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

3.1 TOXICOKINETICS

No studies were located regarding bromodichloromethane toxicokinetics in humans, but there are limited data from studies in animals. These data are summarized below.

- Bromodichloromethane is rapidly absorbed through the gastrointestinal tract and skin and is presumed to be rapidly absorbed through the respiratory tract
- Absorbed bromodichloromethane is distributed throughout the body with the highest concentrations found in the fat, liver, lungs, and kidneys.
- The predominant pathway for bromodichloromethane metabolism is cytochrome P450 oxidation. Bromodichloromethane can also be metabolized via reduction to a dichloromethyl radical or glutathione conjugation catalyzed by glutathione transferase.
- Bromodichloromethane is rapidly excreted; the half-life following a single oral dose was 1.5–2 hours in rats and mice. The major route of excretion is expiration of the parent compound or carbon dioxide through the lung; smaller amounts of bromodichloromethane are excreted in the urine and feces.

3.1.1 Absorption

There are limited data on the bromodichloromethane absorption following inhalation exposure. Based on its physical-chemical properties and by analogy to another trihalomethane (chloroform) (ATSDR 1997), it is assumed that bromodichloromethane will be well absorbed.

Direct evidence of oral and dermal absorption of bromodichloromethane in humans comes from studies measuring blood levels of bromodichloromethane following ingestion or dermal exposure to bromodichloromethane (Leavens et al. 2007) or following ingestion, bathing, or showering with tap water containing trihalomethanes, including bromodichloromethane (Backer et al. 2000; Lynberg et al. 2001). Following oral exposure, bromodichloromethane is rapidly absorbed with peak blood levels of ^{13}C-bromodichloromethane occurring 11 minutes after exposure (Leavens et al. 2007). Following a 1-hour dermal exposure, peak blood levels were observed at the end of the exposure, also suggesting that it is rapidly absorbed through the skin (Leavens et al. 2007).

Animal studies support the findings in humans that bromodichloromethane is rapidly absorbed following oral exposure (Aida et al. 1989; da Silva et al. 1999, 2000; Lilly et al. 1998; Mink et al. 1986; NTP 2006; Smith et al. 1985). In rats, peak blood levels were found 5–15 minutes (NTP 2006) or approximately 30 minutes (da Silva et al. 1999, 2000) after gavage administration. In contrast, a monkey study reported peak blood levels 4 hours after a gavage dose (Smith et al. 1985). Although studies have not quantified percent absorption, Mink et al. (1986) reported 62.7 and 92.7% recovery of radiolabeled bromodichloromethane in expired air, urine, and internal organs of rats and mice, respectively. Several animal studies found vehicle-specific differences in absorption rates. Mathews et al. (1990) found that 87–94% of radioactivity was excreted within 24 hours of single administration of 1–100 mg/kg bromodichloromethane. Lilly et al. (1998) and NTP (2006) found a more rapid initial uptake of bromodichloromethane dissolved in an aqueous solution than when it was dissolved in corn oil. Bromodichloromethane in olive oil administered via gavage was more rapidly absorbed than when the bromodichloromethane was dissolved in olive oil, microencapsulated, and added to the diet (Aida et al. 1989).

3.1.2 Distribution

Absorbed bromodichloromethane is distributed throughout the body. Six hours after a single intravenous administration of 10 mg/kg [^{14}C]-bromodichloromethane in rats, the highest percentage of radioactivity is found in the fat, followed by muscle, liver, skin, blood, small intestine, and kidneys (Smith et al. 1985). In contrast, the highest percentage of radioactivity in rats after a single gavage dose is found in the stomach, followed by the liver, fat, muscle, small intestine, blood, skin, and kidney (Smith et al. 1985). Only a small amount of radioactivity was measured 24 hours after rats received a single gavage dose of 1, 10, or 100 mg/kg [^{14}C]-bromodichloromethane. When tissue levels of radioactivity are compared based on tissue to blood ratios (see Table 3-1), the highest levels were found in the liver, kidney, stomach, small intestine, and large intestine (Mathews et al. 1990). Single administration studies found differences in the liver:blood ratios with the highest ratios present in rats administered 1 mg/kg as compared to 100 mg/kg. No evidence of bioaccumulation of radioactivity was observed in rats administered 10 or 100 mg/kg/day for 10 days. Mathews et al. (1990) examined the kidney and small intestines to examine the relative distribution between different regions. In the kidneys, 6–8 times higher levels of radioactivity were detected in the cortex, as compared to the medulla. No significant differences in radioactivity levels were found between the duodenum, jejunum, and ileum. Although no studies were located regarding distribution following inhalation or dermal exposure, it is expected to be similar to that of oral exposure based on the similarity of the distribution following intravenous and oral exposure (Smith et al. 1985).

