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 copper 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 copper.

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.
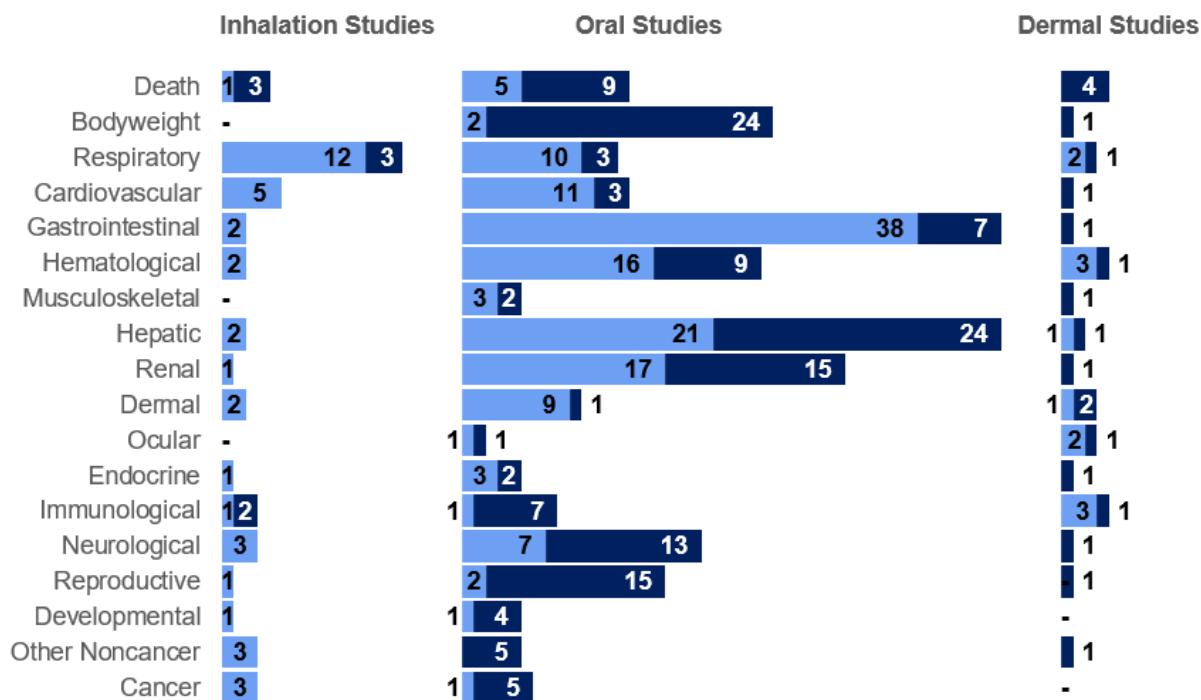
6.1 EXISTING INFORMATION ON HEALTH EFFECTS

Studies evaluating the health effects of inhalation, oral, and dermal exposure of humans and animals to copper 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 copper. 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 exposed to copper primarily apply to oral ingestion. Many of these studies are case reports of individuals who intentionally or accidentally ingested copper or copper-containing substances. Epidemiological and controlled-exposure studies in humans primarily examined effects following ingestion of copper in drinking water. In these studies, gastrointestinal symptoms were the most frequently observed health effect. There are a robust number of experimental studies in animals that examine a wide range of health effects following oral exposure to copper and/or copper compounds, particularly the hepatic and renal toxicity endpoints. Inhalation and dermal studies were limited in both animals and humans, but the results generally support the effects following oral ingestion.

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

Potential gastrointestinal and hepatic effects were the most studied endpoints.
The majority of these studies examined oral exposure in **humans** (versus **animals**).

	Inhalation Studies	Oral Studies	Dermal Studies
Death	1 3	5 9	4
Bodyweight	-	2 24	1
Respiratory	12 3	10 3	2 1
Cardiovascular	5	11 3	1
Gastrointestinal	2	38 7	1
Hematological	2	16 9	3 1
Musculoskeletal	-	3 2	1
Hepatic	2	21 24	1 1
Renal	1	17 15	1
Dermal	2	9 1	1 2
Ocular	-	1 1	2 1
Endocrine	1	3 2	1
Immunological	1 2	1 7	3 1
Neurological	3	7 13	1
Reproductive	1	2 15	1
Developmental	1	1 4	-
Other Noncancer	3	5	1
Cancer	3	1 5	-

*Includes studies discussed in Chapter 2; the number of studies includes those finding no effect.

