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

3.1 TOXICOKINETICS

- Absorption:
  - Respiratory tract: Bromomethane is well absorbed from the respiratory tract. A small study in humans estimated that approximately 52–55% of the inhaled dose was absorbed. Studies in animals estimate a fractional absorption from the respiratory tract of 27–48%.
  - Gastrointestinal tract: The estimated fractional absorption of oral bromomethane in a single study in rats was ≥97%.
  - Dermal: Bromomethane is absorbed following dermal exposure, although quantitative estimates of absorption were not identified.

- Distribution: Based on inhalation exposure studies in laboratory animals, bromomethane undergoes wide distribution throughout the body, including the central nervous system.

- Metabolism: Bromomethane undergoes extensive metabolism. Metabolites include bromide ion, methanol (which can be further metabolized to formaldehyde, formate, and carbon dioxide), S-methyl derivatives, and glutathione conjugates.

- Excretion: Excretion of bromomethane occurs mainly by expiration of carbon dioxide or by urinary excretion of nonvolatile metabolites (Bond et al. 1985; Jaskot et al. 1988; Medinsky et al. 1985). Small amounts of bromomethane undergo biliary excretion and are excreted in the feces.

3.1.1 Absorption

In a small human study, bromomethane uptake was 55.4% and 52.1% when the subjects inhaled 0.018 ppm bromomethane through the nose or mouth, respectively (ARB 1988). A study in nine fumigators accidentally exposed to bromomethane found elevated serum bromide levels 4 hours after exposure (Hustinx et al. 1993). Studies in rats suggest that exposure to airborne bromomethane is rapidly absorbed and distributed (Andersen et al. 1980; Bond et al. 1985; Gargas and Andersen 1982; Jaskot et al. 1988; Medinsky et al. 1985). Andersen et al. (1980) and Gargas and Andersen (1982) suggested that bromomethane absorption followed a rapid first-order uptake kinetics with no measurable saturable kinetics based on studies in rats exposed to 100–10,000 ppm bromomethane for 2 hours. Gas uptake

constants of 0.44 $(kg\text{-}hour)^{-1}$ (Andersen et al. 1980) and 0.55 $(kg\text{-}hour)^{-1}$ (Gargas and Andersen 1982) were calculated from these data. Gargas and Andersen (1982) estimated a first-order rate constant of 0.32 $(kg\text{-}hour)^{-1}$. In contrast, Medinsky et al. (1985) reported nonlinear uptakes, with saturation at ≥170 ppm. Fractional absorption was 48% at 1.6 and 9.0 ppm, 38% at 170 ppm, and 27% at 310 ppm in rats exposed for 6 hours. At high bromomethane levels (310 ppm), the total amount absorbed appears to reach a maximum (62 mg/kg), suggesting that some aspect of uptake (perhaps glutathione availability) becomes limiting (see Section 3.1.3). The first-order rate constant for bromomethane was estimated to be 1.6 $(kg\text{-}hour)^{-1}$. Medinsky et al. (1985) suggested that the higher concentrations tested in the Gargas and Andersen (1982) study may have resulted in glutathione depletion shortly after exposure was initiated and that the glutathione availability was a rate-limiting factor in bromomethane uptake; this is supported by the much higher rate constant estimated in the Medinsky et al. (1985) study compared to the Gargas and Andersen (1982) study. In dogs, an uptake of 39.5% was estimated following a 3-hour exposure to 0.174–0.361 ppm bromomethane (ARB 1986).

No studies were located regarding bromomethane absorption after oral exposure of humans. In rats given a single oral dose of ^{14}C-labeled bromomethane dissolved in corn oil, only about 3% of the label was excreted in the feces (Medinsky et al. 1984). This indicates that at least 97% of the dose was absorbed from the gastrointestinal tract.

No quantitative studies were located regarding bromomethane absorption across the skin of humans. Yamamoto et al. (2000) reported a rapid increase in plasma bromide levels in rats dermally exposed to liquid bromomethane for 0.5–5 minutes.

3.1.2 Distribution

Most information regarding distribution of bromomethane was obtained from inhalation exposure studies in laboratory animals. In rats exposed to ^{14}C-bromomethane in air, radioactive label was widely distributed throughout the body (Bond et al. 1985; Jaskot et al. 1988; Medinsky et al. 1985). Levels were somewhat higher in lungs, adrenals, liver, and kidneys than in other tissues (Bond et al. 1985; Jaskot et al. 1988). The form of the label was not studied by these researchers, but is probably mostly metabolites. However, Honma et al. (1985) showed that low levels of parent bromomethane can be detected for up to 24 hours after an 8-hour exposure to 250 ppm bromomethane. The study found that the highest levels of bromomethane were in the adipose tissue, followed by the blood, muscles, brain, kidneys, and liver. Bromomethane levels in the adipose tissue and blood rapidly declined post-exposure; the levels were