Table 3-1. Tissue to Blood Ratios of Radioactivity 24-Hours After Gavage Administration of [^{14}C]-Bromodichloromethane to Male Rats[a]

	Single administration			10-Day administration	
	1 mg/kg	10 mg/kg	100 mg/kg	10 mg/kg/day	100 mg/kg/day
Adipose	0.83	0.42	0.68	1.01	1.99
Large intestine	3.33	2.30	2.89	1.74	3.03
Small intestine	3.71	2.91	2.45	1.91	3.18
Kidney	4.93	5.98	8.2	6.51	13.64
Liver	44.46	20.05	11.41	14.30	14.72
Muscle	2.38	1.99	1.56	0.59	1.14
Skin	1.28	0.94	0.90	1.23	2.21
Stomach	4.21	3.31	8.33	2.01	2.99

[a]Data from Mathews et al. (1990).

Batterman et al. (2002), Kenyon et al. (2015), and Lilly et al. (1997) determined partition coefficients for bromodichloromethane. In humans, blood:air, blood:urine, and milk:blood partition coefficients of 26.6, 4.13, and 1.26 were calculated (Batterman et al. 2002); Kenyon et al. (2015) estimated blood:air partition coefficients of 17.33 and 14.61 for male and female, respectively. A blood:air partition coefficient of 31.4 was calculated for rats (Lilly et al. 1997).

3.1.3 Metabolism

Three pathways have been identified for the metabolism of bromodichloromethane: (1) cytochrome P450 oxidation to phosgene (Allis and Zhao 2002; Allis et al. 2002; NTP 2006; Lilly et al. 1997; Mathews et al. 1990; Zhao and Allis 2002); (2) reduction to dichloromethyl radical (Lilly et al. 1997; Tomasi et al. 1985); and (3) glutathione conjugation (NTP 2006; Ross and Pegram 2003).

The predominant pathway is oxidation catalyzed by cytochrome P450. *In vivo* studies have identified four cytochrome P450 isozymes that are responsible for metabolizing bromodichloromethane in rats: CYP2E1, CYP2B1/2, CYP1A2, and CYP3A1 (Allis and Zhao 2002). Four isozymes are involved in humans: CYP2E1, CYP1A2, CYP2A6, and CYP3A4 (Allis and Zhao 2002). *In vitro* studies by these investigators showed that 90 and 60% of bromodichloromethane is metabolized by CYP2E1 in rats and humans, respectively; in humans, CYP3A4 accounts for most of the rest of the cytochrome metabolism. Oxidation of bromodichloromethane via CYP2E1 results in the formation of phosgene, which hydrolyzes to produce carbon dioxide (Allis and Zhao 2002; Lilly et al. 1997; Mathews et al. 1990). PBPK modeling

estimates that following oral exposure to bromodichloromethane approximately 97% of the metabolism occurs in the liver; approximately 90% of total metabolism occurs on the first pass through the liver (NTP 2006). Cytochrome P450 oxidation accounts for 99% of the bromodichloromethane metabolism in the liver and 84–88% of the metabolism in the kidney and colon. Cytochrome P450 oxidation in the liver is also the primary metabolism pathway following inhalation exposure to bromodichloromethane (Allis et al. 2001). Several studies have shown that as the bromodichloromethane exposure level increases, the percentage of metabolism due to CYP2E1 decreases (Allis and Zhao 2002; Zhao and Allis 2002).