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 acute-duration oral human database was adequate for the derivation of an acute-duration oral MRL. Gastrointestinal symptoms occur in humans following acute ingestion of excess copper in drinking water (Araya et al. 2001, 2003a, 2003c; Gotteland et al. 2001; Olivares et al. 2001; Pizarro et al. 1999, 2001). Gastrointestinal toxicity is supported by evidence in the oral database in animals where gastrointestinal symptoms and histological changes in the gut were observed (Cheng et al. 2020; Kadammattil et al. 2018; Khushboo et al. 2018). The acute-duration inhalation database was not adequate for the derivation of an inhalation MRL. The database is limited to one study in humans

examining the immunological endpoint which was of insufficient quality as there was no comparable control group (Markert et al. 2016), and a study in animals examining the respiratory and immunological endpoints (Drummond et al. 1986). Two additional studies only examined the death endpoint (Holbert 1990; Rush 1991). Toxicity studies examining gastrointestinal and hepatic toxicity from inhalation of excess copper would be useful to identify the target of toxicity. Additionally, studies examining a possible concentration-response relationship would be useful especially in occupational settings where such exposures are likely to occur.

Intermediate-Duration MRLs. The intermediate-duration oral animal database was not adequate for the derivation of an intermediate oral MRL. The acute oral MRL was adopted to the intermediate oral MRL. Two studies found increased incidence of gastrointestinal symptoms in humans over a 2-month exposure to copper doses ≥0.106 mg Cu/kg/day (Araya et al. 2003b, 2004). However, the reports as related to gastrointestinal responses were episodic and data for systemic effects are limited. Alternatively, no copper-related effects on the gastrointestinal system, liver or on body weight were observed at doses as low as 0.058–0.3 mg Cu/kg/day (O'Connor et al. 2003; Olivares et al. 1998). A study in nine men showed that oral exposure to 0.1 mg Cu/kg/day for 18 days resulted in reduced antibodies to a strain of influenza following immunization, when compared to non-exposed control (Turnlund et al. 2004). Oral studies in animals examined a wide range of endpoints and data indicates that the liver is a sensitive target of copper toxicity (Hashish and Elgaml 2016; Kvietkauskaite et al. 2005; Wu et al. 2020; Epstein et al. 1982; Shen et al. 2005). The intermediate-duration inhalation database was not adequate for the derivation of an inhalation MRL; it is limited to two studies in rabbits that did not report any adverse health outcomes (Johansson et al. 1983, 1984). It would be useful for toxicity studies to identify the target of copper toxicity following intermediate-duration inhalation exposure to copper, as well as establish concentration-response relationships.

Chronic-Duration MRLs. No studies examining chronic-duration inhalation or oral exposure in humans or animals were adequate for the derivation of chronic MRLs. The oral database is limited to a few studies in animals where decreased lifespan, reduced hemoglobin, and mild hepatic effects were reported (Araya et al. 2012; Massie and Aiello 1984). Since serious health effects have been reported at the acute- and intermediate-durations, studies examining the toxicity of exposure to excess chronic doses of copper may be useful to establish chronic doses that would be harmful to humans. The NIH's tolerable upper intake level for copper in 900 µg/day for adults aged 19 to >70 years. Studies would be helpful to determine effects of ingestion exceeding this level. No inhalation studies in either humans or animals provided any data on copper toxicity. Studies in the inhalation database were limited to studies reporting associations; however, the data is suggestive at best. Studies examining toxicity resulting from chronic

exposure to excess copper in air would be useful for populations living near sites currently processing, using, or manufacturing copper that may enter the local environment, and for workers who handle copper in occupational settings.

Health Effects.

Respiratory. Symptoms of respiratory irritation, including coughing, sneezing and thoracic pain, were observed in workers following inhalation of copper in occupational settings (Askergren and Mellgren 1975; Suciu et al. 1981). Inhalation of copper fumes in an occupational case study resulted in persistent sinus pressure and rhinorrhea (Gibson et al. 2011). A copper sulfate mixture was implicated as the etiologic agent in a unique disease observed among vineyard workers spraying an antimildew agent (Pimentel and Marques 1969; Pimentel and Menezes 1975; Stark 1981; Villar 1974; Villar and Nogueira 1980). Drummond et al. (1986) observed decreased cilia beating in hamsters and alveolar thickening in mice that were exposed repeatedly, and toxicity increased with duration of exposure. Further studies are needed to characterize respiratory toxicity of copper, especially in workers who likely inhale copper dust or fumes in occupational settings. Additionally, concentration-response relationships are yet to be established.