decreased by half within the first 30 minutes. The elimination of bromomethane from the brain and liver was slower. A similar distribution pattern was found when bromine (rather than bromomethane) levels were measured. A similar tissue distribution pattern was observed in rats exposed to 250–1,000 ppm bromomethane for 2 hours (Honma et al. 1985), with the exception that bromomethane levels were higher in the liver than in the brain immediately following exposure. Kato et al. (1986) noted concentration-specific differences in bromine tissue distribution in rats 5 days following a 6-week exposure to 200–400 ppm (4 hours/day, 5 days/week). At 200 ppm, the ratio of bromine concentrations in the kidneys, spleen, and liver was 1:0.87:0.16; at 400 ppm, the ratio was 1:0.76:0.56.

In rats exposed to a very high bromomethane concentration (2,000 ppm for 1 hour), there was a rapid increase in relative plasma bromine levels and then a rapid decrease. Using a two-compartment model, a half-time of 9.1 days was calculated for plasma bromine levels (Hori et al. 2002). In rats exposed to 300 ppm bromomethane 6 hours/day for 3 days, a plasma bromine half-time of 5.4 days was calculated (Hori et al. 2002).

Bromomethane's relative hydrophobicity suggests that it can cross the blood-brain barrier (de Souza et al. 2013), which is supported by the elevated brain bromomethane levels measured in the Honma et al. (1985) rat study.

In rats given oral doses of ^{14}C-bromomethane, label was distributed widely throughout the body, with highest levels in liver and kidneys (Medinsky et al. 1984).

In rats dermally exposed to liquid bromomethane, plasma bromide levels rapidly increased in proportion to the exposure duration with peak levels observed 1 hour after a 0.5-, 1-, 3-, or 5-minute exposure (Yamamoto et al. 2000). The plasma bromide levels gradually decreased and returned to baseline levels 4–8 weeks postexposure. Yamamoto et al. (2000) estimated plasma bromide ion half-times (assuming a two-compartment model) of 6.3, 6.5, 5.3, and 5.0 days following the 0.5-, 1-, 3-, and 5-minute exposures, respectively.

3.1.3 Metabolism

Bromomethane undergoes initial metabolism primarily by nucleophilic displacement of the bromide ion. When the attacking species is water, the products are methanol and bromide ion:

$$HOH + CH_3Br \rightarrow CH_3OH + H^+ + Br^-$$

The amount of bromomethane broken down by this reaction in the body is not known, but increased levels of both methanol and bromide have been detected in exposed animals (Gargas and Andersen 1982; Honma et al. 1985). Elevated bromine levels were found in the blood, kidneys, and liver of rats shortly after termination of an 8-hour exposure to 250 ppm (Honma et al. 1985). The peak levels of bromine occurred 4–8 hours after exposure, as compared to the peak levels of bromomethane, which occurred after 1 hour of exposure. Oxidation of methanol leads to formaldehyde and formate, which may enter the one-carbon metabolic pool, be oxidized to carbon dioxide and water, or undergo further reactions in the oxalate or tricarboxylic acid cycles to form amino acids such as cysteine or homocysteine (Bulathsinghala and Shaw 2014). Bromomethane may also react with organic thiols (R-SH) to yield S-methyl derivatives:

$$R\text{-}SH + CH^3Br \rightarrow R\text{-}SCH^3 + H^+ + Br^-$$

This has been shown to result in formation of S-methylcysteine derivatives in hemoglobin of mice exposed to bromomethane (Iwasaki 1988b), and by analogy with methyl chloride (Kornbrust and Bus 1983), is likely to result in formation of S-methyl glutathione (Medinsky et al. 1985). Conjugation with glutathione is supported by the finding of decreased glutathione concentrations in the liver, kidneys, lungs, and brains of mice exposed to bromomethane for 1 hour (Alexeeff et al. 1985). Further metabolism of S-methyl derivatives such as those mentioned above produces methanethiol via intermediates S-methylcysteine and methylthioacetic acid (Bulathsinghala and Shaw 2014). Methanethiol undergoes additional metabolism to formaldehyde and formate, subsequently following the pathways described above. Ultimately, the formation of carbon dioxide accounts for 40–50% of the administered dose and other unidentified nonvolatile metabolites account for about 20–25% of the administered dose (Bond et al. 1985; Jaskot et al. 1988; Medinsky et al. 1985).

