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

3.1 TOXICOKINETICS

- Copper is absorbed in the gastrointestinal tract, primarily by the small intestine. Copper absorption ranges from 12 to 71% in adult humans and from 75 to 84% in infants. Dietary copper intake and copper absorption are tightly regulated by copper homeostasis maintenance.
- Following absorption, copper is distributed by a two-phase process. The first phase distributes copper by transport to portal venous circulation where copper is bound to serum protein and ultimately about 75% of this copper is taken up by the liver. In the second phase, copper is bound primarily to ceruloplasmin in the liver, is released to systemic blood circulation, and is redistributed to other organ tissues including the brain, kidneys, muscles, and connective tissues.
- Copper metabolism is largely regulated by copper-transporting P-Type ATPases: ATP7A and ATP7B. Cu(II) reduces to Cu(I) mediated by reductases for copper to transport through cellular membranes.
- Bile excretion through feces is the major excretory pathway for copper. Copper half-lives have been measured in various tissues and were 3.9–21 days in the liver, 5.4–35 days in the kidney, 23–662 days in the heart, and 457 days in the brain.

3.1.1 Absorption

No studies were located that provided data on the rate or extent of absorption following inhalation exposure of copper in humans or animals.

Oral copper absorption occurs in the gastrointestinal tract, primarily in the stomach and small intestine, mostly from the duodenum (van den Berghe and Klomp 2009). Oral absorption was rapid with the maximum concentration of copper in the plasma (C_{max}) detected 1.5 hours after administration of a single gavage dose of 79.5 mg Cu/kg in rats (given as copper gluconate in water) (García-Martínez et al. 2021). Copper is absorbed from the gastrointestinal tract as ionic copper or bound to amino acids. Evidence indicates that oral copper absorption is dependent on transport proteins, particularly the high-affinity copper transport 1 (Ctr1) and ATP7A. Active mechanisms for copper absorption from the small intestine likely initially involve transport through Ctr1 into enterocytes. Prior to uptake across the apical membrane by Ctr1, the oxidized state Cu(II) is reduced to Cu(I) mediated by reductases activity at the apical membrane of the gastrointestinal enterocytes (Nishito and Kambe 2018; Ohgami et al. 2006). Cuprous, Cu(I), copper transported by Ctr1 concentrates in the apical membrane and early endosomes of the intestinal epithelial cells (Nishito and Kambe 2018). From the epithelial cells, copper is then

transported by the copper chaperone, antioxidant-1, to ATP7A that then readily exports copper into the blood of the portal venous system through which distribution occurs (Nishito and Kambe 2018). To maintain homeostasis and regulation of internal copper levels, copper absorption decreases with increased consumption of dietary copper (van den Berghe and Klomp 2009). In a study of adult men fed low-copper or high-copper diets, copper hemostasis was maintained, and absorption was similar between the groups (Harvey et al. 2003). Another study of 11 young men administered various copper doses in food over a period of 42–98 days found absorption efficiencies of 55–56, 36, and 12% at doses of 0.785, 1.68, and 7.53 mg Cu/day, respectively (Turnlund et al. 1989). In humans, the amount of stored copper does not appear to influence copper absorption (Strickland et al. 1972).

Multiple human studies examined the oral absorption of dietary copper and reported absorption rates ranging from 12 to 71% in presumably healthy adults (Harvey et al. 2003, 2005; Jacob et al. 1987; Johnson et al. 1992; Strickland et al. 1972; Turnlund et al. 1982, 1983, 1985, 1988, 1989, 2005; Weber et al. 1969). Peak copper absorption, estimated through a non-compartment analysis, occurred 1–2 hours after ingestion of a single oral dose of copper gluconate in a controlled study of obese males (Boullata et al. 2017). In infants, higher absorption rates were reported, ranging from 75 to 84% (Araya et al. 2003d; Domellof et al. 2009; Olivares et al. 2002).

As previously stated, infants appear to have higher absorption rates than those reported in adults (Araya et al. 2003d; Domellof et al. 2009; Olivares et al. 2002). Olivares et al. (2002) did not find significant differences in copper absorption between 1- and 3-month-old infants. Conversely, Dörner et al. (1989) found a linear relationship between copper intake and retention in a metabolic balance study of infants (aged 2–16 weeks). An animal study by Varada et al. (1993) reported age-related differences in copper absorption which was linear and nonsaturable in suckling (16 days of age) and weanling (21–22 days of age) rats, whereas in adolescent rats (6 weeks of age), copper absorption was saturable. The levels of copper retained in the intestine were greater in the suckling rats than in the weanling or adolescent rats (Varada et al. 1993).

Evidence showing sex and age differences in absorption rate are mixed. Several studies in adults did not find differences in copper absorption between older male and female adults aged 60–83 years (Johnson et al. 1992) or between older men (65–74 years) and young men (22–30 years) (Turnlund et al. 1982, 1988). Conversely, Johnson et al. (1992) did find that copper absorption was higher in women (71%) than in men (64%) aged 20–59 years. Obesity did not appear to impair copper absorption in adult males (Boullata et al. 2017). In addition, the composition of the diet can influence copper absorption including

plant-based protein diets (Turnlund et al. 1983) and lacto-ovo-vegetarian diets through their impacts on the levels of bioavailable copper ions (Hunt and Vanderpool 2001). One study of organic diets did not find an effect on copper absorption (Mark et al. 2013). Organic diets refer to eating crops grown without synthetic herbicides, pesticides, or fertilizers, or without bioengineered genes.

