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.

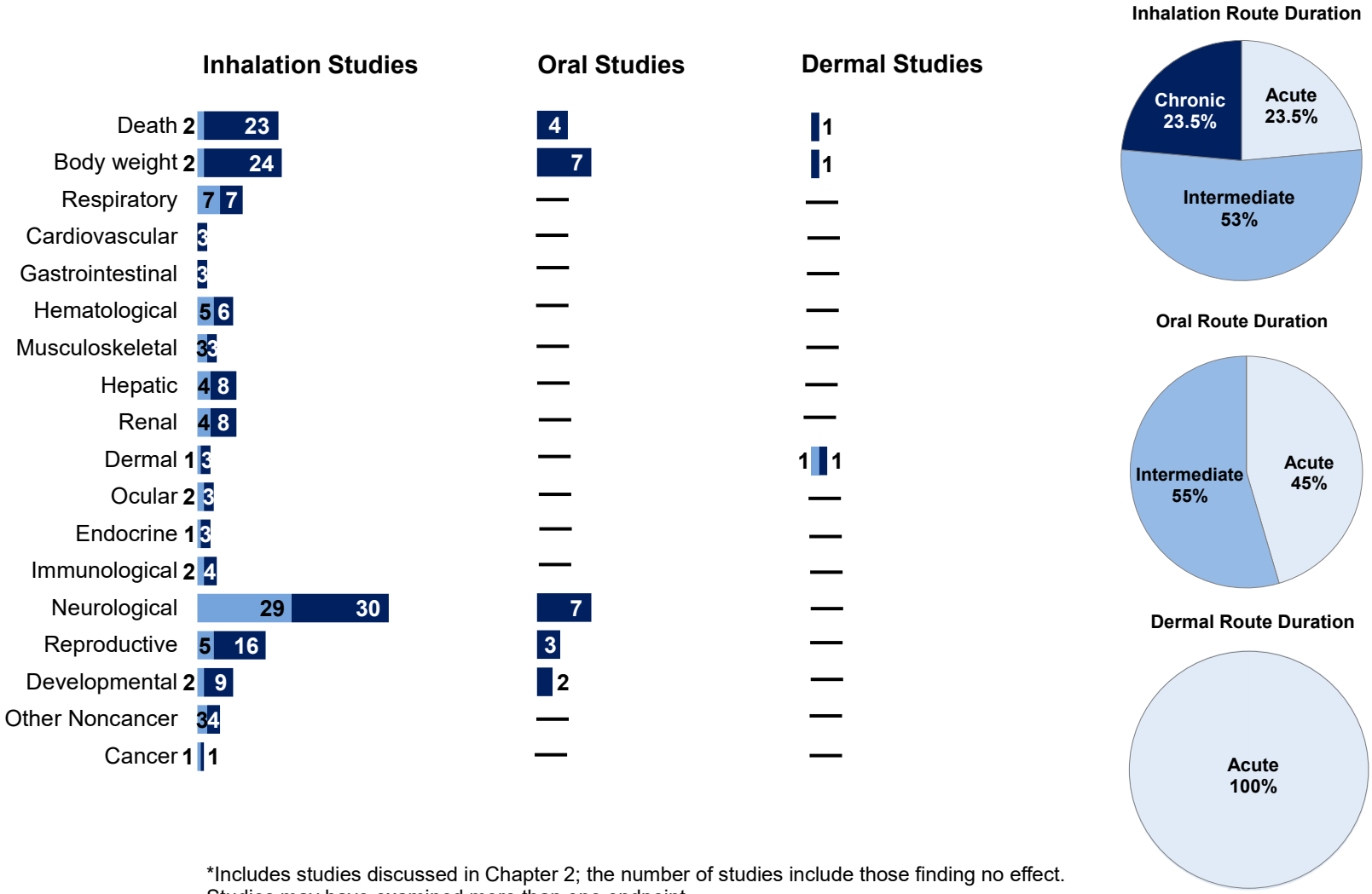
6.1 EXISTING INFORMATION ON HEALTH EFFECTS

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.