006.

Spencer PS, Schaumburg HH, Sabri MI, et al. 1980. The enlarging view of hexacarbon neurotoxicity. Crit Rev Toxicol 7(4):279-356. http://doi.org/10.3109/10408448009037489.

SRI. 1988. n-Hexane. In: 1988 Directory of chemical producers: United States of America. Menlo Park, CA: SRI International, 682-683.

SRI. 1990. n-Hexane. In: 1990 Directory of chemical producers: United States of America. Menlo Park, CA: SRI International, 691.

SRI. 1992. n-Hexane. In: 1992 Directory of chemical producers: United States of America. Menlo Park, CA: SRI International, 693.

SRI. 1994. n-Hexane. In: 1994 Directory of chemical producers: United States of America. Menlo Park, CA: SRI International, 672.

SRI. 1995. n-Hexane. In: 1995 Directory of chemical producers: United States of America. Menlo Park, CA: SRI International, 667.

SRI. 1996. n-Hexane. In: 1996 Directory of chemical producers: United States of America. Menlo Park, CA: SRI International, 657.

Srinivas H, Swamylingappa B, Chand N. 1992. Secondary extraction of soybeans using hexane-acetic acid: Effect on beany flavor removal and physiochemical properties. J Agric Food Chem 40:276-279. http://doi.org/10.1021/jf00014a022.

Stelljes ME, Watkin GE. 1993. Comparison of environmental impacts posed by different hydrocarbon mixtures: A need for site-specific composition analyses. In: Kostecki PT, Calabrese EJ, eds. Hydrocarbon contaminated soils and groundwater. Vol. 3. Chelsea, MI: Lewis Publishers, 549-569.

Stevens P. 1988. Refining. In: Oil and gas dictionary. New York, NY: Nichols Publishing, 174-178.

Stoltenburg-Didinger G, Altenkirch H, Wagner M. 1990. Neurotoxicity of organic solvent mixtures: embryotoxicity and fetotoxicity. Neurotoxicol Teratol 12(6):585-589. http://doi.org/10.1016/0892-0362(90)90066-l.

Sun Y, Wu X, Chen J, et al. 2020. The effect of rehabilitation in patients with polyneuropathy induced by occupational intoxication with n-hexane: a report of 9 cases. Ann Palliat Med 9(6):4179-4186. http://doi.org/10.21037/apm-20-2176.

Sundell J. 1996. What we know, and don't know, about sick building syndrome. ASHRAE Journal 38(6):51-57.

Swann RL, Laskowski DA, McCall PJ, et al. 1983. A rapid method for the estimation of the environmental parameters octanol/water partition coefficient, soil sorption constant, water to air ratio, and water solubility. Residue Rev 85:17-28. http://doi.org/10.1007/978-1-4612-5462-1_3.

Sweeney LM, Gearhart JM. 2020. Examples of physiologically based pharmacokinetic modeling applied to risk assessment. In: Fisher JW, Gearhart JM, Lin Z, eds. Physiologically based pharmacokinetic (PBPK) modeling. Academic Press: 281-299. https://doi.org/10.1016/B978-0-12-818596-4.00011-4.

Takeuchi Y, Ono Y, Hisanaga N, et al. 1980. A comparative study on the neurotoxicity of n-pentane, n-hexane, and n-heptane in the rat. Br J Ind Med 37(3):241-247. http://doi.org/10.1136/oem.37.3.241.

Takeuchi Y, Ono Y, Hisanaga N, et al. 1983. An experimental study of the combined effects of n-hexane and methyl ethyl ketone. Br J Ind Med 40(2):199-203. https://doi.org/10.1136/oem.40.2.199.

Takeuchi Y, Hisanaga N, Ono Y, et al. 1993. Modification of metabolism and neurotoxicity of hexane by co-exposure of toluene. Int Arch Occup Environ Health 65(1 Suppl):S227-230. http://doi.org/10.1007/BF00381347.

Talbott EO, Marshall LP, Rager JR, et al. 2015. Air toxics and the risk of autism spectrum disorder: the results of a population based case-control study in southwestern Pennsylvania. Environ Health 14:80. http://doi.org/10.1186/s12940-015-0064-1.

Tan YM, Chan M, Chukwudebe A, et al. 2020. PBPK model reporting template for chemical risk assessment applications. Regul Toxicol Pharmacol 115:104691. https://doi.org/10.1016/j.yrtph.2020.104691.

Thulasirajah S, Michaud J, Doja A, et al. 2020. Inhaled solvent abuse mimicking chronic inflammatory demyelinating polyradiculoneuropathy. Child Neurol Open 7:2329048x20934914. http://doi.org/10.1177/2329048x20934914.

Toftgard R, Halpert J, Gustafsson JA. 1983. Xylene induces a cytochrome P-450 isozyme in rat liver similar to the major isozyme induced by phenobarbital. Mol Pharmacol 23(1):265-271.

Toftgard R, Haaparanta T, Eng L, et al. 1986. Rat lung and liver microsomal cytochrome P-450 isozymes involved in the hydroxylation of n-hexane. Biochem Pharmacol 35(21):3733-3738. http://doi.org/10.1016/0006-2952(86)90658-1.

Tomei F, Giuntoli P, Biagi M, et al. 1999. Liver damage among shoe repairers. Am J Ind Med 36(5):541-547. http://doi.org/10.1002/(sici)1097-0274(199911)36:5<541::aid-ajim6>3.0.co;2-4.

TRI21. 2023. n-Hexane. TRI explorer: Providing access to EPA's toxics release inventory data. Washington, DC: U.S. Environmental Protection Agency. https://www.epa.gov/enviro/tri-search. January 23, 2023.

Tsai SY, Chen JD, Chao WY, et al. 1997. Neurobehavioral effects of occupational exposure to low-level organic solvents among Taiwanese workers in paint factories. Environ Res 73(1-2):146-155. http://doi.org/10.1006/enrs.1997.3704.

Umicevic N, Kotur-Stevuljevic J, Paleksic V, et al. 2022. Liver function alterations among workers in the shoe industry due to combined low-level exposure to organic solvents. Drug Chem Toxicol 45(4):1907-1914. http://doi.org/10.1080/01480545.2021.1894703.

USITC. 1994. Synthetic organic chemical: United States production and sales, 1992. Washington, DC: U.S. International Trade Commission. USITC Publication 2720. https://usitc.gov/publications/332/pub2720.pdf. February 17, 2023.

Valentino M. 1996. Residual electroneurographic modifications in subjects with n-hexane induced polyneuropathy: a follow-up study. Med Lav 87(4):289-296.

van Engelen JG, Rebel-de Haan W, Opdam JJ, et al. 1997. Effect of coexposure to methyl ethyl ketone (MEK) on n-hexane toxicokinetics in human volunteers. Toxicol Appl Pharmacol 144(2):385-395. http://doi.org/10.1006/taap.1997.8149.

Van Ert MD, Arp EW, Harris RL, et al. 1980. Worker exposures to chemical agents in the manufacture of rubber tires: solvent vapor studies. Am Ind Hyg Assoc J 41(3):212-219. http://doi.org/10.1080/15298668091424627.

Vanacore N, Gasparini M, Brusa L, et al. 2000. A possible association between exposure to n-hexane and parkinsonism. Neurol Sci 21(1):49-52. http://doi.org/10.1007/s100720070118.

Verberk MM, Brons JT, Salle HJ. 2004. Visual evoked potentials in workers with chronic solvent encephalopathy. Int Arch Occup Environ Health 77(5):328-334. http://doi.org/10.1007/s00420-004-0511-8.

Verma DK, Shaw DS, McLean JD. 1992. An evaluation of airborne concentrations of selected hydrocarbons at an oil refinery land reclamation operation. Ann Occup Hyg 36(3):307-315. http://doi.org/10.1093/annhyg/36.3.307.

Veulemans H, Van Vlem E, Janssens H, et al. 1982. Experimental human exposure to n-hexane. Study of the respiratory uptake and elimination, and of n-hexane concentrations in peripheral venous blood. Int Arch Occup Environ Health 49(3-4):251-263. http://doi.org/10.1007/BF00377934.

Veulemans H, Groeseneken D, Masschelein R, et al. 1987. Survey of ethylene glycol ether exposure in Belgian industries and workshops. Am Ind Hyg Assoc J 48(8):671-676. http://doi.org/10.1080/15298668791385390.

Villeneuve PJ, Jerrett M, Su J, et al. 2013. A cohort study of intra-urban variations in volatile organic compounds and mortality, Toronto, Canada. Environ Pollut 183:30-39. http://doi.org/10.1016/j.envpol.2012.12.022.

Wadden RA, Scheff PA, Franke JE, et al. 1995. VOC emission rates and emission factors for a sheetfed offset printing shop. Am Ind Hyg Assoc J 56(4):368-376. http://doi.org/10.1080/15428119591016999.

Wahlberg JE. 1984. Erythema-inducing effects of solvents following epicutaneous administration to man-studied by laser Doppler flowmetry. Scand J Work Environ Health 10(3):159-162. http://doi.org/10.5271/sjweh.2351.

Wahlberg JE, Boman A. 1979. Comparative percutaneous toxicity of ten industrial solvents in the guinea pig. Scand J Work Environ Health 5(4):345-351. http://doi.org/10.5271/sjweh.2647.

Wanasundara JPD, Shahidi F. 1994. Alkanol-ammonia-water/hexane extraction of flaxseed. Food Chem 49(1):39-44. http://doi.org/10.1016/0308-8146(94)90230-5.

Wang JD, Chang YC, Kao KP, et al. 1986. An outbreak of n-hexane induced polyneuropathy among press proofing workers in Taipei. Am J Ind Med 10(2):111-118. http://doi.org/10.1002/ajim.4700100202.

Wang S, Li M, Wang X, et al. 2017. Diallyl trisulfide attenuated n-hexane induced neurotoxicity in rats by modulating P450 enzymes. Chem Biol Interact 265:1-7. http://doi.org/10.1016/j.cbi.2017.01.013.

Watts JO, Holswade W. 1967. Gas chromatographic determination of residual hydrocarbon solvents in solvent-extracted edible oils. J AOAC Int 50(3):717-726. http://doi.org/10.1093/jaoac/50.3.717.

WHO. 1991. n-Hexane. Geneva, Switzerland: World Health Organization. Environmental Health Criteria 122. https://apps.who.int/iris/bitstream/handle/10665/41283/9241571225-eng.pdf?sequence=1&isAllowed=y. February 17, 2023.

WHO. 2010. Guidelines for indoor air quality: Selected pollutants. World Health Organization. http://www.euro.who.int/__data/assets/pdf_file/0009/128169/e94535.pdf. April 25, 2012.

WHO. 2022. Guidelines for drinking-water quality. Fourth edition incorporating the first and second addenda. World Health Organization. https://www.who.int/publications/i/item/9789240045064. June 22, 2022.

Wichmann FA, Muller A, Busi LE, et al. 2009. Increased asthma and respiratory symptoms in children exposed to petrochemical pollution. J Allergy Clin Immunol 123(3):632-638. http://doi.org/10.1016/j.jaci.2008.09.052.

Wilson MP, Hammond SK, Nicas M, et al. 2007. Worker exposure to volatile organic compounds in the vehicle repair industry. J Occup Environ Hyg 4(5):301-310. http://doi.org/10.1080/15459620701258021.

Winer AM, Arey J, Atkinson R, et al. 1992. Emission rates of organics from vegetation in California's Central Valley. Atmos Environ A Gen Top 26(14):2647-2659. http://doi.org/10.1016/0960-1686(92)90116-3.

Yamamura Y. 1969. n-Hexane polyneuropathy. Folia Psychiatr Neurol Jpn 23(1):45-57. http://doi.org/10.1111/j.1440-1819.1969.tb01441.x.

Yin H, Zhang C, Guo Y, et al. 2014. Biological exposure indices of pyrrole adducts in serum and urine for hazard assessment of n-hexane exposure. pLoS ONE 9(1):e86108. https://doi.org/10.1371/journal.pone.0086108.

Yokoyama K, Feldman RG, Sax DS, et al. 1990. Relation of distribution of conduction velocities to nerve biopsy findings in n-hexane poisoning. Muscle Nerve 13(4):314-320. http://doi.org/10.1002/mus.880130406.

Yokoyama K, Araki S, Murata K, et al. 1997. Postural sway frequency analysis in workers exposed to n-hexane, xylene, and toluene: assessment of subclinical cerebellar dysfunction. Environ Res 74(2):110-115. http://doi.org/10.1006/enrs.1997.3769.

Yu RC, Hattis D, Landaw EM, et al. 2002. Toxicokinetic interaction of 2,5-hexanedione and methyl ethyl ketone. Arch Toxicol 75(11-12):643-652. http://doi.org/10.1007/s00204-001-0298-2.

Yücesoy B, Yücel A, Erdem O, et al. 1999. Effects of occupational chronic co-exposure to n-hexane, toluen, and methyl ethyl ketone on NK cell activity and some immunoregulatory cytokine levels in shoe workers. Hum Exp Toxicol 18(9):541-546. http://doi.org/10.1191/096032799678845115.

Zhang Y, Liu Q, Liu Q, et al. 2006. Study on the damage of peripheral nerve in the workers exposed to n-hexane. Biomarkers 11(1):61-69. http://doi.org/10.1080/13547500500451176.

Zhao W, Misumi J, Yasui T, et al. 1998. Effects of methyl ethyl ketone, acetone, or toluene coadministration on 2,5-hexanedione concentration in the sciatic nerve, serum, and urine of rats. Int Arch Occup Environ Health 71(4):236-244. http://doi.org/10.1007/s004200050275.

Zhu X, Suidan MT, Pruden A, et al. 2004. Effect of substrate Henry''s constant on biofilter performance. J Air Waste Manag Assoc 54(4):409-418. https://doi.org/10.1080/10473289.2004.10470918.

Zielinska B, Sagebiel JC, Harshfield G, et al. 1996. Volatile organic compounds up to C20 emitted from motor vehicles: Measurement methods. Atmos Environ 30(12):2269-2286. http://doi.org/10.1016/1352-2310(95)00116-6.

# APPENDIX A. ATSDR MINIMAL RISK LEVEL WORKSHEETS

MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration for a given route of exposure. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified route and duration of exposure. MRLs are based on noncancer health effects only; cancer effects are not considered. These substance-specific estimates, which are intended to serve as screening levels, are used by ATSDR health assessors to identify contaminants and potential health effects that may be of concern at hazardous waste sites. It is important to note that MRLs are not intended to define clean-up or action levels.

MRLs are derived for hazardous substances using the NOAEL/uncertainty factor approach. They are below levels that might cause adverse health effects in the people most sensitive to such chemical-induced effects. MRLs are derived for acute (1–14 days), intermediate (15–364 days), and chronic (≥365 days) durations and for the oral and inhalation routes of exposure. Currently, MRLs for the dermal route of exposure are not derived because ATSDR has not yet identified a method suitable for this route of exposure. MRLs are generally based on the most sensitive substance-induced endpoint considered to be of relevance to humans. LOAELs for serious health effects (such as irreparable damage to the liver or kidneys, or serious birth defects) are not used as a basis for establishing MRLs. Exposure to a level above the MRL does not mean that adverse health effects will occur.

MRLs are intended only to serve as a screening tool to help public health professionals decide where to look more closely. They may also be viewed as a mechanism to identify those hazardous waste sites that are not expected to cause adverse health effects. Most MRLs contain a degree of uncertainty because of the lack of precise toxicological information on the people who might be most sensitive (e.g., infants, elderly, nutritionally or immunologically compromised) to the effects of hazardous substances. ATSDR uses a conservative (i.e., protective) approach to address this uncertainty consistent with the public health principle of prevention. Although human data are preferred, MRLs often must be based on animal studies because relevant human studies are lacking. In the absence of evidence to the contrary, ATSDR assumes that humans are more sensitive to the effects of hazardous substances than animals and that certain persons may be particularly sensitive. Thus, the resulting MRL may be as much as 100-fold below levels that have been shown to be nontoxic in laboratory animals.

APPENDIX A

Proposed MRLs undergo a rigorous review process: Health Effects/MRL Workgroup reviews within the Office of Innovation and Analytics, Toxicology Section, expert panel peer reviews, and agency-wide MRL Workgroup reviews, with participation from other federal agencies and comments from the public. They are subject to change as new information becomes available concomitant with updating the toxicological profiles. Thus, MRLs in the most recent toxicological profiles supersede previously published MRLs. For additional information regarding MRLs, please contact the Office of Innovation and Analytics, Toxicology Section, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road NE, Mailstop S106-5, Atlanta, Georgia 30329-4027.

APPENDIX A

# MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** *n*-Hexane
***CAS Number:*** 110-54-3
***Date:*** May 2024
***Profile Status:*** Draft for Public Comment
***Route:*** Inhalation
***Duration:*** Acute
***MRL*:** 6 ppm (21 mg/m$^3$) (provisional)
***Critical Effects:*** Decreased fetal body weight
***Reference:*** NIEHS 1987
***Point of Departure:*** NOAEL of 200 ppm ($NOAEL_{HEC}$ of 167 ppm)
***Uncertainty Factor:*** 30
***LSE Graph Key:*** 9
***Species:*** Rat

***MRL Summary:*** A provisional acute-duration inhalation MRL of 6 ppm was derived for *n*-hexane based on a NOAEL of 200 ppm and a LOAEL of 1,000 ppm for developmental effects (7.5% decrease in male fetal body weight) in rats exposed on GDs 6–19 (14 days) for 20 hours/day (NIEHS 1987). The NOAEL was adjusted to continuous duration exposure, converted to a human equivalent concentration ($NOAEL_{HEC}$) of 167 ppm, and divided by a total uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustments and 10 for human variability).

***Selection of the Critical Effect:*** Several studies examined the acute-duration inhalation toxicity of *n*-hexane. A summary of the identified LOAELs is presented in Table A-1. Exposure to 1,000 ppm resulted in developmental effects, while exposure to 5,000 ppm resulted in body weight, neurological, developmental, and reproductive effects. The lowest LOAELs were 1,000 ppm for decreased litter weight and fetal body weight (Bus et al. 1979; NIEHS 1987).

***Selection of the Principal Study:*** Bus et al. (1979) and NIEHS (1987) both identified LOAELs of 1,000 ppm for decreased fetal/litter weights. In the Bus et al. (1979) study, a 13.9% decrease in mean litter weight at 3 weeks after birth was observed in the offspring of rats exposed to 1,000 ppm *n*-hexane 6 hours/day on GDs 8–16. No differences were observed in birth weight or pup body weight 7 weeks after birth. In the NIEHS (1987) study, a 7.5% decrease in male fetal body weight was observed in the offspring of rats exposed to 1,000 ppm 20 hours/day on GDs 6–19; a NOAEL of 200 ppm was also identified.

Adjusting the durations to continuous exposure results in a $LOAEL_{ADJ}$ of 250 ppm for Bus et al. (1979) study and a $NOAEL_{ADJ}$ of 167 ppm and $LOAEL_{ADJ}$ of 833 ppm for NIEHS (1987) study. The NIEHS (1987) study was selected as the principal study because it identified a NOAEL for the most sensitive endpoint, used multiple doses, exposed animals for 20 hours/day (close to a continuous exposure), and examined a larger number of litters (23–28/group versus 8–14/group in the Bus et al. [1979] study).

**Table A-1. Summary of NOAEL and LOAEL Values Following Acute-Duration Inhalation Exposure to *n*-Hexane**

| Species (strain, sex) | Duration | NOAEL ($NOAEL_{ADJ}$) (ppm) | LOAEL ($LOAEL_{ADJ}$) (ppm) | Effect | Reference |
|---|---|---|---|---|---|
| Developmental effects | | | | | |
| Rats (Fischer-344, F) | 9 days (GDs 8–16) 6 hours/day | ND | 1,000 (250) | Decreased litter weight (13.9% at 3 weeks after birth) | Bus et al. 1979 |
| Rats (Sprague-Dawley, F) | 14 days (GDs 6–19) 20 hours/day | 200 (167) | 1,000 (833) | Decreased fetal body weight (7.5% in male offspring) | NIEHS 1987 |
| Mice (Swiss, F) | 12 days (GDs 6–17) 20 hours/day | ND | 5,000 (4,167) | Decreased number of live fetuses per litter, increased incidence of late resorptions | NIEHS 1988c |
| Reproductive effects | | | | | |
| Rats (Sprague-Dawley, M) | 2 weeks 6 days/week 16 hours/day | ND | 5,000 (2,857) | Testicular lesions (spermatocyte necrosis, exfoliation of spermatids, and Sertoli cell vacuolization) | De Martino et al. 1987 |
| Rats (Sprague-Dawley, M) | 8 days 16 hours/day | ND | 5,000 (3,333) | Testicular lesions (degeneration of spermatocytes, exfoliation of elongated spermatids, and Sertoli cell vacuolization) | De Martino et al. 1987 |
| Rats (Sprague-Dawley, M) | 24 hours Continuous | ND | 5,000 | Testicular lesions (focal degeneration of spermatocytes and mild exfoliation of elongated spermatids) | De Martino et al. 1987 |
| Neurological effects | | | | | |
| Rats (Sprague-Dawley, M) | 2 weeks 6 days/week 16 hours/day | ND | 5,000 (2,857) | Decreased motor conduction velocity | De Martino et al. 1987 |
| Body weight effects | | | | | |
| Rats (Sprague-Dawley, M) | 2 weeks 6 days/week 16 hours/day | ND | 5,000 (2,857) | Decreased body weight (20–30%) (serious LOAEL) | De Martino et al. 1987 |
| Rats (Sprague-Dawley, F) | 14 days (GDs 6–19) 20 hours/day | 1,000 (833) | 5,000 (4,167) | Decreased body weight (10% in pregnant dams, 12% in virgin females) | NIEHS 1987 |

ADJ = adjusted for continuous exposure; F = females; GD = gestation day; LOAEL = lowest-observed-adverse-effect level; M = males; ND = not determined; NOAEL = no-observed-adverse-effect level

***Summary of the Principal Study:***

NIEHS. 1987. Inhalation developmental toxicology studies: Teratology study of n-hexane in rats: Final report. Washington, DC: National Institute of Environmental Health Sciences. DE88006812. PNL-645. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/DE88006812.xhtml. October 20, 2022.

Timed-pregnant (30/group) and virgin (10/group) Sprague-Dawley rats were exposed to 0, 200, 1,000, or 5,000 ppm *n*-hexane vapor for 20 hours/day for 14 days (GDs 6–19 for pregnant females). All animals were observed daily for mortality, morbidity, and overt signs of toxicity. Adult body weights were monitored throughout the study and at sacrifice (GD 20 for pregnant rats). Uterine, placental, and fetal body weights were measured from pregnant females. Reproductive/developmental parameters evaluated included number of implants, early or late resorptions, number of live fetuses, number of dead fetuses, sex ratios, fetal weight, and malformations.

No maternal deaths or clinical signs of toxicity were observed. Statistically significantly decreased body weights were observed in pregnant and virgin females at 5,000 ppm. Extra-gestational body weight gain (weight gain minus the weight of the gravid uterus) was also statistically significantly decreased at 5,000 ppm. Exposure to *n*-hexane had no effect on the number of implantations, live pups per litter, resorptions per litter, fetal sex ratio, intrauterine death rate, or fetal or skeletal malformations. Fetal body weights were decreased at 1,000 ppm (7.5% for male offspring). At 5,000 ppm, decreased body weights were observed in males and females combined (15%), in males only (15%) and in females only (14%) (Table A-2).

