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

3.1 TOXICOKINETICS

- Absorption:
  - Available data from human and animal studies indicate that carbon disulfide is extensively and rapidly absorbed via inhalation, oral, and dermal routes.
  - Inhalation studies indicate that a minimum of 80% of the inhaled dose in humans is absorbed. Similar results were observed in laboratory animals, with absorption of approximately 70–80% of the administered dose.
  - No information on the oral absorption of carbon disulfide in humans was identified. In rats, at least 63% of an intragastric dose was absorbed, based on measurements of carbon disulfide in exhaled air.
  - Dermal absorption of carbon disulfide occurs in animals and humans; however, accurate quantitative estimates have not been reported.

- Distribution:
  - Absorbed carbon disulfide is distributed throughout the body. Because of its lipophilic nature, its distribution is greatest in organs, such as the brain and liver.
  - Carbon disulfide is also distributed to the developing fetus and into breast milk.

- Metabolism:
  - Carbon disulfide is metabolized by cytochrome P-450 to an unstable oxygen intermediate that either spontaneously degrades to atomic sulfur and carbonyl sulfide or hydrolyzes to form atomic sulfur and monothiocarbonate. Carbonyl sulfide is converted to monothiocarbonate, which degrades to generate carbonyl sulfide or forms carbon dioxide and hydrogen sulfide.
  - Conjugation of carbon disulfide or carbonyl sulfide with endogenous glutathione results in formation of TTCA and 2-oxythiazolidine-4-carboxylic acid, respectively.
  - Species differences exist in the metabolism of carbon disulfide. Oxidation of sulfur to inorganic sulfate occurs in animals but is not a significant metabolic pathway in humans. However, this observation is based on limited data.

- Excretion:
  - Renal excretion is the primary route of excretion of carbon disulfide metabolites.
  - Unmetabolized carbon disulfide is exhaled in air, with small amounts (<1%) excreted in the urine.

- Toxicokinetics models:
  - No pharmacokinetic models for carbon disulfide were identified.

3.1.1 Absorption

Inhalation Exposure. Studies conducted on human subjects reported rapid and extensive absorption of inhaled carbon disulfide. Rapid absorption was demonstrated in a study conducted on volunteers exposed

to 17–51 ppm for 1–4 hours (Teisinger and Soucek 1949). The amounts of carbon disulfide retained in the body and excreted by the lungs and kidneys were determined by measuring the carbon disulfide in inspired and expired air, blood, and urine during and after completion of the experiment until it disappeared from the urine and blood. About 80% of the inhaled carbon disulfide was retained during the first 15 minutes of exposure, which decreased to about 40% after 45 minutes and remained at that level for the rest of the exposure period. Systemic absorption of at least 80% of the total inhaled dose indicate high bioavailability via the inhalation route. The degree of retention did not depend on the exposure concentration. Only 5% of the retained carbon disulfide at the end of the exposure period was subsequently eliminated in the exhaled air. About 0.06% of the retained carbon disulfide was excreted unchanged in the urine and was detectable 24 hours after exposure. In another retention study involving exposure to vapor for an unspecified period (Soucek 1957), about 10–30% of the retained carbon disulfide was exhaled and <1% was excreted in urine as carbon disulfide. The concentration of inhaled carbon disulfide was not reported. About 70–90% of absorbed carbon disulfide was metabolized.

Studies in animals indicate that carbon disulfide is rapidly absorbed following inhalation exposure. Absorption of carbon disulfide was studied by evaluating pulmonary and urinary excretion of carbon disulfide during and after exposure. Studies in rats show rapid uptake of inhaled carbon disulfide during a 180-minute exposure, with a blood half-time of 6–9 minutes (Moorman et al. 1998). Blood levels reached a plateau after approximately 90 minutes, with blood concentration proportional to exposure level at concentrations of 50–800 ppm. However, peak blood levels were lower in females than males (Moorman et al. 1998). Studies in rabbits indicate that an equilibrium concentration of carbon disulfide is reached after inhalation exposure to 20–150 ppm for 1.5–2.0 hours (Toyama and Kusano 1953). About 70–80% of the inhaled carbon disulfide was absorbed. After termination of exposure, 15–30% of the absorbed carbon disulfide was excreted through the lungs and <0.1% was excreted by the kidneys. In dogs exposed to 25–60 ppm carbon disulfide, equilibrium concentrations in blood were attained after 0.5–2.0 hours (McKee et al. 1943). Desaturation of blood carbon disulfide was almost complete within the first 30–60 minutes after exposure. Approximately 8–13% of the retained carbon disulfide was exhaled, <0.5% was excreted in the urine, and none was excreted in the feces. Excretion in the urine occurred within 2 hours of exposure. Freundt et al. (1975) observed that an equilibrium concentration of carbon disulfide in blood was attained after exposure of rats to 400 ppm carbon disulfide for 1 hour. Equilibrium was reached in liver and blood 1–8 hours after exposure. Elimination of free carbon disulfide from these tissues was rapid, with an estimated half-life in the blood of 35 minutes and in the liver of approximately 1 hour.

The data presented above indicate that carbon disulfide is absorbed by humans and animals following inhalation exposure and reaches equilibrium rapidly (0.5–8 hours) across a wide range of doses and exposure durations.

Oral Exposure. No studies were located regarding absorption of carbon disulfide following oral exposure of humans. In rats, intragastric administration of 10 mg/kg ^{14}C-carbon disulfide resulted in exhalation of 63% of the dose within 4 hours as unchanged carbon disulfide (DeMatteis and Seawright 1973). It is evident from these results that a large fraction of orally administered carbon disulfide is absorbed by rats.