Competition with other metals in the body can also affect copper absorption in humans and animals as iron and zinc are potential absorption inhibitors for copper uptake across cellular membranes. Increased levels of zinc in the diet resulted in decreased in copper absorption in humans and rats (Hall et al. 1979; Hoogenraad et al. 1979; Prasad et al. 1978). Turnlund et al. (1988) found that diets with zinc intake slightly above the RDA did not interfere with copper absorption nor increase fecal copper loss. While absorption significantly varied between study groups (48.1% of radiolabeled copper was absorbed when the diet contained 1.3 mg Cu/day and 16.5 mg Zn/day; 37.2–38.5% of radiolabeled copper was absorbed when the diet contained 1.3 mg Cu/day and 5.5 mg Zn/day), both groups had positive copper balance at both levels. A decrease in copper absorption was observed in infants with high intakes of iron (Haschke et al. 1986). Conversely, iron supplements in healthy breastfed infants at 6–9 months of age had no effect on copper absorption (Domellof et al. 2009). Similarly, in adults with an ileostomy, oral iron therapy given as ferrous gluconate did not appear to impair copper absorption even with increasing doses (Troost et al. 2003).

In rats, the absorption of copper appears to be inversely related to the amount of cadmium in the diet (Davies and Campbell 1977). A significant decrease in copper absorption was observed when the copper:cadmium ratio was 1:4. The amount of copper retained in the intestinal mucosal cells was inversely related to cadmium dietary concentration. Conflicting results are reported on the effect of ascorbic acid on copper absorption in humans. Based on a decrease in serum ceruloplasmin levels, Finley and Cerklewski (1983) concluded that a diet high in ascorbic acid resulted in a decrease in copper bioavailability. However, in a study by Jacob et al. (1987), copper absorption was not affected by a high ascorbic acid intake. A decrease in serum ceruloplasmin activity was identified; however, the amount of ceruloplasmin protein was not affected.

The available *in vivo* data do not provide information on the rate and extent of absorption through intact skin following dermal exposure of humans or animals to copper. Following a copper azide explosion that yielded metallic copper and nitrogen fumes, a small increase in serum copper levels was found in the affected worker (Bentur et al. 1988). Animal studies demonstrate that copper can pass through dermal barriers when applied with an appropriate vehicle, (e.g., salicylic acid or phenylbutazone) (Beveridge et

al. 1984; Walker et al. 1977). *Ex vivo* studies on human skin reported mixed results. Less than 6% of copper deposited on *ex vivo* human skin samples was absorbed (Pirot et al. 1996a, 1996b); copper chloride was absorbed to a higher extent than copper sulfate (Pirot et al. 1996b). Copper applied transdermally as a tripeptide on *ex vivo* human skin samples permeated the skin and was retained in the stratum corneum, total epidermis, and dermatomed skin (Hostynek et al. 2010). Retention was significant compared to baseline.

3.1.2 Distribution

One study examining respiratory toxicity in rats measured significantly elevated copper levels in the liver and plasma suggesting distribution into these organs (Romeu-Moreno et al. 1994). Nonsignificant increases in copper were measured in kidneys and lung following daily 1-hour inhalation chamber exposure to aerosol copper sulfate for up to 10 days. These results are consistent with more detailed findings of distribution following oral absorption of copper, which is largely similar between humans and animals.

Copper distribution in the body is considered biphasic where ATP7B, predominantly expressed in hepatocytes, is essential for normal distribution of copper. ATP7B has two primary functions: the transfer of copper to a ceruloplasmin that is secreted into the blood and then other organs, and excretion of copper from the body through bile (Guttmann et al. 2018). The first phase is the absorption of copper by enterocytes in the gut and subsequent absorption and distribution by active transport by way of the portal vein (van den Berghe and Klomp 2009). Subsequently, copper levels in the blood rapidly rise as the copper ions bind tightly to albumin and the transcuprin macroglobulin in blood plasma (Moriya et al. 2008). Albumin carries a large portion of the exchangeable copper in peripheral circulation, releasing it to other carriers for cell-specific uptake (Bost et al. 2016; Weiss and Linder 1985). Although passive cellular transport occurs with other metal ions, the absence of copper absorption in Menkes' disease patients and in mice lacking the copper uptake protein, hCTR1, suggest that under normal conditions passive paracellular transport likely does not occur for copper (van den Berghe and Klomp 2009). Prior to phase 1, some copper passes from the small intestines to the large intestines with indigested dietary materials and is then excreted with the feces. A study evaluating plasma kinetics in rats following a single gavage dose of 79.5 mg Cu/kg in rats (given as copper gluconate in water), reported plasma half-life ($t_{1/2}$) and area under the plasma concentration-time curve (AUC) values of 1.79 hours and 2.48 μg/mL*hours, respectively (García-Martínez et al. 2021).

