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 chloroform 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 chloroform.

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.

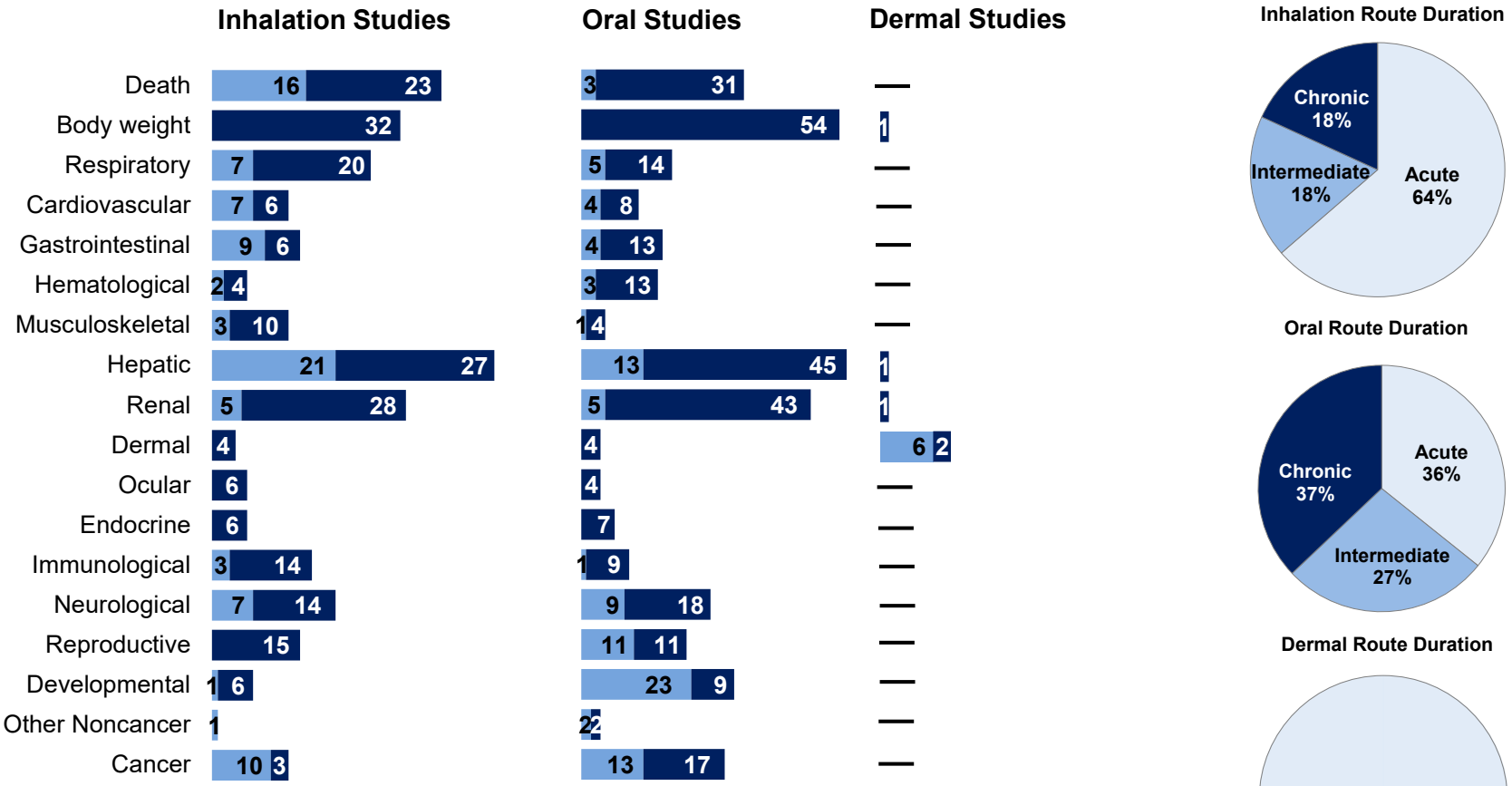
6.1 INFORMATION ON HEALTH EFFECTS

Studies evaluating the health effects of inhalation, oral, and dermal exposure of humans and animals to chloroform 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 chloroform. 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 shown in Figure 6-1, information on the health effects in humans are available from inhalation, oral, and dermal exposure. For the purposes of Figure 6-1, all human studies with exposure to chloroform as a tap water disinfection byproduct were classified as oral, despite potential for multi-route exposure (e.g., inhalation and dermal via showering and bathing activities). Similarly, human studies evaluating exposure to chloroform when swimming in chlorinated pools are classified as inhalation exposure, despite concurrent dermal exposure, because exposure via inhalation is expected to contribute more to body burden. Lastly, human studies that evaluated blood levels of chloroform as a biomarker of exposure but did not have any information pertaining to possible exposure sources are not included in Figure 6-1 due to unknown route(s) of exposure. The organs or systems adversely affected in humans after exposure to chloroform include the respiratory, liver, kidney, and neurological system. Death as well as multisystem damage may occur at sufficiently high exposure levels. Findings pertaining to reproductive, developmental, and carcinogenic effects of chloroform exposure in humans are mixed.

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

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



*Includes studies discussed in Chapter 2. The number of studies include those finding no effect; most studies examined multiple endpoints. Human studies with multi-route exposure were included only once in the figure; the studies were classified based on the most predominant route of exposure (e.g., tap water exposure classified as oral, despite potential for inhalation or dermal exposure via showering/bathing). Human studies with unknown route(s) of exposure (e.g., exposure assessed via biomarker of exposure only) are not included in this figure or the study count reported above.