Dermal Exposure. Dermal exposure of humans to aqueous solutions of carbon disulfide resulted in significant absorption through the skin. A series of experiments were performed to investigate the rate of absorption of carbon disulfide by immersion of the hand in aqueous solutions of increasing concentrations (0.33–1.67 g/L) for 1 hour (Dutkiewicz and Baranowska 1967). Absorption was calculated indirectly by determining carbon disulfide elimination by the lung or directly by measuring carbon disulfide concentration in the solutions before and after immersion of the hand. Rates of absorption of carbon disulfide, determined from analysis of the solutions, ranged from 0.232 to 0.789 mg/cm^2/hour and were about 10 times higher than rates calculated from lung excretion of carbon disulfide. In the former case, 25% of the absorbed dose was exhaled in the desaturation period; in the latter, only 3% was eliminated in the expired air. These findings suggest that carbon disulfide excretion varies with the route of absorption. This study provided only brief details of the experimental procedure, and therefore, factors other than absorption through the skin (e.g., evaporation) may have accounted for the reduced carbon disulfide concentration noted at the end of the experimental period. Nevertheless, these results suggest that rapid absorption of carbon disulfide can occur in humans through skin. Occupational exposure of persons with pathological skin conditions has also been noted to increase the dermal absorption of carbon disulfide (Drexler et al. 1995). *In vitro*, the short-term dermal absorption rates for carbon disulfide through cadaver skin, when applied in isopropyl myristate, were 33.8 µg/cm^2/hour for 10 minutes and 4.38 µg/cm^2/hour for 60 minutes, based on the amount of carbon disulfide on the receptor fluid and in the skin (Fasano and McDougal 2008). A skin permeability coefficient of 0.0033 cm/hour was calculated.

The limited information available on skin absorption in animals indicates that carbon disulfide is appreciably absorbed. Exposure of rabbit skin to high concentrations of the vapor (≥800 ppm) for 1 hour resulted in detectable amounts of carbon disulfide in the breath (Cohen et al. 1958). A linear relationship was noted between the dermal exposure concentration and the amount of carbon disulfide exhaled. No

detectable carbon disulfide was found in the breath of rabbits exposed to 150 ppm vapor by skin contact for 6 hours (Cohen et al. 1958).

3.1.2 Distribution

Absorbed carbon disulfide is taken up by the blood (McKee et al. 1943) and is distributed throughout the body (Brieger 1967). Milk from nursing mothers occupationally exposed to carbon disulfide was found to contain an average of 12.3 µg carbon disulfide/100 mL (Cai and Bao 1981). Exposure concentrations of carbon disulfide ranged from 9.3 to 21.1 ppm for a 6.5-hour period. Exposure to 7.4–40 ppm for a shorter duration (2–4 hours) resulted in a lower average milk concentration of 6.8 µg/100 mL.

The distribution of carbon disulfide following inhalation exposure has been studied in rabbits and rats (Toyama and Kusano 1953). In rabbits, blood equilibrium concentrations of carbon disulfide were reached after exposure to 20–150 ppm for 1.5–2.0 hours. In rats exposed to 60–350 ppm carbon disulfide, distribution was primarily to the brain, kidney, and liver. Blood equilibrium concentrations for various carbon disulfide exposures in rats were not reported. Although carbon disulfide was rapidly eliminated from rat tissues during the first 6–8 hours after exposure, low concentrations of carbon disulfide were still detected in the tissues 20 hours after exposure. A separate study reported that equilibrium concentrations of carbon disulfide in blood were attained in dogs after 0.5–2.0 hours of exposure to 25–60 ppm carbon disulfide (McKee et al. 1943). Desaturation was largely complete within the first 30–60 minutes after inhalation exposure. Anesthetized male Sprague-Dawley rats exposed to 640 ppm carbon disulfide had an exponential increase in carbon disulfide in the blood which reached an apparently steady state after 90 minutes of exposure. In all tissues except fat, the carbon disulfide concentration approached steady state within 4–5 hours of exposure. Loss of free carbon disulfide was rapid from all tissues except the liver and kidneys, which retained 25 and 29%, respectively, at 8 hours postexposure (McKenna and DiStefano 1977).

Inhalation exposure of pregnant mice to carbon disulfide during gestation resulted in rapid absorption and distribution of carbon disulfide and its metabolites in embryonic and fetal tissues within 1 hour (Danielsson et al. 1984). Pregnant mice were exposed via inhalation to 25 microcuries (µCi) ^{35}S- or ^{14}C-carbon disulfide for 10 minutes on GD 9, 14, or 17. The levels of ^{35}S-labelled metabolites in the embryonic neuroepithelium were higher in the fetal brain than in the maternal brain during early gestation (GD 9). The concentrations in the fetal brain, eyes, and skeleton exceeded that of other fetal organs during mid-gestation (GD 14). In late gestation (GD 17), the levels in the fetal and maternal brain were

relatively low, but high uptake of radioactivity was seen in the placenta, fetal blood, liver, and eyes. During early gestation, the distribution of ^{14}C-labelled metabolites was similar to that of ^{35}S-labelled metabolites with an immediate higher uptake in the embryo (including neuroepithelium) than in the maternal serum. On GDs 14 and 17, radioactivity was present in the ventricle of the fetal brain. High levels were detected in the fetal liver and blood at late gestation (GD 17). In contrast to ^{35}S-labelled metabolites, ^{14}C-labelled metabolites were retained longer (up to 24 hours) in the fetal brain and liver. High concentrations of ^{14}C-labelled metabolites were also seen in the fetal urinary tract. Thus, the distribution pattern varied with the age of the conceptus and also with the radiolabel of carbon disulfide. These results indicate that carbon disulfide and its metabolites pass through the placenta at all stages of gestation and localize selectively in various tissues of the body.