**Table A-2. Average Fetal Weights Following Maternal Inhalation Exposure to *n*-Hexane**

| Exposure concentration | 0 | 200 ppm | 1,000 ppm | 5,000 ppm |
|---|---|---|---|---|
| Fetuses examined | 339 | 350 | 392 | 408 |
| Sex ratio (M/F) | 0.53±0.14[a] | 0.48±0.11 | 0.46±0.17 | 0.54±0.14 |
| Fetal body weight (g) | 3.48±0.37 | 3.54±0.36 | 3.27±0.32[b] | 2.97±0.38[b] |
| Male fetal body weight (g) | 3.60±0.39 | 3.66±0.39 | 3.33±0.33[b] | 3.05±0.41[b] |
| Female fetal body weight (g) | 3.33±0.37 | 3.43±0.37 | 3.23±0.32 | 2.86±0.36[b] |

[a]Mean±standard deviation.
[b]Statistically significantly different from controls at p<0.05.

F = female; M = male

Source: NIEHS 1987

***Selection of the Point of Departure for the MRL:*** Benchmark dose (BMD) modeling of the male fetal body weight data could not be attempted because the number of male fetuses was not reported. The NOAEL of 200 ppm for developmental effects (decreased male fetal body weights) in rats exposed for 20 hours/day on GDs 6–19 (NIEHS 1987) was selected as the point of departure (POD) for the MRL.

***Adjustment for Intermittent Exposure:*** The intermittent 20 hours/day NOAEL of 200 ppm was adjusted to a 24-hour continuous exposure using the following equation:

$$NOAEL_{ADJ} = NOAEL \times \frac{20\ hours}{24\ hours} = 200\ ppm \times \frac{20\ hours}{24\ hours} = 167\ ppm$$

***Human Equivalent Concentration:*** The human equivalent concentration (HEC) was calculated by multiplying the $NOAEL_{ADJ}$ by the ratio of the *n*-hexane air:blood partition coefficient for humans and rats. The reported blood:gas (air) partition coefficient ($H_{b/g}$) values for *n*-hexane are 2.29 for rats (Gargas et al. 1989) and 0.8 for humans (Perbellini et al. 1985). Since the ratio of the rat to human blood:gas (air) partition coefficients is >1, a default value of 1 was used.

$$NOAEL_{HEC} = NOAEL_{ADJ} \times \frac{(H_{b/g})_A}{(H_{b/g})_H} = 167\ ppm \times 1 = 167\ ppm$$

***Uncertainty Factor:*** The $NOAEL_{HEC}$ was divided by a total uncertainty factor of 30:

- 3 for extrapolation from animals to humans with dosimetric adjustments
- 10 for human variability

$$MRL = NOAEL_{HEC} \div UFs$$
$$167\ ppm \div 30 = 5.56\ ppm \approx 6\ ppm$$

***Other Additional Studies or Pertinent Information that Lend Support to this MRL:*** Selection of decreased fetal weight in rats as the critical effect is supported by several other studies using similar or higher concentrations or longer durations that also observed effects on fetal weight (Bus et al. 1979; Stoltenburg-Didinger et al. 1990). Additionally, a positive association was observed between ambient *n*-hexane exposure (represented as a unitless exposure intensity) and low birth weight (OR 1.06) in a case-control study in Texas (Gong et al. 2018).

***Agency Contacts (Chemical Managers):*** Obaid Faroon

APPENDIX A

# MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** *n*-Hexane
***CAS Number:*** 110-54-3
***Date:*** May 2024
***Profile Status:*** Draft for Public Comment
***Route:*** Inhalation
***Duration:*** Intermediate
***MRL*:** 0.4 ppm (1.4 $mg/m^3$) (provisional)
***Critical Effects:*** Lesions in the nasal cavity (multifocal regeneration and metaplasia in olfactory epithelium)
***Reference:*** NTP 1991
***Point of Departure:*** LOAEL of 1,099 ppm ($LOAEL_{HEC}$ of 111 ppm)
***Uncertainty Factor:*** 300
***LSE Graph Key:*** 43
***Species:*** Mouse

***MRL Summary:*** A provisional intermediate-duration inhalation MRL of 0.4 ppm was derived for *n*-hexane based on a LOAEL of 1,099 ppm for respiratory effects (nasal cavity lesions) in mice exposed for 22 hours/day, 5 days/week for 13 weeks (NTP 1991). The LOAEL was duration adjusted to continuous duration exposure, converted to a human equivalent concentration ($LOAEL_{HEC}$) of 111 ppm, and divided by a total uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation from animals to humans with dosimetric adjustments, and 10 for human variability).

***Selection of the Critical Effect:*** Several studies have evaluated the intermediate-duration toxicity of inhaled *n*-hexane. A summary of the identified LOAELs is presented in Table A-3. The lowest LOAELs were identified for renal, neurological, musculoskeletal, body weight, and developmental effects with respiratory and reproductive effects observed at higher concentrations. One of the lowest LOAELs identified was 500 ppm for increased relative kidney weights and chronic nephritis in male rats exposed for 22 hours/day, 7 days/week for 6 months (API 1981). The study authors were unable to determine whether exposure to *n*-hexane exacerbated the normal age-related process observed in controls or caused a unique injury. High background rates of chronic nephropathy are commonly observed in male Sprague-Dawley rats and complicate its use as the critical effect. Additionally, the renal system is not a known target of *n*-hexane exposure, and several other rat and mouse studies have failed to identify a similar response (API 1978; Cavender et al. 1984; NTP 1991). Therefore, the renal effects were not selected as the critical effect for derivation of an intermediate-duration inhalation MRL.

The next lowest LOAELs identified were for neurological endpoints, which are a common outcome following inhalation exposure to *n*-hexane. Rats (sex not specified) exposed to 400–600 ppm *n*-hexane continuously for 42–162 days developed central and peripheral neuropathy, footdrop, waddling gait, and limb weakness, while histopathology revealed swollen axons and axonal degeneration (Schaumburg and Spencer 1976). Male rats exposed to 500 ppm for 22 hours/day, 7 days/week for 9 weeks presented with clinical signs of neurotoxicity (narcosis, paralysis) and histopathology (axonal swellings, myelin degradation), while rats exposed to 700 ppm for 8 hours/day, 7 days/week for 40 weeks only had axonal swelling (Altenkirch et al. 1982). Other studies have also shown neurological effects at 500 ppm, with abnormal gait and peripheral neuropathy in male rats exposed for 22 hours/day, 7 days/week for 6 months (API 1981), and decreased grip strength in male rats exposed for 24 hours/day, 5 days/week for 10 weeks (Rebert and Sorenson 1983). While there is consistent evidence of neurological effects, the calculated HECs for these effects were higher than those for respiratory nasal effects; thus, neurological effects were not selected as the critical effect for MRL derivation.

**Table A-3. Selected NOAEL and LOAEL Values Following Intermediate-Duration Inhalation Exposure to *n*-Hexane**

| Species (strain, sex) | Duration | NOAEL ($NOAEL_{ADJ}$) (ppm) | LOAEL ($LOAEL_{ADJ}$) (ppm) | Effect | Reference |
|---|---|---|---|---|---|
| Renal effects | | | | | |
| Rats (Sprague-Dawley, M) | 6 months 7 days/week 22 hours/day | ND | 500 (458) | Increased kidney weight, chronic nephropathy | API 1981 |
| Respiratory effects | | | | | |
| Mice (B6C3F1, B) | 13 weeks 5 days/week 22 hours/day | ND | 1,099 (719) | Lesions in the nasal cavity (multifocal regeneration and metaplasia in olfactory epithelium) | NTP 1991 |
| Rabbit (New Zealand) | 24 weeks 5 days/week 8 hours/day | ND | 3,000 (714) | Upper respiratory tract irritation (nasal discharge and salivation), respiratory difficulties (gasping, lung rales, mouth breathing), histopathology (centrilobular emphysema, pulmonary fibrosis, goblet cell metaplasia, epithelial desquamation) | Lungarella et al. 1984 |
| Mice (B6C3F1, B) | 13 weeks 5 days/week 6 hours/day | 1,109 F (198) 4,421 M (789) | 4,421 F (789) 10,000 M (1,786) | Lesions in the nasal cavity (multifocal regeneration and metaplasia in the olfactory epithelium) | NTP 1991 |
| Neurological effects | | | | | |
| Rats (Wistar, M) | 40 weeks 7 days/week 8 hours/day | ND | 700 (233) | Axonal swelling in the spinal cord | Altenkirch et al. 1982 |
| Rats (Fischer-344, M) | 11 weeks 5 days/week 24 hours/day | ND | 500 (357) | Decreased grip strength | Rebert and Sorenson 1983 |
| Rats (Sprague-Dawley, NS) | 45 days continuously | ND | 400–600 | Central and peripheral neuropathy, footdrop, waddling gait, limb weakness, swollen axons, axonal degeneration | Schaumburg and Spencer 1976 |

**Table A-3. Selected NOAEL and LOAEL Values Following Intermediate-Duration Inhalation Exposure to *n*-Hexane**

| Species (strain, sex) | Duration | NOAEL ($NOAEL_{ADJ}$) (ppm) | LOAEL ($LOAEL_{ADJ}$) (ppm) | Effect | Reference |
|---|---|---|---|---|---|
| Rats (Wistar, M) | 9 weeks 7 days/week 22 hours/day | ND | 500 (458) | SLOAEL: Clinical signs (narcosis, paralysis), multifocal giant axonal swellings, primarily in the calf muscles, breakdown of axons, and myelin degradation | Altenkirch et al. 1982 |
| Rats (Sprague-Dawley, M) | 6 months 7 days/week 22 hours/day | ND | 500 (458) | Abnormal gait, peripheral nerve atrophy | API 1981 |
| Rats (Wistar, M) | 16 weeks 7 days/week 12 hours/day | 500 (250) | 1,200 (600) | Decreased grip strength and motor nerve conduction velocity, paranodal swelling, demyelination, and remyelination of the peripheral nerve | Huang et al. 1989 |
| Mice (B6C3F1, B) | 13 weeks 5 days/week 22 hours/day | ND | 1,099 (719) | Decreased locomotor activity (females), paranodal swellings in tibial nerve | NTP 1991 |
| Rats (Wistar, F) | 63 days (GD 1–PND 42) 7 days/week 23 hours/day | ND | 800 (767) | Hindlimb weakness | Stoltenburg-Didinger et al. 1990 |
| Rats (Wistar, M) | 20 weeks 6 days/week 12 hours/day | ND | 2,000 (857) | Decreased motor conduction velocity | Ichihara et al. 1998 |
| Rats (Sprague-Dawley, M) | 30 weeks 6 days/week 10 hours/day | 500 (179) | 2,500 (892) | Tibial nerve axonal degeneration | Frontali et al. 1981 |
| Rats (Fischer-344, M) | 11 weeks 7 days/week (4 weeks) 6 days/week (7 weeks) 24 hours/day | ND | 1,000 (914) | Decreased hindlimb and forelimb strength, ataxia, increased action potential latency and brainstem auditory-evoked response | Howd et al. 1983 |
| Rats (Fischer-344, M) | 13 weeks 5 days/week 6 hours/day | 3,000 (536) | 6,500 (1,160) | Axonopathy in the sciatic nerve | Cavender et al. 1984 |

**Table A-3. Selected NOAEL and LOAEL Values Following Intermediate-Duration Inhalation Exposure to *n*-Hexane**

| Species (strain, sex) | Duration | NOAEL ($NOAEL_{ADJ}$) (ppm) | LOAEL ($LOAEL_{ADJ}$) (ppm) | Effect | Reference |
|---|---|---|---|---|---|
| Rats (Fischer-344, M) | 14 weeks 7 days/week 14 hours/day | ND | 2,000 (1,167) | Decreased limb grip strength, startle response, and motor activity; increased evoked potential latencies | Pryor et al. 1983 |
| Rats (Sprague-Dawley, M) | 14 weeks 5 days/week 9 hours/day | 1,500 (402) | 5,000 (1,339) | Tibial nerve axonal degeneration | Frontali et al. 1981 |
| Rats (Wistar, M) | 16 weeks 7 days/week 12 hours/day | ND | 3,040 (1,520) | Gait disturbances, decreased motor and mixed nerve conduction velocity, axonal swelling, neurofilament accumulation, denervated neuromuscular junctions | Takeuchi et al. 1980 |
| Mice (B6C3F1, B) | 13 weeks 5 days/week 6 hours/day | 4,421 (789) | 10,000 (1,785) | Decreased locomotor activity (females), paranodal swellings in tibial nerve | NTP 1991 |
| Rats (Wistar, F) | 20 days (GD 1-20) 4 hours/day | 2,500 (417) | 12,500 (2,083) | Irritability, aggression | Li et al. 2014, 2015 |
| Rats (Sprague-Dawley, M) | 6 weeks 6 days/week 16 hours/day | ND | 5,000 (2,857) | SLOAEL: Decreased motor conduction velocity, peripheral neuropathy, and paralysis | De Martino et al. 1987 |
| Musculoskeletal effects | | | | | |
| Rats (Sprague-Dawley, M) | 6 months 7 days/week 22 hours/day | ND | 500 (458) | Skeletal muscle atrophy | API 1981 |
| Rats (Sprague-Dawley, M) | 61 days 7 days/week 18 hours/day | ND | 1,000 (750) | Hindlimb muscular atrophy | Nylen et al. 1989 |
| Rats (Sprague-Dawley, M) | 28 days 7 days/week 21 hours/day | ND | 1,000 (875) | Hindlimb muscular atrophy | Nylen et al. 1989 |

**Table A-3. Selected NOAEL and LOAEL Values Following Intermediate-Duration Inhalation Exposure to *n*-Hexane**

| Species (strain, sex) | Duration | NOAEL ($NOAEL_{ADJ}$) (ppm) | LOAEL ($LOAEL_{ADJ}$) (ppm) | Effect | Reference |
|---|---|---|---|---|---|
| Rats (Wistar, M) | 16 weeks 7 days/week 12 hours/day | ND | 3,040 (1,520) | Muscular atrophy, denervation, irregular fibers, and disordered myofilaments | Takeuchi et al. 1980 |
| Reproductive effects | | | | | |
| Rats (Sprague-Dawley, M) | 61 days 7 days/week 18 hours/day | ND | 1,000 (750) | Testicular atrophy | Nylen et al. 1989 |
| Rats (Sprague-Dawley, M) | 28 days 7 days/week 21 hours/day | ND | 1,000 (875) | Testicular atrophy | Nylen et al. 1989 |
| Rats (Sprague-Dawley, M) | 6 weeks 6 days/week 16 hours/day | ND | 5,000 (2,857) | Testicular lesions (spermatocyte necrosis, exfoliation of spermatids, and Sertoli cell vacuolization) | De Martino et al. 1987 |
| Developmental effects | | | | | |
| Rats (Wistar, F) | 21 days (GDs 1–21) 7 days/week 23 hours/day | ND | 500 (479) | Decreased fetal body weight (22% at 9 days after birth), delayed histogenesis of the cerebellar cortex | Stoltenburg-Didinger et al. 1990 |
| Rats (Wistar, F) | 20 days (GDs 1–20) 7 days/week 4 hours/day | 2,500 (417) | 12,500 (2,083) | SLOAEL: Decreased live pups/litter, decreased percentage of secondary follicles, increased atretic follicles, and alterations in oestrus cycle in female offspring | Li et al. 2014, 2015 |

ADJ = adjusted for continuous exposure; B = both sexes; F = females; GD = gestation day; LOAEL = lowest-observed-adverse-effect level; M = males; ND = not determined; NOAEL = no-observed-adverse-effect level; NS = not specified; PND = postnatal day; SLOAEL = serious LOAEL

Decreased fetal body weights were observed following maternal exposure to 500 ppm (Stoltenburg-Didinger et al. 1990). Body weight and musculoskeletal effects were also observed at exposures between 500 and 1,000 ppm, but these effects were not considered critical effects for MRL derivation because they are thought to be secondary outcomes following injury to the primary neurological targets of *n*-hexane. Muscular atrophy and limb weakness have been shown to result from denervation in the extremities (Nylen et al. 1989; Takeuchi et al. 1980), and loss of body weight is often observed along with decreased food intake, possibly due to an underlying effect resulting in the animal's refusal or inability to eat normally. At concentrations of 1,000 ppm, respiratory effects have been observed. In male and female mice, exposure to 1,099 ppm *n*-hexane for 22 hours/day, 5 days/week for 13 weeks resulted in nasal cavity lesions, including multifocal regeneration, and metaplasia in olfactory epithelium (NTP 1991). Similar results were not observed in two rat studies, suggesting that mice may be more susceptible than rats to point-of-entry effects from *n*-hexane inhalation.

Taken together, the data suggest that respiratory, developmental, and neurological effects are the most sensitive targets following intermediate-duration inhalation exposure to *n*-hexane. A comparison of the HECs of the LOAELs ($LOAEL_{HEC}$) for these endpoints is presented in Table A-4. The lowest $LOAEL_{HEC}$ value is 111 ppm for nasal lesions in mice exposed for 22 hours/day (NTP 1991). Thus, nasal lesions were selected as the critical effect.

**Table A-4. Potential PODs Following Intermediate-Duration Inhalation Exposure to *n*-Hexane**

| Species | Exposure | LOAEL (ppm) | $LOAEL_{ADJ}$ (ppm) | $LOAEL_{HEC}$ (ppm) | Effect (Reference) |
|---|---|---|---|---|---|
| Respiratory effects | | | | | |
| Mice | 22 hours/day 5 days/week | 1,099 | 719 | 111 | Nasal cavity lesions (NTP 1991) |
| Mice | 6 hours/day 5 days/week | 4,421 | 789 | 122 | Nasal cavity lesions (females only) (NTP 1991) |
| Neurological effects | | | | | |
| Rats | 8 hours/day 7 days/week | 700 | 233 | 233 | Axonal swelling in the spinal cord (Altenkirch et al. 1982) |
| Rats | 24 hours/day 5 days/week | 500 | 357 | 357 | Decreased grip strength (Rebert and Sorenson 1983) |
| Developmental effects | | | | | |
| Rats | 23 hours/day 7 days/week | 500 | 479 | 479 | Decreased pup body weight (Stoltenburg-Didinger et al. 1990) |

ADJ = adjusted; HEC = human equivalent concentration; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; POD = point of departure

***Selection of the Principal Study:*** Few studies have evaluated respiratory effects following intermediate-duration inhalation exposure to *n*-hexane. Although rats appear to be more sensitive to *n*-hexane-induced neurotoxicity than mice, inhalation exposure studies in rats have failed to produce respiratory effects, suggesting that mice may be more sensitive to respiratory effects than rats. Male and female mice exposed to 1,099 ppm *n*-hexane for 22 hours/day, 5 days/week for 13 weeks presented with nasal cavity lesions (NTP 1991). In contrast, no histopathology was observed in the nasal cavity of male and female rats exposed up to 10,000 ppm for 6 hours/day, 5 days/week for 13 weeks (Cavender et al. 1984), or in male rats exposed to 500 ppm for 22 hours/day, 7 days/week for 6 months (API 1981).

***Summary of the Principal Study:***

NTP. 1991. Toxicity studies of n-hexane in B6C3F1 (inhalation studies). Research Triangle Park, NC: National Toxicology Program. PB91185322. NIH Publication No. 91-3121 https://ntp.niehs.nih.gov/ntp/htdocs/st_rpts/tox002.pdf. July 25, 2022.

Dunnick JK, Graham DG, Yang RS, et al. 1989. Thirteen-week toxicity study of n-hexane in B6C3F1 mice after inhalation exposure. Toxicology 57(2):163-172. https://doi.org/10.1016/0300-483x(89)90162-5.

Male and female $B6C3F_1$ mice (18/sex/group) were exposed to *n*-hexane for 6 hours/day, 5 days/week for 13 weeks at concentrations of 0, 500, 1,000, 4,000, or 10,000 ppm (mean analytical concentrations of 0, 580, 1,109, and 4,421 ppm; analytical concentration not reported for the 10,000-ppm exposure level). An additional group of mice (18/sex/group) was exposed to 1,000 ppm *n*-hexane for 22 hours/day, 5 days/week for 13 weeks (mean analytical concentration of 1,099 ppm). The core set of animals (10/sex/group) were evaluated for body weights, clinical signs of toxicity, and a complete histopathological assessment. The remaining animals (8/sex/group) were evaluated in a series of neurobehavior and neuropathology studies, including undifferentiated motor activity, forelimb and hindlimb grip strengths, thermal sensitivity, startle response, and foot splay. Four males and four females were randomly selected from the 0, 1,099 (22 hours/day), and 10,000 ppm groups for evaluation of spinal cord and tibial nerve.

*Core group:* Exposure up to 10,000 ppm for 6 hours/day or 1,099 ppm for 22 hour/day had no effect on survival or clinical signs of toxicity. Body weights were decreased in male mice exposed to either 10,000 ppm *n*-hexane for 6 hours/day (17% decrease) or to 1,099 ppm for 22 hours/day (10% decrease), but not in female mice comparably exposed. Sneezing was seen in males and females exposed to 10,000 ppm beginning at week 4 and continuing until the end of the study. Histopathologic evaluations revealed exposure-related lesions in the nasal cavities of male and female mice at 1,099 ppm (22 hours/day) and in female mice at 4,421 ppm. Multifocal regeneration and metaplasia of the olfactory epithelium were the most common lesions observed in both male and female mice. Higher concentrations resulted in more significant damage, including epithelial erosion, subacute inflammation, and focal fibrosis of the submucosa. No other histopathological effects outside of the nasal cavity were observed.

*Neurological group:* The only neurobehavioral finding observed was a decrease in locomotor activity in female mice at 1,099 (22 hours/day) and 10,000 ppm. Paranodal swellings in the tibial nerve were observed in 6/8 mice exposed to 1,099 (22 hours/day) and 10,000 ppm. Nerve damage at 1,099 ppm for 22 hours/day was similar to that observed at 10,000 ppm for 6 hours/day, suggesting that continuous exposure to *n*-hexane is more toxic than intermittent exposure.

***Selection of the Point of Departure for the MRL:*** Lesions in the nasal cavity were observed in male and female mice at 1,099 ppm (22 hours/day) and in female mice at 4,421 ppm (6 hours/day), with continuous-adjusted $LOAEL_{ADJ}$ values of 719 and 789 ppm, respectively. The LOAEL value was selected as the POD. BMD modeling was not conducted because only one concentration was evaluated at this exposure duration.