3.1.4 Excretion

No studies were located regarding excretion of bromomethane in humans after inhalation or oral exposure. In animals exposed to bromomethane vapors, excretion occurs mainly by expiration of carbon dioxide or by urinary excretion of nonvolatile metabolites (Bond et al. 1985; Jaskot et al. 1988; Medinsky et al. 1985). Only small amounts are excreted in the feces. Very little parent bromomethane is exhaled (Jaskot et al. 1988; Medinsky et al. 1985), and tissue levels of parent bromomethane decrease with a half-life of only about 15–30 minutes (Honma et al. 1985; Jaskot et al. 1988). Half-lives for clearance of

metabolites from the body and most tissues range from 2 to 10 hours (Honma et al. 1985; Jaskot et al. 1988).

A significant fraction (about 25–30%) of ^{14}C-radiolabeled bromomethane ($^{14}CH^{3}Br$) remains in tissues after 24–72 hours and a small portion is excreted (7% via exhaled air in 32 hours). The greater excretion rate of $^{14}CO_2$ (43% in exhaled air, 21% in urine, and 2% in feces) indicates rapid metabolism and longer-term retention of bromide ion (Jaskot et al. 1988; Medinsky et al. 1985). This rapid excretion of $^{14}CO_2$ presumably represents turnover of various intracellular metabolites or adducts, although this has not been established. The half-life of bromine in the blood, kidneys, and liver was approximately 5 days in rats exposed to 250 ppm bromomethane for 8 hours (Honma et al. 1985). Following a 1-hour exposure to 220–1,530 ppm bromomethane, 95% of the bromide was eliminated from the blood, kidneys, liver, lungs, and brain of mice after 2.5 days. Saturation of the detoxification mechanism by inhaled bromomethane (which can affect excretion) was proposed by the study authors (Alexeeff et al. 1985).

One study in animals indicates that the rate and pattern of excretion of ^{14}C-label following oral exposure to ^{14}C-bromomethane is similar to that following inhalation exposure: 32% was exhaled as carbon dioxide, 43% was excreted in the urine, 4% of unmetabolized parent compound was exhaled, 2% was excreted in the feces, and 14% remained in the body after 72 hours (95% of the radiolabel was recovered) (Medinsky et al. 1984). In rats with cannulated bile ducts, 46% of the administered dose was excreted in the bile, with much lower amounts exhaled as CO_2 (12%) and excreted in urine (7%) (Medinsky et al. 1984). Given the low fecal excretion seen in rats without bile duct cannulation, these experiments suggest that bromomethane metabolite(s) excreted in bile are reabsorbed and further metabolized prior to excretion in urine or as exhaled CO_2.

3.1.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

PBPK models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models 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 (Clewell and Andersen 1985). 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.

No PBPK models were identified for bromomethane.

3.1.6 Animal-to-Human Extrapolations

In several species, including humans, similar target tissues have been found, namely the respiratory tract and the nervous system. Although the target tissues were similar across species, dose-response differences were noted in several animal studies. Irish et al. (1940) exposed rats, rabbits, and monkeys to the same bromomethane concentrations for 6 months. The respective NOAEL and LOAEL values for neurotoxicity were 66 and 100 ppm (convulsions) for rats, 17 and 33 ppm (paralysis) for rabbits, and 33 and 66 ppm (paralysis) for monkeys. NTP (1992) also noted species-differences in the neurotoxicity of bromomethane; exposure to 120 ppm resulted in alterations in performance on neurobehavioral tests without overt signs of toxicity in rats and severe curling and crossing of hindlimbs and twitching of forelimbs in mice. Although mice were more sensitive to the neurotoxicity of bromomethane, the respiratory tract was more sensitive in rats than in mice. Olfactory epithelial dysplasia was observed in rats exposed to 120 ppm for 13 weeks; no nasal effects were observed in mice also exposed to 120 ppm for 13 weeks (NTP 1992). Reliable dose-response data are not available for humans that would allow for a comparison of adverse effect levels with animal data. In the absence of these data, it is assumed that humans would be as sensitive as animals to the neurological and respiratory toxicity of bromomethane.

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

There are limited data on the toxicity of bromomethane in children. One case report discussed effects observed in an infant accidentally exposed to bromomethane (Langard et al. 1996). Vomiting and severe diarrhea were reported in the infant who died; the cause of death was determined to be acute pneumonia due to aspiration. Vomiting, as well as eye, throat, and mouth irritation, was reported in the parents.

A study in rabbits found increased incidences of a minor malformation and minor variation and decreases in body weights in the offspring of rabbits exposed via inhalation (Breslin et al. 1990). However, other studies in rats and rabbits have not reported developmental effects following inhalation exposure during gestation (Hardin et al. 1981; NIOSH 1980) or oral exposure (Kaneda et al. 1998).