The distribution of free carbon disulfide and bound carbon disulfide liberated by acid hydrolysis was investigated in the tissues of white rats after a large, single subcutaneous dose (approximately 361 mg/kg) of carbon disulfide (Bartonicek 1957, 1959). Results of these studies indicate that following absorption, free carbon disulfide is rapidly removed from the blood and tissues. Negligible blood levels were present 11 hours after the dose was administered (Bartonicek 1957, 1959). Initially, free carbon disulfide accumulated in the blood, adrenals, and brain, but levels in the organs rapidly decreased, and only very small amounts were present after 10–16 hours.

A similar rapid reduction of free carbon disulfide levels in the blood was noted when radiolabelled ^{35}S-carbon disulfide was administered parenterally to guinea pigs (Strittmatter et al. 1950). About 20–50% of intracardially injected ^{35}S-carbon disulfide was retained; the amount of material retained depended on the concentration of dose administered. The largest amount of radiolabel appeared in the liver (0.42–0.56 µg) and the least amount in the brain (0.03–0.05 µg) at 1.5 hours following injection. Only 10% of the labelled compound remained in the tissues after 48 hours. Urinary and fecal excretion was not reported. In guinea pigs exposed to carbon disulfide vapors (13.6–25.7 ppm), the liver contained the most ^{35}S-label, followed by the blood, then the brain. Forty-eight hours later, 30–50% of ^{35}S-label remained in the tissues such as blood, liver, brain, kidney, and skin. The urinalyses revealed that urinary ^{35}S-label was about 30% of the retained sulfur, with about 85 or 90% of it appearing in the first 24-hour output, the larger part of the metabolized material in the urine being excreted as inorganic sulfate. The feces contained about 5–15% metabolized ^{35}S-label, the amount of which increased with the increasing dose of carbon disulfide.

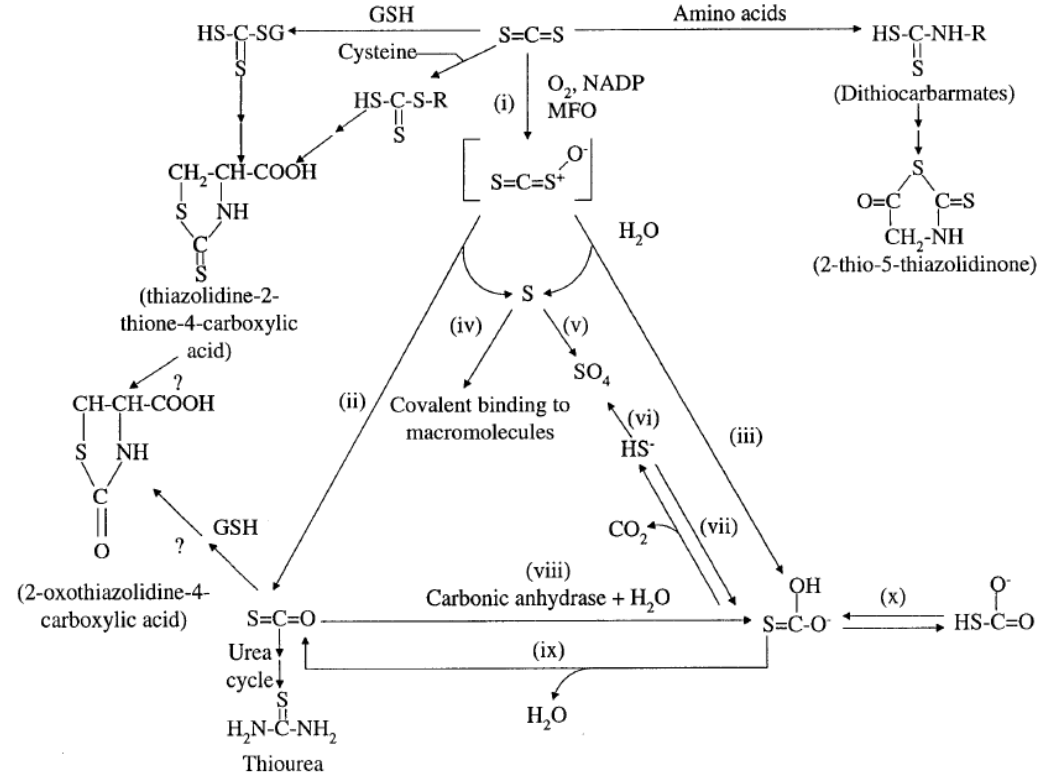
Only metabolites of carbon disulfide were found 3 hours after a dose of ^{14}C- or ^{35}S-labeled carbon disulfide was intraperitoneally administered (Snyderwine and Hunter 1987). Distribution varied with the age of the rat and the radiolabel injected. Following intraperitoneal administration of ^{14}C-carbon disulfide, 4–9% of the dose was metabolized to carbon dioxide depending on age. Significantly more carbon disulfide was metabolized to carbon dioxide by 30- and 40-day-old rats than by 1–20-day-old rats. The biotransformation products of carbon disulfide that were covalently bound remained in tissues from rats of all ages. Twenty-four hours after dosing with ^{35}S-labeled carbon disulfide, up to 13 times more labeled metabolites were covalently bound in organs from 1-day-old rats than in similar organs from 40-day-old rats.

The data presented above indicate that the absorbed carbon disulfide is rapidly distributed via blood to other tissues irrespective of the route of exposure.