***Adjustment for Intermittent Exposure:*** The intermittent 22-hours/day, 5 days/week LOAEL of 1,000 ppm was adjusted to a 24-hour, 7 day/week continuous exposure using the following equation:

$$LOAEL_{ADJ} = LOAEL \times \frac{22\ hours}{24\ hours} \times \frac{5\ days}{7\ days}$$

$$1{,}099\ ppm \times \frac{22\ hours}{24\ hours} \times \frac{5\ days}{7\ days} = 719\ ppm$$

***Human Equivalent Concentration:*** The HEC was calculated by multiplying the $LOAEL_{ADJ}$ by the regional gas dose ratio (RGDR). The RGDR for extrathoracic respiratory tract effects was calculated using the following equation:

$$RGDR_{ET} = \frac{(\frac{V_E}{SA_{ET}})_A}{(\frac{V_E}{SA_{ET}})_H}$$

Where:

$RGDR_{ET}$ = extrathoracic regional gas dose ratio (animal: human)
$V_E$ = minute volume (based on body weight)
$SA_{ET}$ = surface area of the extrathoracic region
A = animal
H = human

The human minute volume (13,800 mL/minute) and extrathoracic surface area (200 $cm^2$) are provided in EPA (1994b) along with the extrathoracic surface area in mice (3 $cm^2$). The mouse minute volume (32 mL/minute) was calculated based on the body weights reported in the study (males 30 g, females 25.4 g, average 27.7 g) (NTP 1991).

$$LOAEL_{HEC} = LOAEL_{ADJ} \times RGDR_{ET}$$

$$LOAEL_{HEC} = 655\ ppm \times \frac{(\frac{32\ ml/min}{3\ cm^2})}{(\frac{13{,}800\ ml/min}{200\ cm^2})} = 719\ ppm \times 0.1546 = 111\ ppm$$

***Uncertainty Factor:*** The $LOAEL_{HEC}$ was divided by a total uncertainty factor of 300:

- 10 for extrapolation from a LOAEL
- 3 for extrapolation from animals to humans with dosimetric adjustments
- 10 for human variability

$$MRL = LOAEL_{HEC} \div UFs$$
$$111\ ppm \div 300 = 0.37\ ppm \approx 0.4\ ppm$$

***Other Additional Studies or Pertinent Information that Lend Support to this MRL:*** The principal study (NTP 1991) was the only study located that examined respiratory histopathology in mice following *n*-hexane exposure. No histopathological effects have been observed in the nasal cavities of rats exposed to *n*-hexane at concentrations up to 10,000 ppm for 6 hours/day or 500 ppm for 22 hours/day (API 1981; Cavender et al. 1984), although the exposure duration or concentration may not have been high enough to elicit a response. Pulmonary effects have also been observed. Relative lung weights were increased in

male rats exposed to 1,000 ppm for 24 hours/day, 6–7 days/week for 11 weeks. However, histology was not performed on the lungs nor the nasal cavity (Howd et al. 1983). Male rabbits exposed to 3,000 ppm for 8 hours/day, 5 days/week for 24 weeks showed signs of respiratory irritation, breathing difficulties, and respiratory track histopathology (centrilobular emphysema, pulmonary fibrosis, goblet cell metaplasia, epithelial desquamation) (Lungarella et al. 1984). Since the NTP (1991) mouse study had the lowest $LOAEL_{HEC}$ for respiratory effects, it was chosen as the principal study.

Neurological effects are the most sensitive outcome evaluated and identified following inhalation exposure to *n*-hexane; however, the MRL based on neurological effects would be higher due to the dosimetric adjustment for systemic effects. Therefore, the MRL based on nasal effects is protective of neurological effects.

***Agency Contacts (Chemical Managers):*** Obaid Faroon

# MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** *n*-Hexane
***CAS Numbers:*** 110-54-3
***Date:*** May 2024
***Profile Status:*** Draft for Public Comment
***Route:*** Inhalation
***Duration:*** Chronic

***MRL Summary:*** The database was not considered adequate for derivation of a provisional chronic-duration inhalation MRL for *n*-hexane. Although there is a large database describing neurological effects in occupational workers resulting from inhalation exposure to *n*-hexane, these studies lack exposure information, or the exposure is confounded by additional compounds that exacerbate *n*-hexane-induced neurological effects. Only a single chronic-duration animal study was located that examined the reproductive toxicity of inhaled *n*-hexane (Leydig cell hyperplasia), but the study did not evaluate other potential sensitive targets such as neurological or respiratory outcomes, so it was not considered sufficient for deriving a chronic-duration MRL.

***Rationale for Not Deriving an MRL:*** The neurotoxicity of *n*-hexane was first observed in the shoe industries of Japan and Italy in the 1960s and early 1970s. A number of epidemiological studies were initiated in response to outbreaks of apparent peripheral neuropathy in shoe workers. While the clinical course of the disease was well described, elucidation of a dose-response relationship has been difficult. In most cases, concentrations of *n*-hexane in the workplace air were not measured until after disease developed. Also, in almost all cases, workers were concurrently exposed to other chemicals which may have affected their response to *n*-hexane.

Few human studies are available that have the exposure and duration information needed to identify a point of departure. Sanagi et al. (1980) evaluated motor nerve conduction velocity in 14 occupationally exposed workers employed in a factory producing tungsten carbide alloys. Personal monitors reported mean 8-hour time weighted concentrations of *n*-hexane (58 ppm) and acetone (39 ppm); no other "solvent vapors" were detected. Chang et al. (1993) reported alterations in motor and sensory nerve amplitudes and latencies in printing press workers diagnosed with peripheral neuropathy. Mean concentrations of *n*-hexane were 63 ppm (background, 30–110 ppm) and 132 ppm (personal samplers, 80–210 ppm), although toluene and isopropyl alcohol were also measured, and additional exposures to lead, toluene, mercury, and diesel were also possible from the solvents used. Mutti et al. (1982a, 1982b) also identified electroneurographic abnormalities in shoe factory workers exposed to *n*-hexane near the recommended threshold limit value, but additional contaminants measured in the breathing zone included cyclohexane, MEK, and ethyl acetate.

Several studies suggest that co-exposures to chemicals including acetone, toluene, and MEK may enhance *n*-hexane metabolism and neurotoxicity (Altenkirch et al. 1977, 1982; Ladefoged et al. 1989, 1994; Ladefoged and Perbellini 1986; Nylen et al. 1994; Nylen and Hagman 1994; Patten et al. 1986; Robertson et al. 1989; Zhao et al. 1998). Due to the known co-exposure to and potential interactions with acetone, the available human studies were not considered adequate for the development of a chronic-duration inhalation MRL. A single chronic-duration animal study examined the male reproductive toxicity of inhaled *n*-hexane (Imai and Omoto 1999). Leydig cell hyperplasia was observed in male rats exposed to 1,000 ppm *n*-hexane for 60 weeks, but this study was not considered adequate due to poor reporting and the lack of supporting studies suggesting that Leydig cells are a target of *n*-hexane exposure. Additionally, this study did not evaluate any other potential targets of *n*-hexane exposure such as neurotoxicity or respiratory effects. Therefore, a chronic-duration inhalation MRL was not derived.

***Agency Contacts (Chemical Managers):*** Obaid Faroon

APPENDIX A

# MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** *n*-Hexane
***CAS Numbers:*** 110-54-3
***Date:*** May 2024
***Profile Status:*** Draft for Public Comment
***Route:*** Oral
***Duration:*** Acute

***MRL Summary:*** The database was not considered adequate for derivation of a provisional acute-duration oral MRL for *n*-hexane. There is limited information on the toxicity of *n*-hexane following acute oral exposure. The only nonlethal effect is developmental toxicity (decreased fetal weight); however, increased maternal mortality has been observed at lower doses.

***Rationale for Not Deriving an MRL:*** Three studies have evaluated the acute oral toxicity of *n*-hexane (Kimura et al. 1971; Linder et al. 1992; Marks et al. 1980). A LOAEL of 7,920 mg/kg/day was identified in mice for decreased fetal weight following oral exposure 3 times/day on GDs 6–15 (10 days) (Marks et al. 1980). In this same dosing regime, a non-statistically significant increase in dam mortality (9%) was observed at 2,830 mg/kg/day. The remaining two studies evaluated the potential reproductive toxicity of oral *n*-hexane exposure but did not identify an effect for any outcome. None of the acute-duration oral studies examined non-reproductive or developmental endpoints, particularly potential neurotoxicity, which is a known sensitive target of *n*-hexane.

***Agency Contacts (Chemical Managers):*** Obaid Faroon

# MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** *n*-Hexane
***CAS Number:*** 110-54-3
***Date:*** May 2024
***Profile Status:*** Draft for Public Comment
***Route:*** Oral
***Duration:*** Intermediate
***MRL*:** 0.1 mg/kg/day (provisional)
***Critical Effects:*** Neurological effects (impaired performance on a test of memory)
***Reference:*** Gao et al. 2019
***Point of Departure:*** LOAEL of 43.5 mg/kg/day
***Uncertainty Factor:*** 300
***LSE Graph Key:*** 12
***Species:*** Mouse

***MRL Summary:*** A provisional intermediate-duration oral MRL of 0.1 mg/kg/day was derived for *n*-hexane based on neurobehavioral effects (impaired performance on a test of memory) in mice administered via gavage 43.5 mg/kg/day for 20 consecutive days (Gao et al. 2019). The minimal LOAEL of 43.5 mg/kg/day was divided by a total uncertainty factor of 300 (3 for the use of a minimal LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).

***Selection of the Critical Effect:*** Several intermediate-duration studies examining the oral toxicity of *n*-hexane are available. A summary of the identified LOAELs is presented in Table A-5. Exposure to 43.5 mg/kg/day resulted in neurobehavioral effects and neuromuscular effects were observed at ≥1,000 mg/kg/day. Other observed adverse effects include decreased body weight gain at ≥570 mg/kg/day and reproductive effects at 4,000 mg/kg/day. Neurological effects were chosen as the critical effect because they occurred at the lowest adverse effect level and the endpoint is well supported by epidemiological and animal studies involving inhalation exposure.

***Selection of the Principal Study:*** Several studies are available that evaluated neurological effects following intermediate-duration oral exposure to *n*-hexane. The lowest adverse effect level of 43.5 mg/kg/day was identified in the Gao et al. (2019) mouse study. At higher doses, signs of clinical neurotoxicity (transient paralysis, abnormal gait, decreased rotarod latency) and decreased motor nerve conduction velocity were observed (Krasavage et al. 1980; Li et al. 2018, 2020a, 2020b; Ono et al. 1981; Wang et al. 2017). The Gao et al. (2019) study was selected as the principal study because it identified the lowest LOAEL for neurological effects.

**Table A-5. Available NOAEL and LOAEL Values Following Intermediate-Duration Oral Exposure to *n*-Hexane**

| Species (strain, sex) | Duration | NOAEL (mg/kg/day) | LOAEL (mg/kg/day) | Effect | Reference |
|---|---|---|---|---|---|
| Body weight effects | | | | | |
| Rats (COBS, M) | 90 days 5 times/week | ND | 570 | Decreased body weight (15%) | Krasavage et al. 1980 |
| Rats (Wistar, M) | 24 weeks 7 times/week | 500 | 1,000 | Decreased body weight (19%) | Li et al. 2020b |
| Rats (Wistar, M) | 10 weeks 6 times/week | 1,000 | 2,000 | Decreased body weight (19%) | Li et al. 2018 |
| Rats (Wistar, M) | 7 weeks 7 times/week | ND | 3,000 | SLOAEL: Decreased body weight (23%) | Li et al. 2020a |
| Neurological effects | | | | | |
| Mice (Kunming, M, F) | 20 days 7 times/week | ND | 43.5 | Impaired response on test of learning and memory (Y-maze) | Gao et al. 2019 |
| Rats (Wistar, M) | 24 weeks 7 times/week | 500 | 1,000 | Transient paralysis, abnormal gait, decreased ability to stay on a rotating rod and motor nerve conduction velocity | Li et al. 2020b |
| Rats (Wistar, M) | 8 weeks 7 times/week | ND | 1,251 | Decreased motor and mixed nerve conduction velocity | Ono et al. 1981 |
| Rats (Wistar, M) | 10 weeks 6 times/week | 1,000 | 2,000 | Abnormal gait, decreased ability to stay on rotating rod | Li et al. 2018 |
| Rats (Wistar, M) | 7 weeks 7 times/week | ND | 3,000 | SLOAEL: Paralysis | Li et al. 2020a |
| Rats (Wistar, M) | 8 weeks 7 times/week | ND | 3,000 | Decreased grip strength, abnormal gait | Wang et al. 2017 |
| Rats (COBS, M) | 90 days 5 times/week | 1,140 | 4,000 | SLOAEL: Hindlimb paralysis, axonal swelling, myelin retraction | Krasavage et al. 1980 |
| Reproductive effects | | | | | |
| Rats (COBS, M) | 90 days 5 times/week | 1,140 | 4,000 | SLOAEL: Testicular atrophy of the germinal epithelium | Krasavage et al. 1980 |

F = females; LOAEL = lowest-observed-adverse-effect level; M = males; ND = not determined; NOAEL = no-observed-adverse-effect level; SLOAEL = serious LOAEL

***Summary of the Principal Study:***

Gao J, Zhang X, Lin Q, et al. 2019. Changes to learning and memory ability and the expression of NGF/NGF-R mRNA in the brain tissue of mice exposed to *n*-hexane. Chin J Ind Hyg Occup Dis. 37:217-221.

Groups of 10 Kunming mice (presumably 5 males and 5 females) were administered *n*-hexane via gavage at 0, 43.5, 86.5, or 173.0 mg/kg/day for 20 days. Learning and memory were evaluated using a Y-maze test. The test was conducted on 2 consecutive days (the day after exposure completion and 24-hours later), the first day to assess learning and the second day to assess memory recall ability.

The investigators noted that decreased activity and reduced food intake were observed in the exposed groups beginning after exposure day 4; more pronounced symptoms were observed in the 173.0 mg/kg/day group. However, no incidence or frequency data were provided. The investigators also noted that the mean body weight, presumably in the 173.0 mg/kg/day group, was decreased by 2.1 g; no additional information was provided. On the first test (learning), significant decreases in the correct response rate were observed in the 86.5 and 173.0 mg/kg/day groups; no statistically significant alterations in total electric shock time or total training sessions were observed. In the second test (memory), significant increases in total electric shock time and decreases in correct response rate were observed in all three groups. Significant decreases in nerve growth factor (NGF) and nerve growth factor receptor (NGF-R) concentrations were observed at 173.0 and 86.5 mg/kg/day, respectively. Decreased levels of NGF messenger ribonucleic acid (mRNA) and NGF-R mRNA levels were also observed at 43.5 and 86.5 mg/kg/day, respectively.

***Selection of the Point of Departure for the MRL:*** The minimal LOAEL of 43.5 mg/kg/day for impaired performance on a test of memory in mice administered *n*-hexane for 20 days was selected as the POD for the MRL. BMD modeling was not conducted because it is unclear whether values presented in Table 2 of the paper are the mean±standard deviation or mean±standard error of the mean. Therefore, the NOAEL/LOAEL approach was used.

***Uncertainty Factor:*** The NOAEL is divided by a total uncertainty factor of 300:

- 3 for the use of a minimal LOAEL
- 10 for extrapolation from animals to humans
- 10 for human variability

$$MRL = LOAEL \div UFs$$
$$43.5\ mg/kg/day \div 300 = 0.1\ mg/kg/day$$

The 43.5 mg/kg/day dose was considered a minimal LOAEL because overt signs of neurotoxicity were not observed at this dose level.

***Other Additional Studies or Pertinent Information that Lend Support to this MRL:*** Several studies are available with similar LOAELs and critical effects (Li et al. 2018; Ono et al. 1981), while more serious neurological signs including lasting paralysis have been observed at higher concentrations (Krasavage et al. 1980; Li et al. 2020a). Additionally, body weight changes occurred at the same dose as the selected LOAEL, so this MRL is protective against decreased body weight as well.

***Agency Contacts (Chemical Managers):*** Obaid Faroon

# MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** *n*-Hexane
***CAS Numbers:*** 110-54-3
***Date:*** May 2024
***Profile Status:*** Draft for Public Comment
***Route:*** Oral
***Duration:*** Chronic

***MRL Summary:*** The database was not considered adequate for derivation of a provisional chronic-duration oral MRL for *n*-hexane.

***Rationale for Not Deriving an MRL:*** No studies were located that describe the effects of chronic-duration oral exposure to *n*-hexane in humans or animals.

***Agency Contacts (Chemical Managers):*** Obaid Faroon

# APPENDIX B. LITERATURE SEARCH FRAMEWORK FOR *n*-HEXANE

The objective of the toxicological profile is to evaluate the potential for human exposure and the potential health hazards associated with inhalation, oral, or dermal/ocular exposure to *n*-hexane.

## B.1 LITERATURE SEARCH AND SCREEN

A literature search and screen were conducted to identify studies examining health effects, toxicokinetics, mechanisms of action, susceptible populations, biomarkers, chemical interactions, physical and chemical properties, production, use, environmental fate, environmental releases, and environmental and biological monitoring data for *n*-hexane. ATSDR primarily focused on peer-reviewed articles without publication date or language restrictions. Foreign language studies are reviewed based on available English-language abstracts and/or tables (or summaries in regulatory assessments, such as International Agency for Research on Cancer [IARC] documents). If the study appears critical for hazard identification or MRL derivation, translation into English is requested. Non-peer-reviewed studies that were considered relevant to the assessment of the health effects of *n*-hexane have undergone peer review by at least three ATSDR-selected experts who have been screened for conflict of interest. The inclusion criteria used to identify relevant studies examining the health effects of *n*-hexane are presented in Table B-1.

**Table B-1. Inclusion Criteria for the Literature Search and Screen**

| |
|---|
| Health Effects |
| Species |
| Human |
| Laboratory mammals |
| Route of exposure |
| Inhalation |
| Oral |
| Dermal (or ocular) |
| Parenteral (these studies will be considered supporting data) |
| Health outcome |
| Death |
| Systemic effects |
| Body weight effects |
| Respiratory effects |
| Cardiovascular effects |
| Gastrointestinal effects |
| Hematological effects |
| Musculoskeletal effects |
| Hepatic effects |
| Renal effects |
| Dermal effects |
| Ocular effects |
| Endocrine effects |
| Immunological effects |
| Neurological effects |
| Reproductive effects |

**Table B-1. Inclusion Criteria for the Literature Search and Screen**

| |
|---|
| Developmental effects |
| Other noncancer effects |
| Cancer |
| Toxicokinetics |
| Absorption |
| Distribution |
| Metabolism |
| Excretion |
| PBPK models |
| Biomarkers |
| Biomarkers of exposure |
| Biomarkers of effect |
| Interactions with other chemicals |
| Potential for human exposure |
| Releases to the environment |
| Air |
| Water |
| Soil |
| Environmental fate |
| Transport and partitioning |
| Transformation and degradation |
| Environmental monitoring |
| Air |
| Water |
| Sediment and soil |
| Other media |
| Biomonitoring |
| General populations |
| Occupation populations |

### B.1.1 Literature Search

The current literature search was intended to update the 1999 Toxicological Profile for *n*-Hexane; thus, the literature search was restricted to studies published between January 1997 and July 2022. The following main databases were searched in July 2022:

- PubMed
- National Technical Reports Library (NTRL)
- Scientific and Technical Information Network's TOXCENTER

The search strategy used the chemical names, Chemical Abstracts Service (CAS) numbers, synonyms, Medical Subject Headings (MeSH) headings, and keywords for *n*-hexane. The query strings used for the literature search are presented in Table B-2.

The search was augmented by searching the Toxic Substances Control Act Test Submissions (TSCATS), NTP website, and National Institute of Health Research Portfolio Online Reporting Tools Expenditures and Results (NIH RePORTER) databases using the queries presented in Table B-3. Additional databases were searched in the creation of various tables and figures, such as the TRI Explorer, the Substance Priority List (SPL) resource page, and other items as needed. Regulations applicable to *n*-hexane were identified by searching international and U.S. agency websites and documents.

Review articles were identified and used for the purpose of providing background information and identifying additional references. ATSDR also identified reports from the grey literature, which included unpublished research reports, technical reports from government agencies, conference proceedings and abstracts, and theses and dissertations.

**Table B-2. Database Query Strings**

| Database search date | Query string |
|---|---|
| **PubMed** | |
| 07/2022 | ((110-54-3 [rn] OR (("hexanes/toxicity"[mh] OR "hexanes/adverse effects"[mh] OR "hexanes/poisoning"[mh] OR "hexanes/pharmacokinetics"[mh] OR ("hexanes"[mh] AND ("environmental exposure"[mh] OR ci[sh])) OR ("hexanes"[mh] AND toxicokinetics[mh:noexp]) OR "hexanes/blood"[mh] OR "hexanes/cerebrospinal fluid"[mh] OR "hexanes/urine"[mh] OR ("hexanes"[mh] AND ("endocrine system"[mh] OR "hormones, hormone substitutes, and hormone antagonists"[mh] OR "endocrine disruptors"[mh])) OR ("hexanes"[mh] AND ("computational biology"[mh] OR "medical informatics"[mh] OR genomics[mh] OR genome[mh] OR proteomics[mh] OR proteome[mh] OR metabolomics[mh] OR metabolome[mh] OR genes[mh] OR "gene expression"[mh] OR phenotype[mh] OR genetics[mh] OR genotype[mh] OR transcriptome[mh] OR ("systems biology"[mh] AND ("environmental exposure"[mh] OR "epidemiological monitoring"[mh] OR analysis[sh])) OR "transcription, genetic "[mh] OR "reverse transcription"[mh] OR "transcriptional activation"[mh] OR "transcription factors"[mh] OR ("biosynthesis"[sh] AND (RNA[mh] OR DNA[mh])) OR "RNA, messenger"[mh] OR "RNA, transfer"[mh] OR "peptide biosynthesis"[mh] OR "protein biosynthesis"[mh] OR "reverse transcriptase polymerase chain reaction"[mh] OR "base sequence"[mh] OR "trans-activators"[mh] OR "gene expression profiling"[mh])) OR "hexanes/antagonists and inhibitors"[mh] OR ("hexanes/metabolism"[mh] AND ("humans"[mh] OR "animals"[mh])) OR ("hexanes/pharmacology"[majr]) OR ("hexanes"[mh] AND ("Neoplasms"[mh] OR "Carcinogens"[mh] OR "Lymphoproliferative disorders"[mh] OR "Myeloproliferative disorders"[mh] OR "Toxicity Tests"[mh] OR ((cancer*[tiab] OR carcinogen*[tiab]) AND (risk*[tiab] OR health[tiab]) AND assessment*[tiab]) OR "Mutagens"[mh] OR "Mutagenicity Tests"[mh] OR "Chromosome Aberrations"[mh] OR "DNA Damage"[mh] OR "DNA Repair"[mh] OR "DNA Replication/drug effects"[mh] OR "DNA/drug effects"[mh] OR "DNA/metabolism"[mh] OR "Genomic Instability"[mh] OR "Salmonella typhimurium/drug effects"[mh] OR "Salmonella typhimurium/genetics"[mh] OR "Sister Chromatid Exchange"[mh] OR strand-break*[tiab]))))) AND (1997:3000[mhda] OR 1997:3000[crdt] OR 1997:3000[edat] OR 1997:3000[dp])) OR ((("Gettysolve-B"[tw] OR "Hexane, n-"[tw] OR "Hexyl hydride"[tw] OR "Skellysolve B"[tw] OR "n-Hexan"[tw] OR "n-Hexane"[tw] OR "hexanes"[tw]) NOT medline[sb]) AND (1997:3000[crdt] OR 1997:3000[edat] OR 1997:3000[dp])))) |
| **NTRL** | |
| 07/2022 | Limited to title or keyword (1997-present)<br>"Hexane" OR "Hexanes" OR "n-Hexane" OR "Hexyl hydride" OR "Hexane, n-" OR "n-Hexan" OR "Gettysolve-B" OR "Skellysolve B" |