For animal data, inhalation and oral studies are available for all health effect and exposure duration categories. The dermal animal database is limited to two acute studies. The organs or systems adversely affected in animals were body weight, respiratory, hepatic, and renal effects. Death as well as multisystem damage may occur at sufficiently high exposure levels. Findings pertaining to cardiovascular, immunological, reproductive, and developmental effects of chloroform exposure in animals are mixed. Chloroform caused hepatic and renal cancer in animals following chronic-duration inhalation or oral exposure.

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 database was adequate to derive acute-duration inhalation and oral MRLs. While there are numerous acute-duration oral studies, most of the studies administered chloroform via gavage, which is considered less relevant to human exposure (due to saturation of detoxification pathways following bolus gavage exposure). Only two acute-duration drinking water studies were available. Additional, multi-dose, acute-duration drinking water studies could decrease uncertainty in the acute-duration oral MRL.

Intermediate-Duration MRLs. The inhalation database was adequate to derive an intermediate-duration inhalation MRL. Additional low-dose studies designed to identify a NOAEL for the critical effect (nasal lesions) could decrease uncertainty in the intermediate-duration inhalation MRL. The oral database is adequate to derive an intermediate-duration oral MRL.

Chronic-Duration MRLs. The inhalation database was adequate to derive a chronic-duration inhalation MRL. The oral database is adequate to derive a chronic-duration oral MRL. Additional low-dose studies designed to identify a NOAEL for the critical effects (nasal lesions via inhalation, hepatotoxicity via oral exposure) could decrease uncertainty in the chronic-duration MRLs.

Health Effects. Identification of data needs for health effects in animal studies is limited to targets included in the systematic review.