3.1.3 Metabolism

Limited information is available on the biotransformation of carbon disulfide in humans, and the metabolic products of carbon disulfide are not completely known. In animals and humans, the proposed metabolic pathways involved in the metabolism of carbon disulfide (Beauchamp et al. 1983) are depicted in Figure 3-1, reactions i–x. Reaction i has been demonstrated in *in vivo* animal studies and in *in vitro* assays. Reactions ii–v were identified by *in vitro* studies, while products of reactions vi–ix are the results of proposed metabolic pathways of carbon disulfide in animals and humans. Carbon disulfide is metabolized by cytochrome P-450 to an unstable oxygen intermediate (reaction i). The intermediate may either spontaneously degrade to atomic sulfur and carbonyl sulfide (reaction ii) or hydrolyze to form atomic sulfur and monothiocarbonate (reaction iii). The atomic sulfur generated in these reactions may either covalently bind to macromolecules (reaction iv) or be oxidized to products such as sulfate (reaction v). The carbonyl sulfide formed in reaction ii may be converted to monothiocarbonate by carbonic anhydrase (reaction viii). Monothiocarbonate may further spontaneously degrade in reaction ix, regenerating carbonyl sulfide or forming carbon dioxide and sulfide bisulfide ion (HS^-) (reaction vii). The HS^- formed in reaction vii can subsequently be oxidized to sulfate or other nonvolatile metabolites (reaction vi).

Figure 3-1. Proposed Metabolic Pathways for Carbon Disulfide



Source: Beauchamp et al. 1983

Dithiocarbamates are the products of the reaction of carbon disulfide with amino acids (Brieger 1967). *In vitro* studies demonstrated that carbon disulfide readily combines with the amino acids in human blood, the half-life of this reaction being approximately 6.5 hours (Soucek 1957). Thiocarbamide has been found in the urine of exposed workers (Pergal et al. 1972b). After inhalation exposure of male subjects, up to 90% of the retained carbon disulfide was metabolized while the remainder was eliminated unchanged by various routes (McKee et al. 1943). High levels of thiocarbamide and trace amounts of 2-thio-5-thiazolidinone were identified by chromatographic analysis of the urine of workers exposed to carbon disulfide by inhalation (Pergal et al. 1972a, 1972b). Van Doorn et al. (1981a, 1981b) reported conjugation of carbon disulfide or carbonyl sulfide with endogenous glutathione to yield TTCA and 2-oxythiazolidine-4-carboxylic acid, respectively. High concentrations (approximately 320 mM) of TTCA were detected in the urine of women exposed to approximately 32 ppm (100 mg/m^3) carbon disulfide through inhalation (refer to Figure 3-1).

The formation of trithiocarbonates has been demonstrated *in vitro* under physiological conditions due to reaction of carbon disulfide with various thiols (Souza et al. 2017). Trithiocarbonates either underwent

slow cyclization to TTCA or decayed to carbon disulfide. The rate of formation of trithiocarbonates was pH-dependent, while decay was pH-independent.

In contrast to the results obtained in animals, oxidation to inorganic sulfate does not appear to contribute significantly to the metabolism of carbon disulfide in humans. A marked increase in inorganic sulfate excretion in the urine was noted in a case study of a young worker with signs of carbon disulfide poisoning because of exposure to high levels of the vapor; no increase was noted in the amount of inorganic sulfate excreted in the urine (Djerassi and Lumbroso 1968). However, exact dose, mode of exposure, and duration were not presented in the study.

Carbon disulfide is oxidized by the liver MFO system to carbonyl sulfide, which then undergoes further desulfurization, releasing elemental sulfur. This reaction has been shown to occur *in vitro* (Dalvi et al. 1974; DeMatteis 1974). *In vivo* studies in rats using ^{14}C-labelled carbon disulfide demonstrated that significant amounts (80%) of $^{14}CO_2$, are exhaled after exposure to carbon disulfide. Following intraperitoneal administration of approximately 100 mg carbon disulfide/kg, about 5% of the total dose was excreted in the breath as carbon dioxide. This amount was increased to 13% in animals pretreated with phenobarbital to induce liver microsomal enzymes (DeMatteis and Seawright 1973). Snyderwine and Hunter (1987) found that 4–9% of an intraperitoneally administered dose of ^{14}C-carbon disulfide was excreted as $^{14}CO_2$ in expired air, with 30- and 40-day-old rats excreting more (9 versus 4%) $^{14}CO_2$, than 1–20-day-old rats. Increased expiration of $^{14}CO_2$ in older rats was attributed to increased hepatic MFO activity at 30–40 days, compared to 1–20 days, resulting in increased metabolism of carbon disulfide to carbon dioxide.

The metabolic formation of carbonyl sulfide from carbon disulfide was confirmed in an *in vivo* study (Dalvi and Neal 1978). After intraperitoneal injection of ^{14}C-carbon disulfide in nonpretreated rats, carbonyl sulfide was excreted by the lung in greater quantities than carbon dioxide. Pretreatment with phenobarbital, however, resulted in a greater amount of excretion of carbon dioxide than carbonyl sulfide. In both experiments, excretion of ^{14}C-carbonyl sulfide and carbon dioxide accounted for 14–43% of the total administered radioactivity, with about twice as much carbon dioxide. These results indicate that phenobarbital treatment caused induction of cytochrome P-450 which catalyzed the conversion of carbon disulfide to carbonyl sulfide faster in pretreated rats than in rats not pretreated with phenobarbital. The role of the cytochrome P-450 monooxygenase system in catalyzing carbonyl sulfide formation was also confirmed by *in vitro* studies (Dalvi et al. 1974, 1975). The rate of carbonyl sulfide formation was NADPH-dependent and increased with microsomes obtained from phenobarbital-treated rats.