## Table B-2. Database Query Strings

| Database search date | Query string |
|---|---|
| **Toxcenter** | |
| 07/2022 | FILE 'TOXCENTER' ENTERED AT 12:36:27 ON 25 JUL 2022<br>CHARGED TO COST=EH038.15.04.LB.04<br>L1 24545 SEA FILE=TOXCENTER 110-54-3<br>L2 24386 SEA FILE=TOXCENTER L1 NOT TSCATS/FS<br>L3 17710 SEA FILE=TOXCENTER L2 NOT PATENT/DT<br>L4 14126 SEA FILE=TOXCENTER L3 AND PY>=1997<br>ACTIVATE TOXQUERY/Q<br>---------<br>L5 QUE (CHRONIC OR IMMUNOTOX? OR NEUROTOX? OR TOXICOKIN? OR BIOMARKER? OR NEUROLOG?)<br>L6 QUE (PHARMACOKIN? OR SUBCHRONIC OR PBPK OR EPIDEMIOLOGY/ST,CT, IT)<br>L7 QUE (ACUTE OR SUBACUTE OR LD50# OR LD(W)50 OR LC50# OR LC(W)50)<br>L8 QUE (TOXICITY OR ADVERSE OR POISONING)/ST,CT,IT<br>L9 QUE (INHAL? OR PULMON? OR NASAL? OR LUNG? OR RESPIR?)<br>L10 QUE ((OCCUPATION? OR WORKPLACE? OR WORKER?) AND EXPOS?)<br>L11 QUE (ORAL OR ORALLY OR INGEST? OR GAVAGE? OR DIET OR DIETS OR DIETARY OR DRINKING(W)WATER?)<br>L12 QUE (MAXIMUM AND CONCENTRATION? AND (ALLOWABLE OR PERMISSIBLE))<br><br>L13 QUE (ABORT? OR ABNORMALIT? OR EMBRYO? OR CLEFT? OR FETUS?)<br>L14 QUE (FOETUS? OR FETAL? OR FOETAL? OR FERTIL? OR MALFORM? OR OVUM?)<br>L15 QUE (OVA OR OVARY OR PLACENTA? OR PREGNAN? OR PRENATAL?)<br>L16 QUE (PERINATAL? OR POSTNATAL? OR REPRODUC? OR STERIL? OR TERATOGEN?)<br>L17 QUE (SPERM OR SPERMAC? OR SPERMAG? OR SPERMATI? OR SPERMAS? OR SPERMATOB? OR SPERMATOC? OR SPERMATOG?)<br>L18 QUE (SPERMATOI? OR SPERMATOL? OR SPERMATOR? OR SPERMATOX? OR SPERMATOZ? OR SPERMATU? OR SPERMI? OR SPERMO?)<br>L19 QUE (NEONAT? OR NEWBORN? OR DEVELOPMENT OR DEVELOPMENTAL?)<br>L20 QUE (ENDOCRIN? AND DISRUPT?)<br>L21 QUE (ZYGOTE? OR CHILD OR CHILDREN OR ADOLESCEN? OR INFANT?)<br>L22 QUE (WEAN? OR OFFSPRING OR AGE(W)FACTOR?)<br>L23 QUE (DERMAL? OR DERMIS OR SKIN OR EPIDERM? OR CUTANEOUS?)<br>L24 QUE (CARCINOG? OR COCARCINOG? OR CANCER? OR PRECANCER? OR NEOPLAS?)<br>L25 QUE (TUMOR? OR TUMOUR? OR ONCOGEN? OR LYMPHOMA? OR CARCINOM?) |

## Table B-2. Database Query Strings

| Database search date | Query string |
|---|---|
| | L26 QUE (GENETOX? OR GENOTOX? OR MUTAGEN? OR GENETIC(W)TOXIC?)<br>L27 QUE (NEPHROTOX? OR HEPATOTOX?)<br>L28 QUE (ENDOCRIN? OR ESTROGEN? OR ANDROGEN? OR HORMON?)<br>L29 QUE (OCCUPATION? OR WORKER? OR WORKPLACE? OR EPIDEM?)<br>L30 QUE L5 OR L6 OR L7 OR L8 OR L9 OR L10 OR L11 OR L12 OR L13 OR L14 OR L15 OR L16 OR L17 OR L18 OR L19 OR L20 OR L21 OR L22 OR L23 OR L24 OR L25 OR L26 OR L27 OR L28 OR L29<br>L31 QUE (RAT OR RATS OR MOUSE OR MICE OR GUINEA(W)PIG? OR MURIDAE OR DOG OR DOGS OR RABBIT? OR HAMSTER? OR PIG OR PIGS OR SWINE OR PORCINE OR MONKEY? OR MACAQUE?)<br>L32 QUE (MARMOSET? OR FERRET? OR GERBIL? OR RODENT? OR LAGOMORPHA OR BABOON? OR CANINE OR CAT OR CATS OR FELINE OR MURINE)<br>L33 QUE L30 OR L31 OR L32<br>L34 QUE (NONHUMAN MAMMALS)/ORGN<br>L35 QUE L33 OR L34<br>L36 QUE (HUMAN OR HUMANS OR HOMINIDAE OR MAMMALS OR MAMMAL? OR PRIMATES OR PRIMATE?)<br>L37 QUE L35 OR L36<br>---------<br>L38 5842 SEA FILE=TOXCENTER L4 AND L37<br>L39 5090 SEA FILE=TOXCENTER L4 AND L30<br>L40 257 SEA FILE=TOXCENTER L39 AND MEDLINE/FS<br>L41 709 SEA FILE=TOXCENTER L39 AND BIOSIS/FS<br>L42 8 SEA FILE=TOXCENTER L39 NOT (MEDLINE/FS OR BIOSIS/FS OR CAPLUS/FS)<br>L43 4116 SEA FILE=TOXCENTER L39 AND CAPLUS/FS<br>L44 4809 DUP REM L40 L41 L42 L43 (281 DUPLICATES REMOVED)<br>D SCAN L44 |

## Table B-3. Strategies to Augment the Literature Search

| Source | Query and number screened when available |
|---|---|
| **TSCATS via ChemView** | |
| 07/2022 | 110-54-3 |
| **NTP** | |
| 07/2022 | "Hexane" "Hexanes" "n-Hexane" "Hexyl hydride" "Hexane, n-" "n-Hexan" "Gettysolve-B" "Skellysolve B" |
| **Regulations.gov** | |
| 07/2022 | Hexane<br>n-Hexane<br>Hexyl hydride |

**Table B-3. Strategies to Augment the Literature Search**

| Source | Query and number screened when available |
|---|---|
| | Gettysolve-B<br>Skellysolve B |
| **NIH RePORTER** | |
| 01/2023 | Search Criteria--Fiscal Year: Active Projects, Text Search: "Hexane" OR "Hexane, n-" OR "Hexanes" OR "Hexyl hydride" OR "n-Hexan" OR "n-Hexane" OR "Gettysolve" OR "Skellysolve" (advanced), Limit to: Project Title, Project Terms, Project Abstracts |
| **Other** | Identified throughout the assessment process |

The 2022 results were:

- Number of records identified from PubMed, NTRL, and TOXCENTER (after duplicate removal): 7,402
- Number of records identified from other strategies: 87
- Total number of records to undergo literature screening: 7,489

### B.1.2 Literature Screening

A two-step process was used to screen the literature search to identify relevant studies on *n*-hexane:

- Title and abstract screen
- Full text screen

***Title and Abstract Screen.*** Within the reference library, titles and abstracts were screened manually for relevance. Studies that were considered relevant (see Table B-1 for inclusion criteria) were moved to the second step of the literature screening process. Studies were excluded when the title and abstract clearly indicated that the study was not relevant to the toxicological profile.

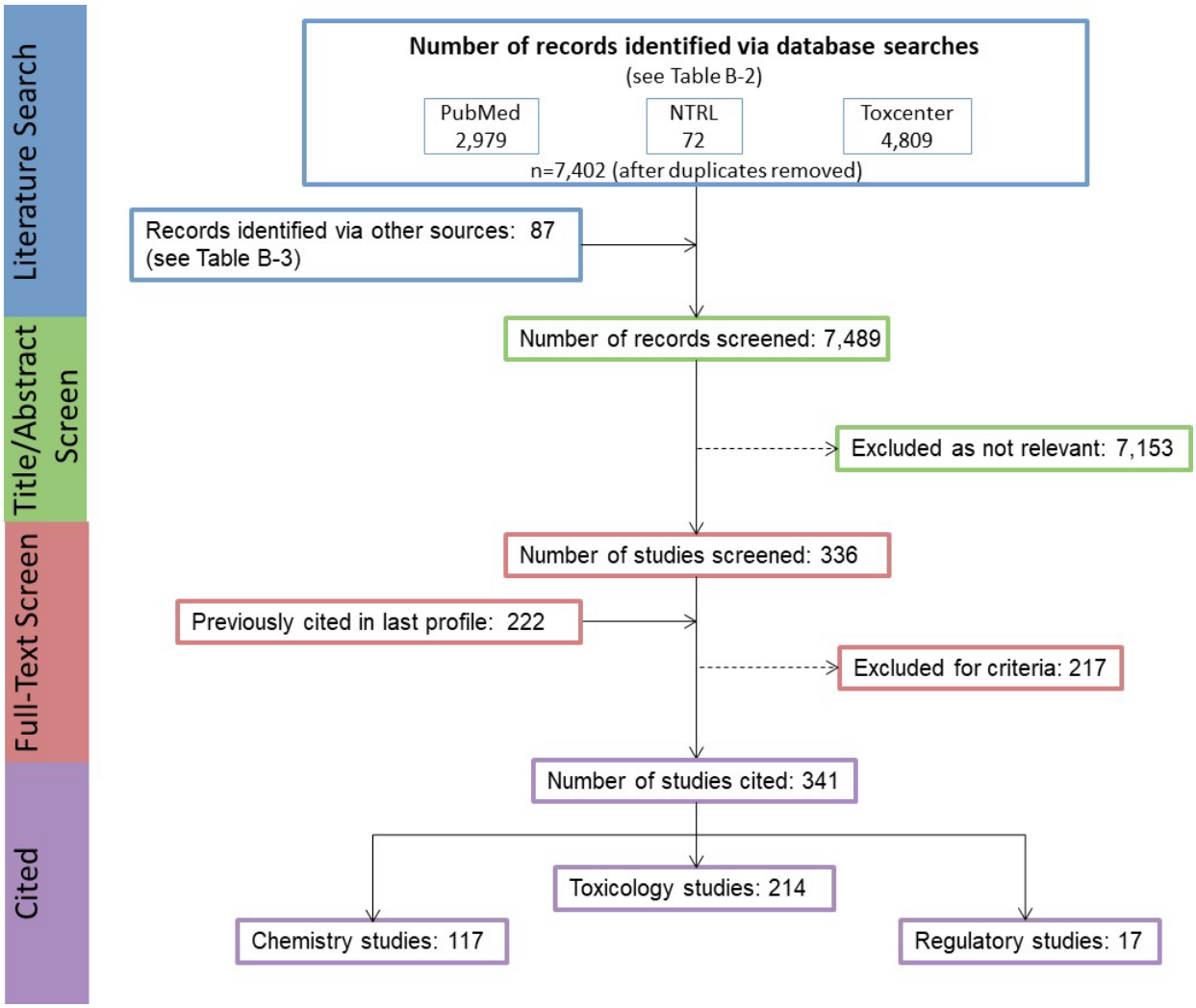
- Number of titles and abstracts screened: 7,489
- Number of studies considered relevant and moved to the next step: 336

***Full Text Screen.*** The second step in the literature screening process was a full text review of individual studies considered relevant in the title and abstract screen step. Each study was reviewed to determine whether it was relevant for inclusion in the toxicological profile.

- Number of studies undergoing full text review: 336
- Number of studies cited in the previous toxicological profile: 222
- Total number of studies cited in the profile: 341

A summary of the results of the literature search and screening is presented in Figure B-1.

## Figure B-1. July 2022 Literature Search Results and Screen for *n*-Hexane



# APPENDIX C. FRAMEWORK FOR ATSDR'S SYSTEMATIC REVIEW OF HEALTH EFFECTS DATA FOR *n*-HEXANE

To increase the transparency of ATSDR's process of identifying, evaluating, synthesizing, and interpreting the scientific evidence on the health effects associated with exposure to *n*-hexane, ATSDR utilized a slight modification of NTP's Office of Health Assessment and Translation (OHAT) systematic review methodology (NTP 2013, 2015; Rooney et al. 2014). ATSDR's framework is an eight-step process for systematic review with the goal of identifying the potential health hazards of exposure to *n*-hexane:

- Step 1. Problem Formulation
- Step 2. Literature Search and Screen for Health Effects Studies
- Step 3. Extract Data from Health Effects Studies
- Step 4. Identify Potential Health Effect Outcomes of Concern
- Step 5. Assess the Risk of Bias for Individual Studies
- Step 6. Rate the Confidence in the Body of Evidence for Each Relevant Outcome
- Step 7. Translate Confidence Rating into Level of Evidence of Health Effects
- Step 8. Integrate Evidence to Develop Hazard Identification Conclusions

## C.1 PROBLEM FORMULATION

The objective of the toxicological profile and this systematic review was to identify the potential health hazards associated with inhalation, oral, or dermal/ocular exposure to *n*-hexane. The inclusion criteria used to identify relevant studies examining the health effects of *n*-hexane are presented in Table C-1.

Data from human and laboratory animal studies were considered relevant for addressing this objective. Human studies were divided into two broad categories: observational epidemiology studies and controlled exposure studies. The observational epidemiology studies were further divided: cohort studies (retrospective and prospective studies), population studies (with individual data or aggregate data), and case-control studies.

**Table C-1. Inclusion Criteria for Identifying Health Effects Studies**

| |
|---|
| Species |
| Human |
| Laboratory mammals |
| Route of exposure |
| Inhalation |
| Oral |
| Dermal (or ocular) |
| Parenteral (these studies will be considered supporting data) |
| Health outcome |
| Death |
| Systemic effects |
| Body weight effects |
| Respiratory effects |
| Cardiovascular effects |

**Table C-1. Inclusion Criteria for Identifying Health Effects Studies**

| |
|---|
| Gastrointestinal effects |
| Hematological effects |
| Musculoskeletal effects |
| Hepatic effects |
| Renal effects |
| Dermal effects |
| Ocular effects |
| Endocrine effects |
| Immunological effects |
| Neurological effects |
| Reproductive effects |
| Developmental effects |
| Other noncancer effects |
| Cancer |

## C.2 LITERATURE SEARCH AND SCREEN FOR HEALTH EFFECTS STUDIES

A literature search and screen were conducted to identify studies examining the health effects of *n*-hexane. The literature search framework for the toxicological profile is discussed in detail in Appendix B.

### C.2.1 Literature Search

As noted in Appendix B, the current literature search was intended to update the 1999 Toxicological Profile for *n*-Hexane; thus, the literature search was restricted to studies published between January 1997 and July 2022. See Appendix B for the databases searched and the search strategy.

A total of 7,489 records relevant to all sections of the toxicological profile were identified (after duplicate removal).

### C.2.2 Literature Screening

As described in Appendix B, a two-step process was used to screen the literature search to identify relevant studies examining the health effects of *n*-hexane.

***Title and Abstract Screen.*** In the Title and Abstract Screen step, 7,489 records were reviewed; 46 documents were considered to meet the health effects inclusion criteria in Table C-1 and were moved to the next step in the process.

***Full Text Screen.*** In the second step in the literature screening process for the systematic review, a full text review of 95 health effect documents (documents identified in the update literature search and documents cited in older versions of the profile) was performed. From those 95 documents (107 studies), 70 documents (90 studies) were included in the qualitative review.

## C.3 EXTRACT DATA FROM HEALTH EFFECTS STUDIES

Relevant data extracted from the individual studies selected for inclusion in the systematic review were collected in customized data forms. A summary of the type of data extracted from each study is presented in Table C-2. For references that included more than one experiment or species, data extraction records were created for each experiment or species.

**Table C-2. Data Extracted from Individual Studies**

| |
|---|
| Citation |
| Chemical form |
| Route of exposure (e.g., inhalation, oral, dermal) |
| Specific route (e.g., gavage in oil, drinking water) |
| Species |
| Strain |
| Exposure duration category (e.g., acute, intermediate, chronic) |
| Exposure duration |
| Frequency of exposure (e.g., 6 hours/day, 5 days/week) |
| Exposure length |
| Number of animals or subjects per sex per group |
| Dose/exposure levels |
| Parameters monitored |
| Description of the study design and method |
| Summary of calculations used to estimate doses (if applicable) |
| Summary of the study results |
| Reviewer's comments on the study |
| Outcome summary (one entry for each examined outcome) |
| No-observed-adverse-effect level (NOAEL) value |
| Lowest-observed-adverse-effect level (LOAEL) value |
| Effect observed at the LOAEL value |

A summary of the extracted data for each study is presented in the Supplemental Document for *n*-Hexane and overviews of the results of the inhalation, oral, and dermal exposure studies are presented in Sections 2.2–2.19 of the profile and in the Levels Significant Exposures tables in Section 2.1 of the profile (Tables 2-1, 2-2, and 2-3, respectively).

## C.4 IDENTIFY POTENTIAL HEALTH EFFECT OUTCOMES OF CONCERN

Overviews of the potential health effect outcomes for *n*-hexane identified in human and animal studies are presented in Tables C-3 and C-4, respectively. Both human and animal studies indicate that the nervous system is the primary target of *n*-hexane exposure. Additionally, the available animal studies suggest that the developmental and respiratory systems may also be sensitive targets of *n*-hexane exposure. Although numerous case reports evaluating *n*-hexane-induced neurotoxicity were available, these studies were not included in this review (discussed in Section 2.15). The remaining epidemiological and animal experimental studies examining these neurological, developmental, and respiratory outcomes were carried through to Steps 4–8 of the systematic review. There were 38 human and 52 animal studies (published in 70 documents) examining these outcomes carried through to Steps 4–8 of the systematic review.

APPENDIX C

## Table C-3. Overview of the Health Outcomes for *n*-Hexane Evaluated In Human Studies

| | Body weight | Respiratory | Cardiovascular | Gastrointestinal | Hematological | Musculoskeletal | Hepatic | Renal | Dermal | Ocular | Endocrine | Immunological | Neurological | Reproductive | Developmental | Other Noncancer | Caner |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Inhalation studies | | | | | | | | | | | | | | | | | |
| Cohort | | | | | | | | | | | | | | | 1<br>1 | | |
| Cross-sectional | 2<br>2 | 6<br>3 | | | 5<br>1 | 1<br>1 | 4<br>2 | 4<br>1 | | 1<br>1 | | 2<br>1 | 24<br>21 | 2<br>2 | | 1<br>0 | |
| Case-control | | | | | | | | | | | | | 5<br>2 | 3<br>3 | 1<br>1 | | 1<br>1 |
| Controlled | | 1<br>0 | | | | | | | | 1<br>0 | | | | | | | |
| Case series | | | | | | 2<br>2 | | | 1<br>1 | | 1<br>0 | | ≥10[a]<br>≥10[a] | | | 2<br>0 | |
| Oral studies | | | | | | | | | | | | | | | | | |
| Cohort | | | | | | | | | | | | | | | | | |
| Case-control | | | | | | | | | | | | | | | | | |
| Controlled | | | | | | | | | | | | | | | | | |
| Case series | | | | | | | | | | | | | | | | | |
| Dermal studies | | | | | | | | | | | | | | | | | |
| Cohort | | | | | | | | | | | | | | | | | |
| Case-control | | | | | | | | | | | | | | | | | |
| Controlled | | | | | | | | | 1<br>1 | | | | | | | | |
| Case series | | | | | | | | | | | | | | | | | |
| Number of studies examining endpoint | | | | 0 | 1 | 2 | 3 | 4 | 5–9 | ≥10 | | | | | | | |
| Number of studies reporting outcome | | | | 0 | 1 | 2 | 3 | 4 | 5–9 | ≥10 | | | | | | | |

[a]Due to the abundant database, case series examining neurological endpoints were not included in the systematic review

APPENDIX C

## Table C-4. Overview of the Health Outcomes for *n*-Hexane Evaluated in Experimental Animal Studies

| | Body weight | Respiratory | Cardiovascular | Gastrointestinal | Hematological | Musculoskeletal | Hepatic | Renal | Dermal | Ocular | Endocrine | Immunological[a] | Neurological[a] | Reproductive[a] | Developmental | Other Noncancer | Caner |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Inhalation studies | | | | | | | | | | | | | | | | | |
| Acute-duration | 6<br>2 | | | | | | | | | | | | 6<br>1 | 5<br>3 | 6<br>3 | | |
| Intermediate-duration | 16<br>11 | 7<br>3 | 3<br>0 | 3<br>0 | 6<br>1 | 3<br>3 | 8<br>0 | 8<br>2 | 3<br>0 | 3<br>1 | 3<br>0 | 4<br>0 | 24<br>22 | 9<br>2 | 3<br>3 | 4<br>0 | 1<br>1 |
| Chronic-duration | 1<br>0 | | | | | | | | | | | | | 1<br>1 | | | |
| Oral studies | | | | | | | | | | | | | | | | | |
| Acute-duration | 2<br>1 | | | | | | | | | | | | | 2<br>0 | 2<br>1 | | |
| Intermediate-duration | 6<br>5 | | | | 1<br>1 | | | | | | | | 7<br>7 | 1<br>1 | | | |
| Chronic-duration | | | | | | | | | | | | | | | | | |
| Dermal studies | | | | | | | | | | | | | | | | | |
| Acute-duration | 1<br>0 | | | | | | | | 1<br>1 | | | | | | | | |
| Intermediate-duration | | | | | | | | | | | | | | | | | |
| Chronic-duration | | | | | | | | | | | | | | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Number of studies examining endpoint | 0 | 1 | 2 | 3 | 4 | 5–9 | ≥10 | |
| Number of studies reporting outcome | 0 | 1 | 2 | 3 | 4 | 5–9 | ≥10 | |

[a]Number of studies examining endpoint includes study evaluating histopathology, but not evaluating function.

## C.5 ASSESS THE RISK OF BIAS FOR INDIVIDUAL STUDIES

### C.5.1 Risk of Bias Assessment

The risk of bias of individual studies was assessed using OHAT's Risk of Bias Tool (NTP 2015). The risk of bias questions for observational epidemiology studies, human-controlled exposure studies, and animal experimental studies are presented in Tables C-5, C-6, and C-7, respectively. Each risk of bias question was answered on a four-point scale:

- **Definitely low risk of bias (++)**
- **Probably low risk of bias (+)**
- **Probably high risk of bias (-)**
- **Definitely high risk of bias (– –)**

In general, "definitely low risk of bias" or "definitely high risk of bias" were used if the question could be answered with information explicitly stated in the study report. If the response to the question could be inferred, then "probably low risk of bias" or "probably high risk of bias" responses were typically used.

**Table C-5. Risk of Bias Questionnaire for Observational Epidemiology Studies**

| |
|---|
| **Selection bias** |
| Were the comparison groups appropriate? |
| **Confounding bias** |
| Did the study design or analysis account for important confounding and modifying variables? |
| **Attrition/exclusion bias** |
| Were outcome data complete without attrition or exclusion from analysis? |
| **Detection bias** |
| Is there confidence in the exposure characterization? |
| Is there confidence in outcome assessment? |
| **Selective reporting bias** |
| Were all measured outcomes reported? |

**Table C-6. Risk of Bias Questionnaire for Human-Controlled Exposure Studies**

| |
|---|
| **Selection bias** |
| Was administered dose or exposure level adequately randomized? |
| Was the allocation to study groups adequately concealed? |
| **Performance bias** |
| Were the research personnel and human subjects blinded to the study group during the study? |
| **Attrition/exclusion bias** |
| Were outcome data complete without attrition or exclusion from analysis? |
| **Detection bias** |
| Is there confidence in the exposure characterization? |
| Is there confidence in outcome assessment? |
| **Selective reporting bias** |
| Were all measured outcomes reported? |

## Table C-7. Risk of Bias Questionnaire for Experimental Animal Studies

| |
|---|
| **Selection bias** |
| Was administered dose or exposure level adequately randomized? |
| Was the allocation to study groups adequately concealed? |
| **Performance bias** |
| Were experimental conditions identical across study groups? |
| Were the research personnel blinded to the study group during the study? |
| **Attrition/exclusion bias** |
| Were outcome data complete without attrition or exclusion from analysis? |
| **Detection bias** |
| Is there confidence in the exposure characterization? |
| Is there confidence in outcome assessment? |
| **Selective reporting bias** |
| Were all measured outcomes reported? |

After the risk of bias questionnaires were completed for the health effects studies, the studies were assigned to one of three risk of bias tiers based on the responses to the key questions listed below and the responses to the remaining questions.