Immunological. Limited evidence in humans and animals suggests that excess copper may be immunotoxic. Reports on humans developing dermatitis after dermal exposure to copper (Barranco 1972; Saltzer and Wilson 1968) suggest that copper is an allergen. This is supported by a report of a woman developing dermatitis after insertion of a copper IUD (Barranco 1972). Increased blood C-reactive protein, an indication of asymptomatic inflammation, was seen in a controlled-study where adult volunteers were exposed to copper-containing welding fumes of 0.53 mg Cu/m^3 for 6 hours, 3 times over 3 weeks (Markert et al. 2016). A study in adult men found that antibodies to an influenza strain were less after immunization when compared to controls following exposure to 0.1 mg Cu/kg/day (Turnlund et al. 2004). Immunological effects were observed in mice following acute inhalation exposure to copper sulfate (Drummond et al. 1986). Copper produced a toxic effect on the antioxidant defense system in mice; decreased percentage of suppressor, natural killer, and its precursor, and increased immunoregulatory index were both reported (Kvietkauskaite et al. 2004). In addition, impaired immune function is observed in mice exposed to copper chloride (Pocino et al. 1991) or copper sulfate (Pocino et al. 1990) in drinking water. Histological changes in the spleen were observed in mice and rats orally exposed to copper compounds (Kadammattil et al. 2018; Khushboo et al. 2018). More studies in humans and animals that examine the immune response to copper exposure would be useful to understand possible dose-response relationships and assess species differences.

Neurological. Neurological impairment was observed in factory workers exposed to copper dust (Donoso et al. 2007). Clinical symptoms of neurotoxicity were observed in a drinking water study of 60 females, with 6 subjects reporting increased salivation (Pizarro et al. 1999). Dizziness, agitation, and drowsiness were noted in case studies of copper ingestion (Malik and Mansur 2011; Du and Mou 2019; Gunay et al. 2006; Yang et al. 2004). No effects on neurobehavioral performance and no histological changes were observed in several oral studies in animals (Kadammattil et al. 2018; Lu et al. 2009; NTP 1993; Seffner et al. 1997). At 4 mg Cu/kg/day, rats showed indications of copper-induced neurotoxicity including neurobehavioral change, impaired muscle strength, and coordination (Kumar et al. 2019). No effects on neurobehavioral performance were observed in rats fed 250 ppm copper in their diet (Murthy et al. 1981). However, this study did find alterations in the levels of a dopamine metabolite, suggesting that copper may adversely affect the nervous system. Serious neurotoxic effects at doses ≥25.5 mg Cu/kg/day included impaired motor coordination and cognitive function (Kalita et al. 2020; Khushboo et al. 2018; Kumar et al. 2015, 2016a, 2016b). Additional studies are needed to further investigate the neurotoxic potential of copper; these studies should assess the potential of copper to perturb dopaminergic pathways and related functions.

A recent *in vitro* study indicates that copper may be critically involved in optimal functioning of the circadian clock by modulating cell metabolism, redox state, transcription, and neuronal activity (Yamada and Prosser, 2020). Alterations in these circadian rhythms have previously been implicated in increased risk for cardiometabolic diseases, cognitive and mood disorders, and sleep disorders (Luojus et al., 2015; Song et al., 2015; Yoshioka et al., 2018; Yukihiro et al., 2020; Abbott et al., 2020). Studies that investigate the effects of inhalation, oral, and dermal exposure to copper that examine its effects on circadian rhythms need to be designed and conducted in animal and human paradigms. These studies need to examine the alterations in circadian machinery and the potential downstream alterations in physiology in the organisms.

There is a growing body of literature that indicates copper may play a role in the development of Alzheimer's disease and other similar neurodegenerative diseases (Pohanka 2019). Medical studies have found evidence that the metabolic balance and distribution of copper is disrupted in individuals with Alzheimer's disease (Coelho et al. 2020). However, the current literature is unclear on how environmental exposures to copper affect the development of Alzheimer's disease. Since the current neurological literature in animals and humans indicate copper can lead to neurological impairment and given that copper can distribute to the brain, epidemiological studies examining the relationship between environmental exposures to copper and Alzheimer's disease would be useful in understanding long term risks of exposure.