Respiratory. Respiratory effects noted in humans include respiratory depression and/or arrest at exposure levels associated with CNS depression; lung damage has been observed in fatal exposure cases (Ago et al. 2011; Featherstone 1947; Giusti and Chiarotti 1981; Harada et al. 1997; Piersol et al. 1933; Royston 1924; Schroeder 1965; Whitaker and Jones 1965). In animals, the nasal epithelium is a sensitive target of toxicity following inhalation and oral exposure (Constan et al. 1999; Dorman et al. 1997; Kasai et al. 2002; Larson et al. 1994c, 1995b, 1996; Mery et al. 1994; Templin et al. 1996a, 1996b; Yamamoto et al. 2002). Damage to the lower respiratory tract in animals was generally only observed at lethal exposure levels (Bowman et al. 1978; Kasai et al. 2002; NCI 1976). However, there is limited evidence of inflammatory responses in the lung at low inhalation exposure levels in mice (de Oliveira et al. 2015). Studies evaluating the potential for nasal effects in humans would be useful. Research on the potential respiratory effects of multi-route exposure to chloroform in residential water supplies estimating exposure levels via respiratory, oral, and dermal exposure routes would also be useful. Of specific interest is inflammatory respiratory responses, if analyses adequately control for confounders, particularly exposures to other known water disinfection byproducts. Additional low-dose animal studies evaluating inflammatory lung effects are needed to confirm findings by de Oliveira et al. (2015).

Hepatic. Numerous studies establish that the hepatic system is a target of chloroform toxicity in humans and animals via inhalation and oral exposure (Section 2.9). However, additional drinking water studies in animals could help better define the dose-response relationship, and how it compares to findings observed in gavage studies. Research on the potential hepatic effects of multi-route exposure to chloroform in residential water supplies would also be useful if analyses adequately control for confounders, particularly exposures to other known water disinfection byproducts.

Renal. Numerous studies establish that the renal system is a target of chloroform toxicity in humans and animals via inhalation and oral exposure (Section 2.10). However, additional drinking water studies in animals could help better define the dose-response relationship, and how it compares to findings observed in gavage studies. Research on the potential renal effects of multi-route exposure to chloroform in residential water supplies would also be useful if analyses

adequately control for confounders, particularly exposures to other known water disinfection byproducts.

Neurological. The CNS is a target organ for chloroform toxicity in humans after inhalation and oral exposure. The neurotoxic effect is well documented in studies of patients exposed to chloroform via anesthesia (Featherstone 1947; Smith et al. 1973; Whitaker and Jones 1965) or of individuals who intentionally and accidentally ingested the chemical (Piersol et al. 1933; Schroeder 1965; Storms 1973). CNS depression has also been clearly demonstrated in animals exposed via inhalation (Constan et al. 1999; EPA 1978; Gehring 1968; Lehmann and Flury 1943) or oral exposure (Bowman et al. 1978; NTP 1988a; Jones et al. 1958). Data pertaining to neurological effects at exposure levels below those associated with frank CNS depression are limited but suggest neurobehavioral changes in humans following occupational exposure (Challen et al. 1958; Li et al. 1993) and in animals following oral exposure (Balster and Borzelleca 1982; Landauer et al. 1982; Wada et al. 2015). Additional studies in humans and/or animals evaluating comprehensive neurological endpoints at low exposure levels via inhalation or oral exposure would be useful to establish dose-response relationships for mild neurological effects. Research on the potential neurological effects of multi-route exposure to chloroform in residential water supplies would also be useful if analyses adequately control for confounders, particularly exposures to other known water disinfection byproducts. More information regarding the mechanism of chloroform-induced neurotoxicity would be helpful.

Developmental. Numerous studies have evaluated potential associations between developmental effects and exposure to disinfection byproducts in chlorinated water, including chloroform. Some of these found associations between impaired growth and measured chloroform level in water, estimated total residential chloroform intake, or chloroform blood levels (Botton et al. 2015; Grazuleviciene et al. 2011; Kramer et al. 1992; Summerhayes et al. 2012; Wright et al. 2004), while others did not (Bonou et al. 2017; Cao et al. 2016; Hinckley et al. 2005; Liu et al. 2021; Porter et al. 2005; Villanueva et al. 2011). A main limitation of these studies is lack of control for other known water disinfection byproducts. Therefore, additional studies evaluating potential associations between residential chloroform exposure and developmental effects with adequate control for exposure to other known water disinfection byproducts could be useful.