Although the metabolism of bromodichloromethane via glutathione conjugation catalyzed by glutathione transferase (GST) is quantitatively minor, the reactive metabolites formed may be toxicologically significant (Ross and Pegram 2003). In humans and rodents, the primary glutathione transferase isoform involved in bromodichloromethane metabolism is glutathione transferase theta 1-1 (GST T1-1) (Leavens et al. 2007; Ross and Pegram 2003). The GST T1-1 reactive metabolites are unstable, react with biomolecules near the site of generation, and have not been detected in circulation (Leavens et al. 2007). The reactive glutathione conjugates may result in the formation of DNA adducts (Ross and Pegram 2003).

Using PBPK modeling, NTP (2006) analyzed the relative contribution of cytochrome P450 and GST metabolism in the liver, kidney, and colon in rats following oral exposure to bromodichloromethane. The ratios of cytochrome P450/GST were 95, 6.9, and 6.0 in the liver, kidney, and colon following administration of 50 mg/kg. Following administration of 100 mg/kg, the ratios were 77, 7.1, and 5.3, respectively. The dose-related changes are likely due to first pass cytochrome P450 saturation in the liver and the higher levels of bromodichloromethane in the blood and availability to other tissues.

3.1.4 Excretion

The major route of excretion of bromodichloromethane in rats, mice, and monkeys is expiration through the lung, either as parent bromodichloromethane, or as volatile metabolites such as carbon dioxide (Mathews et al. 1990; Mink et al. 1986; Smith et al. 1985). Small amounts are excreted in the urine and feces. Twenty-four hours after a single exposure to 1, 10, or 100 mg/kg [^{14}C]-bromodichloromethane, 71–82% of the radiolabel was expired as carbon dioxide, 3–5% as carbon monoxide, and 3–6% as expired volatiles (Mathews et al. 1990). Urinary and fecal excretion accounted for 4 and 0.7–3%, respectively, of the excreted radiolabel (Mathews et al. 1990). Similar excretion patterns were observed in another study of rats (Mink et al. 1986), a study in mice (Mink et al. 1986), and a study in monkeys (Smith et al. 1985).

No route-specific differences in excretion patterns were observed in monkeys administered bromodichloromethane via gavage or intravenous injection (Smith et al. 1985).

The half-lives of bromodichloromethane in rats and mice following a single oral administration were estimated to be 1.5 and 2.5 hours, respectively (Mink et al. 1986), and the half-life in monkeys was 4–8 hours (Smith et al. 1985). This indicates that bromodichloromethane is effectively excreted and that tissue accumulation of bromodichloromethane is unlikely.

In a repeated-dose gavage study in rats (Mathews et al. 1990), the daily excretion of radiolabel (approximately 75%) did not change over the course of the 10-day administration of 10 mg/kg/day. However, administration of 100 mg/kg/day resulted in expiration of 30% of the radiolabel as carbon dioxide during the 8 hours after administration on day 1. On days 2–10, 60% of the label was expired as carbon dioxide.

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.

Single species PBPK models have been developed for bromodichloromethane; however, a PBPK model that could be used to extrapolate from laboratory animals to humans for risk assessment has not been identified for this chemical. Kenyon et al. (2015) developed a human PBPK model that allows for the assessment of the contribution of multiple exposure routes (inhalation, oral, and dermal) to overall internal dose metrics for bromodichloromethane. The model predicts that dermal exposure and inhalation exposure during bathing will substantially contribute to the overall internal dose of bromodichloromethane. Lilly and associates developed a PBPK model in rats that allows predictions of tissue distribution and metabolism following inhalation (Lilly et al. 1997) or gavage administration with an oil or aqueous vehicle (Lilly et al. 1998). The model consists of five compartments (liver, kidney, fat, slowly

perfused tissues, and rapidly perfused tissues) and assumes that 95% of bromodichloromethane metabolism occurs in the liver and the remaining 5% occurs in the kidney. The model accurately predicted the release of bromine.