Some dietary copper is transported to the liver where it is bound to ceruloplasmin (a copper-binding serum ferroxide) and released to circulation for distribution. This is the second phase for post-ingestion copper distribution (van den Berghe and Klomp 2009). The maximum concentration of copper in the liver was reached 12 hours after administration of a single gavage dose in rats (García-Martínez et al. 2021). In the liver, hepatocytes are responsible for the uptake, storage, and regulation of copper; about 75% of copper from the portal vein is taken up by the liver and the rest remains in circulation (Harvey et al. 2005). In an *in vitro* experiment using human hepatic (HepG2) and mammary epithelial (PMC42) cells lines, copper was shown to be transported to Ctr1 in hepatic cells by the plasma protein, α_2-macroglobulin (Moriya et al. 2008). Ceruloplasmin, which tightly binds six or seven copper atoms (Musci et al. 1993; Saenko et al. 1994), is the most abundant copper protein in the plasma, binding 60–95% of serum copper (Harvey et al. 2005; Scott and Turnlund 1994). The remaining 10–18% is bound to albumin or carried as amino-acid bound copper and transported into other tissues (Harris 1993; Hellman and Gitlin 2002; Kodama et al. 2012; van den Berghe and Klomp 2009). Copper can also bind to α_2-macroglobulin and small peptides. Regulatory copper proteins ATP7A and ATP7B are responsible for the transport of copper out of cells (reviewed by Taylor et al. 2020). Excessive hepatic copper is transferred from the liver with bile pigments via ATP7B and ultimately excreted with the feces. The brain is the second major site of copper distribution, and copper is also transported to the kidneys, muscle, and connective tissues (Kodama et al. 2012).

Copper crosses the placental barrier and is primarily found in fetal liver in mammals, as part of normal fetal development (Hardman et al. 2007). The fetus obtains copper from maternal serum, either from copper bound to ceruloplasmin, albumin, or anionic amino acids (McArdle 1995). Although copper is found in human breastmilk, it is unclear if it is dependent on maternal plasma copper concentrations (Domellof et al. 2004; Khaghani et al. 2010; Kim et al. 2012). Pre-term infants appear to have lower copper stores than full-term infants (Kim et al. 2012). Intraperitoneal and intravenous exposure to ^{67}Copper or ^{64}Copper in nonpregnant and lactating rats showed that approximately 60% of copper in the lactating rats went directly to the mammary gland (Donley et al. 2002). Copper isotopes also rapidly appeared in milk. The ceruloplasmin in milk is attributed to copper in circulation that reaches the mammary gland. García-Martínez et al. (2021) provided evidence that copper may also cross the blood-brain barrier. Increased copper concentrations were detected in the striatum of rats, with maximum levels measured at 0.25 hours after a single gavage dose of 79.5 mg Cu/kg. Copper concentrations in the midbrain were not altered by oral copper treatment (García-Martínez et al. 2021).

No studies were located regarding the rate and extent of distribution of copper following dermal exposure of humans or animals to copper.

3.1.3 Metabolism

Copper metabolism is largely regulated by copper-transporting P-type ATPases ATP7A (also known as Menkes' protein) and ATP7B. Several specific other binding proteins for copper have been identified that are important in the uptake, storage, and release of copper from tissues, most notably ceruloplasmin, which is synthesized in the liver (van den Berghe and Klomp 2009).

In the liver and other tissues, copper is stored bound to metallothionein and amino acids and in association with copper-dependent enzymes. Metallothionein, a metal-binding protein, appears to play an important role in the storage of intracellular copper in a safe compartment and cell survival from both normal and excess copper levels (Tapia et al. 2004). Studies have shown that copper exposure induces metallothionein synthesis which is important for copper homeostasis (Mercer et al. 1981; Wake and Mercer 1985).

STEAP4, a six-transmembrane epithelial antigen of prostate 4, acts as a metalloreductase and is involved in the reduction of Cu(II) to Cu(I), which is necessary for copper transport across the membrane (Scarl et al. 2017). This reduction reaction occurs at the apical membrane of intestinal epithelial cells (Ohgami et al. 2006).

3.1.4 Excretion

No studies were located regarding the rate and extent of excretion of copper following inhalation exposure of humans and animals. The half-time of copper sulfate in the lungs was estimated to be 7.5 hours after intratracheal instillation of 20 μg copper in rats (Hirano et al. 1990).

Bile is the major pathway for the excretion of copper, and primarily excreted in feces. Normally, approximately 2.5 mg Cu/day is excreted in bile (van den Berghe and Klomp 2009). Excessive copper in hepatocytes is excreted into bile from the liver via ATP7B; the reabsorption of biliary copper is negligible as copper binds to components that immobilize it (Farrer and Mistilis 1967; van den Berghe and Klomp 2009). Copper in bile is associated with low molecular weight copper binding components as well as macromolecular binding species (Gollan and Deller 1973). After the oral administration of radioactive

copper as copper acetate in healthy humans, 72% was excreted in the feces (Bush et al. 1955). In six adult men fed ^{63}Cu, 27–46% was excreted in the feces (Turnlund et al. 2005). In humans intravenously administered ^{64}Cu, measurements in feces and urine were negligible (Kjaergaard et al. 2020). In a study in 11 adult men, dietary copper intakes of 0.66, 0.38, and 2.49 mg Cu/day resulted in fecal elimination of 0.65, 0.33, and 2.17 mg Cu/day (Turnlund et al. 1998). A study in rats found an increase in fecal excretion of copper in rats fed a high fiber (potato fiber or sugar beet pulp) diet, likely as a result of reduced copper absorption (Gralak et al. 1996). Bile is also the major excretion pathway in children (Olivares et al. 2002).