- Is there confidence in the exposure characterization? (only relevant for observational studies)
- Is there confidence in the outcome assessment?
- Does the study design or analysis account for important confounding and modifying variables? (only relevant for observational studies)

***First Tier.*** Studies placed in the first tier received ratings of "definitely low" or "probably low" risk of bias on the key questions **AND** received a rating of "definitely low" or "probably low" risk of bias on the responses to at least 50% of the other applicable questions.

***Second Tier.*** A study was placed in the second tier if it did not meet the criteria for the first or third tiers.

***Third Tier.*** Studies placed in the third tier received ratings of "definitely high" or "probably high" risk of bias for the key questions **AND** received a rating of "definitely high" or "probably high" risk of bias on the response to at least 50% of the other applicable questions.

The results of the risk of bias assessment for the different types of *n*-hexane health effects studies (observational epidemiology, human-controlled exposure, and animal experimental studies) are presented in Tables C-8, C-9, and C-10, respectively.

## Table C-8. Summary of Risk of Bias Assessment for *n*-Hexane—Observational Epidemiology Studies

| Reference | Risk of bias criteria and ratings | | | | | | Risk of bias tier |
|---|---|---|---|---|---|---|---|
| | Selection bias | Confounding bias | Attrition / exclusion bias | Detection bias | | Selective reporting bias | |
| | Were the comparison groups appropriate? | Did the study design or analysis account for important confounding and modifying variables?* | Were outcome data complete without attrition or exclusion from analysis? | Is there confidence in the exposure characterization?* | Is there confidence in the outcome assessment?* | Were all measured outcomes reported? | |
| ***Outcome: Developmental*** | | | | | | | |
| *Cohort* | | | | | | | |
| Lehmann et al. 2002 | ++ | + | + | + | + | ++ | First |
| *Case-control* | | | | | | | |
| Gong et al. 2018 | ++ | + | ++ | + | + | ++ | First |
| ***Outcome: Respiratory*** | | | | | | | |
| *Cross-sectional* | | | | | | | |
| Buchdahl et al. 2000 | + | + | + | + | + | ++ | First |
| Mustajbegovic et al. 2000 | + | + | ++ | + | + | ++ | First |
| Nijem et al. 2000 | – | – | ++ | – | – | + | Third |
| Nijem et al. 2001 | – | – | ++ | – | – | + | Third |
| Paciencia et al. 2020 | – | – | – | – | – | – | Third |
| Wichmann et al. 2009 | + | + | ++ | – | ++ | ++ | Second |
| ***Outcome: Neurological*** | | | | | | | |
| *Case-control* | | | | | | | |
| Boggess er al. 2016 | ++ | + | ++ | + | ++ | ++ | First |
| Goldman et al. 2012 | + | – | + | – | + | + | Second |
| Issever et al. 2002 | – | – | + | – | – | + | Third |

## Table C-8. Summary of Risk of Bias Assessment for *n*-Hexane—Observational Epidemiology Studies

| Reference | Risk of bias criteria and ratings | | | | | | Risk of bias tier |
|---|---|---|---|---|---|---|---|
| | Selection bias | Confounding bias | Attrition / exclusion bias | Detection bias | | Selective reporting bias | |
| | Were the comparison groups appropriate? | Did the study design or analysis account for important confounding and modifying variables?* | Were outcome data complete without attrition or exclusion from analysis? | Is there confidence in the exposure characterization?* | Is there confidence in the outcome assessment?* | Were all measured outcomes reported? | |
| Talbott et al. 2015 | ++ | ++ | ++ | + | + | ++ | First |
| Verberk et al. 2004 | – | – | + | – | + | + | Third |
| *Cross-sectional* | | | | | | | |
| Bates et al. 2016, 2019 | + | – | – | – | + | ++ | Third |
| Beckman et al. 2016 | + | + | + | – | + | ++ | Second |
| Chang and Yip 1987 | – | – | + | – | – | + | Third |
| Chang 1987 | – | – | + | – | – | + | Third |
| Chang et al. 1993 | – | – | + | + | – | + | Third |
| Gong et al. 2003 | + | + | + | + | + | ++ | First |
| Governa et al. 1987 | – | – | – | + | – | – | Third |
| Huang et al. 1991 | – | – | – | + | – | – | Third |
| Ithnin et al. 2011 | – | – | – | – | – | – | Third |
| Juarez-Perez et al. 2014 | – | – | – | + | – | + | Third |
| Murata et al. 1994 | + | + | – | + | – | + | Second |
| Mutti et al. 1982a | + | – | – | + | + | + | Second |
| Mutti et al. 1982b | + | – | + | + | + | + | Second |
| Neghab et al. 2012 | + | + | + | ++ | + | ++ | First |
| Nijem et al. 2000 | – | – | + | – | + | + | Second |

## Table C-8. Summary of Risk of Bias Assessment for *n*-Hexane—Observational Epidemiology Studies

| Reference | Risk of bias criteria and ratings | | | | | | Risk of bias tier |
|---|---|---|---|---|---|---|---|
| | Selection bias | Confounding bias | Attrition / exclusion bias | Detection bias | | Selective reporting bias | |
| | Were the comparison groups appropriate? | Did the study design or analysis account for important confounding and modifying variables?* | Were outcome data complete without attrition or exclusion from analysis? | Is there confidence in the exposure characterization?* | Is there confidence in the outcome assessment?* | Were all measured outcomes reported? | |
| Nijem et al. 2001 | – | – | + | – | + | + | Third |
| Park et al. 2009 | + | – | + | + | – | + | Third |
| Pastore et al. 1994 | + | – | – | + | + | + | Second |
| Raitta et al. 1978; Seppalainen et al. 1979 | – | – | + | – | + | + | Third |
| Sanagi et al. 1980 | + | + | – | ++ | + | + | First |
| Sliwinska-Kowalska et al. 2005 | + | ++ | + | + | + | + | First |
| Tsai et al. 1997 | + | ++ | + | – | + | ++ | Second |
| Wang et al. 1986 | – | – | – | – | – | + | Third |
| Yokoyama et al. 1997 | + | – | + | – | + | + | Third |

++ = definitely low risk of bias; + = probably low risk of bias; – = probably high risk of bias; – – = definitely high risk of bias

*Key questions used to assign risk of bias tier

APPENDIX C

## Table C-9. Summary of Risk of Bias Assessment for *n*-Hexane – Human-Controlled Exposure Studies

| Reference | Risk of bias criteria and ratings | | | | | | | Risk of bias tier |
|---|---|---|---|---|---|---|---|---|
| | Selection bias | | Performance bias | Attrition/ exclusion bias | Detection bias | | Selective reporting bias | |
| | Was administered dose or exposure level adequately randomized? | Was the allocation to study groups adequately concealed? | Were the research personnel and human subjects blinded to the study group during the study? | Were outcome data complete without attrition or exclusion from analysis? | Is there confidence in the exposure characterization? | Is there confidence in the outcome assessment?* | Were all measured outcomes reported? | |
| ***Outcome: Respiratory*** | | | | | | | | |
| *Inhalation acute exposure* | | | | | | | | |
| Nelson et al. 1943 | – | – | – | – | – – | – | – | Third |

++ = definitely low risk of bias; + = probably low risk of bias; – = probably high risk of bias; – – = definitely high risk of bias

*Key question used to assign risk of bias tier

## Table C-10. Summary of Risk of Bias Assessment for *n*-Hexane—Experimental Animal Studies

| Reference | Risk of bias criteria and ratings: Selection bias: Was administered dose or exposure level adequately randomized? | Selection bias: Was the allocation to study groups adequately concealed? | Performance bias: Were experimental conditions identical across study groups? | Performance bias: Were the research personnel blinded to the study group during the study? | Attrition/ exclusion bias: Were outcome data complete without attrition or exclusion from analysis? | Detection bias: Is there confidence in the exposure characterization? | Detection bias: Is there confidence in the outcome assessment?* | Selective reporting bias: Were all measured outcomes reported? | Risk of bias tier |
|---|---|---|---|---|---|---|---|---|---|
| ***Outcome: Developmental Effects*** | | | | | | | | | |
| *Inhalation acute exposure* | | | | | | | | | |
| API 1979 | – | – | + | + | + | – | + | ++ | First |
| Bus et al. 1979 (GDs 8–12) | – | – | + | + | + | + | + | ++ | First |
| Bus et al. 1979 (GDs 12–16) | – | – | + | + | + | + | + | ++ | First |
| Bus et al. 1979 (GDs 8–16) | – | – | + | + | + | + | + | ++ | First |
| NIEHS 1987 | + | – | ++ | + | + | ++ | + | ++ | First |
| NIEHS 1988c | + | – | ++ | + | + | ++ | + | ++ | First |
| *Inhalation intermediate exposure* | | | | | | | | | |
| Li et al. 2014, 2015 | – | – | – | – | – | – | – | + | Third |
| Stoltenburg-Didinger et al. 1990 (21 days) | – | – | – – | – | – | – | + | – | Third |
| Stoltenburg-Didinger et al. 1990 (63 days) | – | – | – – | – | – | – | + | – | Third |
| *Oral acute exposure* | | | | | | | | | |
| Marks et al. 1980 (1 dose/day) | + | – | + | + | + | + | + | + | Second |
| Marks et al. 1980 (3 doses/day) | + | – | + | + | + | + | + | + | Second |
| ***Outcome: Respiratory Effects*** | | | | | | | | | |
| *Inhalation intermediate exposure* | | | | | | | | | |
| API 1981 | ++ | – | ++ | + | + | ++ | ++ | ++ | First |
| Cavender et al. 1984 | + | – | + | + | ++ | ++ | ++ | ++ | First |

APPENDIX C

## Table C-10. Summary of Risk of Bias Assessment for *n*-Hexane—Experimental Animal Studies

| Reference | Risk of bias criteria and ratings | | | | | | | | Risk of bias tier |
|---|---|---|---|---|---|---|---|---|---|
| | Selection bias | | Performance bias | | Attrition/ exclusion bias | Detection bias | | Selective reporting bias | |
| | Was administered dose or exposure level adequately randomized? | Was the allocation to study groups adequately concealed? | Were experimental conditions identical across study groups? | Were the research personnel blinded to the study group during the study? | Were outcome data complete without attrition or exclusion from analysis? | Is there confidence in the exposure characterization? | Is there confidence in the outcome assessment?* | Were all measured outcomes reported? | |
| Howd et al. 1983 (21 days old) | + | – | + | + | ++ | – | + | ++ | First |
| Howd et al. 1983 (80 days old) | + | – | + | + | ++ | – | + | ++ | First |
| Lungarella et al. 1984 | – | – | + | – | – – | + | + | + | Third |
| NTP 1991 (6 hours/day) | ++ | ++ | + | + | + | ++ | ++ | ++ | First |
| NTP 1991 (22 hours/day) | ++ | ++ | + | + | + | ++ | ++ | ++ | First |
| ***Outcome: Neurological Effects*** | | | | | | | | | |
| *Inhalation acute exposure* | | | | | | | | | |
| Chalansonnet et al. 2013 | – | – | ++ | + | – | ++ | – | + | Third |
| De Martino et al. 1987 (1–2 weeks) | + | – | – | + | – | + | – | ++ | Third |
| NIEHS 1987 | + | – | ++ | + | + | ++ | + | ++ | First |
| NIEHS 1988a | + | – | ++ | + | + | ++ | + | ++ | First |
| NIEHS 1988b | + | – | ++ | + | + | ++ | + | ++ | First |
| NIEHS 1988c | + | – | ++ | + | + | ++ | + | ++ | First |
| *Inhalation intermediate exposure* | | | | | | | | | |
| Altenkirch et al. 1982 (9 weeks) | – | – | ++ | – | + | + | ++ | ++ | Second |
| Altenkirch et al. 1982 (40 weeks) | – | – | ++ | – | + | + | ++ | ++ | Second |
| API 1981 | ++ | – | ++ | – | + | ++ | ++ | ++ | First |
| Cavender et al. 1984 | + | – | + | – | ++ | ++ | ++ | ++ | First |

APPENDIX C

## Table C-10. Summary of Risk of Bias Assessment for *n*-Hexane—Experimental Animal Studies

| Reference | Risk of bias criteria and ratings | | | | | | | | Risk of bias tier |
|---|---|---|---|---|---|---|---|---|---|
| | Selection bias | | Performance bias | | Attrition/ exclusion bias | Detection bias | | Selective reporting bias | |
| | Was administered dose or exposure level adequately randomized? | Was the allocation to study groups adequately concealed? | Were experimental conditions identical across study groups? | Were the research personnel blinded to the study group during the study? | Were outcome data complete without attrition or exclusion from analysis? | Is there confidence in the exposure characterization? | Is there confidence in the outcome assessment?* | Were all measured outcomes reported? | |
| De Martino et al. 1987 (3–6 weeks) | + | – | – | – | – | + | – | ++ | Third |
| Frontali et al. 1981 (9 hours/day, 5 days/week) | ++ | – | + | – | – | + | – | + | Third |
| Frontali et al. 1981 (10 hours/day, 6 days/week) | ++ | – | + | – | – | + | – | + | Third |
| Howd et al. 1983 (21 days old) | + | – | + | – | ++ | – | + | ++ | Second |
| Howd et al. 1983 (80 days old) | + | – | + | – | ++ | – | + | ++ | Second |
| Huang et al. 1989 | + | – | + | – | + | + | + | ++ | Second |
| Ichihara et al. 1998 | + | – | + | + | + | + | + | ++ | First |
| Li et al. 2014, 2015 | – | – | – | – | – | – | – | + | Third |
| Lungarella et al. 1984 | – | – | + | – | – – | + | + | + | Third |
| NTP 1991 (6 hours/day) | ++ | ++ | + | – | + | ++ | ++ | ++ | First |
| NTP 1991 (22 hours/day) | ++ | ++ | + | – | + | ++ | ++ | ++ | First |
| Pryor et al. 1983 | + | + | + | – | – | + | + | + | Second |
| Rebert and Sorenson 1983 | – | – | + | – | + | – | + | + | Third |
| Schaumburg and Spencer 1976 | – | – | – – | – | – | – – | – | – | Third |
| Stoltenburg-Didinger et al. 1990 (21 days) | – | – | – | – | – | – | + | + | Third |
| Takeuchi et al. 1980 | – | – | – | – | – | + | + | + | Third |

APPENDIX C

## Table C-10. Summary of Risk of Bias Assessment for *n*-Hexane—Experimental Animal Studies

| Reference | Risk of bias criteria and ratings: Selection bias: Was administered dose or exposure level adequately randomized? | Selection bias: Was the allocation to study groups adequately concealed? | Performance bias: Were experimental conditions identical across study groups? | Performance bias: Were the research personnel blinded to the study group during the study? | Attrition/ exclusion bias: Were outcome data complete without attrition or exclusion from analysis? | Detection bias: Is there confidence in the exposure characterization? | Detection bias: Is there confidence in the outcome assessment?* | Selective reporting bias: Were all measured outcomes reported? | Risk of bias tier |
|---|---|---|---|---|---|---|---|---|---|
| *Oral intermediate exposure* | | | | | | | | | |
| Gao et al. 2019 | + | - | + | - | + | – | + | + | First |
| Krasavage et al. 1980 | + | – | + | – | + | ++ | + | ++ | First |
| Li et al. 2018 | + | – | + | – | – | – | + | ++ | Second |
| Li et al. 2020a | + | – | + | ++ | – | – | + | ++ | Second |
| Li et al. 2020b | + | – | + | ++ | – | – | + | + | Second |
| Ono et al. 1981 | – | – | + | – | – | – – | + | – | Third |
| Wang et al. 2017 | + | – | + | – | + | – | + | + | Third |

++ = definitely low risk of bias; + = probably low risk of bias; – = probably high risk of bias; – – = definitely high risk of bias

*Key question used to assign risk of bias tier

## C.6 RATE THE CONFIDENCE IN THE BODY OF EVIDENCE FOR EACH RELEVANT OUTCOME

Confidences in the bodies of human and animal evidence were evaluated independently for each potential outcome. ATSDR did not evaluate the confidence in the body of evidence for carcinogenicity; rather, the Agency defaulted to the cancer weight-of-evidence assessment of other agencies including HHS, EPA, and IARC. The confidence in the body of evidence for an association or no association between exposure to *n*-hexane and a particular outcome was based on the strengths and weaknesses of individual studies. Four descriptors were used to describe the confidence in the body of evidence for effects or when no effect was found:

- **High confidence:** the true effect is highly likely to be reflected in the apparent relationship
- **Moderate confidence:** the true effect may be reflected in the apparent relationship
- **Low confidence:** the true effect may be different from the apparent relationship
- **Very low confidence:** the true effect is highly likely to be different from the apparent relationship

Confidence in the body of evidence for a particular outcome was rated for each type of study: case-control, case series, cohort, population, human-controlled exposure, and experimental animal. In the absence of data to the contrary, data for a particular outcome were collapsed across animal species, routes of exposure, and exposure durations. If species (or strain), route, or exposure duration differences were noted, then the data were treated as separate outcomes.

### C.6.1 Initial Confidence Rating

In ATSDR's modification to the OHAT approach, the body of evidence for an association (or no association) between exposure to *n*-hexane and a particular outcome was given an initial confidence rating based on the key features of the individual studies examining that outcome. The presence of these key features of study design was determined for individual studies using four "yes or no" questions, which were customized for epidemiology, human controlled exposure, or experimental animal study designs. Separate questionnaires were completed for each outcome assessed in a study. The key features for observational epidemiology (cohort, population, and case-control) studies, human controlled exposure, and experimental animal studies are presented in Tables C-11, C-12, and C-13, respectively. The initial confidence in the study was determined based on the number of key features present in the study design:

- **High Initial Confidence:** Studies in which the responses to the four questions were "yes".

- **Moderate Initial Confidence:** Studies in which the responses to only three of the questions were "yes".

- **Low Initial Confidence:** Studies in which the responses to only two of the questions were "yes".

- **Very Low Initial Confidence:** Studies in which the response to one or none of the questions was "yes".

## Table C-11. Key Features of Study Design for Observational Epidemiology Studies

| |
|---|
| Exposure was experimentally controlled |
| Exposure occurred prior to the outcome |
| Outcome was assessed on individual level rather than at the population level |
| A comparison group was used |

## Table C-12. Key Features of Study Design for Human-Controlled Exposure Studies

| |
|---|
| A comparison group was used or the subjects served as their own control |
| A sufficient number of subjects were tested |
| Appropriate methods were used to measure outcomes (i.e., clinically-confirmed outcome versus self-reported) |
| Appropriate statistical analyses were performed and reported or the data were reported in such a way to allow independent statistical analysis |

## Table C-13. Key Features of Study Design for Experimental Animal Studies

| |
|---|
| A concurrent control group was used |
| A sufficient number of animals per group were tested |
| Appropriate parameters were used to assess a potential adverse effect |
| Appropriate statistical analyses were performed and reported or the data were reported in such a way to allow independent statistical analysis |

The presence or absence of the key features and the initial confidence levels for studies examining developmental, respiratory, and neurological outcomes observed in the observational epidemiology, human-controlled exposure, and animal experimental studies are presented in Tables C-14, C-15, and C-16, respectively.

## Table C-14. Presence of Key Features of Study Design for *n*-Hexane—Observational Epidemiology Studies

| Reference | Key features | | | | Initial study confidence |
|---|---|---|---|---|---|
| | Controlled exposure | Exposure prior to outcome | Outcomes assessed on an individual level | Comparison group | |
| ***Outcome: Developmental*** | | | | | |
| *Cohort* | | | | | |
| Lehmann et al. 2002 | No | No | Yes | Yes | Low |
| *Case-control* | | | | | |
| Gong et al. 2018 | No | No | Yes | Yes | Low |
| ***Outcome: Respiratory*** | | | | | |
| *Cross-sectional* | | | | | |
| Buchdahl et al. 2000 | No | No | Yes | Yes | Low |
| Mustajbegovic et al. 2000 | No | Yes | Yes | Yes | Moderate |
| Nijem et al. 2000 | No | Yes | Yes | No | Low |
| Nijem et al. 2001 | No | Yes | Yes | No | Low |
| Paciencia et al. 2020 | No | No | Yes | No | Very low |
| Wichmann et al. 2009 | No | No | Yes | Yes | Low |
| ***Outcome:*** | | | | | |
| *Case-control* | | | | | |
| Boggess er al. 2016 | No | No | Yes | Yes | Low |
| Goldman et al. 2012 | No | Yes | Yes | Yes | Moderate |
| Issever et al. 2002 | No | Yes | Yes | Yes | Moderate |
| Talbott et al. 2015 | No | No | Yes | Yes | Low |
| Verberk et al. 2004 | No | Yes | Yes | Yes | Moderate |
| *Cross-sectional* | | | | | |
| Bates et al. 2016, 2019 | No | Yes | Yes | Yes | Moderate |
| Beckman et al. 2016 | No | Yes | Yes | Yes | Moderate |
| Chang and Yip 1987 | No | Yes | Yes | Yes | Moderate |
| Chang 1987 | No | Yes | Yes | Yes | Moderate |
| Chang et al. 1993 | No | Yes | Yes | Yes | Moderate |
| Gong et al. 2003 | No | Yes | Yes | Yes | Moderate |
| Governa et al. 1987 | No | Yes | Yes | No | Low |
| Huang et al. 1991 | No | Yes | Yes | Yes | Moderate |
| Ithnin et al. 2011 | No | Yes | Yes | No | Low |
| Juarez-Perez et al. 2014 | No | Yes | Yes | Yes | Moderate |
| Murata et al. 1994 | No | Yes | Yes | Yes | Moderate |

**Table C-14. Presence of Key Features of Study Design for *n*-Hexane—Observational Epidemiology Studies**

| Reference | Key features: Controlled exposure | Exposure prior to outcome | Outcomes assessed on an individual level | Comparison group | Initial study confidence |
|---|---|---|---|---|---|
| Mutti et al. 1982a | No | Yes | Yes | Yes | Moderate |
| Mutti et al. 1982b | No | Yes | Yes | Yes | Moderate |
| Neghab et al. 2012 | No | Yes | Yes | Yes | Moderate |
| Nijem et al. 2000 | No | Yes | Yes | No | Low |
| Nijem et al. 2001 | No | Yes | Yes | No | Low |
| Park et al. 2009 | No | Yes | Yes | Yes | Moderate |
| Pastore et al. 1994 | No | Yes | Yes | Yes | Moderate |
| Raitta et al. 1978; Seppalainen et al. 1979 | No | Yes | Yes | Yes | Moderate |
| Sanagi et al. 1980 | No | Yes | Yes | Yes | Moderate |
| Sliwinska-Kowalska et al. 2005 | No | Yes | Yes | Yes | Moderate |
| Tsai et al. 1997 | No | Yes | Yes | Yes | Moderate |
| Wang et al. 1986 | No | Yes | Yes | No | Low |
| Yokoyama et al. 1997 | No | Yes | Yes | Yes | Moderate |

**Table C-15. Presence of Key Features of Study Design for *n*-Hexane—Human-Controlled Exposure**

| Reference | Key Features: Comparison group or served as own controls | Sufficient number of subjects tested | Appropriate outcome assessment | Appropriate statistical analysis | Initial study confidence |
|---|---|---|---|---|---|
| ***Outcome: Respiratory effects*** | | | | | |
| *Inhalation acute exposure* | | | | | |
| Nelson et al. 1943 | No | No | Yes | No | Very low |