3.1.6 Animal-to-Human Extrapolations

No studies were identified that provide evidence to suggest differences in the toxicity or toxicokinetics of bromodichloromethane between humans and animals. There are limited human toxicology studies that do not allow for a comparison to rat and mouse toxicity studies. Some species differences have been noted between rats and mice, although the targets of toxicity appear to be similar. The available data suggest that the toxicity of bromodichloromethane is mediated by its reactive metabolites which are most likely formed by cytochrome P450 isoforms, particularly CYP2E1 (Allis and Zhao 2002; Lilly et al. 1997, 1998). CYP2E1 in rats is closely related to human CYP2E1 (Allis and Zhao 2002). Other P450 isoforms may also play an important role in bromodichloromethane metabolism at low concentrations; these isoforms differ in humans and rats (Allis and Zhao 2002; Zhao and Allis 2002); however, the contribution of other isoforms to the overall bromodichloromethane metabolism is not known.

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

There are limited data on the toxicity of bromodichloromethane in children and the toxicity of bromodichloromethane is assumed to be similar to adults. As discussed in Section 2.16, gestational exposure to bromodichloromethane has resulted in full-litter resorption and delays in skeletal ossification in rats (Bielmeier et al. 2001; Christian et al. 2001a; Narotsky et al. 1997; Ruddick et al. 1983) and decreases in birth weight (Wright et al. 2004; Rivera-Nuñez and Wright 2013) and increases in stillbirths or spontaneous abortions (King et al. 2000; Waller et al. 1998) in humans. No human or animal studies assessed the risks associated with childhood exposures. Animal data provide strong evidence that the liver is one of the critical targets of toxicity for bromodichloromethane (see Section 2.9 for details); mechanistic data suggest that a reactive metabolite is the causative agent. In rats (and humans), bromodichloromethane is primarily metabolized in the liver by the cytochrome P450 isoform CYP2E1 (Allis and Zhao 2002). As discussed in EPA (2005b), CYP2E1 levels rapidly increase during the first 24 hours after birth and the levels in children aged 1–10 years are similar to adults, suggesting that the liver toxicity observed in adult rats will be predictive of effects in younger animals.

Persons with existing renal or hepatic disease might also be more susceptible, since these organs are adversely affected by exposure to bromodichloromethane. The elderly may represent an unusually susceptible population because they may have age-related deficiencies of liver and kidney function. They may also be frequently exposed to metabolism-influencing medications.

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 bromodichloromethane 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 http://www.cdc.gov/exposurereport/). If available, biomonitoring data for bromodichloromethane 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 bromodichloromethane 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

Human studies have identified blood, alveolar air, and urine levels of bromodichloromethane as biomarkers of exposure. Since bromodichloromethane is rapidly excreted, these biomarkers assess recent exposure. A number of human studies have found associations between exposure to bromodichloromethane in tap water (Backer et al. 2000; Lynberg et al. 2001; Nuckols et al. 2005; Riederer et al. 2014; Rivera-Nuñez et al. 2012) and blood bromodichloromethane levels. In 2005–2006, the geometric median blood bromodichloromethane level in the United States was 1.52 pg/mL (CDC 2017); see Section 5.6 for a more detailed presentation of the NHANES biomonitoring data. Lynberg et al. (2001) reported that blood bromodichloromethane levels were approximately 1,000-fold lower than the bromodichloromethane level in a resident’s tap water. Exposure to bromodichloromethane in tap water can occur via multiple routes of exposure from several daily activities including consumption of tap water, showering, and bathing. A study comparing the relative contribution of different activities found the highest levels of blood bromodichloromethane in subjects showering for 10 minutes, as compared to those bathing for 10 minutes or drinking 1 L of water in 10 minutes (Backer et al. 2000). A more detailed analysis of water use activity and bromodichloromethane blood levels found increases in blood levels associated with showering, bathing, and hand dish washing, but not with consumption of hot or cold beverages, clothes washing, or hand washing (Nuckols et al. 2005). Bromodichloromethane levels

