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EPA Office of Water

DRINKING WATER CRITERIA DOCUMENT FOR BROMINATED TRIHALOMETHANES

Prepared for

Health and Ecological Criteria Division
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FOREWORD

Section 1412 (b) (3) (A) of the Safe Drinking Water Act, as amended in 1986, requires the Administrator of the Environmental Protection Agency to publish Maximum Contaminant Level Goals (MCLGs) and promulgate National Primary Drinking Water Regulations for each contaminant, which, in the judgment of the Administrator, may have an adverse effect on public health and which is known or anticipated to occur in public water systems. The MCLG is nonenforceable and is set at a level at which no known or anticipated adverse health effects in humans occur and which allows for an adequate margin of safety. Factors considered in setting the MCLG include health effects data and sources of exposure other than drinking water.

This document provides the health effects basis to be considered in establishing the MCLGs for brominated trihalomethanes found in chlorinated drinking water. To achieve this objective, data on pharmacokinetics, human exposure, acute and chronic toxicity to animals and humans, epidemiology and mechanisms of toxicity were evaluated. Specific emphasis is placed on literature data providing dose-response information. Thus, while the literature search and evaluation performed in support of this document was comprehensive, only the reports considered most pertinent in the derivation of the MCLGs are cited in this document. The comprehensive literature search in support of this document includes information published up to January, 2005; however, more recent information may have been added during the review process.

When adequate health effects data exist, Health Advisory values for less than lifetime exposure (One-day, Ten-day and Longer-term, approximately 10% of an individual's lifetime) are included in this document. These values are not used in setting the MCLGs, but serve as informal guidance to municipalities and other organizations when emergency spills or contamination situations occur.

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Acknowledgments

This document is derived and updated/expanded of the *Draft for the Drinking Water Criteria Document on Trihalomethanes* (U.S. EPA, 1994), *Summary of New Health Effects Data on Drinking Water Disinfectants and Disinfection Byproducts (D/DBPs)* for the Notice of Availability (NODA) (U.S.EPA, 1997), and *Draft Drinking Water Criteria Document on Brominated Trihalomethanes* (U.S. EPA, 2003). This document includes and evaluation of literature on brominated trihalomethanes resulting from full literature searches conducted up to January 2005 for toxicity data. In addition, few newer studies identified after the literature search date have been included as available at the time of document preparation.

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I. EXECUTIVE SUMMARY

Brominated trihalomethanes are volatile organic liquids that have a number of industrial and chemical uses. The chief reason for health concern is that they are generated as by-products during the disinfection of drinking water. The brominated trihalomethanes occurring in water are bromoform, dibromochloromethane, and bromodichloromethane. These compounds are formed when hypochlorous acid oxidizes bromide ion present in water to form hypobromous acid, which subsequently reacts with organic material to form the brominated trihalomethanes.

Toxicokinetics

No human data on absorption of brominated trihalomethanes are available. Measurements in mice and rats indicate that gastrointestinal absorption of brominated trihalomethanes is rapid (peak levels attained less than an hour after administration of a gavage dose) and extensive (63% to 93%). Most studies of brominated trihalomethane absorption have used oil-based vehicles. A study in rats found that the initial absorption rate of bromodichloromethane was higher when the compound was administered in an aqueous vehicle than when administered in a corn oil vehicle.

Data for distribution of brominated trihalomethanes in human organs and tissues are limited. Bromoform was found primarily in the stomach and lungs of a human overdose victim, with lower levels detected in intestine, liver, kidney and brain. Dibromochloromethane was found in 1 of 42 samples of human breast milk collected from women living in urban areas. Radiolabeled brominated trihalomethanes or their metabolites were detected in a variety of tissues following oral dosing in rats and mice. Approximately 1 to 4% of the administered dose was recovered in body tissues when analysis was conducted 8 or 24 hours post-treatment. The highest concentrations were detected in stomach, liver, blood, and kidneys when assayed 8 hours after administration of the compounds. Bromodichloromethane was detected at a concentration of 0.38 μg/g in the milk of one of three female rats exposed to approximately 112 mg/kg-day during a reproductive/developmental study. Bromodichloromethane was not detected in placentas, amniotic fluid, or fetal tissue collected on gestation day 21 from rats exposed to doses up to approximately 112 mg/kg-day or in plasma collected from postpartum day 29 weanling pups. Bromodichloromethane was detected at concentrations slightly above the limit of detection in placentas from two litters born to rabbits exposed to 76 mg/kg-day. Bromodichloromethane was detected in one fetus from a rabbit exposed to 76 mg/kg-day "...at a level below the limit of detection". Bromodichloromethane was not detected in placentas from female rabbits exposed to doses of approximately 32 mg/kg-day, or in amniotic fluid or the remaining fetuses from rabbits exposed to doses of approximately 76 mg/kg-day.

Brominated trihalomethanes are extensively metabolized by animals. Metabolism of brominated trihalomethanes occurs via at least two pathways. One pathway predominates in the presence of oxygen (the oxidative pathway) and the other predominates under conditions of low oxygen tension (the reductive pathway). In the presence of oxygen, the initial reaction product is trihalomethanol (CX_3OH), which spontaneously decomposes to yield the corresponding dihalocarbonyl (CX_2O). The dihalocarbonyl species are reactive and may form adducts with

cellular molecules. When intracellular oxygen levels are low, the trihalomethane is metabolized via the reductive pathway, resulting in a highly reactive dihalomethyl radical ($\bullet CHX_2$), which may also form covalent adducts with cellular molecules. The metabolism of brominated trihalomethanes and chloroform appear to occur via the same pathways, although *in vitro* and *in vivo* data suggest that metabolism via the reductive pathway occurs more readily for brominated trihalomethanes. Both oxidative metabolism and reductive metabolism of trihalomethanes appear to be mediated by cytochrome P450 isoforms. The identity of cytochrome P450 isoforms that metabolize brominated trihalomethanes has been investigated in several studies which used bromodichloromethane as a substrate. The available data suggest that the cytochrome P450 isoforms CYP2E1, CYP2B1/2, and CYP1A2 metabolize bromodichloromethane in rats. The human isoforms CYP2E1, CYP1A2, and CYP3A4 show substantial activity toward bromodichloromethane *in vitro* and low but measurable levels of CYP2A6 activity have also been detected. Based on the available data, CYP2E1 and CYP1A2 are the only isoforms active in both rats and humans. CYP2E1 shows the highest affinity for bromodichloromethane in both species and the metabolic parameters K_m and k_{cat} are similar for rat and human CYP2E1. In contrast, the metabolic parameters for CYP1A2 differ in rats and humans. The pattern of results for isozyme activity obtained from an inhalation study of bromodichloromethane was similar to the pattern reported for male F344 rats treated with bromodichloromethane by gavage.

Recent studies suggest that metabolism of brominated trihalomethanes may occur via a glutathione-*S*-transferase (GST) theta-mediated pathway. Based on the existing data, the related trihalomethane chloroform is not metabolized to any significant extent via the GST theta pathway. These data suggest that common pathways of metabolism (and mode of action for health effects) cannot be assumed for chloroform and the brominated trihalomethanes.

The lung is the principle route of excretion in rats and mice. Studies with ^{14}C-labeled compounds indicate that up to 88% of the administered dose can be found in exhaled air as carbon dioxide, carbon monoxide, and parent compound. Excretion in the urine generally appears to be 5% or less of the administered oral dose. Data from one study suggest that fecal excretion accounts for less than 3% of the administered dose.

Human Exposure

Brominated trihalomethanes are found in virtually all water treated for drinking; however, concentrations of individual forms vary widely depending on the type of water treatment, locale, time of year, sampling point in the distribution system, and source of the drinking water. Occurrence data for brominated trihalomethanes are available from 13 national surveys and 9 additional studies that are more restricted in scope. The procedures used for sampling, processing and storage, and calculation of summary statistics should be carefully considered when evaluating and comparing brominated trihalomethane occurrence data. Some methods restrict trihalomethane formation by refrigeration or the use of quenching agents, whereas others maximize trihalomethane formation by storage at room temperature. Approaches to data summarization vary in their treatment of data below the analytical detection level or minimum reporting level.

When all available national survey data are considered, bromodichloromethane concentrations in drinking water range from below the detection limit to 183 μg/L (ppb), while dibromochloromethane and bromoform concentrations range from below the detection limit to 280 μg/L (ppb). When data for the three brominated trihalomethanes are compared, the frequency of detection and measured concentrations of bromodichloromethane in drinking water supplies tend to be higher than those for dibromochloromethane. Bromoform is detected less frequently and at lower concentrations than the other two brominated trihalomethanes, except in some ground waters. Concentrations of all trihalomethanes in drinking water were generally lower when the raw water was obtained from ground water sources rather than surface water sources. The most recent national survey data are those collected by the U.S. EPA under the Information Collection Rule (ICR). Monitoring data were collected over an 18-month period between July 1997 and December 1998 from approximately 300 water systems operating 501 plants and serving at least 100,000 people. Summary occurrence data stratified by raw water source (groundwater or surface water) are available for finished water, the distribution system (DS) average, and the DS high values. The mean, median, and 90th percentile values for surface water DS average concentrations in the ICR survey are 8.6, 70.2, and 20.3 μg/L, respectively, for bromodichloromethane (range of individual values 0 - 65.8 μg/L); 2.4, 4.72, and 13.2 μg/L, respectively, for dibromochloromethane (range 0 - 67.3); and 0, 1.18, and 3.10, respectively, for bromoform (range 0 - 3.43).

Exposure to brominated trihalomethanes via ingestion of drinking water was estimated using data obtained for disinfectants and disinfection byproducts under the Information Collection Rule (ICR). ICR data offer several advantages over other national studies for purposes of estimating national exposure levels of adults in the United States to brominated trihalomethanes via ingestion of drinking water. First, they are recent and reflect relatively current conditions. Second, data of very similar quality and quantity were collected systematically from a large number of plants (501) and systems (approximately 300), including both surface and ground water systems. Third, the mean, median, and 90th percentile value were estimated on the basis of all samples taken, not just the sample detects. Thus, these descriptive statistics are representative of the exposures of the entire populations served by those systems, not just the populations served by systems with higher concentrations of these compounds. However, this study can not be considered representative of smaller public water supplies or water supplies from the most highly industrialized or contaminated areas.

Exposure was calculated by multiplying the concentration of individual brominated trihalomethanes in drinking water by the average daily intake, assuming that each individual consumes two liters of water per day. The annual median, mean, and upper 90th percentile values are presented for both surface and ground water systems. Assuming that the DS High value actually represents the average exposure level of persons served by one plant distribution pipe with the longest water-residence time, the DS High value might be used to estimate a high-end exposure level.

For bromodichloromethane, the median, mean, and 90th percentile population exposures from surface water systems are estimated to be 17, 20, and 40 μg/person/day, respectively. The same values for populations exposed to bromodichloromethane from ground water systems are

lower – 3.6, 8.1, and 22 μg/person/day, respectively. For dibromochloromethane, the median, mean, and 90th percentile population exposures from surface water systems are estimated to be 4.8, 9.4, and 26 μg/person/day, respectively. The corresponding values for populations exposed to dibromochloromethane from groundwater system are lower, with estimates of 2.7, 6.2, and 18 μg/person/day, respectively. For bromoform, the median, mean, and 90th percentile population exposures from surface water systems are estimated to be near 0, 2.4, and 6.2 μg/person/day, respectively. The same values for populations exposed to bromoform from ground water systems are higher, with estimates of 0.65, 3.8, and 9.6 μg/person/day, respectively.

For purposes of comparison, estimates of ingestion exposure to bromodichloromethane, dibromochloromethane, and bromoform in drinking water were also estimated from data collected in other, older studies. Ingestion from ground water supplies was estimated from the median levels found in the Ground Water Supply Survey conducted by U.S. EPA in 1980-81. Based on the range of median levels (1.4–2.1 μg/L (ppb)) and a consumption rate of two liters per day, the median ingestion exposure to bromodichloromethane may range from 2.8 to 4.2 μg/day. Similarly, median exposure to dibromochloromethane may range from 4.2 to 7.8 μg/day, and for bromoform, median exposure may range from 4.8 to 8.4 μg/day. Exposure to bromodichloromethane from surface water supplies can be estimated based on the range of median values observed under different conditions in the National Organics Monitoring Survey conducted by U.S. EPA in 1976-1977, which mainly sampled surface water systems. Based on a range of 5.9 to 14 μg/L (ppb), exposure to bromodichloromethane from surface water is estimated to be between 12 and 28 μg/day. Similarly, based on the range of medians reported for dibromochloromethane concentrations, the median exposure is estimated to be up to 6 μg/day. The median levels of bromoform in the surface water supplies have been found to be less than the EPA Drinking Water minimum reporting levels (MRLs) of 0.5 to 1 μg/L (ppb). An estimate of exposure based on the MRLs will be overly conservative because the actual concentration of bromoform is not detectable. Based on the range of MRLs, 0.5 to 1 μg/L (ppb), the exposure to bromoform is estimated to range from 1 to 2 μg/day for surface water supplies.

Ingestion exposure to brominated trihalomethanes in drinking water can also be estimated from the concentrations found at the tap in the U.S. EPA's Total Exposure Assessment Methodology (TEAM) study. Estimates of the average of the population intakes for ingestion of bromodichloromethane from drinking water range from 0.42 to 42 μg/person/day. The upper 90th percentile estimates range from <2.0 to 90 μg/person/day. Estimates of the average population intake of dibromochloromethane from drinking water range from 0.2 to 56 μg/person/day. The upper 90th percentile estimates range from < 0.9 to 86 μg/person/day. Estimates of the average of the population intakes of bromoform, for those areas in which bromoform was measurable in a majority of the samples, range from 1.6 to 16.2 μg/person/day. The upper 90th percentile estimates range from 2.4 to 26 μg/person/day. Four of the six locations in the TEAM study, however, had a low frequency (less than 10%) of detection of bromoform in measurable quantities.

Sources of uncertainty in these estimates of ingestion exposure include use of different analytical methods, failure to report quantitation limits, using measures near the detection limit, failure to report how nondetects are handled when averaging values (e.g., set to zero or one half

the detection limit), and failure to report sample storage method and duration. In addition, many environmental factors influence the concentrations of these compounds in drinking water at the tap and in vended or bottled waters used for drinking. These factors include season and temperature, geographic location, source of water, residence time in distribution system, and others.

Relatively few studies have analyzed non-beverage foods for the occurrence of brominated trihalomethanes. In the few studies available, bromodichloromethane has been detected in non-beverage foods (i.e., in one sample of butter at 7 ppb, in three samples of ice-cream at 0.6 to 2.3 ppb, in 6 of 10 samples of bean curd at 1.2 to 5.2 ppb, and in one sample of bacon). In addition, bromodichloromethane was detected in one sample each of eleven foods out of 70 tested in 14 Market Baskets for the FDA Total Diet Study. The detected concentrations ranged from 10 to 37 ppb for individual food items. Studies that analyzed non-beverage foods for dibromochloromethane and bromoform detected neither compound in any of the tested samples. Brominated trihalomethanes have been detected in up to a third or one half of the types of prepared beverages examined in some studies, being detected most frequently in colas and other carbonated soft drinks. Bromodichloromethane has been found most frequently of the three compounds and bromoform the least frequently. Bromodichloromethane was detected in approximately half of the prepared beverages examined by McNeal et al. (1995) in the United States and in all of 13 soft drinks that they analyzed. One sampled soft drink contained bromodichloromethane at a concentration of 12 ppb; the remainder of the samples contained less than 4 ppb. Bromodichloromethane was detected in one sample of fruit juice at 5 ppb.

Some data on the occurrence of brominated trihalomethanes in foods and beverages are available from studies conducted in Italy, Japan, and Finland. These studies were also limited in scope to examination of relatively few food or beverage items. Bromodichloromethane, dibromochloromethane, and bromoform concentrations measured in foods and beverages in Italy, Japan and Finland ranged from undetectable to 40 ppb, undetectable to 13.9 ppb, and undetectable to 10.7 ppb, respectively. Because of possible differences in water disinfection or food processing practices, these data may not be representative of concentrations in foods and beverages produced in the U.S.

Measured concentrations of brominated trihalomethanes in outdoor air are variable from site to site. When data from several urban/suburban and source-dominated sites in Texas, Louisiana, North Carolina and/or Arkansas were combined, the resulting average outdoor air concentrations were 110 ppt (0.74 $\mu g/m^3$) for bromodichloromethane, 3.8 ppt (0.032 $\mu g/m^3$) for dibromochloromethane, and 3.6 ppt (0.037 $\mu g/m^3$) for bromoform. A regional study conducted at several sites in southern California found bromodichloromethane, dibromochloromethane, and bromoform in 35%, 17%, and 31% of the samples, respectively. The maximum concentrations observed were 40 ppt (0.27 $\mu g/m^3$) for bromodichloromethane; 290 ppt (2.5 $\mu g/m^3$) for dibromochloromethane; 310 ppt (3.2 $\mu g/m^3$) for bromoform. Bromodichloromethane was detected in 64% (n=11) and 17% (n=6) of personal air samples collected in Texas and North Carolina. The detected concentrations ranged from 0.12 to 4.36 $\mu g/m^3$ (0.017 to 0.65 ppb). Dibromochloromethane was not detected.

Mean concentrations in indoor air ranged from 0.38 to 0.75 $\mu g/m^3$ for bromodichloromethane; 0.44 to 0.53 $\mu g/m^3$ for dibromochloromethane, and 0.29 to 0.35 $\mu g/m^3$ for bromoform, as determined from 15 minute samples collected in 48 New Jersey residences. It was not clear whether these values were based exclusively on detected concentrations. In a separate study, levels of brominated trihalomethanes in indoor air were locally increased (e.g., in shower/bath enclosures and vanity areas) during showering and bathing events. The levels of individual brominated trihalomethanes in air were reported to be consistent with the levels in tap water.

Bromoform and dibromochloromethane have been identified in soil and sediment samples collected at NPL hazardous waste sites. Soils and other unconsolidated surficial materials may become contaminated with bromoform and dibromochloromethane by chemical spills, the landfilling of halomethane-containing solid wastes, or the discharge of chlorinated water. However, no data were located to suggest that land releases are a significant source of the chemicals in the environment (ATSDR, 2003).

The chemical and physical properties of the brominated trihalomethanes indicate that they should volatilize readily from wet or dry soil surfaces. Bromoform and dibromochloromethane have only a minor tendency to be adsorbed by soils and sediments and will tend to be highly mobile. Therefore, ingestion of soil is not expected to be a significant route of exposure (ATSDR, 2003).

Brominated trihalomethanes have been detected in the blood and breast milk of humans. The level of individual brominated trihalomethanes in blood increases shortly after exposure to these compounds in tap water (by dermal contact and/or inhalation of the volatilized compound) during bathing and showering. Dibromochloromethane was detected in one of eight samples of breast milk collected from women living in the vicinity of U.S. chemical manufacturing plants or user facilities.

The RSC (relative source contribution) is the percentage of total daily exposure that is attributable to tap water when all potential sources are considered (e.g., air, food, soil, and water). Ideally, the RSC is determined quantitatively using nationwide, central tendency and/or high-end estimates of exposure from each relevant medium. In the absence of such data, a default RSC ranging from 20% to 80% may be used.

The RSC used in the current and previous drinking water regulations for dibromochloromethane is 80%. This value was established by use of a screening level approach to estimate and compare exposure to dibromochloromethane from various sources. Information considered for use during this process is summarized in Appendix C. There are some uncertainties in the 80% RSC that are related to the availability of adequate concentration data for dibromochloromethane in media other than water. Parallel RSC calculations were not performed for bromodichloromethane and bromoform. The EPA has set the regulatory level for these chemicals in drinking water at zero because it has been determined that they are probable human carcinogens. Therefore, determination of an RSC is not relevant for these chemicals because it is the Agency's policy to perform RSC analysis only for noncarcinogens.

The use of chemicals containing chlorine and bromine to disinfect swimming pools and hot tubs results in the formation of brominated trihalomethanes. Swimming pool and hot tub users are potentially exposed to brominated trihalomethanes via dermal contact, ingestion, and inhalation of compounds released to the overlying air. As a result, swimming pool and hot tub users may experience greater overall exposures to brominated trihalomethanes than the general population. One study indicated that bromodichloromethane, dibromochloromethane, and bromoform concentrations in swimming pool and hot tub water ranged from 1 to 105 μg/L (ppb), from 0.1 to 48 μg/L (ppb), and from less than 0.1 to 62 μg/L (ppb), respectively. Concentrations of the same brominated trihalomethanes in the air two meters above the pool water ranged from less than 0.1 to 14 $\mu g/m^3$ (0.015 to 2.09 ppb), from less than 0.1 to 10 $\mu g/m^3$ (0.011 to 1.2 ppb), and from less than 0.1 to 5.0 $\mu g/m^3$ (0.0097 to 0.48 ppb), respectively. Data from several studies confirm the uptake of brominated trihalomethanes from swimming pools, hot tubs, and environs by dermal and/or inhalation pathways.

Health Effects of Acute and Short-term Exposure of Animals

Large oral doses of brominated trihalomethanes are lethal to mice and rats. Reported acute LD_{50} values range from 450 to 969 mg/kg for bromodichloromethane, 800 to 1200 mg/kg for dibromochloromethane, and 1388 to 1550 mg/kg for bromoform.

Acute oral exposure to sublethal doses of brominated trihalomethanes can also produce effects on the central nervous system, liver, kidney, and heart. Ataxia, anaesthesia, and/or sedation were noted in mice receiving 500 mg/kg bromodichloromethane, 500 mg/kg dibromochloromethane, or 1000 mg/kg bromoform. Renal tubule degeneration, necrosis, and elevated levels of urinary markers of renal toxicity have been observed in rats receiving 200 to 400 mg/kg bromodichloromethane. Elevated levels of serum markers for hepatotoxicity and have been observed in rats at doses of bromodichloromethane ranging from approximately 82 to 400 mg/kg-day, and hepatocellular degeneration and necrosis were observed at 400 mg/kg. Effects on heart contractility were reported in male rats at doses of 333 and 667 mg/kg dibromochloromethane.

Short-term oral exposure of laboratory animals to brominated trihalomethanes has been observed to cause effects on the liver and kidney. Hepatic effects, including organ weight changes, elevated serum enzyme levels, and histopathological changes, became evident in mice and/or rats administered 38 to 250 mg/kg-day bromodichloromethane, 147 to 500 mg/kg-day dibromochloromethane, or 187 to 289 mg/kg-day bromoform for 14 to 30 days. Kidney effects, characterized by decreased p-aminohippurate uptake, histopathological changes, and organ weight changes, became evident in mice and/or rats administered 148 to 300 mg/kg-day bromodichloromethane, 147 to 500 mg/kg-day dibromochloromethane, or 289 mg/kg-day bromoform for 14 days. Evidence for decreased immune function was noted at bromodichloromethane and dibromochloromethane doses of 125 mg/kg-day.

The inhalation toxicity of bromodichloromethane has been evaluated in wild type and p53 heterozygous FVB/N and C57BL/N mice. Dose-related renal tubular degeneration, and associated regenerative cell proliferation were seen in all strains at concentrations of 10 ppm and

above after one week of exposure. Dose-related increases in hepatic degeneration and regenerative cell proliferation were observed at 30 ppm and above. After three weeks of exposure, macroscopic and histologic lesions in the kidney and liver were resolved and cell proliferation indices had returned to near baseline levels. Pathological changes were more severe in the FVB/N compared to the C57BL/N mice and were more severe in the heterozygotes than in the wild type strains.

Health Effects of Subchronic and Chronic Exposure of Animals

The predominant effects of subchronic oral exposure to brominated trihalomethanes occur in the liver and kidney. The effects produced in these two organs are similar in nature to those described for short-term exposures, with liver appearing to be the most sensitive target organ for dibromochloromethane and bromoform exposure. Histopathological changes in the liver were reported in mice and/or rats administered 200 mg/kg-day bromodichloromethane, 50 to 250 mg/kg-day dibromochloromethane, or 50 to 283 mg/kg-day bromoform. Histopathological changes in the kidney were reported in mice and/or rats administered 100 mg/kg-day bromodichloromethane, or 250 mg/kg-day dibromochloromethane.

As observed for shorter durations of exposure, the predominant effects of chronic oral exposure are observed in the liver and kidney. Histopathological signs of hepatic toxicity in mice and/or rats were evident at doses of 6 to 50 mg/kg-day for bromodichloromethane, 40 to 50 mg/kg-day for dibromochloromethane, and 90 to 152 mg/kg-day for bromoform. Signs of bromodichloromethane-induced renal toxicity became evident in mice and rats treated with doses of 25 and 50 mg/kg-day, respectively.

Reproductive/Developmental Effects in Animals

Reproductive and developmental studies of brominated trihalomethanes are summarized in Table V-9. Signs of maternal toxicity (decreased body weight, body weight gain and/or changes in organ weight) were reported in rats administered bromodichloromethane at 25 to 200 mg/kg-day and in rabbits administered 4.9 to 35.6 mg/kg-day. Signs of maternal toxicity were observed in rats or mice administered 17 (marginal) to 200 mg/kg-day dibromo-chloromethane and in mice administered 100 mg/kg-day bromoform. Maternal toxicity was not observed in female rats dosed with up to 200 mg/kg-day of bromoform. Several well-conducted studies on the developmental toxicity of bromodichloromethane gave negative results at doses up to 116 mg/kg-day in rats and 76 mg/kg-day in rabbits when administered in drinking water. However, in other studies, slightly decreased numbers of ossification sites in the hindlimb and forelimb were observed in fetuses of Sprague-Dawley rats administered 45 mg/kg-day in the drinking water on gestation days 6 to 21 and sternebral aberrations were observed in the offspring of Sprague-Dawley rats administered 200 mg/kg-day by gavage in corn oil. Reductions in mean pup weight gain and pup weight were observed when the pups were administered bromodichloromethane in the drinking water at concentrations of 150 ppm and above (biologically meaningful estimates of intake on a mg/kg-day basis could not be calculated for this study). Full litter resorption has been noted in F344 rats, but not Sprague-Dawley rats, treated with bromodichloromethane at doses of 50 to 100 mg/kg-day during gestation.

Additional studies in F344 rats that varied the timing of bromodichloromethane administration indicate that gestation days 6-10 are a critical period for induction of full litter resorption. Chronic oral exposure of male F344 rats to bromodichloromethane resulted in reduced sperm velocities at doses of 39 mg/kg-day. This response was not accompanied by histopathological changes in any reproductive tissue examined. Adverse clinical signs and reduced body weight and body weight gain were observed in parental generation female rats and F_1 male and female rats at 150 ppm (approximately 11.6 to 40.2 mg/kg-day) in a two generation drinking water study of bromodichloromethane. In the same study, small but statistically significant delays in sexual maturation occurred in F_1 males at 50 ppm (approximately 11.6 to 40.2 mg/kg-day) and F_1 females at 450 ppm (approximately 29.5 to 109 mg/kg-day). These delays may have been secondary to dehydration caused by taste aversion to bromodichloromethane in the drinking water.

Two studies on the reproductive or developmental toxicity of dibromochloro-methane gave negative results when tested at doses of up to 200 mg/kg-day. In another separate study, dibromochloromethane administered at 17 mg/kg-day in a multigenerational study resulted in reduced day 14 postnatal body weight in one of two F_2 generation litters. At 171 mg/kg-day, the mid-dose in the study, decreased litter size, viability index, lactation index, and postnatal body weight were observed in some F_1 and/or F_2 generations.

The developmental and reproductive toxicity of bromoform has been examined in two studies. Bromoform administered to Sprague-Dawley rats at 100 mg/kg-day in corn oil by gavage resulted in a significant increase in sternebral aberrations in the apparent absence of maternal toxicity. In a continuous breeding toxicity protocol, gavage doses of 200 mg/kg-day in corn oil resulted in decreased postnatal survival, organ weight changes, and liver histopathology in F_1 ICR Swiss mice of both sexes. No effects on fertility or other reproductive endpoints were noted.

Mutagenicity and Carcinogenicity Studies

In vitro and *in vivo* studies of the mutagenic and genotoxic potential of bromodichloromethane, dibromochloromethane, and bromoform have yielded mixed results. Synthesis of the overall weight of evidence from these studies is complicated by the use of a variety of testing protocols, different strains of test organisms, different activating systems, different dose levels, different exposure methods (gas versus liquid), and, in some cases, problems due to evaporation of the test chemical. Overall, a majority of studies yielded more positive results for bromoform and bromodichloromethane. The genotoxicity and mutagenicity data for dibromochloromethane are inconclusive. Recent studies in strains of *Salmonella* that contain rat theta-class glutathione S-transferase suggest that mutagenicity of the brominated trihalomethanes may also be mediated by glutathione conjugation.

Carcinogenicity and Related Studies in Animals

The carcinogenic potential of individual brominated trihalomethanes administered in oil has been investigated in chronic oral exposure studies in mice and rats. Ingestion of

bromodichloromethane caused liver tumors in female mice, renal tumors in male mice and in male and female rats, and tumors of the large intestine in male and female rats. Ingestion of dibromochloromethane caused liver tumors in male and female mice, and ingestion of bromoform caused intestinal tumors in male and female rats.

Studies of induction of aberrant crypt foci (ACF) show that bromodichloromethane, dibromochloromethane, and bromoform given in drinking water significantly increase the number and focal area of ACF in the colons of male F344 rats, Eker rats, and strain A/J mice, but not in colons of $B6C3F_1$ mice. The biological significance of this induction is unclear, as intestinal tumors have not been observed either in the colons of F344 rats treated with dibromochloromethane by corn oil gavage or in the colons of rats exposed to bromodichloromethane in the drinking water for two years. Administration of individual brominated trihalomethanes in a high animal fat diet did not significantly increase the number of ACF when compared to a diet containing normal levels of fat.

Exposure of male and female rats Eker rats (a rodent hereditary model of renal cancer) to bromodichloromethane did not significantly increase the incidence of urinary bladder epithelial hyperplasia, individual cell hypertrophy, renal tumors, hemangioma of the spleen, or leiomyomas or mesenchymal cell proliferation in the uterus of females.

Other Key Health Effects Data from Animal Studies

The immunotoxicity of brominated trihalomethanes has been investigated in mice and rats. Short-term bromodichloromethane exposure resulted in decreased antibody forming cells in serum, decreased hemagglutinin titers, and/or suppression of Con A-stimulated proliferation of spleen cells at doses of 125 to 250 mg/kg-day.

No studies have been reported for hormonal effects following exposure to dibromochloromethane or bromoform. There is evidence from studies in F344 rats and cultured human placental trophoblasts that bromodichloromethane causes hormonal disruption. Rats exposed to bromodichloromethane on gestation days 8 or 9 show reduced serum levels of LH and progesterone. Serum LH reductions indicate that the mode of action for this strain-specific effect involves altered LH secretion; however, a contributing effect on LH signal transduction has not been ruled out.

Exposure to bromodichloromethane alters the function of human placental trophoblasts, as shown by reduced CG secretion and by changes in morphological differentiation. The mode of action for the observed effects is unknown. Possible mechanisms proposed by the study authors for effects on CG secretion include disruption of CG synthesis at the translational or post-translational level (e.g., by altering glycosylation of CG subunits or disruption of dimerization) or indirect effects on secretion via disruption of gonadotropin releasing hormone activity. The significance of these findings for human health is that placental trophoblasts are the sole source of CG during normal human pregnancy and play a major role in the maintenance of the conceptus. If the observed effect on CG secretion is substantiated in future studies, it may help to explain apparent adverse pregnancy outcomes associated with consumption of

chlorinated drinking water in some epidemiological studies (e.g., increased incidence of spontaneous abortion as reported by Waller et al., 1998).

Limited structure-activity data for brominated trihalomethanes and chloroform suggest that bromination may influence the proportion of compound metabolized via the oxidative and reductive pathways, with brominated compounds being more extensively metabolized by the reductive pathway. Additional evidence suggests that a GST-mediated pathway may play an important role in metabolism of brominated trihalomethanes.

Health Effects in Humans

Limited human health data are available for the brominated trihalomethanes. In the past, bromoform was used as a sedative for children with whooping cough. Doses of 50 to 100 mg/kg-day usually produced sedation without apparent adverse effects. Some rare instances of death or near-death were reported, although these cases were generally attributed to accidental overdoses. No human toxicological data were available for bromodichloromethane or dibromochloromethane.

Numerous epidemiological studies have examined the association between water chlorination and increased cancer incidence. Very few studies have examined the association between cancer and exposure to brominated trihalomethanes, and possible increased cancer incidence in bladder was suggested. Recent studies have examined the association of chlorinated water use with various pregnancy outcomes, including low birth weight, premature birth, intrauterine growth retardation, spontaneous abortion, stillbirth, and birth defects. An association has been reported for exposure to bromodichloromethane (or a closely associated compound) and a moderately increased risk of spontaneous abortion during the first trimester. An association has also been reported for exposure to bromodichloromethane (or a closely associated compound) and 1) stillbirth of fetuses weighing more than 500 g, 2) reduction in birth weight (small for gestational age), and 3) increased risk of neural tube defects in women exposed to $\geq$20 µg/L of bromodichloromethane prior to conception through the first month of pregnancy. An association has been reported for total brominated trihalomethanes and reduced menstrual cycle and follicular phase length in women of child-bearing age. Among the individual brominated trihalomethanes, dibromochloromethane displayed the strongest association with altered menstrual function. A study of semen quality in healthy men found an association between increased exposure to bromodichloromethane in residential tap water and decreased sperm linearity; exposure to dibromochloromethane or bromoform was not associated with decrements in semen quality.

To directly conclude that bromodichloromethane and dibromochloromethane are developmental or reproductive toxicants in humans can be complicated by the fact that there are many disinfection byproducts in chlorinated water. Nevertheless, these studies raise significant concern for possible human health effects. The methodology used to estimate exposure to brominated trihalomethanes in tap water has been examined with the goal of refining estimates of intake of these compounds in epidemiological studies.

Susceptible Populations

There is currently no clear evidence that children or the fetus are at greater risk for adverse effects from exposure to bromoform or dibromochloromethane than are adults. Associations between concentration of bromodichloromethane (or a co-occurring chemical) and spontaneous abortion or still birth have been observed in several epidemiological studies. Evidence in rats indicates that exposure to bromodichloromethane causes full litter resorption in F344 rats but not Sprague-Dawley rats. Full litter absorption appears to result from a maternally-mediated mode of action, rather than from a direct effect on the embryo. A mechanism of action for bromodichloromethane-related pregnancy loss is suggested for the rat (reduced sensitivity of the corpus luteum to luteinizing hormone), but is not without alternative explanation. At present, there is insufficient information on the mode of action leading to full litter resorption in rats to fully evaluate the relevance of this outcome to potential reproductive and/or developmental toxicity in humans. There is presently no evidence that infants, children, or the fetus are at increased risk for brominated trihalomethane toxicity as a result of higher levels of metabolizing enzymes.

Subpopulations with either high levels of glutathione *S*-transferase or low baseline levels of glutathione may potentially be more sensitive than the general population to brominated trihalomethane-induced toxicity, but there are currently no epidemiological or animal data to confirm this speculation. The functional significance of polymorphisms in cytochrome P450 isoforms that metabolize brominated trihalomethanes is also unknown.

Mechanism of Toxicity

It is generally believed that the toxicity of the brominated trihalomethanes is related to their metabolism. This conclusion is based largely on the observation that liver and kidney, the chief target tissues for these compounds, are also the primary sites of their metabolism. In addition, treatments which increase or decrease metabolism also tend to increase or decrease trihalomethane-induced toxicity in parallel.

Metabolism of brominated trihalomethanes is believed to occur via oxidative and reductive pathways. Limited structure-activity data for brominated trihalomethanes and the structurally-related trihalomethane chloroform suggest that bromination may influence the proportion of compound metabolized via the oxidative and reductive pathways, with brominated compounds being more extensively metabolized by the reductive pathway. Additional evidence suggests that a GSH-mediated pathway may also play an important role in metabolism of brominated trihalomethanes. These data raise the possibility that brominated trihalomethanes may induce adverse effects (toxicity and carcinogenicity) via several different pathways.

The precise biochemical mechanisms which link brominated trihalomethane metabolism to toxicity have not been characterized, but many researchers have proposed that toxicity results from the production of reactive intermediates. Reactive intermediates may arise from either the oxidative (dihalocarbonyls) or the reductive (free radicals) pathways of metabolism. Such reactive intermediates are known to form covalent adducts with various cellular molecules, and

may impair the function of those molecules and cause cell injury. Free radical production may also lead to cell injury by inducing lipid peroxidation in cellular membranes. Direct evidence showing a relationship between the level of covalent binding intermediates generated by either pathway and the extent of toxicity is not available for the brominated trihalomethanes. Manipulation of cellular glutathione levels suggests that this compound may play a protective role in brominated trihalomethane-induced toxicity.

Individual brominated trihalomethanes have been shown to induce tumors in laboratory animals. The mode of action by which brominated trihalomethanes induce tumors in target tissues has not been fully characterized. DNA adducts can be formed by interaction of reactive metabolites (resulting from oxidative and reductive metabolism) with DNA. In addition, preliminary evidence suggests that DNA adducts can be formed through conjugation with glutathione and bioactivation of the resulting conjugates. The role of cytotoxicity and associated regenerative cell proliferation in tumorigenicity of brominated trihalomethanes is presently unclear. Comparison of dose-response data for liver and kidney toxicity (including cell proliferation) and tumorigenicity in mice and rats suggests that tumor formation occurs at concentrations lower than those which stimulate cell proliferation.

Interaction with agents which increase or decrease the activity of enzymes responsible for metabolism of brominated trihalomethanes may modify carcinogenicity/toxicity. Pretreatment with inducers of CYP2E1 has been observed to increase the hepatotoxicity of bromodichloromethane and dibromochloromethane in male rats. Pretreatment with m-xylene, an inducer of the CYP2B1/CYP2B2 isoforms, increased the hepatotoxicity of dibromochloromethane in male rats. Conversely, administration of the cytochrome P450 inhibitor 1-aminobenzotriazole prevented bromodichloromethane-induced hepatotoxicity in rats. Recent findings indicating possible glutathione-mediated metabolism of brominated trihalomethanes suggest that treatments or agents which alter glutathione-*S*-transferase activity could potentially modify the toxicity of brominated trihalomethanes.

The severity of brominated trihalomethane toxicity is potentially affected by the vehicle of administration. In a study of vehicle effects on the acute toxicity of bromodichloromethane, a high dose (400 mg/kg) of the chemical was more hepato- and nephrotoxic when given in corn oil compared to aqueous administration, but this difference was not evident at a lower dose (200 mg/kg).

Quantification of Noncarcinogenic Effects

Candidate health effects endpoints were analyzed by benchmark dose (BMD) modeling using a benchmark response of 10% extra risk. The $BMDL_{10}$ was defined as the 95% lower bond on the BMD estimate. For bromodichloromethane, a $BMDL_{10}$ of 30 mg/kg-day identified on the basis of full litter resorption in F344 rats was used to calculate a One-day Health Advisory (HA) value of 1 mg/L. A $BMDL_{10}$ of 18 mg/kg-day for single cell hepatic necrosis, identified in a 30-day drinking water study in rats, was used to calculate a Ten-day HA value of 0.6 mg/L. A $BMDL_{10}$ of 18 mg/kg-day for reduced maternal body weight gain on gestation days 6-9, identified in a developmental study in rats, was used to calculate a Longer-term HA of 0.6 mg/L

for a 10-kg child. A Longer-term HA value of 2 mg/L was calculated for a 70-kg adult based on the same endpoint. The calculations for the Reference Dose (RfD) of 0.003 mg/kg-day and Drinking Water Exposure Level (DWEL) of 90 μg/L employed a duration adjusted $BMDL_{10}$ of 0.8 mg/kg-day for fatty degeneration of the liver, identified in a 24 month dietary study in rats. Because bromodichloromethane is classified as a probable human carcinogen, a Lifetime HA is not recommended.

For dibromochloromethane, no suitable study was located for the calculation of a One-day HA value. Use of the 10-day HA value as a conservative estimate is recommended. The Ten-day HA value of 0.6 mg/L was calculated using a $BMDL_{10}$ of 5.5 mg/kg-day for hepatic cell vacuolization, identified in a 28-day feeding study in rats. A duration-adjusted $BMDL_{10}$ value of 1.7 mg/kg-day for hepatic cell vacuolization, identified in a 13-week gavage study in rats, was used to calculate Longer-term HA values of 0.2 and 0.6 mg/L for a 10-kg child and a 70-kg adult, respectively. A duration-adjusted $BMDL_{10}$ value of 1.6 mg/kg-day for fatty changes identified in a 2 year gavage study in mice was used to calculate a RfD of 0.02 mg/kg-day and a DWEL of 700 μg/L. The Lifetime HA for dibromochloromethane is 60 μg/L. This value was calculated using the default RSC value of 80% for exposure via ingestion of drinking water. Because this compound is classified as a possible human carcinogen, the derivation of the Lifetime HA incorporated an uncertainty factor of 10.

For bromoform, an estimated dose of 54 mg/kg-day that caused sedation in children was used to calculate a One-day HA value of 5 mg/L. A $BMDL_{10}$ of 2.3 mg/kg-day for hepatic vacuolization, identified in a one month dietary study in rats, was used to calculate a value of 0.2 mg/L for the Ten-day HA for the 10-kg child. This value was also recommended for use as the Longer-term HA for a 10 kg child. A duration-adjusted $BMDL_{10}$ value of 2.6 mg/kg-day for hepatic vacuolization, identified in a 13 week gavage study in rats, was also used to calculate a value of 0.9 mg/L for the Longer-term HA for the 70-kg adult. The $BMDL_{10}$ value of 2.6 mg/kg-day was also used to calculate an RfD of 0.03 mg/kg-day and a DWEL of 1000 μg/L. Because bromoform is classified as a probable human carcinogen, a Lifetime HA is not recommended.

Quantification of Carcinogenic Effects

Chronic oral exposure studies performed by the National Toxicology Program in rats and mice provide adequate data to derive quantitative cancer risk estimates for the three brominated trihalomethanes, although the chemicals were administered in a corn oil vehicle. For bromodi-chloromethane, a unit risk of 1.0×10^{-6} $(\mu g/L)^{-1}$ was derived, based on the incidence of renal tumors in male mice. The oral slope factor and concentration for excess cancer risk of 10^{-6} were 3.5×10^{-2} $(mg/kg\text{-}day)^{-1}$ and 1.0 μg/L, respectively. For dibromochloromethane, a unit risk of 1.2×10^{-6} $(\mu g/L)^{-1}$ was derived, based on liver tumors in female mice. The oral slope factor and concentration for excess cancer risk of 10^{-6} were 4.3×10^{-2} $(mg/kg\text{-}day)^{-1}$ and 0.8 μg/L, respectively. For bromoform, a unit risk of 1.3×10^{-7} $(\mu g/L)^{-1}$ was derived, based on tumors of the large intestine in female rats. The oral slope factor and concentration for excess cancer risk of 10^{-6} were 4.6×10^{-3} $(mg/kg\text{-}day)^{-1}$ and 8 μg/L, respectively. These values were calculated using an animal-to-human scaling factor of body $weight^{3/4}$ in accordance with proposed U.S. EPA guidance (U.S. EPA, 1992b; 1999).

In a previous assessment of the carcinogenicity of brominated trihalomethanes, the Carcinogenic Risk Assessment Verification Endeavor (CRAVE) group of the U.S. EPA assigned bromodichloromethane and bromoform to Group B2: probable human carcinogen. CRAVE assigned dibromochloromethane to Group C: possible human carcinogen. Under the proposed 1999 U.S. EPA Guidelines for Cancer Assessment, bromodichloromethane and bromoform are *likely to be carcinogenic to humans* by all routes of exposure. This descriptor is appropriate when the available tumor data and other key data are adequate to demonstrate carcinogenic potential to humans. This finding is based on the weight of experimental evidence in animal models which shows carcinogenicity by modes of action that are relevant to humans. Dibromochloromethane shows *suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential*. This descriptor is used when the evidence from human or animal data is suggestive of carcinogenicity, which raises a concern for carcinogenic effects but is not judged sufficient for a conclusion as to human carcinogenic potential. This finding is based on the weight of experimental evidence in animal models which indicate limited or equivocal evidence of carcinogenicity.

IARC has recently re-evaluated the carcinogenic potential of the brominated trihalomethanes. IARC classified bromodichloromethane as a Group 2B carcinogen: possibly carcinogenic to humans. IARC classified dibromochloromethane and bromoform as Group 3: not classifiable as to carcinogenicity in humans.

Table I-1 summarizes the quantification of noncarcinogenic and carcinogenic effects for brominated trihalomethanes.

Table I-1 Summary of Quantification of Toxicological Effects for Brominated Trihalomethanes

Advisory	Value	Reference
Bromodichloromethane		
One-day HA for 10-kg child	1 mg/L	Narotsky et al. (1997)
Ten-day HA for 10-kg child	0.6 mg/L	NTP (1998)
Longer-term HA for 10-kg child	0.6 mg/L	CCC (2000d)
Longer-term HA for 70-kg adult	2 mg/L	CCC (2000d)
RfD	0.003 mg/kg-day	Aida et al. (1992b)
DWEL	100 µg/L	Aida et al. (1992b)
Lifetime HA	Not applicable	--
Oral Slope Factor [c]	3.5×10^{-2} $(mg/kg\text{-}day)^{-1}$	NTP (1987)
Concentration for excess cancer risk (10^{-6})	1.0 µg/L	NTP (1987)
Unit Risk	$1x10^{-6}$ $(\mu g/L)^{-1}$	NTP (1987)
Dibromochloromethane		
One-day HA for 10-kg child [b]	0.6 mg/L	Aida et al. (1992a)
Ten-day HA for 10-kg child	0.6 mg/L	Aida et al. (1992a)
Longer-term HA for 10-kg child	0.2 mg/L	NTP (1985)
Longer-term HA for 70-kg adult	0.6 mg/L	NTP (1985)
RfD	0.02 mg/kg-day	NTP (1985)
DWEL	700 µg/L	NTP (1985)
Lifetime HA	60 µg/L	NTP (1985)
Oral Slope Factor [c]	4.3×10^{-2} $(mg/kg\text{-}day)^{-1}$	NTP (1985)
Concentration for Excess cancer risk (10^{-6})	0.8 µg/L	NTP (1985)
Unit Risk	1.2×10^{-6} $(\mu g/L)^{-1}$	NTP (1985)
Bromoform		
One-day HA for 10-kg child	5 mg/L	Burton-Fanning (1901)
Ten-day HA for 10-kg child	0.2 mg/L	NTP (1989a)
Longer-term HA for 10-kg child [a]	0.2 mg/L	NTP (1989a)
Longer-term HA for 70-kg adult	0.9 mg/L	NTP (1989a)
RfD	0.03 mg/kg-day	NTP (1989a)
DWEL	1000 µg/L	NTP (1989a)

Table I-1 (cont.)

Advisory	Value	Reference
Lifetime HA	Not applicable	--
Oral Slope Factor [c]	4.6×10^{-3} $(mg/kg\text{-}day)^{-1}$	NTP (1989a)
Concentration for Excess cancer risk (10^{-6})	8 µg/L	NTP (1989a)
Unit Risk	1.3×10^{-7} $(\mu g/L)^{-1}$	NTP (1989a)

[a] The calculated value for the Longer-term HA was slightly higher than the values for the Ten-day HAs. Therefore, use of the Ten-day HA for a 10-kg child is recommended as an estimate of the Longer-term HA for a 10-kg child.
[b] Use of the Ten-day HA recommended as an estimate of the One-day HA for a 10-kg child.
[c] The oral slope factor was calculated using the Linearized Multistage model (extra risk) and an animal-to-human scaling factor of body weight$^{3/4}$

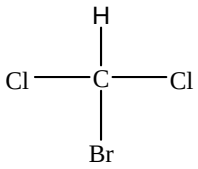
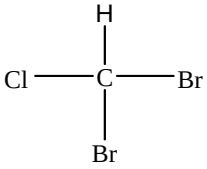
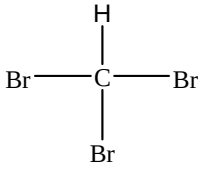
Abbreviations: BW, Body weight; DWEL, Drinking water exposure limit; HA, Health advisory; LMS; Linearized Multistage Model

II. PHYSICAL AND CHEMICAL PROPERTIES

A. Properties and Uses

Bromodichloromethane ($CHBrCl_2$), dibromochloromethane ($CHBr_2Cl$) and bromoform ($CHBr_3$) are clear liquids with higher densities than the structurally-related compound chloroform. They have limited solubility in water but are very soluble in organic solvents (Windholz, 1976). Some important physical and chemical properties of these bromine-containing trihalomethanes are summarized in Table II-1. Brominated trihalomethanes are sufficiently volatile to evaporate from drinking water (Jolley et al., 1978).

Table II-1 Physical and Chemical Properties of the Brominated Trihalomethanes

Property	Chemical		
	Bromodichloromethane	**Dibromochloromethane**	**Bromoform**
Structure			
Chemical Abstracts Registry Number	75-27-4	124-48-1	75-25-2
Registry of Toxic Effects of Chemical Substances Number	PA 5310000	PA 6360000	PB 5600000
Synonyms	dichlorobromomethane	chlorodibromomethane	tribromomethane
Chemical Formula	$CHBrCl_2$	$CHBr_2Cl$	$CHBr_3$
Molecular Weight	163.83	208.29	252.77
Boiling Point	90˚C	116C	149 - 150˚C
Melting Point	-57.1˚C	--	6-7˚C
Specific Gravity (20˚)	1.980	2.38	2.887
Vapor Pressure	50mm (20˚C)	15 mm (10˚C)	5.6 mm (25˚C)
Stability in Water	volatile	volatile	volatile
Water Solubility	3,032 mg/L (30˚C)	1,050 mg/L (30˚C)	3,190 mg/L (30˚C)
Log Octanol:Water Partition Coefficient (K_{ow})	2.09	2.23	2.37

Source: U.S. EPA (1992c; 1994b)

Brominated trihalomethanes also occur in drinking water as by-products of chlorination. Bromide (Br^-), a common constituent of natural waters, is oxidized by hypochlorous acid ($HOCl_3$) to form hypobromous acid (HOBr) in the following reaction:

$$Br^- + HOCl_3 \rightarrow HOBr + Cl^-$$

Hypobromous acid reacts with naturally occurring organic substances in water (e.g., humic and fulvic acids) to yield the bromine-containing trihalomethanes bromoform, dibromochloromethane and bromodichloromethane (in increasing order of formation rates) (Jolley et al., 1978).

Trihalomethanes may also be produced by reaction with endogenous organic material in the gut. Mink et al. (1983) treated adult male Sprague-Dawley rats with a single oral dose of 48 mg Cl (as sodium chloroacetate) and 32 mg Br^- (as potassium bromide). All three brominated trihalomethanes were detected in the stomach contents of nonfasted rats following treatment (Mink et al., 1983). Bromoform and dibromochloromethane were also detected in the plasma.

In the past, bromoform, bromodichloromethane and dibromochloromethane have been used in pharmaceutical manufacturing and chemical synthesis, as ingredients in fire-resistant chemicals and gauge fluids, and as solvents for waxes, greases, resins, and oils (U.S. EPA, 1975). However, use patterns have changed over time. At present, the primary use of bromodichloromethane is as a chemical intermediate for organic synthesis and as a laboratory reagent (ATSDR 1989). Dibromochloromethane is reportedly used in laboratory quantities only (ATSDR 1990). Use of bromoform is limited to performance of geological tests, use as a laboratory reagent, and use in quality assurance programs in the electronics industry (ATSDR 1990).

B. Summary

Brominated trihalomethanes are volatile organic liquids that occur in drinking water as by-products of disinfection with chlorine. The brominated trihalomethanes occurring in water are bromoform, dibromochloromethane and bromodichloromethane. These compounds are formed in water when hypochlorous acid oxidizes bromide ions to form hypobromous acid, which subsequently reacts with organic material. In the past, individual brominated trihalomethanes have been used for a variety of industrial purposes. Currently, these compounds are used as laboratory reagents and, in the case of bromodichloromethane, as an intermediate in chemical synthesis.

III. TOXICOKINETICS

This section summarizes available information on the absorption, distribution, metabolism and excretion of brominated trihalomethanes. Because the toxicokinetic properties of brominated trihalomethanes appear to be generally similar, data in this section are presented for this class of compounds as a group, rather than by individual chemical.

A. Absorption

Mink et al. (1986) compared the absorption of bromodichloromethane, dibromochloromethane, and bromoform in male Sprague-Dawley rats and male B6C3F$_1$ mice. The study animals received single oral doses of ^{14}C-labeled compound in corn oil by gavage at dose levels of 100 mg/kg (rats) or 150 mg/kg (mice). Total recovery of label in exhaled air, urine, or tissues after 8 hours ranged from 62% to 93% (Table III-1), indicating that gastrointestinal absorption was high for all three compounds. The level of radiolabeled carbon monoxide in exhaled air was not quantified in this experiment. Carbon monoxide has since been recognized as a product of brominated trihalomethane catabolism.

Mathews et al. (1990) administered ^{14}C-bromodichloromethane by gavage in corn oil to male Fischer 344 rats at doses of 1, 10, 32, or 100 mg/kg, and monitored the radiolabel in exhaled air, urine, feces, and tissues. Absorption was extensive, with at least 86% of the dose recovered as expired volatiles, carbon dioxide, or carbon monoxide. Only small amounts were recovered in urine (<5%) or in feces (<3%) within 24 hours of administration, regardless of the size of the dose (Table III-2).

Table III-1 Recovery of Label 8 Hours after Oral Administration of ^{14}C-Labeled Brominated Trihalomethanes to Male Sprague-Dawley Rats or Male B6C3F$_1$ Mice

Chemical	Percent of Label					
	Species	Expired CO_2	Expired Parent	Urine	Organs	Total Recovery
Bromodichloromethane	Rat	14.2	41.7	1.4	3.3	62.7
	Mouse	81.2	7.2	2.2	3.2	92.7
Dibromochloromethane	Rat	18.2	48.1	1.1	1.4	70.3
	Mouse	71.6	12.3	1.9	5.0	91.6
Bromoform	Rat	4.3	66.9	2.2	2.1	78.9
	Mouse	39.7	5.7	4.6	12.2	62.2

Adapted from Mink et al. (1986) and U.S. EPA (1994b).

Table III-2 Cumulative Excretion of Label after Oral Administration of ^{14}C-Labeled Bromodichloromethane to Male F344 Rats

Dose	Time (hrs post-treatment)	Percent of Dose					
		Expired CO_2	Expired CO	Expired Volatiles	Urine	Feces	Total Recovery
1 mg/kg	1	9.5±1.1	NR[a]	2.1±1.5	NR	NR	11.6±1.3
	4	37.0±3.2	1.5±0.7	2.7±1.8	NR	NR	41.1±2.8
	8	62.9±2.2	2.7±1.1	NR	NR	NR	68.±1.7
	16	76.4±3.2	NR	NR	NR	NR	81.8±2.9
	24	77.5±3.3	3.3±1.5	3.0±1.6	4.1±0.2	2.7±1.5	90.7±1.8
10 mg/kg	1	8.0±2.0	NR	2.0±0.8	NR	NR	10.0±1.85
	4	39.9±3.2	1.9±0.4	2.7±1.1	NR	NR	44.5±3.0
	8	66.0±4.0	3.4±0.9	NR	NR	NR	72.1±3.9
	16	81.3±1.7	NR	NR	NR	NR	87.4±1.5
	24	82.1±1.8	4.3±1.0	2.8±1.1	4.3±0.2	0.7±0.2	94.2±1.6
100 mg/kg	1	1.9±0.9	0.1±0	1.5±1.2	NR	NR	4.6±1.8
	4	5.5±1.8	0.3±0.1	4.2±1.9	NR	NR	10.0±2.9
	8	NR	NR	NR	0.6±0.4	NR	10.6±3.0
	16	33.4±7.4	2.3±0.7	5.7±2.1	NR	NR	42.0±8.3
	24	71.0±1.7	5.2±0.3	6.3±2.1	4.1±0.2	0.7±0.3	87.3±1.6

Adapted from Mathews et al. (1990) and U.S. EPA (1994b).
[a]Not reported

Lilly et al. (1998) examined the effects of vehicle on the absorption of orally administered bromodichloromethane in an experiment designed to develop and validate a physiologically-based pharmacokinetic model. Male F344 rats (3 animals/dose/vehicle/assay) were gavaged with 0, 50, or 100 mg bromodichloromethane/kg in either corn oil or 10% Emulphor®, and bromodichloromethane levels were monitored in blood and exhaled air. The dose levels approximated doses previously utilized in two-year cancer bioassays of bromodichloromethane (NTP, 1987). Concentrations of bromodichloromethane in blood and exhaled air peaked rapidly, reaching maximal concentrations less than one hour after administration. The vehicle of administration had significant effects on the blood and exhaled air concentrations. Delivery of bromodichloromethane in 10% Emulphor® resulted in faster initial uptake, as inferred from higher blood, tissue and breath chamber concentrations, when compared to corn oil (data presented graphically). At 6 hours after administration, more than 90% and 100% of the administered dose had been absorbed from the corn oil and Emulphor® vehicles, respectively.

B. Distribution

Data on the distribution of brominated trihalomethanes in exposed humans are limited. Roth (1904) measured the bromoform content of tissues of a man who died from an accidental oral overdose of bromoform and found levels in stomach and lung of 130 and 90 mg/kg wet weight, respectively. Lower levels were reported in the intestine, liver, kidney, and brain. Pellizzari et al. (1982) measured trihalomethanes in 42 samples of human milk taken from women in urban areas. Dibromochloromethane was detected in one sample. Neither the level measured nor the detection limit were reported for this study.

Data on the distribution of brominated trihalomethanes in animals are available from studies in rats and mice. Mink et al. (1986) compared the distribution of bromodichloromethane, dibromochloromethane, and bromoform in male Sprague-Dawley rats and male $B6C3F_1$ mice. Single oral doses of ^{14}C-labeled compound in corn oil were administered by gavage at dose levels of 100 mg/kg (rats) or 150 mg/kg (mice). Tissue levels of radioactivity were measured 8 hours after dose administration. The chemical form of the label measured in the tissues (e.g. parent or metabolite, bound or free) was not determined. In the rat, the total organ content of label ranged from 1.4% to 3.6% for the various compounds. The stomach, liver, and kidneys contained higher levels than bladder, brain, lung, muscle, pancreas, and thymus. In mice, 4% to 5% of the administered compound was recovered in the organs. However, an additional 10% of the label associated with bromoform was recovered in the blood of mice, yielding total organ levels of 12% to 14%. The authors attributed this elevated recovery of label to carboxyhemoglobin formation. The levels of carboxyhemoglobin were not measured in this experiment.

Mathews et al. (1990) investigated the distribution of bromodichloromethane following oral exposure in male Fischer 344 rats. Animals were given a single oral gavage dose of 1, 10, 32, or 100 mg/kg of ^{14}C-bromodichloromethane dissolved in corn oil. Approximately 3% to 4% of the administered dose was detected in tissues after 24 hours. The highest levels (1% to 3%) were measured in liver. Repeated doses of 10 or 100 mg/kg-day for 10 days resulted in total

retention of only 0.9% to 1.1% of the administered label, and had no effect on the tissue distribution of bromodichloromethane.

The Chlorine Chemistry Council (CCC, 2000c) sponsored a study which analyzed the levels of bromodichloromethane in parental tissues and fluids and F_1 generation tissues as part of a reproductive and developmental study in Sprague-Dawley rats (see Section V.E.1 for a full study description). Data from this study were summarized in Christian et al. (2001b). Bromodichloromethane was administered in the drinking water at concentrations of 0, 50, 150, 450, or 1350 ppm. The estimated dosage on a mg/kg-day basis varied with the stage of the study (see Table V-7). Plasma and other tissue samples were collected for analysis as described in Table III-3. All samples were maintained frozen and shipped to the analytical lab (Lancaster Laboratories, Lancaster, PA). Analysis of plasma collected from male and female rats prior to mating and from female rats during gestation and lactation did not detect quantifiable levels of bromodichloromethane (limit of detection 0.11 μg). Bromodichloromethane was detected at a concentration of 0.38 μg/g in the milk from one female in the 1350 ppm group. Bromodichloromethane was not detected in placentas, amniotic fluid, or fetal tissue collected on GD 21 or in plasma collected from postpartum day 29 weanling pups.

Table III-3 Over view of Tissue Collection for Analysis of Bromodichloromethane in Sprague-Dawley Rat Tissues and Fluids (CCC, 2000c).

Generation	Sex	Physiological state	Tissue	Day of collection	No. of Samples; Collection freq.	Comments
P	M, F	Pre-mating	Plasma	Day 1 of exposure	3 rats/sex/group; 3 times/day	-
P	M, F	Pre-mating	Plasma	Day 14 of exposure	3 rats/sex/group; 3 times/day	-
P	F	Pregnant	Plasma	GD 20	3 rats/group; 3 times/day	Rats continuously exposed since study day 1
P	F	Pregnant	Placenta amniotic fluid, and fetuses	GD 21	3 litters/day	Tissues pooled by litter
P	F	Lactating	Plasma	LD 15	3 rats/group	3 times/day
P	F	Lactating	Milk	LD 15	3 rats/group	1 IU oxytocin admin. by IV approx. 5 min. before milking
F_1	M, F	Weaning	Plasma	LD 29	3 pups/sex; 3 litters; 3 times/day	-

Modified from CCC (2000c)
Abbreviations: P, parental; M, male; F, female; GD, gestation day; LD, lactation day

The Chlorine Chemistry Council (CCC, 2000a) sponsored a study which analyzed the levels of bromodichloromethane in parental tissues and fluids and F_1 generation tissues as part of a reproductive and developmental study in New Zealand White rabbits (see Section V.E.1 for a full study description). Data from this study were summarized in Christian et al. (2001b). Bromodichloromethane was administered to groups of rabbits (4/concentration) in the drinking water at concentrations of 0, 50, 150, 450, or 1350 ppm. The estimated doses at these concentrations were 0, 4.9, 13.9, 32.3, or 76.3 mg/kg-day, respectively. Blood samples were collected on GD 7 and 28. Amniotic fluid, and placenta samples were collected on GD 29 after collection of a third blood sample, and amniotic fluid and placental tissue were pooled by litter. Blood samples were collected from three randomly selected fetuses per litter. Bromodichloromethane was detected at concentrations of 0.15 and 0.17 μg/g (limit of detection 0.11 μg/g) in placentas from two litters in the 1350 ppm exposure group. Bromodichloromethane was detected in one fetus from the 1350 ppm group "...at a level below the limit of detection". Bromodichloromethane was not detected in placentas from does exposed to concentrations up to 450 ppm, in amniotic fluid from does exposed to concentrations up to 1350 ppm, or in the remaining fetuses of does exposed to concentrations as high as 1350 ppm.

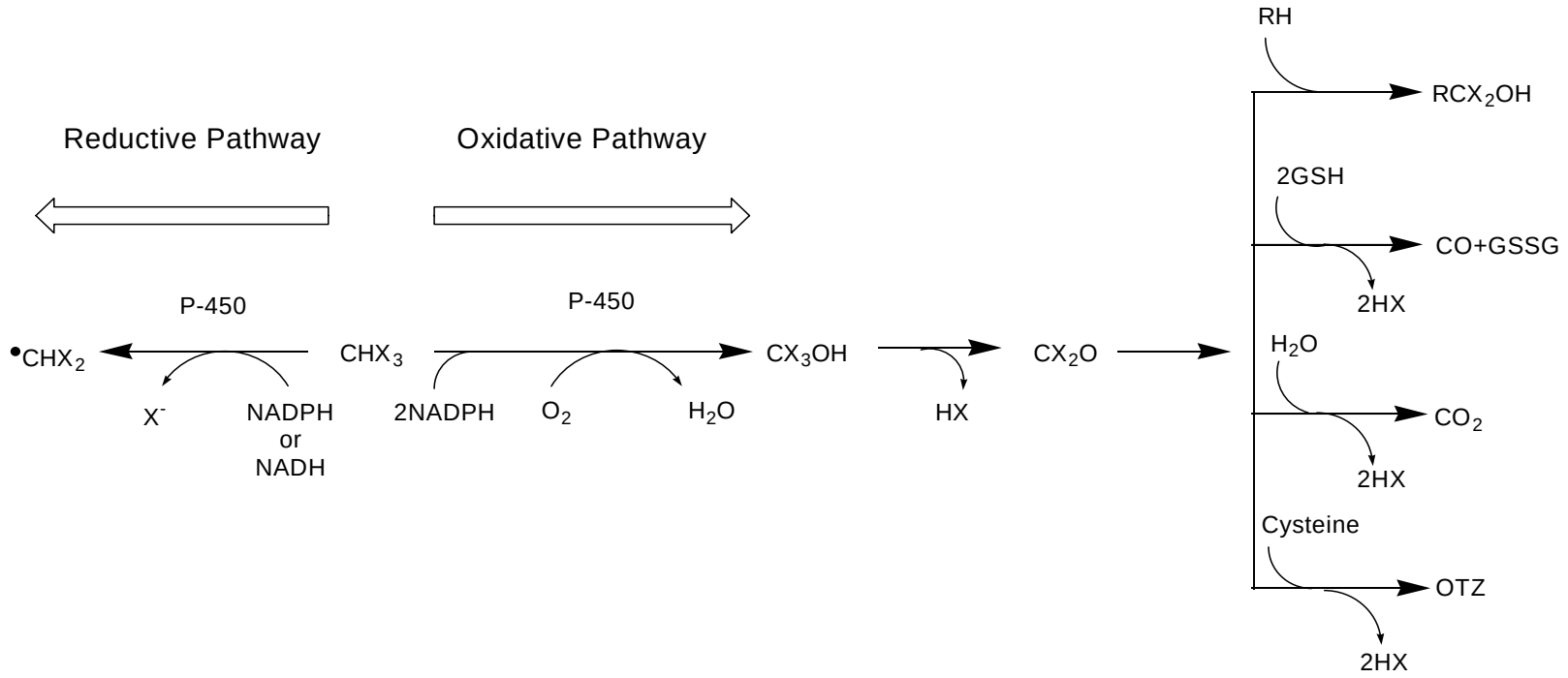
C. Metabolism

1. Overview

The toxicity of the brominated trihalomethanes is mediated by cytochrome P450-mediated bioactivation to reactive metabolites. The pathways for brominated trihalomethane metabolism were initially inferred from studies of the structurally-related trihalomethane chloroform (U.S. EPA, 1994b). Additional details of brominated trihalomethane metabolism have subsequently been elucidated in a number of laboratories using both *in vitro* and *in vivo* approaches and are described below. Figure III-1 presents a general metabolic scheme for chloroform and the brominated trihalomethanes.

The metabolism of brominated trihalomethanes occurs via at least two pathways (U.S. EPA 1994b). The oxidative pathway requires NADPH and oxygen, whereas the reductive pathway can utilize NADPH or NADH and is inhibited by oxygen. Both reactions are believed to be mediated by cytochrome P450 isoforms. In the presence of oxygen (oxidative metabolism), the reaction product is trihalomethanol (CX_3OH), which spontaneously decomposes to yield a reactive dihalocarbonyl (CX_2O) such as phosgene (CCl_2O). Dihalocarbonyls may undergo a variety of reactions, such as adduct formation with various cellular nucleophiles, hydrolysis to yield carbon dioxide, or glutathione-dependent reduction to yield carbon monoxide. When oxygen levels are low (reductive metabolism), the metabolic reaction products appear to be free radical species such as the dihalomethyl radical ($\bullet CHX_2$). These radicals are highly reactive and may also form covalent adducts with a variety of cellular molecules. Evidence supporting this metabolic scheme and information on species differences in the rate and extent of trihalomethane metabolism are presented below. Additional data derived from studies of chloroform are described in U.S. EPA (1994b).

Figure III-1 Proposed Oxidative and Reductive Metabolic Pathways for Brominated Trihalomethanes



X = halogen atom (chlorine or bromine); R = cellular nucleophile (protein, nucleic acid);
GSH = reduced glutathione; GSSG = oxidized glutathione;
OTZ = oxothiazolidine carboxylic acid; P-450 = cytochrome P-450
Adapted from Stevens and Anders (1981); Tomasi et al. (1985)

The metabolism of trihalomethanes (including chloroform and the brominated trihalomethanes) has been most intensively studied using chloroform as a substrate. These studies indicate that many factors influence metabolism, including strain, species, chloroform concentration, and possibly gender. A comprehensive review of chloroform studies is beyond the scope of this document. However, because the P450-mediated metabolism of the brominated trihalomethanes is expected to be similar to that of chloroform, descriptions of a few representative studies of chloroform metabolism are provided to provide additional background information on the metabolism of trihalomethanes.