Developmental studies in animals provide some evidence of fetal malformation after inhalation exposure (Murray et al. 1979; Schwetz et al. 1974). However, these defects were not observed in additional developmental studies in rats exposed via inhalation (Baeder and Hofmann 1988; EPA 1978) or rats or rabbits exposed orally (Ruddick et al. 1983; Thompson et al. 1974). Evidence for delayed ossification and impaired growth after inhalation or oral exposure indicate these findings are generally only observed at maternally toxic doses (Baeder and Hofmann 1988; Murray et al. 1979; Ruddick et al. 1983; Schwetz et al. 1974; Thompson et al. 1974). Additional low-dose studies are needed to confirm findings by Murray et al. (1979) and Schwetz et al. (1974) and/or establish dose-response data for developmental effects below those associated with maternal toxicity. One developmental study reported altered glucose homeostasis in offspring following developmental exposure to very low doses in drinking water (Lim et al. 2004). However, due to reporting deficiencies, there is uncertainty in the exposure estimate. Additional developmental studies evaluating low-dose drinking water exposures with adequate monitoring of body weight and intake are needed to corroborate findings from this study.

Epidemiology and Human Dosimetry Studies. Populations may be exposed to chloroform in the workplace, near hazardous waste sites containing chloroform, from chlorinated water, and from various consumer products that contain chloroform. Limited information was obtained from occupational studies reporting CNS and liver effects in exposed workers (Bomski et al. 1967; Challen et al. 1958; Phoon et al. 1983). However, exposure measurements in these studies were not rigorous. Occupational studies with quality external exposure assessments and/or reliable dosimetry data correlating occupational exposure with signs of toxic effects would be useful. Epidemiology studies suggest an association between elevated chloroform levels in drinking water and certain types of cancer in humans (Bove et al. 2007; Doyle et al. 1997; Font-Ribera et al. 2018; Gao et al. 2014) or impaired growth during development (Botton et al. 2015; Grazuleviciene et al. 2011; Kramer et al. 1992; Summerhayes et al. 2012; Wright et al. 2004). However, all of these studies were limited by a lack of control for important confounders, namely co-exposure to other known disinfection byproducts (e.g., trihalomethanes). Epidemiological studies with adequate control for other disinfection byproducts would be helpful. Since toxicokinetics of lipophilic compounds are expected to be different in lean versus obese individuals (La Merrill and Birnbaum 2011), epidemiological studies stratifying analyses by body mass index (BMI) may be useful in determining if there is increased risk of chloroform-related toxicity in obese individuals.

Biomarkers of Exposure and Effect. Methods for detecting chloroform in exhaled breath, blood, breast milk, urine, and tissues are available. Nevertheless, it is difficult to correlate chloroform levels in

biological samples with exposure, because of the volatility and short half-life of chloroform in biological tissues. Several studies monitored chloroform levels in environmentally exposed populations (Antoine et al. 1986; Hajimiragha et al. 1986; Peoples et al. 1979); however, the measured levels probably reflect both inhalation and oral exposure. Moreover, increased tissue levels of chloroform or its metabolites may reflect exposure to other chlorinated hydrocarbons. Studies designed to determine and validate more reliable biomarkers of exposure to allow for better quantitation of chloroform exposure would enhance the database.

No biomarkers were identified that are particularly useful in characterizing the effects induced by exposure to chloroform. The target organs of chloroform toxicity are the CNS, liver, and kidneys; however, damage to these organs may result from exposure to other chemicals. More effort to identify subtle biochemical changes to serve as biomarkers of effects of chloroform exposure would be useful in detecting early, subtle signs of chloroform-induced damage.