Copper excretion in urine is comparatively low relative to fecal excretion, and normal excretion is expected to be 0.01–0.025 mg Cu/day (Bost et al. 2016). In six adult men fed a diet with ^{63}Cu, 1.3–2.1% was excreted in the urine (Turnlund et al. 2005). One study in humans reported that urinary copper excretion in adult females (mean: 18.7 µg/24 hours) was lower than in adult males (mean: 26.2 µg/24 hours) (Vieira et al. 2012).

The half-life of copper in several tissues was calculated by Levenson and Janghorbani (1994). The study sought to understand the processes by which copper was excreted from several tissues. By restricting copper in the diet of rats, the study authors were able to model the competing processes by which the body tends to excrete copper, while concurrently attempting to retain copper for use in other metabolic processes. These were represented as components, where the first component had a relatively rapid half-life generally unaffected by copper dietary restrictions while the second component half-life was increased substantially by a copper restricted diet. The individual half-life component balance for each organ could not be calculated; however, they could be calculated for some organs. The half-lives for each tissue are presented as the component 1 then component 2 half-lives. The respective calculated copper half-lives were 3.9 and 21 days for the liver, 5.4 and 35 days for the kidney, and 23 and 662 days for the heart; copper turnover in the brain appeared to be monophasic, with a half-life of 457 days.

No studies were located regarding the rate and extent of excretion of copper following dermal exposure of humans or animals to copper.

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).

Human PBPK models have been developed for predicting plasma copper levels following intravenous or oral doses of copper (Harvey et al. 2005; Scott and Turnlund 1994).

Harvey et al. 2005 Model

Model Description. Harvey et al. (2005) developed a model for simulating kinetics of copper in humans. The model consists of eight compartments representing plasma (two compartments), liver (two compartments), other tissues, gastrointestinal tract (two compartments), and feces. The two plasma compartments represent copper transferred from the gastrointestinal tract and copper bound to ceruloplasmin transferred from the liver. In the liver, one compartment exchanges copper with the gastrointestinal tract and one transfers copper ceruloplasmin to plasma. The gastrointestinal tract is divided into two compartments, one that delivers copper to plasma, exchanges copper with liver, and receives copper from other tissues; and one that transfers copper to the lower gastrointestinal tract for excretion in feces. Transfers of copper between compartments are simulated as first order and are governed by rate coefficients (day^{-1}), with delay terms applied to transfer of copper ceruloplasmin from liver to plasma and transfer of copper from gastrointestinal tract to feces.

Model Calibration and Evaluation. Parameters consisted of compartment copper masses, inter-compartment rate coefficients and delay terms, and the volume of distribution of the plasma compartment (for comparing observed and measured concentrations). The volume of distribution was assigned a value of 5,000 mL and the transfer rate from the gastrointestinal tract to the compartment destined to deliver

copper to feces was set to 10 day^{-1}. All other parameters were estimated by fitting the model to data collected in a human clinical study (Harvey et al. 2005). In this study, five adult males received an intravenous dose of ^{65}Cu-labelled copper chloride (0.5 mg), and fecal and urine samples were collected over a period or 14 days. Four weeks later, the same individuals received an oral dose of ^{65}Cu-labelled copper chloride (3 mg) after an overnight fast, and plasma, fecal, and urine samples were collected over a period of 14 days. Data from both studies were used to calibrate model parameters. These included plasma and fecal copper (^{65}Cu) following the oral dose and fecal copper following the intravenous dose. The model predicted concentrations of copper in plasma and feces that were within one standard deviation of observations following the oral or intravenous dose (see Figures 2 and 3 of Harvey et al. 2005). The model predicted that approximately 74% of copper absorbed from the gastrointestinal tract is transferred to the liver (first-pass extraction). Of this, 80% is delivered to plasma as copper ceruloplasmin and 20% is secreted back into the gastrointestinal tract (e.g., biliary transfer). Nearly all plasma copper (99%) was predicted to be copper bound to ceruloplasmin. The model was not evaluated with data not used to calibrate parameters.

Scott and Turnlund 1994 Model

Model Description. Scott and Turnlund (1994) developed a model for simulating kinetics of copper in humans. The model consists of seven compartments representing plasma (two compartments), liver (two compartments), other tissues, feces, and urine. The two plasma compartments represent ceruloplasmin and non-ceruloplasmin (other forms of copper). Plasma copper exchanges with copper in liver and other tissues and delivers copper to urine. The two liver compartments represent: (1) non-ceruloplasmin copper received from plasma and transferred to feces and (2) copper ceruloplasmin, which is transferred to plasma. Transfers of copper between compartments are simulated as first order and are governed by rate coefficients (day^{-1}), with delay terms applied to transfer of copper from other tissues to plasma and from liver to feces.