A key question in hazard characterization is the identity of the P450 isoforms responsible for bioactivation of brominated trihalomethanes to reactive metabolites. This is because individuals or subpopulations with elevated levels of these enzymes may be at greater risk for adverse effects. The identities of the cytochrome P450 isoforms responsible for trihalomethane metabolism have been investigated most intensively in studies of chloroform (studies of brominated trihalomethanes are described in sections 2 and 3 below).

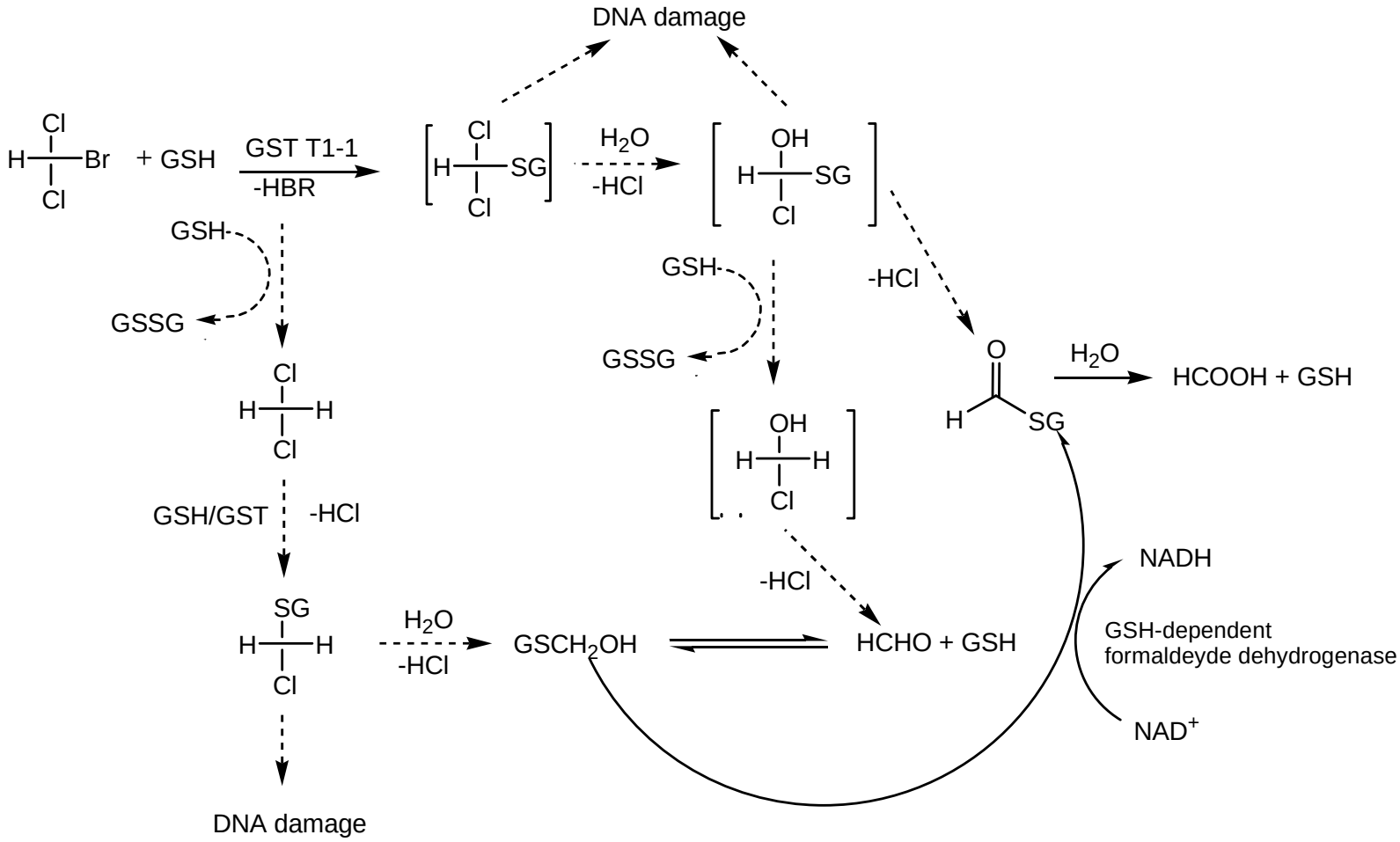
Studies by Nakajima et al. (1995) and Testai et al. (1996) indicate that chloroform concentration plays a critical role in determining the role of different isoforms and the associated effects of metabolic inducers. Nakajima et al. (1995) pretreated male Wistar rats with three inducers of specific P450 isoforms and subsequently administered a single dose of chloroform by gavage in corn oil. The inducers used were phenobarbital (CYP2B1/2), n-hexane (CYP2E1), and 2-hexanone (CYP2B1/2 and CYP2E1). Liver damage (as determined by serum enzyme activity and histopathology) was greatest at the mid-dose in the hexane-treated animals. In contrast, rats pretreated with phenobarbital or 2-hexanone showed a dose-related increase of liver damage at all dose levels. The pattern of damage was consistent in each case with the tissue distribution patterns of the induced cytochrome P450 isoform(s). The study authors concluded on the basis of these results that CYP2E1 catalyzes chloroform metabolism at low doses and that CYP2B1/2 catalyzes chloroform metabolism at higher doses.

While experimental evidence indicates that CYP2E1 and CYPB1/2 catalyze the oxidative pathway, the identities of the cytochrome P450 isoforms which catalyze the reductive pathway have not been established. In general, CYP2E1 protein can catalyze reductive as well as oxidative reactions (Lieber, 1997) and this isoform has been implicated in the production of trichloromethyl radicals from carbon tetrachloride (see Lieber et al. 1997). However, evidence for a dual role of either CYP2E1 or CYP2B1/2 in catalyzing the oxidative and reductive pathways for trihalomethane metabolism has been contradictory, perhaps as a result of the different concentrations of chloroform used in different experiments (summarized in Testai et al. 1996). To address the issue of concentration, Testai et al. (1996) studied the role of different isoforms in chloroform using microsomes prepared from Sprague-Dawley rats pretreated with a variety of cytochrome P450 inducers. The microsomes were incubated under varying conditions of chloroform concentration, oxygenation, and presence of isoform-specific inhibitors or antibodies. Under the conditions utilized in this series of experiments, the authors concluded that the cytochrome P450 isoforms involved in oxidative metabolism of brominated trihalomethanes do not participate in the reductive pathway.

Studies conducted in *Salmonella typhimurium* strains that express the rat GST theta gene (*GSTT1-1*) (Pegram et al., 1997, DeMarini et al., 1997; Landi et al., 1999b) and using liver cytosols isolated from rats, mice, and humans (Ross and Pegram, 2003) provide evidence for a third pathway of metabolism via GST theta-mediated conjugation of bromodichloromethane with glutathione (GSH). Rat GSTT1-1 is closely related to human theta-class GSTs, suggesting that humans are likely to have the capability to conjugate brominated trihalomethanes via this pathway if it is active *in vivo* (Meyer et al., 1991).

Ross and Pegram (2003) studied the characteristics of bromodichloromethane conjugation with GSH in liver cytosols from mice, rats, and humans. Conjugation of bromodichloromethane with GSH in mouse liver cytosol was time- and protein-dependent, was not affected by an inhibitor of alpha-, mu-, and pi-class GSTs, and correlated with activity toward a GSTT1-1-specific substrate (1,2-epoxy-3-(4'-nitrophenoxy)propane). Conjugation activity toward bromodichloromethane in hepatic cytosols of different species followed the rank order mouse, followed by rat, then human. The initial conjugate formed was S-chloromethyl-GSH ($GSCHCl_2$). This compound was unstable and degraded to multiple metabolites including S-hydroxymethyl-GSH ($GSCH_2OH$), S-formyl-GSH, and formic acid (HCOOH). These data indicate that GSTT1-1-mediated glutathione conjugation of bromodichloromethane occurs in mammalian liver cytosol, via reactions consistent with the metabolic scheme presented in Figure III-2.

Figure III-2 Proposed GSTT1-1-Catalyzed Glutathione Conjugation of Bromodichloromethane[a]



[a]Solid arrows represent pathways that are supported by direct experimental evidence. Dashed arrows indicate pathways that are inferred from the experimental evidence and literature precedents. Proposed scheme is from Ross and Pegram (2003).

2. *In Vitro* Studies

Ahmed et al. (1977) investigated the *in vitro* oxidative (aerobic) metabolism of brominated trihalomethanes to carbon monoxide by the rat liver microsomal fraction. Metabolism of bromoform resulted in the highest level of carbon monoxide formation, followed by dibromochloromethane and bromodichloromethane in decreasing order. Glutathione, NADPH and oxygen were required for maximal carbon monoxide production. This activity was inducible by phenobarbital or 3-methylcholanthrene pretreatment (agents which are known to increase cytochrome P-450 activity) and was inhibited by the cytochrome P-450 inhibitor SKF 525-A. Similar results were reported by Stevens and Anders (1979). In addition, Stevens and Anders (1979) reported the formation of 2-oxothiazolidine-4-carboxylic acid (OTZ) when bromoform was incubated in the presence of cysteine. Dihalocarbonyls react with cysteine to form OTZ. Thus, detection of OTZ provides evidence that a dihalocarbonyl intermediate was formed during bromoform metabolism.

Wolf et al. (1977) studied the *in vitro* metabolism of bromoform and chloroform to carbon monoxide under anaerobic conditions using liver preparations from phenobarbital-induced rats. Bromoform metabolism resulted in much greater levels of carbon monoxide production than did the metabolism of chloroform. Gao and Pegram (1992) reported that binding of reactive intermediates to rat hepatic microsomal lipid and protein under reductive (anaerobic) conditions was more than twice as high for bromodichloromethane as for chloroform. These data suggest that reductive metabolism may be a more important pathway for metabolism of brominated trihalomethanes than for chloroform.

Tomasi et al. (1985) studied the anaerobic activation of bromoform, bromodichloromethane, and chloroform to free radical intermediates *in vitro* using rat hepatocytes isolated from phenobarbital-induced male Wistar rats. The production of a free radical intermediate was measured by electron spin resonance (ESR) spectroscopy using the spin trap compound phenyl-t-butylnitrone. The intensity of the ESR signal was greatest for bromoform, followed by bromodichloromethane and then chloroform. The largest ESR signal was detected when hepatocytes were incubated under anaerobic conditions. Incubation in the presence of air resulted in a reduction of the signal, as did addition of cytochrome P-450 inhibitors such as SKF-525A, metyrapone, and carbon monoxide. These data were interpreted to indicate that free-radical formation depended on reductive metabolism of the trihalomethanes mediated by the cytochrome P450 system. Comparison of the ESR spectra for chloroform, deuterated chloroform, and bromodichloromethane indicated that the free radical intermediate produced by chloroform metabolism was $\bullet CHCl_2$. The authors speculated that the brominated trihalomethanes are also metabolized by transfer of an electron directly from the cytochrome to the halocompound with the successive formation of the dihalomethyl radical ($\bullet CHX_2$) and a halide ion (X^-).

As noted in IIIC1, recent evidence from studies in *Salmonella typhimurium* strains that express the rat glutathione *S*-transferase theta 1-1 (*GSTT1-1*) gene suggests that bioactivation of brominated trihalomethanes to mutagenic species is also mediated by one or more glutathione *S*-transferase-mediated conjugation pathways (Pegram et al., 1997; DeMarini et al., 1997; Landi et al., 1999b). Details of these studies are presented in Section V.F.

3. *In Vivo* Studies

Mink et al. (1986) compared the metabolic products of bromodichloromethane, dibromochloromethane, and bromoform in male Sprague-Dawley rats and male B6C3F$_1$ mice (strain not reported). Animals were given a single oral dose of ^{14}C-labeled compound by gavage in corn oil at dose levels of 100 mg/kg for rats and 150 mg/kg for mice. Levels of ^{14}C were measured in exhaled carbon dioxide recovered within 8 hours after dose administration. Expired carbon dioxide accounted for 4.3% to 18.2% of the administered label in rats (Table III-1), suggesting that the parent compound had undergone limited metabolism and oxidation. In mice, the fraction of label excreted as carbon dioxide was higher, ranging from 40% to 81%. These data indicate that oxidative metabolism of brominated trihalomethanes to carbon dioxide was more rapid and extensive (by a factor of four- to ninefold) in mice than in rats. As previously noted in Section III.A, production of carbon monoxide, a known metabolite of brominated trihalomethanes, was not measured in this study.

Anders et al. (1978) investigated the formation of carbon monoxide from brominated trihalomethanes in corn oil administered to Sprague-Dawley rats at doses of 1 mmol/kg (119 to 252 mg/kg) by intraperitoneal injection. Bromoform produced the highest levels of blood carbon monoxide, followed by dibromochloromethane and bromodichloromethane in decreasing order. A dose-response relationship was noted for bromoform following administration of 252, 506, or 1,012 mg/kg. Carbon monoxide production was inducible by pretreatment with phenobarbital, but pretreatment with 3-methylcholanthrene had no effect. Carbon monoxide production was significantly inhibited by SKF-525-A. Administration of ^{3}H-bromoform resulted in decreased carbon monoxide formation when compared to bromodichloromethane and dibromochloromethane, indicating that the carbon-hydrogen bond breakage may be the rate-limiting step under aerobic conditions. Similar results were later reported by Stevens and Anders (1981).

Tomasi et al. (1985) studied the *in vivo* metabolism of chloroform, bromodichloromethane, and bromoform to free radical intermediates in rats. Starved, phenobarbital-induced male Wistar rats (number not stated) were given intraperitoneal injections of 1,100 mg/kg chloroform, 820 mg/kg bromodichloromethane, or 1,260 mg/kg bromoform dissolved in olive oil. The animals were sacrificed and the livers were homogenized. The production of a free radical intermediate by the livers was determined by ESR spectroscopy. The authors reported detection of free radicals in the livers of all treated rats. The intensity of the ESR signal followed a ranking similar to that observed in *in vitro* experiments (bromoform > bromodichloromethane > chloroform), confirming that the reductive formation of free radicals is greater for brominated trihalomethanes than for chloroform.

Mathews et al. (1990) studied the metabolism of ^{14}C-bromodichloromethane in male Fischer 344 rats. Animals (n = 4) were given a single oral dose of 1, 10, 32, or 100 mg/kg of bromodichloromethane dissolved in corn oil. Levels of labeled carbon dioxide and carbon monoxide in exhaled air were measured for 24 hours. Approximately 70% to 80% of the dose was metabolized and exhaled as $^{14}CO_2$ and 3% to 5% of the dose as ^{14}CO. However, $^{14}CO_2$ production was slower following a single dose of 100 mg/kg than after the administration of a single dose of 32 mg/kg or lower, suggesting saturation of metabolism. Repeated doses of 100 mg/kg-day for 10 days resulted in an increased rate of $^{14}CO_2$ production, compared with the initial rate. The authors concluded on the basis of these data that bromodichloromethane may induce its own metabolism.

Thornton-Manning et al. (1994) evaluated the effect of bromodichloromethane exposure on cytochrome P450 isozyme activity in female F344 rats (6/dose). Gavage doses of 75 to 300 mg/kg-day were administered in a solution of 10% Emulphor® (an emulsifier) for five consecutive days. Treatment resulted in decreased activity of the CYP1A and CYP2B isozymes. In contrast, there was no effect on CYP2E1 activity.

Pankow et al. (1997) investigated the metabolism of dibromochloromethane in male Wistar rats following single and repeated gavage doses. Rats receiving a single-dose (6 animals per group) were treated with 0 (vehicle only), 0.4, 0.8, 1.6 or 3.1 mmol dibromochloro-methane/kg dissolved in olive oil. Rats receiving multiple doses were gavaged with 0 (vehicle only) or 0.8 mmol dibromochloromethane/kg once a day for 7 days. The blood or plasma concentrations of parent compound, bromide, and carbon monoxide (as carboxyhemoglobin, COHb) were measured following dibromochloromethane administration. The level of oxidized glutathione (GSSG) in the liver of treated animals was also assayed. Oral administration of dibromochloromethane resulted in a significant elevation of plasma bromide levels at all doses tested. Bromide did not return to baseline levels even after 10 days. Repeated administration of 0.8 mmol dibromochloromethane/kg resulted in significantly higher plasma levels of bromide than were measured following a single dose of 0.8 mmol/kg. COHb was also elevated in a dose-dependent manner following either single or repeated administration of dibromochloromethane, but returned to baseline levels within 24 hours after treatment. GSSG levels were significantly increased at 12- and 24-hour time points following a single 0.8 mmol/kg dose (no other doses were examined). Levels of GSSG returned to baseline levels by 48 hours after treatment.

Pankow et al. (1997) conducted additional experiments to determine whether reduced glutathione (GSH) is a requirement for *in vivo* dibromochloromethane metabolism and to identify P450 isozymes involved in the metabolism of dibromochloromethane. Pretreatment of rats with buthionine sulfoximine (an agent which depletes GSH) reduced GSH concentrations as anticipated and decreased the rate of bromide and COHb production. In contrast, pretreatment with butylated hydroxyanisole (which increases GSH levels) increased the rate of bromide and COHb production. These results suggest that GSH plays a role in dibromochloromethane metabolism. Further studies were conducted to determine which cytochrome P450 isoform(s) participate in the *in vivo* metabolism of dibromochloromethane. Simultaneous exposure to 0.8 mmol dibromochloromethane/kg and diethyldithiocarbamate (a potent inhibitor of P450 isoform CYP2E1) partially inhibited the production of bromide and COHb. In contrast, pretreatment

with isoniazid (an potent inducer of CYP2E1) increased formation of bromide and COHb. These experiments indicate that CYP2E1 is at least partially responsible for dibromochloromethane metabolism. Pretreatment with phenobarbital, an inducer of cytochrome P450 isoforms CYP2B1 and 2B2, increased the concentration of bromide in plasma, suggesting that CYP2B1 and 2B2 may also participate in the catabolism of dibromochloromethane. Pretreatment with m-xylene, which induces both CYP2E1 and CYP2B1/2, resulted in higher bromide levels than inducers of CYP2E1 (isoniazid) or CYP2B1/2 (phenobarbital) administered individually. Pankow et al. (1997) concluded on the basis of these multiple experiments that 1) bromide and carbon monoxide are metabolites of dibromochloromethane; 2) dibromochloromethane is metabolized via the oxidative pathway; 3) the oxidative metabolism of dibromochloromethane is catalyzed by CYP2E1 and CYP2B1/2; and 4) dibromochloromethane plays a role in the induction of CYP2E1.

Allis et al. (2001) investigated the effect of inhalation exposure to bromodichloromethane on the activity and protein levels of CYP1A2, CYP2B1, and CYP2E1 in female F344 rats (6/dose). In addition, the effect of inhalation exposure on the activity level of CYP1A1 was assayed. Serum bromide ion concentration, an indicator of the total metabolism of bromodichloromethane, was measured in samples drawn from a separate set of animals (4/concentration) exposed to the same concentrations. The test animals were exposed for 4 hours to measured bromodichloromethane concentrations of 0, 106, 217, 419, 812, 1620, and 3240 ppm. The microsomal isozyme activities assayed were: *p*-nitrophenol hydrolase (PNP), an indicator of CYP2E1 activity; pentoxyresorufin-*O*-dealkylase (PROD), an indicator of CYP2B1/2 activity; ethoxsyresorufin-O- dealkylase (EROD), an indicator of CYP1A1 activity; and methoxyresorufin-O-dealkylase (MROD), an indicator of CYP1A2 activity. The pattern of results for isozyme activity obtained in this inhalation study was similar to that reported for male F344 rats treated with bromodichloromethane by gavage. CYP2E1 activity as measured by PNP activity was not significantly affected by treatment. MROD, EROD, and PROD activities showed modest increases at low exposure concentrations. The increases were statistically significant for EROD and MROD at the 106 ppm exposure concentration. Decreases were observed at higher exposure concentrations relative to controls. These decreases were statistically significant for PROD at 3240 ppm and for EROD and MROD at concentrations of 800 ppm and greater. The results for isozyme protein levels, as measured by Western blots, were generally consistent with the results for isozyme activity. The study authors speculated that the most dramatic reductions in isozyme activity (PROD and MROD) were a result of suicide inhibition. In addition, they concluded that analyses of the EROD and MROD activity and protein level patterns indicates that CYP1A2 is involved in the metabolism of bromodichloromethane. The study authors noted that it is not typical for this isozyme to metabolize the small molecules (such as chloroform) that are the usual substrates for CYP2E1, but observed that the presence of the large bromide ion may make bromodichloromethane a suitable substrate for CYP1A2. Blood bromide concentration reached a maximum at 200 ppm, indicating that metabolism was saturated a concentrations equal to or greater than 200 ppm.

Allis et al. (2002) reported additional evidence for metabolism of bromodichloromethane by CYP1A2. Induction of CYP1A2 without parallel induction of CYP2E1 or CYP2B1/2 (accomplished by pretreatment with 2,3,7,8-tetrachlorodibenzodioxin, TCDD), increased

hepatotoxicity in male F344 rats administered a gavage dose of 400 mg/kg bromodichloromethane. Hepatotoxicity was assessed by measurement of alanine aminotransferase (ALT) and sorbitol dehydrogenase (SDH) activity. Pretreatment with TCDD increased serum bromide levels (a measure of total bromodichloromethane metabolism) in rats treated with 200 or 400 mg/kg when compared to uninduced controls. The apparent inconsistency between lack of hepatotoxicity and increased total metabolism at 200 mg/kg was explained by effective detoxification at this dose, presumably by glutathione. Selective inhibition of CYP1A2 activity, by administration of isosafrole to TCDD-induced animals prior to treatment with 400 mg/kg bromodichloromethane significantly reduced the hepatotoxic response and serum bromide concentrations.

Allis and colleagues (Allis et al., 2002; Allis and Zhao, 2002; Zhao and Allis, 2002) assessed the ability of various rat and human CYP isoenzymes to metabolize bromodichloromethane and determined kinetic parameters for those showing measurable metabolic activity. Allis and Zhao (2002) tested five rat and six human CYP isoenzymes *in vitro* for metabolism of bromodichloromethane using recombinant systems expressing single isozyme activities. The tested recombinant isoenzymes were rat CYP2E1, CYP2B1/2, CYP1A2, CYP2C11, AND CYP3A1 and human CYP2E1, CYP1A2, CYP2A6, CYP2B6, CYP2D6 and CYP3A4. The results of this study indicate that the principal metabolizing enzymes in rat are those identified previously, namely CYP2E1, CYP2B1/2, CYP1A2. Results for CYP3A1 suggest that it may have weak metabolic activity, but the level of activity was not sufficient for a quantitative assessment. CYP2C11 was not active. Human CYP2E1, CYP1A2, and CYP3A4 showed substantial metabolic activity toward bromodichloromethane. Human CYP2A6 showed lower, but measurable, levels of activity. CYP2B6 and CYP2D6 were not active. Based on these assays, only CYP2E1 and CYP1A2 metabolize bromodichloromethane in both species. CYP2E1 is the high affinity enzyme in both rats and humans, with Km values approximately 27-fold lower than those for the isoenzymes with the next lowest value (CYP2B1 in rats, CYP1A2 in humans). The metabolic parameters K_m and k_{cat} for rat and human CYP2E1 were similar. In contrast, the metabolic parameters for CYP1A2 were not similar across species. The study authors concluded that the results of this study appear consistent with observations *in vivo* for the rat (Allis et al., 2002) and with predictions of the existing PBPK model for bromodichloromethane in the rat (Lilly et al., 1998).

Zhao and Allis (2002) determined kinetic constants for metabolism of bromodichloromethane by CYP2E1, CYP1A2, and CYP3A4 in human liver microsomes. Constants for individual isoenzymes were determined by addition of enzyme-specific inhibitory antibodies for two isoenzymes to the microsomal preparations while measuring the activity of the third. Measurements were performed in microsomes obtained from four donors. CYP2E1 was found to have the lowest K_m (2.9 μM) and the highest catalytic activity. The K_m values for CYP1A2 and CYP3A4 were approximately 20-fold higher (60 μM) and the catalytic activity was lower. Eleven additional human microsomal preparations were characterized for activity of 10 CYP isoenzymes. The initial rate of metabolism in each preparation measured at 9.7 μM bromodichloromethane was compared to the activity of individual isoenzymes. Statistical analysis showed a significant correlation only with CYP2E1 activity at the tested concentration. At the low concentrations expected from drinking water exposure, the results of this study

suggest that CYP2E1 would dominate metabolism. However, the study authors have noted that humans are highly variable in the induction of CYP isoenzymes and that the contributions of the three isoenzymes to metabolism of bromodichloromethane in individuals may not be entirely predictable.

D. Excretion

Mink et al. (1986) compared the excretion of bromodichloromethane, dibromochloromethane, and bromoform in male Sprague-Dawley rats and male $B6C3F_1$ mice. Animals were given single oral doses of ^{14}C-labeled compound in corn oil by gavage at dose levels of 100 mg/kg and 150 mg/kg for rats and mice, respectively. The lung was the principal route of excretion in both species, accounting for 45% to 88% of the administered label, either as carbon dioxide or as parent compound. Small amounts of label (1.1% to 4.9%) were recovered in urine, but the chemical identity of labeled compounds was not investigated.

Mathews et al. (1990) exposed Fischer 344 rats to either a single oral dose of 1, 10, 32, or 100 mg/kg, or 10-day repeated doses of 10 or 100 mg/kg-day bromodichloromethane dissolved in corn oil. Approximately 70% to 80% of the administered dose was excreted in exhaled air as ^{14}C-carbon dioxide, with 3% to 5% as ^{14}C-carbon monoxide. In general, less than 5% of the dose was excreted in the urine or feces.

E. Bioaccumulation and Retention

No data were located regarding the bioaccumulation or retention of brominated trihalomethanes following repeated exposures. However, based on the rapid excretion and metabolism of the brominated trihalomethanes and the low levels of the structurally-related compound chloroform detected in human post-mortem tissue samples, marked accumulation and retention of these compounds are not anticipated.

F. Summary

No data on absorption of brominated trihalomethanes were available for humans. Measurements in mice and rats indicate that gastrointestinal absorption of brominated trihalomethanes is rapid (peak levels attained less than an hour after administration of a gavage dose) and extensive (63% to 93%). Most studies of brominated trihalomethane absorption have used oil-based vehicles. A study in rats found that the initial absorption rate of bromodichloromethane was higher when the compound was administered in an aqueous vehicle when compared to administration in a corn oil vehicle.

Data for distribution of brominated trihalomethanes in human organs and tissues are limited. Bromoform was found primarily in the stomach and lungs of a human overdose victim, with lower levels detected in intestine, liver, kidney and brain. Dibromochloromethane was found in 1 of 42 samples of human breast milk collected from women living in urban areas. Radiolabeled brominated trihalomethanes were detected in a variety of tissues following oral dosing in rats and mice. Approximately 1 to 4% of the administered dose was recovered in body

tissues when analysis was conducted 8 or 24 hours post-treatment. The highest concentrations were detected in stomach, liver, blood, and kidneys when assayed 8 hours after administration of the compounds. Analyses of placentas, amniotic fluid and fetuses from female rats and rabbits administered bromodichloromethane in drinking water indicate that this compound does not accumulate in these tissues or fluids. There are no data which are suggestive of strain specific differences in metabolism.

Brominated trihalomethanes are extensively metabolized by animals. Metabolism of brominated trihalomethanes occurs via at least two pathways. One pathway predominates in the presence of oxygen (the oxidative pathway) and the other predominates under conditions of low oxygen tension (the reductive pathway). In the presence of oxygen, the initial reaction product is trihalomethanol (CX_3OH), which spontaneously decomposes to yield the corresponding dihalocarbonyl (CX_2O). The dihalocarbonyl species are quite reactive and may form adducts with cellular molecules. When intracellular oxygen levels are low, the trihalomethane is metabolized via the reductive pathway, resulting in a highly reactive dihalomethyl radical ($\bullet CHX_2$), which may also form covalent adducts with cellular molecules. The metabolism of brominated trihalomethanes and chloroform appear to occur via the same pathways, although *in vitro* and *in vivo* data suggest that metabolism via the reductive pathway occurs more readily for brominated trihalomethanes. Both oxidative metabolism and reductive metabolism of trihalomethanes appear to be mediated by cytochrome P450 isoforms. The identity of cytochrome P450 isoforms that metabolize brominated trihalomethanes has been investigated in several studies which used bromodichloromethane as a substrate. The available data suggest that the cytochrome P450 isoforms CYP2E1, CYP2B1/2, and CYP1A2 metabolize bromodichloromethane in rats. The human isoforms CYP2E1, CYP1A2, and CYP3A4 show substantial activity toward bromodichloromethane *in vitro* and low but measurable levels of CYP2A6 activity have also been detected. Based on the available data, CYP2E1 and CYP1A2 are the only isoforms active in both rats and humans. CYP2E1 shows the highest affinity for bromodichloromethane in both species and the metabolic parameters K_m and k_{cat} are similar for rat and human CYP2E1. In contrast, the metabolic parameters for CYP1A2 differ in rats and humans. The pattern of results for isozyme activity obtained from an inhalation study of bromodichloromethane was similar to the pattern reported for male F344 rats treated with bromodichloromethane by gavage.

Recent evidence suggests that brominated trihalomethanes are also metabolized by a glutathione-S-transferase theta (GSTT)-mediated pathway. The available evidence indicates that this pathway has a low affinity for chloroform, suggesting that the brominated trihalomethanes could cause health effects by a different mode of action than those caused by chloroform.

The lung is the principle route of excretion in rats and mice. Studies with ^{14}C-labeled compounds indicate that up to 88% of the administered dose can be found in exhaled air as carbon dioxide, carbon monoxide, and parent compound. Excretion in the urine generally appears to be 5% or less of the administered oral dose. Data from one study suggests that fecal excretion is less than 3% of the administered dose.

IV. HUMAN EXPOSURE

A. Occurrence in Drinking Water

The occurrence of brominated trihalomethanes in U.S. drinking water has been determined in both national-scale and localized studies. The occurrence of bromodichloromethane and bromoform has been described in eleven national surveys. Dibromochloromethane occurrence has been described in twelve national surveys. Nine localized studies on the occurrence of brominated trihalomethanes are also described below.

It is important to note that a variety of sampling and preservation techniques are used for collection of occurrence data on brominated trihalomethanes. The addition of chlorine to raw water as a disinfectant at water treatment plants results in the formation of hypochlorous acid in the processed water. The acid in turn reacts with organic materials to produce chloroform and also oxidizes available bromide ions to form hypobromous acid. Hypobromous acid reacts with organic materials in the processed water to form the brominated trihalomethanes. Because these chemical reactions occur over periods of days in treated waters, the method used to sample drinking waters can affect the measured concentrations of trihalomethanes in the water. Therefore, appropriate sampling and preservation methods must be selected to ensure that the analytical data are representative of the desired endpoint. For example, if an investigator wants to know the concentration of trihalomethanes in the water at the time of sampling, a reducing agent is added to the sample containers to "quench" or prevent further formation of trihalomethanes. If an investigator wants to know the maximum amount of trihalomethanes that could occur, no quenching is used and the reactions are allowed to run to completion at room temperature. If a concentration similar to that at a household tap is desired (i.e., after the water spends several days in the distribution system, the samples generally are not quenched but are refrigerated to slow the reactions (Wallace, 1997). Information on sample handling has been included in the discussion of individual studies when available in the materials reviewed for this document.

Spatial and temporal variability exist in the occurrence data reported for brominated trihalomethanes. Multiple factors contribute to this variability. With respect to spatial variability, the geographical distribution of bromide ion in soil is not uniform (Shacklette and Boerngen, 1984). Brominated byproducts may predominate or comprise a substantial proportion of the disinfection byproduct profile in regions with high soil concentrations. Brominated trihalomethanes may continue to form within water distribution systems due to the action of free residual chlorine on remaining humic precursors, resulting in substantial intra-system spatial variability (Chen and Weisel, 1998). Temporal variability may result from seasonal variation in the concentration of brominated trihalomethanes as a result of seasonal fluctuations in precursor material (Brett et al., 1979). Short term variability may be introduced by changes in the demand cycle to individual homes or neighborhoods.

1. National Surveys

The National Organics Reconnaissance Survey (NORS), conducted by U.S. EPA, collected drinking water samples from 80 cities nationwide in 1975 (Symons et al., 1975). The survey sampled for several organics, including brominated trihalomethanes, at the water treatment facilities. The sampling method employed was refrigeration without quenching; therefore, brominated trihalomethane concentrations may have increased following collection. Bromodichloromethane was found in 98% of the systems sampled. The median concentration was 8 μg/L (ppb), and the maximum level was 116 μg/L (ppb). Dibromochloromethane was found in 90% of the systems sampled at a median concentration of 2 μg/L (ppb). The detection limit for dibromochloromethane and bromodichloromethane was 0.1 μg/L (ppb). The median concentration for bromoform was below the detection limit of approximately 5 μg/L (ppb) (Symons et al,. 1975). NORS was performed prior to the promulgation of the total trihalomethane regulation; therefore, these results may be higher than current levels.

The National Organics Monitoring Survey (NOMS) was conducted by the EPA from March 1976 to January 1977 (Wallace, 1997). In NOMS, 113 community water supplies were sampled. Surface water was the major source for 92 of the systems, and ground water was the major source for the remaining 21 systems. The NOMS used three sample storage methods. During Phase 1, all samples were refrigerated. In Phase 2, the samples were allowed to stand at 20 to 25°C for 2 to 3 weeks to maximize trihalomethane formation. Phase 3 had two parts. The samples identified as 3T were allowed to stand an additional 2 to 3 weeks. The samples identified as 3Q were quenched by addition of sodium thiosulfate. As expected, the highest trihalomethane values occurred in Phases 2 and 3T. Bromodichloromethane was detected in over 90% of the systems sampled. The median concentration under the various sample storage conditions ranged from 5.9 to 14 μg/L (ppb), and the maximum concentration was 183 μg/L (ppb). The mean concentrations of bromodichloromethane in Phases 1, 2, 3T, and 3Q were 18, 18, 17, and 9 μg/L (ppb), respectively. Dibromochloromethane was detected in 73% of the systems sampled. The median concentration ranged from below the detection limit to 3 μg/L (ppb), and the maximum value was 280 μg/L (ppb). The mean concentrations of dibromochloromethane in Phases 1, 2, 3T, and 3Q were 8, 12, 11, and 6 μg/L (ppb), respectively. The median bromoform concentration under all sampling conditions was below the detection limit of 0.3 μg/L (ppb); the maximum value was 280 μg/L (ppb). The mean concentrations of bromoform Phases 1, 2, 3T, and 3Q were 3, 4, 4, and 2 μg/L (ppb), respectively. NOMS was conducted before the promulgation of the total trihalomethane regulation; therefore, these results may be higher than current levels.

The Community Water Supply Survey (CWSS) was conducted by the EPA in 1978. The survey examined over 1,100 samples, representing over 450 water supply systems (Brass et al., 1981). The samples were taken at the treatment plants and in the distribution systems. In the CWSS, 94% of the surface water supplies and 33% of the ground water supplies were positive for bromodichloromethane. For surface water supplies, the mean of the positives and the overall median were 12 and 6.8 μg/L (ppb), respectively. The mean of the positives for ground water supplies was 5.8 μg/L (ppb), and the overall median was below the minimum reporting limit of 0.5 μg/L (ppb). For dibromochloromethane, 67% of the surface water supplies and 34% of the

ground water supplies were positive. For surface water supplies, the mean of the positives and the overall median were 5.0 and 1.5 μg/L (ppb), respectively. The mean of the positives for ground water supplies was 6.6 μg/L (ppb), and the overall median was below the minimum reporting limit of 0.5 μg/L (ppb). For bromoform, 13% of the surface water supplies and 26% of the ground water supplies were positive. The mean concentration of the positives in surface water supplies was 2.1 μg/L (ppb), and the overall median was less than 1.0 μg/L (ppb). The mean of the positives for ground water supplies was 11 μg/L (ppb), and the overall median was below the minimum reporting limit of 0.5 μg/L (ppb) (Brass et al., 1981).

The Rural Water Survey (RWS) was conducted between 1978 and 1980 by the EPA to evaluate the status of drinking water in rural America. Samples from over 2,000 households, representing more than 600 rural water supply systems, were examined. In the RWS, 76% of the surface water supplies and 13% of the ground water supplies were positive for bromodichloromethane, 56% of the surface water supplies and 13% of the ground water supplies were positive for dibromochloromethane, and 18% of the surface water supplies and 12% of the ground water supplies were positive for bromoform. For the surface water supplies, the mean of the positives and the overall median concentrations were 17 μg/L (ppb) and 11 μg/L (ppb) for bromodichloromethane, 8.5 μg/L (ppb) and 0.8 μg/L (ppb) for dibromochloromethane, and 8.7 μg/L (ppb) and <0.5 μg/L (ppb) for bromoform. For the ground water supplies, the mean of the positives was 7.7 μg/L (ppb) for bromodichloromethane, 9.9 μg/L (ppb) for dibromochloromethane, and 12 μg/L (ppb) for bromoform. The overall median for ground water supplies was below the minimum reporting limit of 0.5 μg/L (ppb) for all three brominated trihalomethanes (Brass, 1981).

The Ground Water Supply Survey (GWSS) was conducted from December 1980 to December 1981 by the EPA to develop data on the occurrence of volatile organic chemicals in ground water supplies. Out of a total of 945 ground water systems that were sampled, 466 systems were chosen at random, and the remaining 479 systems were chosen on the basis of location near industrial, commercial, and waste disposal activities. Samples were collected at or near the entry to the distribution system, and trihalomethane formation was allowed to continue without quenching after sample collection. For bromodichloromethane, the median of the positives for the randomly chosen systems serving greater than 10,000 people was 1.4 μg/L (ppb), and the occurrence rate was 36%. For the randomly chosen smaller systems, the median positive concentration was 1.6 μg/L (ppb), and the occurrence rate was 54%. The nonrandomly chosen systems had a median positive concentration of 2.1 μg/L (ppb) and an occurrence rate of 51%. For dibromochloromethane, the median positive concentration and the occurrence rate for the randomly chosen systems serving greater than 10,000 people were 2.1 μg/L (ppb) and 31%, respectively; these values for the smaller systems were 2.9 μg/L (ppb) and 52%. The nonrandomly chosen systems had a median positive concentration of 3.9 μg/L (ppb) and an occurrence rate of 46%. For bromoform, the median positive concentration was 2.4 μg/L (ppb) for the randomly chosen systems serving greater than 10,000 and 3.8 μg/L (ppb) for the randomly chosen systems serving fewer than 10,000 people, with occurrence rates of 16% and 31%, respectively. The nonrandomly chosen systems had a median positive concentration of 4.2 μg/L (ppb) and an occurrence rate of 31% (Westrick et al., 1983).

The National Screening Program for Organics in Drinking Water (NSP), sponsored by the EPA, was conducted from June 1977 to March 1981 and sampled 169 systems nationwide. Samples were collected at the treatment facilities. For dibromochloromethane, the mean and median for 130 positives were 17.2 and 10 μg/L (ppb), respectively. The maximum concentration found was 131 μg/L (ppb) (Boland, 1981).

The Technical Support Center (TSC) of the Office of Ground Water and Drinking Water (OGWDW) maintains a ground water contaminant database. For both bromodichloromethane and dibromochloromethane, the database contains 4,439 samples taken at the treatment facilities from nineteen states between 1984 and 1991. For bromodichloromethane, the mean concentration was 5.6 μg/L (ppb), and the median was 3 μg/L (ppb). For dibromochloromethane, the mean concentration was 3.0 μg/L (ppb), and the median was 1.7 μg/L (ppb). For bromoform, the database contains 1409 samples from 19 states taken at treatment facilities between 1984 and 1991. The mean and median concentrations were determined to be 2.5 μg/L (ppb) and 1 μg/L (ppb), respectively (U.S. EPA, 1991).

Thirty-five water utilities nationwide, 10 of which were located in California, were sampled for bromodichloromethane, dibromochloromethane, and bromoform in the clearwell effluent. Samples were taken for four quarters (spring, summer, and fall in 1988 and winter in 1989). The median bromodichloromethane concentration for all four quarters was 6.6 μg/L (ppb), with the medians of the individual quarters reported as 6.9, 10, 5.5 and 4.1 μg/L (ppb), respectively, and with a maximum value of 82 μg/L (ppb). For all four quarters, 75% of the measured concentrations were less than 14 μg/L (ppb). The median dibromochloromethane concentration for all four quarters was 3.6 μg/L (ppb), with the medians of the individual quarters reported as 2.6, 4.5, 3.8 and 2.7 μg/L (ppb), respectively, and with a maximum value of 63 μg/L (ppb). For all four quarters, 75% of the data were below 9.1 μg/L (ppb). The median bromoform concentration for all four quarters was 0.57 μg/L (ppb), with the medians of the individual quarters reported as 0.33, 0.57, 0.88, and 0.51 μg/L (ppb), respectively, and with a maximum value of 72 μg/L (ppb). For all four quarters, 75% of the bromoform concentrations were below 2.8 μg/L (ppb) (Krasner et al., 1989; U.S. EPA 1989a; 1989b).

The EPA's Technical Support Center compiled a database from its disinfection by-products field studies. The studies included a chlorination by-products survey, conducted from October 1987 to March 1989. In this survey, concentrations of bromodichloromethane, dibromochloromethane, and bromoform were determined in finished water from the treatment plant and in the distribution system. Systems using both surface water sources and ground water sources were analyzed.

Mean concentrations of bromodichloromethane, dibromochloromethane, and bromoform in finished water at the treatment plants were determined for surface water systems serving both greater than and less than 10,000 people. Forty-two samples were taken from systems serving more than 10,000 people, and 20 samples were taken from systems serving fewer than 10,000 people. The mean concentration of bromodichloromethane was 12.7 μg/L (ppb) in samples from systems serving more than 10,000 people (90th percentile, 25.0 μg/L (ppb)) and 17.0 μg/L (ppb) for samples from the smaller systems (90th percentile, 29.5 μg/L (ppb)). The mean

dibromochloromethane concentrations was 4.7 μg/L (ppb) for samples from the larger systems (90th percentile, 13.8 μg/L (ppb)) and 6.9 μg/L (ppb) for samples from the smaller systems (90th percentile, 24.2 μg/L (ppb)). The mean concentrations for bromoform were 0.7 μg/L (ppb) (90th percentile, 1.5 μg/L (ppb)) and 0.9 μg/L (ppb) (90th percentile, 4.9 μg/L (ppb)) in samples from the larger systems and samples from the smaller systems, respectively (U.S. EPA, 1992a).

Mean bromodichloromethane, dibromochloromethane, and bromoform concentrations in distribution systems of these surface water systems also were analyzed. Thirty-nine samples were taken from systems serving more than 10,000 people, and 11 samples were from systems serving fewer than 10,000 people. The mean bromodichloromethane concentrations in the larger systems and the smaller systems were 17.4 μg/L (ppb) (90th percentile, 35.3 μg/L (ppb)) and 24.8 μg/L (ppb) (90th percentile, 51.0 μg/L (ppb)), respectively. The mean dibromochloromethane concentrations were 6.3 μg/L (ppb) (90th percentile, 17.3 μg/L (ppb)) and 10.4 μg/L (ppb) (90th percentile, 35.0 μg/L (ppb)), respectively. Mean bromoform concentrations were 0.8 μg/L (ppb) (90th percentile, 3.1 μg/L (ppb)) and 1.4 μg/L (ppb) (90th percentile, 5.1 μg/L (ppb)), respectively (U.S. EPA, 1992a).

Ground water systems serving less than 10,000 people were analyzed for bromodichloromethane, dibromochloromethane, and bromoform in seven finished water samples and in five distribution system samples. Mean bromodichloromethane concentrations in the finished water samples and in the distribution system samples were 1.1 μg/L (ppb) (90th percentile, 2.6 μg/L (ppb)) and 2.2 μg/L (ppb) (90th percentile, 5.4 μg/L (ppb)), respectively. Mean dibromochloromethane concentrations were 0.6 μg/L (ppb) (90th percentile, 1.0 μg/L (ppb)) and 1.8 μg/L (ppb) (90th percentile, 3.6 μg/L (ppb)), respectively. Mean bromoform concentrations were 0.6 μg/L (ppb) (90th percentile, 2.6 μg/L (ppb)) and 2.3 μg/L (ppb) (90th percentile, 10 μg/L (ppb)), respectively.

For ground water systems serving more than 10,000 people, dibromochloromethane and bromoform were not detected in single samples taken at the plant or from the distribution system, based on a detection limit of 0.2 μg/L (ppb). Bromodichloromethane concentrations in the plant and distribution system samples were 0.2 and 0.4 μg/L (ppb), respectively (U.S. EPA, 1992a).

The U.S. Geological Survey conducted an assessment of volatile organic compounds in untreated ambient groundwater of the conterminous United States based on samples collected between 1985 and 1995 from 2948 wells. The sampled wells were located in rural and urban areas and included wells used for drinking and non-drinking water purposes. A minimum reporting level of 0.2 μg/L (ppb) was used for most of the compounds, including bromodichloromethane, dibromochloromethane, and bromoform. In samples from the 406 urban wells assessed, bromodichloromethane, dibromochloromethane, and bromoform were detected in 3.0%, 2.8%, and 2.8% of the wells examined, respectively. In samples from the 2542 rural wells examined, these compounds were detected in 0.8%, 0.6%, and 0.4% of the wells, respectively. The measured concentration of the compounds in well water were reported in summary graphics only. Thus, the values reported here are approximate based on visual inspection of the figures. The median concentrations measured in the positive samples from the

urban wells was approximately 1.0 µg/L (ppb) for all three compounds, while the maximum concentrations of bromodichloromethane, dibromochloromethane, and bromoform in the urban wells were approximately 11, 11, and 13 µg/L (ppb), respectively. The median concentrations measured in the positive samples from the rural wells were approximately 0.4 to 0.5 µg/L (ppb) for all three compounds, while the maximum concentrations of bromodichloromethane, dibromochloromethane, and bromoform in the rural wells were approximately 7, 10, and 18 µg/L (ppb), respectively.

The most recent survey of the occurrence of brominated trihalomethanes in public water supplies (PWSs) serving at least 100,000 persons resulted from the Information Collection Rule (ICR) promulgated in May of 1996 for disinfectants and disinfection byproducts (D/DBPs). The rule covered both surface and ground water systems. Monitoring data were collected from about 300 water systems operating 501 plants over the18-month period between July 1997 and December 1998. At each plant, samples were collected monthly and analyzed for a variety of D/DBPs on a monthly or quarterly basis. Bromodichloromethane, dibromochloromethane, and bromoform were among the analytes evaluated quarterly (U.S. EPA, 2001a). Five samples were taken each quarter at each plant – one of the finished water and four of the water in the distribution system. Of the four samples from the distribution system, one represented a sample with the same residence time as a finished water sample held for a specific period of time, two represented approximate average water residence times in the system, and one sample was taken where water residence time in the system is the longest. For each plant and reporting period, EPA compiled several summary statistics. The Distribution System (DS) Average value is the average of the four distribution system samples. The DS High Value is the highest concentration of the four distribution system samples collected by a plant in a given quarter. The DS High Value might be from any of the four samples and could vary from quarter to quarter depending on which sample yielded the highest concentrations in each quarter (U.S. EPA, 2001a). Table IV-1 summarizes the results of all six of the quarterly reporting periods.

U.S. EPA set a minimum reporting level (MRL) for bromodichloromethane, dibromochloromethane, and bromoform of 1.0 µg/L for the ICR. The MRL is a level below which systems were not required to report their monitoring results, even if there were detectable results. Values below the MRL were assigned a value of zero for the purpose of calculating averages; this assignment affects the calculation of mean values for finished water and DS high results and calculation of all DS average values.

Recent data for concentrations of brominated trihalomethanes are now available for 117 small surface water plants (serving $\leq$10,000 people) from the National Rural Water Association Survey (NWRA) (U.S. EPA 2001b). Most, but not all, plants that participated in the survey took two samples at each of three sampling locations. One sample was taken between November, 1999, and March, 2000, and the other between July and November, 2000, for a total of 217 THM samples. The samples were taken at the finished water location, distribution system average residence time location, and maximum residence time location. These data are summarized in Table IV-2 below.

Table IV-1 Brominated Trihalomethane Concentrations Measured in U.S. Public Drinking Water Systems Serving 100,000 or More Persons

Source	Data Type (a)	Number of Samples	Median (b)	Mean (b)	90th Percentile	Range
Bromodichloromethane (µg/L)						
Suface Water	Finished	1856	6.6	8.2	17.5	<1.0 - 49
	DS Average	1656	8.6	10.2	20.3	0 - 65.8
	DS High	1656	9.9	11.9	23.3	<1.0 - 73
Ground Water	Finished	604	< 1.0	7.9	6.80	<1.0 - 27
	DS Average	603	1.80	4.06	11.2	0 - 35.3
	DS High	603	2.8	5.78	16.0	<1.0 - 110
Dibromochloromethane (µg/L)						
Surface Water	Finished	1853	1.9	4.03	12.0	<1.0 - 55.1
	DS Average	1655	2.40	4.72	13.2	0 - 67.3
	DS High	1655	2.9	5.57	15.0	< 1.0 - 67.3
Ground Water	Finished	604	< 1.0	1.38	4.10	<1.0 - 33
	DS Average	602	1.35	3.09	8.94	0 - 37.5
	DS High	602	2.1	4.60	12.9	<1.0 - 85
Bromoform (µg/L)						
Surface Water	Finished	1853	<1.0	0.998	2.88	<1.0 - 34
	DS Average	1653	0	1.18	3.10	0 - 34.3
	DS High	1653	<1.0	1.48	3.90	<1.0 - 40
Ground Water	Finished	602	<1.0	0.838	2.20	<1.0 - 21
	DS Average	599	0.325	1.92	4.78	0 - 28.8
	DS High	599	1.2	2.95	7.72	<1.0 - 391

(a) Finished = sample location after treatment, before entering the distribution system (DS); DS Average = average of four sample locations in the DS; DS High = the highest concentration of the four distribution system samples collected by a plant in a given quarter. For purposes of calculations, all values below the minimum reporting level (MRL) of 1.0 µg/L for all three compounds were assigned a value of zero.
(b) Median and mean of all samples including those below the MRL.
Source: Disinfectants and Disinfection Byproducts (D/DBPs) ICR Data, U.S. EPA (2001a).

Table IV-2 NRWA Brominated Trihalomethane Results for Small Surface Water Plants

THM	Sample Location	Mean	Median	90th Percentile	Range
Bromodichloromethane (µg/L)	Finished	11.2	6.5	26.6	0 - 84.4
	DS Average	14.3	9.4	32.2	0 - 100.3
	DS Max	15.9	10.2	34.2	0 - 121.1
Dibromochloromethane (µg/L)	Finished	5.0	1.1	13.4	0 - 83.1
	DS Average	6.1	1.5	16.3	0 - 99.0
	DS Max	6.7	1.9	17.1	0 - 91.6
Bromoform (µg/L)	Finished	4.0	0	1.2	0 - 333.4
	DS Average	4.6	0	1.2	0 - 340.5
	DS Max	4.5	0	1.3	0 - 349.7

Median and mean of all samples, including those below the detection limit.
Source: National Rural Water Association Survey U.S. EPA (2001b)

2. Other Studies

Several less comprehensive surveys have analyzed drinking water for one or more of the brominated trihalomethanes. An overview of these studies is provided below.

The EPA Region V Organics Survey sampled finished water from 83 sites in a region that includes Illinois, Indiana, Michigan, Minnesota, Ohio, and Wisconsin. Bromoform was found at a median concentration of the positives of 1 µg/L (ppb) and a maximum level of 7 µg/L (ppb). A total of 14% of the locations sampled contained detectable levels of bromoform (U.S. EPA, 1980). Kelley (1985) surveyed 18 drinking water plants in Iowa for trihalomethanes, and detected bromoform in five water supplies at concentrations ranging from 1.0 to 10 µg/L (ppb).

The EPA's Five-year Total Exposure Assessment Methodology (TEAM) study measured the personal exposures of a probability-based sample of residents in several U.S. cities to various organic chemicals in air and drinking water between 1981 and 1987. As part of the study, running tap water samples were collected from residences of nearly 850 study participants during the morning and the evening to test for brominated trihalomethane concentrations. The samples were quenched with sodium thiosulfate at the time of collection. Tables IV-3, IV-4, and IV-5 show bromodichloromethane, dibromochloromethane, and bromoform concentrations found in drinking water from the six cities surveyed. Samples of water were taken from each participating residence at the household taps and sodium thiosulfate added as a quenching agent.

Table IV-3 Bromodichloromethane Concentrations in Drinking Water from the U.S. EPA TEAM Study (μg/L)

Location	Date Sampled	Sample Size	% Measured	Concentration μg/L (ppb)						
				Mean	Median	Max	Percentiles			
							25%	75%	90%	95%
Elizabeth/ Bayonne, New Jersey	Fall 1981	340	99.7	13.6	13	23	--	15	16	18
	Summer 1982	156	99.8	13.6	12	54	--	15	18	20
	Winter 1983	49	100	5.4	5.8	16	--	7.1	8.3	8.3
Los Angeles, California	Winter 1984	117	93	11	12	23	5.1	16	17	20
	Summer 1984	52	96	20	24	38	7.7	31	33	37
	Winter 1987	9	89	19	24	31	--	--	--	--
	Summer 1987	7	100	26	27	36	--	--	--	--
Antioch/ Pittsburg, California	Spring 1984	71	96	21	17	47	2.4	36	45	47
Devils Lake, North Dakota	Fall 1982	24	73	0.21	0.18	1.0	--	--	--	--
Greensboro, North Dakota	Fall 1982	24	93	7.1	7.8	11	--	9.2	--	--
Baltimore, Maryland	Spring 1987	10	100	10	10	13	--	--	--	--

Adopted from Hartwell, (1987), Wallace et al. (1987), Wallace et al. (1988), and Wallace (1992) by U.S. EPA (1994b). Mean and median values of all samples, including those below the quantitation limit.

Table IV-4 Dibromochloromethane Concentrations in Drinking Water from the U.S. EPA TEAM Study

Location	Date Sampled	Sample Size	% Measured	Concentration μg/L (ppb)						
				Mean	Median	Max	Percentile			
							25%	75%	90%	95%
Elizabeth/ Bayonne, New Jersey	Fall 1981	340	99.7	2.4	2.4	8.4	--	2.7	3.2	3.4
	Summer 1982	156	99.8	2.1	1.9	7.2	--	2.4	3.1	3.8
	Winter 1983	49	93	1.4	1.6	3.0	--	1.8	2.0	2.1
Los Angeles, California	Winter 1984	117	89	9.4	11	19	2.4	15	17	18
	Summer 1984	52	85	28	32	55	15	42	43	48
	Winter 1987	9	89	10	12	17	--	--	--	--
	Summer 1987	7	100	24.7	18	70	--	--	--	--
Antioch/ Pittsburg, California	Spring 1984	71	85	8	6.4	21	0.98	15	18	19
Devils Lake, North Dakota	Fall 1982	24	18	0.09	0.06	0.45	--	0.06	--	--
Greensboro, North Dakota	Fall 1982	24	93	1.2	1.2	1.9	--	1.5	--	--
Baltimore, Maryland	Spring 1987	10	100	2.7	2.6	3.5	--	--	--	--

Adopted from Hartwell, (1987), Wallace et al. (1987), Wallace et al. (1988), and Wallace (1992) by U.S. EPA (1994b). Mean and median values of all samples, including those below the quantitation limit.

Table IV-5 Bromoform Concentrations in Drinking Water from the U.S. EPA TEAM Study

Location	Date Sampled	Sample Size	% Measured	Concentration µg/L (ppb)						
				Mean	Median	Max	Percentile			
							25%	75%	90%	95%
Los Angeles, California	Winter 1984	117	69	0.78	0.54	12	0.34	0.92	1.2	1.5
	Summer 1984	52	90	8.08	3.0	78	2.0	5.9	13	53
	Winter 1987	9	89	3.2	3.2	4.7	--	--	--	--
	Summer 1987	7	100	25.5	9.6	113	--	--	--	--
Antioch/ Pittsburg, California	Spring 1984	71	69	0.78	0.58	2.0	0.19	1.2	1.8	1.9

Adopted from Wallace (1992) by U.S. EPA (1994b). Mean and median values of all samples, including those below the quantitation limit. Bromoform was measured in fewer than 10% of samples from the other four cities in the TEAM study and are not presented here

Furlong and D'itri (1986) reported that a survey of water treatment plants in Michigan detected bromodichloromethane in 35 of 40 plants at a median concentration of 2.7 µg/L (ppb) and a maximum of 54.2 µg/L (ppb); the mean of the positive samples was 7.4 µg/L (ppb).

Dibromochloromethane was also detected in 30 plants at a median concentration of 2.2 µg/L (ppb) and a maximum of 39.6 µg/L (ppb); the mean of the positives was 5.1 µg/L (ppb). Bromoform was detected at three of 40 plants sampled at concentrations of 0.9, 1.3, and 1.6 µg/L (ppb).

Fair et al. (1988) analyzed drinking water from three community water supplies for chlorination by-products. Bromodichloromethane concentrations ranged from 7.5 to 30 µg/L (ppb) in finished water and from 9.9 to 36 µg/L (ppb) in the distribution systems. Dibromochloromethane concentrations ranged from less than 0.5 to 19 µg/L (ppb) in finished water at the plant and from less than 0.5 to 23 µg/L (ppb) in the distribution systems. Bromoform concentrations ranged from less than 0.5 to 2.5 µg/L (ppb) in finished water and from less than 0.5 to 3.1 µg/L (ppb) in the distribution systems.

Wallace et al. (1982) analyzed tap water for bromodichloromethane as part of a study to determine individual exposures to volatile organics during normal daily activities of students at the University of North Carolina, Chapel Hill. Bromodichloromethane was detected in 7 of 7 samples of tap water, at concentrations ranging from 15 to 20 μg/L (ppb), with a mean of 17 μg/L (ppb). The detection limit was 0.1 μg/L (ppb).

Chang and Singer (1984) analyzed the bromoform concentration in drinking water samples prepared by the desalination of seawater. After pretreatment using either activated carbon or ultrafiltration, but prior to reverse osmosis treatment, bromoform concentrations were 13 ± 14 and 110 ± 59 μg/L (ppb), respectively. After reverse osmosis was completed, the finished water product contained bromoform concentrations ranging from 2.0 to 51 μg/L (ppb) (mean, 20.17 μg/L (ppb)) when activated carbon was used as a pretreatment and 127 μg/L (ppb) when ultrafiltration was used. In the reverse osmosis treatment, three reverse osmosis membranes were evaluated. Use of a cellulose triacetate filter resulted in concentrations of 51 μg/L (ppb), while use of a polyether/urea thin film spiral wound membrane or a polysulfone membrane filters which resulted in final concentrations of 5.0 μg/L (ppb) and 2.25 μg/L (ppb), respectively.

Bromodichloromethane, dibromochloromethane, and bromoform were detected in 9.5 to 12.8% of drinking water samples collected in 1987 in Nassau County, New York. The county draws its drinking water from underground aquifers. Bromodichloromethane and dibromochloromethane had similar concentration profiles, being detected in approximately 10% and 8.5% of the samples, respectively, at concentrations less than 4.9 ppb. The detection limit was 1 ppb for each chemical. Bromoform was detected in 8% of the samples at 2 to 4.9 ppb, in 2.5% of the samples at 5 to 9.9 ppb, and in less than 1% of the samples at 10 to 49.9 ppb. The detection limit was 2 ppb. None of the drinking water samples contained more than 50 ppb of any of the trihalomethanes, and less than 1% of the samples contained between 10 and 49.9 ppb of the brominated compounds (Moon et al., 1990).

U.S. EPA conducted a study of contaminants in household water in nine residences as part of a larger study of health risks due to environmental contamination in the Lower Rio Grande Valley (Berry et al., 1997). Samples of water used for drinking were taken once during a 3-day period in the spring and once during a 2-day period in the summer of 1993. Water used for drinking in the nine residences could be traced to one of three sources: the municipal water supply of Brownsville, Texas, vended water supplies (municipal water that had undergone further treatment), and well water. Samples were collected using U.S. EPA protocols, including quality assurance samples and field blanks. The detection and minimum quantitation limits for each analyte were documented in other reports. Bromodichloromethane, dibromochloromethane, and bromoform were detected in the household water of seven of the nine residences during the spring and in five of the nine residences during the summer (Berry et al., 1997). During the spring, the minimum, median, and maximum concentrations of bromodichloromethane for the seven positive samples were 3.2, 5.2, and 24.4 μg/L (ppb), respectively. For dibromochloromethane, the values were 3.3, 5.1, and 17.3 μg/L (ppb), respectively. For bromoform, the values were 1.0, 3.0, and 14.1 μg/L (ppb), respectively. During the summer, the minimum, median, and maximum concentrations of

bromodichloromethane in the five positive samples were 2.3, 7.7, and 34.3 µg/L (ppb), respectively. For dibromochloromethane, the values were 1.8, 7.6, and 49.9 µg/L (ppb), respectively. For bromoform, the values were 1.6, 7.8, and 31.7 µg/L (ppb), respectively.

Weisel et al. (1999) examined concentrations of trihalomethanes in the tap water of the homes of 49 women in New Jersey. The 49 residences were selected so that approximately half would represent the lower extreme of trihalomethane contamination and half the upper extreme of trihalomethane contamination identified in a previous study. Samples were stored unquenched on ice after collection and were analyzed within 24 hours. The three brominated trihalomethanes were detected in all 49 samples. The mean (± standard deviation) concentrations of bromodichloromethane, dibromochloromethane, and bromoform were 5.7 ± 8.6, 2.0 ± 2.1, and 0.73 ± 0.90 µg/L (ppb), respectively. The median values for the three compounds were 2.6, 1.4, and 0.45 µg/L (ppb), respectively. These values are not representative of New Jersey, because of the selection criteria for the residences. The ranges (minimum to maximum) of concentrations measured for each compound were 0.06 to 48 µg/L (ppb) for bromodichloromethane, 0.14 to 9.7 µg/L (ppb) for dibromochloromethane, and 0.03 to 4.21 µg/L (ppb) for bromoform.

3. Estimates of Tap Water Ingestion Exposure to Brominated Trihalomethanes

a. Estimates Based on ICR Data for Disinfection Byproducts

The data from EPA's ICR for disinfectants and disinfection byproducts (U.S. EPA 2001a) offer several advantages over the other national studies for purposes of estimating national exposure levels of adults in the United States to brominated trihalomethanes via ingestion of drinking water. First, they are recent and reflect relatively current conditions. Second, data of very similar quality and quantity were collected systematically from a large number of plants (501) and systems (approximately 300), including both surface and ground water systems. Third, the mean, median, and 90th percentile value were estimated on the basis of all samples taken, not just the sample detects. Thus, these descriptive statistics are representative of the exposures of the entire populations served by those systems, not just the populations served by systems with higher concentrations of these compounds. However, this study can not be considered representative of smaller public water supplies or water supplies from the most highly industrialized or contaminated areas.

Table IV-6 presents estimated drinking water exposures to brominated trihalomethanes of the adult populations served by large public water systems (serving 100,000 or more persons) based on the ICR Occurrence Data (U.S. EPA, 2001a). Exposure was calculated by multiplying the concentration of individual brominated trihalomethanes in drinking water by the average daily intake, assuming that each individual consumes two liters of water per day. The annual median, mean, and upper 90th percentile values are presented for both surface and ground water systems. Assuming that the DS High value actually represents the average exposure level of persons served by one plant distribution pipe with the longest water-residence time, the DS High value might be used to estimate a high-end exposure level. Thus, the 90th percentile of the DS High values are also presented in Table IV-6.

Table IV-6 Estimated Drinking Water Exposures to Brominated Trihalomethanes in U.S. Public Drinking Water Systems Serving More than 100,000 Persons[a]

Source	Median[b]	Mean[b]	90th Percentile[b]	DS High 90th Percentile[c]
Bromodichloromethane (µg/person/day)				
Surface Water	17	20	40	47
Ground Water	3.6	8.1	22	32
Dibromochloromethane (µg/person/day)				
Surface Water	4.8	9.4	26	30
Ground Water	2.7	6.2	18	26
Bromoform (µg/person/day)				
Surface Water	0	2.4	6.2	7.8
Ground Water	0.65	3.8	9.6	15

[a] Source: U.S. EPA (2001a). Assumes that each individual consumes 2 liters of water daily. Also assumes that concentrations at the drinking water tap are similar to concentrations in the distribution system (DS) sampled at locations considered to be representative of average (DS Average) and highest (DS High) retention times (see Table IV-1).
[b] Based on concentrations from the DS Average values.
[c] Based on the 90th percentile of the DS High values to represent a plausible high-end exposure level.

For bromodichloromethane, the median, mean, and 90th percentile population exposures from surface water systems are estimated to be 17, 20, and 40 µg/person/day, respectively. The same values for populations exposed to bromodichloromethane from ground water systems are lower – 3.6, 8.1, and 22 µg/person/day, respectively. For dibromochloromethane, the median, mean, and 90th percentile population exposures from surface water systems are estimated to be 4.8, 9.4, and 26 µg/person/day, respectively. The corresponding values for populations exposed to dibromochloromethane from groundwater system are lower – 2.7, 6.2, and 18 µg/person/day, respectively. For bromoform, the median, mean, and 90th percentile population exposures from surface water systems are estimated to be near 0, 2.4, and 6.2 µg/person/day, respectively. The same values for populations exposed to bromoform from ground water systems are higher – 0.65, 3.8, and 9.6 µg/person/day, respectively.

Average daily intake of dibromochloromethane was also evaluated for determination of the Relative Source Concentration. The details of this evaluation are presented in Appendix C. Intake for ingestion was calculated using mean intake rates of 1.2 or 0.6 L/day for total and direct intake (NRC, 1999), respectively. Direct intake includes consumption of water directly from the tap, but does not include intake of tap water used for preparation of heated items such tea, coffee, or soup. Based on the ICR distribution system average concentration of 4.72 µg/L for dibromochloromethane in surface water, the average daily total and direct and ingestion

intakes would be 5.7 and 2.8 μg/day, respectively. Absorption of dibromochloromethane from tap water was estimated using methodology described in U.S. EPA (1992c), as modified by Vecchia and Bunge (2002). The average dermal uptake of dibromochloromethane was estimated to be 2 μg per shower or bathing event. Intake via inhalation of dibromochloromethane volatilized during household activities (e.g., showering, bathing, dishwashing, toilet flushing, etc.) was estimated using a three-compartment model based on McKone (1987). This model estimated an average daily inhalation exposure of 7 μg/day for the volatilized compound. Parallel calculations were not performed for bromodichloromethane or bromoform, because these compounds are probable carcinogens. Therefore, in accordance with U.S. EPA policy, RSC analysis was not conducted.

b. Estimates of Ingestion Exposure Based on Other National Studies

Exposure to bromodichloromethane, dibromochloromethane, and bromoform in drinking water from ground water supplies can be estimated from the median levels found in the GWSS. Based on the range of median levels (1.4–2.1 μg/L (ppb)) and a consumption rate of two liters per day, the median exposure to bromodichloromethane may range from 2.8 to 4.2 μg/day. Similarly, median exposure to dibromochloromethane may range from 4.2 to 7.8 μg/day, and for bromoform, median exposure may range from 4.8 to 8.4 μg/day. Exposure to bromodichloromethane from surface water supplies can be estimated based on the range of median values observed under different conditions in NOMS, which mainly sampled surface water systems. Based on a range of 5.9–14 μg/L (ppb), exposure to bromodichloromethane from surface water is estimated to be between 12 and 28 μg/day. Similarly, based on the range of medians reported for dibromochloromethane concentrations, the median exposure is estimated to be up to 6 μg/day. The median levels of bromoform in the surface water supplies have been found to be less than the EPA Drinking Water minimum reporting levels (MRLs) of 0.5–1 μg/L (ppb). An estimate of exposure based on the MRLs will be overly conservative because the actual concentration of bromoform is not detectable. Based on the range of MRLs, 0.5–1 μg/L (ppb), the exposure to bromoform is estimated to range from 1 to 2 μg/day for surface water supplies.