Developmental. Developmental studies examining oral exposure to copper in rats (Haddad et al. 1991) and mice (Lecyk 1980) have shown that high copper intakes can result in impaired growth. In rabbit fetuses, mean fetal weight was reduced; 4 fetuses had protrusion of the abdomen; and there was increased incidence of hemivertebrae, delay ossification, and supernumerary ribs compared to controls (Munley 2003a, 2003b). Kadammattil et al. (2018) found no copper-related changes in implantations, non-viable embryos, resorbed embryos, or embryo body weight. The developmental toxicity of copper in humans is not adequately investigated, as animal studies produce strong evidence that copper may be toxic to the reproductive system (Arafa et al. 2019; Babaei et al. 2012; Kadammattil et al. 2018; Khushboo et al. 2018; Liu et al. 2016; Munley 2003a, 2003b; NTP 1993; Sakhaee et al. 2012, 2016a, 2016b). No data were located regarding developmental effects of copper after inhalation or dermal exposures in humans or animals. Multigeneration studies and further investigations in different animal species would provide valuable information on the potential of copper to adversely affect development. Such information might be relevant to humans.

Cancer. Data on the carcinogenicity of copper in humans are limited. A study of copper miners (Chen et al. 1993) and a follow-up to this study (Chen et al. 1995) observed increased risk of cancer, stomach cancer, and lung cancer. Because the workers were also exposed to radon and radon daughters, silica, iron, titanium, sulfur, and arsenic, a causal relationship between copper and increased cancer risk cannot be established. Only one study examined the association between copper ingestion and cancer risk in humans. Odds of leukemia cancer development was not affected by copper levels in carpet dust, which was presumably ingested (Raaschou-Nielsen et al. 2016). This study was very limited in the exposure examined and results are not indicative. A prospective cohort study of populations across Europe found that copper in $PM_{2.5}$ was associated with increased risk of lung cancer (Hazard Ratio = 1.25; 95% CI=1.01-1.53), while PM_{10} was not associated with increased risk of lung cancer (Hazard Ratio = 1.14; 95% CI=0.96-1.35) (Raaschou-Nielsen et al. 2016). These observations were also not indicative of a causal relationship. Several animal studies have examined the carcinogenic potential of ingested copper (Abe et al. 2008; Greene et al. 1987; Kamamoto et al. 1973). These studies are limited in scope; the studies by Green et al. (1987) and Kamamoto et al. (1973) only examined one potential target and tested fairly low doses of copper. No dermal carcinogenicity studies in humans or animals were identified. Additional studies by the inhalation, oral, and dermal routes are needed to assess the carcinogenic potential of copper in humans.

Genotoxicity. No data on the genotoxicity of copper in humans were located; studies of workers or individuals accidentally exposed to high levels of copper would provide value information on its genotoxic potential in humans. The available genotoxicity data suggest that copper is a clastogenic agent

(Agarwal et al. 1990; Bhunya and Jena 1996; Bhunya and Pati 1987; Fahmy 2000; Sideris et al. 1988). However, mixed results are found in point mutation assays (Demerec et al. 1951; Marzin and Phi 1985; Singh 1983; Tso and Fung 1981; Wong 1988). Additional studies are needed to assess copper's potential to induce point mutations. Several studies have also shown that exposure to copper can result in DNA damage (Caicedo et al. 2007; Garrett and Lewtas 1983; Husain and Mahmood 2019; Prasad et al. 2006; Sideris et al. 1988; Sina et al. 1983; Urbino-Cano et al. 2006).

Copper Nanoparticles. The toxicity of copper nanoparticles has not been examined in humans and studies in animals are limited. Data primarily come from *in vivo* and *in vitro* studies examining its genotoxicity and cytology. Oral exposure studies in rats and mice suggest CuNPs cause histological damage to the liver and alter enzyme levels (Anreddy et al. 2018; El Bialy et al. 2020; Chen et al. 2006; De Jong et al. 2019; Tang et al. 2018). Kidney damage is also reported in rats and mice from oral CuNP exposure (El Bialy et al. 2020; Chen et al. 2016; De Jong et al. 2019). These effects are similar to those seen in animals following oral exposure to ionic copper. Further studies are needed to determine if renal and hepatic toxicity is expected at levels lower than those of ionic copper. Additionally, rats and mice show gastrointestinal, respiratory, and neurotoxic effects following oral exposure, which are similar to effects seen in humans following both oral exposure and inhalation exposure, especially in workers. Occupational studies in workers exposed to CuNPs toxicity would help elucidate the effect that particle size has on toxicity, particularly in the respiratory system.