increased approximately 7–16-, 8.2–12-, or 3-fold after showering, bathing, or hand dish washing, respectively. Using NHANES 1999–2006 data, Riederer et al. (2014) examined predictors of blood bromodichloromethane levels. Water concentration was one of the major predictors of blood levels. Other factors that were negatively associated with blood bromodichloromethane levels included diabetes and eating cruciferous vegetables. Backer et al. (2000) examined the possible association of polymorphisms and bromodichloromethane blood levels. A significant association was found for the CYP2D6 *4/*4 enzyme variant (decreased metabolizing activity).

Studies of pool workers and swimmers report increases in bromodichloromethane levels in alveolar air (Aggazzotti et al. 1998; Caro and Gallego 2007, 2008; Lindstrom et al. 1997; Pleil and Linstrom 1997). Alveolar air bromodichloromethane levels increased rapidly during the entire 1–2 hours exposure period (Caro and Gallego 2008). Alveolar air levels rapidly declined and returned to pre-exposure levels within 1 hour post-exposure (Aggazzotti et al. 1998; Caro and Gallego 2008). One study estimated a half-life of 26 minutes (Caro and Gallego 2008). Pleil and Lindstrom (1997) estimated a half-time of 0.45–0.63 minutes in blood based on alveolar elimination in swimmers exposed for 2 hours.

Studies in pool workers and swimmers have also established urinary bromodichloromethane as a biomarker of exposure (Caro and Gallego 2007, 2008). Urinary bromodichloromethane levels increased 1.8 times in workers at an indoor pool for 2 hours and 2.5 times in workers near the pool for 4 hours (Caro and Gallego 2007). Much higher increases in urinary bromodichloromethane were found in swimmers; a 3–4-fold increase in levels were observed following a 1-hour swim, suggesting that increased ventilation rate and dermal exposure increased the amount of bromodichloromethane absorbed. A half-time for bromodichloromethane in urine was estimated to be 45 minutes (Caro and Gallego 2008).

3.3.2 Biomarkers of Effect

There are no specific biomarkers to characterize the effects caused by bromodichloromethane. The available evidence suggests that the hepatoxicity of bromodichloromethane is likely due to oxidative damage from reactive intermediates. Measurement of biomarkers of oxidative stress such as glutathione and oxidative response agents such as NrF2 could be indicative of liver toxicity. However, measurement of these agents would not be specific to bromodichloromethane.

3.4 INTERACTIONS WITH OTHER CHEMICALS

Hewitt et al. (1983) reported that pretreatment of rats with an oral dose of acetone increased the hepatic and renal toxicity of an oral dose of bromodichloromethane given 18 hours later, as evidenced by increased relative liver weight, serum alanine aminotransferase and aspartate aminotransferase activities, increases in relative kidney weight, and increases in blood urea nitrogen levels, as compared to rats pretreated with water.

Several studies have examined toxicokinetic interactions between bromodichloromethane and other trihalomethanes and chloroacetic acids. The blood area under the curve (AUC) obtained in rats receiving a single gavage dose of 0.25 mmol/kg bromodichloromethane was significantly lower than the AUC when bromodichloromethane was administered with three other trihalomethanes (chloroform, dibromochloromethane, and bromoform, 0.25 mmol/kg of each compound); the AUC was 8.15 times higher when administered with other trihalomethanes (da Silva et al. 1999). The investigators suggested that this may be due to metabolic interactions between the compounds. Similar results were found when binary mixtures of trihalomethanes were tested (da Silva et al. 2000). Co-administration via intravenous injection of bromodichloromethane with trichloroacetic acid or monochloroacetic acid also resulted in higher blood bromodichloromethane levels (St. Pierre et al. 2003). *In vitro* studies demonstrated that the increases in bromodichloromethane blood levels were due to metabolic inhibition by other trihalomethanes or trichloroacetic acid (St. Pierre et al. 2005).