Absorption, Distribution, Metabolism, and Excretion. Human data indicate that chloroform absorption from the lungs is rapid and fairly complete after inhalation exposure (Smith et al. 1973). The data also indicate that absorption after oral exposure is close to 100% for both animals and humans (Brown et al. 1974a; Fry et al. 1972; Taylor et al. 1974). Dermal absorption in humans and animals also occurs to some extent, and it is governed by both ambient temperature and its rate of diffusion through the skin (Cammann and Hübner 1995; Fan et al. 2007; Gordon et al. 1998; Islam et al. 1995, 1996, 1999a, 1999b; Lévesque et al. 1994; Tsuruta 1975; Xu and Weisel 2005). Although there are no experimental data regarding dermal absorption in humans, some data have been extrapolated from mouse studies (Tsuruta 1975). The rate of absorption following oral or inhalation exposure is rapid (within 1–2 hours).

Data are available regarding the distribution of chloroform in animals after inhalation, oral, and dermal exposure to chloroform (Brown et al. 1974a; Chenoweth et al. 1962; Cohen and Hood 1969; Corley et al. 1990; Danielsson et al. 1986; Islam et al. 1995; Taylor et al. 1974); however, data regarding the distribution of chloroform in humans are very limited (Feingold and Holaday 1977) and warrant further investigation. Animal studies indicate that distribution following oral exposure is similar to that following inhalation exposure (Brown et al. 1974a; Pfaffenberger et al. 1980; Take et al. 2010); another well-conducted animal study focusing on distribution and excretion after dermal exposure would be useful to assess exposure via this route.

The metabolic pathways of chloroform metabolites are well understood (Ade et al. 1994; Constan et al. 1999; Lipscomb et al. 2004; Liu et al. 2013; Testai et al. 1996). Based on clear differences in toxicity between gavage and drinking water studies in rodents (Larson et al. 1993, 1994b, 1995a, 1995b), it appears that the mode of oral administration affects metabolism. Increased toxicity in rodents following acute-duration gavage exposure, compared to drinking water, is likely due to saturation of detoxification pathways following bolus gavage exposure, which exacerbates toxicity due to accumulation of toxic metabolites in hepatic and renal tissues. Specifically, it is proposed that the reaction of chloroform metabolites with GSH acts as a detoxifying mechanism. This is supported by observations that chloroform doses that caused liver GSH depletion produced liver necrosis (Docks and Krishna 1976). However, increased toxicity is not observed in gavage studies (Dunnick and Melnick 1993; NCI 1976; Roe et al. 1979) following chronic-duration exposure in rodents when compared to drinking water studies (Hard et al. 2000; Jorgenson et al. 1985; Nagano et al. 2006). Several factors may contribute to this differential finding in longer-duration studies, including: (1) adaptive metabolic changes with chronic-duration exposure leading to blunting or attenuation of bolus effects; (2) lack of evaluation at low gavage doses in some studies (which may have potentially identified lower LOAELs); and/or (3) evaluation of different strains in chronic versus shorter-duration studies that may have differential susceptibility. Additional data investigating the impact of mode of oral exposure, duration of administration, and impact of strain would be useful in order to understand the role of these factors in the mechanism of chloroform's toxicity.

The excretion of chloroform and its metabolites is understood, based on human and animal data derived from oral and inhalation studies (Brown et al. 1974a; Corley et al. 1990; Fry et al. 1972; Taylor et al. 1974) and in animals following dermal exposure (Islam et al. 1996, 1999a). The major route of chloroform elimination is pulmonary, but minor pathways are through enterohepatic circulation, urine, and feces as parent compound or metabolites (Corley et al. 1990).

Comparative Toxicokinetics. Target organs for chloroform distribution appear to be similar in humans and animals. Nonetheless, human and animal studies indicate that there are large interspecies differences in chloroform metabolism and tissue partition coefficients (Brown et al. 1974a; Corley et al. 1990). Since hepatic, renal, and nasal toxicity is attributed to reactive metabolites (e.g., phosgene), differential activity of CYP2E1 across species and sexes will confer a difference in susceptibility to toxic effects (Constan et al. 1999). Data on CYP2E1 activity in human olfactory epithelial tissue are limited and conflicting (Green et al. 2001; Longo and Ingelman-Sundberg 1993); studies designed to evaluate the ability of human olfactory mucosa to metabolize chloroform would be useful to decrease the uncertainty