Ingestion exposure to brominated trihalomethanes in drinking water can also be estimated from the concentrations found at the tap in the TEAM studies. Table IV-7 presents median, mean, 90th percentile, and 95th percentile estimates of daily intakes of bromodichloromethane, dibromochloromethane, and bromoform, based on an assumed drinking water ingestion rate of 2 liter per day. Table IV-7 provides estimates for those locations and seasons with a sample size of at least 50, with one exception. Devils Lake, ND, with a sample size of only 24, is added to represent an area with low concentrations. Thus, the influence of small sample size on distributional statistics should be minimized in Table IV-7. The median, mean, and 90th percentile values in Table IV-7 for the TEAM study can be compared with the corresponding values in Table IV-6 for the ICR Occurrence data.

Table IV-7 Estimated Distribution of Drinking Water Exposures to Brominated Trihalomethanes for Populations in U.S. EPA TEAM Study (a)

Location	Season Year	Median (b)	Mean (b)	90th Percentile (b)	95th Percentile
Bromodichloromethane (µg/person/day)					
Elizabeth/Bayone NJ	summer 82	24	27	36	40
	winter 83	12	11	17	17
Los Angeles, CA	summer 84	48	40	66	74
	winter 84	24	22	34	40
Antioch/Pittsburg, CA	spring 84	34	42	90	94
Devils Lake, ND	fall 82	0.36	0.42	< 2.0	< 2.0
Dibromochloromethane (µg/person/day)					
Elizabeth/Bayone NJ	summer 82	3.8	4.2	6.2	7.6
	winter 83	3.2	2.8	4.0	4.2
Los Angeles, CA	summer 84	64	56	86	96
	winter 84	22	19	34	36
Antioch/Pittsburg, CA	spring 84	13	16	36	38
Devils Lake, ND	fall 82	0.12	0.2	< 0.9	< 0.9
Bromoform (µg/person/day)					
Los Angeles, CA	summer 84	6.0	16.2	26	100
	winter 84	1.1	1.6	2.4	3.0
Antioch/Pittsburg, CA	spring 84	1.2	1.6	3.6	3.8

(a) Intakes estimated from data in Tables IV-3, IV-4, and IV-5 assuming a water ingestion rate of 2 liters per day. Selected locations and seasons with samples sizes over 50. Added Devils Lake, ND, to represent an area with low air concentrations.
(b) Median, mean, and upper percentiles estimated for entire population of city.

Table IV-7 demonstrates that concentrations of brominated trihalomethanes are lower in winter than in summer, as would be expected on the basis of temperature. In this sample of geographic locations, estimates of the average of the population intakes of bromodichloromethane from drinking water range from 0.42 to 42 µg/person/day. The upper

90th percentile estimates range from <2.0 to 90 µg/person/day. Estimates of the average population intake of dibromochloromethane from drinking water range from 0.2 to 56 µg/person/day. The upper 90th percentile estimates range from < 0.9 to 86 µg/person/day. Estimates of the average of the population intakes of bromoform, for those areas in which bromoform was measurable in a majority of the samples, range from 1.6 to 16.2 µg/person/day. The upper 90th percentile estimates range from 2.4 to 26 µg/person/day. Four of the six locations in the TEAM study, however, had a low frequency (less than 10%) of detection of bromoform in measurable quantities.

c. Sources of Uncertainty in Estimates of Exposure from Drinking Water

Sources of uncertainty in the estimates of ingestion exposure include use of different analytical methods, failure to report quantitation limits, use of measurements near the detection limit, failure to report how nondetects were handled when averaging values (e.g., set to zero or one half the detection limit), and failure to report sample storage method and duration. In addition, many environmental factors influence the concentrations of these compounds in drinking water at the tap and in vended or bottled waters used for drinking. These factors include season and temperature, geographic location, source of water, residence time in distribution system, and others.

B. Exposure from Sources Other Than Drinking Water

1. Dietary Intake

a. Measured Concentrations in Foods and Beverages

Information on the levels of brominated trihalomethanes in foods and beverages is limited. Chlorine is used in food production for applications such as the disinfection of chicken in poultry plants and the superchlorination of water at soda and beer bottling plants (Borum, 1991). Therefore, the possibility exists for contamination of foodstuffs by disinfection by-products with resulting dietary exposure. The occurrence of bromodichloromethane in foods and beverages is the best characterized of the three compounds. Less information is available concerning the occurrence of dibromochloromethane or bromoform in foods and beverages in the United States. Some information is available from international studies, but may not be relevant to U.S. occurrence because of different water treatment and food processing practices. The available U.S. and international studies are summarized below.

Entz et al. (1982) analyzed food samples from Elizabeth, NJ, Chapel Hill, NC, and Washington, DC, for bromodichloromethane. A total of 39 different food items from each city were collected according to standards set for the FDA's Total Diet Market Basket Study. The Adult Market Basket, representing the diet of a teenage male, is divided into 12 food groups. Individual foods are prepared as generally consumed in the home and foods from each group are blended together in “the proper proportions” to form composites. In this study, foods were blended into four composites representing dairy products; meat, fish and poultry; oils, fats and shortening; and beverages. The estimated limit of quantitation for bromodichloromethane in

each of these composites was 2.3, 4.5, 8.3, and 0.5 ng/g, respectively. Five sets of each composite were tested for a total of 20 composites. Bromodichloromethane was detected in one dairy composite at 1.2 ppb and two beverage composites at 0.3 ppb and 0.6 ppb. Analysis of individual foods from the beverage and dairy composites found bromodichloromethane in three samples of cola soft drinks at concentrations of 2.3 ppb, 3.4 ppb, and 3.8 ppb and in one sample of butter at 7 ppb.

Uhler and Diachenko (1987) sampled 38 food and beverage products from 15 food processing plants in nine states. Plants were chosen on a "worst-case" basis from areas where contaminated water would most likely be used in processing. In addition, processing plants were chosen for study only if they produced high fat content food that came in contact with water during processing or contained a high percentage of added water. Samples containing less than 1 ng/g were considered nondetects. Bromodichloromethane was detected in 6 out of 37 tested food tested at the following levels: two samples of clear sodas at 1.2 and 2.3 ng/g (ppb) and one sample of dark cola at l.2 ng/g (ppb) out of fifteen soft drinks, and three of six samples of ice cream at 0.6 to 2.3 ng/g (ppb). Bromodichloromethane was not found in any of the eight cheese samples analyzed.

U.S. EPA (1985) reported that bromodichloromethane was identified in bacon. No further information on sample size, detection limit, or study methodology was provided.

Abdel-Rahman (1982) analyzed various soft drinks for bromodichloromethane and found average levels ranging from 0.2 to 6.6 μg/L (ppb) for colas and from 0.1 to 0.2 μg/L (ppb) for clear soft drinks (Abdel-Rahman, 1982). In Italy, Cocchioni et al. (1996) analyzed 61 samples of different commercially prepared beverages and 94 samples of mineral waters for volatile organo-halogenated compounds. In the prepared beverages, they found maximum concentrations of bromodichloromethane, dibromochloromethane, and bromoform of 40.6, 13.9, and 10.7 μg/L (ppb), respectively. The frequencies of detection of these three compounds in prepared beverages were 46% (28/61), 43% (26/61), and 11% (7/61), respectively, with detection limits for all three compounds of less than 1 μg/L (ppb). In contrast, the maximum concentration of any of the halogenated organic compounds identified in mineral water, including chloroform, was 5.79 μg/L (ppb).

McNeal et al. (1995) examined 27 different prepared beverages and mineral waters in the United States for bromodichloromethane, dibromochloromethane, and bromoform at detection limits of 0.1, 0.1, and 0.2 ng/g (ppb), respectively. Bromoform was not detected in any of the samples. Bromodichloromethane and dibromochloromethane were detected at 12 and 1 ng/g (ppb), respectively, in only one of seven types of mineral and sparkling waters examined. The positive sample was the only sparkling and flavored water of the group,. Bromodichloromethane was found in 1 of 5 flavored noncarbonated beverages examined, a fruit drink, at a concentration of 5 ng/g (ppb); dibromochloromethane was not detected in any of these five beverages. Bromodichloromethane was found in all 13 of the types of carbonated soft drinks examined, at concentrations ranging from 1 to 4 ng/g (ppb) for 12 of the drinks examined and at 12 ng/g (ppb) for the thirteenth. Dibromochloromethane was detected in only 4 of the 13 carbonated soft

drinks examined at levels of 0.5 to 2 ng/g (ppb). None of the brominated trihalomethanes was detected in either of the two types of beer examined.

McNeal et al. (1995) also examined several types of prepared non-beverage foods and water from canned vegetables in the United States for bromodichloromethane, dibromochloromethane, and bromoform. None of these compounds was detected in any of the samples. The foods examined included two types of canned tomato sauce, canned pizza sauce, canned vegetable juice, vegetable waters from two types of canned green beans and one type of sweet corn, duck sauces, beef extract, and Lite syrup product.

The U.S. Food and Drug Administration (U.S. FDA, 2000) has analyzed for 18 volatile organic hydrocarbons (VOCs), including bromodichloromethane and bromoform, in the Total Diet Study since 1995. Bromodichloromethane and bromoform were analyzed in a subset of 70 food items in 14 Market Baskets. During the period 1995 to 1999, bromodichloromethane was detected in one sample each of 11 non-beverage food items (sliced bologna, fried eggs, canned pork and beans, smooth peanut butter, homemade cornbread, raw orange, canned pineapple, boiled collards, red tomato, green pepper, and fast-food hamburger) (U.S. FDA, 2000). The detected concentrations ranged from 10 to 16 ppb, with the exception of fast food hamburger which contained 37 ppb. Bromodichloromethane was detected in one sample of bottled apple juice at a concentration of 33 ppb. The mean detected concentration of bromodichloromethane in three samples of tap water was 18 ppb. Dibromochloromethane was not included in the list of VOC analytes for the Total Diet Study. Bromoform was listed as an analyte, but no detections were reported in the data summary for 1991 to 1999. The detection limits for bromodichloromethane and bromoform were not reported.

Imaeda et al. (1994) examined bean curd commercially available in Japan for trihalomethanes. Neither bromoform nor dibromochloromethane were detected in any of the samples at a detection limit of 0.1 ppb. Bromodichloromethane was detected in 6 of 10 samples of bean curd at concentrations ranging from 1.2 to 5.2 ppb and in 1 of 10 samples of the water in the bean curd packages at 5.2 ppb.

Kroneld and Reunanen (1990) analyzed for brominated trihalomethanes in samples of pasteurized and unpasteurized cow's milk collected in Turku, Finland. The average concentration of bromodichloromethane measured in pasteurized milk was 0.008 μg/L (ppb) (range, undetectable to 0.03 μg/L (ppb), detection limit not specified). Dibromochloromethane was detected in only one sample of pasteurized milk at 5 μg/L (ppb). Traces of bromoform were detected but not quantified. Brominated trihalomethanes were not detected in unpasteurized milk. Their presence in pasteurized milk was considered to result from use of chlorinated water during processing.

b. Estimated Dietary Intake

Estimates for dietary intake of brominated trihalomethanes by residents of the United States were not identified in the materials reviewed for this document. Furthermore, information on the levels in U.S. foods is too limited to independently calculate a reliable estimate.

However, the available data suggest that the concentrations of brominated trihalomethanes in non-beverage foods are likely low. The apparently low concentrations of brominated trihalomethanes in non-beverage foods are consistent with the physical and chemical properties of these compounds. The levels of individual brominated trihalomethanes in beverages prepared in the United States appear to be less than or about equal to levels measured in disinfected surface water.

Toyoda et al. (1990) analyzed the dietary intake of bromodichloromethane, dibromochloromethane, and bromoform for 30 Japanese housewives in Nagoya and Yokohama, Japan. Duplicate portions of daily meals were collected for three consecutive days and sampled for all three brominated trihalomethanes. The types of food consumed were not reported. This omission prevents a meaningful comparison of the studied diet to that consumed by the U.S. population. The detection limits for bromodichloromethane, dibromochloromethane, and bromoform were reported to be 0.1, 0.2, and 0.5 ppb, respectively. The concentration of bromodichloromethane ranged from undetectable to 1.7 ppb (average, 0.3 ± 0.3 ppb SD). The mean daily intake of bromodichloromethane was estimated to be 0.6 ± 0.5 μg/day. The concentration of dibromochloromethane ranged from undetectable to 0.6 ppb (average, 0.1 ± 0.2 ppb), and the mean dietary intake was estimated to be 0.3 ± 0.3 μg/day. The concentration of bromoform ranged from undetectable to 8.1 ppb (average, 0.5 ± 1.3 ppb). The mean dietary intake of bromoform was estimated to be 0.9 ± 1.3 μg/day.

Brominated trihalomethanes have been detected in a number of beverages. In conducting an exposure assessment, the potential exposures from drinking prepared beverages would not be added to the default assumption of an adult consuming 2 liters of drinking water per day. Instead, the prepared beverages would be considered part of the 2 liters of fluid intake per person per day.

2. Air Intake

a. Concentrations in Outdoor Air

Brominated trihalomethanes are usually found in outdoor air at low concentrations when all data, including nondetects, are considered. Brodzinsky and Singh (1983) reviewed, summarized, and critically evaluated existing data for brominated trihalomethane concentrations in ambient outdoor air for several urban/suburban or source dominated locations across the United States (Table IV-8). No concentration data were available for rural or remote areas. The authors reported mean, median, first and third quartile values, and minimum and maximum values by city. In addition, they reported the same measures when the data were grouped by type of location (i.e., urban/suburban or source dominated), and when all data were combined. Ambient air concentrations were reported for bromodichloromethane at Magnolia, AR, El Dorado, TX, Chapel Hill, NC, and Beaumont, TX. Bromodichloromethane was detected at mean concentrations of 0.76 ppt, 1.40 ppt, 120 ppt, and 180 ppt for those four cities, respectively, where ppt is expressed as parts per trillion by volume. Dibromochloromethane was detected in the air samples from Magnolia, AR, El Dorado, TX, Chapel Hill, NC, Beaumont TX, and Lake Charles, LA at mean concentrations of 0 ppt, 0.48 ppt, 14 ppt, 14 ppt, and 19 ppt,

respectively. Bromoform was detected in air samples from Magnolia, AR, El Dorado, TX, and Lake Charles, LA, at concentrations of 1.5 ppt, 0.81 ppt, and 50 ppt, respectively. Air concentration data from these sites were combined for additional statistical analysis. The study authors indicated that a value of 0.0 was entered for samples below the detection limit. Mean (± standard deviation) outdoor air concentrations in urban/suburban and source dominated locations, respectively, were 160 ± 29 ppt and 1.2 ± 0.4 ppt for bromodichloromethane; 15 ± 4 ppt and 0.28 ± 0.67 ppt for dibromochloromethane; and 50 ± 29 ppt and 1.1 ± 2.1 ppt for bromoform. Brodzinsky and Singh (1983) also calculated overall (grand) means based on data from all sites. Grand mean values for bromodichloromethane, dibromochloromethane, and bromoform were 110 ppt (n = 26, with one nondetect), 3.8 ppt (n = 89, with 63 nondetects), and 3.6 ppt (n = 78, with 60 nondetects), respectively. When expressed on a $\mu g/m^3$ basis, the corresponding mean values for bromodichloromethane, dibromochloromethane, and bromoform are 0.74 $\mu g/m^3$, 0.032 $\mu g/m^3$, and 0.037 $\mu g/m^3$.

Table IV-8 Selected Concentration Data for Individual Brominated Trihalomethanes (ppt) in Outdoor Air as Summarized in Brodzinsky and Singh (1983)[a,b]

City	n	Nondetects	Mean (Std dev.)	Median	3rd Quartile	Maximum	Reference
Bromodichloromethane							
Individual Sites							
Beaumont, TX	11	0	180 (100)	180	180	180	Wallace (1981)
Chapel Hill, NC	6	0	120 (210)	120	120	120	Wallace (1981)
El Dorado, AR	7	1	1.4 (0.35)	1.6	1.6	1.6	Pellizzari and Bunch (1979)
Magnolia, AR	2	0	0.76 (0.0)	0.0	0.0	0.76	Pellizzari and Bunch (1979)
Totals							
Urban/Suburban	17	0	160 (29)	180	180	180	-
Source Areas	9	1	1.2 (0.41)	1.6	1.6	1.6	-
Grand totals	26	1	110 (82)	120	180	180	-
Dibromochloromethane							
Individual Sites							
Beaumont, TX	11	0	14 (0.0)	14	14	14	Wallace (1981)
Chapel Hill, NC	6	0	14 (0.0)	14	14	14	Wallace (1981)
El Dorado, AR	40	35	0.48 (0.82)	0.0	0.82	2.5	Pellizzari et al. (1978)
Lake Charles, LA	4	0	19 (9.6)	21	27	27	Pellizzari (1979)
Magnolia, AR	28	28	0.0 (0.0)	0.0	0.0	0.0	Pellizzari et al. (1978)

Table IV-8 (cont.)

City	n	Nondetects	Mean (Std dev.)	Median	3rd Quartile	Maximum	Reference
Dibromochloromethane (cont.)							
Totals							
Urban/Suburban	21	0	15 (4.2)	14	14	27	-
Source Areas	68	63	0.28 (0.67)	0.0	0.0	2.5	-
Grand Totals	89	63	3.8 (6.7)	0.0	2.5	27	-
Bromoform							
Individual Sites							
El Dorado, AR	46	35	0.81 (0.95)	0.43	1.3	2.7	Pellizzari et al. (1978) Pellizzari and Bunch (1979)
Lake Charles, LA	4	0	50 (29)	62	68	71	Pellizzari (1979)
Magnolia, AR	28	25	1.5 (3.2)	0.0	0.29	8.3	Pellizzari et al. (1978)
Totals							
Urban/Suburban	4	0	50 (29)	62	68	71	-
Source Areas	74	60	1.1 (2.1)	0.0	1.3	8.3	-
Grand Totals	78	60	3.6 (12)	0.0	1.5	71	-

[a] Includes only data considered to be of adequate, good, or excellent quality by the study authors.
[b] Concentrations are reported as parts per trillion by volume

Shikiya et al. (1984) analyzed ambient air samples collected at four urban/industrial locations in the California South Coast Air Basin from November 1982 to December 1983 for the presence of halogenated hydrocarbons. Data for bromodichloromethane, dibromochloromethane, and bromoform were included in this analysis. The sampling locations were El Monte, downtown Los Angeles, Dominguez, and Riverside. The air samples were analyzed using gas chromatography with detection by electron capture. The quantitation limit, defined as a level 10 times greater than the noise level, was 10 ppt by volume for all three brominated trihalomethanes. The detection limit was defined as three times the noise level. Summary data for each compound included monthly means and composite means. The monthly means were calculated as the average of all data at a site that were above the quantitation limit for a single month; samples with concentrations below the limit of detection were not included in the calculations. The composite means were calculated as the average value of all data for each compound above the quantitation limit at each site. Most data in this report were presented graphically. A few additional details were presented in a short summary statement for each chemical. Thirty-five percent of the samples had bromodichloromethane levels above the quantitation limit of 10 ppt (0.067 $\mu g/m^3$). Peaks in the concentration of bromodichloromethane were observed at various sites in June and July, with downtown Los Angeles and Dominguez registering the highest monthly means of approximately 30 ppt (0.20 $\mu g/m^3$). The highest reported concentration was 40 ppt (0.27 $\mu g/m^3$). The highest composite mean of 100 ppt (0.67 $\mu g/m^3$) for bromodichloromethane was observed at El Monte. In comparison, the remaining three locations had a composite mean of 20 ppt (0.08 $\mu g/m^3$). For dibromochloromethane, only seventeen percent of the samples had levels above the quantitation limit of 10 ppt (0.085 $\mu g/m^3$). The highest reported concentration, monthly mean, and mean composite for dibromochloromethane were 290 ppt (2.5 $\mu g/m^3$), 280 ppt (2.4 $\mu g/m^3$), and 50 ppt (0.43 $\mu g/m^3$), respectively; all were recorded in downtown Los Angeles in June. Only two monthly means were above 160 ppt; the remainder of the monthly means were below 60 ppt. For bromoform, thirty-one percent of the samples had concentrations above the quantitation limit of 10 ppt (0.10 $\mu g/m^3$). Peaks in the concentration of bromoform were observed at various sites in May and June, with the downtown Los Angeles site registering the highest composite mean (40 ppt; 0.41 $\mu g/m^3$) and the highest monthly mean (310 ppt; 3.2 $\mu g/m^3$) in June 1983. Only two monthly means were greater than 160 ppt; the remainder of the monthly means were below 60 ppt.

Atlas and Schauffler (1991) collected replicate air samples at various locations on the Island of Hawaii during a month-long field experiment to test an analytical method for determining halocarbons in ambient air. Dibromochloromethane was found at a mean level of 0.27 ppt, and bromoform was found at a mean concentration of 1.9 ppt. Information on sample size and detection limit were not provided in the secondary source that reported this study (U.S. EPA 1994b).

Wallace et al. (1982) conducted a pilot study designed to field test personal air-quality monitoring methods. Personal air samples were collected from students at two universities: Lamar University, Texas, located near a petrochemical manufacturing area, and the University of North Carolina (UNC), located in a nonindustrialized area. The samples were analyzed for a number of volatile organic compounds, including brominated trihalomethanes. Bromodichloromethane was detected in 64% of personal air samples from 11 Lamar students,

with a mean of 1.23 μg/m^3 (0.18 ppb), a median of 1 μg/m^3 (0.15 ppb), and a range of 0.12–3.72 μg/m^3 (0.018–0.56 ppb). The limit of detection was 0.24 μg/m^3 (0.036 ppb). At UNC, 17% of the samples from 6 students had detectable levels of bromodichloromethane. Concentrations ranged from 0.12–4.36 μg/m^3 (0.017–0.65 ppb) (mean, 0.83 μg/m^3 (0.12 ppb); median, 0.12 μg/m^3 (0.017 ppb)). Based on the above information, the average daily intake of bromodichloromethane from air using an inhalation rate of 20 m^3/day was estimated to be 25 μg/day for Lamar students and 17 μg/day for UNC students. Dibromochloromethane was not present above 0.12 μg/m^3 (0.018 ppb) at either site.

b. Concentrations in Indoor Air

Relatively few studies have reported the concentrations of trihalomethanes in the indoor air of homes. Kostiainen (1995) identified over 200 volatile organic compounds in indoor air of 26 houses identified by residents as causing symptoms such as headache, nausea, irritation of the eyes, drowsiness, and fatigue. Bromoform was detected at low (unspecified) levels in 54 percent of the homes, and no mention was made of dibromochloromethane or bromodichloromethane.

Weisel et al. (1999) measured brominated trihalomethane concentrations in indoor air in New Jersey residences selected to examine low and high levels of drinking water contamination with trihalomethanes. Descriptive statistics for trihalomethane concentration in water were provided for the combined high and low concentration groups, but not for the individual categories. One valid 15-minute air sample was collected at each of 48 residences. The indoor air concentrations of bromodichloromethane averaged 0.38 ± 0.82 (SD) μg/m^3 (0.057 ± 0.12 ppb) and 0.75 ± 0.96 μg/m^3 (0.11 ± 0.14 ppb) from the low and high water concentration groups, respectively. The detection frequencies were 12/25 and 16 /23 in the low and high water concentration groups, respectively. The indoor air concentrations of dibromochloromethane averaged 0.44 ± 0.95 μg/m^3 (0.052 ± 0.11 ppb) and 0.53 ± 0.84 μg/m^3 (0.062 ± 0.09 ppb) from the low and high water concentration groups with detection frequencies of 5/25 and 7/23, respectively. For bromoform, the average concentrations from the low and high water concentration groups were 0.29 ± 0.93 μg/m^3 (0.028 ±0.089 ppb) and 0.35 ± 0.94 μg/m^3 (0.034 ± 0.091 ppb), with detection frequencies of 8/25 and 4/23, respectively. It was not clear whether the averages were based on all measured samples or only those samples that were above the detection limit for each compound.

Kerger et al (2000) evaluated the transfer of bromodichloromethane and dibromochloromethane to indoor air in bathrooms during showering and bathing in homes supplied with chlorinated tap water. The test sites were three urban homes containing three bedrooms, a full bath, and approximately 1000 square feet of living space. The compounds were simultaneously measured in hot and cold tap water (drawn from the kitchen sink) and in the shower/bath enclosure and bathroom vanity area. Three shower protocols were examined: 6.8 min unventilated shower; 12 min unventilated shower and 6.8 min ventilated shower. Water flow rate and temperature were monitored but not controlled. Airborne vapor samples were captured by Summa canister and measured by gas chromatography using electron capture detection according to U.S. EPA method TO-14. Air samples were collected before, during and after the water use event, for a total of 16 showers and 7 baths. Data for several events were

eliminated because of technical difficulties. For all shower protocols combined (n = 12), the increase in average airborne concentration (± standard error), expressed as μg/m^3, in shower enclosure or bathroom air per μg/L in water, was 1.8 ± 0.3 for bromodichloromethane and 0.5 ± 0.1 for dibromochloromethane. For baths (n = 4), the average concentration increase during the bath was 0.59 ± 0.21 for bromodichloromethane and 0.15 ± 0.05 for dibromochloromethane. The relative contribution of each chemical was consistent with the relative concentration in water and its chemical and physical properties. The average exposures measured in this study were approximately 30% lower than results reported by other investigators using EPA analytical methods when data were normalized for water concentration, flow rate, shower volume, and duration. This difference may have resulted from differences in the air exchange rate between residential showers and laboratory test showers. These data are not adequate for characterizing levels of individual brominated trihalomethanes in the home because the measurements targeted a specific area of the residences and the sample size consisted of only three homes.

c. Estimates of Exposure from Air

The data available for occurrence of brominated trihalomethanes in air do not permit calculation of a nationally aggregated intake estimate for the U.S. general population. To accurately estimate total daily inhalation exposures, factors including location and season, the fraction of time spent indoors compared with outdoors, potential exposures of individuals while showering or bathing, potential exposure from volatilization of brominated trihalomethanes during other household activities (e.g., use of dishwashers, toilet flushing), exposures of individuals who spend large amounts of time at indoor pools, and potential for occupational exposures (e.g., for laundromat or sewage treatment plant workers) require consideration. Although the existing data do not permit such a refined analysis, they may be used to roughly estimate intake from air. Based on the grand means calculated for multiple sampling locations by Brodzinsky and Singh (1983), exposure to bromodichloromethane, dibromochloromethane and bromoform resulting from inhalation of outdoor air can be roughly estimated assuming an inhalation rate of 20 m^3/day, 100% absorption, and exposure to outdoor air for a full 24 hours per day. Using the mean ambient air concentration of 110 ppt (0.74 μg/m^3) by volume for all sites reported in Brodzinsky and Singh (1983), the daily intake of bromodichloromethane from outdoor air would be 21 μg/day. Assuming a mean air concentration of 3.8 ppt (0.032 μg/m^3) for dibromochloromethane, daily intake would be 0.64 μg/day,. Assuming a mean air concentration of 3.6 ppt (0.037 μg/m^3) by volume or bromoform, the daily intake would be 0.74 μg/day. Because these estimates are based on data from urban/suburban and industrial sites only, they may represent high end exposures.

Adequate, nationally aggregated occurrence data are not available for calculating intake of brominated trihalomethanes from indoor air. The indoor air concentrations measured by Weisel et al. (1999) were not used for intake calculations because it could not be determined how the means for each compound were calculated (i.e., whether all measurements were averaged or only those above the detection limit). In addition, the data were based on a single 15 minute air sample collected from each of 48 homes located in a single state.

While brominated trihalomethane concentrations might be expected to be higher in indoor air than in outdoor air due to confined space and additional indoor air sources (e.g. volatilization from showering, baths, and other household activities), the available data do not allow such a comparison.

Based on data from personal air monitors, Wallace et al. (1982) estimated daily inhalation of bromodichloromethane to be 25 μg/day for 11 students attending a university located near a petrochemical manufacturing area and 17 μg/day for 6 students attending a university in a nonindustrialized area.. The personal air monitors registered bromodichloromethane from both indoor (with the exception of showering and bathing) and outdoor exposures. Dibromochloromethane was not detected and no data were available for bromoform.

3. Concentrations and Exposures Associated with Swimming Pools and Hot Tubs

Numerous studies have reported data for concentrations of brominated trihalomethanes and exposures associated with swimming pools and hot tubs. Exposure of swimmers or hot tub users to brominated trihalomethanes may result from dermal, ingestion, and inhalation exposure. When evaluating these data, it is important to note that additional disinfectants are routinely added to water contained in swimming pools and hot tubs; therefore, the levels of brominated trihalomethanes present may not be representative of those in tap water.

Armstrong and Golden (1986) measured bromodichloromethane, dibromochloromethane, and bromoform concentrations in the water and surrounding air of four indoor swimming pools, five outdoor swimming pools, and four hot tubs. Concentrations in air were measured two centimeters from the water surface. The bromodichloromethane concentrations of water in the outdoor pools ranged from 1 to 72 μg/L (ppb) (mean, 33 μg/L). Levels in the indoor pools ranged from 1 to 90 μg/L (ppb) (mean, 16 μg/L). The levels of bromodichloromethane in the hot tubs ranged from ≤0.1 to 105 μg/L (ppb) (mean, 17 μg/L). Means and ranges of the bromodichloromethane concentration two meters above the water surface for outdoor pools, indoor pools, and hot tubs, respectively, were: <0.1 $\mu g/m^3$ (<0.015 ppb) (range not reported), 1.7 $\mu g/m^3$ (0.25 ppb) (range <0.1–10 $\mu g/m^3$ (0.015 –1.5 ppb)), and 1.4 $\mu g/m^3$ (0.21 ppb) (range <0.1–10 $\mu g/m^3$ (0.015 –1.5 ppb)). The dibromochloromethane concentration of water in the outdoor pools ranged from <0.1 to 8 μg/L (ppb) (mean, 4.2 μg/L (ppb)). Levels in the indoor pools ranged from 0.3 to 30 μg/L (ppb) (mean, 9.5 μg/L (ppb)). The level of dibromochloromethane in the hot tubs ranged from ≤0.1 to 48 μg/L (ppb) (mean, 14.4 μg/L (ppb)). Means and ranges of the dibromochloromethane concentration two meters above the water surface for outdoor pools, indoor pools, and hot tubs, respectively, were: <0.1 $\mu g/m^3$ (<0.01 ppb) (range not reported), 0.9 $\mu g/m^3$ (0.11 ppb) (<0.1–5 $\mu g/m^3$ (0.012–0.59 ppb)), and 0.7 $\mu g/m^3$ (0.08 ppb) (<0.1– 5 $\mu g/m^3$ (0.012–0.59 ppb)). The mean bromoform concentration in the outdoor pools was less than 0.1 μg/L (ppb). Levels in the indoor pools ranged from less than 0.1 to 20 μg/L (ppb) (mean, 6 μg/L (ppb)). The levels of bromoform in the hot tubs ranged from less than 0.1 to 62 μg/L (ppb) (mean, 13 μg/L (ppb)). Means and ranges of the bromoform concentration two meters above the water surface for outdoor pools, indoor pools, and hot tubs, respectively, were: <0.1

μg/m^3 (<9.7 ppt) (range not reported), 9 μg/m^3 (870 ppt) (<0.1–14 μg/m^3 (9.7–1360 ppt)), and 8 μg/m^3 (770 ppt) (<0.1–14 μg/m^3 (9.7–1360 ppt)).

Cammann and Hübner (1995) compared concentrations of trihalomethanes in swimmers' and bath attendants' blood and urine before and after swimming or working in indoor swimming pools. Water and air concentrations were measure in different locations in the pool environment. The purpose was to determine whether blood levels of trihalomethanes would reflect inhalation exposure to trihalomethanes in the pool environment and whether those compounds also would appear in urine. Measured concentrations of bromodichloromethane, dibromochloromethane, and bromoform in samples of swimming pool waters collected at a depth of 10 to 20 cm were 0.69 to 5.64 μg/L (ppb), 0.03 to 6.51 μg/L (ppb), and 0.14 to 2.32 μg/L (ppb) for the three compounds, respectively. Averages (± SD) of the 10 pool water measurements presented in Table 1 of the report were 2.12 ± 1.52 μg/L (ppb), 1.11 ± 2.07 μg/L (ppb), and 0.42 ± 0.73 μg/L (ppb) for bromodichloromethane, dibromochloromethane, and bromoform, respectively. Average (± 1 SD) concentrations in the four air samples taken (location of sampling not specified) were 15.4 ± 7.36 μg/m^3 (2.30 ± 1.10 ppb), 1.94 ± 1.01 μg/m^3 (0.228 ± 0.119 ppb), and below the quantitation limit (QL) (not specified, although probably 0.02 ppb) for bromodichloromethane, dibromochloromethane, and bromoform, respectively.

Measurements of bromodichloromethane in 8 bath attendants' blood before their shifts ranged from below QL for 12/18 measurements (67%) to 0.1 μg/L (ppb) (Camman and Hübner, 1995). After their shifts, the concentrations ranged from below QL in 7/18 measurements (39%) to 0.6 μg/L (ppb). Similarly, measurements of bromodichloromethane in swimmers' blood was higher after than before swimming. Before swimming, blood concentrations of bromodichloromethane ranged from less than the QL in 10/20 (50%) swimmers to 0.2 μg/L (ppb); while after swimming, blood concentrations were above the QL in all 20 swimmers, ranging from ≈ 0.02 to 0.4 μg/L (ppb) in 19 of the swimmers. The twentieth swimmer had a blood concentration of ≈ 1.5 μg/L (ppb). For all but two of the swimmers, blood concentrations of bromodichloromethane had dropped below the QL by the next day (values for the other two swimmers were less than 0.1 μg/L (ppb)). Dibromochloromethane and bromoform were not detected in the blood of either the bath attendants or swimmers. None of the brominated trihalomethanes were detected in the urine of the study subjects. Thus, only exposure to bromodichloromethane by inhalation (bath attendants) or inhalation, dermal absorption, and ingestion (swimmers) is reflected in increased blood levels of the compound. Blood levels of bromodichloromethane usually returned to pre-exposure levels within 24 hours after the exposure .

Aggazzotti et al. (1998) evaluated concentrations of trihalomethanes in the blood and breath of five competitive swimmers regularly training in an indoor swimming pool in Italy. The group included three males and two females between the ages of 17 and 21 years. All were non-smokers. Concurrent sampling of blood, alveolar air, and environmental air occurred at five times for each of four sessions: (a) at the University Department two hours before arriving at the pool, (b) after one hour sitting near the edge of the pool, (c), after one hour of swimming, (d) back at the University one hour after swimming ended, and (e) at the University 1.5 hr after swimming ended. While bromodichloromethane and dibromochloromethane were always found

in water and environmental air samples at the pool immediately before and after the 1-hr swimming session, bromoform was rarely detected in the indoor pool air. None of the three brominated trihalomethanes were detected in the air at the University Department or in the alveolar air of the swimmers at the Department two hours before arriving at the pool. At the pool, prior to the swimming session, the means (± SD) of the four measured ambient air concentrations of bromodichloromethane, dibromochloromethane, and bromoform were10.5 ± 3.1 $\mu g/m^3$ (1.6 ± 0.46 ppb; 4 detects), 5.2 ±1.5 $\mu g/m^3$ (0.61 ± 0.17 ppb; 4 detects), and 1 detect of 0.2 $\mu g/m^3$ (0.02 ppb), respectively. At the pool, just after the 1-hr swimming session, the means (± SD) of the four measured ambient air concentrations of bromodichloromethane, dibromochloromethane, and bromoform were 20.0 ± 4.1 $\mu g/m^3$ (2.99 ± 4.1 ppb; 4 detects), 11.4 ±2.1 $\mu g/m^3$ (1.34 ± 0.23 ppb; 4 detects), and 1 detect of 0.2 $\mu g/m^3$ (0.02 ppb), respectively.

Concentrations of bromodichloromethane and dibromochloromethane in the alveolar air of the swimmers before and after the swimming session indicated inhalation uptake of both compounds (Aggazzotti et al., 1998). At the pool, prior to the swimming session, the means (± SD) of the 20 measured alveolar air concentrations (5 swimmers assessed at each of 4 sessions) of bromodichloromethane and dibromochloromethane were 2.7 ± 1.2 $\mu g/m^3$ (0.40 ± 0.18 ppb) and 0.8 ± 0.8 $\mu g/m^3$ (0.09 ± 0.09 ppb), respectively. Bromoform was not detected in any of the 20 samples. At the pool, after the 1-hr swimming session, the means (± SD) of the alveolar air concentrations of bromodichloromethane and dibromochloromethane were 6.5 ± 1.3 $\mu g/m^3$ (0.97 ± 0.19 ppb) and 1.4 ± 0.9 $\mu g/m^3$ (0.16 ± 0.11 ppb), respectively. Bromoform was not detected in any of the 20 samples. Blood levels of bromodichloromethane and dibromochloromethane before and after swimming, on the other hand, were below detection limits in most samples, and hence showed no trends.

Aggazzotti et al. (1998) estimated uptake of the trihalomethanes of the resting and active swimmers using the following assumptions. At rest, the pulmonary ventilation rate of the women was 6 liters per minute (L/min) while that of men was 7.5 L/min. During swimming, the ventilation rate of the women was 25 L/min while that of the men 36 L/min. The estimated uptake rates of bromodichloromethane for the five swimmers at rest ranged from 2.8 to 3.7 µg per hour (µg/h), with a mean value of 3.3 ± 0.41(SD) µg/h for the three males and two females combined. The estimated uptake rates for the same individuals actively swimming were 20 to 30 µg/h, with a mean value of 26 ± 5.1 µg/h. The estimated uptake rates of dibromochloromethane for the five swimmers at rest ranged from 1.5 to 2.0 µg/h, with a mean value of 1.8 ± 0.23 µg/h. The estimated uptake rates during swimming increased to between 14 and 22 µg/h, with a mean value of 18 ± 3.6 µg/h. Occurrence of dermal uptake was acknowledged but not estimated.

Lindstrom et al. (1997) also assessed exposure of two competitive swimmers to bromodichloromethane during training sessions at an indoor pool. The indoor pool air concentrations of bromodichloromethane collected over 60- and 119-minute intervals were 2.76 and 3.02 $\mu g/m^3$ (0.41 and 0.45 ppb), respectively. Breath samples were collected from the swimmers before, during, and for 3 hours after a training workout. Breath samples collected during the workout demonstrated a rapid uptake of bromodichloromethane to maximum alveolar concentrations of 5 to 6 $\mu g/m^3$ (0.7 to 0.9 ppb), which are higher than the ambient air concentrations. The authors concluded that significant (80% of total exposure) dermal

absorption of the related trihalomethane chloroform from water was occurring, but did not estimate the extent of dermal uptake for bromodichloromethane.

4. Soil Concentrations and Exposure

Data on the concentration of brominated trihalomethanes in soil were not available in the materials reviewed for this document. Based on the measured Henry's Law constant and vapor pressure of the individual compounds, volatilization from both wet and dry soil surfaces should be relatively rapid (U.S. EPA 1987). Therefore. exposure from soil ingestion is not considered to be a significant route for exposure to the brominated trihalomethanes.

C. Overall Exposure

The RSC (relative source contribution) is the percentage of total daily exposure that is attributable to tap water when all potential sources are considered (e.g., air, food, soil, and water). Ideally, the RSC is determined quantitatively using nationwide, central tendency and/or high-end estimates of exposure from each relevant medium. In the absence of such data, a default RSC ranging from 20% to 80% may be used.

The RSC used in the current and previous drinking water regulations for dibromochloromethane is 80%. This value was established by use of a screening level approach to estimate and compare exposure to dibromochloromethane from various sources. Information considered for during this process is summarized in Appendix C. The use of the 80% value for the RSC for dibromochloromethane is supported by limited use of this chemical in industrial applications with potential for direct release to the environment. The use of the 80% value is further supported by apparently low concentrations in foods and soils and the potential for human exposure to dibromochloromethane in tap water via three exposure routes: 1) ingestion as drinking water; 2) inhalation of volatilized dibromochloromethane during use of tap water for household activities; and 3) by dermal exposure during showering, bathing, or other activities. The available data for concentrations of outdoor air and food, although limited, suggest that exposures via these routes are likely to be low when compared to water.

Parallel RSC calculations were not performed for bromodichloromethane and bromoform. The EPA has set the regulatory level for these chemicals in drinking water at zero because it has been determined that they are probable human carcinogens. Therefore, determination of an RSC is not relevant for these chemicals because it is the Agency's policy to perform RSC analysis only for noncarcinogens.

D. Body Burden

1. Blood and Breath Levels

Barkley et al. (1980) analyzed blood samples from nine residents of the old Love Canal area in 1978 for a variety of volatile organic compounds, including all three of the brominated trihalomethanes. Bromodichloromethane was detected in the blood of one individual; its concentration was 14 μg/L (ppb). Dibromochloromethane and bromoform were not detected.

Antoine et al. (1986) analyzed the blood of 250 environmentally sensitive patients for 18 volatile organic compounds. Bromoform concentrations ranged from undetectable to 3.4 μg/L (ppb), with a mean of 0.6 μg/L (ppb).

Ashley et al. (1994) analyzed samples of whole blood of 600 or more people in the United States who participated in the Third National Health and Nutrition Examination Survey (NHANES III) for 32 volatile organic compounds using analytical methods designed to measure extremely low concentrations. Bromodichloromethane, with a detection limit of 0.009 μg/L (ppb) was detected only in 14% of 1072 samples. Dibromochloromethane, with a detection limit of 0.013 μg/L (ppb), was detected in only 12% of 1035 samples. Using unprocessed commercial Vacutainer Tubes, Ashley et al. (1994) initially obtained measures of bromoform concentrations in blood similar to those reported by Antoine et al. (1986). However, using Vacutainer Tubes that had been processed to removed VOCs prior to use, Ashley et al. (1994) detected bromoform in less than 10% of samples analyzed at a detection limit of 0.027 μg/L (ppb). Wallace (1997) obtained the summary statistics for bromodichloromethane and dibromochloromethane, which were not published in Ashley et al. (1994) . The mean (± SD) of the measured blood concentrations were 0.0077 ± 0.0178 and 0.00886 ± 0.00856 μg/L (ppb), respectively. The median values were below the limit of detection. The upper 90^{th} percentile values were 0.0122 and 0.0151 μg/L (ppb), respectively.

Weisel et al. (1999) measured brominated trihalomethanes in the exhaled breath of female subjects after showering. The study authors recruited 49 women who had previously participated in a case-control study on neural tube birth defects from locations throughout the state of New Jersey (Klotz and Pyrch, 1999). The method used to select the subjects provided a wide range of brominated trihalomethane exposures within the home, in contrast to a distribution of exposures that might exist within a single water distribution system or within the general population. Exposure to brominated trihalomethanes was estimated by collection of duplicate cold tap water samples, collection of a 15-minute air sample, and responses to a 48-hour recall questionnaire on water use in the home. Post-shower whole breath samples were collected by having the subject blow into a Tedlar® sampling bag at the conclusion of a shower. Background breath samples were collected at a subsequent home visit by the investigators. Valid samples were obtained from 33 of the subjects. However, the time of post-shower sample collection as reported by the subjects varied from immediately after the shower to 20 minutes later. As noted by the authors, the delay in sample collection is an important determinant in breath concentrations because trihalomethane breath concentration declines exponentially after exposure ceases. As a result, each subject was assigned to one of three groups: 1) breath sample

collected within 5 minutes after completion of shower (Group A; n = 13); 2) breath sample collected within 5 to 20 minutes after completion of shower (Group B; n=14); or 3) breath sample collected more than 20 minutes after showering (Group C; n=6). The breath concentrations on individual brominated trihalomethanes for each group were compared to measured water concentrations and estimates of exposure (calculated as the product of the water concentration and reported duration of the shower; shower duration data and calculated exposure estimates were not reported).

The mean (± standard deviation) concentrations of bromodichloromethane, dibromochloromethane, and bromoform were 5.7 ± 8.6, 2.0 ± 2.1, and 0.73 ± 0.90 µg/L (ppb), respectively. The median values for the three compounds were 2.6, 1.4, and 0.45 µg/L (ppb), respectively. Bromodichloromethane showed significant correlations for breath and water concentration and breath and shower exposure for Groups A and B. Significant correlations for dibromochloromethane and bromoform were found for Group A participants. Analytical variability related to low concentrations of dibromochloromethane and bromoform (near the detection limit) may have obscured trends in the data for Group B. source of in the houses and found significant correlations between the water concentration of each brominated trihalomethane and the concentration of that trihalomethane in expired air if the air samples were collected within 5 minutes of showering. Results of statistical analysis for Group C were not reported because the sample size was small and the authors considered the results questionable. The observed results were considered consistent with showering being a source of exposure to brominated trihalomethanes.

Backer et al. (2000) examined levels of brominated trihalomethanes in whole blood following three types of water use events by adult volunteers: showering for 10 minutes in tap water (n=11); bathing for 10 minutes in a tub filled with tap water (n=10); or consumption of one liter of tap water over a 10 minute period (n=10). Each participant provided a blood sample immediately before exposure, 10 minutes after exposure ended, and 30 minutes (showering and bathing) or one hour (ingestion) after exposure. Tap water and blood samples were analyzed by purge-and-trap/gas chromatography/mass spectrometry with detection capability in the parts per quadrillion range. Bromoform was not detected in either tap water or whole blood. Mean tap water concentrations of bromodichloromethane and dibromochloromethane were 6 µg/L and 1.1 µg/L, respectively. The highest levels of these compounds in whole blood occurred 10 minutes after exposure had ended. The second post-exposure measurements showed that blood levels of both compounds had decreased, but were still above the pre-exposure baseline levels in subjects who took showers or baths. Measurement data are shown in Table IV-9 below.

Table IV-9 Mean Bromodichloromethane Concentrations in Blood Following Three Types of Water Use Events

Water Use Event	Median Bromodichloromethane Concentration in Whole Blood (pg/mL)		
	Pre-exposure	10 minutes post-exposure	30 or 60 minutes post-exposure
10 Minute Shower	3.3	19.4	10.3 (30 min)
10 Minute Bath	2.3	17.0	9.9 (30 min)
Ingestion of 1 L	2.6	3.8	2.8 (60 min)

The study authors reported that similar relative findings were obtained for dibromochloromethane (data shown graphically in the study report). These data indicate a dramatic difference between the whole blood levels resulting from ingestion and those resulting from bathing or showering (including dermal, inhalation, and possibly ingestion exposure). Blood level increases observed for each compound after ingestion of one liter of water were less than 10% of those observed after bathing or showering for 10 minutes. The blood level increases observed for each compound 10 minutes after bathing or showering were significantly increased ($p<0.01$) when compared to the post-ingestion blood levels. Measurable levels of bromodichloromethane and dibromochloromethane in the pre-exposure whole blood samples were attributed to recent prior exposure or to bioaccumulation after repeated exposure to tap water.

In addition to the differences observed in whole blood levels of bromodichloromethane and dibromochloromethane among exposure groups, the study authors observed that the blood concentration data for each chemical occurred in two clusters within each exposure group. The mean increases for the two clusters observed after bathing or showering were significantly different for bromodichloromethane. The same individuals who had greater increases of bromodichloromethane also experienced greater increases of dibromochloromethane and chloroform in the blood after bathing or showering. The underlying basis for the observed clustering is unknown, but was not related to gender. The study authors suggested that polymorphic expression of a metabolizing enzyme (e.g. glutathione-S-transferase theta) or differences in fitness level (resulting in inhalation of larger volumes of air) may have accounted for the observed pattern. However, they noted that differences in fitness level would more likely be expected to result in a continuous distribution.

Lynberg et al. (2001) conducted a field study in Corpus Christi, Texas, and Cobb County, Georgia, to evaluate exposure measures for disinfection by-products, including brominated trihalomethanes. These areas were selected for study based on the following criteria: 1) relatively high trihalomethane concentrations relative to national averages; 2) high intrasystem differences that would result in a potential exposure gradient across the study population; 3) one water distribution system with predominately chlorinated species of trihalomethanes (i.e., chloroform) and one water system with predominately brominated trihalomethanes; and 4) a

water utility service population large enough to allow rapid selection of 25 mothers per geographic area who had given birth to healthy babies from June, 1998 through May, 1999. Exposure to individual trihalomethanes was assessed by collection of blood and water samples and by collection of information on water use patterns and tap water characteristics. Whole blood samples were collected before and after showering. Levels of individual trihalomethanes were determined for samples collected in the home of participants, in the distribution system, and at the water treatment plants. A modified version of the Total Exposure Model (TEM) was used to estimate uptake of trihalomethanes into the bloodstream (data for chloroform exposure were presented for one individual in Corpus Christi). The results of the study indicate that concentration of individual trihalomethanes varied by site and location within the water system (Table IV-10). In Corpus Christi water samples, brominated trihalomethanes accounted for 71% of the total trihalomethane concentration by weight. In contrast, brominated trihalomethanes accounted for only 12% of the trihalomethanes in Cobb County water samples. Significant differences ($p = 0.0001$) in the blood levels of dibromochloromethane and bromoform were observed between study locations (Table IV-11). The differences between locations were evident both before and after showering. The study authors indicated that there was considerable variability in blood levels of trihalomethanes among participants from a single location. For example, pre-shower chloroform blood levels in Cobb County ranged from 130 ppt to 1100 ppt. No data were presented for the brominated trihalomethanes. The variability was tentatively attributed to different patterns of household water use among participants. Significant increases ($p = 0.0001$) in blood levels of all brominated trihalomethanes were observed after showering. The increases in dibromochloromethane and bromoform were significantly greater in Corpus Christi than in Cobb County. No TEM modeling data were presented for brominated trihalomethanes. However, TEM results presented for chloroform exposure for one study participant who consumed bottled water indicated that inhalation exposure in the household accounted for approximately 98% of the calculated 24-hour chloroform dose, with the remainder attributed to the dermal route. Overall, this study demonstrates that blood levels of brominated trihalomethanes vary significantly across populations, with water quality characteristics and water use activities being important variables.

Table IV-10 Median Tap Water Trihalomethane Levels (ppb) in Cobb County and Corpus Christi Homes, Water Treatment Plants, and Distribution Systems

Trihalomethane	Cobb County			Corpus Christi		
	Home (n=25)	Distribution System (n=20)	Water Treatment Plant (n=7)	Home (n=25)	Distribution System (n=30)	Water Treatment Plant (n=20)
Bromodichloromethane	13.5	12.5	9.5	12.2	8.3	9.5
Dibromochloromethane	1.7	2.4	1.4	13.5	12.6	14.3
Bromoform	ND[a]	ND	ND	8.7	9.7	11.9
Chloroform	84.8	79	49.5	8.2	4.6	6.7

[a] ND, not detected (detection limit < 1ppb)

Table IV-11 Between Site Comparison of Median Blood Levels (ppt) and Changes in Blood Levels (ppt) after Showering

Trihalomethane	Before Shower		After Shower		Change in Blood Level after Showering	
	Cobb[a]	Corpus[b]	Cobb	Corpus	Cobb	Corpus
Bromodichloromethane	6.2	6.8	38	43	30	34
Dibromochloromethane	1.2	7.0	6.1	41	5.0	35
Bromoform	0.3	3.5	0.5	17	0.2	12
Chloroform	70	25	280	57	189	25

[a] Cobb County, Georgia
[b] Corpus Christi, TX

2. Mother's Milk

Pellizzari et al. (1982) analyzed the milk of eight nursing mothers for various compounds, including bromodichloromethane and dibromochloromethane. The samples were collected from 49 lactating women living in the vicinity of chemical manufacturing plants and/or industrial user facilities in Bridgeville, PA, Bayonne, NJ, Jersey City, NJ, and Baton Rouge LA. Both compounds were identified in one of the eight samples. Actual concentrations and detection limits were not reported. Kroneld and Reunanen (1990) did not detect any of the brominated trihalomethanes in human milk in a study conducted in Turku, Finland.

E. Summary

Brominated trihalomethanes are found in virtually all water treated for drinking; however, concentrations of individual forms vary widely depending on the type of water treatment, locale, time of year, sampling point in the distribution system, and source of the drinking water. Occurrence data for brominated trihalomethanes are available from 13 national surveys and 9 additional studies that are more restricted in scope. The procedures used for sampling processing and storage and calculation of summary statistics should be carefully considered when evaluating and comparing brominated trihalomethane occurrence data. Some methods restrict trihalomethane formation by refrigeration or the use of quenching agents, whereas others maximize trihalomethane formation by storage at room temperature. Approaches to data summarization vary by study in the treatment of data below the analytical detection level or minimum reporting level.

When all available national survey data are considered, bromodichloromethane concentrations in drinking water range from below the detection limit to 183 µg/L (ppb), while dibromochloromethane and bromoform concentrations range from below the detection limit to 280 µg/L (ppb). When data for the three brominated trihalomethanes are compared, the frequency of detection and measured concentrations of bromodichloromethane in drinking water

supplies tend to be higher than those for dibromochloromethane. Bromoform is detected less frequently and at lower concentrations than the other two brominated trihalomethanes, except in some ground waters. Concentrations of all trihalomethanes in drinking water were generally lower when the raw water is obtained from ground water sources rather than surface water sources. The most recent national survey data are those collected by the U.S. EPA under the Information Collection Rule (ICR). Monitoring data were collected over an 18-month period between July 1997 and December 1998 from approximately 300 water systems operating 501 plants and serving at least 100,000 people. Summary occurrence data stratified by raw water source (groundwater or surface water) are available for finished water, the distribution system (DS) average, and the DS high values. The mean, median, and 90th percentile values for surface water DS average concentrations in the ICR survey are 8.6, 70.2, and 20.3 μg/L, respectively, for bromodichloromethane (range of individual values 0 - 65.8 μg/L); 2.4, 4.72, and 13.2 μg/L, respectively, for dibromochloromethane (range 0 - 67.3); and 0. 1.18, and 3.10, respectively, for bromoform (range 0 - 3.43).

Relatively few studies have analyzed non-beverage foods for the occurrence of brominated trihalomethanes. In the few studies available, bromodichloromethane has been detected in non-beverage foods (i.e., in one sample of butter at 7 ppb, in three samples of ice-cream at 0.6 to 2.3 ppb, in 6 of 10 samples of bean curd at 1.2 to 5.2 ppb, and in one sample of bacon (probably below the minimal quantitation limit)). In addition, bromodichloromethane was detected in one sample each of eleven foods out of 70 tested in 14 Market Baskets for the FDA Total Diet Study. The detected concentrations ranged from 10 to 37 ppb for individual food items. Studies that analyzed non-beverage foods for dibromochloromethane and bromoform detected neither compound in any of the samples. Brominated trihalomethanes have been detected in up to a third or one half of the types of prepared beverages examined in some studies, being detected most frequently in colas and other carbonated soft drinks. Bromodichloromethane has been found most frequently of the three compounds and bromoform the least frequently. Bromodichloromethane was detected in approximately half of the prepared beverages examined by McNeal et al. (1995) in the United States and in all of 13 soft drinks that they analyzed. With the exception of one of the 13 soft drinks examined by McNeal et al. (1995) with a concentration of 12 ppb, none of the at least 18 other measured concentrations of bromodichloromethane in soft drinks described above (three from Entz et al. (1982), three from Uhler and Diachenko (1987), and the remaining 12 from McNeal et al. (1995)) exceeded a value of 4 ppb. Bromodichloromethane was detected in one sample of fruit juice at 5 ppb.

Exposure to brominated trihalomethanes via ingestion of drinking water was estimated using data obtained for disinfectants and disinfection byproducts under the Information Collection Rule (ICR). ICR data offer several advantages over other national studies for purposes of estimating national exposure levels of adults in the United States to brominated trihalomethanes via ingestion of drinking water. First, they are recent and reflect relatively current conditions. Second, data of very similar quality and quantity were collected systematically from a large number of plants (501) and systems (approximately 300), including both surface and ground water systems. Third, the mean, median, and 90th percentile value were estimated on the basis of all samples taken, not just the sample detects. Thus, these descriptive statistics are representative of the exposures of the entire populations served by those systems,

not just the populations served by systems with higher concentrations of these compounds. However, this study can not be considered representative of smaller public water supplies or water supplies from the most highly industrialized or contaminated areas.

Exposure was calculated by multiplying the concentration of individual brominated trihalomethanes in drinking water by the average daily intake, assuming that each individual consumes two liters of water per day. The annual median, mean, and upper 90th percentile values are presented for both surface and ground water systems. Assuming that the DS High value actually represents the average exposure level of persons served by one plant distribution pipe with the longest water-residence time, the DS High value might be used to estimate a high-end exposure level.

For bromodichloromethane, the median, mean, and 90th percentile population exposures from surface water systems are estimated to be 17, 20, and 40 μg/person/day, respectively. The same values for populations exposed to bromodichloromethane from ground water systems are lower – 3.6, 8.1, and 22 μg/person/day, respectively. For dibromochloromethane, the median, mean, and 90th percentile population exposures from surface water systems are estimated to be 4.8, 9.4, and 26 μg/person/day, respectively. The corresponding values for populations exposed to dibromochloromethane from groundwater system are lower – 2.7, 6.2, and 18 μg/person/day, respectively. For bromoform, the median, mean, and 90th percentile population exposures from surface water systems are estimated to be near 0, 2.4, and 6.2 μg/person/day, respectively. The same values for populations exposed to bromoform from ground water systems are higher – 0.65, 3.8, and 9.6 μg/person/day, respectively.

For purposes of comparison, estimates of ingestion exposure to bromodichloromethane, dibromochloromethane, and bromoform in drinking water were also estimated from data collected in other, older studies. Ingestion from ground water supplies was estimated from the median levels found in the Ground Water Supply Survey conducted by U.S. EPA in 1980-81. Based on the range of median levels (1.4–2.1 μg/L (ppb)) and a consumption rate of two liters per day, the median ingestion exposure to bromodichloromethane may range from 2.8 to 4.2 μg/day. Similarly, median exposure to dibromochloromethane may range from 4.2 to 7.8 μg/day, and for bromoform, median exposure may range from 4.8 to 8.4 μg/day. Exposure to bromodichloromethane from surface water supplies can be estimated based on the range of median values observed under different conditions in the National Organics Monitoring Survey conducted by U.S. EPA in 1976-1977, which mainly sampled surface water systems. Based on a range of 5.9–14 μg/L (ppb), exposure to bromodichloromethane from surface water is estimated to be between 12 and 28 μg/day. Similarly, based on the range of medians reported for dibromochloromethane concentrations, the median exposure is estimated to be up to 6 μg/day. The median levels of bromoform in the surface water supplies have been found to be less than the EPA Drinking Water minimum reporting levels (MRLs) of 0.5–1 μg/L (ppb). An estimate of exposure based on the MRLs will be overly conservative because the actual concentration of bromoform is not detectable. Based on the range of MRLs, 0.5–1 μg/L (ppb), the exposure to bromoform is estimated to range from 1 to 2 μg/day for surface water supplies.

Ingestion exposure to brominated trihalomethanes in drinking water can also be estimated from the concentrations found at the tap in the U.S. EPA's Total Exposure Assessment Methodology (TEAM) study. Estimates of the average of the population intakes for ingestion of bromodichloromethane from drinking water range from 0.42 to 42 µg/person/day. The upper 90th percentile estimates range from <2.0 to 90 µg/person/day. Estimates of the average population intake of dibromochloromethane from drinking water range from 0.2 to 56 µg/person/day. The upper 90th percentile estimates range from < 0.9 to 86 µg/person/day. Estimates of the average of the population intakes of bromoform, for those areas in which bromoform was measurable in a majority of the samples, range from 1.6 to 16.2 µg/person/day. The upper 90th percentile estimates range from 2.4 to 26 µg/person/day. Four of the six locations in the TEAM study, however, had a low frequency (less than 10%) of detection of bromoform in measurable quantities.

Sources of uncertainty in these estimates of ingestion exposure include use of different analytical methods, failure to report quantitation limits, using measures near the detection limit, failure to report how nondetects are handled when averaging values (e.g., set to zero or one half the detection limit), and failure to report sample storage method and duration. In addition, many environmental factors influence the concentrations of these compounds in drinking water at the tap and in vended or bottled waters used for drinking. These factors include season and temperature, geographic location, source of water, residence time in distribution system, and others.

Average daily intake of dibromochloromethane via ingestion, dermal contact, and inhalation of compound volatilized during household use were also estimated for determination of the relative source contribution (RSC). Intake for ingestion was calculated using mean intake rates of 1.2 or 0.6 L/day for total and direct intake (NRC, 1999), respectively. Direct intake includes consumption of water directly from the tap, but does not include intake of tap water used for preparation of heated items such tea, coffee, or soup. Based on the ICR distribution system average concentration of 4.72 µg/L for dibromochloromethane in surface water, the average daily total and direct and ingestion intakes would be 5.7 and 2.8 µg/day, respectively. The average dermal uptake of dibromochloromethane was estimated to be 2 µg per shower or bathing event. Average daily intake via inhalation of dibromochloromethane volatilized during showering was estimated to be 7 µg/day. Parallel calculations were not performed for bromodichloromethane or bromoform, because these compounds are probable carcinogens. Therefore, in accordance with U.S. EPA policy, RSC analysis was not conducted.

Some data on the occurrence of brominated trihalomethanes in foods and beverages are available from studies conducted in Italy, Japan, and Finland. These studies were also limited in scope to examination of relatively few food or beverage items. Bromodichloromethane, dibromochloromethane, and bromoform concentrations measured in foods and beverages in Italy, Japan and Finland ranged from undetectable to 40 ppb, undetectable to 13.9 ppb, and undetectable to 10.7 ppb, respectively. Because of possible differences in water disinfection or food processing practices, these data may not be representative of concentrations in foods and beverages produced in the U.S.

Concentrations in outdoor air were variable from site to site. When data from several urban/suburban and source-dominated sites in Texas, Louisiana, North Carolina and/or Arkansas were combined, the resulting average outdoor air concentrations were 110 ppt (0.74 $\mu g/m^3$) for bromodichloromethane, 3.8 ppt (0.032 $\mu g/m^3$) for dibromochloromethane, and 3.6 ppt (0.037 $\mu g/m^3$) for bromoform. A regional study conducted at several sites in southern California found bromodichloromethane, dibromochloromethane, and bromoform in 35%, 17%, and 31% of the samples, respectively. The maximum concentrations observed were 40 ppt (0.27 $\mu g/m^3$) for bromodichloromethane; 290 ppt (2.5 $\mu g/m^3$) for dibromochloromethane; 310 ppt (3.2 $\mu g/m^3$) for bromoform. Bromodichloromethane was detected in 64% (n=11) and 17% (n=6) of personal air samples collected in Texas and North Carolina. The detected concentrations ranged from 0.12 to 4.36 $\mu g/m^3$ (0.017 to 0.65 ppb). Dibromochloromethane was not detected.

Mean concentrations in indoor air ranged from 0.38 to 0.75 $\mu g/m^3$ for bromodichloromethane; 0.44 to 0.53 $\mu g/m^3$ for dibromochloromethane, and 0.29 to 0.35 $\mu g/m^3$ for bromoform, as determined from 15 minute samples collected in 48 New Jersey residences. In a separate study, levels of brominated trihalomethanes in indoor air were locally increased (e.g., in shower/bath enclosures and vanity areas) during showering and bathing events. The levels of individual brominated trihalomethanes in air were reported to be consistent with the levels in tap water.

The use of chlorine to disinfect swimming pools and hot tubs results in the formation of brominated trihalomethanes. Swimming pool and hot tub users are potentially exposed to brominated trihalomethanes via dermal contact, ingestion, and inhalation of compounds released to the overlying air. As a result, swimming pool and hot tub users may experience greater overall exposures to brominated trihalomethanes than the general population. One study indicated that bromodichloromethane, dibromochloromethane, and bromoform concentrations in swimming pool and hot tub water ranged from 1 to 105 $\mu g/L$ (ppb), from 0.1 to 48 $\mu g/L$ (ppb), and from less than 0.1 to 62 $\mu g/L$ (ppb), respectively. Concentrations of the same brominated trihalomethanes in the air two meters above the pool water ranged from less than 0.1 to 14 $\mu g/m^3$ (0.015 to 2.09 ppb), from less than 0.1 to 10 $\mu g/m^3$ (0.011 to 1.2 ppb), and from less than 0.1 to 5.0 $\mu g/m^3$ (0.0097 to 0.48 ppb), respectively. Data from several studies confirm the uptake of brominated trihalomethanes from swimming pools and environs by dermal and/or inhalation pathways.

No data for occurrence of brominated trihalomethanes in soil were available in the materials reviewed for this document. The chemical and physical properties of the brominated trihalomethanes indicate that they should volatilize readily from wet or dry soil surfaces. Therefore, ingestion of soil is not expected to be a significant route of exposure.

Exposure to brominated trihalomethanes via ingestion of drinking water was estimated using data obtained for disinfectants and disinfection byproducts under the Information Collection Rule (ICR). ICR data offer several advantages over other national studies for purposes of estimating national exposure levels of adults in the United States to brominated trihalomethanes via ingestion of drinking water. First, they are recent and reflect relatively current conditions. Second, data of very similar quality and quantity were collected

systematically from a large number of plants (501) and systems (approximately 300), including both surface and ground water systems. Third, the mean, median, and 90th percentile value were estimated on the basis of all samples taken, not just the sample detects. Thus, these descriptive statistics are representative of the exposures of the entire populations served by those systems, not just the populations served by systems with higher concentrations of these compounds. However, this study can not be considered representative of smaller public water supplies or water supplies from the most highly industrialized or contaminated areas.

Exposure was calculated by multiplying the concentration of individual brominated trihalomethanes in drinking water by the average daily intake, assuming that each individual consumes two liters of water per day. The annual median, mean, and upper 90th percentile values are presented for both surface and ground water systems. Assuming that the DS High value actually represents the average exposure level of persons served by one plant distribution pipe with the longest water-residence time, the DS High value might be used to estimate a high-end exposure level.

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concentration of bromoform is not detectable. Based on the range of MRLs, 0.5–1 µg/L (ppb), the exposure to bromoform is estimated to range from 1 to 2 µg/day for surface water supplies.

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The RSC is the percentage of total daily exposure that is attributable to tap water when all potential sources are considered (e.g., air, food, soil, and water). Ideally, the RSC is determined quantitatively using nationwide, central tendency and/or high-end estimates of exposure from each relevant medium. In the absence of such data, a default RSC ranging from 20% to 80% may be used.

The RSC used in the current and previous drinking water regulations for dibromochloromethane is 80%. This value was established by use of a screening level approach to estimate and compare exposure to dibromochloromethane from various sources. Information considered for during this process is summarized in Appendix C. There are some uncertainties in the 80% RSC that are related to the availability of adequate concentration data for dibromochloromethane in media other than water. Parallel RSC calculations were not performed for bromodichloromethane and bromoform. The EPA has set the regulatory level for these chemicals in drinking water at zero because it has been determined that they are probable human carcinogens. Therefore, determination of an RSC is not relevant for these chemicals because it is the Agency's policy to perform RSC analysis only for noncarcinogens.

Brominated trihalomethanes have been detected in the blood and breast milk of humans. A national survey of volatile organic compounds in whole blood detected bromodichloro-methane, dibromochloromethane, and bromoform in 14%, 12%, and less than 10% of samples,

respectively, when highly sensitive analytical methods were applied. Several studies have demonstrated that the level of individual brominated trihalomethanes in blood or breath increases shortly after exposure to these compounds in tap water during bathing and showering. Exposure during these events may occur by ingestion, dermal contact and/or inhalation of the volatilized compound. In studies which examined households with differing concentrations of brominated trihalomethanes in tap water, the levels of individual brominated trihalomethanes in blood or exhaled breath paralleled the tap water concentration. The studies of showering and bathing indicate that water use patterns and water quality characteristics are important variables in determining the blood levels of brominated trihalomethanes. Dibromochloromethane was detected in one of eight samples of breast milk collected from women living in the vicinity of U.S. chemical manufacturing plants or user facilities.

V. HEALTH EFFECTS IN ANIMALS

A. Acute Exposures

This section presents data on the acute effects of brominated trihalomethanes. Acute lethality values for the brominated trihalomethanes are summarized in Table V-1. Additional acute toxicity data are summarized in Table V-2.