Epidemiology and Human Dosimetry Studies. Several studies have examined the toxicity of inhaled copper in workers (Askergren and Mellgren 1975; Finelli et al. 1981; Suciu et al. 1981). These studies have primarily focused on the respiratory tract, although health examinations revealed other adverse effects (e.g., hepatomegaly). Chen et al. (1993, 1995) examined the carcinogenic potential of inhaled copper. In general, these studies are limited by poor exposure characterization, co-exposure to several toxic and/or carcinogenic compounds (e.g., arsenic, cadmium, radon, lead), and limited number of endpoints examined. Occupational exposure studies examining populations of workers exposed to copper and with minimal exposure to other metals would be useful in assessing the toxicity of inhaled copper. These studies should examine a wide variety of endpoints, particularly the gastrointestinal tract, liver, and kidneys, which are well established targets of toxicity following oral exposure. There are numerous reports of accidental or intentional ingestion of copper, and the most commonly reported effect in these studies is gastrointestinal upset, followed by liver effects. There have been several experimental studies designed to examine gastrointestinal upset following short-term (2 weeks or less) exposure to copper in drinking water (Araya et al. 2001, 2003a, 2003c, 2004; Gotteland et al. 2001; Olivares et al. 2001; Pizarro et al. 1999, 2001). Several studies have examined health effects, mainly gastrointestinal but also hepatic,

in populations exposed to elevated level copper levels in drinking water (Buchanan et al. 1999; Dieter et al. 1999; Eife et al. 1999; Knobeloch et al. 1994, 1998; Pettersson et al. 2003; Pizarro et al. 2007; Zietz et al. 2003a, 2003b). Studies in both children and adults could elucidate the understanding of possible age-related differences in toxicity.

Biomarkers of Exposure and Effect.

Exposure. Copper levels can be measured in tissues, body fluids, excreta, hair, and nails. Whole blood, serum, and urine copper levels have been established in healthy individuals. It has been demonstrated that copper levels in the body increase with increased exposure after acute poisoning. Similarly, increased copper levels were observed in workers after occupational exposure. Serum and urine copper levels, plasma ceruloplasmin levels, and clinical manifestations are specific indicators of copper status. Current biomarkers appear sufficient for assessing copper exposure.

Effect. There are no specific biomarkers for copper toxicity. Individuals with Wilson's disease are usually diagnosed by examining serum and urine copper levels, plasma ceruloplasmin levels, and clinical manifestations. However, the relationship between serum and urine levels of copper and health effects is not known. Studies examining the possible correlation between blood levels or excreta levels of copper with effects would facilitate medical surveillance. Liver enzyme levels can indicate liver damage resulting from copper toxicity; however, these are not specific to copper-induced liver damage.

Absorption, Distribution, Metabolism, and Excretion. The absorption, distribution, metabolism, and excretion of copper administered orally have been studied in animals and, to some extent, in humans. Furthermore, alterations in copper absorption, distribution, and excretion have been studied in deficiency and toxicity states. Despite the information on copper absorption, there is very little information on differences between absorption rates of the various compounds and differences between the bioavailability of copper from food and water.

There is very limited information on copper absorption following inhalation exposure, and data on the absorption of copper through the skin are limited. Further studies in animals on the rate and extent of copper absorption following exposure from both the inhalation route and the dermal route would more fully characterize copper toxicokinetics in animals and by extrapolation in humans.

Comparative Toxicokinetics. The metabolism of copper has been studied in rats, pigs, hamsters, and humans. However, there are no comparative studies on the effects of high copper intakes on the distribution of copper in the body or the development of tolerance to continued high intakes of copper.

Furthermore, the animal species that might serve as the best model for extrapolating results to humans is not known.

Children's Susceptibility.   There are some data on the toxicity of copper in infants and children. Severe liver damage has been reported in infants and children. These effects are typically clustered in geographically regions and have been grouped into two syndromes: Indian childhood cirrhosis and idiopathic copper toxicosis. Both of these syndromes have been connected to elevated copper intakes and are believed to have a genetic component. Very high levels of copper are found in the livers of affected children, suggesting that the mechanism of action is related to impaired copper efflux. Additional studies are needed to determine the mechanism of toxicity and to ascertain copper's role in the observed effects.