Model Calibration and Evaluation. Parameters consisted of compartment masses, inter-compartment rate coefficients and delay terms and the plasma volume (for comparing observed and measured concentrations). The plasma volume was based on average body-weight-standardized blood volume and hematocrit for humans. All other parameters were estimated by fitting the model to data collected in a human clinical study (Scott and Turnlund 1994). In this study, five adult males were placed on three copper diets that were adequate (1.68 mg/day), low (0.785 mg/day) or high (7.53 mg day), using copper sulfate to supplement the high-copper diet. During each dietary period, the subjects received an

intravenous dose (392 μg) and oral doses of ^{65}Cu-labelled copper chloride (1.02–7.66 mg). Plasma samples were collected at various times during each dietary period. Plasma data (^{65}C) from all dietary periods were used to calibrate model parameters to each subject. The model predicted observed spikes in plasma concentration following intravenous and oral dosing and the concentrations between doses (see Figures 2 and 3 of Scott and Turnlund 1994). The model predicted that 4.1% of the total copper burden was in plasma and that 65% of plasma copper was bound to ceruloplasmin, which was within the range of observations (56–68%). Two rate coefficients were affected by dietary copper levels. The rate of transfer of copper from liver to plasma ceruloplasmin was lower during the low copper period compared to the adequate and high copper periods. The rate of transfer from plasma ceruloplasmin to other tissues increased with increasing dietary copper. The model was not evaluated with data not used to calibrate parameters.

3.1.6 Animal-to-Human Extrapolations

NTP (1993) demonstrated that mice appeared less sensitive than rats to the hepatotoxicity of copper based on the observation that no hepatic effects occurred in mice given doses much higher than rats, which showed liver damage at much lower doses. The cause of this apparent difference in toxicity between the species has not been examined.

The dietary requirements for copper in rats and mice are 5 and 6 mg Cu/kg diet, respectively (corresponding to a dose of ~0.5 mg Cu/kg body weight/day in rats and ~1 mg Cu/kg-body weight/day in mice), (NRC 1995). It is unlikely that humans would tolerate prolonged exposure to a copper dose that is about 40 times higher than the dietary requirement (0.9 mg Cu/day, corresponding to ~0.013 mg Cu/kg body weight/day for a 70-kg human). Thus, the applicability of these animal data to humans is not known.

The Long-Evans Cinnamon rat is often used as a model for Wilson's disease. This rat strain shares many characteristics associated with Wilson's disease: accumulation of copper in the liver, decreased serum copper and ceruloplasmin levels, and impaired biliary excretion of copper (Sugawara et al. 1991, 1992, 1994; Suzuki et al. 1995).

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 copper are discussed in Section 5.7, Populations with Potentially High Exposures.

Wilson's disease, an autosomal recessive disorder that causes liver dysfunction, typically has a childhood onset. Affected individuals can develop toxic tissue accumulations of copper, even with low levels of dietary exposure (reviewed by Taylor et al. 2020). They require lifelong medical treatment combined with a low-copper diet. Without medical treatment, Wilson's disease is fatal, usually early in life.

Another copper-related genetic disorder, ICT, is largely believed to be caused by an autosomal recessive genetic susceptibility causing excess copper accumulation and subsequent liver damage; however, it is unclear whether exposure to excess copper plays a role in disease manifestation or if it merely exacerbates symptoms (Müller et al. 1998; Nayak and Chitale 2013). Another disorder, ICC, is characterized by severe liver damage in infants and children (<5 years of age). It is suspected to be caused by a genetic predisposition due to its random occurrence in siblings and higher liver disease mortality in second-line family members (Nayak and Chitale 2013; Pandit and Bhave 1996). However, data are inconclusive on whether ICC is caused by external exposure to copper, such as the consumption of milk stored in copper or brass vessels, or endogenously through copper dysregulation in the body (Nayak and Chitale 2013; Tanner 1998). ICC was previously considered endemic to India, but it has been documented in children of non-Indian origin in other countries (Nayak and Chitale 2013). ICT and ICC lead to a loss of copper homeostasis in diagnosed children and may make them more susceptible to excess copper accumulation

especially early in life. In early stages of postnatal development, the mechanisms of copper intestinal absorption and excretion through bile are not fully developed causing children to be susceptible to even small excesses of copper in water (Puchkova et al. 2018).

Gastrointestinal upset, the most commonly reported adverse health effect in adults, has also been reported in infants and children. It is manifested as nausea, vomiting, abdominal pain, and/or diarrhea. Symptoms usually occur shortly after ingesting a copper-contaminated beverage or drinking water containing a high level of copper. In most of the reports of gastrointestinal upset in children, no reliable information on copper concentration or dose was reported (Gill and Bhagat 1999; Karlsson and Noren 1965; Knobeloch et al. 1994; Spitalny et al. 1984; Walsh et al. 1977). In one report where school-age children ingested a beverage stored in an old urn, the concentration of copper in the beverage was estimated to be 300 mg/L (Gill and Bhagat 1999). Another study reported vomiting in infants ingesting a single dose of 7.5 mg/L copper sulfate (Karlsson and Noren 1965). Knobeloch et al. (1994) noted that children appear to be more sensitive to the gastrointestinal effects of copper than adults. This statement was based on two surveys of residents with elevated copper levels in the drinking water. In the first survey, it appears that children who were categorized as having gastrointestinal upsets, were described as “unusually irritable” or had recurrent headaches. In a second survey, mothers were asked to recall the frequency of gastrointestinal effects for all family members (Knobeloch et al. 1994). A significantly higher percentage of children, as compared to adults, were reported to have gastrointestinal effects. Recall bias can be affected by self-reporting or adult reporting of symptoms in children in the household. The available data are inadequate to assess accurately whether there is an age-related difference in the gastrointestinal toxicity of copper.