1. Bromodichloromethane

Acute lethality of bromodichloromethane has been investigated in mice and rats. LD_{50} values for male and female ICR Swiss mice were 450 and 900 mg/kg, respectively (Bowman et al., 1978). Chu et al. (1980) determined LD_{50} values of 916 and 969 mg/kg for male and female Sprague-Dawley rats, respectively.

Bowman et al. (1978) administered bromodichloromethane in a single gavage dose in Emulphor®:alcohol:saline (1:1:8) to ICR Swiss mice (10/sex/group). The administered doses ranged from 500 to 4,000 mg/kg (individual doses not reported). Sedation and anesthesia occurred at 500 mg/kg. Males were more sensitive to the lethal effects of bromodichloromethane than females.

Table V-1 Summary of LD_{50} Values for Brominated Trihalomethanes

Compound	LD_{50} Values (mg/kg)			
	ICR Swiss Mouse [a]		Sprague-Dawley Rat [b]	
	Male	Female	Male	Female
Bromodichloromethane	450	900	916	969
Dibromochloromethane	800	1200	1186	848
Bromoform	1400	1550	1388	1147

[a]Bowman et al. (1978)
[b]Chu et al. (1980)

Table V-2 Summary of Acute Toxicity Studies for Brominated Trihalomethanes

Reference	Species	Route	Sex	Number per dose group	Duration	Dose (mg/kg-day)	Results
Bromodichloromethane							
Bowman et al. (1978)	Mouse ICR Swiss	Gavage (aqueous)	M, F	10	Single dose	500 - 4000	Anesthesia, sedation at 500 mg/kg
NTP (1987)	Rat F344/N	Gavage (corn oil)	M, F	5	Single dose	150 300 600 1,250 2,500	Increased mortality at 600 mg/kg-day. Lethargy, labored breathing at 1250 mg/kg-day and above, 100% mortality at the two highest dose groups
NTP (1987)	Mouse $B6C3F_1$	Gavage (oil)	M, F	5	Single dose	150 300 600 1,250 2,500	100% mortality at the two highest dose groups
Lilly et al. (1994)	Rat F344	Gavage (corn oil) (aqueous)	M	6	Single dose	0 200 (LOAEL) 400	Renal tubule degeneration and necrosis; alteration in markers of renal function
Lilly et al. (1996)	Rat F344	Gavage (corn oil or water)	M	6	Single dose	0 200 (LOAEL) 400	Renal tubule necrosis; alteration in markers of renal function
Lilly et al. (1997)	Rat F344	Gavage (aqueous)	M	5	Single dose	0 123 164 (NOAEL) 246 (LOAEL) 328 492	Decreased body weight; elevated liver and renal markers
Keegan et al. (1998)	Rat F344	Gavage (aqueous)	M	6	Single dose	0 21 31 41 (NOAEL) 82 (LOAEL) 123 164 246	Elevated liver markers; decreased liver weight and body weight
Dibromochloromethane							
Bowman et al. (1978)	Mouse ICR Swiss	Gavage (aqueous)	M, F	10	Single dose	500 - 4000	Anesthesia, sedation
NTP (1985)	Rat F344/N	Gavage (corn oil)	M, F	5	Single dose	160 310 630 1250 2,500	Increased mortality at 630 mg/kg and above, with 100% mortality in high-dose group

Table V-2 (cont.)

Reference	Species	Route	Sex	Number per dose group	Duration	Dose (mg/kg-day)	Results
NTP (1985)	Mouse $B6C3F_1$	Gavage (corn oil)	M, F	5	Single dose	160 310 630 1250 2500	Increased mortality in females at 630 mg/kg-day and above and in males at 310 mg/kg-day and above; 100% mortality in males in two highest dose groups and females in two highest dose groups
Müller et al. (1997)	Rat Wistar	Gavage (olive oil)	M	6	Single dose	0 83 167 333 667	Transient decrease in blood pressure and heart rate; decreased activity; effects on heart muscle contractility and changes in some cardiac parameters
Bromoform							
Bowman et al. (1978)	Mouse ICR Swiss	Gavage (aqueous)	M, F	10	Single dose	500 - 4000	Ataxia, sedation, and anesthesia at 500 mg/kg
Chu et al. (1980)	Rat SD*	Gavage (corn oil)	M, F	10	Single dose	546 765 1071 1500 2100	Sedation, ataxia, liver and kidney congestion
NTP (1989a)	Rat F344/N	Gavage (corn oil)	M, F	5	Single dose	125 250 500 1,000 2000	No deaths at 500 and lower; 60% mortality at 1,000; 100% mortality at 2,000; shallow breathing in two highest dose groups
NTP (1989a)	Mouse $B6C3F_1$	Gavage (corn oil)	M, F	5	Single dose	125 250 500 1,000 2000	10% mortality at 500 mg/kg-day

* SD, Sprague-Dawley

NTP (1987) administered single gavage doses of bromodichloromethane in corn oil to male and female F344/N rats and $B6C3F_1$ mice (5/sex/dose) at 150, 300, 600, 1,250, or 2,500 mg/kg. Animals were observed for 14 days, and a necropsy was performed on at least one male and one female in each dose group. All animals dosed with 1,250 or 2,500 mg/kg died before the end of the study. At 600 mg/kg, deaths occurred in two of five male rats, one of five female rats, five of five male mice, and two of five female mice. Clinical signs observed in rats at 1,250 or 2,500 mg/kg included lethargy and labored breathing. Clinical signs observed in mice at or above 600 mg/kg included lethargy, with the exception that this sign was not observed

in high-dose male mice. At necropsy, the liver from animals dosed with 1,250 or 2,500 mg/kg appeared pale. No dose-related effects were seen on body weight gain in animals that survived.

Lilly et al. (1994) examined the effect of vehicle on the toxicity of bromodichloromethane. Male F344 rats (6/dose) were administered a single dose of 0, 200, or 400 mg/kg bromodichloromethane by gavage in corn oil or in an aqueous 10% Emulphor® solution. Body weights were significantly decreased at 400 mg/kg only in the animals receiving the aqueous gavage. Absolute and relative kidney weights were significantly increased at 400 mg/kg in both vehicles, with a significantly greater increase observed in the animals gavaged with oil compared to those gavaged with 10% Emulphor® solution. Serum markers of hepatotoxicity were significantly increased at 400 mg/kg in both vehicles with one nonsignificant increase in the aqueous vehicle. The increases were significantly greater for two of these markers in animals receiving the oil vehicle compared to those receiving the aqueous vehicle. Clinical observations were supported by histopathology findings. Hepatocellular degeneration and necrosis were observed at 400 mg/kg in animals receiving either vehicle. The difference in vehicles was reflected in more severe hepatocellular degeneration and a higher incidence of centrilobular necrosis in animals receiving the oil gavage compared to those receiving the aqueous gavage. Numerous increases in urinary markers of renal toxicity were observed 24 hours after dosing. Based on the differences observed, renal toxicity at 200 mg/kg was similar or greater in the aqueous vehicle. Renal toxicity at 400 mg/kg, however, was greater in the oil vehicle. The time to peak toxicity was both dose- and vehicle-dependent. At 200 mg/kg, peak damage was observed at 24 hours in animals receiving either vehicle. At 400 mg/kg, peak damage was observed at 48 hours following oil gavage and at 24-36 hours following aqueous gavage. Histopathology revealed both renal tubule degeneration and necrosis at both dose levels. The incidence of renal tubule degeneration was greater in animals receiving the aqueous gavage at the low dose; however, the severity of renal degeneration and necrosis was greater in the animals receiving the oil gavage at the high dose. The authors attributed the vehicle differences to slower gastrointestinal uptake of bromodichloromethane from the oil vehicle compared to the aqueous vehicle. At the high dose, more bromodichloromethane would be converted to a reactive metabolite following oil dosing, while saturation would occur following aqueous dosing. At the low dose, the difference in uptake would have less of an effect. Overall, this study found that the kidney was more sensitive than the liver to a single dose of bromodichloromethane. A LOAEL of 200 mg/kg was identified for each vehicle based on minimal renal tubule degeneration and changes in markers of renal function.

Lilly et al. (1996) investigated the effect of subchronic pretreatment with corn oil on the toxicity of bromodichloromethane. Prior to initiation of dosing with bromodichloromethane, male Fischer 344 rats (6 animals/group) were gavaged with oral doses of corn oil or water for six weeks (5 days/week) at a constant volume of 5 mL/kg. Following pretreatment, the animals were gavaged with a single dose of 0, 200, or 400 mg bromodichloromethane/kg in 10% Emulphor®. Urine was collected at 24, 36, and 48 hours following bromodichloromethane administration. The rats were sacrificed at 48 hours and necropsies were performed. Activities of the hepatotoxicity indicators alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and sorbitol dehydrogenase (SDH) were measured in the serum, and the renal toxicity indicators alkaline phosphatase (ALK), AST, and LDH, were

measured in the urine. Additional analyses included determination of serum levels of bile acids, triglycerides, cholesterol and albumin, and urine levels of N-acetylglucosaminidase and gamma glutamyl transpeptidase activity. Enzymatic activities of cytochrome P450 isoforms CYP2E1 and CYP2B1/B2 were measured in the microsomal fraction of the liver to investigate whether corn oil was an inducer of bromodichloromethane metabolizing enzymes.

Liver weight was significantly reduced only in the water pretreatment group at the high dose. Kidney weight was reduced in both pretreatment groups at the high dose. Activities of serum AST and LDH were significantly elevated in both pretreatment groups at 400 mg/kg. ALT levels increased in a dose-dependent manner in the water pretreatment group, but significant elevations were noted only at the 400 mg/kg dose in animals pretreated with corn oil. Activities of urinary AST and LDH were greater than controls in both pretreatment groups after 24, 36, and 48 hours. ALK levels were significantly increased in both pretreatment groups at 24 hours. At 36 and 48 hours, ALK levels were elevated only in water-pretreated animals. High incidences of renal tubular necrosis occurred at 200 and 400 mg/kg in both pretreatment groups. There were no significant differences in the histopathological lesion scores between the pretreatment groups. No significant differences were noted in the hepatic activity of CYP2E1 or CYP2B1/B2 in the corn oil pretreated animals compared to the water controls. Although a number of differences between the pretreatment groups were noted in results for specific endpoints, the overall results from this study indicate that 6 weeks of pretreatment with corn oil did not significantly enhance the acute hepato- or nephrotoxicity of bromodichloromethane. In addition, the reported data suggest that vehicle-related differences in toxicity observed in other bromodichloromethane studies are most likely due to pharmacokinetic differences in absorption rather than altered enzyme activity induced by corn oil. This study confirms the acute LOAEL of 200 mg/kg-day previously identified by Lilly et al. (1994) for renal toxicity.

Lilly et al. (1997) administered single doses of bromodichloromethane by gavage in aqueous 10% Emulphor® solution to male F344 rats at dose levels of 0, 123, 164, 246, 328, or 492 mg/kg. Groups of 5 animals/dose were sacrificed at 24 and 48 hours post-dosing. Body weights were significantly decreased at or above 246 mg/kg after 48 hours. At 24 hours, absolute and relative kidney weights were significantly increased at or above 328 mg/kg and 246 mg/kg, respectively. At 48 hours, only relative kidney weight at the high dose was significantly increased. At 24 hours, serum markers of liver damage (ALT and AST) were significantly increased at or above 246 mg/kg with one marker (SDH) increased at all dose levels. Although smaller statistically significant increases were observed at the low doses at 24 hours for ALT (123 and 164 mg/kg) and AST (164 mg/kg), the biological significance of these increases is unclear. After 48 hours, serum levels of these markers were decreased from 24-hour levels with statistically significant changes noted only at the higher doses. No effects in urinary markers of kidney damage were found at either 123 or 164 mg/kg. These markers, however, were significantly elevated after 24 hours for doses at or above 246 mg/kg with few exceptions. No histopathological examination was conducted. These results were generally consistent with earlier results (Lilly et al., 1994), although the present study was conducted at doses low enough to identify a NOAEL. In contrast to the earlier results of Lilly et al. (1994), this study did not find that the kidney was more sensitive than the liver to the toxic effects of

bromodichloromethane. Based on hepatotoxicity and nephrotoxicity, this study identified a NOAEL of 164 mg/kg and a LOAEL of 246 mg/kg.

Keegan et al. (1998) investigated the acute toxicity of bromodichloromethane administered orally in an aqueous vehicle. Male Fischer 344 rats (6 animals/group) were gavaged with a single dose of 0, 0.125, 0.1875, 0.250, 0.5, 0.75, 1.0 or 1.5 mmol/kg dissolved in a 10% aqueous solution of Alkamuls EL-620. These doses of bromodichloromethane are equivalent to 0, 20.5, 30.7, 41.0, 81.9, 122.9, 163.8, and 245.7 mg/kg, respectively. Control animals were dosed with vehicle only (10% Alkamuls EL-620). Gavage volumes were kept constant at 5 ml/kg body weight. Animals were sacrificed 24 hours after dose administration and the liver, kidneys, and serum were harvested. Significant decreases in body weight were observed in animals treated with 0.75, 1.0, or 1.5 mmol/kg. Decreases in absolute liver weights were observed in the 0.5, 0.75, 1.0, or 1.5 mmol/kg animals. No change was noted in relative liver weights. Absolute kidney weights were not affected by bromodichloromethane treatment, but relative kidney weights were significantly increased in the two highest dose groups (1.0 and 1.5 mmol/kg). Serum levels of ALT, SDH, and AST were assessed as an indication of liver toxicity. Dose-dependent elevations in ALT (45% to 239% increase), AST (25% to 130% increase) and SDH (74% to 378% increase) were observed in the 0.5, 0.75, 1.0, and 1.5 mmol dose groups. Based on these findings, 0.25 mmol/kg (41.0 mg/kg) represents the NOAEL and 0.5 mmol/kg (81.9 mg/kg) represents the LOAEL for orally administered bromodichloromethane in an aqueous vehicle. The study authors used the NOAEL of 41.0 mg/kg to calculate One-Day Health Advisories for drinking water of 4 mg/L for a 10-kg child and 14 mg/L for a 70-kg adult.

2. Dibromochloromethane

The acute oral lethality of dibromochloromethane has been assessed in rats and mice of both sexes. Chu et al. (1980) reported LD_{50} values in male and female Sprague-Dawley rats of 1,186 and 848 mg/kg-day for males and females, respectively. Bowman et al. (1978) reported LD_{50} values in mice of 800 and 1,200 mg/kg-day, respectively.

Bowman et al. (1978) investigated the acute oral toxicity of dibromochloromethane in ICR Swiss mice (10/sex/group). Doses of 500 to 4000 mg/kg (individual doses not reported) were administered by gavage in Emulphor®:alcohol:saline (1:1:8) to fasted animals. Sedation and anesthesia occurred at 500 mg/kg. Males were more sensitive than females to the acute lethal effects of dibromochloromethane.

NTP (1985) evaluated the acute toxicity of dibromochloromethane in male and female F344/N rats. The rats (5 animals/sex/dose) received single doses of 160, 310, 630, 1,250, or 2,500 mg/kg dibromochloromethane by gavage in corn oil. The observation period following treatment was 14 days. Mortality in high-dose rats was 100% by day 3. At the 1,250 mg/kg dose, four male rats and one female rat died. One female rat died in the 630 mg/kg group. Doses of 310 mg/kg or greater produced lethargy in all animals for 3 hours after dosing. A gross necropsy was conducted on one or two animals from each group. No treatment-related effects were observed in rats selected for gross necropsy.

In a concurrent study, NTP (1985) evaluated the acute toxicity of dibromochloromethane in male and female B6C3F$_1$ mice (5/sex/dose). The mice received single doses of 160, 310, 630, 1,250, or 2,500 mg/kg dibromochloromethane by gavage in corn oil. The observation period following treatment was 14 days. All male mice receiving the 2,500 mg/kg and 1,250 mg/kg doses died. Three male mice receiving the 630 mg/kg dose died, while a single male mouse died at the 310 mg/kg dose. All female mice receiving the 2500 mg/kg dose died. Four of the female mice administered the 1,250 mg/kg dose died between days 2 and 8 post-treatment. No female mice died at doses of 630 mg/kg or lower. A gross necropsy was conducted on one or two animals from each group. At necropsy, aberrations of the kidney (dark red or pale medullae) and liver (discolored foci) were reported to be more frequently observed in treated animals than in control animals (raw data were not presented in the study).

Müller et al. (1997) investigated the cardiotoxic effects of acute dibromochloromethane exposure. Male Wistar rats were administered a single dose of dibromochloromethane by gavage in olive oil at dose levels of 0, 83, 167, 333, or 667 mg/kg. Telemetric measurements of cardiovascular parameters (heart rate, blood pressure, body temperature, and physical activity) were recorded in conscious rats (6/group) from 24 hours prior to administration to 72 hours following administration. Heart rate and blood pressure were also measured in urethane-anesthetized rats (10/group) 25 minutes following administration. For these rats, contractility parameters, such as the Krayenbühl index, were also calculated. Treatment-related arrhythmias were not observed in conscious rats dosed with 83 to 333 mg/kg of dibromochloromethane, while rats in the high-dose group exhibited premature ventricular contractions one minute following administration. Heart rate and body temperature were initially decreased in all treatment groups following administration, but returned to control values 24 hours post-exposure in rats administered 83 to 333 mg/kg. In the high-dose rats, heart rate remained depressed up to 48 hours post-exposure, and body temperature decreased 4.5°C below control values by 72 hours post-exposure. Blood pressure was initially increased in all treatment groups following administration, but began to return to control values within 48 hours post-exposure in rats administered 83 to 333 mg/kg. Blood pressure in the high-dose group, however, decreased below control values 72 hours post-exposure. Physical activity was decreased in conscious rats administered 333 and 667 mg/kg during the entire observation period. In urethane-anesthetized rats, negative effects on muscle contractility were observed at dose levels of 333 and 667 mg/kg, negative chronotropic (rate of contraction) effects were observed at the 333 mg/kg dose level, and negative dromotropic (defined as influencing the velocity of conduction of excitation, as in nerve or cardiac muscle fibers) effects were observed at dose levels of 167 to 667 mg/kg. Heart rate, blood pressure, and several contractility parameters, however, did not exhibit dose-related trends.

3. Bromoform

Bowman et al. (1978) assessed the acute oral toxicity of bromoform in ICR Swiss mice. Groups of ten male (30 to 35 g) and ten female (25 to 30 g) mice were treated with single doses ranging from 500 to 4,000 mg/kg. Compounds were solubilized in Emulphor®:alcohol:saline (1:1:8) and administered by gavage to fasted animals. The period of observation following treatment was 14 days. LD_{50} values were 1400 and 1550 mg/kg for males and females,

respectively. Ataxia, sedation, and anesthesia occurred within 60 minutes of treatment at doses of 1000-mg/kg and above. Sedation lasted approximately 4 hours.

Chu et al. (1980) evaluated the acute toxicity of bromoform in male and female Sprague-Dawley rats. Fasted adult rats (10/sex/dose) received doses of 546, 765, 1071, 1500, or 2100 mg/kg bromoform dissolved in corn oil by gavage. Clinical observations were made for 14 days after treatment. The LD_{50} values for male and female rats were 1388 and 1147 mg/kg, respectively. Clinical signs observed in treated rats included sedation, flaccid muscle tone, ataxia, piloerection, and hypothermia. Gross pathological examination revealed liver and kidney congestion in treated animals. Chu et al. (1982a) reported results for growth, food intake, organ weight, histopathology, hematological indices, liver microsome aniline hydroxylase activity and serum chemistry in surviving rats. Bromoform treatment increased liver protein concentration in the serum of male rats at doses of 765 and 1071 mg/kg. Lymphocyte counts were decreased in male (765 and 1071 mg/kg doses) and female (765 mg/kg) rats but the effect was not dose-dependent. Female rats at the 765 mg/kg dose had elevated aniline hydroxylase levels.

NTP (1989a) investigated the acute oral toxicity of bromoform in male and female F344/N rats. The rats (5/sex/group) were administered a single oral dose of bromoform (by gavage, in corn oil) at dose levels of 125, 250, 500, 1,000, or 2,000 mg/kg. Control groups were not included in the study design. Mortality was 10/10 at 2,000 mg/kg, 6/10 at 1,000 mg/kg, and 0/10 at 500 mg/kg or lower. Shallow breathing was observed in rats that received the 1000 or 2000 mg/kg doses. No other clinical signs were reported.

NTP (1989a) investigated the acute oral toxicity of bromoform in male and female $B6C3F_1$ mice. The mice received a single oral dose of bromoform (by gavage, in corn oil) at dose levels of 125, 250, 500, 1,000, or 2,000 mg/kg. There were no controls. Mortality was 0/10 at 2,000 mg/kg, 6/10 at 1,000 mg/kg, 1/10 at 500 mg/kg, and 0/10 at 250 mg/kg or lower. The final mean body weight of mice that survived to the end of the study period was unaffected by bromoform exposure. Male mice that received doses of 500, 1,000, or 2,000 mg/kg and females that received 1,000 or 2,000 mg/kg were lethargic. Shallow breathing was noted in male mice administered the 1,000 or 2,000 mg/kg dose.

B. Short-Term Exposures

This section summarizes short-term studies (less than approximately 90 days) on the health effects of brominated trihalomethanes in animals. Details of these studies are summarized in Table V-3.

Table V-3 Summary of Short Term Toxicity Studies for Brominated Trihalomethanes

Reference	Species	Route	Sex	Number per dose group	Duration	Dose (mg/kg-day)	Results
Bromodichloromethane							
Oral Exposure							
Chu et al. (1982a)	Rat SD*	Drinking water	M	10	28 days	0 0.8 8 68 (NOAEL)	No signs of toxicity observed.
Munson et al. (1982)	Mouse CD-1	Gavage (aqueous)	M, F	8-12	14 days	0 50 (NOAEL) 125 (LOAEL) 250	Decreased immune function; increased liver weight, decreased absolute and relative spleen wt. (females)
Condie et al. (1983)	Mouse CD-1	Gavage (corn oil)	M	8-16	14 days	0 37 74 (NOAEL) 148 (LOAEL)	Liver and kidney histopathology
NTP (1987)	Rat F344/N	Gavage (corn oil)	M, F	5	14 days	0 38 75 150 (NOAEL) 300 (LOAEL) 600	Decreased body weight gain; renal pathology
NTP (1987)	Mouse $B6C3F_1$	Gavage (corn oil)	M, F	5	14 days	0 19 38 75 (NOAEL) 150 (LOAEL) 300	Mortality, renal histopathology
Aida et al. (1992a)	Rat Wistar	Diet	M	7	1 month	0 21 62 (NOAEL) 189 (LOAEL)	Liver histopathology
Aida et al. (1992a)	Rat Wistar	Diet	F	7	1 month	0 21 66 (NOAEL) 204 (LOAEL)	Liver histopathology
Thornton-Manning et al. (1994)	Rat F344	Gavage (aqueous)	F	6	5 days	0 75 (NOAEL) 150 (LOAEL) 300	Liver histopathology, renal histopathology; increased liver and kidney wt.; elevated markers of hepatotoxicity
Thornton-Manning et al. (1994)	Mouse C57BL/6J	Gavage (aqueous)	F	6	5 days	0 75 (NOAEL) 150 (LOAEL)	Increased serum markers of hepatotoxicity

Table V-3 (cont.)

Reference	Species	Route	Sex	Number per dose group	Duration	Dose (mg/kg-day)	Results
Potter et al. (1996)	Rat F344	Gavage (aqueous)	M		1, 3, or 7 days	123 246 (NOAEL)	No effect in hyaline droplet formation or cell proliferation
Melnick et al. (1998)	Mouse $B6C3F_1$	Gavage (corn oil)	F	10	3 weeks (5 d/wk)	0 75 (NOAEL) 150 (LOAEL) 326	Increased abs. and relative liver weight; increased serum markers of hepatotoxicity; hepatocyte degeneration; increased labeling index
NTP (1998)	Rat SD	Drinking water	M, F	6	2 weeks	0 11 45 (NOAEL) 91 (LOAEL) 124	Transient reduction in weight gain
NTP (1998)	Rat SD	Drinking water	M, F	5-13	35 days	Group A males 0 9 (NOAEL) 38 (LOAEL) 67	Single cell hepatic necrosis in Group A males
Coffin et al. (2000)	Mouse $B6C3F_1$	Gavage (Corn oil) Drinking water	F	10	11 days 11 days	0 150 (LOAEL) 300 0 138 (LOAEL)	Hydropic degeneration in liver (corn oil gavage and drinking water); increased relative liver weight (gavage); increased proliferating cell nuclear antigen labeling index (gavage)
Lock et al. (2004)	Rat F344	Gavage	M	5	28 days (5 d/wk)	0 50 (LOAEL) 100	Increased formic acid excretion by the kidneys; decrease urinary pH; increased labeling index in renal cells (only at 100 mg/kg)
Lock et al. (2004)	Mouse $B6C3F_1$	Gavage	M	6	28 days (5 d/wk)	0 25 50 (LOAEL)	Increased formic acid excretion by the kidneys. At both doses, no increased cell proliferation or clinical/histological evidence of renal damage.
Inhalation Exposure							
Torti et al. (2001)	Mouse C57BL/6 FVB/N (wild-type)	Vapor	M	6	1 week (6 hr/day)	0 ppm 1 ppm (NOAEL) 10 ppm (LOAEL) 30 ppm 100 ppm 150 ppm	Dose-dependent marginal increase in renal tubular degeneration in C57BL/6 mice; mild increase in renal tubular degeneration and marginal increase in hepatic degeneration in FVB/N mice; sign. increased labeling index

Table V-3 (cont.)

Reference	Species	Route	Sex	Number per dose group	Duration	Dose (mg/kg-day)	Results
Torti et al. (2001)	Mouse C57BL/6 (p53 hetero-zygous)	Vapor	M	6	1 week (6 hr/day)	0 1 ppm (NOAEL) 10 ppm (LOAEL) 30 ppm 100 ppm 150 ppm	Dose-dependent marginal to mild increase in renal tubular degeneration; sign. increased labeling index
Torti et al. (2001)	FVB/N (p53 hetero-zygous)	Vapor	M	6	1 week (6 hr/day)	0 ppm 0.3 ppm 1 ppm 3 ppm (NOAEL) 10 ppm (LOAEL) 30 ppm	Dose-dependent mild increase in renal tubular degeneration and marginal increase in nephrosis; marginal increase in hepatic degeneration; sign. increased relative kidney wt. and labeling index.
Torti et al. (2001)	Mouse C57BL/6 FVB/N (wild-type)	Vapor	M	6	3 weeks (6 hr/day)	0 ppm 0.3 ppm 1 ppm 3 ppm (NOAEL) 10 ppm (LOAEL) 30 ppm	Marginal increase in renal tubular degeneration
Torti et al. (2001)	Mouse C57BL/6 FVB/N (p53 hetero-zygous)	Vapor	M	6	3 weeks (6 hr/day)	0 ppm 0.3 ppm 1 ppm 3 ppm (NOAEL) 10 ppm (LOAEL) 30 ppm	Marginal or mild increase in renal tubular degeneration in both strains; marginal increase in hepatic degeneration in FVB/N heterozygous strain
Dibromochloromethane							
Munson et al. (1982)	Mouse CD-1	Gavage (aqueous)	M, F	8-12	14 days	0 50 (NOAEL) 125 (LOAEL) 250	Decreased immune function
Chu et al. (1982a)	Rat SD	Drinking water	M	10	28 days	0 0.7 8.5 68 (NOAEL)	No effect on growth, clinical signs, biochemical or histopathological endpoints
Condie et al. (1983)	Mouse CD-1	Gavage (corn oil)	M	8-16	14 days	0 37 74 (NOAEL) 147 (LOAEL)	Decreased PAH uptake, moderate liver and kidney histopathology

Table V-3 (cont.)

Reference	Species	Route	Sex	Number per dose group	Duration	Dose (mg/kg-day)	Results
NTP (1985)	Rat F344/N	Gavage (corn oil)	M, F	5	14 days	0 60 125 250 (NOAEL) 500 (LOAEL) 1,000	Mortality; liver and renal gross pathology
NTP (1985)	Mouse $B6C3F_1$	Gavage (corn oil)	M, F	5	14 days	0 30 60 (NOAEL) 125 (LOAEL) 250 500	Liver and kidney gross pathology
Aida et al. (1992a)	Rat Wistar	Diet	M	7	1 month	0 18 (NOAEL) 56 (LOAEL) 173	Liver histopathology
Aida et al. (1992a)	Rat Wistar	Diet	F	7	1 month	0 34 (NOAEL) 101 (LOAEL) 333	Liver histopathology; increased relative liver weight
Potter et al. (1996)	Rat F344	Gavage (aqueous)	M		1, 3, or 7 days	156 312 (NOAEL)	No effect on hyaline droplet formation or cell proliferation
Melnick et al. (1998)	Mouse $B6C3F_1$	Gavage (corn oil)	F	10	3 weeks (5 d/wk)	0 50 100 (NOAEL) 192 (LOAEL) 417	Liver histopathology; increased serum enzymes and liver weight. Significant increase of hepatocyte labeling index only at the highest dose of 417 mg/kg-day
Coffin et al. (2000)	Mouse $B6C3F_1$	Gavage (Corn oil) Drinking water	F	10	11 days 11 days	0 100 (LOAEL) 300 0 171 (LOAEL)	Increased proliferating cell nuclear antigen labeling index , increased relative liver wt. (gavage); Mild lobular ballooning hepatocytes (gavage & drinking water)
Bromoform							
Munson et al. (1982)	Mouse CD-1	Gavage (aqueous)	M, F	6-12	14 days	0 50 125 (NOAEL) 250 (LOAEL)	Increased serum enzyme activity (AST); decrease in antibody forming cells and delayed-type hypersensitivity response
Chu et al. (1982a)	Rat SD	Drinking water	M	10	28 days	0.7 8.5 80 (NOAEL)	No effect on growth, clinical signs, biochemical or histopathological endpoints

Table V-3 (cont.)

Reference	Species	Route	Sex	Number per dose group	Duration	Dose (mg/kg-day)	Results
Condie et al. (1983)	Mouse CD-1	Gavage (corn oil)	M	8-16	14 days	0 72 145 (NOAEL) 289 (LOAEL)	Decreased PAH uptake, moderate histopathological changes
NTP (1989a)	Rat F344/N	Gavage (corn oil)	M, F	5	14 days	0 100 200 (NOAEL) 400 (LOAEL) 600 800	Decreased body weight gain; 1/5 died at 400 mg/kg-day;100% mortality at two highest doses
NTP (1989a)	Mouse $B6C3F_1$	Gavage (corn oil)	M	5	14 days	0 50 100 200 (NOAEL) 400 (LOAEL) 600	Stomach nodules; ataxia, lethargy; 1/5 died at high dose
Aida et al. (1992a)	Rat Wistar	Diet	M	7	1 month	0 62 (NOAEL) 187 (LOAEL) 618	Hepatic vacuolization, serum chemistry/biochemistry
Aida et al. (1992a)	Rat Wistar	Diet	F	7	1 month	0 56 (NOAEL) 208 (LOAEL) 728	Hepatic vacuolization, serum chemistry/biochemistry
Potter et al. (1996)	Rat F344	Gavage (aqueous)	M		1, 3, or 7 days	190 379 (NOAEL)	No effect on hyaline droplet formation or cell proliferation
Melnick et al. (1998)	Mouse $B6C3F_1$	Gavage (corn oil)	F	10	3 weeks (5 d/wk)	0 200 (NOAEL) 500 (LOAEL)	Increase in absolute and relative liver wt.; marginally significant increase in LI at highest dose
Coffin et al. (2000)	Mouse $B6C3F_1$	Gavage (corn oil) Drinking Water	F	10	11 days 11 days	0 200 (LOAEL) 500 0 301 (LOAEL)	Liver histopathology; increased proliferating cell nuclear antigen labeling index (gavage and drinking water); increased relative liver wt (gavage).

* SD, Sprague-Dawley

1. Bromodichloromethane

Munson et al. (1982) administered bromodichloromethane by aqueous gavage to male and female CD-1 mice (8 to 12/sex/group) for 14 days at levels of 0, 50, 125, or 250 mg/kg-day. Endpoints evaluated included body and organ weights, hematology, serum enzyme levels (SGOT, SGPT), and humoral and cell-mediated immune system functions. At 250 mg/kg-day, body weights were significantly decreased. Significant organ weight changes included increased relative liver weight (mid- and high-dose groups), decreased absolute spleen weight (high-dose males and mid- and high-dose females), and decreased relative spleen weight (mid- and high-dose females).

Among the hematology endpoints, only fibrinogen levels were significantly decreased in high- dose males and in mid- and high-dose females. Significant clinical chemistry findings included decreased glucose levels (high-dose males), increased ALT and AST activities (high-dose groups), and increased blood urea nitrogen (BUN) levels (high-dose groups). Bromodichloromethane appeared to affect the humoral immune system, as judged by significantly decreased antibody-forming cells (high-dose males and mid- and high-dose females) and hemagglutination titers (mid- and high-dose males and high-dose females). This study identified a NOAEL of 50 mg/kg-day and a LOAEL of 125 mg/kg-day for bromodichloromethane on the basis of decreased immune function in females.

Chu et al. (1982a) administered bromodichloromethane to male Sprague-Dawley rats (10/group) in drinking water for 28 days at dose levels of 0, 5, 50, or 500 ppm. These levels corresponded to doses of 0, 0.8, 8.0, or 68 mg/kg-day, as calculated by the authors based on recorded fluid intake. The authors observed no effects on growth rate or food consumption and no signs of toxicity throughout the exposure. No dose-related biochemical or histologic changes were detected (no data were provided). This study identified a NOAEL of 68 mg/kg-day, but the reported data were too limited to allow an independent verification.

Condie et al. (1983) investigated the renal and hepatic toxicity of bromodichloromethane in male CD-1 mice (8 to 16/group). Bromodichloromethane was administered by gavage in corn oil for 14 days at dose levels of 0, 37, 74 or 148 mg/kg-day. Biochemical evidence of liver damage (significantly elevated ALT) was observed at the high dose, while biochemical evidence of kidney damage (significantly decreased p-aminohippurate (PAH) uptake by kidney slices) was observed at the mid and high dose. Significantly decreased BUN levels were observed in the low- and mid-dose groups, but not in the high-dose group. Histopathology revealed no consistent or important changes at the low or mid-level doses, with minimal to moderate liver and kidney injury observed in the majority of animals at the high dose. Liver lesions included centrilobular pallor and focal inflammation. Kidney lesions included intratubular mineralization, epithelial hyperplasia, and cytomegaly. Although the severity of these lesions was primarily minimal to slight, a few animals in the high-dose group exhibited moderate to moderately severe intratubular mineralization and/or epithelial hyperplasia. This study identified a NOAEL value of 74 mg/kg-day and a LOAEL value of 148 mg/kg-day for bromodichloromethane, based on histopathology.

NTP (1987) administered doses of 0, 38, 75, 150, 300, or 600 mg/kg-day of bromodichloromethane in corn oil by gavage to male and female F344/N rats (5/sex/dose) for 14 days. One low-dose and one high-dose female died before study termination. All high-dose animals were hyperactive after dosing and either lost weight or gained no weight during the study. Final mean body weights were not significantly affected in groups given 38, 75, or 150 mg/kg-day. At 300 mg/kg, body weights of males and females were decreased by 21% and 7%, respectively, relative to vehicle controls. At 600 mg/kg-day, body weights of males and females were decreased by 44% and 22%, respectively, relative to vehicle controls. Necropsy was performed on all animals. Renal medullae were reddened in all high-dose males and in one female in each of the control, low-dose, and high-dose groups. This study identified a NOAEL of 150 mg/kg-day and a LOAEL of 300 mg/kg-day in rats, based on decreased body weight gain.

In a parallel experiment, NTP (1987) administered doses of 0, 19, 38, 75, 150, or 300 mg/kg-day bromodichloromethane in corn oil by gavage to male and female B6C3F_1 mice (5/sex/dose) for 14 days. All male mice that received 150 or 300 mg/kg-day bromodichloromethane died before study termination. Clinical signs included lethargy, dehydration, and hunched posture. The final mean body weights of the mice that survived were not significantly different from the controls. The renal medullae were reddened in four males in the 150 mg/kg-day group, all males in the 300 mg/kg-day group, and one female in the 150 mg/kg-day group. Based on behavior, appearance, gross necropsy, and mortality, this study identified a NOAEL of 75 mg/kg-day and a frank effect level (FEL) of 150 mg/kg-day in male mice. An interesting point to note is that this study and the study by Condie et al. (1983) were conducted under similar conditions (mice administered bromodichloromethane by gavage in corn oil for 14 days), but with dramatically different results. In contrast to the 100% mortality observed in this study for male mice, Condie et al. (1983) found only moderately severe histopathology in male CD-1 mice at 148 mg/kg-day with no deaths occurring. The reason for this difference is unclear, but may be related to strain-specific differences in sensitivity.

Aida et al. (1992a) administered bromodichloromethane to Slc:Wistar rats (7/sex/group) for one month at dietary levels of 0%, 0.024%, 0.072%, or 0.215% for males and 0%, 0.024%, 0.076%, or 0.227% for females. The test material was microencapsulated and mixed with powdered feed; placebo granules were used for the control groups. Based on the mean food intakes, the study authors reported the mean compound intakes for the one-month period as 0, 20.6, 61.7, or 189.0 mg/kg-day for males and 0, 21.1, 65.8, or 203.8 mg/kg-day for females. Clinical effects, body weight, food consumption, hematology parameters, serum chemistry, and histopathology of all major organs were determined. Body weights were significantly decreased in the high-dose groups relative to the controls. The high-dose animals also exhibited slight piloerection and emaciation. Relative liver weight was increased only in high-dose females. Significant, dose-related biochemical findings at the low dose were limited to decreased LDH levels in males, but the biological significance of this effect is unclear. Serum LDH levels were also significantly decreased at the low and high dose in females. Other statistically significant, changes included decreased glucose (high-dose males), decreased serum triglycerides (high-dose groups), decreased serum cholinesterase activity (high-dose males and mid- and high-dose females), and increased total cholesterol (mid- and high-dose males). The changes in cholinesterase activity and cholesterol levels in males were not dose-related. The cholesterol

levels were within normal ranges at all doses. Treatment-related histopathological lesions were limited to the liver and were rated as very slight or slight. The lesions were mostly confined to the high-dose groups. Vacuolization observed in mid-dose females and in a single low-dose male was not considered an adverse effect. Other observed effects included swelling of hepatocytes, single cell necrosis, hepatic cord irregularity, and bile duct proliferation. These lesions were observed only in high-dose males and females with the exception of very slight to slight changes in individual low-dose males. No effect was observed on any hematology parameter. Based on the histopathology observed in high-dose males and females, the LOAELs identified in this study for bromodichloromethane in rats were 189.0 mg/kg-day in males and 203.8 mg/kg-day in females; the NOAELs were 61.7 mg/kg-day in males and 65.8 mg/kg-day in females.

Thornton-Manning et al. (1994) administered bromodichloromethane at dose levels of 0, 75, 150, or 300 mg/kg-day by gavage to female F344 rats (6 animals/dose) for five consecutive days. The dosing vehicle consisted of an aqueous 10% Emulphor® solution. Animals were sacrificed on day 6. Two animals in the high-dose group died on day 5. Final body weights of the high-dose group were significantly decreased compared to the controls. Absolute and relative kidney and liver weights were significantly increased at 150 and 300 mg/kg-day with the exception of a nonsignificant increase in absolute liver weight at 150 mg/kg-day. Toxic effects on the kidney and liver were reflected in significantly increased LDH, AST, SDH, creatinine, and BUN at 300 mg/kg-day. These results were supported by the histopathology findings. In the liver, centrilobular vacuolar degeneration was observed at both 150 and 300 mg/kg-day with the severity of the effect increased with increasing dose. Centrilobular hepatocellular necrosis was also observed in one high-dose animal. In the kidney, renal tubular vacuolar degeneration and renal tubule regeneration were observed at 150 and 300 mg/kg-day with the incidence and severity increased with increasing dose. While minimal renal tubule necrosis was observed in only one animal at the mid dose, all animals at the high dose exhibited mild to moderate renal tubule necrosis. Significant decreases in the hepatic activity of the CYP1A and CYP2B markers ethoxyresorufin-O-dealkylase (EROD) and pentoxyresorufin-O-dealkylase (PROD), were observed at all doses. The effect, however, was not dose-related. No effect on the CYP2E1 marker paranitrophenol hydroxylase was observed. Based on kidney and liver lesions observed at the mid dose, this study identified a NOAEL of 75 mg/kg-day and a LOAEL of 150 mg/kg-day.

Thornton-Manning et al. (1994) conducted an analogous experiment with female C57BL/6J mice. Six mice per group were administered an aqueous solution (10% Emulphor®) of bromodichloromethane by gavage for five consecutive days at dose levels of 0, 75 and 150 mg/kg-day. Animals were sacrificed on day 6. All mice survived to the termination of the experiment. No effect on body, kidney, or liver weight was observed with the exception of a significant increase in absolute liver weight at 150 mg/kg-day. No change in cytochrome P450 activity was observed, although a nonsignificant dose-related decrease in total P450 content was observed. ALT was significantly increased at 150 mg/kg-day, and a significant dose-related increase in SDH activity was observed. Creatinine and BUN were not significantly increased. No kidney or liver lesions were observed at either dose. Based on increases in serum enzyme

activity, a LOAEL of 150 mg/kg-day and a NOAEL of 75 mg/kg-day were identified for this study.

Potter et al. (1996) investigated hyaline droplet formation and cell proliferation in the kidney of male F344 rats. Test animals (4/dose) received 0.75 or 1.5 mmol/kg of bromodichloromethane in 4% Emulphor® by gavage for 1, 3, or 7 days. The administered doses corresponded to 123 or 246 mg/kg-day. No exposure-related increase in hyaline droplet formation was observed at either dose. Binding of bromodichloromethane to α_{2u}-globulin was not measured. Cell proliferation in the kidney was assessed *in vivo* by [^{3}H]-thymidine incorporation. No statistically significant effect of bromodichloromethane on tubular cell proliferation was observed following exposures of up to 7 days, although high labeling levels were observed in 3 of 4 rats at the 246 mg/kg-day dose.

Melnick et al. (1998) exposed female B6C3F$_1$ mice (10 animals/group) to bromodichloromethane in corn oil via gavage for 3 weeks (5 days/week). Doses of bromodichloromethane used in this study were 0 (vehicle only), 75, 150, or 326 mg/kg-day. The doses were selected on the basis that high incidences of hepatocellular adenoma and carcinoma were previously seen in female mice exposed at 75 mg/kg or 150 mg/kg-day in a dose-dependent manner (NTP 1987). There were no treatment-related signs of overt toxicity observed during the study. Body weight and water intake were not significantly altered at any dose tested. However, a significant dose-related increase in absolute liver weight and liver weight/body weight ratio was noted for the 150 and 326 mg/kg-day dose groups. Serum ALT activity was significantly increased in the two highest dose groups and serum SDH activity was elevated at all doses tested. At necropsy, there was clear evidence of hepatocyte hydropic degeneration in animals treated with 150 and 326 mg/kg-day. BrdU was administered to the animals during the last 6 days of the study, and hepatocyte labeling index (LI) analysis was conducted. The two highest (150 and 326 mg/kg-day) doses resulted in significantly elevated hepatocyte proliferation as measured by the LI. NOAEL and LOAEL values of 75 and 150 mg/kg-day were identified on the basis of elevated serum enzyme activity, increased liver weight, increased cell proliferation, and histological findings.

NTP (1998) evaluated the effect of bromodichloromethane on food and water consumption by Sprague-Dawley rats in the course of a range-finding experiment for a study of developmental and reproductive effects. This study was conducted in compliance with Good Laboratory Practice Regulations as described in 21 CFR 58. Bromodichloromethane was administered to test animals (6 animals/sex/dose) at nominal concentrations of 0, 100, 500, 1000, and 1500 ppm in the drinking water for 2 weeks. The average doses of bromodichloromethane estimated based on water consumption were 11, 45, 91 and 124 mg/kg-day for the 100, 500, 1000 and 1500 ppm dose groups, respectively. All animals were observed twice daily for signs of toxicity. Body weight data were obtained twice weekly and at termination of the experiment. Feed and water consumption were measured twice weekly. Animals were euthanized at termination of the experiment without necropsy. No mortality or treatment-related clinical signs were observed in any dose group. Body weights and weight gains were comparable among all dose groups, except for body weight gains on Study Day 5 (the first day of compound administration) in the 1000 and 1500 ppm dose groups which were decreased 127.5% and

118.5%, respectively. Feed consumption was also comparable across dose groups, with the exception of male rats dosed with 1000 and 1500 ppm. Male rats in these dose groups showed decreases in consumption of 31% and 41% , respectively, on Study Days 1 to 5. Water consumption was reduced in the 500, 1000, and 1500 ppm dose groups, suggesting that bromodichloromethane is unpalatable at higher concentrations. The greatest reduction in water intake was noted on Study Days 1 to 5 (61% and 62% for males in the 1000 ppm and 1500 ppm dose groups, respectively, and 38%, 40% and 52% for females in the 500, 1000 and 1500 ppm dose groups, respectively).

NTP (1998) conducted a short-term reproductive and developmental toxicity screen in Sprague-Dawley rats to evaluate the potential toxicity of bromodichloromethane administered in drinking water for 35 days. This study was conducted in compliance with the Good Laboratory Practice Regulations as described in 21 CFR 58. Groups of male and female rats (5-13/sex/dose) were exposed to drinking water concentrations of 0, 100, 700 and 1300 ppm bromodichloromethane using the study design described in Table V-6 (Section V.E.1). Feed and water consumption, body weight, hematology, clinical chemistry, cell proliferation, and pathology were evaluated in addition to developmental and reproductive endpoints. Based on water consumption and analytical measurements of bromodichloromethane in the provided drinking water, the calculated average daily doses were 0, 9, 38, and 67 mg/kg-day for Group A males (not treated with bromodeoxyuridine); 0, 7, 43, and 69 mg/kg-day for Group B males (bromodeoxyuridine-treated); and 0, 14, 69, or 126 mg/kg-day for Group C females (peri-conception exposure, bromodeoxyuridine-treated).

The results for reproductive and developmental effects are reported in Section V.E.1. Alterations in hematological endpoints or clinical chemistry were not observed following bromodichloromethane exposure, with the exception of a 14% drop in creatinine in the 100 ppm Group A males and a 43% increase in 5'-nucleotidase in the 1300 ppm Group A males when compared to controls. An increase in 5'-nucleotidase is an indication of hepatobiliary dysfunction in which there is interference with the secretion of bile, and should be accompanied by a parallel change in alkaline phosphatase activity. Since alkaline phophatase activity was unaltered in this study, the toxicological significance of the observed increase in 5'-nucleotidase was considered uncertain. Organ weight and organ/body weight ratios reported by NTP (1998) were comparable in all treatment groups for both males and females. Histopathological examination identified three tissue changes that were potentially treatment-related. Cytoplasmic vacuolization of hepatocytes and mild liver necrosis were observed in Group A males (see Table V-6 for details of group assignment) treated with 700 and 1300 ppm bromodichloromethane and in Group B males treated with 1300 ppm bromodichloromethane. Hepatic necrosis was dose-dependent, with incidences of 0/10, 0/10, 4/9, and 10/10 observed at 0, 100, 700, and 1300 ppm, respectively. These changes were not accompanied by an increase in alkaline phosphatase activity. Hematopoietic cell proliferation in the spleen was observed in Group A males at all doses of bromodichloromethane. However, the biological significance of this finding with respect to bromodichloromethane treatment was unclear, since cell proliferation in the spleen may occur as a response to general stress. Evidence of mild kidney necrosis was evident in Group A males in the 1300 ppm dose group, but may have resulted from decreased water intake. BrdU labeling index (LI), a measurement of cell proliferation, was unchanged in the livers and

kidneys of Group B males in all dose groups. A small but statistically significant increase in the LI was noted in the livers and kidneys of Group C females in the 1300 ppm dose group.

As discussed in Sections V.E.1, results from this study indicate that bromodichloromethane did not result in reproductive or developmental toxicity at drinking water concentrations up to 1300 ppm. However, exposure to concentrations of 700 ppm and 1300 ppm produced changes in liver histopathology in male rats and resulted in decreases in body weight and food and water consumption in both sexes. On the basis of these results, NTP (1998) concluded that bromodichloromethane is unpalatable at these concentrations and is a possible general toxicant in male and female rats at concentrations of 700 ppm and above. Although not accompanied by changes in alkaline phosphatase activity, the occurrence of individual hepatocyte cell necrosis was clearly dose-related and thus considered appropriate for identification of NOAEL and LOAEL values. Based on calculated average daily doses for Group A males at the 100 and 700 ppm concentrations (Table 6A in NTP, 1998), these data identify NOAEL and LOAEL values of 9 mg/kg-day and 38 mg/kg-day, respectively, for occurrence of hepatic cell necrosis.

Coffin et al. (2000) examined the effect of bromodichloromethane administered by corn oil gavage or in drinking water on cell proliferation and DNA methylation in the liver of female B6C3F1 mice. Gavage doses of 0, 0.92, or 1.83 mmol/kg (0, 150, or 300 mg/kg, respectively) were administered to test animals (7-8 weeks old; 10/group) daily for five days, off for two days, and then again daily for four days. It had previously been shown that bromodichloromethane administered by corn oil gavage at either 75 mg/kg or 150 mg/kg-day caused significant increases in the incidence of hepatocellular adenoma and adenocarcinoma, in a dose-dependent manner, in female mice (NTP 1987). In a separate experiment, bromodichloromethane was administered in drinking water for 11 days at approximately 75% of the saturation level, resulting in an average daily dose of 0.85 mmol/kg (138 mg/kg). The mice were sacrificed 24 hours after the last gavage dose and the livers were removed, weighed, and processed for histopathological examination, proliferating cell nuclear antigen - labeling index (PCNA-LI) analysis, and determination of *c-myc* methylation status. A significant, dose-dependent increase in relative liver weight was observed in animals dosed by gavage; however, relative liver weight was unaffected in animals administered the compound in drinking water, when compared to controls. Histopathological findings in gavage-dosed animals consisted of hydropic degeneration at the low dose and necrosis, fibrosis, and giant cell reaction at the high dose. No severity or incidence data were provided. The histopathology findings for animals receiving bromodichloromethane in the drinking water were similar to those observed in the low-dose gavage group. Bromodichloromethane administered by gavage caused a dose-dependent increase in the PCNA-LI which was significant at each dose tested when compared to the control. There was no significant effect when the compound was administered in drinking water. Administration of bromodichloromethane by gavage or in drinking water decreased methylation of the *c-myc* gene. A LOAEL of 150 mg/kg, the lowest dose tested, was identified on the basis of liver toxicity (hydropic degeneration) and increased cell proliferation in animals administered bromodichloromethane by corn oil gavage. A NOAEL was not identified. The results of the single-dose drinking water experiment suggest a slightly lower LOAEL of 138 mg/kg-day, based on hydropic degeneration of the liver.

Lock et al. (2004) administered bromodichloromethane by gavage in corn oil to male F344 rats (5/group) at doses of 0, 50, or 100 mg/kg and male B6C3F1 mice (6/group) at doses of 0, 25, or 50 mg/kg-day for 5 days per week over a 28-day period. A dose-dependent increase in incidences of large intestine and kidney tumors has previously been shown in male rats exposed at 50 or 100 mg/kg-day in dose-dependent manner (NTP 1987). A dose-dependent increase in the incidence of kidney tumors has also been shown in male mice exposed at 25 or 50 mg/kg-day (NTP 1987). Body weights were measured on each day of dosing and a 24-hour urine sample was collected on days 4 to 5, 11 to 12, and 18 to 19. Urine samples were analyzed for creatinine, total protein, urinary pH, and formic acid concentration. Osmotic mini-pumps containing bromodeoxyuridine were implanted subcutaneously 5 days prior to sacrifice. Animals were sacrificed by overdose with halothane anaesthesia on day 29 and blood was obtained by cardiac puncture for clinical chemistry analysis, including alanine aminotransferase activity, aspartate aminotransferase activity, and the concentration of blood urea nitrogen and creatinine. Kidneys were removed, weighed and prepared for histopathological analysis and determination of the labeling index.

Large increases in formic acid excretion were observed in rats at both doses, and this was accompanied by a decrease in urinary pH. In mice, increases in formic acid excretion were much smaller, and urinary pH was not measured. No change was observed in body weight or clinical chemistry markers of liver or kidney injury in exposed rats and mice. Kidney histopathology was not altered in mice, but mild renal tubule injury was observed in 2 of 5 rats exposed to the highest dose of bromodichloromethane (100 mg/kg-day). Increased cell proliferation was observed in all rats exposed to the highest dose, but was not seen in low-dose rats or in mice at either dose level.

Torti et al. (2001) conducted a one week inhalation exposure study of bromodichloromethane in male wild-type ($p53^{+/+}$) and genetically engineered p53 heterozygous ($p53^{+/-}$) mice. The objective of this study was to evaluate the role of genotype in the toxic response of mice to inhalation of bromodichloromethane. Heterozygous and wild type C57BL/6 mice (6 mice/type/concentration) and wild-type FVB/N mice (6 mice/concentration) were exposed to target exposure concentrations of 0, 1, 10, 30, 100, or 150 ppm for six hours per day for seven days. Heterozygous FVB/N $p53^{+/-}$ mice (6 mice/concentration) were exposed to concentrations of 0, 0.3, 1, 10, or 30 ppm for six hours per day for seven days. The test animals were evaluated for clinical and pathological changes and induced regenerative cell proliferation in kidney and liver. Osmotic pumps for delivery of bromodeoxyuridine for determination of labeling index were implanted at 3.5 days prior to scheduled termination. Test animals were euthanized approximately 18 hours after the last scheduled exposure. With the exception of the highest target concentration (150 ppm), the average measured concentrations were 102 to 114% of the target concentrations The average high dose concentration was 78.8% of the target concentration (150 ppm) as a result of technical problems with the metering system. The effects observed in all mouse groups (i.e., wild-type and heterozygous) exposed to concentrations of 30 ppm or greater included; mortality, clinical signs (i.e., reddened skin and eyes), reduced body weight gain, increased liver and kidney weight, histopathological lesions in the liver and kidney, and increased labeling index in the kidney. Clinical signs in mice surviving exposure at 100 and 150 ppm included lethargy and labored breathing. Histopathologic evaluation revealed severe

renal damage consisting of nephrosis, tubular degeneration, and associated regeneration. Centrilobular degeneration and necrosis were observed in the livers of moribund mice sacrificed before study termination and in animals surviving for 1 week of exposure. Regenerative cell-proliferation in the kidney cortex was significantly increased in all mouse groups (i.e., wild-type and heterozygous) exposed to concentrations of 10 ppm and above. Regenerative cell proliferation in the liver was less pronounced than in the kidney.

A comparison of the data for each wild-type strain indicates that FVB/N mice were more susceptible to mortality, increased liver weight, kidney degeneration and nephrosis, and hydropic degenerationin the liver as compared to C57BL/6 mice. For C57BL/6 mice, mortality, body weight loss, kidney degeneration and nephrosis, and the liver labeling index were greater in the heterozygous $p53^{+/-}$ than in the corresponding wild-type strain. For FVB/N mice, increased kidney weight occurred at a lower dose (10 ppm) in heterozygous $p53^{+/-}$ mice, while other effects were similar that occurring in the corresponding wild-type strain. Bromodichloromethane did not induce cellular proliferation in the transitional epithelium of the bladder. No histopathologic lesions were observed in the bladder. These data identify NOAEL and LOAEL values of 1 and 10 ppm, respectively, based on histopathological changes in the liver and kidney of male p53 wild-type and heterozygous C57BL/6 and FVB/N mice.

Torti et al. (2001) also conducted a three week inhalation exposure study of bromodichloromethane in wild-type ($p53^{+/+}$) and genetically engineered p53 heterozygous ($p53^{+/-}$) male mice. C57BL/6, FVB/N, C57BL/6 $p53^{+/-}$, and FVB/N $p53^{+/-}$ mice (6 mice/type/concentration) were exposed to target exposure concentrations of 0.3, 1, 3, 10, or 30 ppm for six hours per day, seven days per week. The test protocol and endpoints measured were the same as those used for the one week study described above. Test animals were euthanized approximately 18 hours after the last scheduled exposure. Average measured concentrations were 92 to 97% of the target concentrations in all exposure groups. Mortality was observed in all 30 ppm dose groups with the exception of wild type C57BL/6 mice. No clinical signs of toxicity were reported. Body weight gain was significantly reduced only in C57BL/6 wild type mice exposed at 30 ppm. Relative kidney weights in exposed groups did not differ significantly from the control values. Significantly increased relative liver weight was observed only in heterozygous C57BL/6 and wild type FVB/N mice exposed at 30 ppm. Histopathologic evaluation revealed near-normal kidney architecture. Minimal to moderate degenerative tubular change and regenerative tubules were observed in the 10 and 30 ppm groups, but the acute tubular nephrosis observed in the one week study was not evident. Minimal hepatocyte degeneration was observed in heterozygous C57BL/6 mice exposed at 30 ppm and in heterozygous FVB/N mice exposed at 10 or 30 ppm. These observations suggest that the liver and severe renal toxicity observed in the one week experiment conducted by Torti et al. (2001) are transient and were resolving by three weeks. No histopathologic lesions were observed in the bladder. Regenerative cell-proliferation in the kidney cortex was near baseline levels, with only the 30 ppm groups showing small elevations. These elevations were statistically significant in all 30 ppm groups except C57BL/N wild type mice. No increases in regenerative cell proliferation were evident in the liver or bladder. The NOAEL and LOAEL values in this study are 3 and 10 ppm, respectively, based on histopathologic changes in the liver and kidney of male p53 wild type and heterozygous C57BL/6 and FVB/N mice.

Taken together, the 1-week and 3-week inhalation studies (Torti et al., 2001) illustrate both strain and genotypic difference in bromodichloromethane toxicity. A comparison of wild-type strains indicates that FVB/N mice are more susceptible to kidney toxicity and mortality following inhalation exposure. Differences between wild-type and p53+/- mice were observed in mortality and morbidity, body weight changes, and the severity of liver and kidney toxicity. The C57BL/6 p53+/- mice were more susceptible than wild-type mice to bromodichloromethane toxicity as measured by mortality, histopathology, and liver labeling index. The same relationship was not observed in FVB/N mice. In this strain the wild-type mice were more susceptible to toxicity as evidenced by the kidney labeling index. The role of p53 gene expression in bromodichloromethane metabolism and toxicity remains to be elucidated.

2. Dibromochloromethane

Chu et al. (1982a) administered dibromochloromethane to male Sprague-Dawley rats (10/group) in drinking water for 28 days at dose levels of 0, 5, 50, or 500 ppm. Based on recorded fluid intake, these levels corresponded to doses of 0, 0.7, 8.5, or 68 mg/kg-day, as calculated by the authors. The authors observed no effects on growth rate or food consumption and no signs of toxicity throughout the exposure. No dose-related biochemical or histologic changes were detected (no data provided). This study identified a NOAEL of 68 mg/kg-day, but the reported data were too limited to allow an independent verification.

Munson et al. (1982) administered dibromochloromethane by aqueous gavage to male and female CD-1 mice (8 to 12/sex/group) for 14 days at dose levels of 0, 50, 125, or 250 mg/kg-day. Endpoints measured included body and organ weights, hematology, clinical chemistry, and humoral and cell-mediated immune system function. At 250 mg/kg-day, body weights were significantly decreased only in high-dose males. Significant organ weight changes included increased absolute liver weight (high-dose females), increased relative liver weight (mid- and high-dose groups), and decreased absolute and relative spleen weight (high-dose males). The only hematology parameter significantly affected by treatment was fibrinogen concentration, which was decreased in high-dose males and females. Significant clinical chemistry findings were limited to the high-dose groups. Specifically, glucose levels were significantly decreased in both males and females, and ALT and AST activities were significantly increased in both males and females. Dibromochloromethane appeared to affect the humoral immune system, as judged by significantly decreased antibody-forming cells (mid- and high-dose groups) and hemagglutination titers (high-dose groups). The cell-mediated immune system also appeared to be affected in male animals, as judged by a significant decrease in the popliteal lymph node stimulation index at the high dose. This study identified a NOAEL of 50 mg/kg-day and a LOAEL of 125 mg/kg-day, based on decreased immune function.

Condie et al. (1983) investigated the renal and hepatic toxicity of dibromochloromethane. Male CD-1 mice (8 to 16/group) were administered 0, 37, 74, or 147 mg/kg-day of dibromochloromethane by gavage in corn oil for 14 days. Biochemical evidence of liver damage (elevated ALT) and kidney damage (decreased PAH uptake by kidney slices) was observed at the high dose, but not at the mid-level or low doses. Similarly, histopathology revealed no consistent or important changes at the low or mid doses, with minimal to moderate liver and

kidney injury at the high dose. Liver lesions included mitotic figures, focal inflammation, and cytoplasmic vacuolation, while kidney lesions included epithelial hyperplasia and mesangial nephrosis. The NOAEL and LOAEL values in this study were 74 mg/kg-day and 147 mg/kg-day, respectively.

NTP (1985) administered 0, 60, 125, 250, 500, or 1,000 mg/kg-day of dibromochloromethane to male and female F344/N rats (5/sex/dose) by gavage in corn oil for 14 days. Animals were observed twice daily for mortality and were weighed once per week. Necropsies were performed on all animals. All high-dose rats and all females that received 500 mg/kg-day died by day 6. Three males at 500 mg/kg-day died between days 5 and 8. No deaths occurred at or below 250 mg/kg-day. At 500 or 1000 mg/kg-day, clinical observations included lethargy, ataxia, and labored breathing. Treatment-related macroscopic findings included mottled livers and darkened renal medullae in animals administered 500 or 1,000 mg/kg-day. This study identified a NOAEL of 250 mg/kg-day and a LOAEL of 500 mg/kg-day based on behavior, gross pathology, and mortality.

In a parallel study (NTP, 1985), male and female $B6C3F_1$ mice (5/sex/dose) were administered 0, 30, 60, 125, 250, or 500 mg/kg-day of dibromochloromethane in corn oil by gavage for 14 days. Treatment-related deaths occurred in 80% of the males and in 60% of the females at the high dose. Clinical signs at this dose included lethargy, ataxia, and labored breathing. Treatment-related macroscopic findings included mottled livers and darkened renal medullae in high-dose males and females. White papillomatous nodules in the stomach were also observed in males at 125, 250, or 500 mg/kg-day and in female mice at 250 or 500 mg/kg-day. The NOAEL and LOAEL in this study were 60 mg/kg-day and 125 mg/kg-day, respectively, based on gross lesions.

Aida et al. (1992a) administered dibromochloromethane to Slc:Wistar rats (7/sex/group) for one month at dietary levels of 0%, 0.020%, 0.062%, or 0.185% for males, and 0%, 0.038%, 0.113%, or 0.338% for females. The test material was microencapsulated and mixed with powdered feed; placebo granules were used for the control groups. Based on the mean food intakes, the study authors reported calculated doses of 0, 18.3, 56.2, or 173.3 mg/kg-day for males and 0, 34.0, 101.1, or 332.5 mg/kg-day for females. Clinical effects, body weight, food consumption, hematology parameters, serum chemistry, and histopathology of all major organs were determined. Body weights were significantly reduced in high-dose females relative to the controls. High-dose females also exhibited slight piloerection and emaciation. Dose-related increases in both absolute and relative liver weights were observed in males (significant at the high dose) and females (significant at all dose levels with the exception of a nonsignificant increase in absolute liver weight at the low dose). Relative kidney weights were also significantly increased in the high-dose females. Significant decreases in alkaline phosphatase (mid- and high-dose males and all female dose groups) and LDH (all female dose groups) were observed, but the biological significance of these changes is unclear. Significant, dose-related changes in serum biochemistry included reduced nonesterified fatty acids in high-dose males, reduced triglycerides in high-dose groups, and increased cholesterol in mid- and high-dose males and in females at all dose levels. The cholesterol levels, however, were within normal ranges at all dose levels. Serum cholinesterase activity was also significantly decreased in high-dose

males and mid- and high-dose females with the trend clearly dose-related in females. Liver cell vacuolization was generally noted at a similar incidence in the controls and all dosing groups, but dose-related increases in severity were observed in mid- and high-dose males and females. The incidence and very slight severity of the effects at the low dose were similar to those observed in the control groups and were not considered adverse. The severity of the liver cell vacuolization at the mid-dose was rated as very slight to slight, while the severity at the high-dose was rated as moderate to remarkable. Swelling and single cell necrosis were also observed, primarily in the high-dose groups. No effect was observed on any hematology parameter. NOAELs of 18.3 (males) and 34.0 (females) mg/kg-day and LOAELs of 56.2 (males) and 101.1 (females) mg/kg-day were identified for this study based on the histopathology findings.

Potter et al. (1996) evaluated hyaline droplet formation and cell proliferation in the kidney of male F344 rats following exposure to dibromochloromethane. The rats (4/dose)were dosed with 0.75 or 1.5 mmol/kg (156 or 312 mg/kg-day, respectively) of dibromochloromethane in 4% Emulphor® by gavage for 1, 3, or 7 days. No exposure-related increase in hyaline droplets was observed in dosed rats. Binding to α_{2u}-globulin was not measured. Changes in kidney tubule cell proliferation were assessed by *in vivo* incorporation of [^{3}H]-thymidine. No statistically significant effect of dibromochloromethane exposure on this endpoint was noted following exposures of up to 7 days duration.

Melnick et al. (1998) exposed female B6C3F$_1$ mice (10/dose) to dibromochloromethane in corn oil via gavage for 3 weeks (5 days/week). The doses of dibromochloromethane in this study were 0 (vehicle only), 50, 100, 192, or 417 mg/kg-day. The doses were selected on the basis that increases in the incidence of hepatocellular adenoma and carcinoma were previously seen in female mice exposed at 50 mg/kg or 100 mg/kg-day in a dose-dependent manner (NTP 1985). The corresponding time-weighted doses were 0, 37, 71, 137, and 298 mg/kg-day. No treatment-related signs of overt toxicity were observed during the study. Body weight and water intake were not significantly altered at any dose tested. However, a statistically significant and dose-related increase in liver weight/body weight ratio was seen in the 100, 192 and 417 mg/kg-day dose groups. Serum ALT activity was significantly increased in the two highest dose groups. The activity of serum SDH was significantly elevated at all doses tested except 50 mg/kg-day. However, the increase in activity (shown graphically) was very small relative to the control at the 100 and 192 mg/kg-day doses. At necropsy, there was clear evidence of hepatocyte hydropic degeneration in the 192 and 417 mg/kg-day dose groups. BrdU was administered to the animals during the last 6 days of the study, and hepatocyte labeling index (LI) analysis was conducted. Only the highest dose tested (417 mg/kg-day) resulted in significantly elevated hepatocyte proliferation as measured by the LI. Evaluation of the data in this study suggest a LOAEL of 192 mg/kg-day, based on a consistent pattern of positive results for indicators of hepatotoxicity at this dose.

Coffin et al. (2000) examined the effect of dibromochloromethane administered by corn oil gavage or in drinking water on cell proliferation and DNA methylation in the liver of female B6C3F1 mice. Gavage doses of 0, 0.48, or 1.44 mmol/kg (0, 100, or 300 mg/kg, respectively) were administered to test animals (7-8 weeks old; 10/group) daily for five days, off for two days, and then again daily for four days. Dose-dependent increases in the incidence of hepatocellular

adenoma and carcinoma were previously seen in female mice exposed at 50 mg/kg or 100 mg/kg-day (NTP 1985). The high dose was selected on the basis that it had previously been demonstrated to be carcinogenic in female mice. Dibromochloromethane was administered in drinking water at approximately 75% of the saturation level, resulting in an average daily dose of 0.82 mmol/kg (171 mg/kg). The mice were sacrificed 24 hours after the last gavage dose and the livers were removed, weighed, and processed for histopathological examination, proliferating cell nuclear antigen - labeling index (PCNA-LI) analysis, and determination of *c-myc* methylation status. For histopathological analysis, stained liver sections were evaluated for toxicity using a semi-quantitative procedure using the following severity scoring system: Grade 1 consisted of mid lobular ballooning hepatocytes; Grade 2 consisted of mid lobular ballooning hepatocytes extending to the central vein; Grade 3 consisted of centrilobular necrosis with ballooning hepatocytes; and Grade 4 consisted of necrosis extending from the central vein to the mid lobule zone. A significant, dose-dependent increase in relative liver weight was observed in animals dosed by gavage; however, relative liver weight was unaffected in animals administered the compound in drinking water, when compared to controls. At the low gavage dose, liver toxicity consisted mainly of a Grade 1 response. At the high dose, liver toxicity consisted mainly of a Grade 2 response. No incidence data were provided in the study report, nor was a severity grade reported for the control group. The histopathology findings for animals receiving bromodichloromethane in the drinking water were similar to those observed in the low-dose gavage group. Dibromochloromethane administered by gavage caused a dose-dependent increase in the PCNA-LI. There was no significant effect on PCNA-LI when the compound was administered in drinking water. Administration of dibromochloromethane by gavage or in drinking water decreased methylation of the *c-myc* gene. A LOAEL of 100 mg/kg, the lowest dose tested, was identified on the basis of liver toxicity (ballooning hepatocytes) and increased cell proliferation in gavaged animals.