In a study designed to examine the effect of P-450 induction on the metabolism of carbon disulfide to TTCA, rats were treated with nothing, ethanol, phenobarbital, 3-methylcholanthrene, or phenobarbital and ethanol before being exposed to carbon disulfide at 50 ppm for 6 hours (Kivistö et al. 1995). After 7 days, the pretreatment regimens were repeated in the same rats, and the rats were again exposed to carbon disulfide at 500 ppm for 6 hours. None of the inducers had any effect on urinary excretion of TTCA. About 7.6 and 2.3% of the dose was excreted as TTCA at 50 and 500 ppm, respectively, suggesting saturation. However, the investigators speculated that saturation may not have occurred because the physical activity level of the rats was reduced at 500 ppm, suggesting that carbon disulfide uptake at 500 ppm may also have been reduced because of the lowered respiratory rate. They also noted that the saturation observed in rats is not likely to occur in humans at the prevailing occupational exposure concentrations. Saturation of TTCA production was observed in an oral study in rats (Kivistö et al. 1995). In rats treated with a single gavage dose of 1, 10, 30, or 100 mg/kg, 4.6, 2.4, 1.7, and 0.8%, respectively, of the dose was excreted in the urine as TTCA. A 13-week study in rats also indicates saturation of carbon disulfide metabolism at high inhalation exposure levels, with plateauing of blood carbon disulfide and urinary TTCA levels at concentrations ≥500 ppm (Moorman et al. 1998).

The effect of P-450 induction or glutathione depletion on carbon disulfide metabolism to TTCA in rats following oral exposure has also been studied (Kivistö et al. 1995). The rats were pretreated with nothing, acetone, phenobarbital, 3-methylcholanthrene, or three inhibitors of glutathione production, namely phorone, diethylmaleate, or buthionine sulfoximine, before being given a single gavage dose of carbon disulfide at 26–34 mg/kg. Phenobarbital decreased the output of TTCA by 21% during the first 12 hours of the urine collection. None of the other P-450 inducers had any effects on TTCA excretion, and the investigators suggested that the effect of phenobarbital may have been a result of cytochrome P-450 aggregation. Buthionine sulfoximine, an inhibitor of glutathione production, reduced the total output of TTCA by 37%. Phorone and diethylmaleate pretreatment, which transiently reduce glutathione, decreased TTCA excretion.

3.1.4 Excretion

Following inhalation exposure, the primary route of excretion of unmetabolized carbon disulfide in humans is exhalation. In one study, it was estimated that 6–10% of the carbon disulfide that was taken up was excreted by the lungs (McKee et al. 1943). In a study conducted on humans, carbon disulfide levels in the exhaled breath decreased rapidly on cessation of exposure (Soucek 1957). The excretion by the

lung accounted for 10–30% of the absorbed carbon disulfide. Less than 1% was excreted unchanged in the urine. The remaining 70–90% of the dose was metabolized. The details regarding carbon disulfide exposure levels were not available. A correlation was established between carbon disulfide exposure of rayon workers and urinary excretion of a metabolite or metabolites that catalyzed the reaction of iodine with sodium azide (Djuric 1967). This test indicated exposures to carbon disulfide above 16 ppm but failed to identify specific urinary metabolites. The failure to detect carbon disulfide exposure <16 ppm may be because of interference with the reaction by dietary sulfur containing compounds.

An occupational study in 10 rayon factory workers in China showed that the carbon disulfide metabolite, TTCA, undergoes first-order elimination kinetics, based on urinary excretion studies (Chang et al. 2002). First-order elimination kinetics for TTCA was also observed in rats (Cox et al. 1996). Mean urinary elimination half-times following inhalation exposure in rats for TTCA and total thioesters were 5.2 and 8.5 hours, respectively (Cox et al. 1996).

In dogs exposed to 25–60 ppm carbon disulfide for 0.5–2.0 hours, approximately 8–13% of the carbon disulfide that was taken up was exhaled; <0.5% was excreted in the urine (McKee et al. 1943). Experimental details and control information are limited in this study. Inhalation exposure of rabbits to 20–150 ppm carbon disulfide for 1.5–2 hours resulted in excretion of 15–30% of the absorbed carbon disulfide via the lung and <0.1% by the kidney after termination of exposure (Toyama and Kusano 1953). In rats exposed to 500–800 ppm for 180 minutes, absorbed carbon disulfide was rapidly eliminated from the blood with elimination half-times of 41–77 minutes; elimination was biphasic (Moorman et al. 1998).

In guinea pigs, carbon disulfide metabolites are excreted as inorganic sulfur compounds in the urine (Strittmatter et al. 1950). Inhalation exposure to 25.7 ppm ^{35}S-carbon disulfide for 40 hours resulted in excretion of the 61% absorbed dose within 48 hours, mainly in the urine (33% of absorbed dose) with smaller amounts in expired air (15%) and feces (13%). The remaining 39% of the labelled compound was found in the carcass, skin, liver, and brain. The metabolized material was excreted in the urine predominantly in the form of inorganic sulfur compounds; some organosulfur derivatives were also present. Most of the unmetabolized carbon disulfide was excreted in the expired air.

The studies discussed above indicate that the lungs are the primary route of excretion of unmetabolized carbon disulfide in humans and animals exposed by inhalation, whereas the kidneys are the primary route of excretion of carbon disulfide metabolites.

No studies were located regarding excretion of carbon disulfide in humans after oral exposure. Rats administered 10 mg ^{14}C-carbon disulfide/kg by gavage excreted 63.2% of the dose as unchanged carbon disulfide in the breath (DeMatteis and Seawright 1973).

Following dermal exposure of humans to aqueous solutions of carbon disulfide of increasing concentrations (0.33–1.67 g/L) for 1 hour, only 3% of the absorbed carbon disulfide was eliminated by the lungs (Dutkiewicz and Baranowska 1967). For details and study limitations, see Section 3.1.1.