in the dosimetry extrapolation in the inhalation MRLs. Marked sex-related differences in tissue distribution and covalent binding to tissue macromolecules in mice also have been observed (Taylor et al. 1974). Excretion data indicate that humans and nonhuman primates excrete chloroform in the breath primarily as unchanged chloroform; mice eliminate almost 80% of an oral chloroform dose as CO_2 (Brown et al. 1974a). Thus, toxicokinetic data indicate that it may be difficult to compare the toxicokinetics of chloroform in animals with that in humans. There are many oral studies, relatively few inhalation studies, and a limited number of dermal studies regarding the toxicokinetics of chloroform. Quantitative toxicokinetic studies in several animal species involving exposure to chloroform via all three routes, especially inhalation and dermal, would help complete the database. In addition, further refining of the existing PBPK/PD models and/or additional PBPK/PD model development would further advance our understanding of chloroform tissue dosimetry in humans and animals. For example, current nasal dosimetry models are limited to rats (Sarangapani et al. 2002); a PBPK/PD model for nasal dosimetry in humans would reduce uncertainty in dosimetry extrapolation in the inhalation MRLs. For oral MRLs, current PBPK/PD models extrapolate the internal doses from delivery of a large bolus dose. PBPK/PD models based on more environmentally-relevant exposure scenarios (drinking water) may be useful and could reduce uncertainty in dosimetry extrapolation for the acute-duration oral MRL. PBPK/PD models in additional species (e.g., dogs) would reduce uncertainty in dosimetry extrapolation for the intermediate- and chronic-duration oral MRLs.

Children's Susceptibility. It is unknown if developing fetuses, infants, or children are uniquely susceptible to chloroform toxicity. There may be age-related susceptibility to chloroform, as observed in rodent lethality studies (Deringer et al. 1953; Kimura et al. 1971). As discussed above (under Developmental Toxicity), developmental findings in human studies are mixed. In mice, it has been shown that chloroform passes the placenta (Danielsson et al. 1986). However, available evidence suggests that developmental effects in rodents are most likely to occur only at exposure levels associated with maternal toxicity. Serious effects (cleft palate, imperforate anus) were only observed in one study each, and both were at or above concentrations associated with maternal toxicity (Murray et al. 1979; Schwetz et al. 1974). These defects were not observed in additional developmental studies in rats exposed via inhalation (Baeder and Hofmann 1988; EPA 1978) or rats or rabbits exposed orally (Ruddick et al. 1983; Thompson et al. 1974), even at maternally toxic exposure levels. Similarly, many studies reported delayed ossification and/or impaired growth at maternally toxic inhalation or oral doses (Baeder and Hofmann 1988; Murray et al. 1979; Ruddick et al. 1983; Schwetz et al. 1974; Thompson et al. 1974). Additional studies at low, non-maternally toxic doses, are needed to fully evaluate children's susceptibility.

Physical and Chemical Properties. As reported in Table 4-2, the physical and chemical properties of chloroform have been characterized sufficiently to permit estimation of its environmental fate.

Production, Import/Export, Use, Release, and Disposal. Data regarding the production methods, production capacity volumes (current, past, projected future), and current import and export volumes are available (EPA 2023a; Holbrook 2003; Ohligschläger et al. 2019; USITC 2023). However, these statistics will generally not include all instances where chloroform is generated as a chemical intermediate or waste product. Except for the partial coverage provided in the TRI, comprehensive information regarding current release and disposal patterns, are lacking. General disposal information is adequately detailed in the literature, and information regarding disposal regulations of chloroform is available (EPA 1988a, 1988b). Production, release, and disposal data are useful to determine where environmental exposure to chloroform may be high.

Environmental Fate. Chloroform partitions mainly into the atmosphere and into groundwater. Experimental data are available regarding the transport and partitioning properties of chloroform in surface waters (Bean et al. 1985; Clark et al. 1982; Class and Ballschmidter 1986; Dilling 1977; Ferrario et al. 1985; Piwoni et al. 1986; Sawhney 1989). Chloroform can be transported long distances in air.