## Table C-16. Presence of Key Features of Study Design for *n*-Hexane—Experimental Animal Studies

| Reference | Key features | | | | Initial study confidence |
|---|---|---|---|---|---|
| | Concurrent control group | Sufficient number of animals per group | Appropriate parameters to assess potential effect | Adequate data for statistical analysis | |
| ***Outcome: Developmental Effects*** | | | | | |
| *Inhalation acute exposure* | | | | | |
| API 1979 | Yes | Yes | Yes | Yes | High |
| Bus et al. 1979 (GDs 8–12) | Yes | No | Yes | Yes | Moderate |
| Bus et al. 1979 (GDs 12–16) | Yes | No | Yes | Yes | Moderate |
| Bus et al. 1979 (GDs 8–16) | Yes | No | Yes | Yes | Moderate |
| NIEHS 1987 | Yes | Yes | Yes | Yes | High |
| NIEHS 1988c | Yes | Yes | Yes | Yes | High |
| *Inhalation intermediate exposure* | | | | | |
| Li et al. 2014, 2015 | Yes | No | Yes | Yes | Moderate |
| Stoltenburg-Didinger et al. 1990 (21 days) | Yes | No | Yes | No | Low |
| Stoltenburg-Didinger et al. 1990 (63 days) | Yes | No | Yes | No | Low |
| *Oral acute exposure* | | | | | |
| Marks et al. 1980 (1 dose/day) | Yes | No | Yes | Yes | Moderate |
| Marks et al. 1980 (3 doses/day) | Yes | No | Yes | Yes | Moderate |
| ***Outcome: Respiratory Effects*** | | | | | |
| *Inhalation intermediate exposure* | | | | | |
| API 1981 | Yes | Yes | Yes | Yes | High |
| Cavender et al. 1984 | Yes | Yes | Yes | Yes | High |
| Howd et al. 1983 (21 days old) | Yes | Yes | Yes | Yes | High |
| Howd et al. 1983 (80 days old) | Yes | Yes | Yes | Yes | High |
| Lungarella et al. 1984 | Yes | Yes | Yes | No | Moderate |
| NTP 1991 (6 hours/day) | Yes | Yes | Yes | Yes | High |
| NTP 1991 (22 hours/day) | Yes | Yes | Yes | Yes | High |
| ***Outcome: Neurological Effects*** | | | | | |
| *Inhalation acute exposure* | | | | | |
| Chalansonnet et al. 2013 | Yes | No | Yes | No | Low |
| De Martino et al. 1987 (1–2 weeks) | Yes | No | Yes | No | Low |
| NIEHS 1987 | Yes | Yes | Yes | No | Moderate |
| NIEHS 1988a | Yes | Yes | Yes | No | Moderate |

## Table C-16. Presence of Key Features of Study Design for *n*-Hexane—Experimental Animal Studies

| Reference | Key features | | | | Initial study confidence |
|---|---|---|---|---|---|
| | Concurrent control group | Sufficient number of animals per group | Appropriate parameters to assess potential effect | Adequate data for statistical analysis | |
| NIEHS 1988b | Yes | Yes | Yes | No | Moderate |
| NIEHS 1988c | Yes | Yes | Yes | No | Moderate |
| *Inhalation intermediate exposure* | | | | | |
| Altenkirch et al. 1982 (9 weeks) | Yes | No | Yes | No | Low |
| Altenkirch et al. 1982 (40 weeks) | Yes | No | Yes | No | Low |
| API 1981 | Yes | Yes | Yes | Yes | High |
| Cavender et al. 1984 | Yes | Yes | Yes | Yes | High |
| De Martino et al. 1987 (3–6 weeks) | Yes | No | Yes | No | Low |
| Frontali et al. 1981 (9 hours/day, 5 days/week) | Yes | No | Yes | No | Low |
| Frontali et al. 1981 (10 hours/day, 6 days/week) | Yes | No | Yes | No | Low |
| Howd et al. 1983 (21 days old) | Yes | Yes | Yes | Yes | High |
| Howd et al. 1983 (80 days old) | Yes | Yes | Yes | Yes | High |
| Huang et al. 1989 | Yes | No | Yes | Yes | Moderate |
| Ichihara et al. 1998 | Yes | No | Yes | Yes | Moderate |
| Li et al. 2014, 2015 | Yes | No | Yes | Yes | Moderate |
| Lungarella et al. 1984 | Yes | Yes | Yes | No | Moderate |
| NTP 1991 (6 hours/day) | Yes | Yes | Yes | Yes | High |
| NTP 1991 (22 hours/day) | Yes | Yes | Yes | Yes | High |
| Pryor et al. 1983 | Yes | Yes | Yes | Yes | High |
| Rebert and Sorenson 1983 | Yes | No | Yes | Yes | Moderate |
| Schaumburg and Spencer 1976 | No | No | Yes | No | Low |
| Stoltenburg-Didinger et al. 1990 (21 days) | Yes | No | Yes | No | Low |
| Stoltenburg-Didinger et al. 1990 (63 days) | Yes | No | Yes | No | Low |
| Takeuchi et al. 1980 | Yes | No | Yes | Yes | Moderate |
| *Oral intermediate exposure* | | | | | |
| Gao et al. 2019 | Yes | Yes | Yes | Yes | High |
| Krasavage et al. 1980 | Yes | No | Yes | No | Low |
| Li et al. 2018 | Yes | Yes | Yes | Yes | High |
| Li et al. 2020a | Yes | Yes | Yes | Yes | High |

**Table C-16. Presence of Key Features of Study Design for *n*-Hexane—Experimental Animal Studies**

| Reference | Key features: Concurrent control group | Sufficient number of animals per group | Appropriate parameters to assess potential effect | Adequate data for statistical analysis | Initial study confidence |
|---|---|---|---|---|---|
| Li et al. 2020b | Yes | No | Yes | Yes | Moderate |
| Ono et al. 1981 | Yes | No | Yes | Yes | Moderate |
| Wang et al. 2017 | Yes | Yes | Yes | Yes | High |

A summary of the initial confidence ratings for each outcome is presented in Table C-17. If individual studies for a particular outcome and study type had different study quality ratings, then the highest confidence rating for the group of studies was used to determine the initial confidence rating for the body of evidence; any exceptions were noted in Table C-17.

**Table C-17. Initial Confidence Rating for *n*-Hexane Health Effects Studies**

| | Initial study confidence | Initial confidence rating |
|---|---|---|
| ***Outcome: Developmental Effects*** | | |
| *Inhalation acute exposure* | | |
| Animal studies | | |
| Bus et al. 1979 (GDs 8–12) | Moderate | High |
| Bus et al. 1979 (GDs 12–16) | Moderate | |
| Bus et al. 1979 (GDs 8–16) | Moderate | |
| API 1979 | High | |
| NIEHS 1987 | High | |
| NIEHS 1988c | High | |
| *Inhalation intermediate exposure* | | |
| Animal studies | | |
| Li et al. 2014, 2015 | Moderate | Moderate |
| Stoltenburg-Didinger et al. 1990 (21 days) | Low | |
| Stoltenburg-Didinger et al. 1990 (63 days) | Low | |
| *Inhalation chronic exposure* | | |
| Human studies | | |
| Lehmann et al. 2002 | Low | Low |
| Gong et al. 2018 | Low | |

## Table C-17. Initial Confidence Rating for *n*-Hexane Health Effects Studies

| | Initial study confidence | Initial confidence rating |
|---|---|---|
| *Oral acute exposure* | | |
| Animal studies | | |
| Marks et al. 1980 (1 dose/day) | Moderate | Moderate |
| Marks et al. 1980 (3 doses/day) | Moderate | |
| ***Outcome: Respiratory Effects*** | | |
| *Inhalation acute exposure* | | |
| Human studies | | |
| Nelson et al. 1943 | Very Low | Very Low |
| *Inhalation intermediate exposure* | | |
| Animal studies | | |
| API 1981 | High | High |
| Cavender et al. 1984 | High | |
| Howd et al. 1983 (21 days old) | High | |
| Howd et al. 1983 (80 days old) | High | |
| Lungarella et al. 1984 | Moderate | |
| NTP 1991 (6 hours/day) | High | |
| NTP 1991 (22 hours/day) | High | |
| *Inhalation chronic exposure* | | |
| Human studies | | |
| Buchdahl et al. 2000 | Low | Low |
| Mustajbegovic et al. 2000 | Moderate | |
| Nijem et al. 2000 | Low | |
| Nijem et al. 2001 | Low | |
| Paciencia et al. 2020 | Very low | |
| Wichmann et al. 2009 | Low | |
| Nelson et al. 1943 | Very low | |
| ***Outcome: Neurological Effects*** | | |
| *Inhalation acute exposure* | | |
| Animal studies | | |
| Chalansonnet et al. 2013 | Low | Moderate |
| De Martino et al. 1987 (1–2 weeks) | Low | |
| NIEHS 1987 | Moderate | |
| NIEHS 1988a | Moderate | |
| NIEHS 1988b | Moderate | |
| NIEHS 1988c | Moderate | |
| *Inhalation intermediate exposure* | | |
| Animal studies | | |
| API 1981 | High | High |
| Altenkirch et al. 1982 (9 weeks) | Low | |
| Altenkirch et al. 1982 (40 weeks) | Low | |

## Table C-17. Initial Confidence Rating for *n*-Hexane Health Effects Studies

| | Initial study confidence | Initial confidence rating |
|---|---|---|
| Cavender et al. 1984 | High | |
| De Martino et al. 1987 (3–6 weeks) | Low | |
| Frontali et al. 1981 (9 hours/day, 5 days/week) | Low | |
| Frontali et al. 1981 (10 hours/day, 6 days/week) | Low | |
| Howd et al. 1983 (21 days old) | High | |
| Howd et al. 1983 (80 days old) | High | |
| Huang et al. 1989 | Moderate | |
| Ichihara et al. 1998 | Moderate | |
| Li et al. 2014, 2015 | Moderate | |
| Lungarella et al. 1984 | Moderate | |
| NTP 1991 (6 hours/day) | High | |
| NTP 1991 (22 hours/day) | High | |
| Pryor et al. 1983 | High | |
| Rebert and Sorenson 1983 | Moderate | |
| Schaumburg and Spencer 1976 | Low | |
| Stoltenburg-Didinger et al. 1990 (21 days) | Low | |
| Takeuchi et al. 1980 | Moderate | |
| *Inhalation chronic exposure* | | |
| Human studies | | |
| Boggess er al. 2016 | Low | |
| Goldman et al. 2012 | Moderate | |
| Issever et al. 2002 | Moderate | |
| Talbott et al. 2015 | Low | |
| Verberk et al. 2004 | Moderate | |
| Bates et al. 2016, 2019 | Moderate | |
| Beckman et al. 2016 | Moderate | |
| Chang and Yip 1987 | Moderate | |
| Chang 1987 | Moderate | |
| Chang et al. 1993 | Moderate | |
| Gong et al. 2003 | Moderate | Moderate |
| Governa et al. 1987 | Low | |
| Huang et al. 1991 | Moderate | |
| Ithnin et al. 2011 | Low | |
| Juarez-Perez et al. 2014 | Moderate | |
| Murata et al. 1994 | Moderate | |
| Mutti et al. 1982a | Moderate | |
| Mutti et al. 1982b | Moderate | |
| Neghab et al. 2012 | Moderate | |
| Nijem et al. 2000 | Low | |
| Nijem et al. 2001 | Low | |

**Table C-17. Initial Confidence Rating for *n*-Hexane Health Effects Studies**

| | Initial study confidence | Initial confidence rating |
|---|---|---|
| Park et al. 2009 | Moderate | |
| Pastore et al. 1994 | Moderate | |
| Raitta et al. 1978; Seppalainen et al. 1979 | Moderate | |
| Sanagi et al. 1980 | Moderate | |
| Sliwinska-Kowalska et al. 2005 | Moderate | |
| Tsai et al. 1997 | Moderate | |
| Wang et al. 1986 | Low | |
| Yokoyama et al. 1997 | Moderate | |
| *Oral intermediate exposure* | | |
| Animal studies | | |
| Gao et al. 2019 | High | |
| Krasavage et al. 1980 | Low | |
| Li et al. 2018 | High | |
| Li et al. 2020a | High | High |
| Li et al. 2020b | Moderate | |
| Ono et al. 1981 | Moderate | |
| Wang et al. 2017 | High | |

## C.6.2 Adjustment of the Confidence Rating

The initial confidence rating was then downgraded or upgraded depending on whether there were substantial issues that would decrease or increase confidence in the body of evidence. The nine properties of the body of evidence that were considered are listed below. The summaries of the assessment of the confidence in the body of evidence for developmental, respiratory, and neurological effects are presented in Table C-18. If the confidence ratings for a particular outcome were based on more than one type of human study, then the highest confidence rating was used for subsequent analyses. An overview of the confidence in the body of evidence for all health effects associated with *n*-hexane exposure is presented in Table C-19.

## Table C-18. Adjustments to the Initial Confidence in the Body of Evidence

| | Initial confidence | Adjustments to the initial confidence rating | Final confidence |
|---|---|---|---|
| **Outcome: Developmental Effects** | | | |
| Human studies | Low | -1 indirectness | Very low |
| Animal studies | High | +1 consistency | High |
| **Outcome: Respiratory Effects** | | | |
| Human studies | Low | -1 risk of bias | Very low |
| Animal studies | High | | High |
| **Outcome: Neurological Effects** | | | |
| Human studies | Moderate | -1 risk of bias<br>+1 consistency<br>+1 large magnitude of effect<br>+1 Residual bias | High |
| Animal studies | High | +1 consistency<br>+1 dose response<br>+1 large magnitude of effect | High |

**Table C-19. Confidence in the Body of Evidence for *n*-Hexane**

| | Confidence in body of evidence | |
|---|---|---|
| Outcome | Human studies | Animal studies |
| Developmental | Very low | High |
| Respiratory | Very low | High |
| Neurological | High | High |

Five properties of the body of evidence were considered to determine whether the confidence rating should be downgraded:

- **Risk of bias.** Evaluation of whether there is substantial risk of bias across most of the studies examining the outcome. This evaluation used the risk of bias tier groupings for individual studies examining a particular outcome (Tables C-8, C-9, and C-10). Below are the criteria used to determine whether the initial confidence in the body of evidence for each outcome should be downgraded for risk of bias:
    - No downgrade if most studies are in the risk of bias first tier
    - Downgrade one confidence level if most studies are in the risk of bias second tier
    - Downgrade two confidence levels if most studies are in the risk of bias third tier

- **Unexplained inconsistency.** Evaluation of whether there is inconsistency or large variability in the magnitude or direction of estimates of effect across studies that cannot be explained. Below are the criteria used to determine whether the initial confidence in the body of evidence for each outcome should be downgraded for unexplained inconsistency:
    - No downgrade if there is little inconsistency across studies or if only one study evaluated the outcome
    - Downgrade one confidence level if there is variability across studies in the magnitude or direction of the effect
    - Downgrade two confidence levels if there is substantial variability across studies in the magnitude or direct of the effect

- **Indirectness.** Evaluation of four factors that can affect the applicability, generalizability, and relevance of the studies:
    - Relevance of the animal model to human health—unless otherwise indicated, studies in rats, mice, and other mammalian species are considered relevant to humans
    - Directness of the endpoints to the primary health outcome—examples of secondary outcomes or nonspecific outcomes include organ weight in the absence of histopathology or clinical chemistry findings in the absence of target tissue effects
    - Nature of the exposure in human studies and route of administration in animal studies—inhalation, oral, and dermal exposure routes are considered relevant unless there are compelling data to the contrary
    - Duration of treatment in animal studies and length of time between exposure and outcome assessment in animal and prospective human studies—this should be considered on an outcome-specific basis

  Below are the criteria used to determine whether the initial confidence in the body of evidence for each outcome should be downgraded for indirectness:
    - No downgrade if none of the factors are considered indirect
    - Downgrade one confidence level if one of the factors is considered indirect

  - Downgrade two confidence levels if two or more of the factors are considered indirect

- **Imprecision.** Evaluation of the narrowness of the effect size estimates and whether the studies have adequate statistical power. Data are considered imprecise when the ratio of the upper to lower 95% CIs for most studies is ≥10 for tests of ratio measures (e.g., odds ratios) and ≥100 for absolute measures (e.g., percent control response). Adequate statistical power is determined if the study can detect a potentially biologically meaningful difference between groups (20% change from control response for categorical data or risk ratio of 1.5 for continuous data). Below are the criteria used to determine whether the initial confidence in the body of evidence for each outcome should be downgraded for imprecision:
  - No downgrade if there are no serious imprecisions
  - Downgrade one confidence level for serious imprecisions
  - Downgrade two confidence levels for very serious imprecisions

- **Publication bias.** Evaluation of the concern that studies with statistically significant results are more likely to be published than studies without statistically significant results.
  - Downgrade one level of confidence for cases where there is serious concern with publication bias

Four properties of the body of evidence were considered to determine whether the confidence rating should be upgraded:

- **Large magnitude of effect.** Evaluation of whether the magnitude of effect is sufficiently large so that it is unlikely to have occurred as a result of bias from potential confounding factors.
  - Upgrade one confidence level if there is evidence of a large magnitude of effect in a few studies, provided that the studies have an overall low risk of bias and there is no serious unexplained inconsistency among the studies of similar dose or exposure levels; confidence can also be upgraded if there is one study examining the outcome, provided that the study has an overall low risk of bias

- **Dose response.** Evaluation of the dose-response relationships measured within a study and across studies. Below are the criteria used to determine whether the initial confidence in the body of evidence for each outcome should be upgraded:
  - Upgrade one confidence level for evidence of a monotonic dose-response gradient
  - Upgrade one confidence level for evidence of a non-monotonic dose-response gradient where there is prior knowledge that supports a non-monotonic dose-response and a non-monotonic dose-response gradient is observed across studies

- **Plausible confounding or other residual biases.** This factor primarily applies to human studies and is an evaluation of unmeasured determinants of an outcome such as residual bias towards the null (e.g., "healthy worker" effect) or residual bias suggesting a spurious effect (e.g., recall bias). Below is the criterion used to determine whether the initial confidence in the body of evidence for each outcome should be upgraded:
  - Upgrade one confidence level for evidence that residual confounding or bias would underestimate an apparent association or treatment effect (i.e., bias toward the null) or suggest a spurious effect when results suggest no effect

- **Consistency in the body of evidence.** Evaluation of consistency across animal models and species, consistency across independent studies of different human populations and exposure

scenarios, and consistency across human study types. Below is the criterion used to determine whether the initial confidence in the body of evidence for each outcome should be upgraded:

- Upgrade one confidence level if there is a high degree of consistency in the database

## C.7 TRANSLATE CONFIDENCE RATING INTO LEVEL OF EVIDENCE OF HEALTH EFFECTS

In the seventh step of the systematic review of the health effects data for *n*-hexane, the confidence in the body of evidence for specific outcomes was translated to a level of evidence rating. The level of evidence rating reflected the confidence in the body of evidence and the direction of the effect (i.e., toxicity or no toxicity); route-specific differences were noted. The level of evidence for health effects was rated on a five-point scale:

- **High level of evidence:** High confidence in the body of evidence for an association between exposure to the substance and the health outcome
- **Moderate level of evidence:** Moderate confidence in the body of evidence for an association between exposure to the substance and the health outcome
- **Low level of evidence:** Low confidence in the body of evidence for an association between exposure to the substance and the health outcome
- **Evidence of no health effect:** High confidence in the body of evidence that exposure to the substance is not associated with the health outcome
- **Inadequate evidence:** Low or moderate confidence in the body of evidence that exposure to the substance is not associated with the health outcome OR very low confidence in the body of evidence for an association between exposure to the substance and the health outcome

A summary of the level of evidence of health effects for *n*-hexane is presented in Table C-20.

**Table C-20. Level of Evidence of Health Effects for *n*-Hexane**

| Outcome | Confidence in body of evidence | Direction of health effect | Level of evidence for health effect |
|---|---|---|---|
| **Human studies** | | | |
| Developmental | Very low | Health effect | Inadequate |
| Respiratory | Very low | Health effect | Inadequate |
| Neurological | High | Health effect | High |
| **Animal studies** | | | |
| Developmental | High | Health effect | High |
| Respiratory | High | Health effect | High |
| Neurological | High | Health effect | High |

## C.8 INTEGRATE EVIDENCE TO DEVELOP HAZARD IDENTIFICATION CONCLUSIONS

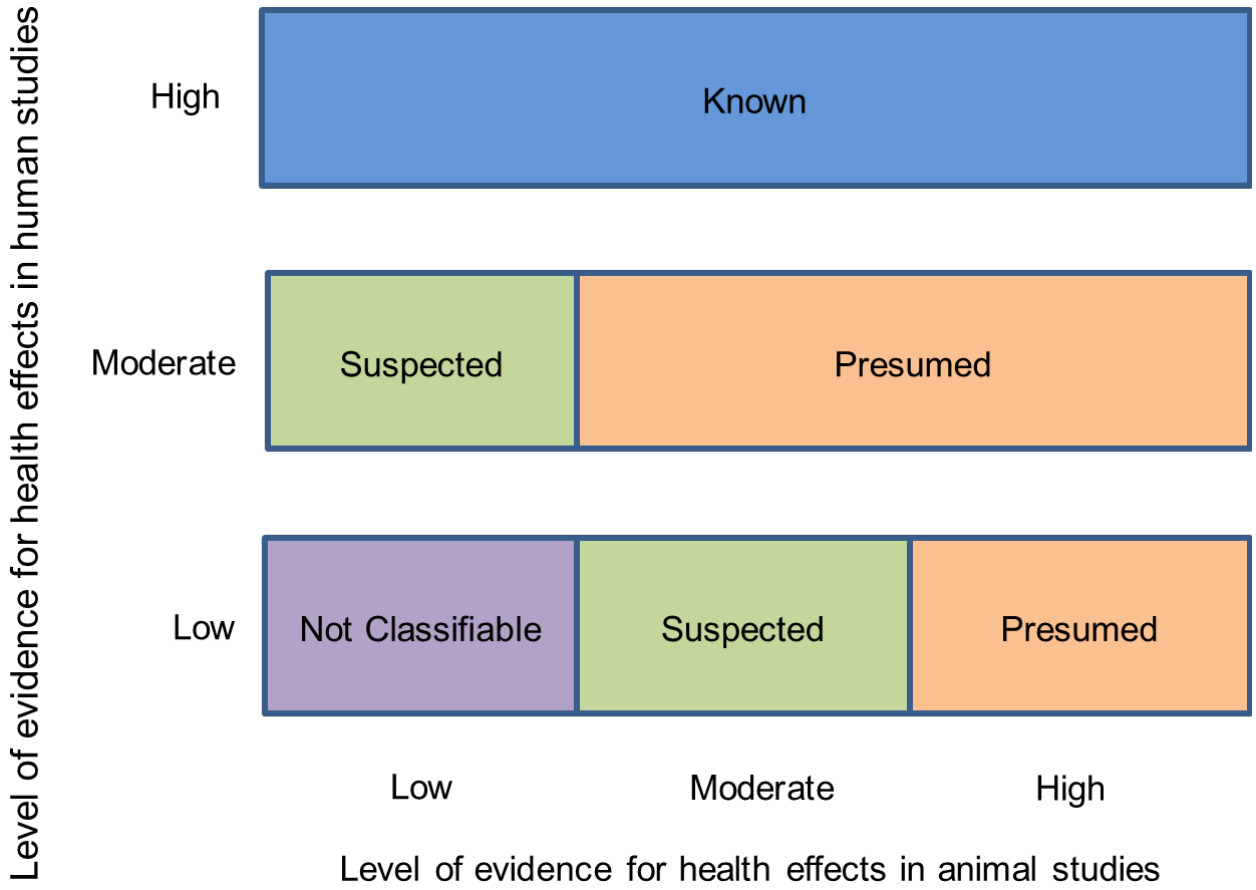
The final step involved the integration of the evidence streams for the human studies and animal studies to allow for a determination of hazard identification conclusions. For health effects, there were four hazard identification conclusion categories:

- **Known** to be a hazard to humans
- **Presumed** to be a hazard to humans
- **Suspected** to be a hazard to humans
- **Not classifiable** as to the hazard to humans

The initial hazard identification was based on the highest level of evidence in the human studies and the level of evidence in the animal studies; if there were no data for one evidence stream (human or animal), then the hazard identification was based on the one data stream (equivalent to treating the missing evidence stream as having low level of evidence). The hazard identification scheme is presented in Figure C-1 and described below:

- **Known:** A health effect in this category would have:
    - High level of evidence for health effects in human studies **AND** a high, moderate, or low level of evidence in animal studies.
- **Presumed:** A health effect in this category would have:
    - Moderate level of evidence in human studies **AND** high or moderate level of evidence in animal studies **OR**
    - Low level of evidence in human studies **AND** high level of evidence in animal studies
- **Suspected:** A health effect in this category would have:
    - Moderate level of evidence in human studies **AND** low level of evidence in animal studies **OR**
    - Low level of evidence in human studies **AND** moderate level of evidence in animal studies
- **Not classifiable:** A health effect in this category would have:
    - Low level of evidence in human studies **AND** low level of evidence in animal studies

**Figure C-1. Hazard Identification Scheme**




Other relevant data such as mechanistic or mode-of-action data were considered to raise or lower the level of the hazard identification conclusion by providing information that supported or opposed biological plausibility.