Exposure of rabbit skin to high concentrations of carbon disulfide vapor (800 ppm and above) for 1 hour resulted in detectable amounts of carbon disulfide in the breath of animals (Cohen et al. 1958). A linear relationship was noted between the exposure concentration and the amount of carbon disulfide in the exhaled breath.

Appreciable amounts of absorbed carbon disulfide are excreted unchanged in breath regardless of the route of exposure. Small amounts of carbon disulfide are excreted in the sweat and saliva of exposed individuals. In mice injected intraperitoneally with 30.2–41.9 μg of ^{35}S-carbon disulfide, about 13–23% of the radiolabel was excreted via the lung (Strittmatter et al. 1950). Rats receiving 10 mg ^{14}C-carbon disulfide/kg by intraperitoneal injection excreted about 70% of the dosed material as unchanged carbon disulfide in the breath (DeMatteis and Seawright 1973). Rats receiving 19 mg/kg ^{14}C-carbon disulfide intraperitoneally excreted 58–83% free carbon disulfide in expired air in the 3 hours following dosing (Snyderwine and Hunter 1987). Younger rats expired significantly more free carbon disulfide than older rats. In another study (Dalvi and Neal 1978), intraperitoneal administration of ^{14}C-carbon disulfide to rats resulted in excretion of carbonyl sulfide by the lungs in greater quantities than carbon dioxide. Pretreatment of rats with phenobarbital, however, resulted in a greater amount of excretion of carbon dioxide than carbon disulfide. In both experiments, excretion of ^{14}C-carbonyl sulfide and carbon dioxide accounted for 14–43% of the total administered radioactivity, with about twice as much carbon dioxide.

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).

No PBPK models for carbon disulfide were identified.

3.1.6 Animal-to-Human Extrapolations

Toxicokinetics studies show that absorption, distribution, and excretion of carbon disulfide are similar in humans and animals, although limited quantitative data are available particularly in humans and non-primates. However, metabolism of carbon disulfide differs slightly between humans and animals, adding some uncertainty in extrapolations from animals to humans. In animals, oxidation of sulfur to inorganic sulfate occurs (Beauchamp et al. 1983); whereas limited data indicate that it is not a significant metabolic pathway in humans (Djerassi and Lumbroso 1968). Available data on this apparent difference are inadequate to quantify the impact of extrapolation between species. Additionally, this reported difference would only be relevant for animal-to-human extrapolations for endpoints potentially mediated through this specific metabolic pathway.

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

There have been no human studies to determine the health effects of exposure to carbon disulfide in children, or whether children are more or less susceptible to the potential health effects of carbon disulfide at a given exposure level and duration of exposure. There is no information on whether the effects reported in adults following occupational exposures would be similarly observed in children.

Since there are limited data on the toxicity of carbon disulfide in children, it is assumed that the toxicity of carbon disulfide in children is similar to the toxicity observed in adults. Available developmental toxicity data from animal studies indicate that developmental toxicity in rats was generally observed at high inhalation and oral exposure levels associated with maternal toxicity (Holson 1992; NCTR 1984a; Saillenfait et al. 1989). However, a series of inhalation studies in rats reported effects below maternally toxic concentrations (Tabacova and Balabaeva 1980; Tabacova et al. 1978, 1983). Additionally, in rabbits, developmental effects were noted at exposure levels lower than those associated with maternal toxicity, particularly in oral studies (Denny and Gerhart 1991; NCTR 1984b). Therefore, it is unclear based on unavailable data if the developing fetus or infant will be more (or less) susceptible to carbon disulfide toxicity compared to an adult.

There are studies that have investigated particular metabolic traits that may result in hyper-susceptibility to carbon disulfide (Djuric et al. 1973; Stokinger and Scheel 1973). The study conducted by Djuric et al. (1973) reported on 72 workers who had been divided into three groups: 18 exposed to carbon disulfide at levels below the industrial air limit of 20 ppm (60 mg/m^3) (control group), 21 who had been exposed to levels >20 ppm but had shown no signs or symptoms of carbon disulfide intoxication (resistant group), and 33 who had polyneuritis or other signs of overexposure and had been removed from exposure (susceptible group). All individuals were administered an oral dose of 0.5 g of disulfiram (Antabuse), a compound that produces carbon disulfide when metabolized. It was assumed that carbon disulfide and disulfiram are metabolized by the same or similar enzyme system, and determination of diethyl dithiocarbamates (DDC) in urine after disulfiram administration was used to evaluate the rate at which sulfur compounds are metabolized. The excretion of DDC was significantly lowest in the susceptible

group (49.70 μg/mg creatinine) when compared to both the control (160.05 μg/mg creatinine) and resistant (90.04 μg/mg creatinine) groups. These results led to the suggestion that the reduced ability of the symptomatic workers to metabolize this compound would lead to hyper susceptibility to carbon disulfide and would thus be associated with the clinical signs observed in that group. No supporting data have been located, however.

The study authors (Djuric et al. 1973) suggested that carbon disulfide exposure causes a decrease in excretion of DDC, especially in once-poisoned workers; thus, carbon disulfide exposure produced a disturbance in the metabolism of sulfur compounds. They also suggested that in the susceptible worker group, this decreased metabolic conversion appeared to persist even 5–10 years after exposure, and carbon disulfide exposure may therefore have led to an irreversible metabolic disturbance. The study authors did not speculate on the mechanism of actual metabolic inhibition, nor did they propose any genetic hypothesis.