3. Bromoform

Chu et al. (1982a) administered bromoform to male Sprague-Dawley rats (10/group) in drinking water for 28 days at dose levels of 0, 5, 50, or 500 ppm. Based on recorded fluid intake, these levels corresponded to doses of 0, 0.7, 8.5, or 80 mg/kg-day, as calculated by the authors. The authors observed no effects on growth rate or food consumption and no signs of toxicity throughout the exposure. No dose-related biochemical or histologic changes were detected (no data provided). This study identified a NOAEL of 80 mg/kg-day, but the reported data were too limited to allow an independent confirmation.

Munson et al. (1982) administered bromoform by aqueous gavage to male and female CD-1 mice (6 to 12/sex/group) for 14 days at levels of 0, 50, 125, or 250 mg/kg-day. Parameters evaluated included body and organ weights, hematology, clinical chemistry, and humoral and cell-mediated immune system functions. Body weights were significantly decreased in high-dose females, while body weights in males were significantly increased at the mid and high doses. Absolute and relative liver weights were significantly increased in males at the mid -and high dose and in females at the high dose. Absolute spleen weight was also decreased in mid- and high-dose females. Hematologic effects included significantly decreased fibrinogen in males at the high dose and significantly decreased prothrombin time in all treated males. The

changes in prothrombin time, however, were not dose-related. Significant clinical chemistry findings included decreased glucose levels (high-dose males), increased AST activity (high-dose males and females), and decreased BUN levels (high-dose males). Both the humoral and cell-mediated immune systems appeared to be affected in males at the high dose with a significant decrease in antibody-forming cells and a significant decrease in delayed-type hypersensitivity response. The authors stated that no treatment-related effects on the immune system in females were observed (no data were reported). Based on changes in clinical chemistry parameters, this study identified a NOAEL of 125 mg/kg-day and a LOAEL of 250 mg/kg-day.

Condie et al. (1983) investigated the renal and hepatic toxicity of bromoform. Male CD-1 mice (8 to 16/group) were administered 0, 72, 145, or 289 mg/kg-day of bromoform by gavage in corn oil for 14 days. Biochemical evidence of liver damage (elevated ALT) and kidney damage (decreased PAH uptake by kidney slices) was observed at the high dose, but not at the mid or low dose. Histopathological examination revealed no consistent or important changes at the low or mid doses, with minimal to moderate liver and kidney injury at the high dose. Specific microscopic changes included intratubular mineralization, epithelial hyperplasia, mesangial hypertrophy and mesangial nephrosis in the kidney, and centrilobular pallor, mitotic figures, focal inflammation, and cytoplasmic vacuolation in the liver. This study identified a NOAEL value of 145 mg/kg-day and a LOAEL value of 289 mg/kg-day based on histopathologic changes in the liver.

NTP (1989a) investigated the short term oral toxicity of bromoform in F344/N rats and B6C3F_1 mice. Groups of male and female rats (5/sex/group) and female mice (5/group) were administered doses of 0, 100, 200, 400, 600, or 800 mg/kg-day of bromoform in corn oil by gavage for 14 days. Male mice were administered 0, 50, 100, 200, 400, or 600 mg/kg-day. All rats that were dosed at 600 or 800 mg/kg-day died before the end of the study. At 400 mg/kg-day, only one male rat died before study termination. These rats exhibited lethargy, labored breathing, and ataxia. At 400 mg/kg-day, final body weights were decreased by 14% in male rats and by 4% in female rats relative to controls. In mice, one male and one female administered the high dose died before study termination. Ataxia and lethargy were noted at 600 mg/kg-day. Final body weights of mice were comparable to those of the controls. Raised stomach nodules were observed in males at 400 and 600 mg/kg-day and in females at 600 and 800 mg/kg-day. This study identified a NOAEL of 200 mg/kg-day and a LOAEL of 400 mg/kg-day. based on decreased body weight and mortality in rats and on stomach nodules in mice,

Aida et al. (1992a) administered bromoform to Slc:Wistar rats (7/sex/group) for one month at dietary levels of 0%, 0.068%, 0.204%, or 0.612% for males and 0%, 0.072%, 0.217%, or 0.651% for females. The test material was microencapsulated and mixed with powdered feed; placebo granules were used for the control groups. Based on the mean food intakes, the study authors reported the mean compound intakes as 0, 61.9, 187.2, or 617.9 mg/kg-day for males and 0, 56.4, 207.5, or 728.3 mg/kg-day for females. Clinical effects, body weight, food consumption, hematology parameters, serum chemistry, and histopathology of all major organs were determined. Body weights were significantly reduced in high-dose males relative to the controls. High-dose animals of both sexes exhibited slight piloerection and emaciation. Relative liver weight was significantly increased in mid- and high-dose males and females. Significant

changes in serum chemistry were primarily observed in the mid- and high-dose animals with the females more significantly affected. These changes included significant decreases in (a) serum glucose in low- and high-dose males and in mid- and high-dose females, (b) triglycerides in high-dose males and in mid- and high-dose females, (c) cholinesterase activity in high-dose males and in all female treatment groups, (d) LDH in mid- and high-dose females, and (e) BUN in mid- and high-dose females. All of these changes in the groups noted exhibited strong dose-related trends with the exception of serum glucose in males. Creatinine levels and alkaline phosphatase activity were also significantly decreased in all female treatment groups, but the changes were not dose-related. Significant increases, although not dose-related, were observed for phospholipids and cholesterol in mid- and high-dose animals with the exception of a nonsignificant increase in phospholipids in high-dose females. The only change of clear biological significance at the low dose was a decrease in cholinesterase activity in females. No effect was observed on any hematology parameter. Microscopic and macroscopic findings were limited to the liver. Specifically, discoloration was observed in all males and females in the high-dose group. The incidence and severity of liver cell vacuolization and swelling were dose-related. Severe hepatic cell vacuolization was observed in 5/7 high-dose males and in 6/7 females at the mid and high dose. Slight to moderate liver cell swelling was observed in three high-dose males, while all high-dose females displayed slight signs of liver cell swelling. Females appeared to be more sensitive for development of histopathological effects, but the changes observed in low-dose females were not considered an adverse effect. Based on the histopathology and serum chemistry changes in the mid-dose animals, this study identified NOAELs of 61.9 mg/kg-day for males and 56.4 mg/kg-day for females, and LOAELs of 187.2 mg/kg-day for males and 207.5 mg/kg-day for females.

Potter et al. (1996) evaluated the effect of bromoform on hyaline droplet formation and cell proliferation in the kidney of male F344 rats. Animals (4/dose) received doses of 0.75 or 1.5 mmol/kg of bromoform in 4% Emulphor® by gavage for 1, 3, or 7 days. These doses correspond to 190 or 379 mg/kg-day, respectively. No exposure-related increase in hyaline droplet formation was observed. Cell proliferation in the kidney following bromoform exposure was measured *in vivo* by [^{3}H]-thymidine incorporation. No statistically significant effects were noted following exposures of up to 7 days duration. Binding of bromoform to α_{2u}-globulin was not measured.

Melnick et al. (1998) exposed female B6C3F$_1$ mice (10 animals/group) to bromoform in corn oil via gavage for 3 weeks (5 days/week). Doses of bromoform used in this study were 0 (vehicle only), 200, or 500 mg/kg-day. There were no treatment-related signs of overt toxicity observed during the study. Body weight and water intake were not significantly altered at any dose tested. However, a dose-related increase in absolute liver weight and liver weight/body weight ratio was noted in both tested doses. Neither serum ALT nor serum SDH activity were significantly elevated at either dose of bromoform. At necropsy, there was no evidence of hepatocyte hydropic degeneration in animals treated with either dose. BrdU was administered to the animals during the last 6 days of the study, and hepatocyte labeling index (LI) analysis was conducted. Only the 500 mg/kg-day dose resulted in marginally significant increase in hepatocyte proliferation as measured by the LI. These data suggest a NOAEL of 200 mg/kg-day and a LOAEL of 500 mg/kg-day based on increased hepatocyte proliferation.

Coffin et al. (2000) examined the effect of bromoform administered by corn oil gavage or in drinking water on liver toxicity, cell proliferation and DNA methylation in female B6C3F1 mice. Gavage doses of 0, 0.79, or 1.98 mmol/kg (0, 200, or 500 mg/kg, respectively) were administered to test animals (7-8 weeks old; 10/group) daily for five days, off for two days, and then again daily for 5 days. Bromoform was not shown to be carcinogenic to female mice exposed at doses up to 200 mg/kg-day (NTP 1989a). The high dose was selected on the basis that it had previously been demonstrated to be carcinogenic in female mice. Bromoform was administered in drinking water at approximately 75% of the saturation level, resulting in an average daily dose of 1.19 mmol/kg (301 mg/kg). The mice were sacrificed 24 hours after the last gavage dose and the livers were removed, weighed, and processed for histopathological examination, proliferating cell nuclear antigen - labeling index (PCNA-LI) analysis, and determination of *c-myc* methylation status. For histopathological analysis, stained liver sections were evaluated for toxicity using a semi-quantitative procedure using the following severity scoring system: Grade 1 consisted of mid lobular ballooning hepatocytes; Grade 2 consisted of mid lobular ballooning hepatocytes extending to the central vein; Grade 3 consisted of centrilobular necrosis with ballooning hepatocytes; and Grade 4 consisted of necrosis extending from the central vein to the mid lobule zone. A significant, dose-dependent increase in relative liver weight was observed in animals dosed by gavage; however, relative liver weight was unaffected in animals administered the compound in drinking water, when compared to controls. At the low gavage dose, liver toxicity consisted mainly of a Grade 1 response. At the high dose, liver toxicity consisted mainly of a Grade 2 response. No incidence data were provided in the study report, nor were severity data presented for the control group. The histopathology findings for animals receiving bromoform in the drinking water were similar to those observed in the low-dose gavage group. Bromoform administered by gavage caused a significant, dose-dependent increase in the PCNA-LI. Bromoform also significantly enhanced cell proliferation when the compound was administered in drinking water. Administration of bromoform by gavage or in drinking water decreased methylation of the *c-myc* gene. A LOAEL of 200 mg/kg, the lowest dose tested, was identified on the basis of liver toxicity in gavaged animals.

C. Subchronic Exposure

This section addresses studies of brominated trihalomethanes that are of approximately 90 days in duration. Table V-4 summarizes the details of these subchronic studies.

Table V-4 Summary of Subchronic Toxicity Studies for Brominated Trihalomethanes

Reference	Species	Route	Sex	Number per dose group	Duration	Dose (mg/kg-day)	Results
Bromodichloromethane							
Oral Exposure							
Chu et al. (1982b)	Rat SD*	Drinking water	M	20	90 days	0 0.57 6.5 53 212	Non dose-dependent hepatic and thyroid lesions
Chu et al. (1982b)	Rat SD	Drinking water	F	20	90 days	0 0.75 6.9 57 219	Non dose-dependent hepatic and thyroid lesions
NTP (1987)	Rat F344/N	Gavage (corn oil)	M, F	10	13 weeks (5 d/wk)	0 19 38 75 (NOAEL) 150 (LOAEL) 300	Reduced body weight gain
NTP (1987)	Mouse $B6C3F_1$	Gavage (corn oil)	M	10	13 weeks (5 d/wk)	0 6.3 13 25 50 (NOAEL) 100 (LOAEL)	Focal necrosis of proximal renal tubular epithelium
NTP (1987)	Mouse $B6C3F_1$	Gavage (corn oil)	F	10	13 weeks (5 d/wk)	0 25 50 100 (NOAEL) 200 (LOAEL) 400	Hepatic microgranulomas
Inhalation Exposure							
Torti et al. 2001	Mouse C57BL/6 FVB/N p53 (hetero-zygous)	Vapor	M	Not reported	13 weeks (6 h/day)	0 ppm 0.5 ppm 3 ppm 10 ppm 15 ppm	Text reported minimal cortical scarring and occasional regenerative tubules in C56BL/6 mice and mild renal cortical tubular karyocytomegaly. Concentrations at which these effects occurred were not reported.

Table V-4 (cont.)

Reference	Species	Route	Sex	Number per dose group	Duration	Dose (mg/kg-day)	Results
Dibromochloromethane							
Chu et al. (1982b)	Rat SD	Drinking water	M	20	90 days	0 0.57 6.1 49 (NOAEL) 224 (LOAEL)	Hepatic lesions
Chu et al. (1982b)	Rat SD	Drinking water	F	20	90 days	0 0.64 6.9 55 (NOAEL) 236 (LOAEL)	Hepatic lesions
NTP (1985)	Rat F344/N	Gavage (corn oil)	M, F	10	13 weeks (5 d/wk)	0 15 30 (NOAEL) 60 (LOAEL) 125 250	Hepatic vacuolization indicative of fatty metamorphosis (males)
NTP (1985)	Mouse $B6C3F_1$	Gavage (corn oil)	M, F	10	13 weeks (5 d/wk)	0 15 30 60 125 (NOAEL) 250 (LOAEL)	Fatty liver and toxic nephropathy in males
Daniel et al. (1990)	Rat SD	Gavage (corn oil)	M, F	10	90 days	0 50 (LOAEL) 100 200	Hepatic vacuolization (males); renal lesions (females)
Bromoform							
Chu et al. (1982b)	Rat SD	Drinking water	M	20	90 days	0 0.65 6.1 57 (NOAEL) 218 (LOAEL)	Hepatic lesions and vacuolation
Chu et al. (1982b)	Rat SD	Drinking water	F	20	90 days	0 0.64 6.9 55 (NOAEL) 283 (LOAEL)	Hepatic lesions and vacuolation
NTP (1989a)	Rat F344/N	Gavage (corn oil)	M, F	10	13 weeks (5 d/wk)	0 12 25 (NOAEL) 50 (LOAEL) 100 200	Hepatic vacuolation in males

Table V-4 (cont.)

Reference	Species	Route	Sex	Number per dose group	Duration	Dose (mg/kg-day)	Results
NTP (1989a)	Mouse $B6C3F_1$	Gavage (corn oil)	M, F	10	13 weeks (5 d/wk)	0 25 50 100 (NOAEL) 200 (LOAEL) 400	Hepatic vacuolation in males

* SD, Sprague-Dawley

1. Bromodichloromethane

Chu et al. (1982b) administered bromodichloromethane to male and female weanling Sprague-Dawley rats (20/sex/dose) in drinking water at levels of 0, 5, 50, 500, or 2,500 ppm for 90 days. Half of each group (10/sex/dose) was sacrificed at the end of the exposure period, and the remaining animals were given tap water for another 90 days. As calculated by the authors (using data on water consumption and the average initial and final body weights in the vehicle controls and the high-dose groups), these levels corresponded to doses of approximately 0, 0.57, 6.5, 53, and 212 mg/kg-day for males and 0, 0.75, 6.9, 57, and 219 mg/kg-day for females. At 2,500 ppm, food consumption was significantly depressed and significant growth suppression occurred in both males and females. Mild histologic changes were observed in the liver and thyroid of the male animals. Neither incidence nor severity were clearly dose-related. Specifically, the incidence of hepatic lesions was increased in males at concentrations equal to or greater than 50 ppm, with similar statistically significant increases in the severity of these lesions in these dose groups compared to the control. The author noted that the hepatic lesions were mild and similar to the control following the 90-day recovery period. Increased incidence of thyroid lesions was also observed in males at concentrations equal to or greater than 50 ppm. The severity of these lesions was similar to that observed in the control group. These lesions were also mild and similar in nature to those of the control after the 90-day recovery period. The incidence of hepatic lesions in the female treatment groups (3-5/10) was slightly increased compared to that of the control group (0/10) with the severity significantly increased in the 50 and 2,500 ppm treatment groups, but not in the 500 ppm group. No significant numbers of females were reported as having thyroid lesions. Lack of a clear dose-response relationship for either incidence or severity of lesions prevented identification of reliable NOAEL or LOAEL values.

NTP (1987) administered doses of 0, 19, 38, 75, 150, or 300 mg/kg-day of bromodichloromethane to male and female F344/N rats (10/sex/dose) by gavage in corn oil for 5 days/week for 13 weeks. The low-dose group was administered 1.9 mg/kg-day for the first 3 weeks of the study. A necropsy was performed on all animals. Before study termination, 50% of the males and 20% of the females in the high-dose group died. Although food consumption was not recorded, animals in the high-dose groups appeared to eat less food. These animals were

also emaciated. At 300 mg/kg-day, final body weights of the males and females were decreased by 55% and 32%, respectively, relative to the controls. At 150 mg/kg-day, final body weights of the males and females were decreased by 30% and 12%, respectively, relative to the controls. Treatment-related lesions were observed only at the high dose. At 300 mg/kg-day in males, centrilobular degeneration of the liver and occasional necrotic cells were observed in 4/9 animals. Mild bile duct hyperplasia was also observed in these animals. Kidney lesions in high-dose males consisted of degeneration of renal proximal tubular epithelial cells (4/9) and definite foci of coagulative necrosis of the tubular epithelium (2/9). High-dose males (4/9) also exhibited lymphoid degeneration of the thymus, spleen, and lymph nodes, and mild to moderate atrophy of the seminal vesicles and/or prostate. Enlarged hepatocytes were observed in females (2/9) at 300 mg/kg-day. Although degeneration of the spleen, thymus, and lymph nodes was noted in high-dose females, the extent of the atrophy was much less than that observed in males. This study identified a NOAEL of 75 mg/kg-day and a LOAEL of 150 mg/kg-day based on reduced body weight gain.

In a parallel experiment, NTP (1987) administered bromodichloromethane in corn oil by gavage to male and female $B6C3F_1$ mice (10/sex/dose) for 5 days/week for 13 weeks. Doses were 0, 6.25, 12.5, 25, 50, or 100 mg/kg-day for males and 0, 25, 50, 100, 200, or 400 mg/kg-day for females. All animals survived to the end of the study. The final body weights of high-dose males were decreased by 9% relative to the controls. The final body weights of females that received 200 and 400 mg/kg-day were decreased 5% and 6%, respectively, relative to the controls. No treatment-related clinical signs were noted. Treatment-related lesions were observed only at 100 mg/kg-day in males and at 200 and 400 mg/kg-day in females. Kidney lesions in high-dose males included focal necrosis of the proximal renal tubular epithelium (6/10) and nephrosis of minimal severity (2/10). Microgranulomas were observed in the liver of 70% of the females that received the 200 mg/kg-day dose. NOAEL and LOAEL values for female mice were 100 and 200 mg/kg-day, respectively, based on occurrence of microgranulomas. This study identified a NOAEL of 50 mg/kg-day and a LOAEL of 100 mg/kg-day for male mice on the basis of liver histopathology.

Torti et al. (2001) reported results from a 13-week interim sacrifice conducted as part of an inhalation cancer bioassay in p53 heterozygous C57BL/6 and FVB/N male mice. Test animals were exposed to vapor concentrations of 0, 0.5, 3, 10, or 15 ppm, 6 hours/day for 13 weeks. Osmotic pumps for delivery of bromodeoxyuridine for determination of labeling index were implanted at 3.5 days prior to scheduled termination. Test animals were euthanized approximately 18 hours after the last scheduled exposure. No exposure-related effects were noted for mortality, morbidity, relative body weight, relative kidney or liver weight, or cell proliferation in liver, kidney or bladder. Histopathologic lesions were limited to the kidney. The study authors reported minimal cortical scarring and occasional regenerative tubules in the C57BL/6 strain. The only lesion reported for the FVB/N strain was limited to mild renal cortical tubular karyocytomegaly. No incidence data were presented for these lesions and the concentrations at which they occurred were not stated. Cell proliferation was not increased over baseline in the liver, kidney or bladder.

2. Dibromochloromethane

Chu et al. (1982b) administered dibromochloromethane to male and female weanling Sprague-Dawley rats (20/sex/dose) in drinking water at levels of 0, 5, 50, 500, or 2,500 ppm for 90 days. Half of each group (10/sex/dose) was sacrificed at the end of the exposure period, and the remaining animals were given tap water for another 90 days. Based on calculations by the authors, these levels corresponded to doses of approximately 0, 0.57, 6.1, 49, and 224 mg/kg-day for males and 0, 0.64, 6.9, 55, and 236 mg/kg-day for females. At 2,500 ppm, food consumption was depressed in both males and females, with the decrease reaching statistical significance in the males. Body weight gain was also decreased at the high-dose, but not significantly. Mild histologic changes occurred in the liver and thyroid in both males and females. Neither the incidence nor severity exhibited clear dose-response trends, with the possible exception of the incidence and severity of hepatic lesions in the males. The severity of hepatic lesions was significantly increased at 50 ppm in females and at 2,500 ppm in both males and females. Hepatic lesions included increased cytoplasmic volume and vacuolation due to fatty infiltration. Lesions of the thyroid included decreased follicular size and colloid density and occasional focal collapse of follicles. The severity of these lesions was not significantly different from that of the control. The authors noted that histological changes were mild and similar to controls when evaluated after the 90-day recovery period. These data identified a NOAEL of 49 mg/kg-day and a LOAEL of 224 mg/kg-day for males, and a NOAEL of 55 mg/kg-day and a LOAEL of 236 mg/kg-day for females.

NTP (1985) administered dibromochloromethane by gavage in corn oil to male and female F344/N rats (10/dose/sex). Doses of 0, 15, 30, 60, 125, or 250 mg/kg were given 5 days/week for 13 weeks. Animals were weighed weekly. All animals were submitted for gross necropsy, while histopathology was conducted on animals in the control and high-dose groups with the exception that the liver was examined in all males and in females at 125 mg/kg-day, and that the kidney and salivary glands were examined in males and females at 125 mg/kg-day. Only one male and one female in the high-dose group survived, with most deaths occurring during weeks 8 to 10. At 125 mg/kg-day, final body weights of males were decreased 7% relative to controls. Histopathological examination revealed severe lesions and necrosis in kidney, liver, and salivary glands, primarily at the high-dose. Males exhibited a dose-dependent increase in the frequency of clear cytoplasmic vacuoles indicative of fatty metamorphosis in the liver; this effect was statistically significant at doses of 60 mg/kg-day or higher. This study identified a NOAEL of 30 mg/kg-day and a LOAEL of 60 mg/kg-day in rats on the basis of histopathological effects in the liver.

NTP (1985) performed a similar 13-week gavage study with dibromochloromethane in male and female B6C3F_1 mice (10/sex/dose). The doses and dosing schedule were the same as for the rat study. No treatment-related effects on body weight or histopathology were observed at doses of 125 mg/kg-day or lower. At the high dose, final body weights of males and females were decreased by 6% relative to controls. Fatty metamorphosis of the liver and toxic nephropathy were observed in high-dose males, but not in high-dose females. This study identified a NOAEL of 125 mg/kg-day and a LOAEL of 250 mg/kg-day based on histopathological lesions in male mice.

Daniel et al. (1990) administered gavage doses (in corn oil) of 0, 50, 100, or 200 mg/kg-day of dibromochloromethane to male and female Sprague-Dawley rats (10/sex/dose) for 90 consecutive days. Individual dosages were adjusted weekly based on individual body weights. During the final week of the study, urinalysis was conducted following an overnight fast. Ophthalmoscopic examinations were performed prior to treatment and during the last week of the study. Hematology, serum clinical chemistry, and a thorough histopathologic examination were also conducted. No deaths, clinical signs of toxicity, or treatment-related changes in the ophthalmoscopic examinations or hematology were observed. Final body weights were significantly reduced in the high-dose groups by 32% in males and by 13% in females. Body weight decreases in the other groups were less than 10% of control weights. A dose-related increase was observed in liver weight in females that reached statistical significance at the high dose. Clinical chemistry values indicative of hepatotoxicity and suggestive of nephrotoxicity included increased levels of alkaline phosphatase (high-dose males and females), ALT (mid- and high-dose males), and creatinine (mid- and high-dose males and high-dose females), and decreased potassium levels (high-dose males). Centrilobular lipidosis (vacuolization) was observed in the liver of almost all high-dose males and females and all mid- and low-dose males (with one exception at each level), but in only one mid-dose female. The severity of the effect was dose-related. Centrilobular necrosis was also observed in high-dose males and females. Slight-to-moderate degeneration within the kidney proximal tubular cells occurred in all high-dose males and females and to a lesser extent in mid-dose males and low- and mid-dose females. Based on the liver histopathology in males and kidney histopathology in females, the LOAEL for dibromochloromethane in this study was 50 mg/kg-day, the lowest dose tested.

3. Bromoform

Chu et al. (1982b) administered bromoform to male and female weanling Sprague-Dawley rats (20 rats/sex/group) for 90 days in drinking water at levels of 0, 5, 50, 500, or 2,500 ppm. Half of each group (10/sex/dose) was sacrificed at the end of the exposure period, and the remaining animals were given tap water for a 90-day recovery period. Based on calculations by the authors, the administered drinking water levels corresponded to doses of approximately 0, 0.65, 6.1, 57, and 218 mg/kg-day for males and 0, 0.64, 6.9, 55, and 283 mg/kg-day for females. At 2,500 ppm, food consumption was depressed in both males and females, with the decrease reaching statistical significance in males. Body weight gain was also decreased at the high-dose, but not significantly. Lymphocyte counts were significantly decreased in high-dose males and females when evaluated 90-days after cessation of treatment. The only change in serum biochemistry was a significant decrease in LDH in both males and females at the high dose. This effect was also noted 90-days after cessation of treatment. Mild histologic changes occurred in the liver and thyroid of male and female animals. Although neither incidence nor severity were clearly dose-related, these parameters tended to increase with dose. The severity of hepatic lesions was significantly increased in high-dose males and in females at 500 and 2,500 ppm. Hepatic lesions included increased cytoplasmic volume and vacuolation due to fatty infiltration. Lesions of the thyroid included decreased follicular size and colloid density and occasional focal collapse of follicles. The severity of these lesions in the treated animals was not significantly different from that in the controls. Although the authors

noted that histologic changes were mild and similar to controls when evaluated after the 90-day recovery period, males in the high-dose group continued to exhibit an increased incidence of hepatic lesions with greater severity relative to the control. These data identified a NOAEL of 57 mg/kg-day and a LOAEL of 218 mg/kg-day for males, and a NOAEL of 55 mg/kg-day and a LOAEL of 283 mg/kg-day for females.

NTP (1989a) exposed male and female F344/N rats to bromoform by gavage for 5 days/week for 13 weeks. Animals (10/sex/dose) received doses of 0, 12, 25, 50, 100, or 200 mg/kg-day. None of the rats died before the end of the study, and body weights were not significantly affected. All high-dose animals, as well as males dosed with 100 mg/kg-day, were lethargic. At sacrifice, tissues were examined for gross and histologic changes. A dose-dependent increase in the frequency of hepatocellular vacuolation was observed in male rats, which reached statistical significance at 50 mg/kg-day (IRIS, 1993b). These hepatic effects were not observed in females. This study identified a NOAEL of 25 mg/kg-day and a LOAEL of 50 mg/kg-day, on the basis of the hepatic vacuolation seen in male rats.

In a parallel study, NTP (1989a) exposed male and female $B6C3F_1$ mice to bromoform by gavage for 5 days/week for 13 weeks. Animals (10/sex/dose) received doses of 0, 25, 50, 100, 200, or 400 mg/kg-day. One female died at 100 mg/kg-day, but no other deaths at any other dose level occurred. At sacrifice, tissues were examined for gross and histologic changes. Body weights were not significantly affected, although males receiving 400 mg/kg-day had body weights about 8% less than controls. A dose-related increase in the number of hepatocellular vacuoles was seen in male mice (incidence of 5/10 at 200 mg/kg and 8/10 at 400 mg/kg reported in text; incidence in controls not explicitly stated), but not in females. This study identified a NOAEL of 100 mg/kg-day and a LOAEL of 200 mg/kg-day in male mice, based on hepatocellular vacuolation.

D. Chronic Exposure

This section addresses studies on the health effects of brominated trihalomethanes that are of one to two years in duration. These studies are summarized in Table V-5.

Table V-5 Summary of Chronic Toxicity Studies for Brominated Trihalomethanes

Reference	Species	Route	Sex	Number per dose group	Duration	Dose (mg/kg-day)	Results
Bromodichloromethane							
NTP (1987)	Rat F344/N	Gavage (corn oil)	M, F	50	2 years (5 d/wk)	0 50 (LOAEL) 100	Renal and hepatic histopathology
NTP (1987)	Mouse $B6C3F_1$	Gavage (corn oil)	M	50	2 years (5 d/wk)	0 25 (LOAEL) 50	Renal and hepatic histopathology
NTP (1987)	Mouse $B6C3F_1$	Gavage (corn oil)	F	50	2 years (5 d/wk)	0 75 (LOAEL) 150	Reduced body weight gain
Aida et al. (1992b); Tobe et al. (1982)	Rat Wistar	Diet	M	40	2 years	0 6 (LOAEL) 26 138	Hepatic vacuolization, serum chemistry
Aida et al. (1992b); Tobe et al. (1982)	Rat Wistar	Diet	F	40	2 years	0 8 (NOAEL) 32 (LOAEL) 168	Hepatic vacuolization, serum chemistry
Klinefelter et al. (1995)	Rat F344	Drinking water	M	7	1 year	0 22 39 (NOAEL)	No evidence of treatment-related histopathological or organ weight effects (see reproductive effects, section V.E for additional data from this study)
George et al. (2002)	Rat F344	Drinking water	M	54	2 years	0 6.4 32.6 (NOAEL) 58.9 (LOAEL)	No evidence of treatment-related histopathological effects. Significant negative trend for relative kidney weight; significantly reduced kidney weight at high dose
George et al. (2002)	Mouse $B6C3F_1$	Drinking water	M	50	2 years	0 8.1 (NOAEL) 27.2 (LOAEL) 43.4	Decreased absolute and relative kidney weight. Mild treatment-related histopathological effects in liver.
Dibromochloromethane							
Tobe et al. (1982)	Rat Wistar SPF	Diet	M	40	2 years	0 12 (NOAEL) 49 (LOAEL) 196	Serum biochemistry, liver appearance at necropsy; decreased body weight gain
Tobe et al. (1982)	Rat Wistar SPF	Diet	F	40	2 years	0 17 (NOAEL) 70 (LOAEL) 278	Serum biochemistry, liver appearance at necropsy; decreased body weight gain

Table V-5 (cont.)

Reference	Species	Route	Sex	Number per dose group	Duration	Dose (mg/kg-day)	Results
NTP (1985)	Rat F344	Gavage (corn oil)	M, F	50	2 years (5 d/wk)	0 40 (LOAEL) 80	Histologic changes in liver, including fat accumulation and ground glass appearance, and altered basophilic staining
NTP (1985)	Mouse $B6C3F_1$	Gavage (corn oil)	M, F	50	105 weeks (5 d/wk)	0 50 (LOAEL) 100	Fatty metamorphosis in liver and follicular cell hyperplasia in thyroid
Bromoform							
Tobe et al. (1982)	Rat Wistar SPF	Diet	M	40	2 years	0 22 (NOAEL) 90 (LOAEL) 364	Enzyme changes and altered liver appearance at necropsy
Tobe et al. (1982)	Rat Wistar SPF	Diet	F	40	2 years	0 38 (NOAEL) 152 (LOAEL) 619	Enzyme changes and altered liver appearance at necropsy
NTP (1989a)	Rat F344/N	Gavage (corn oil)	M, F	50	103 weeks (5 day/wk)	0 100 (LOAEL) 200	Decreased body weight, lethargy, mild hepatotoxicity
NTP (1989a)	Mouse $B6C3F_1$	Gavage (corn oil)	M	50	103 weeks (5 day/wk)	0 50 100 (NOAEL)	No observed effects on body weight or hepatotoxicity
NTP (1989a)	Mouse $B6C3F_1$	Gavage (corn oil)	F	50	103 weeks (5 day/wk)	0 100 (LOAEL) 200	Decreased body weight, minimal to mild fatty changes in liver

* SD, Sprague-Dawley

1. Bromodichloromethane

Tobe et al. (1982) evaluated the chronic effects of bromodichloromethane administered in the diet to male and female Slc:Wistar SPF rats (40/sex/group) for 24 months. The histopathology data for the animals exposed to bromodichloromethane in this study were reported by Aida et al. (1992b). The animals were 5 weeks old at the start of the study and weighed approximately 100 g. Bromodichloromethane was microencapsulated, and an appropriate amount was mixed with powdered feed. The concentrations administered were 0.0 0.014, 0.055, or 0.22%. Control groups (70 rats/sex) received microcapsules without the test compound. Body weight and food consumption were monitored weekly for the first 6 months, every 2 weeks from 6 to 12 months, and every 4 weeks during the second year of the study.

Interim sacrifices of at least 9 animals/sex/control group and 5 animals/sex/dose group were conducted at 6, 12, and 18 months. All surviving animals were sacrificed at 2 years. Necropsies, hematology, and serum biochemistry were conducted at each time of sacrifice. Based on mean food intakes, the reported average doses were approximately 0, 6, 26, or 138 mg/kg-day for males and 0, 8, 32, or 168 mg/kg-day for females (Aida et al., 1992b). Marked suppression of body weight gain was seen in males and females of the high-dose group. Males and females of the high-dose group exhibited mild piloerection and emaciation. Relative liver weight was significantly increased in the mid- and high-dose groups, while relative kidney weight was significantly increased only in the high-dose groups. At 18 months, dose-dependent reductions in serum cholinesterase activity and increases in γ-glutamyl transpeptidase (γ-GTP) activity (indicative of bile duct proliferation) were observed in males, with the changes significant at the high dose. The mid- and high-dose males also displayed a 27% and 65% reduction, respectively, in total serum triglycerides (T-Gly) levels when compared to the control group. At 18 months, serum cholinesterase levels were significantly decreased, while total cholesterol levels were significantly increased, in all dose groups for the treated females. Serum T-Gly levels (decreased) and γ-GTP activity (increased) were also reported to deviate significantly from control values in the mid- and high-dose females. The most sensitive markers at 24 months were T-GLY and serum cholinesterase, with significant changes seen in all of the male treatment groups. Gross necropsy revealed dose-related yellowing and roughening of the liver surface. Treatment-related lesions were limited to the liver. At 24 months, fatty degeneration and granuloma were observed in all dose groups with the exception of granulomas in low-dose females. Specifically, fatty degeneration and granulomas were observed in low-dose males, but not control males, and fatty degeneration was observed in low-dose females at a higher rate (8/19) than in control females (2/32). Cholangiofibrosis was also observed in the high-dose groups. Bile duct proliferation was observed in most high-dose animals at 6 months, and was prevalent in the controls and all dose groups by 24 months. Histopathology was observed in all dose groups as early as 6 months with the exception of low-dose females. Based on the results of Tobe et al. (1982) alone, the NOAEL was 6 mg/kg-day in males and 8 mg/kg-day in females. The LOAEL was identified as 26 (males) to 32 (females) mg/kg-day, based on serum enzyme changes and altered liver appearance. Based on the histopathology data reported for this study by Aida et al. (1992b), however, the entire study identified a LOAEL of 6 mg/kg-day in male rats and 8 mg/kg-day in female rats.

NTP (1987) administered doses of 0, 50, or 100 mg/kg-day of bromodichloromethane in corn oil by gavage to male and female F344/N rats (50/sex/dose), 5 days/week for 102 weeks. The authors observed all animals for clinical signs and recorded body weights (by cage) once per week for the first 12 weeks of the study and once per month thereafter. A necropsy was performed on all animals, including those found dead, unless they were excessively autolyzed or cannibalized. During necropsy, all organs and tissues were examined for grossly visible lesions. Complete histopathology was performed on all female rats and on high-dose and vehicle-control male rats. Male rats in the low-dose group that died early in the study were also examined histologically. Survival of dosed rats was comparable to that of vehicle controls. Mean body weight of high-dose male and female rats was decreased during the last 1.5 years of the study; body weight gain of high-dose male and female rats was 86% and 70% of the corresponding vehicle-control values. Body weight gain of low-dose male and female rats was comparable to

that of the vehicle-control group. No treatment-related clinical signs were observed. In males, treatment-related nonneoplastic effects included renal cytomegaly, tubular cell hyperplasia, hepatic necrosis, and fatty metamorphosis. In females, changes included eosinophilic cytoplasmic change, clear cell change, focal cellular change, fatty metamorphosis of the liver, and tubular cell hyperplasia of the kidney. This study identified a LOAEL of 50 mg/kg-day based on histologic findings in the liver.

NTP (1987) administered bromodichloromethane in corn oil by gavage to male and female $B6C3F_1$ mice (50/sex/dose), 5 days/week for 102 weeks. For males, doses were 0, 25, or 50 mg/kg-day. For females, doses were 0, 75, or 150 mg/kg-day. Final survival of treated male mice was comparable to that of vehicle controls. At week 84, survival of female mice was greater than 50% in all dose groups. After week 84, survival of dosed and vehicle-control female mice was reduced (final survival: 26/50, 13/50, and 15/50 for the 0, 5, and 50 mg/kg-day groups, respectively), and this decreased survival was associated with ovarian abscesses (8/50, 19/47, 18/49). Body weight gain of high-dose male mice was 87% of that of the vehicle-control group; the body weight gain of low-dose male mice was comparable to that of the vehicle-control group. Mean body weight of high-dose female mice was decreased during the last 1.5 years of the study. The body weight gain was reduced 55% compared to the controls at the high dose and by 25% among low-dose females. In males, treatment-related nonneoplastic changes included fatty metamorphosis of the liver, renal cytomegaly, and follicular cell hyperplasia of the thyroid gland. In females, hyperplasia of the thyroid gland was observed. This study identified a LOAEL of 25 mg/kg-day, based on histopathological findings in male mice.

Klinefelter et al. (1995) reported interim (52-week) necropsy data from a cancer bioassay in which male F344 rats were administered average concentrations of 0, 330, or 620 mg/L bromodichloromethane in drinking water. Corresponding doses of 0, 22, and 39 mg/kg-day were calculated by the authors using water consumption and body weight data. For the interim sacrifice, 7 animals per dose group were killed, and the testis, epididymis, liver, spleen, kidney, thyroid, stomach, intestine, and bladder were evaluated histopathologically. Bromodichloromethane had no effect on body weight or on the kidney, liver, spleen, or thyroid weight. There was no histopathological evidence of bromodichloromethane-related noncancer or cancer effects on any of the examined organs. High levels of nephropathy and interstitial cell hyperplasia were observed, but these lesions were not treatment-related. The NOAEL and LOAEL for this study are based on reproductive endpoints. These reproductive effects are summarized in Section V.E.1.

George et al. (2002) exposed male F344/N rats (78 animals/dose) to bromodichloromethane via drinking water for 104 weeks. Nominal concentrations of 0, 0.07, 0.35, or 0.70 g/L were administered in drinking water containing 0.25% Emulphor®. The study authors indicated that testing of higher concentrations was prevented by refusal of the test animals to drink solutions containing more than 0.7 g/L. Six animals per exposure concentration were sacrificed at 13, 26, 52, and 78 weeks for gross observation and histopathological examination of the thyroid, liver, stomach, duodenum, jejunum, ileum, colon, rectum, spleen, kidneys, urinary bladder, and testes. A complete rodent necropsy was performed at terminal

sacrifice (104 weeks) and representative samples of the tissues listed above were examined microscopically. A complete pathological examination was performed on five rats from the high-dose group. Serum profiles of LDH, ALT, ALP, AST, SDH, BUN, total protein, creatine, and total antioxidant activities were determined at 26, 52, and 104 weeks. Hepatocyte and renal tubular cell proliferation were measured at each sacrifice by bromodeoxyuridine labeling.

The measured drinking water concentrations of bromodichloromethane were 0.06, 0.38, and 0.76 g/L. When corrected for loss of bromodichloromethane as a result of volatility, instability, or adsorption to glass surfaces during treatment, the corresponding administered concentrations were 0.06, 0.33, and 0.62 g/L. Based on measured water consumption, these levels correspond to mean daily doses for the entire study of 3.9, 20.6, and 36.3 mg/kg-day as calculated by the study authors. No significant differences were observed among groups for feed consumption or survival. Twenty-one to 22 unscheduled deaths were observed in each treatment group. Mononuclear cell leukemia was seen in all dose groups and was reported to be the primary cause of morbidity and mortality prior to 104 weeks. Exposure to bromodichloromethane did not affect the growth rate of test animals when compared to the control. Kidney weight was significantly depressed at the high dose and a significant negative trend was observed for relative kidney weight. No significant changes were observed in clinical chemistry parameters. Observed nonneoplastic changes in the liver (e.g., biliary fibrosis, bile duct inflammation, and chronic inflammation) were considered to be age-related background changes, since neither the incidence nor severity of the lesions differed from the control values. Bromodichloromethane had no effect on hepatocyte proliferation as measured by bromodeoxyuridine labeling. Renal tubular cell hyperplasia was significantly decreased in the 3.9 mg/kg-day group (-75%) and significantly increased in the 36.3 mg/kg-day group (15.8%) relative to the control value (8.7%). Renal tubule cell proliferation was decreased in the 36.3 mg/kg-day group at 52 and 78 weeks of exposure. These data identify NOAEL and LOAEL values for this study of 20.6 mg/kg-day and 36.6 mg/kg-day based on significant reductions in absolute and relative kidney weight. Tumor data for this study are reported in Section V.G.1.

In a parallel study, George et al. (2002) exposed male B6C3F$_1$ mice (78 animals/dose) to bromodichloromethane via drinking water for 100 weeks. Nominal concentrations of 0.05, 0.25, or 0.50 g/L were administered in drinking water containing 0.25% Emulphor®. The vehicle control solution consisted of 0.25% Emulphor®. Seven animals per exposure concentration were sacrificed at 13, 26, 52, and 78 weeks for gross observation and histopathological examination of the liver, stomach, duodenum, jejunum, ileum, colon, rectum, spleen, kidneys, urinary bladder, and testes. A complete rodent necropsy was performed at terminal sacrifice (100 weeks) and representative samples of the tissues listed above were examined microscopically. A complete pathological examination was performed on five rats from the high-dose group. Serum profiles of LDH, ALT, ALP, AST, SDH, BUN, total protein, creatine, and total antioxidant activities were determined at 26, 52, and 100 weeks. Hepatocyte and renal tubular cell proliferation were measured by bromodeoxyuridine labeling at each sacrifice.

The measured drinking water concentrations of bromodichloromethane were 0.06, 0.30, and 0.55 g/L. When corrected for loss of bromodichloromethane as a result of volatility, instability, or adsorption to glass surfaces during treatment, the corresponding administered

concentrations were 0.06, 0.28, and 0.49 g/L. Based on measured water consumption, these levels correspond to mean daily doses of 8.1, 27.2, and 43.4 mg/kg-day as calculated by the study authors. Water consumption was significantly reduced at the mid- and high doses; the study authors attributed the reduced intake to taste aversion. No significant differences were observed among groups for feed consumption or survival. Exposure to bromodichloromethane did not affect the growth rate of test animals when compared to the control. Absolute and relative kidney weights were significantly depressed at 27.2 and 43.4 mg/kg-day when compared to the control values. No significant changes were observed in clinical chemistry parameters. Mild, treatment-related nonneoplastic hepatic lesions were observed in the 27.2 and 43.4 mg/kg-day dose groups (identity and prevalence not reported). Increased incidences of hepatocellular karyomegaly and necrosis with inflammation (prevalence and severity not reported) were not dose-related. Bromodichloromethane treatment did not alter hepatocyte or renal tubule cell proliferation. The NOAEL and LOAEL for this study are 8.1 and 27.2 mg/kg-day, respectively, based on significantly decreased kidney weight. Tumor data for this study are reported in Section V.G.1.

2. Dibromochloromethane

Tobe et al. (1982) evaluated the chronic effects of dibromochloromethane administered in the diet to male and female Slc:Wistar SPF rats (40/sex/group) at concentrations of 0.0%, 0.022%, 0.088%, or 0.35% for 24 months. The animals were 5 weeks old at the start of the test and weighed approximately 100 g. Dibromochloromethane was microencapsulated, and an appropriate amount was mixed with powdered feed. Control groups (70 rats/sex) received microcapsules without test compound. Body weight and food consumption were monitored weekly for the first 6 months, every 2 weeks from 6 to 12 months, and every 4 weeks during the second year of the study. Data were reported from the sacrifices of 9 animals/sex/control group and 5/sex/dose group at 18 months; all surviving animals were sacrificed at 24 months. Necropsies, hematology, and serum biochemistry were conducted at the time of sacrifice. No histopathology data for dibromochloromethane have been published from this study. Based on reported body weights (150 to 475 g for males and 100 to 215 g for females) and food consumption (15 to 20 g/day for males and 10 to 15 g/day for females), these levels corresponded to doses of approximately 0, 12, 49, and 196 mg/kg-day for males and 0, 17, 70, and 278 mg/kg-day for females. Marked suppression of body weight gain was seen in males and females at the high dose, and mild suppression of body weight gain (about 10%) was seen in males and females at the mid dose. Decreased T-GLY and serum cholinesterase activity and increased γ-GTP were seen in the mid- and high-dose males and females. Yellowing of the liver surface was noted in the mid- and high-dose groups, and roughening of the liver surface was noted in high-dose males. This study suggests NOAELs of 12 mg/kg-day (males) and 17 mg/kg-day (females), and LOAELs of 49 mg/kg-day (males) and 70 mg/kg-day (females), based on serum biochemistry data, decreased body weight, and gross necropsy findings.

NTP (1985) investigated the chronic oral toxicity of dibromochloromethane in male and female F344/N rats. Groups of 50 animals/sex/dose were administered doses of 0, 40, or 80 mg/kg-day by gavage in corn oil for 5 days/week for 104 weeks. Survival was comparable in all dose groups. Body weight gain was decreased in high-dose males after week 20; final weight

gain was 88% of the control value. Females in both dose groups gained more weight than did the controls. Histologic lesions in the liver were observed in males and females at both dose levels. Changes included fat accumulation, "ground glass" appearance of the cytoplasm, and altered basophilic staining. This study identified a LOAEL of 40 mg/kg-day for dibromochloromethane based on liver lesions.

NTP (1985) performed a similar chronic oral exposure study of dibromochloromethane toxicity in male and female B6C3F_1 mice. Groups of 50 animals/sex/dose were administered doses of 0, 50, or 100 mg/kg-day by gavage in corn oil for 5 days/week for 105 weeks. Survival in females was not different from controls, while survival in high-dose males was significantly decreased. An overdosing accident at week 58 killed 35/50 male mice in the low-dose group, and this group was not considered further. Mean body weight was decreased in high-dose males and females, but not in low-dose females. Treatment-related hepatocytomegaly and focal necrosis were observed in livers of high-dose males. Females showed liver calcification at the high dose and fatty metamorphosis at both the low and high doses. An increased incidence of follicular cell hyperplasia in the thyroid was observed in low- and high-dose females relative to the control. Thyroid lesions were not observed in treated males. This study identified a LOAEL of 50 mg/kg-day for dibromochloromethane in mice.

3. Bromoform

Tobe et al. (1982) evaluated the chronic effects of bromoform administered in the diet to male and female Slc:Wistar SPF rats (40/sex/group) for 24 months. The animals were 5 weeks old at the start of the test and weighed approximately 100 g. Bromoform was microencapsulated, and administered at dietary levels of 0.0%, 0.04%, 0.16%, or 0.65%. Control groups (70 rats/sex) received microcapsules without test article. Body weights and food consumption were monitored weekly for the first 6 months, every 2 weeks from 6 to 12 months, and every 4 weeks during the second year of the study. Data were reported from the sacrifices of 9 animals/sex in the control group and 5/sex/dose in the exposure groups at 18 months; all surviving animals were sacrificed at 24 months. At each time of sacrifice, necropsies, hematology, and serum biochemistry were conducted. No histopathology data for bromoform have been published from this study. Based on reported body weights (150 to 475 g for males and 100 to 215 g for females) and food consumption (15 to 20 g/day for males and 10 to 20 g/day for females), these levels corresponded to doses of about 0, 22, 90, and 364 mg/kg-day for males and 0, 38, 152, and 619 mg/kg-day for females. Marked suppression of body weight gain was seen in males and females at the high dose, and mild suppression of body weight gain (about 15%) was seen in males and females at the mid dose. Dose-related decreases in non-esterified fatty acids were observed in all treated males and in females at the mid and high dose. Females also exhibited a dose-related increase in levels of γ-GTP with the increases significant at the mid and high dose. Other serum biochemistry changes in the high-dose groups included decreased serum triglyceride (T-GLY) and increased AST and ALT activity. Specifically, T-GLY levels significantly decreased by 86% and 80% in the male and female high-dose groups, respectively, by study termination. AST and ALT activities at study termination were significantly increased 1.6 to 2.6-fold in animals at the high dose compared to controls, with the exception that the increase in AST activity in males was statistically nonsignificant. Yellowing and small white

spots, and roughening of the surface were seen in the livers of the mid- and high-dose animals. Roughening of the liver surface was observed in the high-dose groups. Based on the necropsy findings and the serum biochemistry data, this study indicated NOAELs of 22 mg/kg-day for males and 38 mg/kg-day for females, and LOAELs of 90 mg/kg-day for males and 152 mg/kg-day for females.

NTP (1989a) exposed male and female F344/N rats (50/sex/group) to bromoform by gavage in oil for 103 weeks (5 days/week) at doses of 0, 100, or 200 mg/kg-day. Animals were observed for clinical signs throughout the study (2 days/week). At termination, necropsy and histopathological examination were performed on all animals. Body weight gain was decreased by 37% in high-dose females and by 29% in high-dose males relative to the respective controls. Survival of the high-dose males was also decreased. Both males and females were lethargic. Hepatic fatty change and chronic inflammation were noted in both males and females at both doses, and minimal necrosis was increased in high-dose males. Nonneoplastic changes were not reported in other tissues. This study identified a LOAEL of 100 mg/kg-day in both male and female rats.

NTP (1989a) exposed groups of 50 male B6C3F_1 mice by gavage in oil to doses of 0, 50, or 100 mg/kg-day of bromoform for 103 weeks (5 days/week). Groups of 50 female mice were administered doses of 0, 100, or 200 mg/kg-day. Animals were observed for clinical signs 2 days/week throughout the study. At termination, all animals were necropsied, and a thorough histological examination of tissues was performed. Decreased survival was observed in females, but not males. This was at least partly due to a utero-ovarian infection. No clinical signs were noted. Body weight gains were 82% and 72% of the control values for low- and high-dose females, respectively, but body weight gain was not affected in males. Increased incidences of minimal to mild fatty changes were noted in the livers of both low- and high-dose females, but not males. Nonneoplastic changes were not detected in other tissues. This study identified a LOAEL of 100 mg/kg-day for female mice, based on decreased body weight and fatty changes of the liver. No NOAEL for females was identified. For males, a NOAEL of 100 mg/kg-day was identified.

E. Reproductive and Developmental Effects

Studies that have examined the reproductive and developmental toxicity of the brominated trihalomethanes are summarized in Table V-9 at the end of this section.

1. Bromodichloromethane

a. Studies in Rats

Ruddick et al. (1983) investigated the teratogenicity and developmental toxicity of bromodichloromethane in Sprague-Dawley rats. Pregnant dams (15/dose group) were administered 0, 50, 100, or 200 mg/kg-day by gavage in corn oil on gestation days (GD) 6 to 15. Body weights were measured on GD 1, on GD 1 through GD 15, and before and after fetuses

were removed by caesarean section on GD 22. On GD 22, females were sacrificed and body tissues (including the uterus) were removed for pathological examination. Females were evaluated for the number of resorption sites, and number of fetuses. Maternal blood samples were collected and evaluated for standard hematology and clinical chemistry parameters. The liver, heart, brain, spleen, and one kidney were weighed. Standard histopathology was conducted on control and high-dose females (5/group). All fetuses were individually weighed, and evaluated for viability and external malformations. Histopathologic examination was performed on two pups per litter. Of the remaining live fetuses, approximately two-thirds were examined for skeletal alterations and one-third for visceral abnormalities.

Although 15 inseminated females per dose group were exposed to bromodichloro-methane, not all females became pregnant and/or delivered litters. Therefore, the number of litters per dose group ranged from 9 to 14. One animal died in the control group, but no deaths occurred in any of the exposed groups. In the high-dose group, maternal weight gain was significantly depressed by 38% as compared with controls. Although maternal weight gains were also reduced in the low- and mid-dose groups (13% and 15%, respectively, as compared with controls), these differences were not reported as statistically significant. Relative maternal liver weight was significantly increased in all exposed groups (110%, 110%, and 117% for the low-, mid-, and high-dose groups, respectively as compared with control values). Relative kidney and brain weights were also statistically increased in the high-dose group only. These increases in relative organ weights may have been associated with the decreased body weight gains in treated females. No treatment-related changes in hematology, clinical chemistry, histopathology, number of resorptions, and the number of fetuses per litter were noted. No differences between treated and control groups were reported for fetal weights, gross malformations (terata), and visceral abnormalities. However, an increase in the incidence of sternebral anomalies was observed in all treated groups. The number of affected fetuses/number of affected litters were 2/2, 8/4, 9/7, 10/6 for the control, low-, mid-, and high-dose groups, respectively. Statistical significance of fetotoxic endpoints was not reported by the study authors. An independent statistical analysis (using the Fisher Exact test) was conducted on the published data for development of this Criteria Document and demonstrated that none of these increases differed significantly from control values ($p>0.05$). A trend test showed a statistically significant dose-related trend ($p=0.03$); stepwise analysis indicated that the trend became nonsignificant if the high-dose (200 mg/kg-day) was omitted from the analysis. These findings suggest that the LOAEL and NOAEL for developmental toxicity are 200 and 100 mg/kg-day, respectively. However, it should be noted that the small sample sizes (the sampling unit is the litter) limited the statistical power of the experiment to detect possible significant differences at lower doses. Based on significantly decreased maternal body weight gain, the LOAEL and NOAEL for maternal toxicity are 200 and 100 mg/kg-day, respectively.

Klinefelter et al. (1995) evaluated the effects of bromodichloromethane exposure on male reproduction during a chronic cancer bioassay study in which F344 rats were administered bromodichloromethane in drinking water at concentrations of 0, 330, or 620 mg/L. The authors estimated the doses to be 0, 22, and 39 mg/kg-day. At 52 weeks, the authors conducted an interim sacrifice, which included an evaluation of epididymal sperm motion parameters and

histopathology of the testes and epididymides. No histologic alterations were observed in any reproductive tissue. Sperm velocities (mean straight-line, average path, and curvilinear), however, were significantly decreased at 39 mg/kg-day. No effect on sperm motility was observed at 22 mg/kg-day. The NOAEL and LOAEL for reproductive effects are thus 22 and 39 mg/kg-day, respectively.

The results for sperm velocity in the study by Klinefelter et al. (1995) are of interest because personal exposure to bromodichloromethane in tap water at home showed a weak but statistically significant inverse association with significantly decreased sperm linearity in an epidemiological study of semen quality (Fenster et al., 2003; see Section VI.B.2.b for summary), suggesting the possibility of similar male reproductive effects in humans and in F344 rats treated at a higher dose than anticipated in human exposures. Treatment-related effects on sperm characteristics were not observed in two other reproductive studies (NTP, 1998; Christian et al., 2002) of Sprague-Dawley rats exposed to bromodichloromethane in the drinking water at concentrations similar to or higher than those used in the Klinefelter et al. (1995) study. However, the differences in outcome may have occurred as a result of the strain tested or differences in methodology. In some male reproductive studies, the use of F344 rats has been associated with considerable variability in endpoints such as epididymal sperm motility (Zenick et al., 1994), although Klinefelter et al. (1995) reported use of techniques designed to reduce this variability. NTP (1998) used a shorter duration of exposure (35 days) that did not span the entire period of spermatogenesis in rats (approximately 52 days) and Christian et al. (2002) did not measure the sensitive sperm motility parameters (mean straight-line, average path, and curvilinear velocities) that were affected in the Klinefelter et al. (1995) study. Neither Christian et al. (2002) nor NTP (1998) observed treatment-related effects on fertility, but fertility is considered to be a less sensitive indicator of male reproductive function than effects on sperm motility.

Narotsky et al. (1997) examined both the developmental toxicity and the effect of dosing vehicle on the developmental toxicity of bromodichloromethane. F344 rats (12 to 14/group) were administered bromodichloromethane by gavage, in either corn oil or an aqueous vehicle containing 10% Emulphor®, at dose levels of 0, 25, 50, or 75 mg/kg-day on GD 6 to 15. Dams were allowed to deliver naturally, and pups were evaluated postnatally. Maternal body weights were assessed on GD 5, 6, 8, 10, 13, and 20, and all rats were observed for clinical signs of toxicity throughout the test period. Postnatal day (PND) 1 was defined as GD 22 irrespective of the actual time of parturition. All pups were examined externally for gross malformations and weighed on PND 1 and 6. Skeletal and visceral anomalies in the pups were not evaluated. Following PND 6 examination, the dams were sacrificed and the number of uterine implantation sites per female was recorded. The uteri of females that did not deliver litters were stained and evaluated histopathologically to detect any cases of full-litter resorption (FLR). In order to compare the kinetics of dosing vehicles, a separate experiment was conducted in which pregnant females (3 to 4 animals per vehicle per time point) were administered a single dose of 75 mg/kg on GD 6 and whole blood samples were collected at 30 minutes, 90 minutes, 4.5 hours, or 24 hours postdosing. Following blood collection, the animals were sacrificed, blood concentrations of bromodichloromethane were measured, and pregnancy status was confirmed at necropsy.

In the developmental toxicity study, one animal that received 75 mg/kg-day in corn oil died before study termination. In the mid- and high-dose groups, clinical signs of toxicity were evident among animals administered bromodichloromethane in either dosing vehicle. At 75 mg/kg-day, kyphosis (humpback) was observed in animals receiving the oil vehicle, and piloerection was observed in animals receiving either vehicle. At 50 mg/kg-day, piloerection was observed in animals receiving the aqueous gavage, and chromodacryorrhea/lacrimation was observed in animals receiving the oil gavage. Maternal weight gain was significantly decreased in all dosed groups receiving the aqueous vehicle and in the 50 and 75 mg/kg-day groups in animals receiving the oil vehicle on GD 6 to 8 (data not reported for other time periods). Although maternal weight gain was also reduced at 25 mg/kg-day in animals given the oil vehicle, this decrease was not statistically significant. However, a two-way analysis of variance (ANOVA) indicated that there was no interaction between vehicle and dose for this maternal endpoint. All control and 25 mg/kg-day litters survived the test period; however, FLR was observed at 50 and 75 mg/kg-day with both dosing vehicles. Statistical analysis (ANOVA) of FLR incidence showed a significant vehicle-dose interaction. For females receiving bromodichloromethane in corn oil, FLR was reported in 8 and 83% of the litters at 50 and 75 mg/kg-day, respectively; an additional high-dose litter was carried to term but was delivered late (GD 23), and all pups died by PND 6. For females receiving the aqueous vehicle, FLR was observed in 17 and 21% of the litters at 50 and 75 mg/kg-day, respectively. There were no effects on gestation length, pre- or postnatal survival, or pup morphology in surviving litters, with the exception noted above in the 75 mg/kg-day oil vehicle group. Based on full litter resorption, the LOAEL for developmental toxicity is 50 mg/kg-day for both vehicles, and the corresponding NOAEL is 25 mg/kg-day. Based on significantly reduced body weight gain during GD 6 to 8 in dams receiving the aqueous vehicle, the LOAEL for maternal toxicity is the lowest dose tested, 25 mg/kg-day, and a NOAEL could not be determined.

Analysis of bromodichloromethane concentrations in blood indicated that circulating levels decreased over time with both vehicles, but tended to be higher following corn oil administration. Bromodichloromethane blood concentrations were thus vehicle-dependent and differed statistically at both 4.5 and 24 hours postdosing (mean of 3.1 ng/mL versus 0.4 ng/mL for oil and aqueous vehicles, respectively, at 24 hours). The elimination half-life of bromodichloromethane was estimated to be 3.6 hours when administered in corn oil and 2.7 hours when given in the aqueous vehicle.

Narotsky et al. (1997) also calculated both an ED_{05} (i.e., the effective dose producing a 5% increase in response rate above background) and a benchmark dose (BMD; as defined by the authors, the BMD is the lower confidence interval of the ED_{05}) for each vehicle. For the corn oil vehicle, the ED_{05} and BMD were 48.4 and 39.3 mg/kg-day, respectively. For the aqueous vehicle, the ED_{05} and BMD were 33.3 and 11.3 mg/kg-day, respectively. The study authors noted that the greater BMD value for the corn oil vehicle seemed counterintuitive in view of the higher FLR response rate in the 75 mg/kg-day aqueous vehicle group (83% for aqueous vehicle versus 21% for corn oil vehicle). However, the dose response for bromodichloromethane-induced FLR differed markedly between vehicles, and the response rate in the 50 mg/kg-day corn oil vehicle group (8%) closely approximated 5%, the effect level defined by the ED_{05}.

According to the study authors, this resulted in a smaller confidence interval around the ED_{05} for the corn oil vehicle, yielding a less conservative (i.e., higher) BMD. These findings are consistent with the pharmacokinetic data demonstrating a slower elimination of bromodichloromethane following a single dose of 75 mg/kg in corn oil as compared with the same dose in aqueous vehicle, and suggest that the influence of vehicle on FLR rate is dose-dependent.

NTP (1998) conducted a short-term reproductive and developmental toxicity screen in Sprague-Dawley rats to evaluate the effects of bromodichloromethane administered in drinking water. The study was designed to identify the physiological endpoints most sensitive to bromodichloromethane exposure, and assessed development, female reproduction, male reproduction, hematology, clinical chemistry, and pathology. In males, the reproductive endpoints evaluated included testis and epididymis weight, sperm morphology, density and motility. The female reproductive parameters evaluated included mating index, pregnancy index, fertility index, gestation index, number of live births, number of resorptions, implants per litter, corpora lutea and pre-and post- implantation loss. Concentrations of 0, 100, 700 and 1300 ppm bromodichloromethane were selected for use in this study based on decreased water consumption observed in a preliminary 14-day range-finding study (see Section V.B.1). Two groups of male Sprague-Dawley rats and three groups of female Sprague-Dawley rats were assigned to treatment groups as indicated in Table V-6.

Table V-6 NTP (1998) Study Design

Gender	Group	Description	# Animals per Dose Group			
			0 ppm*	100 ppm	700 ppm	1300 ppm
Male	A	non-BrdU treated	10	10	10	10
	B	BrdU treated	5	5	5	8
Female	A	peri-conception exposure	10	10	10	10
	B	gestational exposure	13	13	13	13
	C	BrdU treated, peri-conception exposure	5	5	5	8

* Control animals received deionized water

Test animals were dosed for 25 to 30 days, with the exception of Group B females which were dosed from GD 6 to evidence of littering/birth (total duration approximately 15 to 16 days). Based on measured water consumption, the nominal concentrations of 0, 100, 700 and 1300 ppm were equivalent to doses of 0, 8, 41, and 68 mg bromodichloromethane/kg-day for all male rats and 0, 14, 72 and 116 mg bromodichloromethane/kg-day for all female rats in groups A and C. The calculated doses for Group B females were 0, 13, 54, and 90 mg/kg-day. All animals

survived the treatment period, with the exception of one Group A male in the 700 ppm dose group. Body weight and food and water consumption were decreased at many time points for animals dosed with 700 and 1300 ppm bromodichloromethane. Body weights in the dosed groups were decreased from 5% to 13%, food consumption was decreased from 14% to 53%, and water consumption was decreased from 7% to 86% relative to control animals. However, bromodichloromethane exposure did not alter any reproductive parameter investigated in males or females, with the exception of a non-dose-related increase in the number of live fetuses per birth at the 100 ppm concentration in Group C females, and a slight decrease in the number of corpora lutea at the 700 ppm concentration in Group A females. On the basis of these results, NTP (1998) concluded that bromodichloromethane was not a short-term developmental or reproductive toxicant any of the doses tested in the study. The reproductive/developmental NOAELs are 68 and 116 mg/kg-day for male and female rats, respectively. The adult NOAEL and LOAEL for this study were identified on the basis of hepatic effects, which are discussed in detail in Section V.B.1.

Bielmeier et al. (2001) conducted a series of experiments to investigate the mode of action for bromodichloromethane-induced full litter resorption (FLR) in F344 rats. This series of experiments included a strain comparison of F344 and Sprague-Dawley (SD) rats, a critical period study, and two hormone profile studies. The strain comparison and critical period studies are summarized in Table V-7 and discussed below. The hormone profile studies and related follow-on studies reported by Bielmeier et al. (2004) are discussed in Section V.H.2 (Hormonal Disruption).

In the strain comparison experiment, female SD rats (13 to 14/dose group) were dosed with 0, 75, or 10 mg/kg-day by aqueous gavage in 10% Emulphor® on GD 6 to 10. F344 rats (12 to 14/dose group) were concurrently dosed with 0 or 75 mg/kg-day administered in the same vehicle. The incidence of FLR in the bromodichloromethane-treated F344 rats was 62%, while the incidence of FLR in SD rats treated with 75 or 100 mg/kg-day of bromodichloromethane was 0%. Both strains of rats showed similar signs of maternal toxicity, and the percent body weight loss after the first day of dosing was comparable for SD rats (no resorption observed) and the F344 rats that resorbed their litters. F344 rats that maintained their pregnancies generally did not lose weight after the first dose, although they did experience significantly less weight gain than the controls. Both strains of rats had similar incidences of piloerection. However, the strains showed different ocular responses to compound administration. One half (7/14) of the treated F344 rats showed lacrimation and/or excessive blinking shortly after dosing during the first two days of compound administration. In comparison, only 1/28 of the SD rats exhibited this response. The study authors reported that lacrimation was not predictive of FLR in F344 rats. The rats were allowed to deliver and pups were examined on postnatal days 1 and 6. Surviving litters appeared normal and no effect on post-natal survival, litter size, or pup weight was observed.

Table V-7 Summary of Experiments Conducted by Bielmeier et al. (2001)[a]

Study/Strain	Dose (mg/kg-day)	Treatment Period	Number of animals			% FLR
			Treated	Pregnant	Resorbed	
Strain Comparison						
F344	0	GD 6-10	12	11	0	0
F344	75	GD 6-10	14	13	8	62**
SD	0	GD 6-10	13	13	0	0
SD	75	GD 6-10	14	14	0	0
SD	100	GD 6-10	14	14	0	0
Critical Study Period						
F344	0	GD 6-15	8	8	0	0
F344	75	GD 6-15	10	10	5	50*
F344	75	GD 6-10	12	12	9	75**
F344	75	GD 11-15	13	13	0	0

Source: Table 1 in Bielmeier et al. (2001)
Abbreviations: GD, gestation day; FLR, full litter resorption; SD, Sprague-Dawley
[a] Additional experiments to characterize profiles for selected hormones are discussed in section V.H.2
* $p<0.05$; ** $p<0.01$; *** $p<0.001$for significant differences from controls (Fisher's Exact Test)

Bielmeier et al. (2001) conducted a second experiment to identify the critical period for bromodichloromethane-induced FLR in F344 rats. Two different five day periods during organogenesis were compared. Pregnant rats (12 to 13/dose group) were dosed with 75 mg/kg-day by gavage in 10% Emulphor® on GD 6 to 10 (which includes the luteinizing hormone-dependent period of pregnancy) or GD 11 to 15 (a luteinizing hormone-independent period). Rats (8 to 10/dose group) dosed with 0 or 75 mg/kg-day on GD 6 to 15 served as negative and positive controls, respectively. FLR occurred only in rats treated on GD 6 to 10 or GD 6 to 15 (incidences of 75% and 50%, respectively). In contrast, all rats treated with bromodichloromethane on GD 11 to 15 maintained their litters. Surviving litters appeared normal and no effect on post-natal survival, litter size, or pup weight was observed. This finding was interpreted by the study authors as evidence for an effect of bromodichloromethane on luteinizing hormone secretion or signal transduction.