Physical and Chemical Properties.   In general, the available data on the physical and chemical properties of elemental copper and copper sulfate are sufficient for estimating their environmental fate. In general, experimental confirmation is required for predicting copper's fate in the environment. The factors which determine the copper species present or the material to which copper may be bound and the strength of the binding can be site-specific. If the level of detail requires knowledge of, for example, the percentage of copper associated with iron oxides or that which is easily exchangeable, experimental confirmation is necessary.

Production, Import/Export, Use, Release, and Disposal.   Information on the production, use, release, and disposal of metallic copper and copper sulfate is generally available. These two forms of copper account for most of the copper used. This information is tabulated by the U.S. Geological Survey every year in the Minerals Yearbook and predictions of future trends in production and use are available. Information on the future of copper demand and implications on copper recycling and production are also available (Ciacci et al. 2020; Schipper et al. 2018). Such information is not available for other copper compounds. The major uses of copper and where these uses occur (e.g., the home, workplace, etc.) is also available.

Environmental Fate.   Reliable information on how copper and its compounds partition in the environment (i.e., to soil and sediment), and the type of transformations that occur in different media is extensively available. Data on its transport in the environment is also reliable. Although information on the fate of copper in air, water, and soil is available, the fate of copper is both species- and site-specific. Information concerning the forms of copper (i.e., specific compound, to what it is bound or complexed, or, in the case of air, the particle size) or the lability of the copper in particular media is available from only a few studies. These are sufficient to identify numerous contributors to the fate of copper and its

compounds, but they are insufficiently comprehensive for developing accurate fate maps. In addition, studies of how fate data relate to human exposures, especially in regard to projecting copper toxicity in children, is inadequate.

Bioavailability from Environmental Media. Copper is found in food, water, ambient air, and soil. The bioavailability of copper from food and water has been investigated in animals and humans. Studies on the bioavailability of copper from soil and ambient air would be useful in assessing potential toxicity to people living near a hazardous waste site. The form and lability of copper in the environment is known in only a few site-specific cases that do not include hazardous waste sites. More information on the forms of copper found at industrial sites and hazardous waste sites would be useful. Monitoring groundwater near industries that use highly acid, copper-containing solutions, such as electroplating, electrowinning, and ore leaching industries, is important for the protection of human populations at risk of exposure to their highly mobile and highly bioavailable copper.

Food Chain Bioaccumulation. Because copper occurs in different forms in the environment, its bioaccumulation is expected to vary according to site and species. Data are available on the bioconcentration of copper in aquatic organisms, plants, and animals, as well as biomagnification in food chains. This information is useful in assessing the potential for exposure from ingesting food originating from contaminated areas. However, little information is available on the potential for intoxication from foodstuffs from apparently nonpolluted areas or where they may have accumulated toxic levels of copper through biomagnification resulting from foraging in polluted areas.

Exposure Levels in Environmental Media. Data are available regarding the concentrations of copper in environmental media, including the concentration of copper in soil at some hazardous waste sites. Since copper is naturally present in soil, trace quantitative analytical and statistical techniques can be used to determine whether the copper found at these sites is elevated above normal levels. Monitoring data are reasonably current and human intake of copper from food, water, and air can be estimated.

Exposure Levels in Humans. There are reasonably current data on levels of copper in human tissue and human milk. However, few studies address specific U.S. populations living around hazardous waste sites. There are some quantitative data relating occupation, level, and route of exposure to the form of copper to which people are exposed. There is some limited information correlating copper concentration and form to body burden in the general population. However, more information is needed for occupational and other at-risk populations.

Exposures of Children. Data on copper intake in infants and children is generally up to date. Information on copper intake by infants from human milk also is available. Exposure of children to copper in drinking water has been assessed and methods to decrease this exposure have been identified and implemented. However, only limited information on inhalation is available. Some information on exposure of children to copper near mining, smelting, refining, manufacture facilities, waste sites, and other hazardous sites is available, but not for U.S. populations. This information is needed to better estimate exposures of children in U.S. populations living near these facilities and sites. The use of copper concentrations in toenails and hair has been investigated as a surrogate measure of copper exposure in children and adults, and more research into establishing the validity of these surrogates is underway.

6.3 ONGOING STUDIES

No ongoing studies were identified for copper.