Because it appears that one common mechanism of the cerebral, cardiovascular, and hepatic effects may be an acceleration of the arteriosclerotic process, individuals at risk for arteriosclerosis or those with early arteriosclerosis would probably be at increased risk for health effects following exposure to carbon disulfide (NIOSH 1978). The mechanism for carbon disulfide acceleration of arteriosclerotic plaque formation involves direct injury to the vessel endothelium and changes in lipid homeostasis and metabolism. Studies in animals indicate that ingestion of high-fat diets increases susceptibility to atherosclerotic changes associated with carbon disulfide exposure (Antov et al. 1985; Lewis et al. 1999; Wrońska-Nofer et al. 1980).

Three other groups are recognized as being unusually susceptible to carbon disulfide: individuals with alcohol use disorder (including those treated with Antabuse), those with neuropsychic disorders, and those with vitamin B_6 deficiency (Djuric et al. 1973; Lefaux 1968; Peters et al. 1982). Individuals experiencing ethanol intoxication may also have increased susceptibility to acute-duration carbon disulfide exposures. Carbon disulfide reduces the levels of vitamin B_6, which in turn upsets carbohydrate metabolism, particularly the cerebral carbohydrates (Lefaux 1968).

There is limited evidence that genetic differences based on ethnicity may infer differential susceptibility based on occupational data for retinal microaneurysms (Sugimoto et al. 1977). While almost all available cohort studies reported retinal microaneurysms in workers exposed to >10 ppm (NIOSH 1984a; Sugimoto

et al. 1976, 1977, 1978; Vanhoorne et al. 1996), a cohort in Finnish workers exposed to concentrations ranging from 5–60 ppm did not observe this association (Raitta and Tolonen 1975; Sugimoto et al. 1977).

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).

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 carbon disulfide 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 carbon disulfide 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

The most sensitive biomarker for carbon disulfide that correlates best with exposure is urinary levels of the metabolite, TTCA. TTCA is formed when carbon disulfide reacts with glutathione in the body.

Based on occupational exposure scenarios, TTCA has been shown to be associated with carbon disulfide exposure and uptake (Beauchamp et al. 1983; Campbell et al. 1985; Drexler et al. 1994). The American Conference of Governmental Industrial Hygienists (ACGIH) established a biological exposure index (BEI) for carbon disulfide based on urinary TTCA levels (ACGIH 1994); the BEI is a guidance value for evaluating biological monitoring data. TTCA correlates well with personal air sampling concentrations of carbon disulfide ranging from 0.2 to 30 ppm (Drexler et al. 1994; Göen et al. 2014; Meuling et al. 1990). Several studies reported increased excretion of TTCA in the urine of rayon factory workers or workers in facilities that utilize carbon disulfide (Kivistö 2000; Meuling et al. 1990; Tan et al. 2000; Thienpont et al. 1990; van Poucke et al. 1990). Cox et al. (1998) compared urinary TTCA from workers in a Virginia viscose rayon plant with those in a Tennessee rubber product facility and found that those with higher exposures to carbon disulfide had correspondingly higher urinary levels of TTCA.

One limitation of urinary TTCA levels is that this compound has been detected at low concentrations (range, 0.005–0.15 mg/g creatinine) in persons not exposed to carbon disulfide (Kivistö 2000; Lee et al. 1995). The source of this TTCA is thought to be from dietary intake, especially the consumption of brassica vegetables (e.g., cabbage, Brussels sprouts) (Kivistö 2000; Simon et al. 1994). Therefore, in persons who eat large amounts of these vegetables, measurements of urinary TTCA may overestimate carbon disulfide exposure. Baseline sampling is therefore necessary to correct for nonworkplace exposure sources.

Due to the limitations in the methodology for measuring carbon disulfide directly in blood, exhaled breath, and urine of exposed individuals, direct measurement of this compound is not the most sensitive test for determining the extent of exposure (Beauchamp et al. 1983; Campbell et al. 1985; Djuric 1967; McKee et al. 1943; WHO 1979). Additionally, these biomarkers often did not correlate well with external exposures, especially at low concentrations. Measuring urinary carbon disulfide thiometabolites (iodine-azide test) or total concentration of urinary thio compounds (including mercapturic acids and other sulfur-containing carbon disulfide metabolites) may be potential biomarkers; however, these compounds are not specific for carbon disulfide exposure and the sensitivity of the detection methods is poor (Beauchamp et al. 1983; Tan et al. 2000; Van Doorn et al. 1981a).

3.3.2 Biomarkers of Effect

The battery of biomarkers discussed here may be used as indicators of probable carbon disulfide exposure. However, the physiological effects of carbon disulfide poisoning are numerous and range from

mild to severe. Their utilization as biomarkers of effect are confounded by their occurrence in response to other epidemiological, nutritional, and environmental factors. Their significance as biomarkers is further reduced by the fact that these effects occur with great variance in the cohort-exposed population.

The following are proposed as likely biomarkers of effect for carbon disulfide; however, more information about their possible correlation with actual carbon disulfide exposure and their reliability and consistency is necessary before they can be utilized to indicate level or duration of exposure or predict potential health effects.

Changes in lipid homeostasis and metabolism are the most obvious biomarkers of carbon disulfide's vasculopathic effects. Hypercholesterolemia (Toyama and Sakurai 1967) and high β-lipoproteins in the blood (Prerovska and Drdkova 1967) have been observed by investigators following long-term occupational carbon disulfide exposure. Elevated blood lipid concentrations following long-term carbon disulfide exposure in humans may be an appropriate indicator of ensuing arteriosclerosis, clinical vasculopathy, and increased risk of cardiovascular disease (El-Sobkey et al. 1979). However, the accuracy and reliability of this parameter as a potential biomarker of exposure for carbon disulfide are in question since many things can cause changes in lipid homeostasis. Additionally, the usefulness of this biomarker of effect may be concentration-dependent. In the studies discussed above, exposure concentrations were estimated to be 40–50 ppm with occasional peaks of ≥300 ppm (Toyama and Sakurai 1967) and 200 ppm (Prerovska and Drdkova 1967). In an industrial setting where concentrations of carbon disulfide were <5 ppm, no association was seen between urinary TTCA levels in workers and total serum cholesterol or its subfractions (Domergue et al. 2016).