It may be expected that the young, the elderly, and people with lung, kidney, or neurological disease might be more readily affected than healthy adults. In addition, humans with a congenital deficiency in glutathione transferase suffered more severe effects of bromomethane exposure, further supporting a detoxifying action of glutathione conjugation under conditions of acute exposure (reviewed by de Souza et al. 2013). Studies in animals reveal that there are differences in sensitivity between species (such as respiratory toxicity, neurotoxicity, and mortality) (e.g., Gotoh et al. 1994; Irish et al. 1940), and some studies have noted small differences in sensitivity between males and females (Eustis et al. 1988). It is not known if these differences apply to humans.

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 1989).

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 1989). 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 bromomethane are discussed in Section 3.3.1. 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 CDC (2018), http://www.cdc.gov/exposurereport/. If available, biomonitoring data for bromomethane from this report are discussed in Section 5.6, General Population Exposure.

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 1989). 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 bromomethane 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

Measurement of parent bromomethane (e.g., in expired air, blood, or urine) has not been investigated as a possible biomarker of exposure in humans, mainly because studies in animals suggest that bromomethane is cleared so rapidly (half-lives of 15–30 minutes) that this is unlikely to be useful for monitoring environmental exposures. Similarly, methanol and other organic metabolites are also cleared with short half-lives (Honma et al. 1985; Jaskot et al. 1988), so they are also unlikely to be useful in biomonitoring.

In contrast, the bromide ion level in blood or serum has been used as a biomarker of bromomethane exposure. The relationship between bromide ion concentrations and the severity of effects in exposed people was investigated by Alexeeff and Kilgore (1983), who assembled and evaluated data from a large number of case reports. Serum bromide levels are usually below 15 ppm in unexposed people. In bromomethane-exposed people, levels up to 80 ppm may occur without any obvious clinical signs, while levels of 150–400 ppm are observed in people with moderate to severe symptoms. Bromide is cleared from blood with a half-life of about 12 days in healthy people, and half-lives of 3–15 days have been

observed in bromomethane-exposed people (Alexeeff and Kilgore 1983). Consequently, the correlation between serum bromide levels and severity of effects is most apparent within the first 1–2 days of exposure, and there may be little correlation later. Bromide ion is cleared mainly by excretion in the urine, and may be a candidate biomarker of bromomethane exposure. Tanaka et al. (1991) observed a significant correlation ($r=0.596$, $p<0.01$) between bromine levels in urine and personal air samples for bromomethane (concentrations ranging up to 390 ppm) in a group of 41 plant fumigators wearing gas masks with respirator canisters. The authors postulated three potential routes of exposure to bromomethane in the workers, including dermal absorption, leakage through an incomplete seal of the gas mask, and breakthrough in the respiratory canister (Tanaka et al. 1991). Further investigation is needed to better establish whether urinary bromine is a reliable biomarker of exposure to bromomethane.

Formation of stable methylated adducts such as S-methylcysteine in hemoglobin is known to occur in animals following inhalation exposure to bromomethane (Iwasaki 1988a, 1988b), and has been demonstrated *in vitro* using both human and mouse hemoglobin (Bamgbose and Bamgbose 2008), but the potential use of this endpoint for biomonitoring in humans has not been fully explored.

Neither elevated serum bromide levels nor formation of methylated adducts are, by themselves, specific for bromomethane exposure. For example, increased bromide levels could result from exposure to bromide in the diet or ingestion of bromate- or bromide-containing medicines, and increased methyl adducts might result from exposure to other methyl halides, various methyl nitrosamines, or other alkylating agents. However, the combination of these two methods (i.e., a finding of increased bromide and increased methylation) would strongly indicate that bromomethane exposure had occurred.

3.3.2 Biomarkers of Effect

As discussed in Chapter 2, the effects that are most often observed in humans exposed to bromomethane vapor are central nervous system injury (disturbed vision, tremor, convulsions, coma), lung irritation (edema, impaired respiration), and renal injury (oliguria or anuria). Of these, neurological or neurobehavioral signs often appear to be the most sensitive indication of effect, since preclinical symptoms can be observed in humans exposed to low levels of bromomethane in the workplace (Anger et al. 1986; Kishi et al. 1988; Verberk et al. 1979). Of course, positive findings for endpoints of this sort (headache, weakness, ataxia, nausea, double vision, abnormal electroencephalogram) are not specific indicators of bromomethane exposure, since other chemicals or diseases may produce similar neurological changes.

3.4 INTERACTIONS WITH OTHER CHEMICALS

No studies were located regarding the interaction of bromomethane with other chemicals. Since it seems likely that cellular glutathione may serve a protective function by reacting with bromomethane (Kornbrust and Bus 1983), other chemicals (electrophilic xenobiotics, reactive intermediates) that lead to decreases in glutathione levels might increase the toxicity of bromomethane, but this has not been investigated. Similarly, bromomethane might be expected to have additive or synergistic interactions with other alkylating agents, but this has not been investigated.