The experiments conducted by Bielmeier et al. (2001) identified a LOAEL of 75 mg/kg-day (the lowest dose tested) based on FLR in F344 rats. A NOAEL was not identified.

The Chlorine Chemistry Council sponsored a range finding reproductive toxicity study of bromodichloromethane in rats (CCC, 2000c), which was conducted according to standard U.S. EPA test guidelines (U.S. EPA, 1998c) and GLP standards. This study is summarized in Christian (2001b). Male and female Sprague Dawley rats (10/sex/group) were randomly assigned to five exposure groups. Additional rats (6 males/group and 15 females/group) were assigned to satellite groups for collection of samples for analysis of bromodichloromethane concentrations in selected tissues and fluids (see Section III.B). Bromodichloromethane was administered to parental rats (P generation) in drinking water at concentrations of 0, 50, 150, 450, or 1350 ppm. Exposure began 14 days before cohabitation and continued until the day of sacrifice. Female estrous cycle evaluations were performed daily, beginning 14 days before exposure initiation and continuing for 14 days after the first day of exposure. Clinical observations were recorded daily during the exposure period.

Male body weights were recorded weekly during the entire exposure period and at sacrifice; female body weights were recorded weekly during precohabitation and cohabitation, on GD 0, 7, 14, 21, and 25, and on lactation days (LD) 1, 5, 8, 11, 15, 22, and 29. Lactation was extended for one week (LD 22-29) beyond the normal 3-week period because F_1 pup body weights in the three highest dose groups were significantly reduced on LD 21 relative to control values (results are described below). Water and feed consumption were recorded weekly and at sacrifice for males during the entire exposure period (except for feed consumption during cohabitation), and more frequently for females during gestation and lactation. On LD 29, two F_1 pups per sex were selected from each litter for an additional week of postweaning observation, provided *ad libitum* access to water containing the same concentration of bromodichloromethane administered to their parents (P generation), and sacrificed on Day 8 postweaning. P generation female rats were assessed for duration of gestation, fertility index, gestation index, number and sex of offspring per litter, number of implantation sites, and clinical signs of toxicity during the postpartum period. During lactation, maternal behavior was observed and recorded on LD 1, 5, 8, 11, 22, and 29. Litters were externally examined following delivery to identify the number and sex of pups, stillbirths and live births, and gross external malformations. Litters were observed at least twice daily during the preweaning and postweaning period for pup deaths and clinical signs of toxicity. Litter size and viability, viability indices, lactation indices, percent survival, and sex ratios were calculated. During the postweaning period of observation, body weights and feed consumption were recorded at weaning and on day 8 postweaning; water consumption was recorded daily.

At the end of the parental exposure periods (64 days for males and a maximum of 74 days for females), all P generation rats were sacrificed and a gross necropsy of the thoracic, abdominal, and pelvic viscera was performed. In addition, testes and epididymides were excised from males and paired organ weights were measured. F_1 pups exposed to bromodichloromethane in their drinking water for one week following weaning were sacrificed on Day 8 postweaning and examined for gross lesions. No histopathology was performed on either the P or F_1 generation.

The consumption of bromodichloromethane was calculated from measured water intake and measured concentrations of the test article. Mean consumed dosages of bromodichloromethane for P generation male rats during the entire exposure period, P generation female rats during different physiologic stages, and F_1 postweaning rats are summarized in Table V-8. Males and nonpregnant female rats tended to consume similar amounts of bromodichloromethane. Progressively higher dosages were consumed by female rats in the premating, gestation, and lactation periods, respectively. The highest dosages among all groups were consumed by F_1 female rats during the one-week postweaning observation period. A possible source of error in the estimates for lactating females was consumption of the dams' drinking water by their pups.

In the P generation, all male rats and all females except one survived to scheduled sacrifice. Exposure-dependent reductions in both absolute (g/day) and relative (g/kg body weight-day) water consumption were observed in all rats of both sexes and were attributed to taste aversion. Reduced water consumption was most pronounced during the first week of exposure, and was evident during precohabitation and cohabitation in both sexes, and during post-cohabitation in males and gestation in females. However, the decrease in water consumption during these times was not as severe as that observed during the first week of exposure. Decreased water consumption was not clearly noted in females during lactation, presumably reflecting the physiologic demands for high fluid consumption during this period. Exposure-related decreases in feed consumption were noted for males and females in the 150, 450, and 1350 ppm exposure groups, and persisted in the 450 and 1350 ppm females during gestation and lactation. Treatment-related clinical signs of toxicity were observed in both sexes in the 1350 ppm exposure groups and were considered to be generally associated with reduced water consumption. Males exhibited dehydration, emaciation, chromorhinorrhea, and chromodacryorrhea during the pre-mating, cohabitation and post-cohabitation periods; however, the most severe symptoms resolved within the first 17 days of exposure. Among females, urine-stained fur was observed in one or more animals in the three highest dose groups during lactation and was considered to be treatment-related. Reductions in mean body weight gain and body weight were observed in male rats in the 450 and 1350 ppm exposure groups relative to controls. These effects were most severe during the first week of exposure. Mean body weight gains for the 450 ppm and 1350 ppm male groups over the entire exposure period were 91.3% and 76.3% of the control values, respectively. At study termination, mean male body weights were 96.5% and 91.6 % for the 450 ppm and 1350 ppm, respectively, relative to control values. In female rats, reductions in body weight gain and body weight occurred in 150, 450, and 1350 ppm groups. These effects were most severe during the first week of exposure, but also persisted throughout gestation and lactation. During gestation, the mean reductions in female body weight in the 150, 450, and 1350 ppm groups were 95.8%, 95.3%, and 85.3% of the control values, respectively. Mean body weights for the entire lactation period were not presented in the study report; however, inspection of the data, presented separately for LD 1, 8, 15, 22, and 29, indicated that female body weights were decreased relative to controls in a dose-dependent manner in the three highest dose groups at all time points.

Table V-8 Mean Consumed Doses (mg/kg-day) of Bromodichloromethane in the Range Finding Study Conducted by CCC (2000c) and Summarized in Christian et al. (2001b)

Gen.	Sex	Exposure Interval	0 ppm	50 ppm	150 ppm	450 ppm	1350 ppm
P	M	Full study Study Days 1-64	0.0	4.2 ± 0.4	11.8 ± 1.8	27.5 ± 3.4	67.2 ± 5.6
P	F	Pre-mating Study days 1-15	0.0	4.7 ± 0.8	13.3 ± 2.0	23.5 ± 5.3	70.8 ± 1.8
P	F	Gestation days 0-21	0.0	5.4 ± 0.7	16.3 ± 2.2	41.7 ± 6.4	111.7 ± 6.2
P	F	Lactation days 1-15	0.0	11.0 ± 1.9	31.4 ± 2.6	90.3 ± 7.3	222.4 ± 19.9
F_1	M	Postweaning days 1-8	0.0	13.6 ± 3.5	41.4 ± 7.1	106.9 ± 20.8	297.8 ± 113.8
F_1	F	Postweaning days 1-8	0.0	13.9 ± 2.6	40.1 ± 6.8	117.9 ± 42.7	333.6 ± 110.6

No gross lesions attributable to bromodichloromethane were observed in the P generation male or female rats at necropsy. The absolute paired epididymal weights were slightly reduced (93.2% and 92.5%, respectively) in the 450 and 1350 ppm exposure groups. However, relative paired epididymal weights were unaffected, suggesting that the decreased absolute values were associated with the reduced terminal body weights in these groups. Absolute and relative testes weights were not altered by exposure to bromodichloromethane. No effects of bromodichloromethane were observed on any of the measured reproductive parameters in P generation male or female rats. However, bromodichloromethane exposure was associated with a concentration-dependent reduction in F_1 pup body weights in the 150, 450, and 1350 ppm exposure groups. Pup weights were reported for postpartum days 1, 5, 8, 15, 22, and 29. The mean litter pup weights in treated groups were comparable to the mean litter pup weight of the control group on LD 1. Beginning on LD 5, reductions in mean pup weights in the three highest dose groups increased with increasing dose and duration of the postpartum period. On LD 29, pup weights averaged 7, 12, and 29% less than controls in the 150, 450, and 1350 ppm exposure groups, respectively. Reduced body weight gain continued to occur in the F_1 pups administered parental concentrations of bromodichloromethane in drinking water for one week postweaning. No reductions in either body weight gain or body weight were observed in F_1 pup litters in the 50 ppm group during lactation or the one-week postweaning period.

Statistical analysis was not conducted in this range finding study. Based on decreased pup weight and pup weight gain, the LOAEL for developmental toxicity is 150 ppm, and the corresponding NOAEL is 50 ppm. Although the effect of reduced water consumption on the decreases in feed consumption, body weight gain, and body weight observed in the P generation adults is unclear, the LOAEL for parental toxicity is considered to be 150 ppm and the NOAEL is 50 ppm. Due to the marked changes in drinking water consumption by P generation female rats during different physiological stages (pre-mating, mating, gestation, and lactation), it is not

possible to convert the administered drinking water concentrations into biologically meaningful average daily doses.

The Chlorine Chemistry Council sponsored a developmental toxicity study of bromodichloromethane in rats (CCC, 2000d). Data from this study are summarized in Christian et al. (2001a). This study was conducted in accordance with U.S. EPA Health Effects Test Guidelines OPPTS 870.3700: Prenatal Developmental Toxicity Study (U.S. EPA, 1998c) and U.S. EPA Good Laboratory Practice Standards (40 CFR Part 160/792). Female Sprague-Dawley rats (25/exposure group) were exposed to bromodichloromethane in the drinking water at concentrations of 0, 50, 150, 450, and 900 ppm on days 6 to 21 of gestation (GD 6 to 21). The rats were examined daily during the exposure period for clinical signs related to exposure, abortions, premature deliveries and deaths. Body weights, water consumption, and feed consumption were recorded at intervals throughout the exposure period. All study animals were sacrificed on GD 21 and caesarean-sectioned. A gross necropsy of the thoracic, abdominal, and pelvic viscera was performed. Data was collected for gravid uterus weight (with cervix), number of corpora lutea/per ovary, evidence of pregnancy, number and distribution of implantation sites, live and dead fetuses, early and late resorption, and placental abnormalities (size, color, or shape). Individual fetuses were weighed, sexed, and examined for gross external abnormalities. Approximately one-half of the fetuses in each litter were examined for soft tissue alterations and the heads of these fetuses were examined by free-hand sectioning. The remaining fetuses in each litter were examined for skeletal alterations.

Consumed dosages for GD 6 to 21 were calculated from measured water consumption and measured body weights and averaged 0, 2.2, 18.4, 45.0, and 82.0 mg/kg-day, respectively. No abortions, premature deliveries, deaths or treatment-related clinical signs were observed during the study and all rats survived until scheduled sacrifice. No treatment-related gross lesions were identified at autopsy. Exposure-related decreases in maternal body weight gains occurred in all groups administered bromodichloromethane in the drinking water on the first day of exposure (GD 6 to 7). The reduction in maternal body weight gain reached statistical significance in the 150, 450, and 900 ppm groups. The effect was most severe on these days and appeared to be related to taste aversion. The effect on maternal body weight gain was persistent in the 450 and 900 ppm exposure groups. In contrast, the effect was transient in the 50 and 150 ppm exposure groups. Average body weights were significantly reduced in the 450 and 900 ppm exposure groups on GD 7 to 21. Average maternal body weights in the same groups were significantly reduced at terminal sacrifice when corrected for gravid uterine weight.

Statistically significant, exposure-related decreases in absolute (g/day) and relative (g/kg-day) water consumption were observed in all groups exposed to bromodichloromethane. This effect was evident for the entire exposure period (GD 6 to 21) and the entire gestation period (GD 0 to 21). Within the exposure period, the effects were most pronounced on the first two days of exposure and gradually decreased in severity with continued exposure. Exposure-related decreases in absolute and relative feed consumption were observed in the 150, 450, and 900 ppm groups. In the 150 ppm group, the effects were statistically significant only on GD 12 to 15 and thus were considered to be of little biological importance by the study authors. In the 450 ppm

and 900 ppm groups, absolute and relative feed consumption was significantly reduced for the entire exposure period (GD 6 to 21), the entire gestation period (GD 0 to 21), and at many intervals within the exposure period. The effect of bromodichloromethane on feed consumption tended to be most severe during the first two days of compound administration.

Caesarean section and litter parameters were unaffected by exposure of the dams to bromodichloromethane concentrations up to 900 ppm. Litter averages for corpora lutea, implantations, litter sizes, proportion of live fetuses, early or late resorptions, fetal body weights, percent reabsorbed conceptuses, and percent live fetuses were comparable among all study groups and no significant differences were observed. No cases of full litter resorption were observed and there were no dead fetuses. Late resorption occurred in one control group litter. All placentae appeared normal. All values for the examined litter parameters were within the historical range of the test facility (Argus Research Laboratories, Horsham, PA) or litter incidences of any gross external or soft tissue alterations. With respect to skeletal alterations, no skeletal malformations were observed in any fetus. The only statistically significant ($p<0.01$) changes in the occurrence of skeletal variations were reversible delays in ossification. These included an increased fetal incidence (fetal incidence: 0 ppm, 1/182; 50 ppm, 0/199; 150 ppm, 0/200; 450 ppm, 0/188; 900 ppm, 4/194; litter incidence: 0 ppm, 1/23; 50 ppm, 0/25; 150 ppm, 0/25; 450 ppm, 0/25; 900 ppm, 2/25) of wavy ribs in the 900 ppm exposure group and a decreased number of ossification sites per fetus per litter for the forelimb phalanges (Mean number ± SD of ossification sites: 8.14 ± 0.91, 8.30 ± 0.65, 8.09 ± 0.63, 7.92 ± 0.78, 7.46 ± 0.78) and the hindlimb metatarsals (Mean number ± SD of ossification sites: 4.81 ± 0.25, 4.86 ± 0.23, 4.78 ± 0.27, 4.71 ± 0.28, 4.53 ± 0.33) and phalanges (Mean number ± SD of ossification sites: 6.20 ± 1.19, 6.20 ± 1.17, 5.84 ± 0.94, 5.86 ± 0.79, 5.29 ± 0.54). The increased fetal incidence of wavy ribs was considered unrelated to bromodichloromethane exposure by the study authors because the litter incidence (the more relevant measure of effect) did not differ significantly from the control and was within the historical range for this alteration at the test facility.

The concentration-based maternal NOAEL and LOAEL for this study were 150 ppm and 450 ppm, respectively, based on statistically significant, persistent reductions in maternal body weight and body weight gains. Based on the mean consumed dosage of bromodichloromethane, these concentrations correspond to doses of 18.4 mg/kg-day and 45.0 mg/kg-day, respectively. The concentration-based developmental NOAEL and LOAEL were 450 ppm and 900 ppm, respectively, based on a significantly decreased number of ossification sites per fetus for the forelimb phalanges and the hindlimb metatarsals and phalanges. These concentrations correspond to mean consumed doses of 45.0 mg/kg-day and 82.0 mg/kg-day, respectively.

Bielmeier et al. (2004) conducted a series of hormone profile and hormone replacement experiments to elucidate the mode of action for full litter resorption in F344 rats. These studies are discussed in section V.H.2 (Hormonal Disruption).

b. Studies in Rabbits

The Chlorine Chemistry council sponsored a range-finding developmental toxicity study in New Zealand White rabbits (CCC, 2000a). The data from this study have been summarized in Christian et al. (2001b). This study was conducted in accordance with U.S. EPA Health Effects Test Guidelines OPPTS 870.3700: Prenatal Developmental Toxicity Study (U.S. EPA, 1998c) and U.S. EPA Good Laboratory Practice Standards (40 CFR Part 160/792). Bromodichloromethane was provided to New Zealand White presumed pregnant rabbits (5/group) in the drinking water at concentrations of 0, 50, 150, 450, and 1350 ppm on GD 6 to 29. Additional rabbits (4/group) were similarly assigned to satellite treatment groups for use in the collection of samples for analysis of tissue concentrations of bromodichloromethane (discussed in Section III.B). Body weights were recorded on GDs 0 and 4, daily during the exposure period, and on the day of sacrifice. Feed and water consumption data were recorded daily. The rabbits were sacrificed on GD 29 and gross necropsy of the thoracic, pelvic, and abdominal viscera were performed. The gravid uterus was excised and weighed. Examinations were made for number and distribution of corpora lutea, implantation sites, early and late resorptions, and live and dead fetuses. Each fetus was examined for gross external alterations and sex (by internal examination).

The mean consumed daily doses of bromodichloromethane for GDs 6 to 29 were 0.0, 4.9, 13.9, 32.3, and 76.3 mg/kg-day, as determined from measured body weights and measured water consumption. Absolute (g/day) and relative (g/kg-day) maternal water intake for the exposure period was decreased in each group administered bromodichloromethane. The relative consumption values were 92%, 87%, 67%, and 53% of the control group value, respectively. Absolute and relative feed consumption values were reduced in a time (onset of reductions delayed in the 50 and 150 ppm exposure groups) and exposure-dependent manner. The relative values for feed consumption were 96%, 96%, 90%, and 82% of the control group value for the exposure period. No deaths, abortions, or premature deliveries occurred during the study. No treatment-related clinical signs or gross lesions were observed. Maternal body weight gains for the exposure period were 82%, 80%, 73%, and 50%, respectively, relative to the controls. The study authors questioned whether these reductions were associated with bromodichloromethane exposure since similar changes did not occur in the satellite exposure group, and suggested that the reduced body weight gains were artifacts of the small sample size used in the study. When body weights were corrected for gravid uterus weight, all exposed groups in the main study experienced body weight loss while body weight gain occurred in the control group. Absolute uterine weights were reduced in the 450 and 1350 ppm groups. This finding was most likely associated with reduced body weight in these groups, since relative gravid uterine weights in all dosed groups were similar to that of the control.

Litter averages for corpora lutea, implantations, litter sizes, live and dead fetuses, early and late resorptions, percent dead or resorbed conceptuses, fetal body weights, and percent live male fetuses were comparable for the control and all exposure groups and within the historical ranges for the test facility (Argus Laboratories, Horsham, PA). All placentas were normal in appearance. No gross external fetal alterations were observed in the control or treatment groups.

In the satellite study (described in Section III.B), analytical analyses detected trace amounts of bromodichloromethane in placental samples from two litters in the 1350 ppm group and in one fetus from the 1350 ppm group. Bromodichloromethane was not detected in amniotic fluid or maternal plasma. One litter in the 450 ppm satellite exposure group consisted of only early resorptions. The concentration-based LOAEL for maternal toxicity in this study is 50 ppm, the lowest concentration tested, based on reduced body weight gain. This concentration corresponds to a mean daily dose of approximately 4.9 mg/kg-day. The concentration-based NOAEL for developmental effects was 1350 ppm (the highest dose tested). This corresponds to a mean daily intake of approximately 76.3 mg/kg-day.

The Chlorine Chemistry Council (CCC, 2000b) sponsored a developmental toxicity study in New Zealand White rabbits. Data from this study were summarized in Christian et al. (2001a). Bromodichloromethane was provided to pregnant rabbits (25/dose group) at concentrations of 0, 15, 150, 450, and 900 ppm in the drinking water on GD 6-29. Consumed doses were calculated from measured water intake and measured body weights and averaged 0, 1.4, 13.4, 35.6, and 55.3 mg/kg-day, respectively, over the 14 day treatment period. Feed consumption, water intake, and body weight were monitored daily during the exposure period. The rabbits were sacrificed on GD 29 and examined for gross lesions of the thoracic, abdominal, and pelvic viscera. Uterine weight, number of implantation sites, uterine contents, and number of corpora lutea were recorded. Each fetus was examined for weight, gross external alterations, skeletal alterations, and sex. Visceral alterations and cavitated organs were evaluated by dissection. One rabbit in the 900 ppm dose group was sacrificed moribund with hindlimb paralysis caused by a back injury. Another rabbit in the 900 ppm exposure group had a dead litter as a result of a non-treatment related uterine abnormality. No treatment-related clinical signs or necropsy results were observed. The 450 and 900 ppm exposure groups had significantly reduced feed and water consumption rates throughout the exposure period. These groups also had significantly reduced body weight gains and corrected (for weight of gravid uterus) body weight gains for both the bromodichloromethane exposure period (GD 6 to 29) and the entire gestation period (GD 0 to 29). Bromodichloromethane had no observable effect on implantations, corpora lutea, live litter size, early or late resorptions, percentage of male fetuses, percentage of resorbed conceptuses, or fetal body weight. The number of litters with any alteration, the number of fetuses with any alteration, the average percentage of fetuses with any alteration did not differ significantly from the control. Although statistically significant increases in the number of fused sterna centra were observed in the 150 and 450 ppm groups, this effect was not dose-related and the observed incidences were within the historical range for the testing facility. Litter averages for ossification sites per fetus did not differ significantly from the control and were within historical range for the testing facility. The NOAEL and LOAEL identified for maternal toxicity in this study were 13.4 mg/kg-day (150 ppm) and 35.6 mg/kg-day (450 ppm), respectively, based on decreased body weight gain. The developmental NOAEL was 55.3 mg/kg-day (900 ppm) based on absence of statistically significant, dose-related effects at any tested concentration.

Christian et al. (2002) summarized the results of a two-generation reproductive toxicity study on bromodichloromethane conducted in Sprague-Dawley rats. The study was sponsored

by the Chlorine Chemistry Council (CCC, 2002) and was conducted in accordance with U.S. EPA Health Effects Test Guideline OPPTS 870.3800: Reproduction and Fertility Effects (U.S. EPA, 1998b) and U.S. EPA Good Laboratory Practice Standards (40 CFR Part 160/792). Bromodichloromethane was continuously provided to test animals in the drinking water at concentrations of 0, 50, 150, or 450 ppm. Drinking water solutions were prepared at least once weekly and precautions were taken to prevent contamination of the solutions by extraneous sources of chlorine. Concentrations were verified analytically at the beginning and end of each exposure period. The tested concentrations were selected on the basis of results obtained in the developmental toxicity screening study conducted by NTP (1998) and data obtained in a range-finding study (CCC, 2000c; Christian et al., 2001b). Exposure of the parental generation (30 rats/sex/concentration) was initiated when the test animals were approximately 43 days of age and continued through a 70-day pre-mating period and a cohabitation period of up to 14 days. Parental generation males were exposed for approximately 106 days prior to sacrifice. Exposure of parental generation female rats continued through gestation and lactation for a total exposure period of approximately 118 days. F_1 generation rats were exposed to bromodichloromethane in utero and by consumption of the dam's drinking water during the lactation period. At weaning, F_1 rats (30/sex/concentration) were selected for a postweaning/premating exposure period of at least 64 days, followed by a cohabitation period of up to 14 days. Exposure continued through gestation and lactation. F_1 generation females delivered litters and the F_2 litters were sacrificed on lactation day 22.

During the course of the experiment, parental and F_1 generation rats were evaluated for viability, clinical signs, water and feed consumption, and body weight. Parental and F_1 generation females were evaluated for estrous cycling (premating and during cohabitation until mating confirmed and at sacrifice), abortions, premature deliveries, duration of gestation, gestation index, fertility index, number and sex of offspring per litter, general postpartum condition of dam and litter, litter size, viability index, lactation index, percent survival, sex ratio, and maternal behavior. Litters were examined for number and sex of pups, stillbirths, live births, and gross external alterations. F_1 rats selected for continued evaluation were assessed for age at vaginal patency or preputial separation. At sacrifice, test animals were examined for gross pathology, organ weights, and histopathology (control and high-dose groups, 10 parental animals/sex; reproductive organs of 50 and 150 ppm rats suspected of reduced fertility). Male rats were evaluated for sperm concentration, percent motile sperm, sperm morphology, total number of sperm, and testicular spermatid counts. Females were evaluated for number and distribution of implantation sites. F_1 weanlings not selected for continued evaluation (3 pups/sex/litter, when available) and all F_2 weanling rats were evaluated for gross lesions, terminal body weight, and organ weights.

Key findings in the two-generation study reported by CCC (2002) and Christian et al. (2002) include the following. The bromodichloromethane dose-equivalent for each drinking water concentration varied by sex and reproductive status. Average daily doses estimated for the 50, 150 and 450 ppm concentrations were 4.1 to 12.6, 11.6 to 40.2, and 29.5 to 109 mg/kg-day, respectively, as calculated by the study authors. One death in the 150 ppm group and three deaths (including one humane sacrifice) in the 450 ppm group were associated with reduced

water consumption, weight loss and/or adverse clinical signs and may have been compound-related. Adverse clinical signs occurred in parental generation female rats and F_1 male and female rats in the 150 and 450 ppm exposure groups. Compound-related signs included chromorhinorrhea, pale extremities, urine-stained abdominal fur, and coldness to touch. The study authors attributed these signs to reduced water consumption.

Body weight and body weight gain were significantly reduced in the 450 ppm parental generation males and females and 150 and 450 ppm F_1 generation males and females. The significantly reduced final body weight in 450 ppm parental generation females was associated with decreased absolute organ weights and increased relative organ weights when expressed as a percentage of body or brain weight. Absolute and relative water consumption rates were significantly reduced in parental and F_1 generation males and females at all concentrations of bromodichloromethane. Water intake by parental and F_1 animals was generally reduced by 10 to 20 percent in the 150 and 450 ppm groups when compared to the controls. Absolute and relative feed consumption rates were reduced in males and females of both generations at 150 and 450 ppm when compared with the controls. There were no gross pathological or histopathological indications of compound-related toxicity.

Most indicators of reproductive or developmental toxicity examined by Christian et al. (2002) were not significantly affected by bromodichloromethane treatment. However, F_1 and F_2 generation pup body weights were reduced in the 150 and 450 ppm groups during the lactation period after the pups began to drink the water provided to the dams. The F_1 generation had statistically significant reductions in pup body weight at weaning on lactation day 22. Reductions in F_2 pup body weight did not reach statistical significance. Small ($\leq$6%), but statistically significant, delays in F_1 generation sexual maturation occurred at 150 (males) and 450 ppm (males and females) as determined by timing of vaginal patency or preputial separation. The study authors attributed these delays to significant reductions in body weight at weaning. The values for sexual maturation endpoints in the 150 and 450 ppm exposure groups did not differ significantly from control values when body weight at weaning was included as a covariate in the analysis. Females rats with vaginal patency not evident until 40 or 41 days postpartum (i.e., the most delayed) in the 150 and 450 ppm groups had normal estrus cycles, mated, and produced litters. Estrous cycling in parental generation females was not affected by exposure to bromodichloromethane. A marginal effect on estrous cyclicity was observed in F_1 females in the 450 ppm exposure group. This effect was reported to be associated with a higher incidence of rats in the 450 ppm group (5/30) with six or more consecutive days of diestrus relative to the controls (2/30). The study authors considered this effect to be a secondary response associated with reduced pup weights and possible inadvertent stimulation of the uterine cervix during the performance of vaginal smears. Averages for estrous cycles per 21 days, cohabitation, mating indices, and fertility indices were unaffected by exposure to bromodichloromethane. Exposure to bromodichloromethane had no effect on anogenital distances in male or female F_2 pups.

The results of this study appear to identify NOAEL and LOAEL values for reproductive effects of 50 ppm (4.1 to 12.6 mg/kg-day) and 150 ppm (11.6 to 40.2 mg/kg-day), respectively,

based on delayed sexual maturation. However, the study authors have questioned whether delayed sexual maturation in F_1 males associated with reduced body weight should be treated as reproductive toxicity or general toxicity, since the root cause appears to be dehydration brought about by taste aversion to the compound. The parental NOAEL and LOAEL are also 50 and 150 ppm, respectively, based on reduced body weight and body weight gain in F_0 females and F_1 males and females.

2. Dibromochloromethane

Borzelleca and Carchman (1982) evaluated the reproductive toxicity of dibromochloromethane in a two-generation study with ICR Swiss mice. The authors used a modified multi-generation study protocol for this investigation. Groups of 10 males and 30 females (F_0 generation) were exposed to dibromochloromethane in drinking water at concentrations of 0, 0.1, 1.0, or 4.0 mg/mL for seven weeks. The study authors did not estimate average daily doses for all treated groups. However, they did indicate that the highest drinking water concentration (4.0 mg/mL) corresponded to an average daily dose of 685 mg/kg-day. Using this conversion factor, drinking water concentrations of 0.1 and 1.0 mg/mL dibromochloromethane were estimated to correspond to average daily doses of 17 and 171 mg/kg-day, respectively. Following the initial exposure period, the F_0 mice were mated to produce the F_{1a} litters. Each male mouse was co-housed for seven days with three randomly selected females. Two weeks after weaning of the F_{1a} litters, the F_0 mice were randomly re-mated to produce the F_{1b} litters. A similar protocol was followed for the F_{1c} litters. After a 21-day postnatal period, the F_{1a} and F_{1c} litters were sacrificed and necropsied. The F_{1b} generation was culled. The surviving males and females (10 males and 30 females) were exposed for 11 weeks to dibromochloromethane in drinking water at concentrations of 0, 0.1, 1.0 or 4.0 mg/mL, and then randomly mated to produce the F_{2a} and F_{2b} generations. A two-week interval occurred between weaning of the F_{2a} generation and remating of the F_{1b} generation to produce the F_{2b} generation. Thus, parental generations (F_0 and F_{1b}) were exposed continuously to dibromochloromethane in drinking water throughout the pre-mating, mating, gestation, and lactation periods for a total of 27 and 25 weeks, respectively. Following weaning of their final litters, both parental generations were sacrificed and necropsied. The F_{2a} and F_{2b} generations were sacrificed and necropsied following a 21-day postpartum survival period. Additionally, a selected number of pups from the final matings of each generation (i.e., F_{1c} and F_{2b}) were screened for either dominant lethal mutations or teratologic abnormalities.

Body weight gain and drinking water consumption were recorded weekly and semi-weekly for the F_0 and F_{1b} generations, respectively. Mating, gestation, gestation survival, and lactation survival indices were calculated for each mating. During the 21-day postpartum period, pups were counted on days 0, 4, 7, 14, and 21, and sexed on days 7, 14, and 21. Viability and lactation indices were calculated. After sacrifice of all litters except F_{1b} on day 21, one male and one female pup per litter were randomly selected for necropsy. For teratology screening, treated females from the F_0 and F_{1b} generations were sacrificed on GD 18. The number of implantations, resorptions, and live and dead fetuses were counted. Fetuses were individually weighed and examined for gross malformations; a selected subset of fetuses were examined for either skeletal or visceral anomalies. Statistical analysis was conducted on all endpoints, using parametric or nonparametric procedures, as appropriate for different endpoints. For statistical

analyses performed on the pups, the sampling unit was the litter. Treatment-related effects were considered to be statistically significant if the p value $\leq$ 0.05.

As compared with concurrent controls, final body weights were significantly reduced in the high-dose males and the mid- and high-dose females of the F_0 and F_{1b} generations. Water consumption was unaffected by treatment, indicating that taste aversion was not a factor in the observed decreases in body weight. Animals in both the F_0 and F_{1b} generations exhibited enlarged livers with gross morphological changes, interpreted by the authors as indicative of hepatotoxicity. The incidence and the severity of these alterations increased with increasing dose, with 0, 25, 70, and 100% of the F_0 animals and 0, 18, 64, and 100% of the F_{1b} animal exhibiting hepatic discoloration, fat accumulation, and/or lesions at 0, 0.1, 1.0, and 4.0 mg/mL, respectively. Fertility (mating index) was significantly decreased in the high-dose group (4.0 mg/mL) only for the F_{2a} generation. The gestational index was significantly decreased in the high-dose group for all three F_1 generations. Parental ingestion of 4.0 mg/mL dibromochloromethane resulted in (1) decreased litter size in all generations (F_{1a}, F_{1b}, F_{1c}, F_{2a}, and F_{2b}); (2) decreased viability index in four of the five generations (F_{1a}, F_{1b}, F_{1c} and F_{2a}); (3) decreased lactation index in the F_{2b} generation; and (4) decreased postnatal body weight in the F_{2b} generation. Parental ingestion of 1.0 mg/mL dibromochloromethane produced (1) decreased litter size in the F_{1c} generation; (2) decreased viability index in the F_{1b} generation; (3) decreased lactation index in the F_{1b} and F_{2b} generations; and (4) decreased postnatal body weight in the F_{2b} generation. The only statistically significant effect observed at the lowest dose tested (0.1 mg/mL) was a reduction in postnatal body weight in the F_{2b} generation on PND 14; this effect was not noted on PND 7 or 21. No statistically significant increases in dominant lethal or teratogenic effects were reported in either the F_{1c} or F_{2b} generations.

Based on decreased postnatal body weight in the F_{2b} generation, the marginal LOAEL for reproductive/developmental toxicity is 17 mg/kg-day and a NOAEL could not be determined. The developmental LOAEL is considered to be marginal because (1) this effect was only noted in one of the two litters in the F_2 generation; (2) no other adverse effects were observed at this dose level; and (3) it was unclear from the report how many litters and pups per litter were examined for postnatal body weight. For parental toxicity, liver alterations indicative of hepatotoxicity were clearly evident at the two higher doses in both parental generations. At the lowest dose tested, hepatic changes were mainly limited to discoloration, presumably due to the accumulation of fat deposits (Borzelleca and Carchman, 1982); gross morphology was normal, and histopathologic examination was not conducted. Therefore, the adversity of this effect was uncertain. Based on these considerations, the lowest dose, 17 mg/kg-day, is considered to be a marginal LOAEL for parental toxicity (both F_0 and F_{1b} generations) and a NOAEL could not be determined.

Ruddick et al. (1983) investigated the reproductive and developmental toxicity of dibromochloromethane in Sprague-Dawley rats. Pregnant dams (10 to 12 animals per dose group) were administered gavage doses of 0, 50, 100, or 200 mg/kg-day in corn oil on GD 6-15. Body weights were measured on GD 1, on GD 1 through GD 15, and before and after caesarean section on GD 22. On GD 22, females were anesthetized, exsanguinated, and viscera (including the uteri) were examined for pathological changes. The fetuses were removed, weighed

individually, and examined for viability and external malformations. Two pups per dam were placed in fixative for histopathological examination. Approximately two-thirds of the remaining live fetuses were preserved for examination for skeletal abnormalities. The remaining fetuses were preserved for examination for visceral alterations. Maternal blood was analyzed for standard hematological and clinical biochemistry parameters. Following gross pathological examination of the dams, organ weights were collected for liver, heart, brain, spleen, and one kidney. Tissues from control and high-dose dams (5 animals/group) were subject to histopathological examination. Where chemical related effects were observed, the affected tissues were also examined in the mid-dose group.

Maternal weight gain was depressed by 25% in the high-dose group relative to controls. No significant effects on maternal organ weights, hematology and clinical chemistry, number of resorption sites, number of fetuses per litter, and mean fetal body weight gain were observed in any of the dose groups. No treatment-related histopathology was noted in either dams or fetuses. There were no skeletal or visceral fetal anomalies attributed to dibromochloromethane treatment. Statistical analysis of fetal endpoints was not conducted by the study authors. However, inspection of the data indicated that there were no dose-related effects (e.g., the number of affected fetuses/number of affected litters for sternebral aberrations was 3/2, 2/1, 1/1, and 1/1 for control, low-, mid-, and high-dose groups, respectively). The power of this experiment was limited by the small number of litters per dose group. In the absence of observed fetal effects, the NOAEL for developmental toxicity was 200 mg/kg-day, the highest dose tested, and a LOAEL could not be determined. Based on significantly decreased maternal body weight gain, the LOAEL and NOAEL for maternal toxicity were 200 and 100 mg/kg-day, respectively.

NTP (1996) conducted a short-term reproductive and developmental toxicity screen in Sprague-Dawley rats. Dibromochloromethane was administered in drinking water at concentrations of 0, 50, 150, or 450 ppm during a study period of 35 days. Males (10/group) were treated from study days 6 through 34. At study termination, males were submitted for a thorough examination, which included hematology, clinical chemistry, gross necropsy, histopathology, and a complete sperm evaluation (count, density, motility, and morphology). Group A females (10/group) were treated from study days 1 through 34. These females were mated to treated males on study days 13 through 18 and necropsied on study day 34. Group B females (13/group) were mated on study day 1 to untreated males, treated from GD 6 through parturition, and necropsied on postnatal day 5. No hematology, clinical chemistry, or histopathology was conducted on the females.

Based on measured water consumption, the authors estimated dose levels for the males as 4.2, 12.4, and 28.2 mg/kg-day, for Group A females as 6.3, 17.4, and 46.0 mg/kg-day, and for Group B females as 7.1, 20.0, and 47.8 mg/kg-day. A few changes in clinical chemistry were noted for the males. Alkaline phosphatase and 5' nucleotidase were increased at all dose levels in males, but reached statistical significance only at the low dose for alkaline phosphatase and at the mid and high dose for 5' nucleotidase. Total serum proteins were also decreased at the high dose in males. The study authors noted that these changes could reflect mild liver damage. However, no treatment-related microscopic lesions were observed. No statistically significant effects were observed on any sperm parameter investigated. No effect was observed on any

reproductive or fertility measure in Group A or B females at any dose. The proportion of male pups was significantly decreased in Group B females at the high dose compared to the control value. The study authors did not consider this result to be treatment-related because the control value (0.61) was unusually high compared to historical values, and the result for the high-dose group (0.44) was within historical background. Based on these data, the authors noted that dibromochloromethane was not a reproductive toxicant at doses up to the high dose in either males (28.2 mg/kg-day) or females (46.0 to 47.8 mg/kg-day). Based on the clinical chemistry changes, the authors stated that administration of dibromochloromethane may have resulted in general toxicity at all doses in the male treatment groups. The observed changes in clinical chemistry, however, would not be considered adverse for the following reasons: absence of clear dose-related response, small magnitude of the changes, and absence of supporting histopathology data. Therefore, this study identified NOAEL values of 28.2 mg/kg-day and 47.8 mg/kg-day for males and females, respectively, for reproductive and systemic effects.

3. Bromoform

Ruddick et al. (1983) investigated the reproductive and developmental toxicity of bromoform in Sprague-Dawley rats. Pregnant dams (14 to 15 animals/dose group) were administered gavage doses of 0, 50, 100, or 200 mg/kg-day in corn oil on GD 6 to 15. Body weights were measured on GD 1, on GD 6 through GD 15, and before and after pups were delivered by caesarean section on GD 22. On GD 22, females were sacrificed and body tissues (including the uterus) were removed for pathological examination. Females were evaluated for the number of resorption sites and the number of fetuses. Maternal blood samples were collected and evaluated for standard hematology and clinical chemistry parameters. The liver, heart, brain, spleen, and one kidney were weighed. Standard histopathology was conducted on control and high-dose females (5/group). All fetuses in all groups were individually weighed, and evaluated for viability and external malformations. Histopathologic examination was performed on two pups per litter. Of the remaining live fetuses, approximately two-thirds were examined for skeletal alterations and one-third for visceral abnormalities.

Maternal weight gain, organ weights, hematology, and clinical chemistry were unaffected by bromoform treatment. No significant differences between exposed and control groups were observed for the number of resorption sites, the number of fetuses per litter, fetal weights, fetal gross malformations, and visceral abnormalities. No treatment-related histopathological effects were noted in either the dams or fetuses. However an elevation in the incidence of skeletal anomalies, including the presence of a 14th rib, wavy ribs, and interparietal bone deviations was reported in treated animals. An increase in wavy ribs was only observed in the high-dose group. The number of affected fetuses /number of affected litters for the presence of a 14th rib was 3/3, 4/3, 4/3 and 7/5 in the 0, 50, 100, and 200 mg/kg-day groups, respectively. The incidence of sternebral aberrations (number of affected fetuses/number of affected litters) was 1/1, 5/3, 6/5, 13/8 in the 0, 50, 100, and 200 mg/kg-day groups, respectively. Statistical significance for fetotoxic endpoints was not reported by the study authors. A statistical analysis (using the Fisher Exact test) was conducted on the published data and demonstrated that the increase in sternebral anomalies was significantly different from controls at the highest dose tested (200 mg/kg-day). A trend test showed a statistically significant dose-related trend ($p < 0.002$) for this endpoint;

stepwise analysis indicated that the trend was no longer significant when the two highest doses (i.e., 200 and 100 mg/kg-day) were omitted from the analysis. These findings suggest that the LOAEL and NOAEL for developmental toxicity were 100 and 50 mg/kg-day, respectively. In the absence of observed maternal effects, the NOAEL for maternal toxicity was 200 mg/kg-day, and a LOAEL could not be determined.

NTP (1989b) investigated the effect of bromoform on fertility and reproduction in Swiss CD-1 mice using a continuous breeding protocol. Twenty male-female pairs were administered daily doses of 50, 100, or 200 mg/kg-day by gavage in corn oil and forty male-female pairs were dosed with the corn oil vehicle only. Dose selection was based on a 14-day range-finding study. The 105-day dosing period included a seven-day precohabitation phase and a 98-day cohabitation phase. The parameters evaluated for this study were fertility, litters per pair, live pups per litter, proportion of pups born alive, sex of live pups, or pup body weights. The last litter born (generally the fifth litter) in the control and 200 mg/kg-day groups during a holding period following the continuous breeding phase were reared by the dams, weaned and raised to sexual maturity (approximately 74 days) while receiving the same treatment (vehicle control of 200 mg/kg-day bromoform) as their parents. At sexual maturity, males and females from different litters within the same treatment group were cohabited for seven days and then housed individually until delivery. The endpoints for this mating trial were the same as for the parental generation. At study termination, the F_1 mice were weighed, necropsied and evaluated for selected organ weights, epididymal sperm motility, and sperm morphology. Selected organs were fixed for histopathological examination.

In the 200 mg/kg-day treatment group, the body weights of dams at delivery were consistently less than the control group value. The reduction in body weight was statistically significant after delivery of the first, second, fourth, and fifth litters. The fertility index for the parental generation was 100% for the control and treated groups (a breeding pair was designated as fertile if they produced at least one live or dead pup). There was no detectable effect of treatment on the number of litters per pair, the number of live pups per litter, the proportion of pups born alive, the sex of live pups, or pup body weights. The gestational period was similar across groups. However, postnatal survival of F_1 pups in the 200 mg/kg-day group was significantly lower than in the control group. The study authors reported that this difference was primarily attributable to three dams who lost all of their pups by postnatal day 4. One dam in the control group also lost her litter by postnatal day 4. The study authors noted that this result is consistent with a treatment effect on early maternal behavior, early lactational failure, and/or the postnatal developmental processes. When F_1 mice were cohabited for one week, no effect of treatment on mating index or fertility was observed. There were no significant differences relative to control values for the number of live pups per litter (male, female, or combined), the proportion of live pups, the proportion of male pups, or pup weight at birth. At sacrifice, male and female F_1 mice administered 200 mg/kg-day exhibited increased relative liver weights and decreased relative kidney weights as compared with control values. The mean body weight for F_1 males was significantly less than the mean weight of the male control group. Histopathological evaluation revealed minimal to moderate hepatocellular degeneration in the livers of high-dose F_1 male and female mice. Bromoform treatment had no effect on epididymal

sperm density, motility, or morphology in F_1 males. No treatment-related histologic lesions were observed in the seminal vesicles, coagulating glands, or prostate glands of males, or in the lung, kidney, or thyroid gland of males or females. Based on liver histopathology, decreased postnatal survival, and other signs of toxicity (e.g., increased relative liver and decreased relative kidney weights) in F_1 mice of both sexes at the highest dose tested, the LOAEL for developmental toxicity is 200 mg/kg-day, and the NOAEL is 100 mg/kg-day. Based on consistently decreased body weights of pregnant dams at delivery, the LOAEL for maternal toxicity is 200 mg/kg-day and the NOAEL is 100 mg/kg-day.

Table V-9 Summary of Reproductive Studies of Brominated Trihalomethanes

Reference	Species	Route	Sex	Number per dose group	Duration	Dose (mg/kg-day)	Results
Bromodichloromethane							
Ruddick et al. (1983)	Rat Sprague-Dawley	Gavage (Corn oil)	F	9-14	GD 6-15	0 50 100 (NOAEL) [1] 200 (LOAEL)	Developmental toxicity study. Statistically decreased maternal wt. gain at high dose (38%); increased litter incidence of sternebral aberrations. Statistical significance not evaluated for fetotoxic endpoints by study authors; statistical analysis conducted on published data for fetal effects. Trend test indicated statistical effect for sternebral anomalies at highest dose tested. Maternal LOAEL and NOAEL are 200 and 100 mg/kg-day, respectively.
Klinefelter et al. (1995)	Rat F344	Drinking Water	M	7	52 weeks	0 22 (NOAEL) 39 (LOAEL)	Reproductive toxicity study. Decreased sperm velocity at 39 mg/kg-day. No histopathological alterations noted in any reproductive tissue examined.
Narotsky et al. (1997)	Rat F344	Gavage (Corn oil)	F	12-14	GD 6-15	0 25 (NOAEL) 50 (LOAEL) 75	Developmental toxicity study comparing the use of different gavage dosing vehicles. Reduced maternal weight gain GD 6-8 and lacrimation at 50 and 75 mg/kg-day. Full litter resorption (FLR) observed at 50 and 75 mg/kg-day (8% and 83%, respectively). No effects on postnatal survival, pup weight, or pup survival in surviving litters. ED05 and BMD for FLR calculated by study authors as 48.4 and 39.3 mg/kg-day, respectively. Maternal LOAEL and NOAEL are 50 and 25 mg/kg-day, respectively.
Narotsky et al. (1997)	Rat F344	Gavage (Aqueous)	F	12-14	GD 6-15	0 25 (NOAEL) 50 (LOAEL) 75	Developmental toxicity study comparing the use of different gavage dosing vehicles. Reduced maternal weight gain GD 6-8 at all dose levels. Full litter resorption (FLR) observed at 50 and 75 mg/kg-day (17 and 21%, respectively). No effects observed on postnatal survival, pup weight, or pup survival in surviving litters. ED05 and BMD for FLR calculated by study authors as 33.3 and 11.3 mg/kg-day, respectively. Maternal LOAEL is 25 mg/kg-day; maternal NOAEL not determined.
NTP (1998)	Rat Sprague-Dawley	Drinking Water	M	5-10	25-30 days	0 8 41 68 (NOAEL)	Reproductive/developmental toxicity study. Decreased food and water consumption; decreased body weight. No dose-related changes in reproductive/developmental parameters

Table V-9 (cont.)

Reference	Species	Route	Sex	Number per dose group	Duration	Dose (mg/kg-day)	Results
NTP (1998)	Rat Sprague-Dawley	Drinking Water	F	5-10	25-30 days	0 14 72 116 (NOAEL)	Reproductive/developmental toxicity study. Decreased food and water consumption; decreased body weight. No dose-related changes in reproductive/developmental parameters
Bielmeier et al. (2001)	Rat F344	Gavage (Aqueous)	F	8-13	GD 6-15 GD 6-10 GD 11-15	0 75 (LOAEL)	Critical exposure period study. Full litter resorption observed in animals treated on GD 6-10, but not in animals treated on GD 11-15.
Bielmeier et al. (2001)	Rat F344/ Sprague-Dawley	Gavage (Aqueous)	F	12-14	GD 6-10	F344 0 75 (LOAEL) Sprague-Dawley 0 75 100 (NOAEL)	Strain comparison study. Full litter resorption observed in F344 rats but not in concurrently dosed Sprague-Dawley rats.
Bielmeier et al. (2001)	Rat F344	Gavage (Aqueous)	F	8-11	GD 9	0 75 (LOAEL) 100	Hormone profile study. Full litter resorption observed at both doses. Decreased serum progesterone levels in F344 rats which experienced FLR.
CCC (2000c) Christian et al. (2001b)	Rat Sprague-Dawley	Drinking Water	F, M	10	64-74 days Males[2] (days 1-64 i.e., for 14 days premating, during mating, and for ~ 6 weeks following mating) Females[2] (days 1-74, i.e., for 14 days premating, during mating, GD 0-21, lactation days 1-29)	0 ppm 50 ppm (NOAEL) 150 ppm (LOAEL) 450 ppm 1350 ppm	Range finding reproductive/developmental toxicity study. Decreased body weight gain and terminal body weight (>10%) in males at highest dost tested but no apparent effects on reproductive endpoints at any dose. Maternal toxicity (reduced body weight and body weight gain and decreased food and water consumption) at 150 ppm and higher. Dose-dependent decreases in mean pup weight gain and pup weights beginning on lactation day 5-29 in 3 highest dose groups. Decreased pup body weight gain and body weight also observed in 3 highest dose groups in pups treated for one week postweaning at parental drinking water concentrations. Reproductive/developmental LOAEL and NOAEL are 150 and 50 ppm, respectively, based on decreased pup wt and wt gain; parental LOAEL and NOAEL are 150 and 50 ppm, respectively, based on reduced body wt gain and wt. Findings confounded by effects of decreased water consumption at various time points during treatment.

Table V-9 (cont.)

Reference	Species	Route	Sex	Number per dose group	Duration	Dose (mg/kg-day)	Results
CCC (2000d) Christian et al. (2001a)	Rat Sprague-Dawley	Drinking Water	F	25	GD 6-21	0 2.2 18.4 45.0 (NOAEL) 82.0 (LOAEL)	Developmental toxicity study. Decreased maternal body weight and body weight gain at 45.0 mg/kg-day. Developmental LOAEL based on slightly decreased number of ossification sites in the hindlimb (metatarsals and phalanges) and forelimb (phalanges). Maternal LOAEL and NOAEL are 82.0 and 45.0 mg/kg-day, respectively.
CCC (2000a) Christian et al. (2001b)	Rabbit New Zealand White	Drinking Water	F	5	GD 6-29	0.0 4.9 13.9 32.3 76.3 (NOAEL)	Range finding developmental study. Decreased maternal body weight gain and water and feed consumption at all tested doses. No treatment-related changes in reproductive or developmental endpoints. Study authors considered maternal LOAEL to be < 4.9 mg/kg-day, based on significantly reduced body weight gain.
CCC (2000b) Christian et al. (2001a)	Rabbit New Zealand White	Drinking Water	F	25	GD 6-29	0 1.4 13.4 35.6 55.3 (NOAEL)	Developmental toxicity study. Reduced maternal weight gain at 35.3 mg/kg-day. No dose-related changes in reproductive or developmental parameters. Maternal LOAEL and NOAEL are 35.3 and 13.4 mg/kg-day, respectively.
CCC (2002) Christian et al. (2002)	Rat Sprague-Dawley	Drinking Water	M, F	30	Up to 118 days	Males F_0: 106 d (incl. 70 d pre-mating) F_1: 64 d post-weaning, 14 d cohab. Females F_0: 118 d (incl. gest., lactation F_1 64 d post-weaning, 14 d cohab., gest., lactation 0 50 (NOAEL) 150 (LOAEL) 450	Reproductive/developmental LOAEL and NOAEL are 150 and 50 ppm, respectively, based on delayed sexual maturation in F_1 males; parental LOAEL and NOAEL are 150 and 50 ppm, respectively, based on reduced body wt gain and wt in F_0 females and F_1 males and females. Findings confounded by effects of decreased water consumption as a result of taste aversion to the test compound.

Table V-9 (cont.)

Reference	Species	Route	Sex	Number per dose group	Duration	Dose (mg/kg-day)	Results
Bielmeier et al. (2004)	Rat F344	Gavage (Aqueous)	F	5-13	GD 9	0 75 (LOAEL) 100	Hormone profile study. BDCM-induced pregnancy loss was associated with decreased LH followed by decreased progesterone on GD 10. All control dams maintained their litters, whereas 8/9 dams exposed to bromodichloromethane had pregnancy loss.
Bielmeier et al. (2004)	Rat F344	Gavage (Aqueous)	F	5-13	GD 6-10	0 75 (LOAEL) 100	Hormone supplementation study. BCDM-induced pregnancy loss was prevented by progesterone and hCG supplementation. Five of seven dams (71%) treated with 100 mg/kg-day of bromodichloromethane plus the hormone vehicles (corn oil or saline injected subcutaneously) on GD 6-10 lost their pregnancies. In contrast, dams dosed with 100 mg/kg-day of bromodichloromethane on GD 6-10 and concurrently given progesterone had a 0/8 (0%) incidence of pregnancy loss. Dams dosed with 100 mg/kg-day of bromodichloromethane on GD 8-10 and concurrently given hCG by injection had a 1/9 (11%) incidence of pregnancy loss. LH mediated mode of action was suggested.

Table V-9 (cont.)

Reference	Species	Route	Sex	Number per dose group	Duration	Dose (mg/kg-day)	Results
Dibromochloromethane							
Borzelleca and Carchman (1982)	Mouse ICR Swiss	Drinking Water	M F	10 30	25-27 weeks	0 17 (marginal LOAEL) 171 685	Multi-generation reproductive toxicity study. Significant high-dose effects include decreased gestational index in F_1 generation at high dose and decreased litter size in F_1 and F_2 generations. Significant mid-dose effects include decreased litter size, decreased viability index, decreased lactation index, and decreased postnatal body weight in some F_1 and/or F_2 generations. Only significant low-dose effect is reduced postnatal body wt in F_{2b} generation on postnatal day 14. Hepatic effects observed in both parental generations at all doses; liver effects marginal at low dose. Parental marginal LOAEL is 17 mg/kg-day; parental NOAEL not determined.
Ruddick et al. (1983)	Rat Sprague-Dawley	Gavage (Corn oil)	F	10-12	GD 6-15	0 50 100 200 (NOAEL)	Developmental toxicity study. Significantly depressed maternal wt. gain at high dose (25%); increased maternal relative liver wt. and kidney Statistical significance of fetal endpoints not evaluated by study authors. Based on data inspection, no dose-related skeletal or visceral effects observed in litters. Maternal LOAEL and NOAEL are 200 and 100 mg/kg-day, respectively.
NTP (1996)	Rat Sprague-Dawley	Drinking Water	M	10	29 days	4.2 12.4 28.2 (NOAEL)	Reproductive/developmental toxicity study. No treatment-related effects on measured sperm parameters
NTP (1996)	Rat Sprague-Dawley	Drinking Water	F	10	35 days	6.3 17.4 46.0 (NOAEL)	Reproductive/developmental toxicity study. Exposure occurred during a 6-day mating period and most/all of gestation. No clearly adverse effect on any reproductive or developmental endpoint at tested doses
NTP (1996)	Rat Sprague-Dawley	Drinking Water	F	13	~16 days (GD 6-parturit-ion)	7.1 20.0 47.8 (NOAEL)	Reproductive/developmental toxicity study. No clearly adverse effect on any reproductive or developmental endpoint at tested doses

Table V-9 (cont.)

Reference	Species	Route	Sex	Number per dose group	Duration	Dose (mg/kg-day)	Results
Bromoform							
Ruddick et al. (1983)	Rat Sprague-Dawley	Gavage (Corn oil)	F	14-15	GD 6-15	0 50 (NOAEL) 100 (LOAEL) 200	Developmental toxicity study. No statistically significant maternal effects. Apparent treatment-related increases in sternebral aberrations and other skeletal endpoints. Statistical significance of fetotoxic endpoints not evaluated by study authors. Statistical analysis conducted on published data indicated significant increase in sternebral aberrations at two highest doses. Maternal NOAEL is 200 mg/kg-day; LOAEL not determined.
NTP (1989b)	Mouse ICR Swiss	Gavage (oil)	M F	20 20	105 days	0 50 100 (NOAEL) 200 (LOAEL)	Continuous breeding reproductive toxicity protocol. Maternal body weights significantly decreased at highest dose tested. Decreased postnatal survival, organ wt. changes, and liver histopathology observed in F_1 mice of both sexes in high-dose group. No noted effects on fertility, litters/pair, live pups/litter; proportion of live births, sex of live pups, or pup body weight. Maternal LOAEL and NOAEL are 200 and 100 mg/kg-day, respectively.

[1] NOAEL and LOAEL values reported in this column are for developmental/reproductive toxicity effects. The NOAEL and LOAEL values for parental toxicity are reported in the "Results" column.

[2] Doses for this study are presented as ppm in drinking water; due to marked changes in adult female water consumption during different physiologic stages (i.e., pre-mating, mating, gestation, and lactation), it is not possible to convert administered drinking water concentrations into biologically meaningful average daily doses.

F. Mutagenicity and Genotoxicity

1. Bromodichloromethane

The results of *in vivo* and *in vitro* tests conducted to evaluate the mutagenicity, genotoxicity, and neoplastic transformation potential of bromodichloromethane are summarized in Table V-10 at the end of this section.

a. *In Vitro* Assays

Simmon and Tardiff (1978) reported that nonactivated bromodichloromethane was mutagenic in *S. typhimurium* strain TA100 when assayed in a desiccator containing the test compound in the atmosphere. The minimum amount of bromodichloromethane required to elicit a mutagenic response following addition to the desiccator was 600 μmol.

Ishidate et al. (1982) assayed the mutagenicity of bromodichloromethane in *S. typhimurium* strain TA100 in the presence and absence of rat liver S9 fraction. Increased mutation frequencies were observed only in the absence of S9 activation. In contrast, chromosomal aberrations in Chinese hamster fibroblasts were observed in the presence, but not the absence, of S9 fraction. The concentrations tested in these assays were not reported.

Nestmann and Lee (1985) investigated the mutagenicity of bromodichloromethane at 12 to 1,200 μM in *S. cerevisiae* strains D7 and XV185-14C in the presence or absence of S9 activation. No clear increase in convertants or in revertants of strain XV185-14C was observed for bromodichloromethane in the presence or absence of S9 activation.

NTP (1987) reported that bromodichloromethane was not mutagenic when tested using a preincubation protocol in *S. typhimurium* strains TA1535, TA1537, TA98, or TA100 at concentrations reaching cytotoxic levels (20 μmol/plate; 3,333 μg/plate). Testing was done in the absence of S9 and in the presence of S9 prepared from Aroclor-induced male hamster or rat liver. NTP concluded that the negative results may have been due to volatilization of the test compound from the test system. Bromodichloromethane was not mutagenic in the mouse lymphoma L5178Y/TK$^{+/-}$ assay in the absence of S9, but did induce dose-related increases in forward mutations at S9-activated concentrations greater than or equal to 2,000 μM (300 μg/mL). Cytogenetic tests with Chinese hamster ovary cells (CHO) cells were reported in this study and also by Anderson et al. (1990). There was no evidence of induction of chromosomal aberrations following treatment with up to 30,000 μM (5,000 μg/mL) in either the presence or absence of exogenous metabolic activation. There was also no evidence of sister chromatid exchanges induced by the nonactivated material. In the presence of S9 activation, one of three assays resulted in a positive response at doses greater than or equal to 24,400 μM (4,000 μg/mL). These results are difficult to evaluate because cytotoxicity was observed at similar concentrations in the other trials.

Varma et al. (1988) tested bromodichloromethane for mutagenicity in *S. typhimurium* strains TA1535, TA1537, TA98, and TA100. In the absence of S9 fraction, bromodichloromethane at nonactivated concentrations of 2.4 to 3.2 μmol/plate induced mutations in strain TA1537. There was no effect in the other strains.

Bromodichloromethane was positive for the induction of DNA damage in the presence and absence of exogenous activation, based on the results of the SOS chromotest (LeCurieux et al., 1995). Bromodichloromethane gave a negative result in the fluctuation test modification of the *S. typhimurium* reverse mutation assay.

Several studies have evaluated induction of sister chromatid exchanges following exposure to bromodichloromethane. Morimoto and Koizumi (1983) investigated the ability of bromodichloromethane to induce sister chromatid exchanges in human lymphocytes *in vitro* in the absence of S9 activation. Bromodichloromethane caused a dose-dependent increase in sister chromatid exchanges. The increased incidence was significant ($p < 0.05$) at concentrations

greater than or equal to 400 μM. The potential of S9-activated bromodichloromethane to induce sister chromatid exchanges *in vitro* was also investigated by Sobti (1984). A dose of 100 μM increased the frequency of sister chromatid exchange in rat liver cells. In human lymphocytes, a 100-fold greater concentration of bromodichloromethane was required to elicit the same effect on sister chromatid exchange when compared to dibromochloromethane (100 μM vs. 1 μM.). Fujie et al. (1993) observed a statistically significant, dose-related increase in sister chromatid exchange in rat erythroblastic leukemia K_3D cells treated with bromodichloromethane in the absence of exogenous activation. Bromodichloromethane also appeared to give a positive response in the presence of exogenous metabolic activation, although the study protocol and results with negative controls were described less clearly for this phase of testing.

Bromodichloromethane was tested in the mouse lymphoma assay as part of an international collaborative program under the auspices of the Japanese Ministry of Health and Welfare (Sofuni et al., 1996). The results of this assay were equivocal. One laboratory obtained a positive result in the activated phase, but this result was not confirmed by a second laboratory. Results in the nonactivated phase were negative or equivocal due to poor viability of the solvent control cell cultures.

Matsuoka et al. (1996) conducted a chromosome aberration assay with Chinese hamster lung fibroblast (CHL/IU) cells exposed to bromodichloromethane in tightly capped flasks. A weak induction of chromosome aberrations was observed for bromodichloromethane in the presence and absence of exogenous metabolic activation.

Geter et al. (2004a) examined the ability of bromodichloromethane to induce DNA strand breaks (as assessed by the alkaline unwinding assay) in CCRF-CEM human lymphoblastic leukemia cells and primary F344/N rat hepatocytes. Exposure to 5 or 10 mM significantly induced DNA strand breaks in CCRF-CEM cells after a two-hour exposure. Cells exposed to 1 or 5 mM bromodichloromethane showed 100% DNA strand break recovery when assayed after incubation in bromodichloromethane-free medium for 22 hours. No evidence of strand breaks was observed in primary rat hepatocytes exposed to 1, 5, or 10 mM bromodichloromethane for 4 hours.

Landi et al. (2003) demonstrated that bromodichloromethane induced DNA damage in primary cultures of human lung epithelial cells as measured by tail extent movement in the Comet assay. Human lung epithelial cells were collected by scraping the large airways of four volunteers. DNA genotyping was used to identify 2 subjects that were *GSTT1-1*$^+$ and 2 subjects that were *GSTT1-1*$^-$. Cells were maintained in culture and were exposed to 0, 10, 100 or 1000 μM bromodichloromethane for 3 hours prior to being flash frozen for analysis. DNA damage was observed at each concentration tested. Variation in response among subjects was not related to the *GSTT1-1* genotype.

Robbiano et al. (2004) evaluated the ability of bromodichloromethane to cause DNA fragmentation and micronuclei formation in primary cultures of rat and human kidney cells. Human kidney tissue was obtained from surgical patients where a portion of the kidney was

removed for health reasons. Cultured cells were exposed to 0, 0.5, 1, 2, or 4 mM bromodichloromethane for 20 hours and DNA damage was measured as tail length in the Comet assay. Statistically significant increases in DNA damage were observed in cells from rats and humans at all exposure levels. For the micronucleus assay, cells were exposed to 0, 1, 2, or 4 mM bromodichloromethane for 48 hours. Bromodichloromethane caused increased micronuclei formation in primary cultures of rat kidney cells at all exposure levels and in human kidney cells at all exposure levels except the lowest (1.0 mM).

b. *In Vivo* Assays

Ishidate et al. (1982) investigated the *in vivo* clastogenicity of bromodichloromethane in ddY mice, MS mice, and Wistar rats. Doses of 125 to 500 mg/kg-day were administered in olive oil by intraperitoneal injection, and the animals were sacrificed at 18, 24, 30, 48, and 72 hours after dosing. No significant induction of micronucleus formation in bone marrow was observed in either mice or rats.

Morimoto and Koizumi (1983) investigated the potential of bromodichloromethane to induce sister chromatid exchanges in male ICR/SJ mice. Animals were given doses of 0, 25, 50, 100, or 200 mg/kg-day for four days by olive oil gavage. Bromodichloromethane produced a roughly linear dose-dependent increase in sister chromatid exchange frequency. The increase was statistically significant ($p < 0.05$) at 50 mg/kg-day. The authors noted that the concentrations required to produce an increased incidence of sister chromatid exchange were on the order of 1,000 to 10,000 times higher than the concentrations typically found in drinking water.

Hayashi et al. (1988) measured induction of micronucleated polychromatic erythrocytes in ddY mice by intraperitoneal administration of bromodichloromethane at single doses up to 500 mg/kg in corn oil. No evidence of clastogenicity was observed. There was no clear evidence of toxicity or cytotoxicity in the target tissue.

Fujie et al. (1990) analyzed chromosome aberrations in bone marrow from Long-Evans rats (3/sex/dose) following oral (males only) or intraperitoneal (males and females) exposure to bromodichloromethane. Oral administration was by gavage in saline for five consecutive days, and the animals were sacrificed 18 hours after the last dose. Bromodichloromethane induced dose-related increases in chromatid and chromosome breaks. A more pronounced increase in clastogenic activity was observed following a single intraperitoneal dose, with significant ($p < 0.05$) effects at 16.4 mg/kg.

Hayashi et al. (1992) evaluated induction of micronuclei in mouse peripheral blood erythrocytes by bromodichloromethane. Groups of four male ddY mice received an intraperitoneal injection of 0, 25, 50, or 100 mg/kg bromodichloromethane in physiological saline once a week for 5 weeks. Micronuclei were evaluated 1 week after the last dose. No evidence of micronucleus induction was observed. One low-dose mouse died, and weight loss was observed in all treatment groups during exposure.

Robbiano et al. (2004) measured DNA damage and micronuclei formation in kidney cells of Sprague Dawley rats sacrificed 20 hours after a single oral gavage dose of 458 mg/kg bromodichloromethane in 0.5% dimethyl sulfoxide. The frequency of micronucleated kidney cells was increased and DNA damage was observed, as measured by increased tail length in the Comet assay.

Potter et al. (1996) observed no significant increase in DNA strand breakage in male F344 rats one day after a single gavage doses of 0.75 or 1.5 mmol/kg (123 or 246 mg/kg-day) of bromodichloromethane in 4% Emulphor® using the alkaline unwinding procedure. Geter et al. (2004a) likewise found no significant induction of DNA strand breaks using the alkaline unwinding assay in the liver, kidney, or duodenum epithelial cells of male F344/N rats when assayed four hours after administration of a single oral 0.3 or 0.6 mmol/kg (49 or 98 mg/kg) gavage dose of bromodichloromethane. No significant induction of DNA strand breaks was observed in male F344/N rats exposed to 0.6, 1.2, or 2.4 g/L of bromodichloromethane in the drinking water for up to five weeks.

Stocker et al. (1997) investigated the *in vivo* genotoxicity of bromodichloromethane in an unscheduled DNA synthesis assay in the livers of bromodichloromethane treated rats. Male Sprague-Dawley rats (4 animals per group) were administered a single dose of 0 (control), 135 or 450 mg/kg bromodichloromethane via gavage in aqueous 1% methylcellulose. These doses were selected by the authors to correspond to 30% and 100% of the calculated maximum tolerated dose (MTD) for this compound. Analysis of hepatocytes for unscheduled DNA synthesis was conducted 2 and 14 hours after treatment. There was no evidence of increased DNA synthesis in hepatocytes at any tested dose of bromodichloromethane.

c. Mechanistic Studies of Genotoxicity

A potential mechanism for mutagenicity of bromodichloromethane has been studied in strains of *Salmonella typhimurium* that express the rat theta-class glutathione S-transferase *T1-1* gene (*GSTT1-1*). These studies provide evidence for a distinctmechanism of brominated trihalomethane activation and thus are discussed in detail. Pegram et al. (1997) utilized two new strains of TA1535-derived *Salmonella* to investigate glutathione S-transferase-mediated bioactivation of bromodichloromethane. One strain had been transfected with the *GSTT1-1* gene (+GST) and the other strain had the same gene inserted in a non-functioning orientation (-GST). These strains were used in base-substitution revertant colony assays following 24 hour exposures to concentrations of bromodichloromethane ranging from 200 to 4,800 ppm. The agar concentration resulting from a 24 hour exposure to 4,800 ppm bromodichloromethane was 0.67 mM. Bromodichloromethane increased the number of revertant colonies in each strain of *Salmonella* tested (+GST, -GST and TA1535). The frequency of the revertants in TA1535 was significantly increased above the spontaneous level at the three highest concentrations tested (highest concentration 4,800 ppm; intermediate concentrations not explicitly stated), while frequency was increased in the -GST strain only at the highest concentration. In contrast, there was a dramatic, dose-dependent increase in bromodichloromethane-induced mutations in the +GST strain when compared to the -GST control strain (an 18-fold increase at the 4800 ppm bromodichloromethane

concentration). When chloroform was tested for comparative purposes, a positive response was observed only at the two highest concentrations tested (19,200 and 25,600 ppm). These results provide evidence that the mutagenicity of bromodichloromethane is enhanced by GST-mediated conjugation with GSH. The comparatively low affinity of the GST-mediated pathway for chloroform suggests that different trihalomethanes can induce mutations by different mechanisms.