Two hazard identification conclusion categories were used when the data indicated that there may be no health effect in humans:

- **Not identified** to be a hazard in humans
- **Inadequate** to determine hazard to humans

If the human level of evidence conclusion of no health effect was supported by the animal evidence of no health effect, then the hazard identification conclusion category of "not identified" was used. If the human or animal level of evidence was considered inadequate, then a hazard identification conclusion category of "inadequate" was used. As with the hazard identification for health effects, the impact of other relevant data was also considered for no health effect data.

The hazard identification conclusions for *n*-hexane are listed below and summarized in Table C-21.

**Known Health Effects**

- Neurological
  - High level of evidence from human studies: numerous human studies are available that establish the neurotoxicity of *n*-hexane (Chang et al. 1993; Gong et al. 2003; Huang et al. 1991; Murata et al. 1994; Mutti et al. 1982a, 1982b; Pastore et al. 1994; Raitta et al. 1978; Seppalainen et al. 1979; Sanagi et al. 1980; Wang et al. 1986; Yokoyama et al. 1997).
  - High level of evidence from animal studies: abundant data are available demonstrating the neurological effects of *n*-hexane inhalation (Altenkirch et al. 1982; API 1981; Cavender et al. 1984; De Martino et al. 1987; Frontali et al. 1981; Howd et al. 1983; Huang et al. 1989; Ichihara et al. 1998; NTP 1991; Schaumburg and Spencer 1976; Takeuchi et al. 1980) and oral exposure (Gao et al. 2019; Krasavage et al. 1980; Li et al. 2018, 2020a, 2020b; Ono et al. 1981; Wang et al. 2017) in rodents.

**Suspected Health Effects**

- Developmental
  - Inadequate evidence from human studies: A case-control study showed a positive association between ambient *n*-hexane exposure and low birth weight (Gong et al. 2018), while a cohort study reported an association between maternal *n*-hexane exposure and alterations of the neonatal immune system (Lehmann et al. 2002). No other developmental studies were available.
  - High level of evidence from animal studies: several rodent studies have reported decreased fetal/litter weights following inhalation (Bus et al. 1979; NIEHS 1987, 1988c; Stoltenburg-Didinger et al. 1990) or oral exposure (Marks et al. 1980) to *n*-hexane.
- Respiratory
  - Inadequate evidence from human studies: human survey studies have identified higher incidences of self-reported respiratory symptoms (i.e., cough, phlegm, bronchitis, chest tightness) in solvent-exposed workers (Mustajbegovic et al. 2000; Nijem et al. 2001). In a study evaluating the effects of ambient *n*-hexane exposure in children, reduced lung function was observed in children living near point sources (Wichmann et al. 2009); however, two additional studies found no associations between *n*-hexane exposure and hospital visits for breathing problems (Buchdahl et al. 2000) or the incidence of rhinitis (Paciencia et al. 2020) in children.
  - High level of evidence from animal studies: an inhalation study observed nasal lesions in mice following intermediate-duration exposure (NTP 1991); two similar studies did not find lesions in the nasal turbinates of rats (API 1981; Cavender et al. 1984). Additional studies have observed increased lung weights and lung lesions (Howd et al. 1983; Lungarella et al. 1984; NTP 1991).

**Table C-21. Hazard Identification Conclusions for *n*-Hexane**

| Outcome | Hazard identification |
|---|---|
| Developmental | Suspected |
| Respiratory | Suspected |
| Neurological | Known |

# APPENDIX D. USER'S GUIDE

## Chapter 1. Relevance to Public Health

This chapter provides an overview of U.S. exposures, a summary of health effects based on evaluations of existing toxicologic, epidemiologic, and toxicokinetic information, and an overview of the minimal risk levels. This is designed to present interpretive, weight-of-evidence discussions for human health endpoints by addressing the following questions:

1. What effects are known to occur in humans?

2. What effects observed in animals are likely to be of concern to humans?

3. What exposure conditions are likely to be of concern to humans, especially around hazardous waste sites?

### Minimal Risk Levels (MRLs)

Where sufficient toxicologic information is available, ATSDR derives MRLs for inhalation and oral routes of entry at each duration of exposure (acute, intermediate, and chronic). These MRLs are not meant to support regulatory action, but to acquaint health professionals with exposure levels at which adverse health effects are not expected to occur in humans.

MRLs should help physicians and public health officials determine the safety of a community living near a hazardous substance emission, given the concentration of a contaminant in air or the estimated daily dose in water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

MRL users should be familiar with the toxicologic information on which the number is based. Section 1.2, Summary of Health Effects, contains basic information known about the substance. Other sections, such as Section 3.2 Children and Other Populations that are Unusually Susceptible and Section 3.4 Interactions with Other Substances, provide important supplemental information.

MRL users should also understand the MRL derivation methodology. MRLs are derived using a modified version of the risk assessment methodology that the Environmental Protection Agency (EPA) provides (Barnes and Dourson 1988) to determine reference doses (RfDs) for lifetime exposure.

To derive an MRL, ATSDR generally selects the most sensitive endpoint which, in its best judgement, represents the most sensitive human health effect for a given exposure route and duration. ATSDR cannot make this judgement or derive an MRL unless information (quantitative or qualitative) is available for all potential systemic, neurological, and developmental effects. If this information and reliable quantitative data on the chosen endpoint are available, ATSDR derives an MRL using the most sensitive species (when information from multiple species is available) with the highest no-observed-adverse-effect level (NOAEL) that does not exceed any adverse effect levels. When a NOAEL is not available, a lowest-observed-adverse-effect level (LOAEL) can be used to derive an MRL, and an uncertainty factor of 10 must be employed. Additional uncertainty factors of 10 must be used both for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and for interspecies variability (extrapolation from animals to humans). In deriving an MRL, these individual uncertainty factors are multiplied together. The product is then divided into the inhalation concentration or oral dosage selected from the study. Uncertainty factors used in developing a

substance-specific MRL are provided in the footnotes of the levels of significant exposure (LSE) tables that are provided in Chapter 2. Detailed discussions of the MRLs are presented in Appendix A.

**Chapter 2. Health Effects**

**Tables and Figures for Levels of Significant Exposure (LSE)**

Tables and figures are used to summarize health effects and illustrate graphically levels of exposure associated with those effects. These levels cover health effects observed at increasing dose concentrations and durations, differences in response by species and MRLs to humans for noncancer endpoints. The LSE tables and figures can be used for a quick review of the health effects and to locate data for a specific exposure scenario. The LSE tables and figures should always be used in conjunction with the text. All entries in these tables and figures represent studies that provide reliable, quantitative estimates of NOAELs, LOAELs, or Cancer Effect Levels (CELs).

The legends presented below demonstrate the application of these tables and figures. Representative examples of LSE tables and figures follow. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

**TABLE LEGEND**

**See Sample LSE Table (page D-5)**

(1) Route of exposure. One of the first considerations when reviewing the toxicity of a substance using these tables and figures should be the relevant and appropriate route of exposure. Typically, when sufficient data exist, three LSE tables and two LSE figures are presented in the document. The three LSE tables present data on the three principal routes of exposure (i.e., inhalation, oral, and dermal). LSE figures are limited to the inhalation and oral routes. Not all substances will have data on each route of exposure and will not, therefore, have all five of the tables and figures. Profiles with more than one chemical may have more LSE tables and figures.

(2) Exposure period. Three exposure periods—acute (<15 days), intermediate (15–364 days), and chronic (≥365 days)—are presented within each relevant route of exposure. In this example, two oral studies of chronic-duration exposure are reported. For quick reference to health effects occurring from a known length of exposure, locate the applicable exposure period within the LSE table and figure.

(3) Figure key. Each key number in the LSE table links study information to one or more data points using the same key number in the corresponding LSE figure. In this example, the study represented by key number 51 identified NOAELs and less serious LOAELs (also see the three "51R" data points in sample LSE Figure 2-X).

(4) Species (strain) No./group. The test species (and strain), whether animal or human, are identified in this column. The column also contains information on the number of subjects and sex per group. Chapter 1, Relevance to Public Health, covers the relevance of animal data to human toxicity and Section 3.1, Toxicokinetics, contains any available information on comparative toxicokinetics. Although NOAELs and LOAELs are species specific, the levels are extrapolated to equivalent human doses to derive an MRL.

(5) Exposure parameters/doses. The duration of the study and exposure regimens are provided in these columns. This permits comparison of NOAELs and LOAELs from different studies. In this case (key number 51), rats were orally exposed to “Chemical X” via feed for 2 years. For a

more complete review of the dosing regimen, refer to the appropriate sections of the text or the original reference paper (i.e., Aida et al. 1992).

(6) Parameters monitored. This column lists the parameters used to assess health effects. Parameters monitored could include serum (blood) chemistry (BC), biochemical changes (BI), body weight (BW), clinical signs (CS), developmental toxicity (DX), food intake (FI), gross necropsy (GN), hematology (HE), histopathology (HP), immune function (IX), lethality (LE), neurological function (NX), organ function (OF), ophthalmology (OP), organ weight (OW), reproductive function (RX), urinalysis (UR), and water intake (WI).

(7) Endpoint. This column lists the endpoint examined. The major categories of health endpoints included in LSE tables and figures are death, body weight, respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, dermal, ocular, endocrine, immunological, neurological, reproductive, developmental, other noncancer, and cancer. "Other noncancer" refers to any effect (e.g., alterations in blood glucose levels) not covered in these systems. In the example of key number 51, three endpoints (body weight, hematological, and hepatic) were investigated.

(8) NOAEL. A NOAEL is the highest exposure level at which no adverse effects were seen in the organ system studied. The body weight effect reported in key number 51 is a NOAEL at 25.5 mg/kg/day. NOAELs are not reported for cancer and death; with the exception of these two endpoints, this field is left blank if no NOAEL was identified in the study.

(9) LOAEL. A LOAEL is the lowest dose used in the study that caused an adverse health effect. LOAELs have been classified into "Less Serious" and "Serious" effects. These distinctions help readers identify the levels of exposure at which adverse health effects first appear and the gradation of effects with increasing dose. A brief description of the specific endpoint used to quantify the adverse effect accompanies the LOAEL. Key number 51 reports a less serious LOAEL of 6.1 mg/kg/day for the hepatic system, which was used to derive a chronic exposure, oral MRL of 0.008 mg/kg/day (see footnote "c"). MRLs are not derived from serious LOAELs. A cancer effect level (CEL) is the lowest exposure level associated with the onset of carcinogenesis in experimental or epidemiologic studies. CELs are always considered serious effects. The LSE tables and figures do not contain NOAELs for cancer, but the text may report doses not causing measurable cancer increases. If no LOAEL/CEL values were identified in the study, this field is left blank.

(10) Reference. The complete reference citation is provided in Chapter 8 of the profile.

(11) Footnotes. Explanations of abbreviations or reference notes for data in the LSE tables are found in the footnotes. For example, footnote "c" indicates that the LOAEL of 6.1 mg/kg/day in key number 51 was used to derive an oral MRL of 0.008 mg/kg/day.

**FIGURE LEGEND**

**See Sample LSE Figure (page D-6)**

LSE figures graphically illustrate the data presented in the corresponding LSE tables. Figures help the reader quickly compare health effects according to exposure concentrations for particular exposure periods.

(12) Exposure period. The same exposure periods appear as in the LSE table. In this example, health effects observed within the chronic exposure period are illustrated.

(13) Endpoint. These are the categories of health effects for which reliable quantitative data exist. The same health effect endpoints appear in the LSE table.

(14) Levels of exposure. Concentrations or doses for each health effect in the LSE tables are graphically displayed in the LSE figures. Exposure concentration or dose is measured on the log scale "y" axis. Inhalation exposure is reported in $mg/m^3$ or ppm and oral exposure is reported in mg/kg/day.

(15) LOAEL. In this example, the half-shaded circle that is designated 51R identifies a LOAEL critical endpoint in the rat upon which a chronic oral exposure MRL is based. The key number 51 corresponds to the entry in the LSE table. The dashed descending arrow indicates the extrapolation from the exposure level of 6.1 mg/kg/day (see entry 51 in the sample LSE table) to the MRL of 0.008 mg/kg/day (see footnote "c" in the sample LSE table).

(16) CEL. Key number 59R is one of studies for which CELs were derived. The diamond symbol refers to a CEL for the test species (rat). The number 59 corresponds to the entry in the LSE table.

(17) Key to LSE figure. The key provides the abbreviations and symbols used in the figure.

APPENDIX D

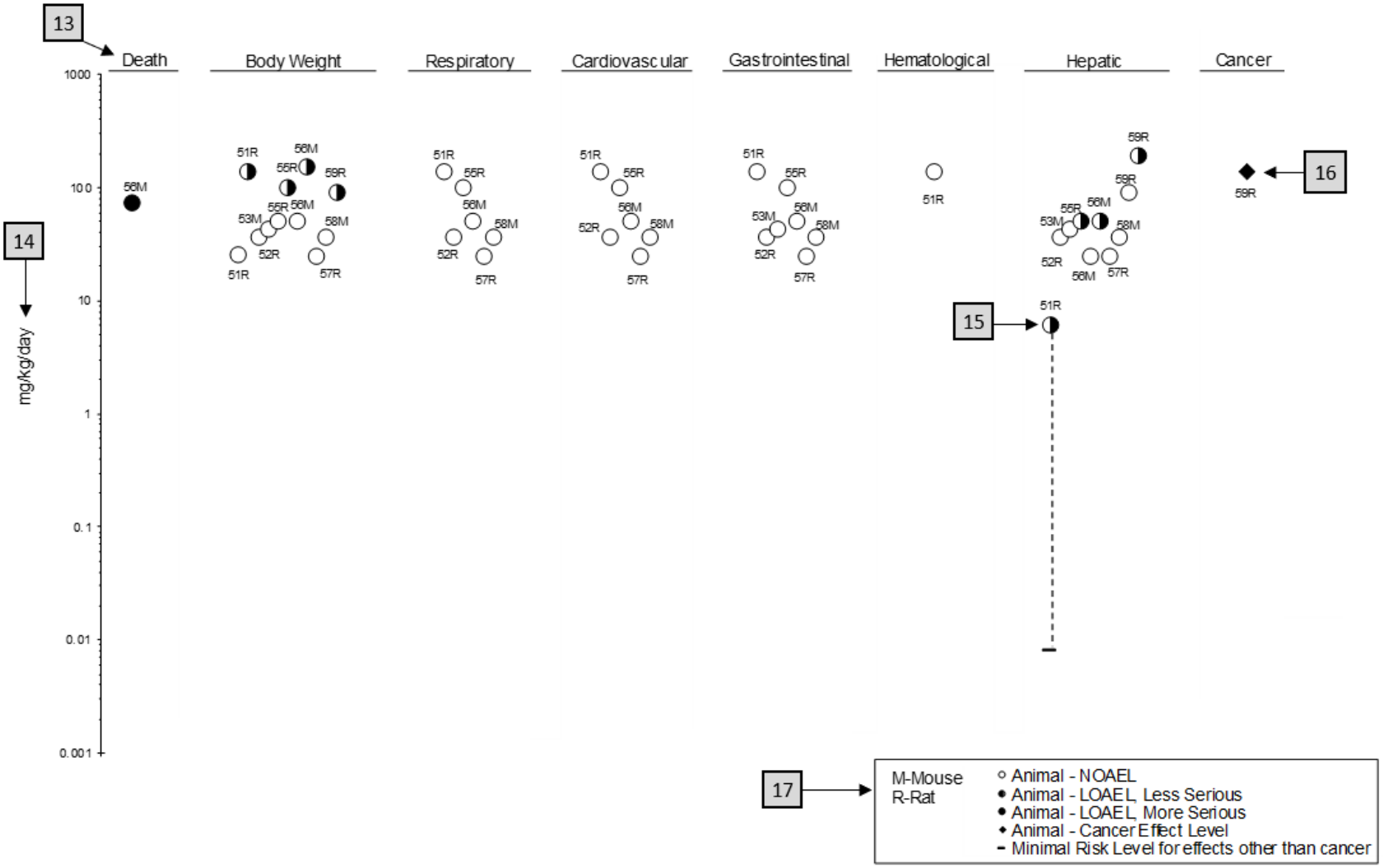
**Table 2-X. Levels of Significant Exposure to [Chemical X] – Oral** (1)

| Figure key[a] (3) | Species (strain) No./group (4) | Exposure parameters (5) | Doses (mg/kg/day) (5) | Parameters monitored (6) | Endpoint (7) | NOAEL (mg/kg/day) (8) | Less serious LOAEL (mg/kg/day) (9) | Serious LOAEL (mg/kg/day) (9) | Effect |
|---|---|---|---|---|---|---|---|---|---|
| **CHRONIC EXPOSURE** (2) | | | | | | | | | |
| 51 | Rat (Wistar) 40 M, 40 F | 2 years (F) | M: 0, 6.1, 25.5, 138.0 F: 0, 8.0, 31.7, 168.4 | CS, WI, BW, OW, HE, BC, HP | Bd wt | 25.5 | 138.0 | | Decreased body weight gain in males (23–25%) and females (31–39%) |
| | | | | | Hemato | 138.0 | | | |
| | | | | | Hepatic | | 6.1[c] | | Increases in absolute and relative weights at ≥6.1/8.0 mg/kg/day after 12 months of exposure; fatty generation at ≥6.1 mg/kg/day in males and at ≥31.7 mg/kg/day in females, and granulomas in females at 31.7 and 168.4 mg/kg/day after 12, 18, or 24 months of exposure and in males at ≥6.1 mg/kg/day only after 24 months of exposure |
| **Aida et al. 1992** (10) | | | | | | | | | |
| 52 | Rat (F344) 78 M | 104 weeks (W) | 0, 3.9, 20.6, 36.3 | CS, BW, FI, BC, OW, HP | Hepatic | 36.3 | | | |
| | | | | | Renal | 20.6 | 36.3 | | Increased incidence of renal tubular cell hyperplasia |
| | | | | | Endocr | 36.3 | | | |
| **George et al. 2002** | | | | | | | | | |
| 59 | Rat (Wistar) 58M, 58F | Lifetime (W) | M: 0, 90 F: 0, 190 | BW, HP | Cancer | | 190 F | | Increased incidence of hepatic neoplastic nodules in females only; no additional description of the tumors was provided |
| **Tumasonis et al. 1985** | | | | | | | | | |

[a]The number corresponds to entries in Figure 2-x.
[b]Used to derive an acute-duration oral minimal risk level (MRL) of 0.1 mg/kg/day based on the $BMDL_{05}$ of 10 mg/kg/day and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability). (11)
[c]Used to derive a chronic-duration oral MRL of 0.008 mg/kg/day based on the $BMDL_{10}$ of 0.78 mg/kg/day and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

APPENDIX D

**Figure 2-X. Levels of Significant Exposure to [Chemical X] - Oral**



# APPENDIX E. QUICK REFERENCE FOR HEALTH CARE PROVIDERS

Toxicological Profiles are a unique compilation of toxicological information on a given hazardous substance. Each profile reflects a comprehensive and extensive evaluation, summary, and interpretation of available toxicologic and epidemiologic information on a substance. Health care providers treating patients potentially exposed to hazardous substances may find the following information helpful for fast answers to often-asked questions.

---

***Primary Chapters/Sections of Interest***

**Chapter 1: Relevance to Public Health**: The Relevance to Public Health Section provides an overview of exposure and health effects and evaluates, interprets, and assesses the significance of toxicity data to human health. A table listing minimal risk levels (MRLs) is also included in this chapter.

**Chapter 2: Health Effects**: Specific health effects identified in both human and animal studies are reported by type of health effect (e.g., death, hepatic, renal, immune, reproductive), route of exposure (e.g., inhalation, oral, dermal), and length of exposure (e.g., acute, intermediate, and chronic).
***NOTE***: Not all health effects reported in this section are necessarily observed in the clinical setting.

**Pediatrics**:
**Section 3.2 Children and Other Populations that are Unusually Susceptible**
**Section 3.3 Biomarkers of Exposure and Effect**

---

***ATSDR Information Center***

***Phone:*** 1-800-CDC-INFO (800-232-4636) or 1-888-232-6348 (TTY)
***Internet***: http://www.atsdr.cdc.gov

ATSDR develops educational and informational materials for health care providers categorized by hazardous substance, clinical condition, and/or by susceptible population. The following additional materials are available online:

*Clinician Briefs and Overviews* discuss health effects and approaches to patient management in a brief/factsheet style. They are narrated PowerPoint presentations with Continuing Education credit available (see https://www.atsdr.cdc.gov/emes/health_professionals/clinician-briefs-overviews.html).

*Managing Hazardous Materials Incidents* is a set of recommendations for on-scene (prehospital) and hospital medical management of patients exposed during a hazardous materials incident (see https://www.atsdr.cdc.gov/MHMI/index.html).

*Fact Sheets (ToxFAQs™)* provide answers to frequently asked questions about toxic substances (see https://www.atsdr.cdc.gov/toxfaqs/Index.asp).

---

***Other Agencies and Organizations***

*The National Center for Environmental Health* (NCEH) focuses on preventing or controlling disease, injury, and disability related to the interactions between people and their environment outside the workplace. Contact: NCEH, Mailstop F-29, 4770 Buford Highway, NE, Atlanta, GA 30341-3724 • Phone: 770-488-7000 • FAX: 770-488-7015 • Web Page: https://www.cdc.gov/nceh/.

*The National Institute for Occupational Safety and Health* (NIOSH) conducts research on occupational diseases and injuries, responds to requests for assistance by investigating problems of health and safety in the workplace, recommends standards to the Occupational Safety and Health Administration (OSHA) and the Mine Safety and Health Administration (MSHA), and trains professionals in occupational safety and health. Contact: NIOSH, 395 E Street, S.W., Suite 9200, Patriots Plaza Building, Washington, DC 20201 • Phone: 202-245-0625 or 1-800-CDC-INFO (800-232-4636) • Web Page: https://www.cdc.gov/niosh/.

*The National Institute of Environmental Health Sciences* (NIEHS) is the principal federal agency for biomedical research on the effects of chemical, physical, and biologic environmental agents on human health and well-being. Contact: NIEHS, PO Box 12233, 104 T.W. Alexander Drive, Research Triangle Park, NC 27709 • Phone: 919-541-3212 • Web Page: https://www.niehs.nih.gov/.