Copper accumulation in fetal tissues primarily occurs in the second half of pregnancy (Chernenkov et al. 2018). Approximately half of the copper in the fetus is stored in the liver, mostly bound to metallothionein. During that phase of a pregnancy, the rate of transfer of copper from the liver to the bile or blood is decreased due to the immaturity of the fetal liver. The magnitude of the amount of copper in the fetal liver is similar to levels observed in Wilson’s disease; however, the fetal and neonatal liver can tolerate these high concentrations (Olivares et al. 2000). Copper levels are imbalanced in early stages of postnatal development for all infants, as the mechanisms for excreting copper through bile and controlling copper absorption in the small intestine have not developed fully (Puchkova et al. 2018). After birth, copper levels decrease to normal levels in infants lacking a genetic defect (Chernenkov et al. 2018; Olivares et al. 2000).

Copper, likely bound to albumin, is found in human breastmilk and is necessary for infant development.

Copper was measured in breastmilk at concentrations of 0.12–0.69 mg/L (Choi et al. 2016; Domellof et al. 2004; Khaghani et al. 2010; Yalcin et al. 2015). Maternal dietary copper intake is not likely to affect copper concentrations in breastmilk (Choi et al. 2016); thus, excess dietary maternal copper intake may not impact infant copper intake from breastmilk. A study in lactating rats suggested that transport of copper to the mammary gland is about 60% following intraperitoneal or intravenous injection of ionic copper (Donley et al. 2002). Subsequently, the labeled isotopes rapidly appeared in milk and milk ceruloplasmin. These results were not found in nonpregnant rats, where transport was primarily to the liver and kidney.

The potential age-related differences in the toxicity of copper have been assessed in rats exposed to 120 mg Cu/kg/day as copper sulfate in the diet for 12 weeks (Fuentealba et al. 2000). The observed liver effects of enzyme activity alterations and hepatitis were more severe in young rats (exposed *in utero*, during lactation, and for 12 weeks post weaning) as compared to the effects observed in adult rats. Copper levels in the liver of young rats, 1,553–1,635 μg/g, were higher than in adult rats, 472–534 μg/g. It is uncertain if these data in rats would be suggestive of sensitivity in human infants and children.

Several studies investigated the potential developmental toxicity of excess dietary copper sulfate and copper hydroxide. Some results suggest that *in utero* exposure to copper can result in delays in growth and development in the offspring of mice (Lecyk 1980). However, some studies testing similar or lower doses in mice and mink observed no developmental effects in offspring (Aulerich et al. 1982; Kadammattil et al. 2018).

Some health conditions may influence sensitivity to the gastrointestinal effects of oral exposure to copper. For example, health conditions that reduce the pH of gastric secretions (e.g., acute *Heliobacter pylori* infection, some neuroendocrine tumors or gastrinomas, rebound acid hypersecretion after stopping proton pump inhibitor therapy) may result in higher concentrations of free copper ions in contact with the gastrointestinal tract than those seen in healthy individuals at the same dose. In addition, health conditions that result in damage to the integrity of the gastrointestinal tract (ulcers, acid reflux) may also increase a person's sensitivity to oral copper exposure.

A number of populations were identified as unusually susceptible to copper toxicity due to genetic defects that impair copper homeostatic mechanisms. Wilson's disease, also referred to as hepatolenticular degeneration, is an autosomal recessive disorder with an estimated prevalence of 1 case per 30,000 live

births among most populations (Schilsky 2019). The primary genetic defect in Wilson's disease is in the ATP7B gene that encodes a P-type ATPase (Wilson protein), which delivers copper to ceruloplasmin. The genetic defect results in impaired biliary excretion of copper and an accumulation of copper in the liver. The progression of the disorder begins with an accumulation of copper in the liver, structural damage to the liver, and subclinical liver cirrhosis (Rodriguez-Castro et al. 2015). Over time, the individual will develop hepatic, neurological, and psychiatric symptoms. The hepatic effects are characterized by jaundice, hypoalbuminemia, ascites, coagulation defects, hyperammonemia, hepatic encephalopathy, and/or liver failure. In the cases with massive liver failure, large amounts of copper are released from the liver, impacting red blood cells and leading to hemolytic anemia. Neurological symptoms include tremors, other movement disorders, and speech abnormalities. Psychiatric and behavioral symptoms are often found in individuals who also manifest neurological symptoms. The psychiatric symptoms include reduced performance in school or work, inability to cope, depression, very labile moods ranging from mania to depression, sexual exhibitionism, and frank psychosis. Individuals with Wilson's disease have low serum ceruloplasmin levels, elevated urinary copper levels, and elevated liver copper levels. Kayser-Fleischer rings, which result from corneal copper deposits, are also present in some individuals with Wilson's disease. Individuals who are heterozygotes for Wilson's disease may also be more susceptible to the toxicity of copper. Increases in urinary copper and hepatic concentrations and decreased copper incorporation into ceruloplasmin have been observed in heterozygotes. These findings suggest that long-term exposure to elevated levels of copper could result in copper overload.