DeMarini et al. (1997) further investigated the role of glutathione S-transferase activity in mediating the mutagenicity of bromodichloromethane in *Salmonella typhimurium.* Strains of *Salmonella* utilized in this investigation included RSJ100, which expresses the *GSTT1-1* gene and TPT100, which has the *GSTT1-1* gene inserted in a non-functioning orientation. Mutagenicity was assayed using a Tedlar bag vaporization technique. Bromodichloromethane (3,200 ppm) induced a 44-fold increase in revertant colonies in the RSJ100 strain of *Salmonella* when compared to background revertant frequency. The spectrum of bromodichloromethane-induced mutations at the *his*G46 allele in strain RSJ100 was analyzed using the colony probe hybridization method. This analysis revealed that 99% of the mutations were GC→AT. A non-brominated halomethane, dichloromethane, was used in *S. typhimurium* strain TA100 (which does not contain the *GSST1-1* gene) for comparison. In contrast to bromodichloromethane-induced mutations in RSJ100, only 15% of the mutations induced by dichloromethane in the non-GST-expressing strain TA100 were GC→AT type mutations. This result suggests that over-expression of *GSTT1-1* in strain RSJ100 enhanced the mutagenicity of bromodichloromethane and induced a specific type of mutational lesion in *Salmonella*. The mutagenicity of dibromochloromethane and bromoform was also markedly enhanced in the GST-expressing strain (discussed in sections V.G.2.c and V.G.3.c below), suggesting that the brominated trihalomethanes are bioactivated by a similar pathway. In contrast, the mutagenicity of chloroform was not enhanced, indicating that chloroform and the brominated trihalomethanes may be activated via different mechanisms.

Proposed metabolic routes for GST-mediated bioactivation of bromodichloromethane to mutagenic species are illustrated in Figure III-2 in section III.C.1.

Table V-10 Summary of Mutagenicity, Genotoxicity, and Neoplastic Transformation Data for Bromodichloromethane

Endpoint	Assay System	Results (with/without activation)[d]	References
***In Vitro* Studies**			
Gene mutation	*Salmonella typhimurium* TA100[a]	NT/+	Simmon and Tardiff (1978)
	TA100[b]	-/+	Ishidate et al. (1982)
	TA98, TA100, TA1535, TA1537[b]	-/-	NTP (1987)
	TA1537 TA1535, TA98, TA100[b]	-/+ -/-	Varma et al. (1988)
	RSJ100	NT/+	DeMarini et al. (1997)
	TA1535, +GST, -GST	NT/+	Pegram et al. (1997)
	Mouse lymphoma cells[b]	+/-	NTP (1987)
	Mouse lymphoma cells	+[c]/-[c]	Sofuni et al. (1996)
Chromosome aberration	Chinese hamster fibroblasts [b]	+/-	Ishidate et al. (1982)
	Chinese hamster ovary cells [b]	-/-	NTP (1987); Anderson et al. (1990)
	Chinese hamster lung fibroblasts [a]	+/+ (weak)	Matsuoka et al. (1996)
DNA damage	*Saccharomyces cerevisiae* [a]	-/-	Nestmann and Lee (1985)
	SOS chromotest	+/+	LeCurieux et al. (1995)
	Human lung epithelial cells	NT/+	Landi et al. (2003)
	Rat and human kidney cells	NT/+	Robbiano et al. (2004)
Micronuclei formation	Rat and human kidney cells	NT/+	Robbiano et al. (2004)
Sister chromatid exchange	Human lymphocytes [a]	NT/+	Morimoto and Koizumi (1983)
	Human lymphocytes [a]	+/NT	Sobti (1984)
	Rat liver cells [a]	+/NT	Sobti (1984)
	Chinese hamster ovary cells [b]	-[c]/-	NTP (1987); Anderson et al. (1990)

Table V-10 (cont.)

Endpoint	Assay System	Results (with/without activation)[d]	References
DNA strand breaks	CCRF-CEM human lymphoblastic leukemia cells	NT/+	Geter et al. (2004a)
	F344/N primary rat hepatocytes	NT/-	Geter et al. (2004a)
In Vivo Studies			
Micronuclei	Mouse bone marrow cells and rat cells	-	Ishidate et al. (1982)
	Mouse bone marrow cells	-	Hayashi et al. (1988)
	Mouse peripheral blood erythrocytes (ip)	-	Hayashi et al. (1992)
	Rat kidney cells	+	Robbiano et al. (2004)
DNA damage	Rat kidney cells	+	Robbiano et al. (2004)
Chromosome aberrations	Rat bone marrow cells (oral)	+	Fujie et al. (1990)
	Rat bone marrow cells (ip)	+	Fujie et al. (1990)
Unscheduled DNA synthesis	Rat liver cells	-	Stocker et al. (1997)
Sister chromatid exchange	Mouse bone marrow cells	+	Morimoto and Koizumi (1983)
DNA strand breaks	Rat kidney cells	-	Potter et al. (1996)
	Rat liver, kidney, and duodenum epithelial cells	-	Geter et al. (2004a)

NT = Not Tested

[a] Assay was conducted in a closed system.

[b] Authors did not specify whether or not the assay was conducted in a closed system.

[c] Equivocal results were obtained.

[d] With/without activation applies to *in vitro* tests only.

2. Dibromochloromethane

The results of *in vivo* and *in vitro* tests conducted to evaluate the mutagenicity, genotoxicity, and neoplastic transformation potential of dibromochloromethane are summarized in Table V-11 at the end of this section.

a. *In Vitro* Assays

Simmon and Tardiff (1978) reported that nonactivated dibromochloromethane was mutagenic in *S. typhimurium* strain TA100 when assayed in a desiccator containing the test compound in the atmosphere. The minimum amount of dibromochloromethane required to elicit a mutagenic response following addition to the desiccator was 57 μmol.

Ishidate et al. (1982) assayed the mutagenicity of dibromochloromethane in *S. typhimurium* strain TA100 in the presence and absence of rat liver S9 fraction. Increased mutation frequencies were observed only in the absence of S9 activation. In contrast, chromosomal aberrations in Chinese hamster fibroblasts were observed in the presence, but not the absence, of S9 fraction. The concentrations tested in these assays were not reported.

NTP (1985) reported that dibromochloromethane (0.5 to 50 μmol/plate; 100 to 10,000 μg/plate) was not mutagenic in strains TA1535, TA1537, TA98, or TA100 when tested in the presence or absence of Aroclor-induced Sprague-Dawley rat or Syrian hamster liver S9 fractions. Volatilization of the test compound was proposed as a possible explanation for the negative results.

Nestmann and Lee (1985) investigated the mutagenicity of dibromochloromethane at concentrations of 11 to 5,700 μM in *S. cerevisiae* strains D7 and XV185-14C in the presence or absence of S9 activation. No clear increase in convertants or in revertants of strain XV185-14C were observed in the presence of S9-activated dibromochloromethane. In the absence of S9 activation, an increased incidence of gene convertants in strain D7 was observed at concentrations greater than 1,140 μM. There was no effect on the incidence of revertants under the same conditions. The high dose of dibromochloromethane was cytotoxic.

Varma et al. (1988) tested dibromochloromethane for mutagenicity in *S. typhimurium* strains TA1535, TA1537, TA98, and TA100. Dibromochloromethane produced a significantly increased mutation frequency at the lowest S9-activated concentration (0.12 μmol/plate) in all four strains. Dibromochloromethane at the same concentration also resulted in increased mutation frequencies in strains TA1535 and TA1537 in the absence of S9 fraction. Higher concentrations had no clear effect on mutation frequency. This spike in mutation frequency at the low dose with similar responses in strains that detect frameshifts and those that detect base substitutions is very unusual. It is possible that the reported data may have resulted from cytotoxicity, although the number of revertants at the nonmutagenic doses was comparable to background levels.

Dibromochloromethane induced mutations at the tk locus of L5178Y mouse lymphoma cells when tested at concentrations greater than or equal to 480 μM in screw-capped tubes. The material was tested only in the absence of S9 activation (McGregor et al., 1991).

Loveday et al. (1990) found that dibromochloromethane did not induce chromosome aberrations in CHO cells with S9-activation at concentrations that caused cell-cycle delay (12,200 μM) or in the absence of S9-activation at concentrations that were cytotoxic with a standard harvest time (6,000 μM). Sister chromatid exchange was induced in CHO cells by S9-activated dibromochloromethane at 3,600 μM with a delayed cell harvest, while the nonactivated test material had no effect at concentrations up to cytotoxic levels (1,200 μM; 247 μg/mL).

Morimoto and Koizumi (1983) investigated the ability of dibromochloromethane to induce sister chromatid exchanges (SCE) in human lymphocytes *in vitro* in the absence of S9 activation. Addition of dibromochloromethane resulted in a dose-dependent increase in SCE. The increased incidence was significant ($p < 0.05$) at concentrations greater than or equal to 400 μM.

The potential of S9-activated dibromochloromethane to induce sister chromatid exchanges *in vitro* was also investigated by Sobti (1984). A dose of 100 μM produced an increased frequency of sister chromatid exchange in rat liver cells. In human lymphocytes, 1 μM dibromochloromethane produced the same effect as 100 μM bromodichloromethane.

Fujie et al. (1993) observed a statistically significant, dose-related increase in sister chromatid exchange in rat erythroblastic leukemia K_3D cells treated with dibromochloromethane in the absence of exogenous activation. Dibromochloromethane had the weakest response among the brominated trihalomethanes tested. Dibromochloromethane also appeared to give a positive response in the presence of exogenous metabolic activation, although the study protocol and results with negative controls were less clear for this phase of testing.

LeCurieux et al. (1995) evaluated the induction of DNA damage by dibromochloromethane in the presence and absence of exogenous activation using the SOS chromotest. Dibromochloromethane exposure gave a positive result for induction. Dibromochloromethane gave negative results in the fluctuation test modification of the *S. typhimurium* reverse mutation assay.

Matsuoka et al. (1996) conducted a chromosome aberration assay with Chinese hamster lung fibroblast (CHL/IU) cells exposed to dibromochloromethane in tightly capped flasks. Dibromochloromethane induced polyploidy in the absence of S9 fraction, but not in the presence of S9. The study authors considered activated dibromochloromethane marginally positive for chromosome aberrations. However, there was no effect under the utilized test conditions when gaps were excluded from consideration. There was no evidence of structural chromosome aberration induction by dibromochloromethane in the absence of exogenous metabolic activation.

Dibromochloromethane was tested in the mouse lymphoma assay as part of an international collaborative program under the auspices of the Japanese Ministry of Health and Welfare (Sofuni et al., 1996). Dibromochloromethane yielded clearly positive results with or without exogenous metabolic activation in two laboratories.

Geter et al. (2004a) examined the ability of dibromochloromethane to induce DNA strand breaks in CCRF-CEM human lymphoblastic leukemia cells and primary F344/N rat hepatocytes *in vitro*. Strand breaks were measured using the alkaline unwinding assay. Exposure to 5 or 10 mM dibromochloromethane significantly induced DNA strand breaks in CCRF-CEM cells after a two-hour exposure. Cells exposed to 1 mM dibromochloromethane showed 100% recovery after incubation in dibromochloromethane-free medium for 22 hours. Cells exposed to 10 mM dibromochloromethane showed increased levels of DNA strand breaks, indicating a lack of recovery and possible inhibition of DNA repair. No evidence of strand breaks in the absence of cytotoxicity was observed in primary rat hepatocytes exposed to 1, 5, or 10 mM dibromochloromethane.

Landi et al. (2003) evaluated the ability of dibromochloromethane to cause DNA damage in primary cultures of human lung epithelial cells as measured by tail extent movement in the Comet assay. Human lung epithelial cells were collected by scraping the large airways of four volunteers. Cells were maintained in culture and were exposed to 0, 10, 100 or 1000 μM dibromochloromethane for 3 hours prior to being flash frozen for analysis. DNA damage was not observed at any concentration tested.

b. *In Vivo* Assays

Fujie et al. (1990) analyzed chromosome aberrations in bone marrow from Long-Evans rats (3/sex/dose) following oral (males only) or intraperitoneal (males and females) exposure to dibromochloromethane. Oral administration was by gavage in saline for five consecutive days, and the animals were sacrificed 18 hours after the last dose. Dibromochloromethane induced dose-related increases in chromosome breaks. A more pronounced increase in clastogenic activity was observed following a single intraperitoneal dose, with significant ($p < 0.05$) effects at 20.8 mg/kg. Regardless of the route, the predominant types of induced aberrations were chromatid and chromosome breaks.

Hayashi et al. (1988) measured induction of micronucleated polychromatic erythrocytes in ddY mice by intraperitoneal administration of dibromochloromethane at single doses of up to 500 mg/kg in corn oil. No evidence of clastogenicity was observed. However, the sampling time utilized in this experiment was insufficient (U.S. EPA, 1994b). There was no clear evidence of toxicity or cytotoxicity in the target tissue.

Ishidate et al. (1982) investigated the *in vivo* clastogenicity of dibromochloromethane in ddY and MS mice and Wistar rats. Doses of 125 to 500 mg/kg-day were administered in olive oil by intraperitoneal injection, and the animals were sacrificed at 18, 24, 30, 48, and 72 hours after dosing. No significant induction of micronucleus formation was observed in either mice or rats.

Morimoto and Koizumi (1983) investigated the potential of dibromochloromethane to induce sister chromatid exchanges in male ICR/SJ mice. Animals were given doses of 0, 25, 50, 100, or 200 mg/kg-day for four days by olive oil gavage. Dibromochloromethane produced a roughly linear dose-dependent increase in sister chromatid exchange frequency. The increase was statistically significant ($p < 0.05$) at 25 mg/kg-day. The authors noted that the concentrations required to produce an increased incidence of sister chromatid exchange were on the order of 1,000 to 10,000 times higher than the concentrations typically found in drinking water.

Potter et al. (1996) observed no significant effect on number of DNA strand breaks in the kidneys of male F344 rats dosed with 0.75 or 1.5 mmol/kg (156 or 312 mg/kg-day)of dibromochloromethane in 4% Emulphor® when analyzed using the alkaline unwinding procedure one day following treatment. Geter et al. (2004a) found no significant induction of DNA strand breaks in liver, kidney, or duodenum epithelial cells of male F344/N rats when assayed using the alkaline unwinding assay four hours after a single oral gavage dose (0.3 or 0.6 mmol, or approximately 62 or 125 mg/kg) of dibromochloromethane in 0.25% Emulphor. No significant induction of DNA strand breaks was observed in liver, kidney, or duodenum epithelial cells of male F344/N rats exposed to 0.6, 1.2, or 2.4 g/L dibromochloromethane in the drinking water for two weeks.

Stocker et al. (1997) investigated the *in vivo* genotoxicity of dibromochloromethane in an unscheduled DNA synthesis assay in the livers of dibromochloromethane treated rats. Male Sprague-Dawley rats (4 animals per group) were administered a single dose of 0 (control), 600 or 2000 mg/kg via gavage in aqueous 1% methylcellulose. These doses were selected by the authors to correspond to 30% and 100% of the calculated maximum tolerated dose (MTD) for this compound. Analysis of hepatocytes for unscheduled DNA synthesis was conducted 2 and 14 hours after treatment. There was no evidence of increased DNA synthesis in hepatocytes from rats treated with any tested dose of dibromochloromethane.

Sekihashi et al. (2002) obtained positive results for dibromochloromethane genotoxicity using the Comet assay. The authors indicated that doses were selected to avoid confounding of the results by cytotoxicity. In Wistar rats, positive (statistically significant differences in mean migration) results were obtained for stomach, colon, liver, kidney, bladder, or lung tissues removed 8 or 24 hours following administration of 200 mg/kg oral dose of. using. In ddY mice, positive results were obtained for liver and brain samples harvested 8 or 24 hours, respectively, after administration of a 400 mg/kg oral dose. Although a statistically significant increase in migration was also noted for the eight hour colon sample, the study authors did not identify this finding as a positive response.

c. Mechanistic Studies of Genotoxicity

DeMarini et al. (1997) investigated the role of glutathione S-transferase activity in the mutagenicity of dibromochloromethane in *Salmonella typhimurium.* Strains of *Salmonella* utilized in this investigation included RSJ100, which expresses the rat glutathione S-transferase theta 1-1 (*GSTT1-1*) gene and TPT100, which has the *GSTT1-1* gene inserted in a non-

functioning orientation. Dibromochloromethane (400 ppm) induced an 85-fold increase in revertant colonies in the RSJ100 strain of *Salmonella* compared to background revertant formation. The mutational spectra for dibromochloromethane-induced mutations at the *his*G46 allele in strain RSJ100 were analyzed using the colony probe hybridization method. This analysis revealed that 100% of the mutations were GC→AT. A non-brominated dihalomethane, dichloromethane, was tested in TA100 (which does not express GSTT1-1) for comparison. In contrast to dibromochloromethane-induced mutations in RSJ100, only 15% of the mutations induced by dichloromethane in TA100 were GC→AT type mutations. This result suggests that over-expression of *GSTT1-1* in strain RSJ100 mediated the mutagenicity of dibromochloromethane and induced a specific type of mutational lesion in *Salmonella*. Proposed pathways of bioactivation of dibromochloromethane and other brominated trihalomethanes are shown in Figure 4-2.

Landi et al. (1999b) investigated the role of GSST1-1 in the mutagenicity of dibromochloromethane in *Salmonella* by using one strain that expressed rat GSST1-1 (RSJ100) and one strain that did not (TPT100). Mutagenicity of dibromochloromethane was assessed by revertant colony formation with or without S9 metabolic activation. The addition of 800 ppm dibromochloromethane greatly increased revertant numbers in the RSJ100 but not the TPT100 strain of *Salmonella*. Addition of the rat liver S9 fraction had no effect on the number of revertants induced by dibromochloromethane exposure in either strain. These data provide further support for the hypothesis that GSST1-1 plays a role in the mutagenicity of dibromochloromethane. Additional experiments were conducted to investigate the effects of exogenously added GSST1-1 on the mutagenic potency of dibromochloromethane. Red blood cells (RBC), which express GSST1-1, were added to the experimental system to address this question. RBC had no effect on results obtained with the TPT100 strain, but completely suppressed the mutagenicity of dibromochloromethane in the RSJ100 strain. However, the 'protective' effect of RBC did not appear to be related to GSST1-1 activity, as this suppression occurred even with the addition of RBC from individuals who do not express GSST1-1. The underlying mechanism of RBC suppression of dibromochloromethane mutagenicity was not investigated. The authors of this study speculated that tissues potentially exposed to dibromochloromethane via the blood may be at less genotoxic risk (due to protection afforded by the RBC) than tissues which are directly exposed to oral bromodichloromethane (such as tissues in the gastrointestinal tract).

Proposed metabolic routes for GST-mediated bioactivation of dibromochloromethane to mutagenic species are shown in Figure III-2 in section III.C1.

Table V-11 Summary of Mutagenicity, Genotoxicity, and Neoplastic Transformation Data for Dibromochloromethane

Endpoint	Assay System	Results (with/without activation)[d]	References
***In Vitro* Studies**			
Gene mutation	*Salmonella typhimurium* TA100[a]	NT/+	Simmon and Tardiff (1978)
	TA100[b]	-/+	Ishidate et al. (1982)
	TA98, TA100, TA1535, TA1537[b]	-/-	NTP (1985)
	TA1535, TA1537[b] TA98, TA100[b]	+/+ -/+	Varma et al. (1988)
	RSJ100	NT/+	DeMarini et al. (1997)
	RSJ100 TPT100	+/+ -/-	Landi et al. (1999b)
	Mouse lymphoma cells[a]	NT/+	McGregor et al. (1991)
	Mouse lymphoma cells	+/+	Sofuni et al. (1996)
Chromosome aberration	Chinese hamster fibroblasts[b]	+/-	Ishidate et al. (1982)
	Chinese hamster ovary cells[b]	-/-	Loveday et al. (1990)
	Chinese hamster lung fibroblasts[a]	-/+ (see text)	Matsuoka et al. (1996)
DNA damage	*Saccharomyces cerevisiae*[a]	-/+	Nestmann and Lee (1985)
	SOS chromotest *S. typhimurium* fluctuation test	+/+ -	LeCurieux et al. (1995)
	Human lung epithelial cells	NT/-	Landi et al. (2003)
Sister chromatid exchange	Human lymphocytes[a]	NT/+	Morimoto and Koizumi (1983)
	Human lymphocytes[a]	+/NT	Sobti (1984)
	Rat liver cells[b]	+/NT	Sobti (1984)
	Chinese hamster ovary cells[b]	+/-	Loveday et al. (1990)
	Rat erythroblastic leukemia cells	-[c]/+	Fujie et al. (1993)

Table V-11 (cont.)

Endpoint	Assay System	Results (with/without activation)[d]	References
DNA strand breaks	CCRF-CEM human lymphoblastic leukemia cells	NT/+	Geter et al. (2004a)
	F344/N primary rat hepatocytes	NT/-	Geter et al. (2004a)
In Vivo Studies			
Micronuclei	Mouse bone marrow cells	-	Ishidate et al. (1982)
	Mouse bone marrow cells	-	Hayashi et al. (1988)
Chromosome aberrations	Rat bone marrow cells	+	Fujie et al. (1990)
	Rat bone marrow cells	+	Fujie et al. (1990)
Sister chromatid exchange	Mouse bone marrow cells	+	Morimoto and Koizumi (1983)
DNA strand breaks	Rat kidney cells	-	Potter et al. (1996)
	Rat liver, kidney, duodenum epithelial cells	-	Geter et al. (2004a)
DNA damage (comet assay)	Rat stomach, colon, liver, kidney, bladder, lung tissue	+	Sekihashi et al. (2002)
	Mouse liver and brain tissue	+	Sekihashi et al. (2002)
Unscheduled DNA synthesis	Rat hepatocytes	-	Stocker et al. (1997)

NT = Not Tested
[a] Assay was conducted in a closed system.
[b] Authors did not specify whether or not the assay was conducted in a closed system.
[c] Equivocal results reported.
[d] With/without activation applies to *in vitro* data only.

3. Bromoform

The results of *in vivo* and *in vitro* tests conducted to evaluate the mutagenicity, genotoxicity, and neoplastic transformation potential of bromoform are summarized in Table V-12 at the end of this section.

a. *In Vitro* Assays

Simmon and Tardiff (1978) reported that nonactivated bromoform was mutagenic in *S. typhimurium* strain TA100 when assayed as vapor in a desiccator. The minimum amount of bromoform required to elicit a mutagenic response following addition to the desiccator was 570 μmol.

Ishidate et al. (1982) assayed the mutagenicity of bromoform in *S. typhimurium* strain TA100 in the presence and absence of rat liver S9 fraction. Increased mutation frequencies were observed only in the absence of S9 activation. In contrast, chromosomal aberrations in Chinese hamster fibroblasts were observed in the presence, but not the absence, of S9 fraction. The concentrations tested in these assays were not reported.

Maddock and Kelly (1980) reported that bromoform did not induce an increase in sister chromatid exchanges when toadfish leukocytes were exposed to concentrations of 0.4 to 400 μM.

Herren-Freund and Pereira (1986) assessed the initiating activity of bromoform using the rat liver GGT-foci assay. The authors reported that a 250 mg/kg (1 mmol/kg) oral dose in an unspecified vehicle did not initiate GGT-foci in this test.

NTP (1989a) evaluated the genotoxic potential of bromoform in multiple test systems. Concentrations of 0.04 to 13 μmol/plate (10 to 3,333 μg/plate) produced no evidence of mutagenicity in *S. typhimurium* strains TA1535 or TA1537, when assayed with or without exogenous metabolic activation by rat or hamster liver S9 fraction. Equivocal evidence of mutagenicity was noted in strain TA100 without activation, and in strains TA97 and TA98 in the presence of liver microsomes prepared from Aroclor-induced Syrian hamsters. Exposure of mouse L5178Y cells to bromoform concentrations greater than or equal to 2,300 μM in the absence of S9 activation or S9-activated concentrations of at least 300 μM with S9 activation resulted in forward mutations at the thymidine kinase (tk) locus. One of two laboratories conducting the assays reported increased sister chromatid exchanges (SCE) in CHO cells exposed to 3,800 μM bromoform in the absence of exogenous activation. Neither laboratory observed increased incidence of SCE in the presence of S9. S9-activated bromoform did not induce chromosome aberrations in CHO cells; results for SCE and chromosome aberrations in the absence of exogenous activation were equivocal.

Zeiger (1990) found that bromoform was mutagenic in *S. typhimurium* strain TA98 when tested as a vapor in a closed system, but not when tested in an open system using a preincubation protocol. Positive results were observed at levels of at least 114 μmol/desiccator, in the presence and absence of S9 prepared from rat or hamster liver. Bromoform was negative in the closed system with strains TA100 and TA1538 with or without rat or hamster liver S9 fraction

Roldan-Arjona and Pueyo (1993) evaluated bromoform in the *S. typhimurium Ara* forward mutation assay at concentrations up to 25 μmol/plate (6.3 mg/plate). A preincubation protocol was employed for the assay. Although a clear dose-related response was observed in the absence

of activation, the results were classified as questionable because a doubling of background levels was not achieved. There was no evidence of mutagenicity in the presence of exogenous metabolic activation. Although no attempt was made to minimize volatilization of the test compound, cytotoxicity at the high exposure level indicated that the test material reached the cells.

Geter et al. (2004a) examined the ability of bromoform to induce DNA strand breaks (as assessed by the alkaline unwinding assay) in CCRF-CEM human lymphoblastic leukemia cells and primary F344/N rat hepatocytes. Exposure to 5 or 10 mM bromoform significantly induced DNA strand breaks in CCRF-CEM cells after a two-hour incubation. Cells exposed to 1 mM bromoform showed increased levels of DNA strand breaks when assayed after incubation in bromoform-free medium for 22 hours, indicating a lack of recovery; recovery at higher concentrations could not be assessed because of cytotoxicity. No evidence of strand breaks in the absence of cytotoxicity was observed in primary rat hepatocytes exposed to 1, 5, or 10 mM bromoform.

Landi et al. (2003) evaluated the ability of bromoform to induce DNA damage in primary cultures of human lung epithelial cells as measured by tail extent movement in the Comet assay. Human lung epithelial cells were collected by scraping the large airways of four volunteers. Cells were maintained in culture and were exposed to 0, 10, 100 or 1000 μM bromodichloromethane for 3 hours prior to being flash frozen for analysis. DNA damage was observed at each concentration tested.

b. *In Vivo* Assays

NTP (1989a) studied the genotoxic potential of bromoform in several test systems. Adult male *Drosophila* fed with a 1,000-ppm solution of bromoform exhibited increased frequency of sex-linked recessive lethal mutations, but no significant effect on reciprocal translocations was observed. Intraperitoneal injection of mice with 200 to 800 mg/kg bromoform caused an increase in sister chromatid exchange but not in chromosomal aberrations in bone marrow cells.

Fujie et al. (1990) analyzed chromosome aberrations in bone marrow from Long-Evans rats (3/sex/dose) following oral (males only) or intraperitoneal (males and females) exposure to bromoform. Oral administration was by gavage in saline for five consecutive days, and the animals were sacrificed 18 hours after the last dose. Bromoform induced a dose-related increase in the incidence of aberrant cells, with a significant ($p < 0.01$) increase at 253 mg/kg-day. A more pronounced increase in clastogenic activity was observed following a single intraperitoneal dose, with a significant ($p < 0.05$) effect at 25.3 mg/kg. Regardless of the route, the predominant types of induced aberrations were chromatid and chromosome breaks.

Morimoto and Koizumi (1983) investigated the ability of bromoform and other brominated trihalomethanes to induce sister chromatid exchanges in human lymphocytes *in vitro* in the absence of S9 activation. All three brominated trihalomethanes caused a dose-dependent increase in sister chromatid exchanges. Bromoform was more potent than bromodichloromethane

or dibromochloromethane. The increases were significant ($p < 0.05$) at concentrations greater than or equal to 400 μM, 400 μM, and 80 μM for bromodichloromethane, dibromochloromethane, and bromoform, respectively.

Potter et al. (1996) evaluated the effect of bromoform on incidence of DNA strand breaks in the kidney. Male F344 rats received 0.75 or 1.5 mmol/kg of bromoform in 4% Emulphor® by gavage for 1, 3, or 7 days. These doses corresponded to 190 or 379 mg/kg. No effect was observed on strand breaks when evaluated using the alkaline unwinding procedure one day after a single dose.

Stocker et al. (1997) investigated the *in vivo* genotoxicity of bromoform in the mouse bone marrow micronuclei assay and by analysis of unscheduled DNA synthesis in the liver of bromoform-treated rats. In the first assay, Swiss CD mice (5/sex/dose) were treated by gavage with doses of 0, 250, 500, or 1,000 mg/kg bromoform dissolved in aqueous 1% methylcellulose. Micronuclei analysis was conducted 24 and 48 hours after dosing, and was negative in all dose groups. In the second assay, male Sprague-Dawley rats (4 animals/dose) received single doses of 0, 324 or 1,080 mg/kg bromoform by gavage in aqueous 1% methylcellulose. These doses were selected by the authors to correspond to 30% and 100% of the calculated MTD for this compound. Analysis of hepatocytes for unscheduled DNA synthesis was conducted 2 and 14 hours after treatment. There was no evidence of increased DNA synthesis in hepatocytes from rats treated with any tested dose of bromoform.

Geter et al. (2004a) did not observe significant induction of DNA strand breaks in liver, kidney, or duodenum epithelial cells of male F344/N rats when evaluated using the alkaline unwinding assay four hours after administration of a single oral gavage dose (0.3 or 0.6 mmol, or approximately 76 and 152 mg/kg) of bromoform in 0.25% Emulphor. No evidence of induction of DNA strand breaks was observed in liver, kidney, or duodenum epithelial cells of male F344/N rats exposed to 0.6, 1.2, or 2.4 g/L bromoform in the drinking water for two weeks.

c. Mechanistic Studies of Genotoxicity

DeMarini et al. (1997) investigated the role of glutathione S-transferase activity in the mutagenicity of bromoform in *Salmonella typhimurium.* Strains of *Salmonella* utilized for this investigation included RSJ100, which expresses the rat glutathione *S*-transferase theta 1-1 (*GSTT1-1*) gene and TPT100, which has the *GSTT1-1* gene inserted in a non-functioning orientation. Exposure to1,600 ppm bromoform induced a 95-fold increase in revertant colonies in the RSJ100 strain of *Salmonella* compared to background revertant formation. The mutational spectra for bromoform-induced mutations at the *his*G46 allele in strain RSJ100 were analyzed using the colony probe hybridization method. This analysis revealed that 96% of the mutations were GC→AT transitions. Bromoform also induced a smaller percentage (2.8%) of GC→TA mutations. A non-brominated halomethane, dichloromethane, was used in *S. typhimurium* strain TA100 (which does not express GSST1-1) for comparison. In contrast to bromoform-induced mutations in RSJ100, only 15% of the mutations induced by dichloromethane in TA100 were GC→AT type mutations. This result suggests that over-expression of GSTT1-1 in strain RSJ100

mediated the mutagenicity of bromoform and induced a specific type of mutational lesion in *Salmonella*.

Landi et al. (1999a) investigated the mutagenicity of bromoform in *in vitro* exposed human lymphocytes from both glutathione-S-transferase theta positive (*GSST1-1+*) and negative (*GSST1-1-*) individuals. Whole blood cultures were exposed to bromoform (10^{-2} to 10^{-4} M) and assayed for DNA breaks with the cometassay. The DNA-damaging potency of bromoform was not significantly different in lymphocytes (the target cell for the comet assay) from *GSST1-1+* and *GSST1-1-* individuals. However, lymphocytes do not express *GSST1-1*, even in *GSST1-1+* individuals, so interpretation of these data is problematic. When data were combined from both genotypic groups, there was a weak but statistically significant induction of comets observed following treatment with bromoform.

Proposed pathways for bioactivation of bromoform to mutagenic species are shown in Figure III-2 in section III.C.1.

Table V-12 Summary of Mutagenicity, Genotoxicity, and Neoplastic Transformation Data for Bromoform

Endpoint	Assay System	Results (with/without activation)[d]	References
***In Vitro* Studies**			
Gene mutation	*Salmonella typhimurium* TA100[a], TA1535	NT/+	Simmon and Tardiff (1978)
	TA1535, TA1537[b] TA100 TA97, TA98	-/- -/±[c] ±[c]/-	NTP (1989a)
	TA100[b]	-/+	Ishidate et al. (1982)
	TA98 TA100, TA1538[a]	+/+ -/-	Zeiger (1990)
	S. typhimurium Ara	-/+[c]	Roldan-Arjona and Pueyo (1993)
	RSJ100	NT/+	DeMarini et al. (1997)
	Mouse lymphoma cells [b]	+/+	NTP (1989a)
Chromosome aberration	Chinese hamster fibroblasts [b]	+/-	Ishidate et al. (1982)
	Chinese hamster ovary cells [b]	-/±	NTP (1989a)
DNA damage	Human lymphocytes	NT/+	Landi et al. (1999a)
	Human lung epithelial cells	NT/+	Landi et al. (2003)
Sister chromatid exchange	Toadfish leukocytes [a]	NT/-	Maddock and Kelly (1980)
	Human lymphocytes [b]	NT/+	Morimoto and Koizumi (1983)
	Chinese hamster ovary cells [b]	-/±	NTP (1989a)
Initiation	Rat liver GGT-foci assay	-	Herren-Freund and Pereira (1986)
DNA strand breaks	CCRF-CEM human lymphoblastic leukemia cells	NT/+	Geter et al. (2004a)
	F344/N primary rat hepatocytes	NT/-	Geter et al. (2004a)
In Vivo Studies			
Micronuclei	Mouse bone marrow cells	-	Ishidate et al. (1982)
	Mouse bone marrow cells	-	Hayashi et al. (1988)

Table V-12 (cont.)

Endpoint	Assay System	Results (with/without activation)[d]	References
	Mouse bone marrow cells	-	Stocker et al. (1997)
Chromosome aberrations	Mouse bone marrow cells	-	NTP (1989a)
	Rat bone marrow cells (oral) Rat bone marrow cells (ip)	+ +	Fujie et al. (1990)
DNA strand breaks	Rat renal cells	-	Potter et al. (1996)
	Rat liver, kidney, or duodenum epithelial cells	-	Geter et al. (2004a)
Unscheduled DNA synthesis	Rat hepatocytes	-	Stocker et al. (1997)
Sister chromatid exchange	Mouse bone marrow cells	+	Morimoto and Koizumi (1983)
	Mouse bone marrow cells (ip)	+	NTP (1989a)
Sex-linked recessive lethal mutations	*Drosophila*	+	NTP (1989a)

NT = Not Tested
[a] Assay was conducted in a closed system.
[b] Authors did not specify whether or not the assay was conducted in a closed system.
[c] Equivocal results obtained.
[d] With/without activation applies to *in vitro* assays only.

G. Carcinogenicity

1. Bromodichloromethane

a. Two-Year Oral Cancer Bioassays

NTP (1987) evaluated the carcinogenic potential of bromodichloromethane in F344/N rats in a two-year oral exposure study. Additional details of this study are provided in Section V.D.1. Groups of male and female rats (50/sex/group) were administered bromodichloromethane in corn oil via gavage at doses of 0, 50, or 100 mg/kg-day for 5 days/week for 102 weeks. All animals were examined grossly and microscopically for neoplastic lesions. Survival of all dosed animals was comparable to or greater than the corresponding control group. Mean body weights of high-dose male and female rats were decreased during the last 1.5 years of the study. Body weight gains of high-dose male and female rats at study termination were 86% and 70% of the corresponding vehicle control group, respectively. Statistically significant increases in the

incidences of neoplasms of the large intestine and kidney were observed in male and female rats (Table V-13). The study authors noted that neoplasms of the large intestine and kidney are uncommon tumors in F344/N rats based on historical control data for NTP studies. They concluded that under the conditions of these 2-year gavage studies, clear evidence of carcinogenicity existed in male and female rats.

NTP (1987) also evaluated the potential toxic and carcinogenic effects of bromodichloromethane mice in a two-year oral exposure study. Additional details of this study are provided in Section V.D.1. Groups of male and female $B6C3F_1$ mice (50/sex/dose) were administered doses of 0, 25, or 50 mg/kg-day (males) or 0, 75, or 150 mg/kg-day (females) for 5 days/week for 102 weeks. All animals were examined grossly and microscopically for neoplastic lesions. Survival of dosed male mice was comparable to the corresponding control group. Survival of dosed and vehicle control females was decreased after week 84 as a result of ovarian abscesses. Body weight gain in high-dose males was decreased by 13% when compared to the vehicle control group. Body weight gain in low- and high-dose females was reduced by 25% and 55%, respectively. Statistically significant increases were observed in the incidences of neoplasms of the kidney in male mice and the liver in female mice (Table V-13). The study authors noted that neoplasms of the kidney are uncommon in $B6C3F_1$ mice based on NTP historical control data. They concluded that under the conditions of these 2-year gavage studies, clear evidence of carcinogenic activity existed in male and female mice.

Tumasonis et al. (1987) exposed groups of 58 male and female Wistar rats to bromodichloromethane in drinking water from weaning until death occurred in all of the animals (approximately 185 weeks). The exposure level was 2,400 mg/L for 72 weeks and was reduced to 1,200 mg/L for the remaining 113 weeks. Based on a graph presented by the authors, the average dose over the course of the experiment was probably about 150 mg/kg-day for females and about 100 mg/kg-day for males. Exposed animals of both sexes gained significantly less weight (approximately 30 to 40%) than control animals. There was a statistically significant ($p < 0.01$) increase in the incidence of hepatic neoplastic nodules in exposed females compared to control females (32% versus 0%). Significant increases were also reported for the occurrence of hepatic adenofibrosis (12% versus 0%) and lymphosarcoma (17% versus 11%) in females. No statistically significant increase in the incidence of any tumor was reported in males. Two males and one female among the treated animals were observed to have renal adenoma or carcinoma, while no renal tumors were observed in the controls. Statistically significant decreases in the incidence of mammary tumors and pituitary tumors in females and lymphosarcomas in males were observed.

Table V-13 Tumor Frequencies in F344/N Rats and B6C3F$_1$ Mice Exposed to Bromodichloromethane in Corn Oil for 2 Years - Adapted from NTP (1987)

Animal	Tissue/Tumor		Tumor Frequency		
			Control	**50 mg/kg**	**100 mg/kg**
Male Rat	Large intestine [a]	Adenomatous polyp	0/50	3/49	33/50[b]
		Adenocarcinoma	0/50	11/49[b]	38/50[b]
		Combined	0/50	13/49[b]	45/50[b]
	Kidney [a]	Tubular cell adenoma	0/50	1/49	3/50
		Tubular cell adenocarcinoma	0/50	0/49	10/50[b]
		Combined	0/50	1/49	13/50[b]
			Control	**50 mg/kg**	**100 mg/kg**
Female Rat	Large intestine [c]	Adenomatous polyp	0/46	0/50	7/47[b]
		Adenocarcinoma	0/46	0/50	6/47[b]
		Combined	0/46	0/50	12/47[b]
	Kidney	Tubular cell adenoma	0/50	1/50	6/50[b]
		Tubular cell adenocarcinoma	0/50	0/50	9/50[b]
		Combined	0/50	1/50	15/50[b]
			Control	**25 mg/kg**	**50 mg/kg**
Male Mouse	Kidney [d]	Tubular cell adenoma	1/46	2/49	6/50
		Tubular cell adenocarcinoma	0/46	0/49	4/50
		Combined	1/46	2/49	9/50[b]
			Control	**75 mg/kg**	**150 mg/kg**
Female Mouse	Liver	Hepatocellular adenoma	1/50	13/48[b]	23/50[b]
		Hepatocellular carcinoma	2/50	5/48	10/50[b]
		Combined	3/50	18/48[b]	29/50[b]

[a] One rat in the low-dose group died at week 33 and was eliminated from the cancer risk calculation.
[b] Statistically significant at $p<0.05$, compared to controls.
[c] Intestine not examined in four rats from control group and three rats from high-dose group.
[d] In the control group, two mice died during the first week, one mouse died during week nine and one escaped in week 79. One mouse in the low-dose group died in the first week. All of these mice were eliminated from the cancer risk calculations.

Aida et al. (1992b) administered bromodichloromethane to Slc:Wistar rats (40/sex/treatment group and 70/sex/controls) at dietary levels of 0%, 0.014%, 0.055%, or 0.22% for up to 24 months. The test material was microencapsulated and mixed with powdered feed. Based on the mean food intakes, the mean doses were 0, 6.1, 25.5, or 138.0 mg/kg-day for males and 0, 8.0, 31.7, or 168.4 mg/kg-day for females. The only neoplastic lesions observed were three cholangiocarcinomas and two hepatocellular adenomas in the high-dose females, one hepatocellular adenoma in a control female, one cholangiocarcinoma in a high-dose male, and one hepatocellular adenoma each in a low-dose male and a high-dose male. Based on these results, the study authors concluded that there was no clear evidence that microencapsulated bromodichloromethane administered in the diet was carcinogenic in Wistar rats.

Voronin et al. (1987) assessed the carcinogenic potential of bromodichloromethane in male and female CBA x C57Bl/6 mice. Groups of mice (50-55/sex/concentration) were exposed to bromodichloromethane provided in drinking water at concentrations of 0.04, 4.0, or 400 mg/L for 104 weeks. Untreated control groups of 75 male and 50 female mice were also included in the study design. No significant differences were observed in total tumor incidence when evaluated by Chi square analysis. The study authors concluded that, under the conditions of this bioassay, bromodichloromethane was not carcinogenic in mice.

George et al. (2002) evaluated the carcinogenicity of bromodichloromethane in male F344/N rats (78 animals/dose) exposed to the compound via drinking water for 104 weeks. Nominal concentrations of 0.07, 0.35, or 0.70 g/L were administered in drinking water containing 0.25% Emulphor®. The vehicle control solution consisted of 0.25% Emulphor®. The study authors indicated that testing of higher concentrations was prevented by refusal of the test animals to drink solutions containing more than 0.7 g/L. Six animals per exposure concentration were sacrificed at 13, 26, 52, and 78 weeks for gross observation and histopathological examination of the thyroid, liver, stomach, duodenum, jejunum, ileum, colon, rectum, spleen, kidneys, urinary bladder, and testes. A complete rodent necropsy was performed at terminal sacrifice and representative samples of the tissues listed above were examined microscopically. A complete pathological examination was performed on five rats from the high-dose group. Serum profiles of LDH, ALT, ALP, AST, SDH, BUN, total protein, creatine, and total antioxidant activities were determined at 26, 52, and 104 weeks. Hepatocyte and renal tubular cell proliferation were measured at each sacrifice by bromodeoxyuridine labeling.

The measured drinking water concentrations of bromodichloromethane were 0.06, 0.38, and 0.76 g/L. When corrected for loss of bromodichloromethane as a result of volatility, instability, or adsorption to glass surfaces during treatment, the corresponding administered concentrations were 0.06, 0.33, and 0.62 g/L. Based on measured water consumption, these levels correspond to mean daily doses for the entire study of 3.9, 20.6, and 36.3 mg/kg-day as calculated by the study authors. Mean daily doses of 6.4, 32.6, and 58.9 mg/kg-day were calculated for the first 13 weeks of the study when the growth rate of the test animals was highest. No significant differences were observed among groups for feed consumption or survival. Twenty-one to 22 unscheduled deaths were observed in each treatment group. Mononuclear cell leukemia was seen in all dose groups and was reported to be the primary cause of morbidity and

mortality prior to 104 weeks. Exposure to bromodichloromethane did not affect the growth rate of test animals when compared to the control. Kidney weight was significantly depressed at the high dose and a significant negative trend was observed for relative kidney weight. No significant changes were observed in clinical chemistry parameters. Observed nonneoplastic changes in the liver (e.g., biliary fibrosis, bile duct inflammation, and chronic inflammation) were considered to be age-related background changes, since neither the incidence nor severity of the lesions differed from the control values. Bromodichloromethane had no effect on hepatocyte proliferation as measured by bromodeoxyuridine labeling. Renal tubular cell hyperplasia was significantly decreased in the 3.9 mg/kg-day group and significantly increased in the 36.3 mg/kg-day group (15.8%) relative to the control value (8.7%).

The absence of effect on body weight and other examined endpoints suggests that a maximum toxic dose may not have been achieved in this study. However, the dosing regimen used by George et al. (2002) was sufficient to increase the incidence of hepatocellular neoplasia (Table V-14). The data for hepatic tumors indicate a biphasic pattern of dose-response. The prevalence and multiplicity of hepatocellular adenoma and combined hepatocellular adenoma and carcinoma were significantly increased at 3.9 mg/kg-day, nonsignificantly increased at 20.6 mg/kg-day, and comparable to the control values at 36.3 mg/kg-day. The prevalence and multiplicity of hepatocellular carcinoma were increased at 20.6 mg/kg-day when compared to control values, but the response did not reach statistical significance. The underlying basis for the biphasic response is unknown, but the study authors noted that the observed pattern of response could be explained by inhibition of the hepatic metabolism of bromodichloromethane by the compound itself. Exposure to bromodichloromethane decreased the prevalence of basophilic (control, 67%; 3.6 mg/kg-day, 62.2%; 20.6 mg/kg-day, 46%; 36.6 mg/kg-day, 34.7%) and clear cell (17.8%, 2.2%, 2.1%, 4.1%) altered foci of cells (AFC) in a dose-dependent manner, but had no significant effect on the prevalence of eosinophilic AFCs when compared to the controls. The decreases in prevalence were statistically significant at the mid and high doses for basophilic AFCs and at all doses for clear cell AFCs. One renal tubular adenoma was observed in the 3.6 mg/kg-day group and two tumors were observe in the 36.3 mg/kg-day (Table V-14). . The historical incidence of renal tubular adenomas in male F344/N rats is very low (2/327 or 0.6%), as determined from control groups in NTP drinking water studies. Therefore, the occurrence of these tumors in the present study may be of biological significance. No increased incidences of neoplasia were evident in the five high-dose animals selected for a histopathological examination of all organs.

On the basis of the increased prevalence and multiplicity of hepatocellular neoplasms in the 3.9 and 20.6 mg/kg-day groups, the study authors concluded that bromodichloromethane was carcinogenic in male F344/N rats under the conditions of the bioassay. A source of uncertainty in this conclusion is lack of knowledge on the biological mechanism underlying the dose-response relationship observed for hepatic tumors.

Table V-14 Hepatic and Renal Tumors in Male F344/N Rats Administered Bromodichloromethane in the Drinking Water for Two Years (George et al., 2002)

Tumor Type	Mean Daily Dose of Bromodichloromethane (mg/kg-day)			
	Vehicle Control	3.9	20.6	36.3
Liver				
Hepatocellular adenoma	1/45 (2.2%)[a] 0.02 ± 0.02[b,c]	7/45 (15.5%)* 0.16 ± 0.04*	3/48 (6.2%) 0.06 ± 0.02	2/49 (4.1%) 0.04 ± 0.02
Hepatocellular carcinoma	1/45 (2.2%) 0.02 ± 0.02	1/45 (2.2%) 0.02 ± 0.01	4/48 (8.3%) 0.10 ± 0.03	2/49 (4.1%) 0.04 ± 0.02
Hepatocellular adenoma and carcinoma (combined)	2/45 (4.4%) 0.04 ± 0.02	8/45 (17.8%)* 0.19 ± 0.00*	7/48 (14.6%) 0.17 ± 0.04	4/49 (8.2%) 0.08 ± 0.28
Kidney				
Tubular cell adenoma	0/46 (0%)	1/45 (2.2%)	0/51 (0%)	2/44 (4.5%)
Tubular cell carcinoma	0/46 (0%)	0/45 (0%)	0/51 (0%)	0/44 (0%)
Tubular cell adenoma or carcinoma (combined)	0/46 (0%)	1/45 (2.2%)	0/51 (0%)	2/44 (4.5%)

Source: George et al. (2002)
* Statistically significant when compared to the control value, $p \leq 0.05$
[a] Prevalence (percentage of animals with tumor)
[b] Multiplicity, number of tumors per animal
[c] Mean ± standard deviation

George et al. (2002) also evaluated the carcinogenicity of bromodichloromethane in male $B6C3F_1$ mice (78 animals/dose) exposed via drinking water for 100 weeks. Nominal concentrations of 0.05, 0.25, or 0.50 g/L were administered in drinking water containing 0.25% Emulphor®. The vehicle control solution consisted of 0.25% Emulphor®. Seven animals per exposure concentration were sacrificed at 13, 26, 52, and 78 weeks for gross observation and histopathological examination of the liver, stomach, duodenum, jejunum, ileum, colon, rectum, spleen, kidneys, urinary bladder, and testes. A complete rodent necropsy was performed at terminal sacrifice and representative samples of the tissues listed above were examined microscopically. A complete pathological examination was performed on five rats from the high-dose group. Serum profiles of LDH, ALT, ALP, AST, SDH, BUN, total protein, creatine, and total antioxidant activities were determined at 26, 52, and 100 weeks. Hepatocyte and renal tubular cell proliferation were measured by bromodeoxyuridine labeling at each sacrifice.

The measured drinking water concentrations of bromodichloromethane were 0.06, 0.30, and 0.55 g/L. When corrected for loss of bromodichloromethane as a result of volatility,

instability, or adsorption to glass surfaces during treatment, the corresponding administered concentrations were 0.06, 0.28, and 0.49 g/L. Based on measured water consumption, these levels correspond to mean daily doses of 8.1, 27.2, and 43.4 mg/kg-day as calculated by the study authors. Water consumption was significantly reduced at the mid- and high doses; the study authors attributed the reduced intake to taste aversion. No significant differences were observed among groups for feed consumption or survival. Exposure to bromodichloromethane did not affect the growth rate of test animals when compared to the control. Kidney weight was significantly depressed at 27.2 and 43.4 mg/kg-day when compared to the control values. No significant changes were observed in clinical chemistry parameters. Mild, treatment-related nonneoplastic hepatic lesions were observed in the 27.2 and 43.4 mg/kg-day dose groups (identity and prevalence not reported). Increased incidences of hepatocellular karyomegaly and necrosis with inflammation (prevalence and severity not reported) were not dose-related. The prevalence of renal tubular hyperplasia was 3%, 0%, 6% and 0% for the vehicle control, 8.1, 27.2, and 43.4 mg/kg-day groups, respectively. Other observed preneoplastic and neoplastic lesions (identity and prevalence not reported) were considered background events for the male $B6C3F_1$ mouse. BrdU labeling index in hepatocytes and renal tubular cells was not altered at any time point. Hepatocellular adenomas and carcinomas were observed in all treatment groups. Neither the prevalence nor multiplicity of these tumors was significantly increased by exposure to bromodichloromethane. Renal tubular cell neoplasia was not observed in any treatment group. No increased incidences of neoplasia were evident in the five high-dose animals subject to a full histopathological examination. On the basis of these data, the study authors concluded that bromodichloromethane was not carcinogenic to male mice under the conditions employed in this study. However, it is not evident that an adequately high dose was tested in this study.

b. Studies of Induction of Aberrant Crypt Foci

DeAngelo et al., (2002) evaluated the ability of bromodichloromethane administered in drinking water to induce aberrant crypt foci (ACF), putative early preneoplastic lesions, in the colons of male F344/N rats. Groups of weanling rats (6 animals/group) were exposed to distilled water, 0.25% Alkamuls EL-620®, or 0.7 g/L bromodichloromethane in 0.25% Alkamuls EL-620 for 13 weeks. A single intraperitoneal injection of 30 mg/kg azoxymethane (AOM) served as the positive control. Body weight and water consumption were measured twice during the first week of the study and once per week thereafter. Colons were collected at study termination, fixed, stained with 0.2% methylene blue, divided into three equal segments, and scanned for ACF. The measured concentration of bromodichloromethane averaged 0.64 ± 0.06 g/L (mean and standard error) over the course of the study. When adjusted for volatilization and adherence to glass, the corrected concentration was 0.51 g/L. Water consumption was significantly reduced (39%) in the bromodichloromethane exposure group when compared to the 0.25% vehicle control. The average daily dose was 45 mg/kg-day as calculated by the study authors. Average terminal body weights of the rats exposed to bromodichloromethane were within 10% of the control values. No ACF were observed in colons from control animals. ACF were observed in 5/6 colons from bromodichloromethane-exposed animals. The total number of AC/focus (30), average number of AC/focus (3.33 ± 0.47), ACF/colon (1.50 ± 0.56), and total and average focal area (550 and 61.11 μm^2, respectively) were significantly increased relative to the combined deionized water and

vehicle controls. All observed ACF were located in the distal (rectal) segment of the colon. For comparison, 807 ACF and 4.95 ± 0.25 crypts per focus (mean and standard error) were observed in the AOM positive control group. Eight percent, 42% and 50% of the ACF induced by AOM were located in the proximal, middle, and distal segment of the colon, respectively. The study authors reported that the localization of ACF in the distal segment of the colon is consistent with the observed sites for tumor formation in the large intestine of rats administered bromodichloromethane in corn oil for two years (NTP, 1987). However, the study authors noted that ACF induced by bromodichloromethane administered in drinking water at the study test dose do not appear to progress to neoplasia, as judged by the absence of colon neoplasms in the two-year drinking water study conducted by George et al. (2002).

De Angelo et al. (2002) evaluated the ability of bromodichloromethane administered in drinking water to induce ACF in the colons of male $B6C3F_1$ (6 animals/group) and A/J mice (9 animals/group; sex not specified). Mice of the A/J strain are sensitive to chemical induction of ACF. Test animals were exposed to distilled water, 0.25% Alkamuls EL-620®, or a target concentration of 0.7 g/L bromodichloromethane in 0.25% Alkamuls EL-620 for 13 weeks (both strains) or 30 weeks (A/J mice only). A single intraperitoneal injection of 50 mg/kg 4-aminobiphenyl or 10 mg/kg AOM served as the positive controls for the $B6C3F_1$ and A/J strains, respectively. Body weight and water consumption were measured twice during the first week of the study and once per week thereafter. Colons were collected at study termination, fixed, stained with 0.2% methylene blue, divided into three equal segments, and scanned for ACF. The study report did not provide results for measured concentration of bromodichloromethane in drinking water solutions or an estimated dose. No differences were observed in between the control and any treatment group for body weight or water and feed consumption. ACF development was not observed in the colons of $B6C3F_1$ mice treated with bromodichloromethane in the drinking water or injected with 4-aminobiphenyl. Bromodichloromethane did not induce ACF in A/J mice. Injection of A/J mice with the positive control compound AOM induced 47.4 ± 4.9 ACF/cm^2 (mean and standard error) and 7.2 ± 1.1 tumors/cm^2 after 13 weeks and 17.8 ± 2.6 tumors/cm^2 after 30 weeks of treatment. In comparison, 807 ACF and 4.95 ± 0.25 crypts per focus were observed in the AOM positive control group.

Geter et al. (2004b) investigated the influence of vehicle and mode of administration on the induction of ACF in the colons of male F344/N rats exposed to bromodichloromethane. Twenty-eight-day-old male F344/N rats (6/treatment) received either 0 or 50 mg/kg-day of bromodichloromethane in corn oil by gavage (five days/week) or 0 or 0.7 g/L (equivalent to 0 or 63 mg/kg-day) of bromodichloromethane in drinking water containing 0.25% Emulphor® for 26 weeks. Animals in the positive control group received a single 15 mg/kg intraperitoneal injection of AOM. Body weight and water consumption were measured twice weekly for the first week and biweekly for the remainder of the experiment. Details of treatment are provided in the related publication by George et al. (2002). At sacrifice, the colon was removed from each animal and divided into proximal, medial, and distal segments. ACF were identified by staining with 0.2% methylene blue.

Significant reductions in water consumption were observed in the positive controls and bromodichloromethane treatment groups, but body weights in these groups were similar to the controls. Exposure to AOM alone or with corn oil resulted in significant increases in the total number of ACF, ACF/colon, total focal area, and total number of aberrant crypts. ACF were observed in 4/6 animals receiving bromodichloromethane by gavage or in the drinking water compared to 0/6 controls. Exposure to bromodichloromethane in the drinking water significantly increased the total number of ACF (control: 0; bromodichloromethane: 8), ACF/colon (0, 1.33), total focal area 0, 670 μm^2), and total number of aberrant crypts (0, 26). Gavage exposure to bromodichloromethane in corn oil increased the total number of ACF (control: 1; bromodichloromethane: 9), ACF/colon (0.16, 1.5), total focal area (0, 650 μm^2), and total number of aberrant crypts (11, 33), but none of these responses were statistically significant when compared to the control. No effect was noted in the mean number of crypts per focus or the distribution of ACF in the proximal, medial, and distal segments for any treatment when compared to the corresponding controls. The study authors concluded that ACF formation is independent of the route of bromodichloromethane exposure, but noted that a longer exposure (i.e., 52 weeks) might demonstrate differences between drinking water and corn oil as vehicles for administration. A limitation of this study is the small sample size (6 animals/treatment), which may have limited the power of the study to detect differences in ACF induction between the vehicles. Geter et al. (2004c) investigated the effects of a diet containing a high amount of animal fat on the induction of ACF in the colon of male F344/N rats exposed to bromodichloromethane. The study was conducted because a high-fat diet is regarded as an important nutritional influence on colon cancer development. Twenty-eight-day-old male F344/N rats (6/treatment) received 0.7 g/L (equivalent to 56 - 63 mg/kg-day) of bromodichloromethane in drinking water containing 0.25% Emulphor® for 26 weeks. Animals in the negative control group received water containing 0.25% Emulphor®. Animals in the positive control group received a single 15 mg/kg intraperitoneal injection of AOM. All animals were fed a standard laboratory diet (Purina 5001), with half receiving the normal feed containing 4.5% fat and half receiving feed supplemented with 19% animal fat. Body weight and water consumption were measured twice weekly for the first week and biweekly for the remainder of the experiment. At sacrifice, the colon was removed from each animal and divided into proximal, medial, and distal segments. ACF were identified by staining with 0.2% methylene blue.

Water consumption was significantly reduced in the positive control (normal diet only) and bromodichloromethane (normal and high-fat diets) treatment groups, but mean body weights in these groups were similar to or greater than the controls. The incidences of ACF (i.e., number of colons with ACF/number of colons scored) for the control, control + high fat, bromodichloromethane, and bromodichloromethane + high fat groups were 0/6, 3/6, 4/6, and 5/6, respectively. The incidence of ACF in the positive control groups was 6/6. Exposure to bromodichloromethane significantly increased the total number of ACF, ACF/colon, total and mean focal area, and total number of aberrant crypts in animals receiving the normal diet relative to the control group. Similar values for these endpoints were observed for animals receiving the high-fat diet, but the incidence of ACF in the high-fat control group was higher and thus no significant differences were observed between the high-fat control and bromodichloromethane-exposed groups. No significant differences were noted in the number or characteristics of ACF

between animals fed normal or high-fat diets and exposed to either AOM or bromodichloromethane.

The induction of ACF in the colon has been investigated in *Tsc2* mutant Long-Evans (Eker) rats, a rodent model of hereditary renal cancer. The basic details of this study (e.g., in-life and chemical consumption data) are described in Hooth et al. (2002) and the results for induction of ACF are reported in McDorman et al. (2003a). Male Eker rats (8-10/concentration) received drinking water containing 0, 0.07, or 0.7 g/L of bromodichloromethane continuously for 10 months. The administered concentrations resulted in average daily doses of approximately 0, 3.5 and 35.0 mg/kg-day, respectively, as calculated by the study authors. At necropsy, the colon was removed, fixed, and processed for histopathological evaluation. Fixed segments of the proximal, middle, and distal colon were stained for identification of ACF and counting of the individual crypts in each focus. Colons from control and high-dose rats were analyzed for crypt cell proliferation using proliferating cell nuclear antigen (PCNA).

Exposure to bromodichloromethane increased the incidence of ACF (0 mg/L, 0/10; 0.07 g/L, 7/8; 0.70 g/L, 6/8), total number of ACF (0, 9, 10), mean ACF/colon (0, 1.13, 1.25), total crypts/ACF (0, 29, 27), mean crypts/ACF (0, 3.22, 2.7), and mean size of ACF (0, 0.36, 0.26 mm^2) in the colons of male rats. There were no statistically significant responses with treatment, which may reflect in part the small number of animals tested and evaluated in this study. At the low dose, approximately 67% of the ACF were in the proximal colon and 33% were in the distal colon. At the high dose, 30% of the ACF were in the proximal colon, 40% were in the middle colon, and 30% were in the distal colon. Exposure to bromodichloromethane did not significantly increase crypt cell proliferation in the colon as evaluated by the LI or % mitoses in individual segments of the colon or the entire colon.

c. Cancer and Cancer-Related Studies in Cancer-Susceptible Rodent Strains

Theiss et al. (1977) examined the carcinogenic potential of bromodichloromethane in Strain A mice (6 to 8 weeks old). Male animals (20 mice/group) were injected intraperitoneally up to three times weekly over a period of 8 weeks. Three dose levels (20, 40, or 100 mg/kg bromodichloromethane) were used with concurrent positive and negative control groups that contained 20 animals each. Mice were sacrificed 24 weeks after the first injection, and the frequency of lung tumors in each test group was compared with vehicle-treated controls. No statistically significant increase in the incidence of lung tumors/mouse was reported.

The induction of transitional cell hyperplasia, and carcinogenicity of bromodichloromethane has been investigated in *Tsc2* mutant Long-Evans (Eker) rats, a rodent model of hereditary renal cancer. The results of this study have been reported in three publications (McDorman et al., 2003a,b; Hooth et al., 2002). The Eker rat model is characterized by a spontaneous germ-line insertion mutation in the tuberous sclerosis complex (Tsc2) tumor-suppressor gene. This mutation predisposes Eker rats to develop multiple spontaneous renal cell carcinomas, as well as splenic hemangiosarcomas and uterine leiomyosarcomas, as early as four

months of age. As a result, Eker rats are highly susceptible to the effects of renal carcinogens (McDorman et al., 2003b).

The basic details of this study (e.g., in-life and chemical consumption data) are described in Hooth et al. (2002). Male and female Eker rats (8-10/sex/concentration) received drinking water containing 0, 0.07, or 0.7 g/L of bromodichloromethane daily for 4 or 10 months. At sacrifice, the test animals were 6 or 12 months of age, respectively. Complete necropsies were performed on all animals at sacrifice. Tissues collected for microscopic examination included the adrenal glands, gross lesions, kidneys, large intestine, liver, spleen, testicles (including surrounding membranes), thyroid gland, urinary bladder, and uterus.

The average daily doses of bromodichloromethane were 3.5 and 35.0 mg/kg-day for males and 6.5 and 55.6 mg/kg-day for females, as estimated by the study authors. Survival, mean body weight, and water consumption of male and female rats exposed to bromodichloromethane were similar to the controls. Centrilobular hypertrophy was observed in the livers of 5/8 high-dose males. Clear cell foci of cellular atypia were observed in 3/8 high-dose males and 1/8 high-dose males had basophilic foci. None of these lesions were present in control rats. After four months of treatment, nearly all males in the study had at least one renal tumor. More adenomas and total renal tumors were observed in the high-dose males than in the low dose males (adenomas: 0 g/L, 1.9 ± 1.9; 0.07 g/L, 2.1 ± 1.1; 0.7 g/L, 2.9 ± 2.0; total tumors: 0 g/L, 2.1 ± 2.0; 0.07 g/L, 2.3 ± 1.0; 0.7 g/L, 3.0 ± 1.9), but the differences were not statistically significant. The same pattern was evident after 10 months of treatment (adenomas: 0 g/L, 5.6 ± 3.2; 0.07 g/L, 8.6 ± 7.2; 0.7 g/L, 9.6 ± 5.9; total tumors: 0 g/L, 5.6 ± 3.2; 0.07 g/L, 8.8 ± 7.2; 0.7 g/L, 9.8 ± 6.1), but again the differences were not statistically significant. The numbers of adenomas and total renal tumors in female rats did not show a dose-related trend after 4 or 10 months of treatment. There were no dose-related trends for incidence or lesion burden (total number of proliferative lesions/total number of rats in the group) of hemangioma of the spleen in male or females or for leiomyomas or mesenchymal cell proliferation in the uterus of females.

McDorman et al. (2003b) reported the analysis of preneoplastic and neoplastic renal lesions in Eker rats exposed to 0, 0.07 or 0.7 g/L bromodichloromethane in the study described by Hooth et al. (2002). At necropsy, a midsagittal section of each kidney was collected, preserved, and processed for histopathological evaluation. Two sagittal sections (one per kidney) from each animal were subsequently examined for the presence and number of atypical renal tubules, atypical tubular hyperplasia, and renal epithelial tumors (adenomas and carcinomas) and the severity of chronic progressive neuropathy. Characterization of renal lesions in the Eker rat is based on the morphology and size of the lesions. Atypical tubules are normal sized tubules with a patent lumen lined by a single layer of one or more altered cells, characterized by combinations of cell swelling with an increase in cytoplasm, basophilia, altered nucleus to cytoplasm ratio, and megalocytosis. Atypical hyperplasias are tubules filled with multiple layers of altered cells that occlude the lumen and expand the normal tubule size but remain smaller than three average tubules. Adenomas are solid, cystic, or cystopapillary foci of altered tubular epithelial cells greater than or equal to the size of three average tubules and less than 10 mm in diameter. They may or may not breach the basement membrane. Renal carcinomas are solid, cystic, or

cystopapillary foci of altered tubular epithelial cells greater than or equal to 10 mm in diameter. Atypical tubules are the first recognizable preneoplastic lesion in this series. Some atypical tubules may progress to the more advanced lesions of atypical hyperplasia, adenoma, or carcinoma. Once an atypical tubule has progressed to atypical hyperplasia, it is assumed that (given sufficient time) it will progress to an adenoma.

After 4 months of continuous exposure, the mean number of atypical tubules per rat was significantly increased in low- and high-dose males (0 g/L, 13.5 ± 4.1; 0.07 g/L, 24.8 ± 7.8; 0.7 g/L, 35.9 ± 11.5) and high-dose females (0 g/L, 49.3 ± 13.9; 0.07 g/L, 71.0 ± 21.9; 0.7 g/L, 77.3 ± 19.9). High-dose males (0 g/L, 5.1 ± 2.5; 0.07 g/L, 3.9 ± 1.8; 0.7 g/L, 9.8 ± 2.8) and low- and high-dose females (0 g/L, 7.6 ± 3.4; 0.07 g/L, 13.1 ± 5.5; 0.7 g/L, 11.0 ± 4.3) had higher mean numbers of atypical hyperplasias per rat, but the response was not statistically significant when compared to the controls. There were no significant increases in the number of tumors or combined hyperplasias and tumors per rat for either sex. After 10 months of exposure, no significant differences were observed in male or female rats for atypical tumors, atypical hyperplasias, adenomas and carcinomas (combined), or hyperplasias and tumors, although the mean values for each category of lesion were consistently greater than the control values. There were no significant changes in the severity of chronic progressive nephropathy in males or females after 4 months or 10 months of continuous treatment.

McDorman et al. (2003a) examined induction of transitional cell hyperplasia in the urinary bladder of Eker rats continuously exposed to 0, 0.07 or 0.7 g/L bromodichloromethane for 10 months in the study described by Hooth et al. (2002). Changes in the urinary bladder were examined to determine whether bromodichloromethane would contribute to effects on this organ as one component of a mixture of disinfection byproducts. At necropsy, the bladder was removed, fixed, and processed for histopathological evaluation. Midsagittal sections of fixed bladder from male and female rats were used for preparation of slides. No evidence of urinary bladder epithelial hyperplasia or individual cell hypertrophy was observed in male or female rats exposed to bromodichloromethane.