---

***Clinical Resources (Publicly Available Information)***

*The Association of Occupational and Environmental Clinics* (AOEC) has developed a network of clinics in the United States to provide expertise in occupational and environmental issues. Contact: AOEC, 1010 Vermont Avenue, NW, #513, Washington, DC 20005 • Phone: 202-347-4976 • FAX: 202-347-4950 • e-mail: AOEC@AOEC.ORG • Web Page: http://www.aoec.org/.

*The American College of Occupational and Environmental Medicine* (ACOEM) is an association of physicians and other health care providers specializing in the field of occupational and environmental medicine. Contact: ACOEM, 25 Northwest Point Boulevard, Suite 700, Elk Grove Village, IL 60007-1030 • Phone: 847-818-1800 • FAX: 847-818-9266 • Web Page: http://www.acoem.org/.

*The American College of Medical Toxicology* (ACMT) is a nonprofit association of physicians with recognized expertise in medical toxicology. Contact: ACMT, 10645 North Tatum Boulevard, Suite 200-111, Phoenix AZ 85028 • Phone: 844-226-8333 • FAX: 844-226-8333 • Web Page: http://www.acmt.net.

*The Pediatric Environmental Health Specialty Units* (PEHSUs) is an interconnected system of specialists who respond to questions from public health professionals, clinicians, policy makers, and the public about the impact of environmental factors on the health of children and reproductive-aged adults. Contact information for regional centers can be found at http://pehsu.net/findhelp.html.

*The American Association of Poison Control Centers* (AAPCC) provide support on the prevention and treatment of poison exposures. Contact: AAPCC, 515 King Street, Suite 510, Alexandria VA 22314 • Phone: 701-894-1858 • Poison Help Line: 1-800-222-1222 • Web Page: http://www.aapcc.org/.

# APPENDIX F. GLOSSARY

**Absorption—**The process by which a substance crosses biological membranes and enters systemic circulation. Absorption can also refer to the taking up of liquids by solids, or of gases by solids or liquids.

**Acute Exposure—**Exposure to a chemical for a duration of ≤14 days, as specified in the Toxicological Profiles.

**Adsorption—**The adhesion in an extremely thin layer of molecules (as of gases, solutes, or liquids) to the surfaces of solid bodies or liquids with which they are in contact.

**Adsorption Coefficient ($K_{oc}$)—**The ratio of the amount of a chemical adsorbed per unit weight of organic carbon in the soil or sediment to the concentration of the chemical in solution at equilibrium.

**Adsorption Ratio (Kd)—**The amount of a chemical adsorbed by sediment or soil (i.e., the solid phase) divided by the amount of chemical in the solution phase, which is in equilibrium with the solid phase, at a fixed solid/solution ratio. It is generally expressed in micrograms of chemical sorbed per gram of soil or sediment.

**Benchmark Dose (BMD) or Benchmark Concentration (BMC)—**is the dose/concentration corresponding to a specific response level estimate using a statistical dose-response model applied to either experimental toxicology or epidemiology data. For example, a $BMD_{10}$ would be the dose corresponding to a 10% benchmark response (BMR). The BMD is determined by modeling the dose-response curve in the region of the dose-response relationship where biologically observable data are feasible. The BMDL or BMCL is the 95% lower confidence limit on the BMD or BMC.

**Bioconcentration Factor (BCF)—**The quotient of the concentration of a chemical in aquatic organisms at a specific time or during a discrete time period of exposure divided by the concentration in the surrounding water at the same time or during the same period.

**Biomarkers—**Indicators signaling events in biologic systems or samples, typically classified as markers of exposure, effect, and susceptibility.

**Cancer Effect Level (CEL)—**The lowest dose of a chemical in a study, or group of studies, that produces significant increases in the incidence of cancer (or malignant tumors) between the exposed population and its appropriate control.

**Carcinogen—**A chemical capable of inducing cancer.

**Case-Control Study—**A type of epidemiological study that examines the relationship between a particular outcome (disease or condition) and a variety of potential causative agents (such as toxic chemicals). In a case-control study, a group of people with a specified and well-defined outcome is identified and compared to a similar group of people without the outcome.

**Case Report—**A report that describes a single individual with a particular disease or exposure. These reports may suggest some potential topics for scientific research, but are not actual research studies.

**Case Series—**Reports that describe the experience of a small number of individuals with the same disease or exposure. These reports may suggest potential topics for scientific research, but are not actual research studies.

**Ceiling Value—**A concentration that must not be exceeded.

**Chronic Exposure—**Exposure to a chemical for ≥365 days, as specified in the Toxicological Profiles.

**Clastogen—**A substance that causes breaks in chromosomes resulting in addition, deletion, or rearrangement of parts of the chromosome.

**Cohort Study—**A type of epidemiological study of a specific group or groups of people who have had a common insult (e.g., exposure to an agent suspected of causing disease or a common disease) and are followed forward from exposure to outcome, and who are disease-free at start of follow-up. Often, at least one exposed group is compared to one unexposed group, while in other cohorts, exposure is a continuous variable and analyses are directed towards analyzing an exposure-response coefficient.

**Cross-sectional Study—**A type of epidemiological study of a group or groups of people that examines the relationship between exposure and outcome to a chemical or to chemicals at a specific point in time.

**Data Needs—**Substance-specific informational needs that, if met, would reduce the uncertainties of human health risk assessment.

**Developmental Toxicity—**The occurrence of adverse effects on the developing organism that may result from exposure to a chemical prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation. Adverse developmental effects may be detected at any point in the life span of the organism.

**Dose-Response Relationship—**The quantitative relationship between the amount of exposure to a toxicant and the incidence of the response or amount of the response.

**Embryotoxicity and Fetotoxicity—**Any toxic effect on the conceptus as a result of prenatal exposure to a chemical; the distinguishing feature between the two terms is the stage of development during which the effect occurs. Effects include malformations and variations, altered growth, and *in utero* death.

**Epidemiology—**The investigation of factors that determine the frequency and distribution of disease or other health-related conditions within a defined human population during a specified period.

**Excretion—**The process by which metabolic waste products are removed from the body.

**Genotoxicity—**A specific adverse effect on the genome of living cells that, upon the duplication of affected cells, can be expressed as a mutagenic, clastogenic, or carcinogenic event because of specific alteration of the molecular structure of the genome.

**Half-life—**A measure of rate for the time required to eliminate one-half of a quantity of a chemical from the body or environmental media.

**Health Advisory—**An estimate of acceptable drinking water levels for a chemical substance derived by EPA and based on health effects information. A health advisory is not a legally enforceable federal standard, but serves as technical guidance to assist federal, state, and local officials.

**Immediately Dangerous to Life or Health (IDLH)—**A condition that poses a threat of life or health, or conditions that pose an immediate threat of severe exposure to contaminants that are likely to have adverse cumulative or delayed effects on health.

**Immunotoxicity—**Adverse effect on the functioning of the immune system that may result from exposure to chemical substances.

**Incidence—**The ratio of new cases of individuals in a population who develop a specified condition to the total number of individuals in that population who could have developed that condition in a specified time period.

**Intermediate Exposure—**Exposure to a chemical for a duration of 15–364 days, as specified in the Toxicological Profiles.

***In Vitro*—**Isolated from the living organism and artificially maintained, as in a test tube.

***In Vivo*—**Occurring within the living organism.

**Lethal Concentration$_{(LO)}$ ($LC_{LO}$)—**The lowest concentration of a chemical in air that has been reported to have caused death in humans or animals.

**Lethal Concentration$_{(50)}$ ($LC_{50}$)—**A calculated concentration of a chemical in air to which exposure for a specific length of time is expected to cause death in 50% of a defined experimental animal population.

**Lethal Dose$_{(LO)}$ ($LD_{Lo}$)—**The lowest dose of a chemical introduced by a route other than inhalation that has been reported to have caused death in humans or animals.

**Lethal Dose$_{(50)}$ ($LD_{50}$)—**The dose of a chemical that has been calculated to cause death in 50% of a defined experimental animal population.

**Lethal Time$_{(50)}$ ($LT_{50}$)—**A calculated period of time within which a specific concentration of a chemical is expected to cause death in 50% of a defined experimental animal population.

**Lowest-Observed-Adverse-Effect Level (LOAEL)—**The lowest exposure level of chemical in a study, or group of studies, that produces statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.

**Lymphoreticular Effects—**Represent morphological effects involving lymphatic tissues such as the lymph nodes, spleen, and thymus.

**Malformations—**Permanent structural changes that may adversely affect survival, development, or function.

**Metabolism—**Process in which chemical substances are biotransformed in the body that could result in less toxic and/or readily excreted compounds or produce a biologically active intermediate.

**Minimal LOAEL—**Indicates a minimal adverse effect or a reduced capacity of an organ or system to absorb additional toxic stress that does not necessarily lead to the inability of the organ or system to function normally.

**Minimal Risk Level (MRL)—**An estimate of daily human exposure to a hazardous substance that is likely to be without an appreciable risk of adverse noncancer health effects over a specified route and duration of exposure.

**Modifying Factor (MF)—**A value (greater than zero) that is applied to the derivation of a Minimal Risk Level (MRL) to reflect additional concerns about the database that are not covered by the uncertainty factors. The default value for a MF is 1.

**Morbidity—**The state of being diseased; the morbidity rate is the incidence or prevalence of a disease in a specific population.

**Mortality—**Death; the mortality rate is a measure of the number of deaths in a population during a specified interval of time.

**Mutagen—**A substance that causes mutations, which are changes in the DNA sequence of a cell's DNA. Mutations can lead to birth defects, miscarriages, or cancer.

**Necropsy—**The gross examination of the organs and tissues of a dead body to determine the cause of death or pathological conditions.

**Neurotoxicity—**The occurrence of adverse effects on the nervous system following exposure to a hazardous substance.

**No-Observed-Adverse-Effect Level (NOAEL)—**The dose of a chemical at which there were no statistically or biologically significant increases in frequency or severity of adverse effects seen between the exposed population and its appropriate control. Although effects may be produced at this dose, they are not considered to be adverse.

**Octanol-Water Partition Coefficient ($K_{ow}$)—**The equilibrium ratio of the concentrations of a chemical in *n*-octanol and water, in dilute solution.

**Odds Ratio (OR)—**A means of measuring the association between an exposure (such as toxic substances and a disease or condition) that represents the best estimate of relative risk (risk as a ratio of the incidence among subjects exposed to a particular risk factor divided by the incidence among subjects who were not exposed to the risk factor). An odds ratio that is greater than 1 is considered to indicate greater risk of disease in the exposed group compared to the unexposed group.

**Permissible Exposure Limit (PEL)—**An Occupational Safety and Health Administration (OSHA) regulatory limit on the amount or concentration of a substance not to be exceeded in workplace air averaged over any 8-hour work shift of a 40-hour workweek.

**Pesticide—**General classification of chemicals specifically developed and produced for use in the control of agricultural and public health pests (insects or other organisms harmful to cultivated plants or animals).

**Pharmacokinetics—**The dynamic behavior of a material in the body, used to predict the fate (disposition) of an exogenous substance in an organism. Utilizing computational techniques, it provides the means of studying the absorption, distribution, metabolism, and excretion of chemicals by the body.

**Pharmacokinetic Model—**A set of equations that can be used to describe the time course of a parent chemical or metabolite in an animal system. There are two types of pharmacokinetic models: data-based and physiologically-based. A data-based model divides the animal system into a series of compartments, which, in general, do not represent real, identifiable anatomic regions of the body, whereas the physiologically-based model compartments represent real anatomic regions of the body.

**Physiologically Based Pharmacodynamic (PBPD) Model—**A type of physiologically based dose-response model that quantitatively describes the relationship between target tissue dose and toxic endpoints. These models advance the importance of physiologically based models in that they clearly describe the biological effect (response) produced by the system following exposure to an exogenous substance.

**Physiologically Based Pharmacokinetic (PBPK) Model—**A type of physiologically based dose-response model that is comprised of a series of compartments representing organs or tissue groups with realistic weights and blood flows. These models require a variety of physiological information, including tissue volumes, blood flow rates to tissues, cardiac output, alveolar ventilation rates, and possibly membrane permeabilities. The models also utilize biochemical information, such as blood:air partition coefficients, and metabolic parameters. PBPK models are also called biologically based tissue dosimetry models.

**Prevalence—**The number of cases of a disease or condition in a population at one point in time.

**Prospective Study—**A type of cohort study in which a group is followed over time and the pertinent observations are made on events occurring after the start of the study.

**Recommended Exposure Limit (REL)—**A National Institute for Occupational Safety and Health (NIOSH) time-weighted average (TWA) concentration for up to a 10-hour workday during a 40-hour workweek.

**Reference Concentration (RfC)—**An estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer health effects during a lifetime. The inhalation RfC is expressed in units of $mg/m^3$ or ppm.

**Reference Dose (RfD)—**An estimate (with uncertainty spanning perhaps an order of magnitude) of the daily oral exposure of the human population to a potential hazard that is likely to be without risk of deleterious noncancer health effects during a lifetime. The oral RfD is expressed in units of mg/kg/day.

**Reportable Quantity (RQ)—**The quantity of a hazardous substance that is considered reportable under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). RQs are (1) ≥1 pound or (2) for selected substances, an amount established by regulation either under CERCLA or under Section 311 of the Clean Water Act. Quantities are measured over a 24-hour period.

**Reproductive Toxicity—**The occurrence of adverse effects on the reproductive system that may result from exposure to a hazardous substance. The toxicity may be directed to the reproductive organs and/or the related endocrine system. The manifestation of such toxicity may be noted as alterations in sexual behavior, fertility, pregnancy outcomes, or modifications in other functions that are dependent on the integrity of this system.

**Retrospective Study—**A type of cohort study based on a group of persons known to have been exposed at some time in the past. Data are collected from routinely recorded events, up to the time the study is undertaken. Retrospective studies are limited to causal factors that can be ascertained from existing records and/or examining survivors of the cohort.

**Risk—**The possibility or chance that some adverse effect will result from a given exposure to a hazardous substance.

**Risk Factor—**An aspect of personal behavior or lifestyle, an environmental exposure, existing health condition, or an inborn or inherited characteristic that is associated with an increased occurrence of disease or other health-related event or condition.

**Risk Ratio/Relative Risk—**The ratio of the risk among persons with specific risk factors compared to the risk among persons without risk factors. A risk ratio that is greater than 1 indicates greater risk of disease in the exposed group compared to the unexposed group.

**Serious LOAEL—**A dose that evokes failure in a biological system and can lead to morbidity or mortality.

**Short-Term Exposure Limit (STEL)—**A STEL is a 15-minute TWA exposure that should not be exceeded at any time during a workday.

**Standardized Mortality Ratio (SMR)—**A ratio of the observed number of deaths and the expected number of deaths in a specific standard population.

**Target Organ Toxicity—**This term covers a broad range of adverse effects on target organs or physiological systems (e.g., renal, cardiovascular) extending from those arising through a single limited exposure to those assumed over a lifetime of exposure to a chemical.

**Teratogen—**A chemical that causes structural defects that affect the development of an organism.

**Threshold Limit Value (TLV)—**An American Conference of Governmental Industrial Hygienists (ACGIH) concentration of a substance to which it is believed that nearly all workers may be repeatedly exposed, day after day, for a working lifetime without adverse effect. The TLV may be expressed as a Time-Weighted Average (TLV-TWA), as a Short-Term Exposure Limit (TLV-STEL), or as a ceiling limit (TLV-C).

**Time-Weighted Average (TWA)—**An average exposure within a given time period.

**Toxicokinetic—**The absorption, distribution, metabolism, and elimination of toxic compounds in the living organism.

**Toxics Release Inventory (TRI)—**The TRI is an EPA program that tracks toxic chemical releases and pollution prevention activities reported by industrial and federal facilities.

**Uncertainty Factor (UF)—**A factor used in operationally deriving the Minimal Risk Level (MRL), Reference Dose (RfD), or Reference Concentration (RfC) from experimental data. UFs are intended to account for (1) the variation in sensitivity among the members of the human population, (2) the uncertainty in extrapolating animal data to the case of human, (3) the uncertainty in extrapolating from data obtained in a study that is of less than lifetime exposure, and (4) the uncertainty in using lowest-observed-adverse-effect level (LOAEL) data rather than no-observed-adverse-effect level (NOAEL) data. A default for each individual UF is 10; if complete certainty in data exists, a value of 1 can be used; however, a reduced UF of 3 may be used on a case-by-case basis (3 being the approximate logarithmic average of 10 and 1).

**Xenobiotic—**Any substance that is foreign to the biological system.

# APPENDIX G. ACRONYMS, ABBREVIATIONS, AND SYMBOLS

| | |
|---|---|
| AAPCC | American Association of Poison Control Centers |
| ACGIH | American Conference of Governmental Industrial Hygienists |
| ACOEM | American College of Occupational and Environmental Medicine |
| ACMT | American College of Medical Toxicology |
| ADI | acceptable daily intake |
| ADME | absorption, distribution, metabolism, and excretion |
| AEGL | Acute Exposure Guideline Level |
| AIC | Akaike's information criterion |
| AIHA | American Industrial Hygiene Association |
| ALT | alanine aminotransferase |
| AOEC | Association of Occupational and Environmental Clinics |
| AP | alkaline phosphatase |
| AST | aspartate aminotransferase |
| atm | atmosphere |
| ATSDR | Agency for Toxic Substances and Disease Registry |
| AWQC | Ambient Water Quality Criteria |
| BCF | bioconcentration factor |
| BMD/C | benchmark dose or benchmark concentration |
| $BMD_X$ | dose that produces a X% change in response rate of an adverse effect |
| $BMDL_X$ | 95% lower confidence limit on the $BMD_X$ |
| BMDS | Benchmark Dose Software |
| BMR | benchmark response |
| BUN | blood urea nitrogen |
| C | centigrade |
| CAA | Clean Air Act |
| CAS | Chemical Abstract Services |
| CDC | Centers for Disease Control and Prevention |
| CEL | cancer effect level |
| CERCLA | Comprehensive Environmental Response, Compensation, and Liability Act |
| CFR | Code of Federal Regulations |
| Ci | curie |
| CI | confidence interval |
| cm | centimeter |
| CPSC | Consumer Products Safety Commission |
| CWA | Clean Water Act |
| DNA | deoxyribonucleic acid |
| DOD | Department of Defense |
| DOE | Department of Energy |
| DWEL | drinking water exposure level |
| EAFUS | Everything Added to Food in the United States |
| ECG/EKG | electrocardiogram |
| EEG | electroencephalogram |
| EPA | Environmental Protection Agency |
| ERPG | emergency response planning guidelines |
| F | Fahrenheit |
| F1 | first-filial generation |
| FDA | Food and Drug Administration |
| FIFRA | Federal Insecticide, Fungicide, and Rodenticide Act |
| FR | Federal Register |

| | |
|---|---|
| FSH | follicle stimulating hormone |
| g | gram |
| GC | gas chromatography |
| gd | gestational day |
| GGT | γ-glutamyl transferase |
| GRAS | generally recognized as safe |
| HEC | human equivalent concentration |
| HED | human equivalent dose |
| HHS | Department of Health and Human Services |
| HPLC | high-performance liquid chromatography |
| HSDB | Hazardous Substance Data Bank |
| IARC | International Agency for Research on Cancer |
| IDLH | immediately dangerous to life and health |
| IRIS | Integrated Risk Information System |
| Kd | adsorption ratio |
| kg | kilogram |
| kkg | kilokilogram; 1 kilokilogram is equivalent to 1,000 kilograms and 1 metric ton |
| $K_{oc}$ | organic carbon partition coefficient |
| $K_{ow}$ | octanol-water partition coefficient |
| L | liter |
| LC | liquid chromatography |
| $LC_{50}$ | lethal concentration, 50% kill |
| $LC_{Lo}$ | lethal concentration, low |
| $LD_{50}$ | lethal dose, 50% kill |
| $LD_{Lo}$ | lethal dose, low |
| LDH | lactate dehydrogenase |
| LH | luteinizing hormone |
| LOAEL | lowest-observed-adverse-effect level |
| LSE | Level of Significant Exposure |
| $LT_{50}$ | lethal time, 50% kill |
| m | meter |
| mCi | millicurie |
| MCL | maximum contaminant level |
| MCLG | maximum contaminant level goal |
| MF | modifying factor |
| mg | milligram |
| mL | milliliter |
| mm | millimeter |
| mmHg | millimeters of mercury |
| mmol | millimole |
| MRL | Minimal Risk Level |
| MS | mass spectrometry |
| MSHA | Mine Safety and Health Administration |
| Mt | metric ton |
| NAAQS | National Ambient Air Quality Standard |
| NAS | National Academy of Science |
| NCEH | National Center for Environmental Health |
| ND | not detected |
| ng | nanogram |
| NHANES | National Health and Nutrition Examination Survey |
| NIEHS | National Institute of Environmental Health Sciences |

| | |
|---|---|
| NIOSH | National Institute for Occupational Safety and Health |
| NLM | National Library of Medicine |
| nm | nanometer |
| nmol | nanomole |
| NOAEL | no-observed-adverse-effect level |
| NPL | National Priorities List |
| NR | not reported |
| NRC | National Research Council |
| NS | not specified |
| NTP | National Toxicology Program |
| OR | odds ratio |
| OSHA | Occupational Safety and Health Administration |
| PAC | Protective Action Criteria |
| PAH | polycyclic aromatic hydrocarbon |
| PBPD | physiologically based pharmacodynamic |
| PBPK | physiologically based pharmacokinetic |
| PEHSU | Pediatric Environmental Health Specialty Unit |
| PEL | permissible exposure limit |
| PEL-C | permissible exposure limit-ceiling value |
| pg | picogram |
| PND | postnatal day |
| POD | point of departure |
| ppb | parts per billion |
| ppbv | parts per billion by volume |
| ppm | parts per million |
| ppt | parts per trillion |
| REL | recommended exposure limit |
| REL-C | recommended exposure limit-ceiling value |
| RfC | reference concentration |
| RfD | reference dose |
| RNA | ribonucleic acid |
| SARA | Superfund Amendments and Reauthorization Act |
| SCE | sister chromatid exchange |
| SD | standard deviation |
| SE | standard error |
| SGOT | serum glutamic oxaloacetic transaminase (same as aspartate aminotransferase or AST) |
| SGPT | serum glutamic pyruvic transaminase (same as alanine aminotransferase or ALT) |
| SIC | standard industrial classification |
| SLOAEL | serious lowest-observed-adverse-effect level |
| SMR | standardized mortality ratio |
| sRBC | sheep red blood cell |
| STEL | short term exposure limit |
| TLV | threshold limit value |
| TLV-C | threshold limit value-ceiling value |
| TRI | Toxics Release Inventory |
| TSCA | Toxic Substances Control Act |
| TWA | time-weighted average |
| UF | uncertainty factor |
| U.S. | United States |
| USDA | United States Department of Agriculture |
| USGS | United States Geological Survey |

| | |
|---|---|
| USNRC | U.S. Nuclear Regulatory Commission |
| VOC | volatile organic compound |
| WBC | white blood cell |
| WHO | World Health Organization |

| | |
|---|---|
| > | greater than |
| ≥ | greater than or equal to |
| = | equal to |
| < | less than |
| ≤ | less than or equal to |
| % | percent |
| α | alpha |
| β | beta |
| γ | gamma |
| δ | delta |
| μm | micrometer |
| μg | microgram |
| $q_1^*$ | cancer slope factor |
| – | negative |
| + | positive |
| (+) | weakly positive result |
| (–) | weakly negative result |