Individuals with a common deficiency of the enzyme glucose-6-phosphate dehydrogenase (G6PD) could be more susceptible to the toxic effects of oxidative stressors such as copper (Calabrese and Moore 1979; Chugh and Sakhuja 1979; Sansinanea et al. 1996). Red blood cell models were used to analyze the effects of copper chloride on oxidative markers while measuring G6PD activity (Swastika et al. 2020). There was a negative correlation between G6PD activity and copper chloride dose. In the blood, most of the copper is bound to ceruloplasmin. With the exception of ingestion of a very large dose of a copper salt, the levels of non-ceruloplasmin-bound copper remain low. Thus, it is unlikely that this relatively small change in free copper would alter the survival of glucose-6-phosphate dehydrogenase deficient red blood cells.

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 copper 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 copper 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 copper 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

Copper levels can be readily measured in tissues, body fluids, and excreta. Depending on the dose and exposure duration, inhalation and/or oral exposure to copper can result in increased levels of copper in serum, urine, hair, and nails.

The normal serum copper level in human adults is 10–25 μmol/L (64–160 μg/dL) (IOM 2006). Serum copper levels can be used to evaluate copper toxicity, deficiency, or the possibility of copper metabolism disorders. Increased serum copper levels (>25 μmol/L) were reported in several human case studies following intentional ingestion of copper compounds, such as copper sulfate biocides (Chuttani et al. 1965; Franchitto et al. 2018; Yang et al. 2004). Serum copper levels reported in these studies ranged from 37 to 140 μmol/L. Elevated plasma copper was also measured in a 23-month-old child who had accidentally ingested an unknown amount of a disinfectant agent containing an unknown concentration of copper sulfate (Mortazavi and Jafari-Javid 2009). Fifteen days after admission, the patient's plasma copper level was 216 μg/dL (33.9 μmol/L) (normal range in 6-month-old to 6-year-old children: 14–30 μg/dL). Whole-blood copper levels measured in humans following intentional ingestion of copper sulfate ranged from 60.3 to 107.6 μmol/L, while in non-exposed individuals, the whole-blood copper was 34.1 μmol/L (Chuttani et al. 1965). Following chronic-duration inhalation exposure to 111–464 mg Cu/m^3 copper in dust, serum copper levels >31.8 μmol/L were observed in 16% of exposed factory workers (Suciu et al. 1981). However, increased serum copper levels may only be reflective of recent exposure. Chuttani et al. (1965) observed that serum ionic copper rapidly diminished within a few days to normal levels following ingestion of an acute bolus dose. Mortazavi and Jafari-Javid (2009) observed that in a 23-month-old child, copper levels took about 2 months to fall to within normal range, even after treatment with a chelating agent. A relationship between blood copper levels and the severity of symptoms has not been established. Among individuals intentionally ingesting a single dose of copper sulfate (1–30 g), there did not appear to be a correlation between serum copper levels and symptom severity (Chuttani et al. 1965). In contrast, whole-blood copper levels did have a significant relationship with the severity of symptoms.

Serum ceruloplasmin, a copper-related carrier protein, is a biomarker for copper exposure. Based on a significant correlation of serum copper with serum ceruloplasmin levels, it has been suggested that serum ceruloplasmin is a reliable biomarker for chronic-duration occupational exposure to copper (Saha et al. 2008). A human dietary study by Turnlund et al. (2004) reported that a high copper intake resulted in an increase of ceruloplasmin in subjects given supplements, when compared to controls. Nine men had been

exposed to 0.02 and 0.1 mg Cu/kg/day during separate 18-day period in a metabolic research unit (Turnlund et al. 2004). A metabolism study in rats observed increases in ceruloplasmin with copper exposure, as over 90% of the copper dose was found primarily in ceruloplasmin as opposed to other serum proteins (Weiss and Linder 1985).

Similar to serum, copper can be measured in urine, but this is primarily used to test for diseases affecting copper homeostasis and the liver. In one patient who intentionally ingested a copper sulfate containing fungicide, the urine copper level 3 days after admission was 112 µg/dL and decreased to 16 µg/dL in follow-up 11 days after admission (Yang et al. 2004).

Copper levels in hair and nails can also be used to assess copper exposure; however, the reliability of these biomarkers has not been established. In a study of preschool children, the levels of copper in hair and toenail samples were log-normally distributed (Wilhelm et al. 1991). The geometric mean concentrations of copper in hair and toenails were 10.6 µg/g (range of 5.4–20.7 µg/g) and 7.5 µg/g (range of 3.0–18.6 µg/g), respectively. A study by Hopps (1977) calculated that for a hair growth rate of 10 mm per month, the copper levels in the first 2 cm proximal to the scalp would represent copper intake over 2 months. In an occupational study of workers exposed to unspecified levels of copper from fossil fuel combustion, oil distribution workers had a mean hair copper level of 69.6 µg/g, which was significantly higher than in controls (defined in the study as non-exposed "healthy individuals living far from hazardous exposure with age and weight matching the test group") who had a mean hair copper level of 36.8 µg/g (Jaccob 2020). The study author suggested that hair levels may be a useful biomarker for copper and heavy metal exposure. Increased hair copper levels have been reported in workers exposed to 0.64–1.05 mg Cu/m^3 of an unspecified copper compound; the concentration of copper in their hair was 705.7 µg Cu/g, as compared to a concentration of 8.9 µg Cu/g in non-exposed workers (Finelli et al. 1981).