Data are available regarding the degradation of chloroform in the atmosphere, but less is known about degradation rates in water and soil (Anderson et al. 1991; Bouwer et al. 1981a, 1981b; Dilling et al. 1975; DOT 1980; Henson et al. 1988; Jeffers et al. 1989; Park et al. 1988; Singh et al. 1981; Tabak et al. 1981; Wilson et al. 1981). Hydrolysis and direct photodegradation are not significant removal processes. Although data regarding biodegradation rates in natural media are lacking, volatilization is expected to dominate over biodegradation as a removal process from surface water and near-surface soil. Chloroform seems relatively persistent in the atmosphere and groundwater. The environmental fate of chloroform is sufficiently determined by the available data. Considering the documented occurrence (Class and Ballschmidter 1986) of chloroform in remote, often pristine areas, further study is warranted to help quantify the relative role of long-range transport processes. These more localized processes could include the reaction of naturally generated chlorinated oxidants with organic materials to yield chloroform. More data would be useful on the half-lives of chloroform in media.

Bioavailability from Environmental Media. Chloroform is absorbed following inhalation, oral, and dermal contact. Toxicity studies of exposure to chloroform in air, water, and food demonstrated the

bioavailability of chloroform by these routes. Data are lacking on the bioavailability of chloroform following ingestion of contaminated soils. However, near-surface soil concentrations can be expected to be low due to volatilization (Piwoni et al. 1986; Wilson et al. 1981), suggesting that soil ingestion is not a likely route of exposure.

Food Chain Bioaccumulation. Data are available that indicate that chloroform does not bioconcentrate in aquatic organisms (Barrows et al. 1980; Veith et al. 1980). Bioconcentration studies are lacking for plants and other animals (e.g., plants, macroinvertebrates). Similarly, no studies were located regarding the biomagnification potential of chloroform in terrestrial and aquatic food chains. Additional information on bioconcentration and biomagnification could be useful in establishing the significance of food chain bioaccumulation as a route of human exposure.

Exposure Levels in Environmental Media. All humans are exposed to at least low levels of chloroform via inhalation of contaminated air, and most humans are exposed by drinking contaminated water. Estimates from intake via inhalation and ingestion of drinking water, based on limited data, are available (EPA 2023d; USGS 2015a, 2015b; WQP 2024). The quantitation of chloroform levels in food has been studied (Cao et al. 2024; Fleming-Jones and Smith 2003; Huang and Batterman 2009, 2010). Current information on exposure to chloroform for workers or people who live near manufacturing and use facilities, water and wastewater-treatment plants, municipal and industrial incinerators, hazardous waste sites, and other sources of significant release would be useful. Likewise, current indoor air exposure levels would be valuable.

Exposure Levels in Humans. Data regarding occupational exposure levels in humans are incomplete and are usually the result of limited, special studies. Studies designed to obtain better, current estimates of expected chloroform exposures in various workplace settings would be useful, including industrial settings (facilities that manufacture or use chloroform, drinking-water plants, wastewater-treatment plants, paper and pulp plants), indoor pools and spas, and industrial and domestic cleaning scenarios. Chloroform has been found in human blood of adults in the U.S. population (CDC 2022a, 2022b). A detailed database of exposure would be helpful in determining the current exposure levels, thus allowing an estimation of the average daily dose associated with various scenarios, such as living near a point source of release, drinking contaminated water, or working in a contaminated place. This information is necessary for assessing the need to conduct health studies on these populations.

Exposures of Children. Although NHANES data does include information on ages 12–19, more studies are needed on exposure levels to children at all stages of development. This information would be useful in determining how exposure of children differs from adults.

6.3 ONGOING STUDIES

No ongoing studies were identified in the National Institute of Health (NIH) RePORTER (2024) database.