More specific blood lipid parameters, however, may prove to be useful in the future. Changes have been observed in lipid homeostasis when a cytochemical enzymological examination of leukocytes and platelets was carried out for >600 exposed workers (Micu et al. 1985). Researchers found high levels of lymphocytic lipids and low levels of granulocytic lipids. Another investigator found elevated serum cholesterol and fatty acids and low cholesterol ester levels in an 11-week study of dogs. However, only the experimental animal group fed a high-fat diet showed altered lipid homeostasis. The exposed groups on normal and high-carbohydrate diets had normal serum lipid content (Lewey et al. 1941).

Several neurological parameters may be useful as more specific biomarkers of polyneuropathy from carbon disulfide exposure. CT scans, magnetic resonance imaging, and pneumoencephalography (PEG) may indicate early cerebral/cerebellar atrophy in humans (Beauchamp et al. 1983; Peters et al. 1988).

EMGs have detected signs of neurogenic lesions in humans, and changes in brain EEG patterns in animals have accompanied carbon disulfide-induced central nervous system toxicity. Moreover, neurophysiological methods may be utilized to detect decreasing nerve conduction velocity, which is a biomarker of peripheral nervous system effects (WHO 1981).

In studying the effects of carbon disulfide exposure on enzyme systems of carbohydrate metabolism, McKee et al. (1943) observed that the succinic-oxidase system was inhibited. They noted a 10% decrease in the activity of this system. Carbohydrate metabolism is crucial in proper neural function; thus, succinic-oxidase activity may serve as an appropriate biomarker of nervous system effects (McKee et al. 1943).

The concentration of crosslinked red blood cell spectrin has been suggested as a marker of nerve protein crosslinking damage (Valentine et al. 1993, 1997). The proposed sequence of events is formation of dithiocarbamate protein adducts that subsequently decompose to form isothiocyanate adducts. These latter adducts can then cause the actual crosslinking of both spectrin and nerve protein. Crosslinking leads to slower nerve conduction velocities. As new red blood cells must be made to replace the damaged spectrin, the crosslinking of this protein may serve as a longer-term biomarker of carbon disulfide exposure.

In conclusion, the following summarizes possible correlative biological markers of early carbon disulfide poisoning: (1) abnormal lipid homeostasis/metabolism as indicated by hypercholesterolemia; (2) electromyographical indications of neural lesions; (3) decreased nerve conduction velocity; (4) lower succinic-oxidase enzyme activity; and (5) erythrocyte spectrin. While these biological markers are not specific for carbon disulfide, one or more of these markers in combination may prove to be a useful biomarker for carbon disulfide effects.

3.4 INTERACTIONS WITH OTHER CHEMICALS

There is limited information on compounds that interact with carbon disulfide to alter its toxicity. Agents that induce hepatic microsomal enzymes (e.g., phenobarbital, various alcohols, chlordane) can increase toxicity of carbon disulfide exposure in rodents (Dalvi et al. 2008; El-Masry et al. 1976; Freundt et al. 1974a; Magos and Butler 1972; Magos et al. 1973; Snyderwine et al. 1988). Co-exposure to ethanol and carbon disulfide, in particular, appears to result in greater-than-additive neurotoxicity and hepatotoxicity in rats (Opacka et al. 1984; Wrońska-Nofer et al. 1986). Also, concurrent exposure of carbon disulfide

and ethanol had adverse effects on the cardiovascular system (decreased heart rate and increased QRS duration) in rats (Morvai et al. 2005).

Exposure to combinations of air toxics in ambient air, including carbon disulfide, may increase severity of childhood asthma outcomes. In a population-based study in New York, New Jersey, and Connecticut, a machine-learning based study of various combinations of air toxic exposure levels during a child's birth year (by zip code) and childhood asthma outcomes in 151 children with mild to severe asthma revealed an increased risk of emergency room visits due to asthma with combined exposure to acetaldehyde, carbon disulfide, and polychlorinated biphenyls (Li et al. 2021). Risk of emergency room visit was not associated with acetaldehyde or carbon disulfide alone; it was associated with exposure to polychlorinated biphenyls alone, but the association was stronger with combined exposure.

Many studies have shown suppression of hepatic microsomal enzymes in laboratory animals following inhalation exposure to carbon disulfide (Bond and DeMatteis 1969; El-Masry et al. 1976; Freundt et al. 1974b; Järvisalo et al. 1977a; Masuda and Yasoshima 1988; Masuda et al. 1986). Due to this, carbon disulfide could potentiate toxicity of compounds that require cytochrome P-450 microsomal metabolism for detoxification or decrease toxicity for compounds that require microsomal metabolism in the liver to exert a toxic effect. For example, data from human studies support inhibition of ethanol and amidopyrine metabolism following exposure to carbon disulfide (Freundt et al. 1976; Mack et al. 1974). Carbon disulfide-induced inhibition of ethanol metabolism in humans occurred when carbon disulfide exposure was combined with moderate intake of alcohol, resulting in an increase in blood acetaldehyde levels. Similarly, oxidative N-demethylation of amidopyrine was inhibited in humans co-exposed to carbon disulfide (Mack et al. 1974).