Based on a toenail growth rate of 1 mm/month, toenail samples would represent copper intakes over 12–18 months (Fleckman 1985). Increased hair and fingernail copper levels were observed in children with ICC (Sharda and Bhandari 1984). An epidemiological study found that mean toenail copper concentrations were significantly higher among residents who lived in copper-mining towns than those who did not (Ndilila et al. 2014). Among adults in the copper-mining town, the mean copper concentration in toenails was 132 mg/kg. The study authors suggested that copper levels in toenails may be an indicator of exposure.

3.3.2 Biomarkers of Effect

No copper-specific biomarkers of effects resulting from copper toxicity have yet been identified.

3.4 INTERACTIONS WITH OTHER CHEMICALS

Numerous studies demonstrate the interaction between copper and metals such as cadmium, iron, and tin. Dietary zinc strongly affects copper absorption, and a diet high in zinc can result in copper deficiency by upregulating metallothionein, which binds to copper in enterocytes and decreases its absorption into plasma (Igic et al. 2002; Myint et al. 2018). Uptake of copper from the small intestine is susceptible to competition from other transition metals including zinc. Increased dietary zinc results in induction of metallothionein synthesis in the intestine. Since metallothionein has a greater binding capacity for copper than for zinc, dietary copper is sequestered in the intestinal mucosal cell metallothionein and is eventually excreted in the feces when the mucosal cell is sloughed off (Hall et al. 1979; Whanger and Weswig 1971). Because exposure to excess dietary zinc results in both decreased copper absorption and decreased serum levels, it is considered an effective therapy for Wilson's disease (Ranucci et al. 2014).

Animal studies demonstrate that ingestion of copper and zinc ions simultaneously results in reduction of systemic copper toxicity because it decreases systemic uptake (Kheirandish et al. 2014). Mice given both zinc sulfate and copper sulfate had less histological damage in the testis compared to mice give copper sulfate only (Kheirandish et al. 2014). Similar results were observed in rats, as improvements in sperm counts, viability, and motility were observed in rats given copper sulfate and zinc sulfate, while no such recovery was seen over the same time period of rats only given copper sulfate (Babaei and Abshenas 2013).

A study in rats found that exposure to sodium arsenate resulted in increased copper concentration in the kidney (Cui and Okayasu 2008). Rats were orally exposed to varying doses of sodium arsenate daily for 4 and 16 weeks. Exposure to manganese in rats also increased copper uptake as demonstrated when a 7-day exposure to manganese in diet, water, or gavage resulted in increased copper levels in the liver (Mercadante et al. 2016). Exposure to manganese by diet and gavage resulted in decreased copper levels in bile; both effects suggest a relationship between manganese and copper hepatobiliary excretion. Several other divalent cations compete with copper for intestinal absorption. Exposure to dietary cadmium (Evans et al. 1970), iron (Ha et al. 2016), and stannous tin (Pekelharing et al. 1994; Wapnir et al. 1993) can result in decreased copper absorption. In the case of cadmium, the copper ion decrease is

related to cadmium's induction of metallothionein synthesis and the binding of copper to it. Tetrathiomolybdate is used for the treatment of Wilson's disease (Brewer et al. 2006); thus, excessive dietary molybdenum can also result in decreased copper uptakes and, therefore, alterations in copper utilization and toxicity. Two mechanisms of action of tetrathiomolybdate have been proposed: (1) it reacts with copper-metallothionein to form a soluble complex that is excreted (Ogra et al. 1996), and (2) it can complex with non-ceruloplasmin-bound plasma copper, impeding its cellular absorption (Brewer et al. 2006). Interactions with copper sulfate may differ, as molybdenum may lower the activity of sulfide oxidase, resulting in the accumulation of copper sulfide (Vyskocil and Viau 1999).

Vitamin C, also known as ascorbic acid, interferes with intestinal copper absorption resulting in reduced copper concentration in various tissue (Van Den Berg and Beynen 1992). This suggests that a diet high in vitamin C can result in copper deficiency.

Several other natural substances have been tested in animals, and studies suggest that they may protect against copper toxicity. In mice, copper-induced toxicity changes in the liver, kidneys, and stomach were less pronounced in mice treated with copper sulfate and coriander, or copper sulfate, coriander, and zinc, compared to mice treated only with copper sulfate (Hashimyousif et al. 2019). Curcumin, the main active ingredient in turmeric and a natural anti-inflammatory agent, appeared to alleviate the hepatic and renal toxicity of copper sulfate (Hashish and Elgaml 2016). This was based on a comparison of hepatic enzyme levels, and liver and kidney antioxidant levels, between rats orally exposed to copper sulfate only and rats exposed to copper sulfate and curcumin at the same time or in succession. Resveratrol, an antioxidative compound, was observed to possibly attenuate copper sulfate-induced liver injury by decreasing oxidative stress and the concentrations of liver transaminases (Tian et al. 2019). An *in vivo* genotoxicity study using mouse blood cells reported that orange juice appeared to have a modulating effect on the action of metallic sulfate salts and was both restorative and protective of the copper-induced genotoxic effects (Franke et al. 2006). The study authors hypothesized that the genotoxic effects could be mediated by the interaction of unspecified orange juice components or that the juice's antioxidant byproducts can interact with transition metals.