2. Dibromochloromethane

a. Two-Year Oral Cancer Bioassays

NTP (1985) administered dibromochloromethane at doses of 0, 40, or 80 mg/kg-day (in corn oil) to groups of 50 male and 50 female F344/N rats via gavage 5 times/week for 104 to 105 weeks. Details on the protocol of this study are provided in section V.D.2. Survival of dosed male and female rats was comparable to that of the vehicle-control groups. High-dose males had lower body weights when compared with the vehicle control. Compound-related nonneoplastic lesions (fatty metamorphosis and ground-glass cytoplasmic changes) were found in the livers of both sexes (See section V.D.2). Nephrosis was observed in female rats. No statistically significant increase in the incidence of any neoplastic lesion was observed. Based on the results of this study, the authors concluded that there was no evidence of carcinogenicity in rats administered dibromochloromethane.

NTP (1985) administered dibromochloromethane to groups of 50 male and 50 female $B6C3F_1$ mice via gavage in corn oil 5 times/week for 104 to 105 weeks. Details on the protocol of this study are provided in section V.D.2. The administered doses were 0, 50, or 100 mg/kg-day. Survival of female mice was comparable to that of the vehicle-control group. High-dose male mice, however, had lower survival rates than the vehicle controls. At week 82, nine high-dose male mice died of an unknown cause. An inadvertent overdose of dibromochloromethane given to low-dose male and female mice at week 58 killed 35 male mice, but apparently did not affect the females. The low-dose male mouse group was, therefore, considered to be unsuitable for analysis of neoplasms. Compound-related nonneoplastic lesions were found primarily in the livers of male mice (hepatocytomegaly, necrosis, fatty metamorphosis) and female mice (calcification and fatty metamorphosis). Nephrosis was observed in male mice. In females, a statistically significant increase in the incidence of hepatocellular adenomas and adenomas and carcinomas combined was observed in the high-dose group. In male mice, a statistically significant increase in the incidence of hepatocellular carcinomas and adenomas and carcinomas combined was observed in the high-dose group. A summary of the incidence of these tumors is presented in Table V-15. A negative trend in the incidence of malignant lymphomas was evident in dibromochloromethane-exposed male mice when compared to the vehicle control. The study authors concluded that the results of this study provided equivocal evidence of dibromochloromethane carcinogenicity in male $B6C3F_1$ mice and some evidence of carcinogenicity in female $B6C3F_1$ mice.

In other bioassays, Voronin et al. (1987) observed no significant tumor increases in CBAxC57B1/6 mice (50/sex/dose) treated with dibromochloromethane in the drinking water at concentrations of 0, 0.04, 4.0, or 400 mg/L (approximately 0, 0.008, 0.76, or 76 mg/kg-day) for 104 weeks. In an unpublished report of a two-year dietary study, Tobe et al. (1982) reported no increase in gross tumors in male rats dosed with up to 210 mg/kg-day or female rats treated with up to 350 mg/kg-day.

Table V-15 Frequencies of Liver Tumors in $B6C3F_1$ Mice Administered Dibromochloromethane in Corn Oil for 105 Weeks - Adapted from NTP (1985)

Treatment (mg/kg-day)	Sex	Adenoma	Carcinoma	Adenoma or Carcinoma (combined)
Vehicle Control	M F	14/50 2/50	10/50 4/50	23/50 6/50
50	M F	--[a] 4/49	-- 6/49	-- 10/49
100	M F	10/50 11/50[b]	19/50[b] 8/50	27/50[c] 19/50[d]

[a] Male low-dose group was inadequate for statistical analysis.
[b] $p < 0.05$ relative to controls.
[c] $p < 0.01$ (life table analysis); $p = 0.065$ (incidental tumor test) relative to controls.
[d] $p < 0.01$ relative to controls.

b. Studies of Induction of Aberrant Crypt Foci

De Angelo et al. (2002) evaluated induction ACF in the colons of male F344/N rats exposed to dibromochloromethane in drinking water. Groups of weanling rats (6 animals/group) were exposed to distilled water, 0.25% Alkamuls EL-620®, or 0.9 g/L dibromochloromethane in 0.25% Alkamuls EL-620 for 13 weeks. A single intraperitoneal injection of 30 mg/kg azoxymethane (AOM) served as the positive control. Body weight and water consumption were measured twice during the first week of the study and once per week thereafter. Colons were collected at study termination, fixed, stained with 0.2% methylene blue, divided into three equal segments, and examined for ACF.

The measured concentration of dibromochloromethane averaged 0.80 ± 0.05 g/L (mean and standard error) over the course of the study. When adjusted for volatilization and adherence to glass, the corrected concentration was 0.63 g/L. Water consumption was significantly reduced (34%) in the dibromochloromethane exposure group when compared to the 0.25% vehicle control. The average daily dose of dibromochloromethane was 60 mg/kg-day as calculated by the study authors. Average terminal body weight of the rats exposed to dibromochloromethane was within 10% of the control values. No ACF were observed in colons from control animals. ACF were observed in three of six colons from dibromochloromethane-exposed animals. The total number of ACF (17) and number of aberrant crypts per focus (2.43 ± 0.61) were significantly increased relative to the combined deionized water and vehicle controls. Fourteen percent of the observed ACF were located in the middle segment of the colon and 86% were located in the distal (rectal) segment. In comparison, 807 ACF and 4.95 ± 0.25 crypts per focus were observed in the AOM positive control group. The total and average focal areas were 10,390 and 63.74 ± 3.06 μm^2 respectively. Eight percent, 42% and 50% of the ACF induced by AOM were located in the proximal, middle, and distal segment of the colon, respectively. The biological significance of the observed increase in ACF in the colon of dosed rats is unclear, as treatment with dibromochloromethane did not induce tumors in this site in two-year bioassays conducted in rats or mice (Tobe et al., 1982; NTP, 1985; Voronin et al., 1987).

De Angelo et al. (2002) tested the ability of dibromochloromethane administered in drinking water to induce ACF in the colons of male $B6C3F_1$ (6 animals/group). Test animals were given distilled water, 0.25% Alkamuls EL-620®, or a target concentration of 0.9 g/L dibromochloromethane in 0.25% Alkamuls EL-620 for 13 weeks. Animals in the positive control group received a single 50 mg/kg intraperitoneal injection of 4-aminobiphenyl. Body weight and water consumption were measured twice during the first week of the study and once per week thereafter. Colons were collected at study termination, fixed, stained with 0.2% methylene blue, divided into three equal segments, and scanned for ACF. The study report did not provide results for measured concentration of dibromochloromethane in drinking water solutions or an estimated average daily dose. No differences were reported between the control and treatment group for body weight or water and feed consumption (data not shown). Development of ACF was not observed in the colons of mice exposed to dibromochloromethane in the drinking water or injected with the positive control 4-aminobiphenyl.

Geter et al. (2004c) investigated the effects of a diet containing a high amount of animal fat on the induction of ACF in the colon of male F344/N rats exposed to dibromochloromethane. The study was conducted because a high-fat diet is regarded as an important nutritional influence on colon cancer development in humans. Twenty-eight-day-old male F344/N rats (6/treatment) received 0.9 g/L (equivalent to 54-65 mg/kg-day) of dibromochloromethane in drinking water containing 0.25% Emulphor® for 26 weeks. Animals in the negative control group received water containing 0.25% Emulphor®. Animals in the normal and high fat-diet positive control groups received a single 15 mg/kg intraperitoneal injection of AOM. All animals were fed a standard laboratory diet (Purina 5001), with half receiving the normal feed containing 4.5% fat and half receiving feed supplemented with 19% animal fat. Body weight and water consumption were measured twice weekly for the first week and biweekly for the remainder of the experiment. At sacrifice, the colon was removed from each animal and divided into proximal, medial, and distal segments. ACF were identified by staining with 0.2% methylene blue.

Water consumption was significantly reduced in the positive control (normal diet only) and dibromochloromethane (normal and high fat diets) treatment groups, but mean body weights in these groups were similar to or greater than the controls. The incidences of ACF (i.e., number of colons with ACF/number of colons scored) for the control, control + high fat, dibromochloromethane, and dibromochloromethane + high fat groups were 0/6, 3/6, 5/6, and 5/6, respectively. The incidence of ACF in the positive control groups was 6/6. Exposure to dibromochloromethane resulted in significantly increased total numbers of ACF and ACF/colon (normal diet only), increased total and mean focal area (normal and high fat diets), and total number of aberrant crypts (normal and high fat diets). However, no significant differences were noted in the number or characteristics of ACF between animals fed the normal or high-fat diets and exposed to either AOM or dibromochloromethane. The ability of this study to detect differences in induction of ACF related to diet may have been limited by the small sample size and short duration of exposure.

3. Bromoform

a. Two-Year Oral Cancer Bioassays

NTP (1989a) exposed male and female F344/N rats (50/sex/dose) to bromoform doses of 0, 100, or 200 mg/kg-day via gavage in oil for 103 weeks (5 days/week). At study termination, all animals were necropsied, and a thorough histological examination of tissues was performed. Adenomatous polyps or adenocarcinomas of the large intestine were noted in three high-dose male rats, eight high-dose female rats, and one low-dose female rat (Table V-16). Although the number of tumors found was small, the incidence was considered to be significant because these intestinal tumors are very rare in the rat. The NTP concluded that there was some evidence for carcinogenic activity in male rats and clear evidence in female rats. Additional details of this study are provided in Section V.D.3.

In a parallel study, NTP (1989a) exposed male B6C3F_1 mice (50/dose) to bromoform via gavage in corn oil at doses of 0, 50, or 100 mg/kg-day for 103 weeks (5 days/week). Female

mice (50 dose) received doses of 0, 100, or 200 mg/kg-day by the same protocol. At termination, all animals underwent gross necropsy and thorough histological examinations of tissues. Survival in both treated female groups was reduced; however, the authors attributed this reduction in survival partly to utero-ovarian infection. A statistically significant increase in the incidence of thyroid follicular cell hyperplasia was noted in high-dose females; however, there were no statistically significant increases in the incidence of any neoplastic lesion in any dose group compared to controls.

Table V-16 Tumor Frequencies in the Large Intestine of F344/N Rats Exposed to Bromoform in Corn Oil for 2 Years - Adapted from NTP (1989a)

Tumor	Tumor Frequency		
Male rat	**Control**	**100 mg/kg**	**200 mg/kg**
Adenocarcinoma	0/50	0/50	1/50
Polyp (adenomatous)	0/50	0/50	2/50
Female rat	**Control**	**100 mg/kg**	**200 mg/kg**
Adenocarcinoma	0/48	0/50	2/50
Polyp (adenomatous)	0/48	1/50	6/50

Based on the results of this study, the study authors concluded there was no evidence for carcinogenicity of bromoform in male or female mice. Additional details of this study are provided in Section V.D.3.

Kurokawa (1987) observed no evidence of carcinogenicity in male or female rats exposed to microencapsulated bromoform at concentrations of 400, 1600, or 6500 ppm in the diet for 24 months.

b. Studies of Induction of Aberrant Crypt Foci

De Angelo et al. (2002) evaluated induction of ACF in the colons of male F344/N rats exposed to bromoform in drinking water. Groups of weanling rats (6 animals/group) were exposed to distilled water, 0.25% Alkamuls EL-620®, or a target concentration of 1.10 g/L bromoform in 0.25% Alkamuls EL-620 for 13 weeks. A single intraperitoneal injection of 30 mg/kg azoxymethane (AOM) served as the positive control. Body weight and water consumption were measured twice during the first week of the study and once per week thereafter. Colons were collected at study termination, fixed, stained with 0.2% methylene blue, divided into three equal segments, and examined for ACF.

The measured concentration of bromoform averaged 0.98 ± 0.08 g/L (mean and standard error) over the course of the study. When adjusted for volatilization and adherence to glass, the

corrected concentration was 0.77 g/L. Water consumption was significantly reduced (32%) in the bromoform exposure group when compared to the vehicle control. The average daily dose of bromoform was 76 mg/kg-day, as calculated by the study authors. Average terminal body weights of the rats exposed to bromoform were within 10% of the vehicle control value. No ACF were observed in colons from control animals. ACF were observed in 4/6 bromoform-exposed animals. The number of ACF/colon (1.17 ± 0.40), total focal area (470 μm^2), average focal area (67.14 ± 8.57 μm^2), total number of ACF (26), and number of aberrant crypts per focus (3.71 ± 0.36) were significantly increased relative to the combined deionized water and vehicle controls. Fourteen percent of the observed ACF were located in the middle segment of the colon and 86% were located in the distal (rectal) segment. In comparison, 807 ACF and 4.95 ± 0.25 crypts per focus were observed in the AOM positive control group. The total and average focal areas were 10,390 and 63.74 ± 3.06 μm^2 respectively. Eight percent, 42% and 50% of the ACF induced by AOM were located in the proximal, middle, and distal segment of the colon, respectively.

De Angelo et al. (2002) tested the ability of bromoform administered in drinking water to induce ACF in the colons of male $B6C3F_1$ (6 animals/group). Test animals were given distilled water, 0.25% Alkamuls EL-620®, or a target concentration of 1.10 g/L bromoform in 0.25% Alkamuls EL-620 for 13 weeks. Animals in the positive control group received a single 50 mg/kg intraperitoneal injection of 4-aminobiphenyl. Body weight and water consumption were measured twice during the first week of the study and once per week thereafter. Colons were collected at study termination, fixed, stained with 0.2% methylene blue, divided into three equal segments, and scanned for ACF. The study report did not provide results for the measured concentration of bromoform in drinking water solutions or an estimated average daily dose. No differences were reported between the control and treatment group for body weight or water and feed consumption (data not shown). Development of ACF was not observed in the colons of mice exposed to bromoform in the drinking water or injected with the positive control 4-aminobiphenyl.

Geter et al. (2004c) investigated the effect of a diet containing a high amount of animal fat on the induction of ACF in the colon of male F344/N rats exposed to bromoform. The study was conducted because a high-fat diet is regarded as an important nutritional influence on colon cancer development in humans. Twenty-eight-day-old male F344/N rats (6/treatment) received 1.0 g/L (equivalent to 71-73 mg/kg-day) of bromoform in drinking water containing 0.25% Emulphor® for 26 weeks. Animals in the negative control group received water containing 0.25% Emulphor®. Animals in the positive control group received a single 15 mg/kg intraperitoneal injection of AOM. All animals were fed a standard laboratory diet (Purina 5001), with half receiving the normal feed containing 4.5% fat and half receiving feed supplemented with 19% animal fat. Body weight and water consumption were measured twice weekly for the first week and biweekly for the remainder of the experiment. Additional details of treatment are provided in the related publication by George et al. (2002). At sacrifice, the colon was removed from each animal and divided into proximal, medial, and distal segments. ACF were identified by staining with 0.2% methylene blue.

Water consumption was significantly reduced in the positive control (normal diet only) and bromoform (normal and high-fat diets) treatment groups, but mean body weights in these groups were similar to or greater than the controls. The incidences of ACF (i.e., number of colons with ACF/number of colons scored) for the control, control + high fat, bromoform, and bromoform + high fat groups were 0/6, 3/6, 6/6, and 6/6, respectively. The incidence of ACF in the positive control groups was 6/6. Exposure to bromoform significantly increased the total number of ACF, ACF/colon, total and mean focal area, and total number of aberrant crypts in animals receiving the normal diet or high-fat diets relative to the respective control groups. Concurrent exposure to the high-fat diet and bromoform resulted in a statistically significant increase in the number of ACF/colon (normal diet: 2.83 ± 1.05, high-fat diet 5.33 ± 1.17).

Geter et al. (2005) investigated the effect of folate deficiency on the bromoform-induced formation of aberrant crypt foci (ACF) in the colon of the male F344 rat. Male F344 rats(28 days old) were give bromoform in the drinking water at a concentration of 500 mg/L for 26 weeks. A single i.p. dose of azoxymethane was used as a positive control. Two groups of rats (control and bromoform exposed) were fed an amino acid deficient diet containing either 0 or 2 mg folic acid/kg. Food and water were given ad libitum. Water consumption was measured weekly throughout the study and body weights were determined weekly through week 16 and then biweekly until the end of the study. After 26 weeks of exposure, rats were sacrificed and colons were removed and prepared for analysis of ACF. For each ACF found, the size, location and number of individual crypts within the focus was noted. Blood samples were analyzed for serum folate and homocysteine levels.

Water consumption and weight gain were not affected by bromoform administration in either control or folate-deficient rats. Bromoform dose levels were calculated to be approximately 96 mg/kg-day for rats given the standard diet and 99 mg/kg-day for rats given the folate deficient diet. Folate deficient rats experienced a decrease in serum folate levels and an increase in serum homocysteine levels. Bromoform administration in drinking water increased the number of ACF and aberrant crypts in rats given a standard diet and in rats fed a diet deficient in folic acid, when compared to controls. For bromoform-exposed rats, folate deficiency resulted in a larger increase in ACF formation, when compared to rats given the standard diet. Azoxymethane also induced the formation of ACF; however folate deficiency reduced the formation of ACF for rats exposed to this chemical. The results of this study suggest that dietary factors may play a modulatory role in the bromoform-induced formation of ACF in the colon of male F344 rats.

c. Cancer and Cancer-Related Studies in Cancer-Susceptible Rodent Strains

Theiss et al. (1977) examined the carcinogenic activity of bromoform in Strain A mice. Twenty mice per group (6 to 8 weeks old) were injected intraperitoneally up to three times weekly over a period of 8 weeks with 4, 48, or 100 mg/kg bromoform. A positive and a negative control group were included in the study design and each contained 20 animals. Mice were sacrificed 24 weeks after the first injection and the frequency of lung tumors in each test group was compared with vehicle-treated controls. Bromoform produced a significant increase ($p = 0.041$)

in tumor frequency only at the intermediate dose; this increase was considered indicative of a carcinogenic response by the study authors.

H. Other Key Health Effects

1. Immunotoxicity

a. Bromodichloromethane

Munson et al. (1982) administered bromodichloromethane by gavage to CD-1 male and female mice (8-12/sex/dose) for 14 days at levels of 0, 50, 125, or 250 mg/kg-day. Bromodichloromethane appeared to affect the humoral immune system, as judged by decreased antibody-forming (ABF) cells in serum and by decreased hemagglutination titers. These changes were clearly significant ($p < 0.05$) at the high dose in both males and females, and decreased ABF cells were also noted at the mid dose (125 mg/kg-day) in females. This study identified a NOAEL of 50 mg/kg-day and a LOAEL of 125 mg/kg-day for bromodichloromethane on the basis of decreased immune function in females. Additional information on other endpoints measured in this study is provided in Section V.B.1.

French et al. (1999) investigated the immunotoxicity of bromodichloromethane in a series of four experiments conducted in mice and rats. Immunotoxicity in mice was examined following exposure via ingestion of drinking water or by gavage. The immunological parameters examined were antibody response to injected sheep red blood cells and T and B lymphocyte proliferation. Mitogens used in the proliferation assay were concanavalin A (Con A) or phyto-hemagglutinin-p (PHA) for T cells and lipopolysaccharide (LPS) for B cells. Female C57BL/6 mice (6 animals per group) were treated for 14 or 28 days with drinking water containing 0, 0.05, 0.25 or 0.5 g/L bromodichloromethane. All drinking water (including controls) contained 0.25% Emulphor® to reduce volatilization of bromodichloromethane. Based on measured water consumption, these concentrations were estimated by the authors to be equivalent to 0, 10, 37 or 62 mg/kg-day. There were no significant differences in the number of antibody forming cells, antibody production, or spleen weights in any treatment group. Likewise, splenic and mesenteric lymph node cell proliferative responses to T and B cell mitogens were similar in all groups. Continuation of this study for an additional 2 weeks did not affect any measured parameter. These data identify a NOAEL of 62 mg/kg-day for short-term exposure.

French et al. (1999) conducted a second experiment in which female C57BL/6 mice were dosed by gavage with bromodichloromethane in 10% Emulphor® once a day for 16 days. Treatment groups (6 animals per group) included controls (deionized water or10% Emulphor®), 50, 125 or 250 mg/kg-day bromodichloromethane. As in the previous experiment, there were no differences in ABF cells, antibody titers or mitogen-induced proliferation in any treatment groups. A decrease in spleen weight and spleen-to-weight ratio was observed in the 125 mg/kg-day group when compared to the Emulphor® control. However, spleen weights in the Emulphor® control were significantly higher than those in the deionized water control group, making this finding difficult to interpret.

French et al. (1999) investigated the immunotoxicity of bromodichloromethane in male Fisher 344 rats following two different *in vivo* exposure regimens: ingestion of drinking water containing bromodichloromethane and gavage. The immunological parameters examined were antibody response to injected sheep red blood cells and T and B lymphocyte proliferation. The mitogens used in the proliferation assay were concanavalin A (Con A) or phyto-hemagglutin-p (PHA) for T cells and *S. typhimurium* mitogen (STM) for B cells. Six rats per treatment group were exposed for 26 weeks to drinking water containing 0, 0.07 or 0.7 g/L bromodichloromethane and 0.25% Emulphor®. Based on water consumption measurements, these concentrations were estimated by the authors to be equivalent to average daily doses of 0, 5 or 49 mg/kg-day. There was a significant suppression of Con A-stimulated proliferation of spleen cells observed in the 49 mg/kg-day dose group. No effect on other immunological parameters was reported. These data suggest NOAEL and LOAEL values of 5 and 49 mg/kg-day, respectively, for immunotoxic effects.

French et al. (1999) also examined the effect of short-term exposure to relatively large doses of bromodichloromethane on immune function. Female F344 rats (6 animals/group) received gavage doses of deionized water, 10% Emulphor®, or 75, 150, or 300 mg bromodichloromethane/kg in 10% Emulphor® for 5 days. Surviving high-dose animals had decreased body, spleen, and thymus weights. Con A and PHA responses were depressed in spleen cells isolated from high-dose animals. Two of the six rats in the 300 mg/kg-day group died during the exposure period. The remaining high-dose animals had significantly decreased body, spleen and thymus weights compared to both control groups. Thymus weight, but not spleen or body weight, was also decreased in the 150 mg/kg-day group. Con A responses were significantly depressed in both spleen and mesenteric lymph node (MLN) cells in the 300 mg/kg-day treatment group. All three (75, 150 and 300 mg/kg-day) dose groups exhibited suppression of PHA stimulated MLN cells when compared to the vehicle (but not the water) controls. This discrepancy was due to the fact that Emulphor® alone significantly elevated the proliferative response to PHA in MLN cells relative to the deionized water group. In contrast to the T cell responses, there was a significant increase in antibody production and proliferative responses to STM (B cells) from spleen cells at the highest dose tested (300 mg/kg-day dose group). These data suggest a marginal NOAEL of 150 mg/kg-day and a LOAEL of 300 mg/kg-day for acute exposure based on depression of immune response.

b. Dibromochloromethane

Munson et al. (1982) administered dibromochloromethane by gavage to CD-1 male and female mice (8 to 12/sex/dose) for 14 days at levels of 0, 50, 125, or 250 mg/kg-day and evaluated humoral and cell-mediated immune system functions. Dibromochloromethane appeared to affect the humoral immune system, as judged by decreased antibody-forming (ABF) cells in serum and by decreased hemagglutination titers. These changes were significant ($p < 0.05$) at the high dose in both males and females. Decreased ABF cells were also noted at the mid dose (125 mg/kg-day) in females. This study identified a NOAEL of 50 mg/kg-day and a LOAEL of 125 mg/kg-day for dibromochloromethane on the basis of decreased immune function in females. Additional information on this study is provided in Section V.B.2.

c. Bromoform

Munson et al. (1982) administered bromoform (aqueous) by gavage to CD-1 male and female mice (6 to 12/sex/dose) for 14 days at levels of 0, 50, 125, or 250 mg/kg-day. Endpoints evaluated included humoral immune system function. The authors judged that the humoral immune system was not significantly affected by bromoform, although a decrease in antibody forming (ABF) cells was reported for high-dose males. These data suggest a NOAEL of 250 mg/kg-day for effects of bromoform on the immune system. Additional information on this study is provided in Section V.B.3.

2. Hormonal Disruption

No studies or case reports were identified that described hormonal disruption by dibromochloromethane or bromoform.

a. Bromodichloromethane - *In Vivo* Studies

Oral exposure to 50 - 100 mg/kg-day of bromodichloromethane causes full litter resorption (FLR) in F344 rats, but not in Sprague-Dawley rats (Narotsky et al., 1997; Bielmeier et al. 2001; see Section V.E.1.a for study details). Bielmeier et al. (2001) characterized luteinizing hormone (LH) and progesterone serum profiles in F344 rats exposed to bromodichloromethane during gestation in two experiments (Table V-17) as part of a larger study on FLR. The objective of these experiments was to determine whether changes in the LH and progesterone profiles were associated with the occurrence of FLR. These hormones were selected for study because progesterone is necessary for the maintenance of pregnancy and LH participates in the maintenance of the corpora lutea which secrete progesterone.

Table V-17 Summary of Hormone Profile Experiments (Bielmeier et al., 2001)

Study/Strain	Dose (mg/kg-day)	Treatment Period	Number of animals			% FLR
			Treated	Pregnant	Resorbed	
Hormone Profile I[a]						
F344	0	GD 8-9	8	7	0	0
F344	100	GD 8	10	10	6	60*
F344	100	GD 9	10	9	9	100***
Hormone Profile II[b]						
F344	0	GD 9	8	8	0	0
F344	75	GD 9	11	11	7	64*
F344	100	GD 9	10	10	9	90***

Source: Table 1 in Bielmeier et al. (2001)
Abbreviations: GD, gestation day; FLR, full litter resorption
[a] Tail blood collected once daily on GD 9 to 12.
[b] Tail blood collected at 0, 6, 12, and 24 hours after dosing.
* $p<0.05$; ** $p<0.01$; *** $p<0.001$for significant differences from controls (Fisher's Exact Test).

In the first experiment conducted by Bielmeier et al. (2001), F344 rats (7 to 10/treatment group) received a single 100 mg/kg dose by aqueous gavage on gestation day 8 or 9. Hormone levels in samples of tail blood were determined on GD 9 through 12. FLR was observed in 0, 60 and 100% of the control, GD 8-dosed, and GD 9-dosed animals, respectively. A marked reduction in progesterone levels was noted 24 hours after dosing in all rats that resorbed their litters when compared to controls and to bromodichloromethane-treated animals that retained their litters. The mean progesterone levels in animals dosed on GD 9 decreased from 137.94 ng/mL ± 11.44 ng/mL to 48.45 ± 23.57 ng/mL within 24 hours (n = 9). For animals treated on GD 8, the mean progesterone level 24 hours after bromodichloromethane treatment was 67.01 ± 16.22 ng/mL in animals that resorbed litters (n = 6) and 127.19 ±14.89 in controls (n=7). The resorbed groups had reduced progesterone levels comparable to the progesterone levels in non-pregnant animals (n = 2) when assayed three days after compound administration. In contrast to the effect noted on progesterone levels, administration of bromodichloromethane had no apparent effect on LH when measured 24 hours after dosing. However, elevated LH concentrations were observed on GD 11 to 12 in animals experiencing resorption. LH levels in these groups increased from approximately 0.20 ng/mL on GD 10 to approximately 0.80 ng/mL on GD 11 and remained elevated through GD 12. In contrast, LH levels in the controls decreased from 0.31 to 0.14 ng/mL over the same time period.

In the second experiment conducted by Bielmeier et al. (2001), F344 rats (8-11/treatment group) were dosed with 0, 75, or 100 mg/kg by aqueous gavage on GD 9. Blood samples were

collected at 0, 6, 12, and 24 hours after dosing. The incidence of FLR was 0, 64%, and 90% in the 0, 75, and 100 mg/kg dose groups, respectively. The progesterone levels peaked in all dose groups (including controls) at 6 hours. At 12 and 24 hours, the progesterone levels in bromodichloromethane-treated animals that resorbed their litters were progressively reduced. Progesterone levels in bromodichloromethane-treated animals that retained their litters remained comparable to levels observed in the control group. No significant differences in LH concentration were noted among dose groups at any time point.

In a follow-on study, Bielmeier et al. (2004) conducted additional experiments to investigate the mode of action for bromodichloromethane-induced pregnancy loss observed in the F344 rat (Narotsky et al., 1997; Bielmeier et al., 2001). The experiments conducted by Bielmeier et al. (2004) were designed to 1) re-examine maternal LH profiles during exposure to levels of bromodichloromethane known to cause pregnancy loss, using a more sensitive assay for LH than used by Bielmeier et al. (2001); 2) assess the temporal pattern of serum LH and progesterone decreases that occur during bromodichloromethane administration at a critical time period during gestation; and 3) test the ability of exogenously administered progesterone and hCG to prevent pregnancy loss induced by bromodichloromethane. The experiments performed are summarized in Table V-18.

In the first experiment, F344 rats received a single dose of 0, 75, or 100 mg/kg bromodichloromethane via oral gavage in 10% Alkamuls EL-620 in the morning of assigned treatment days. Maternal body weights were measured on GD 5-15 and GD 20. Pups were individually examined and weighed on PD 1 and 6. PD 1 was defined as GD 22, independent of the actual time of parturition, so that all pups were examined at the same time postcoitus. The dams and pups were euthanized after PD 6. The number of uterine implantation sites was recorded; the uteri of females that did not deliver were stained with 2% ammonium sulfide to enhance detection of resorption sites. Blood samples for hormone analysis were collected once daily on GD 6-11 from rats in the control and 75 mg/kg groups.

Two additional experiments using the same treatment regimen, but with more frequent blood collection, were conducted to further characterize the changes in hormone levels on GD 9 and 10. In experiment A, tail blood samples were collected on GD 9 once before dosing and then 1.5, 3, 4.5, and 6 hours after treatment. Experiment B continued the collection schedule from experiment A. The first blood samples were collected approximately 8 hours after the dose on GD 9 and four additional samples were collected at 4-hour intervals. This study design was utilized to minimize the impacts of repeated blood collection on a single animal. Progesterone and estradiol concentrations were measured using a direct solid-phase enzyme-linked immunosorbent assay. Serum LH levels were determined using the rat dissociation enhanced lanthanide fluorometric immunoassay (DELPHIA). This method is reported to be 10- to 50-fold more sensitive for detection of LH than the traditional radiolabeled immunoassay used by Bielmeier et al. (2001), with a limit of detection of 0.014 μg for a 25 μL sample. In the hormone replacement experiments, bromodichloromethane (100 mg/kg-day by gavage) and progesterone (10 mg/kg, twice daily by subcutaneous injection in corn oil) were administered on GD 6-10. A

Table V-18 Summary of Bielmeier et al. (2004) Study in Female F344 Rats

Experiment	Dose (mg/kg-day)	Number of Animals			
		Treated	Pregnant	Fully Resorbed	With Live Litters
Hormone Profiles					
Daily Sampling	0	12	9	0	9
	75	13	9	8***	1
Frequent Sampling A	0	6	6	1	5
	75	11	10	8*	2
Frequent Sampling B	0	5	5	0	5
	75	10	9	3	6
Hormone Supplementation					
Control	0	12	8	0	8
BDCM + hormone vehicles	100	9	7	5***	2
BDCM + Progesterone	100	12	8	0	8
BDCM + hCG	100	12	9	1	8

Source: Bielmeier et al. (2004)
* significantly different from control, $p<0.05$; *** significantly different from control, $p<0.001$
Abbreviations: BDCM, bromodichloromethane; hCG, human chorionic gonadotropin

second group of animals received bromodichloromethane (100 mg/kg-day by gavage) and hCG (0.5 IU/rat by subcutaneous injection in saline) on GD 8-10. Control groups received the gavage and injection vehicles via the same route as the experimental groups.

In the daily sampling experiment, bromodichloromethane-induced pregnancy loss was associated with marked reductions in serum progesterone and LH on GD 10. All control dams maintained their litters, whereas 8/9 dams exposed to bromodichloromethane had pregnancy loss. Serum progesterone levels in the control group were greater than 100 ng/mL throughout the study. Serum LH levels in control dams generally ranged from 0.11 to 0.34 ng/mL through GD 9, then fell to mean ± SE values of 0.061 ± 0.03 ng/mL by GD 11. Progesterone levels in dams treated with bromodichloromethane that lost their litters were comparable to those of the controls on GD 6-9. However, these dams had significantly reduced serum progesterone levels on GD10 when compared to the control and all measured concentrations were less than 40 ng/mL. Serum LH levels in treated dams were less than the controls from GD 7 to GD 10, but the response was statistically significant only on GD 7 and 10. The daily sampling protocol used in this experiment did not distinguish which hormone was affected first. No differences between groups were

reported for serum estradiol levels, number of implantations, postnatal loss, or number or weight of live pups on PND 1 or 6.

In frequent sampling experiment A, 8/10 (80%) of the dosed animals had pregnancy loss compared to 1/6 (17%) in the control group. The control animal with pregnancy loss maintained LH levels that were consistent with the other control dams; progesterone levels were also comparable until a sharp decline was observed in the animal with pregnancy loss on GD 10. In contrast to typical bromodichloromethane-induced resorptions, the resorption sites in the control animals were not visible without staining (a possible indication of earlier resorption). In frequent sampling experiment B, 3/9 (33%) of the dosed animals had pregnancy loss compared to 0/5 (0%) of the controls. In both experiments, animals that resorbed their litters displayed a decrease in serum LH concentration (relative to the controls) prior to a reduction in progesterone levels. In experiment A, serum LH levels were already significantly reduced in dosed animals in the first samples collected on GD 9, but serum progesterone levels were not significantly reduced until two hours after administration of the GD 9 dose of bromodichloromethane. In experiment B, the six bromodichloromethane-treated animals that retained their litters also had significantly decreased levels of LH and progesterone. No differences between groups were reported for number of implantations, postnatal loss, or number or weight of live pups on PND 1 or 6 in either experiment.

In the hormone supplementation experiments, administration of either progesterone or hCG significantly reduced the incidence of bromodichloromethane-induced pregnancy loss. Five of seven dams (71%) treated with 100 mg/kg-day of bromodichloromethane plus the hormone vehicles (corn oil or saline injected subcutaneously) on GD 6-10 lost their pregnancies. In contrast, dams dosed with 100 mg/kg-day of bromodichloromethane on GD 6-10 and concurrently given progesterone had a 0/8 (0%) incidence of pregnancy loss. Dams dosed with 100 mg/kg-day of bromodichloromethane on GD 8-10 and concurrently given hCG by injection had a 1/9 (11%) incidence of pregnancy loss.

The study authors had previously hypothesized that bromodichloromethane induces pregnancy loss in F344 rats by altering luteal responsiveness to LH (Bielmeier et al., 2001). The reduction in serum LH level with a corresponding reduction in progesterone concentration detected in the current study suggests that bromodichloromethane alters LH secretion rather than altering luteal responsiveness alone. The timing of the decreases in LH and progesterone levels support the hypothesis that reduction in serum LH is a prerequisite for decreased progesterone concentration, which subsequently results in pregnancy loss. However, other data obtained by Bielmeier et al. (2004) indicate that a significant decrease in serum LH concentration cannot be the sole determinant in pregnancy loss. Specifically, exposure to bromodichloromethane caused decreases in serum LH levels in all dosed animals, but some dosed rats maintained their pregnancies despite serum LH levels that were lower than observed in some animals that lost their pregnancies. In discussing these results, the study authors noted that LH is released in an hourly pulsatile pattern during pregnancy and that the severity and disruption of this pulsatility may be a better predictor of pregnancy loss than single daily values of serum LH.

Administration of exogenous progesterone or hCG reduced the incidence of bromodichloromethane-induced pregnancy loss. Prevention of pregnancy loss by exogenous progesterone supports the conclusion that the mode of action for bromodichloromethane is maternally mediated rather than the result of direct effects on the embryo. The ability of hCG, an LH agonist, to prevent pregnancy loss suggests that full litter resorption is mediated (at least in part) by an effect of bromodichloromethane on maternal LH secretion. These data do not rule out a possible effect of bromodichloromethane on luteal responsiveness to progesterone as previously suggested by Bielmeier et al. (2001).

b. Bromodichloromethane - *In Vitro* Studies

Chen et al. (2003) studied the effect of bromodichloromethane on chorionic gonadotropin (CG) secretion by human placental trophoblast cultures. This in vitro model was used to evaluate possible effects of bromodichloromethane on the placenta. Cytotrophoblast cells were isolated from term human placentas, plated in 24-well cluster dishes or chamber slides, and stimulated to differentiate and produce syncytiotrophoblast-like colonies by culture for 48 hours in Keratinocyte Growth Medium (KGM) containing 10% calf serum. The culture medium was removed and replaced with KGM containing nominal concentrations of 0, 0.020, 20, or 2000 μM bromodichloromethane (actual concentrations were not measured). The culture containers were sealed and incubated for an additional 24 hours, after which levels of immunoreactive and bioactive chorionic gonadotropin were determined in the culture medium. Replicate cultures were processed for morphological evaluation or determination of lactate dehydrogenase. Cultured adherent cells were fixed in methanol and stained for immunocytochemical determination of CG.

Exposure to bromodichloromethane caused a significant dose-dependent decrease in the secretion of immunoreactive and bioactive CG. Decreased CG secretion was observed at each concentration tested, with maximum reductions of 37% (immunoreactive CG) and53% (bioreactive CG) at the highest concentration of 2000 μM. This lowest concentration of 0.020 μM is approximately 100-fold greater than maximum baseline blood levels and 35-fold higher than the maximum peak level of bromodichloromethane measured by Miles et al. (2002) in the blood of human subjects after showering. An effect on intracellular CG levels in syncytiotrophoblast cultures was not detected by immunocytochemical staining. According to the study authors, the failure to detect intracellular changes might have been related to use of different antibodies for detection of extracellular and intracellular CG, resolution of the image analysis procedure, or the fact that the effect on CG secretion was modest. There was no effect of bromodichloromethane on the ratio of bioactive to immunoreactive CG, cellular protein, levels of LDH in culture supernatants, or morphological features of the trophoblast cultures.

The results from this study suggest that bromodichloromethane affects the function of human placental trophoblasts, as shown by reduced CG secretion in primary cultures after 24 hours of exposure to the compound. The mode of action for the effect on CG secretion is unknown. Possible mechanisms proposed by the study authors include disruption of CG synthesis at the translational or post-translational level (e.g., by altering glycosylation of CG

subunits or disruption of dimerization) or indirect effects on secretion via disruption of gonadotropin releasing hormone activity.

Chen et al. (2004) evaluated the effect of bromodichloromethane on the morphological differentiation of human mononucleated cytotrophoblast cells to multinucleated syncytiotrophoblast-like colonies. The objective of this study was to evaluate the mechanism of reduced CG secretion in human placental trophoblast cultures observed in the previous study (Chen et al., 2003). Addition of 20 to 2000 μM bromodichloromethane to primary cytotrophoblast cultures during the differentiation process inhibited the subsequent formation of multinucleated colonies in a dose-dependent manner, as determined by immunocytochemical staining for desmosomes and nuclei. Quantitative image analysis indicated that the number of multinucleated colonies was significantly reduced at bromodichloromethane concentrations of 200 μM and above, with a reduction of 80% observed at the highest tested concentration of 2000 μM. Secretion of immunoreactive and bioreactive CG was significantly reduced in a dose-dependent manner under the same culture conditions, but showed a different dose-response pattern than observed for differentiation (i.e., was effected at lower concentrations). Secretion of immunoreactive CG was significantly reduced (by 30%) at 5×10^{-4} μM, the lowest dose tested. This concentration is within the range of bromodichloromethane concentration observed in human blood following showering with disinfected tap water (1.3×10^{-6} to 5.7×10^{-4} μM). Secretion of immunoreactive and bioreactive CG was near completeat 2000 μM (90% reduction for immunoreactive CG, 95% reduction for bioreactive CG) Intracellular levels of CG were significantly reduced in a dose-dependent manner at concentrations of 20 μM and above, as determined by quantitative immunocytochemical staining. This lack of CG accumulation within the trophoblast, suggests that secretion is blocked at an earlier stage (i.e., transcription or translatin) rather than at later stages (i.e., exocytosis). Trophoblast viability was unaffected by bromodichloromethane at the concentrations tested in this study, as determined by cellular protein levels and by LDH activity in culture supernatants. These findings support the idea that bromodichloromethane affects the placenta and reduces CG production by preventing formation of syncytiotrophoblasts, the major CG-producing cell type. The differences in the dose-response curves observed for CG secretion and differentiation may indicate a dual effect of bromodichloromethane on these processes. The mechanisms of action for the observed effects of bromodichloromethane are unknown. The observation of reduced intracellular CG suggests disruption of production at an earlier (e.g., transcription or translation) rather than a later (e.g., exocytosis) stage of CG production. Other possible mechanisms include an effect of bromodichloromethane on post-translational processing (glycosylation or subunit dimerization) or interference with GnRH activity. An effect on GnRH activity, if substantiated, would parallel one proposed mechanism for BDCM-induced pregnancy loss in F344 rats (Bielmeier et al., 2004).

The significance of the findings reported by Chen et al. (2003, 2004) for human health is that placental trophoblasts are the sole source of CG during normal human pregnancy and play a major role in the maintenance of the conceptus. If the observed effect on CG secretion is substantiated in future studies, it may help to explain adverse pregnancy outcomes that appear to be associated with consumption of chlorinated drinking water in some epidemiological studies (e.g., increased incidence of spontaneous abortion as reported by Waller et al., 1998).

3. Structure-Activity Relationships

Although the mechanism of brominated trihalomethane toxicity is not known with certainty, there is abundant evidence to indicate that adverse effects are secondary to metabolism. Bromine is generally a better leaving group than chlorine, suggesting that bromine substitution could potentially influence the pathway and rate of trihalomethane metabolism. Multiple studies (described in Section III.C) indicate that metabolism of chloroform and the brominated trihalomethanes can occur through one or both of two cytochrome P450-mediated pathways: reductive metabolism to free radical intermediates or oxidative metabolism to dihalocarbonyls (Figure 4-1). Although comparative data are limited, there is some evidence to indicate that chloroform and the brominated trihalomethanes are metabolized to a different extent by these pathways. Tomasi et al. (1985) examined the reductive metabolism of chloroform, bromodichloromethane, and bromoform in rats and obtained the following rank order for free radical formation: bromoform>bromodichloromethane>chloroform. Wolf et al. (1977) reported that bromoform was more extensively metabolized under anaerobic conditions *in vitro* than was chloroform. Gao and Pegram (1992) observed that binding of reactive intermediates to rat hepatic microsomal lipids and proteins was more than twice as high for bromodichloromethane as for chloroform when assayed under anaerobic conditions. These results collectively suggest that reductive metabolism may be a more important metabolic pathway for brominated trihalomethanes than for chloroform. At present, this apparent difference in metabolism has not been linked to specific differences in toxicity.

Two mutagenicity studies provide additional information on structure-activity relationships among the trihalomethanes. Additional details of these studies are presented in Section V.F. Examination of mutagenicity in a strain of *Salmonella typhimurium* that expresses rat theta-class glutathione-S-transferase (GST) indicated the following order for mutagenic potency (number of revertants/ppm) of the brominated trihalomethanes: bromoform≈dibromochloromethane>bromodichloromethane (DeMarini et al., 1997). The potency of the first two compounds was several times greater than that observed for bromodichloromethane. Analysis of the mutational spectra of the brominated trihalomethanes indicated that all three compounds have similar mutational spectra (predominately GC→AT transitions) and site specificity (middle C of a CCC sequence in target DNA). These observations suggest that a common reactive intermediate or class of intermediates is likely to mediate the mutagenicity of these compounds.

In the second study, Pegram et al.(1997) compared the glutathione *S*-transferase-mediated mutagenicity of bromodichloromethane and chloroform in a GST+ strain of *S. typhimurium* (See section V.F.1). Revertants were produced in a dose-related manner in the presence of low as well as high concentrations of bromodichloromethane. In contrast, chloroform induced a doubling of the number of revertants only at high concentrations. This result provides evidence that bromine substitution of trihalomethanes confers the capability for GST-catalyzed transformation to mutagenic intermediates at low substrate concentrations. These data further suggest that chloroform and the brominated trihalomethanes may induce adverse effects via different modes

of action, and indicate the need for care in extrapolating the characteristics of chloroform metabolism and toxicity to brominated trihalomethanes.

I. Summary

1. Health Effects of Acute and Short Term Exposure of Animals

Large oral doses of brominated trihalomethanes are lethal to laboratory animals. Reported acute LD_{50} values range from 450 to 969 mg/kg for bromodichloromethane, 800 to 1,200 mg/kg for dibromochloromethane, and 1,388 to 1,550 mg/kg for bromoform. Acute lethality values are summarized in Table V-1.

Acute oral exposure to sublethal doses of brominated trihalomethanes can also produce effects on the central nervous system, liver, kidney, and heart. Acute duration studies investigating endpoints other than death are summarized in Table V-2. Ataxia, anaesthesia, and/or sedation were noted in mice receiving 500 mg/kg bromodichloromethane, 500 mg/kg dibromochloromethane, or 1,000 mg/kg bromoform. Renal tubule degeneration, necrosis, and elevated levels of urinary markers of renal toxicity have been observed in rats receiving 200 to 400 mg/kg bromodichloromethane. Elevated levels of serum markers for hepatotoxicity and have been observed in rats at doses of bromodichloromethane ranging from approximately 82 to 400 mg/kg-day, and hepatocellular degeneration and necrosis were observed at 400 mg/kg. Effects on heart contractility were reported in male rats at doses of 333 and 667 mg/kg dibromochloromethane.

Short term studies of brominated trihalomethanes are summarized in Table V-3. Short-term exposure of laboratory animals to brominated trihalomethanes has been observed to cause effects on the liver and kidney. Hepatic effects, including organ weight changes, elevated serum enzyme levels, and histopathological changes, became evident in mice and/or rats administered 38 to 250 mg/kg-day bromodichloromethane, 147 to 500 mg/kg-day dibromochloromethane, or 187 to 289 mg/kg-day bromoform for 14 to 30 days. Kidney effects, characterized by decreased p-aminohippurate uptake, histopathological changes, and organ weight changes, became evident in mice and/or rats administered 148 to 300 mg/kg-day bromodichloromethane, 147 to 500 mg/kg-day dibromochloromethane, or 289 mg/kg-day bromoform for 14 days. Evidence for decreased immune function was noted at bromodichloromethane or dibromochloromethane doses of 125 mg/kg-day. Studies examining strain differences in response to short-term brominated trihalomethane exposure have not been reported.

2. Health Effects of Longer-term Exposure of Animals

Subchronic studies of brominated trihalomethanes are summarized in Table V-4. The predominant effects of subchronic oral exposure occur in the liver and kidney. The effects produced in these two organs are similar in nature to those described for short-term exposures, with liver appearing to be the most sensitive target organ for dibromochloromethane and

bromoform exposure. Histopathological changes in the liver were reported in mice and/or rats administered 200 mg/kg-day bromodichloromethane, 50 to 250 mg/kg-day dibromochloromethane, or 50 to 283 mg/kg-day bromoform. Histopathological changes in the kidney were reported in mice and/or rats administered 100 mg/kg-day bromodichloromethane, or 250 mg/kg-day dibromochloromethane. Studies examining strain differences in response to subchronic brominated trihalomethane exposure have not been reported.

Chronic toxicity studies of brominated trihalomethanes are summarized in Table V-5. As observed for exposure for shorter durations, the predominant effects of chronic oral exposure are observed in the liver and kidney. Histopathological signs of hepatic toxicity in mice and/or rats became evident at doses of 6 to 50 mg/kg-day for bromodichloromethane, 40 to 50 mg/kg-day for dibromochloromethane, and 90 to 152 mg/kg-day for bromoform. Signs of bromodichloromethane-induced renal toxicity became evident in mice and rats treated with doses of 25 and 50 mg/kg-day, respectively. Studies examining strain differences in response to chronic brominated trihalomethane exposure have not been reported.

3. Reproductive and Developmental Effects

Reproductive and developmental studies of brominated trihalomethanes are summarized in Table V-9. Signs of maternal toxicity (decreased body weight, body weight gain and/or changes in organ weight) were reported in rats administered bromodichloromethane at 25 to 200 mg/kg-day and in rabbits administered 4.9 to 35.6 mg/kg-day. Signs of maternal toxicity were observed in rats or mice administered 17 (marginal) to 200 mg/kg-day dibromochloromethane and in mice administered 100 mg/kg-day bromoform. Maternal toxicity was not observed in female rats dosed with up to 200 mg/kg-day of bromoform. Several well-conducted studies on the developmental toxicity of bromodichloromethane gave negative results at doses up to 116 mg/kg-day in rats and 76 mg/kg-day in rabbits when administered in drinking water. However, in other studies, slightly decreased numbers of ossification sites in the hindlimb and forelimb were observed in fetuses of rats administered 45 mg/kg-day in the drinking water on gestation days 6 to 21and sternebral aberrations were observed in the offspring of rats administered 200 mg/kg-day by gavage in corn oil. Reductions in mean pup weight gain and pup weight were observed when the pups were administered bromodichloromethane in the drinking water at concentrations of 150 ppm and above (biologically meaningful estimates of intake on a mg/kg-day basis could not be calculated for this study). Full litter resorption has been noted in F344 rats, but not Sprague-Dawley rats, treated with bromodichloromethane at doses of 50 to 100 mg/kg-day during gestation days 6 to 10. Additional studies in F344 rats that varied the timing of bromodichloromethane administration indicate that gestation days 6-10 are a critical period for induction of full litter resorption. Chronic oral exposure to bromodichloromethane resulted in reduced sperm velocities at doses of 39 mg/kg-day. This response was not accompanied by histopathological changes in any reproductive tissue examined. Adverse clinical signs and reduced body weight and body weight gain were observed in parental generation female rats and F_1 male and female rats at 150 ppm (approximately 11.6 to 40.2 mg/kg-day) in a two generation study of bromodichloromethane administered in drinking water. In the same study, small but statistically significant delays in sexual maturation occurred in F_1 males at 50 ppm

(approximately 11.6 to 40.2 mg/kg-day) and F_1 females at 450 ppm (approximately 29.5 to 109 mg/kg-day). These delays may have been secondary to dehydration caused by taste aversion to bromodichloromethane in the drinking water.

Four of five studies on the reproductive or developmental toxicity of dibromochloromethane gave negative results when tested at doses of up to 200 mg/kg-day. In the fifth study, dibromochloromethane administered at 17 mg/kg-day in a multigenerational study resulted in reduced body weight on postnatal day 14 in one of two F_2 generation litters. At 171 mg/kg-day, the mid-dose in the study, decreased litter size, viability index, lactation index, and postnatal body weight were observed in some F_1 and/or F_2 generation. The developmental and reproductive toxicity of bromoform was examined in two studies.

Bromoform administered to rats at 100 mg/kg-day in corn oil by gavage resulted in a significant increase in sternebral aberrations in the apparent absence of maternal toxicity. In a continuous breeding toxicity protocol, gavage doses of 200 mg/kg-day in corn oil resulted in decreased postnatal survival, organ weight changes, and liver histopathology in F_1 mice of both sexes. No effects on fertility or other reproductive endpoints were noted.

4. Mutagenicity and Genotoxicity

In vitro and *in vivo* studies of the mutagenic and genotoxic potential of bromodichloromethane, dibromochloromethane, and bromoform have yielded mixed results. Synthesis of the overall weight of evidence from these studies is complicated by the use of a variety of testing protocols, different strains of test organisms, different activating systems, different dose levels, different exposure methods (gas versus liquid), and in some cases, problems due to evaporation of the test chemical. Overall, a majority of studies yielded more positive results for bromoform and bromodichloromethane. The genotoxicity and mutagenicity data for dibromochloromethane are variable. Recent mutagenicity studies in strains of *Salmonella* that contain rat theta-class glutathione S-transferase suggest that mutagenicity of the brominated trihalomethanes may be mediated by glutathione conjugation.

5. Carcinogenicity and Related Studies in Animals

The carcinogenic potential of individual brominated trihalomethanes administered in oil has been investigated in chronic oral exposure studies in mice and rats. Ingestion of bromodichloromethane caused liver tumors in female mice, renal tumors in male mice and in male and female rats, and tumors of the large intestine in male and female rats. Ingestion of dibromochloromethane caused liver tumors in male and female mice, and ingestion of bromoform caused intestinal tumors in male and female rats.

Studies of induction of aberrant crypt foci (ACF) show that bromodichloromethane, dibromochloromethane, and bromoform given in drinking water significantly increase the number and focal area of ACF in the colons of male F344 rats, Eker rats, and strain A/J mice, but not in colons of $B6C3F_1$ mice. The biological significance of this induction is unclear, as intestinal

tumors have not been observed either in the colons of F344 rats treated with dibromochloromethane by corn oil gavage or in the colons of rats exposed to bromodichloromethane in the drinking water for two years. Administration of individual brominated trihalomethanes in a high animal fat diet did not significantly increase the number of ACF when compared to a diet containing normal levels of fat.

Exposure of male and female Eker rats (a rodent hereditary model of renal cancer) to bromodichloromethane at drinking water concentrations up to 0.7 g/L did not significantly induce urinary bladder epithelial hyperplasia, individual cell hypertrophy, renal tumors, hemangioma of the spleen, or leiomyomas or mesenchymal cell proliferation in the uterus of females.

6. Other Key effects

The immunotoxicity of brominated trihalomethanes has been investigated in mice and rats. Short-term bromodichloromethane exposure resulted in decreased antibody forming cells in serum, decreased hemagglutinin titers, and/or suppression of Con A-stimulated proliferation of spleen cells at doses of 125 to 250 mg/kg-day.

No studies have been reported for hormonal effects following exposure to dibromochloromethane or bromoform. There is evidence from studies in F344 rats and cultured human placental trophoblasts that bromodichloromethane causes hormonal disruption. Rats exposed to bromodichloromethane on gestation days 8 or 9 show reduced serum levels of LH and progesterone. Serum LH reductions indicate that the mode of action for this strain-specific effect involves altered LH secretion; however, a contributing effect on LH signal transduction has not been ruled out.

Exposure to bromodichloromethane alters the function of human placental trophoblasts, as shown by reduced CG secretion and by changes in morphological differentiation. The mode of action for the observed effects is unknown. Possible mechanisms proposed by the study authors for effects on CG secretion include disruption of CG synthesis at the translational or post-translational level (e.g., by altering glycosylation of CG subunits or disruption of dimerization) or indirect effects on secretion via disruption of gonadotropin releasing hormone activity. The significance of these findings for human health is that placental trophoblasts are the sole source of CG during normal human pregnancy and play a major role in the maintenance of the conceptus. If the observed effect on CG secretion is substantiated in future studies, it may help to explain apparent adverse pregnancy outcomes associated with consumption of chlorinated drinking water in some epidemiological studies (e.g., increased incidence of spontaneous abortion as reported by Waller et al., 1998).

Limited structure-activity data for brominated trihalomethanes and chloroform suggest that bromination may influence the proportion of compound metabolized via the oxidative and reductive pathways, with brominated compounds being more extensively metabolized by the reductive pathway. Additional evidence suggests that a GSH-mediated pathway may play an important role in metabolism of brominated trihalomethanes.

VI. HEALTH EFFECTS IN HUMANS

A. Clinical Case Studies

1. Bromodichloromethane

No clinical reports or short term studies were located on the effects in humans from ingestion of bromodichloromethane.

2. Dibromochloromethane

No clinical case reports or short term studies were located on the effects in humans from ingestion of dibromochloromethane.

3. Bromoform

In the past, bromoform was used as a sedative for children with whooping cough. Typical doses were approximately one drop (about 180 mg), given three to six times/day (Burton-Fanning, 1901). This dosing usually resulted in mild sedation in children, although a few rare instances of death or near-death were reported (e.g., Dwelle, 1903; Benson, 1907). These cases were believed to be due to accidental overdoses. Based on these clinical observations, the estimated lethal dose for a 10- to 20-kg child is approximately 300 mg/kg, and the LOAEL for mild sedation is approximately 54 mg/kg-day.

B. Epidemiological Studies

The brominated trihalomethanes occur as disinfection byproducts in water disinfected with chlorine for the prevention of disease. The primary routes of human exposure to brominated trihalomethanes are via ingestion of disinfected tap water; dermal contact with disinfected tap water during bathing, showering, and other activities; and inhalation of brominated trihalomethanes released during showering, bathing or household activities using disinfected tap water. Multiple epidemiological studies have investigated the relationship between exposure to disinfection byproducts in chlorinated drinking water and adverse health effects. These studies fall into two basic categories: studies of association with cancer (Table VI-1) and studies of association with adverse pregnancy or birth outcomes or alteration of reproductive function (Table VI-2). Because the purpose of this document is to isolate the health effects of individual brominated trihalomethanes, a detailed examination of all available studies on disinfection byproducts is beyond the scope of this report. Epidemiologic studies published prior to 1994 are discussed in greater detail in the Drinking Water Criteria on Chlorine (U.S. EPA, 1994a). A number of recent publications have reviewed the association between chlorination disinfection byproducts and cancer and adverse reproductive or developmental outcomes (e.g., Reif et al., 1996; Mills et al., 1998; Nieuwenhuijsen et al., 2000; Bove et al., 2002).

Very few studies have examined the association between cancer and exposure to brominated trihalomethanes. A possible increased cancer incidence in bladder was suggested (Cantor et al., 1978), while negative findings were reported for childhood acute lymphoblastic leukemia (Infante-Rivard et al., 2001, 2002).

Table VI-1 Epidemiological Studies Investigating an Association Between Chlorinated Drinking Water and Cancer

Reference	Study Description	Observation
Alavanja et al. (1978)	Case control study in seven New York State counties.	Greater risk of gastrointestinal and urinary tract cancer mortality, both sexes, in chlorinated water areas of the counties.
Cantor et al. (1978)	Ecological study using age-standardized cancer mortality rates, 1968-1971; and halomethane levels from U.S. EPA surveys.	Strongest correlation between bromine-containing trihalomethanes and bladder cancer.
Struba (1979)	Case-control study of mortality in North Carolina, 1975-1978.	Small but significant odds ratios for rectum, colon and bladder cancers in rural areas but not in urban areas.
Brenniman et al. (1980)	Case-control study in 70 Illinois communities, 1973-1976. Questionnaires sent to water treatment plants to verify 1963 inventory data on chlorine levels.	Statistically significant relative risks of cancer of gall bladder, large intestine, and total gastrointestinal and urinary tract in females served by systems with chlorinated versus nonchlorinated ground water. Due to many uncontrolled confounding factors, authors concluded that chlorination was not a major factor in the etiology of gastrointestinal and urinary tract cancers.
Gottlieb et al. (1981)	Case-control study using mortality data in Louisiana and estimations of exposure.	Rectal cancer significantly elevated with respect to surface or Mississippi River water consumption.
Young et al. (1981)	Case-control study in Wisconsin, 1972-1977. Questionnaires sent to waterworks superintendents on chlorine content.	Colon cancer showed significant ($p<0.05$) association with chlorine intake in all three dosage categories.
Cragle et al. (1985)	Case-control study using colon cancer cases from seven hospitals in North Carolina.	Consumption of chlorinated water strongly associated with colon cancer, above age 60.
Young et al. (1987)	Case-control study of colon cancer cases in Wisconsin. Water consumption was determined by interview, and chloroform levels by historical records and measurement.	No association found between trihalomethane exposure and colon cancer incidence.

Table VI-1 (cont.)

Reference	Study Description	Observation
Morris et al. (1992)	Meta-analysis of nine case-control studies and one cohort study analyzing cancer and consumption of chlorinated water or water containing high chloroform levels.	Statistically significant relative risk of rectal cancer and bladder cancer in exposed groups. No colon cancer.
McGheehin et al. (1993)	Population-based case-control study	Association between bladder cancer risk and exposure to chlorinated water and trihalomethanes.
King and Marret (1996)	Case-control study conducted by Health Canada	Increased risk of bladder cancer associated with total trihalomethane exposure.
Hildesheim et al. (1998)	Population-based case-control study of colon and rectal cancer risk. Iowa, 1986-1989.	Rectal cancer risk associated with duration of chlorinated water use. No association of colon cancer risk with duration of chlorinated water use or trihalomethane estimates.
Cantor et al. (1998)	Population-based case-control study of bladder cancer risk. Iowa, 1986-1989.	Positive findings for risk restricted to men and to current or former smokers. In men, smoking and exposure to chlorinated water enhanced the risk of bladder cancer.
Marcus et al. (1998)	Ecologic study of association between TTHM in 71 North Carolina public water supplies and incidence of histologically confirmed female invasive breast cancer obtained from cancer registry data.	TTHM levels not associated with breast cancer risk when adjusted for potential confounding factors. Data were consistent with TTHMs being unrelated or weakly related to breast cancer risk.
Infante-Rivard et al. (2001, 2002)	Population-based case-control study comparing 491 cases of childhood acute lymphoblastic leukemia (ages 0-9 yrs) with 491 age, sex, and region of residence-matched population-based controls. Quebec, 1980-1993.	Odds ratios for exposure to bromoform, chlorodibromomethane and dibromochloromethane were generally less than one (range from 0.42 to 1.02). Postnatal exposure to bromoform at concentrations exceeding the 95% percentile was associated with an OR of 1.3 (95% CI = 0.71 to 2.71). The most important limitation of this study is the potential for exposure misclassification.
Villanueva et al. (2003)	Meta-analysis of epidemiologic study data extracted from six case-control and two cohort studies that examined the relationship between exposure to chlorinated water and bladder cancer.	Consumption of chlorinated water was associated with sex-specific combined risk estimates for bladder cancer of 1.4 (95% CI 1.1 to 1.9) for men and 1.2 (95% CI 0.7 to 1.8) for women. The authors suggest that this indicates a moderately increased risk for bladder cancer in men.

Table VI-1 (cont.)

Reference	Study Description	Observation
Villanueva et al. (2004)	Pooled data from 6 case-control studies of bladder cancer in subjects exposed to disinfection byproducts in the United States, Canada, France, Italy, and Finland. Detailed data on water consumption and THM exposure were required for inclusion.	Consumption of chlorinated water was associated with sex-specific combined risk estimates for bladder cancer of 1.4 (95% CI 1.2 to 1.7) for men and 1 (95% CI 0.8 to 1.2) for women.

Table VI-2 Epidemiological Studies Investigating an Association Between Chlorinated Drinking Water and Adverse Pregnancy, Altered Menstrual Function, or Sperm Quality

Reference	Study Description	Observation
Aschengrau et al. 1989	Hospital-based case-control study of spontaneous abortion and multiple water quality parameters in Boston, MA area.	After adjustment for potential confounders and chemical constituents, frequency of spontaneous abortion was increased for consumption of surface water when compared to use of mixed surface and ground water (OR 2.2, 95% C.I. 1.3 - 3.6) The association between surface water and increased risk of spontaneous abortion was not confirmed by a comparison of chlorinated vs. chloraminated surface water. Chloraminated water was used as a surrogate for low exposure to disinfection byproducts.
Kramer et al. (1992)	Population-based case-control study that examined potential associations between pregnancy outcome and exposure to trihalomethanes in tap water. Data on pregnancy outcomes for cases and controls were collected from Iowa birth certificates for non-Hispanic white singleton births during the period January 1, 1989 to June 30, 1990.	A possible association was noted between exposure to bromodichloromethane concentrations $\geq$10 µg/L and intrauterine growth retardation (OR = 1.7; 95% C.I. 0.9, 2.9) when compared to drinking water sources without detectable levels.
Aschengrau et al. 1993	Case-control study of drinking water quality and occurrence of late adverse effects among women who delivered infants during August 1977 - March 1980 in Massachusetts	After adjustment for confounding, frequency of stillbirths was increased for women exposed to chlorinated surface water (OR 2.6, 95% CI 0.9-7.5).

Table VI-2 (cont.)

Reference	Study Description	Observation
Nuckols et al. (1995)	Cross-sectional study in Colorado of populations drinking chlorinated and chloraminated water	No statistically significant effects of exposure, although odds ratio was elevated for risk of low birth weight infants.
Bove et al. (1995)	Cross-sectional study in New Jersey	An association was reported between total trihalomethane levels and "small for gestational age."
Savitz et al. (1995)	Population based case-control study in North Carolina	Statistically significant association of miscarriage with increasing concentration of TTHM and with the highest sextile of exposure(OR=2.8, 95% C.I. 1.1, 2.7), but no relationship with ingested dose or water source. Small increase in risk of low birth rate.
Gallagher et al. (1998)	Retrospective cohort study of relationship between THM exposure during third trimester of pregnancy and low birthweight, low term birth weight, and preterm delivery. Colorado birth certificate data matched to historical water data based on census block groups. 1990-1993.	Possible association of trihalomethane concentration in tap water at maternal residence during third trimester and risk of term low birth weight deliveries. Little association with preterm delivery. Weak association with low birth weight.
Waller et al. (1998)	Prospective study of association between total and individual THM exposure and spontaneous abortion. Concurrent THM data obtained from public water supplies.	Women who drank $\geq$ 5 glasses/day of cold tap water containing $\geq$ 75 µg/L TTHMs had an adjusted odds ratio of 1.8 for spontaneous abortion. Of individual THMs, only consumption of $\geq$5 glasses of water containing $\geq$ 18 µg/L bromodichloromethane (or a compound co-occurring with bromodichloromethane) was associated with spontaneous abortion.
Klotz et al. (1998), Klotz and Pyrch (1999)	Case-control study of association between drinking water contaminants (including disinfection byproducts) and neural tube defects. Births with neural tube defects reported to New Jersey's Birth Defects Registry in 1993 and1994 were matched against control births chosen randomly from across the State.	Elevated odds ratios, generally between 1.5 and 2.1, for the association of neural tube defects with total THMs (TTHMs). The only statistically significant results were seen when the analysis was isolated to those subjects with the highest THM exposures (greater than 40 ppb) and was limited to those subjects with neural tube defects in which there were no other malformations (OR = 2.1, 95% CI = 1.1–4.0).

Table VI-2 (cont.)

Reference	Study Description	Observation
Dodds et al. (1999)	Retrospective cohort study in Nova Scotia women with singleton births, 1988-1995.	Little association between TTHM level and fetal weight- or gestational age-related outcomes. Elevated relative risk for stillbirths for exposure to >100 μg/L TTHM levels during pregnancy. Little evidence for increased prevalence or dose-response for congenital abnormalities with possible exception of chromosome aberrations for exposure >100 μg/L.
Magnus et al. (1999)	Ecologic study in Norway of chlorinated water consumption and birth defects observed in births during period 1993-1995. 1994 data on water quality and disinfection practice. Water color used as an indicator for natural organic matter content.	Among 141,077 births, 1.8% had birth defects. Adjusted odds ratios (high color, chlorination vs. low color, no chlorination) of 1.14 (0.99-1.31) for any malformation; 1.26 (0.61-2.62) for neural tube defects; and 1.9 (1.10-3.57) for urinary tract defects.
Yang et al. (2000)	Study in Taiwan of association between chlorination of drinking water and low birth weight.	Examination of 18,025 births showed no association between consumption of chlorinated drinking water and low birth weight.
King et al. (2000)	Population-based retrospective cohort study in Nova Scotia, Canada to examine the relationship between TTHM or individual THMs and risk for stillbirth of fetuses greater than 500 grams. Study cohort assembled from a perinatal database and consisted of 49,756 singleton births that occurred between 1988 and 1995.	Risk doubled for women exposed to a bromodichloromethane level ≥ 20 μg/L when compared to women consuming concentrations of less than 5 μg/L (relative risk = 1.98, 95% confidence interval of 1.23 - 3.49). When categories of stillbirth (unexplained deaths and asphyxia-related deaths) were examined, relative risk estimates for asphyxia-related deaths increased by 32% for each 10 μg/L increase in bromodichloromethane concentration.
Dodds and King (2001)	Retrospective cohort study conducted using data from a population-based perinatal database in Nova Scotia, Canada and routine water monitoring data. The cohort consisted of women who had a singleton birth in Nova Scotia between 1988 and 1995 and who lived in an area with a municipal water supply.	Exposure to bromodichloromethane at concentrations of 20 μg/L and over was associated with increased risk of neural tube defects (adjusted relative risk = 2.5; 95% confidence interval 1.2 to 5.1) and decreased risk of cardiovascular anomalies (adjusted relative risk = 0.3; 95% confidence interval 0.2 to 0.7). No association observed for bromodichloromethane and cleft defects.

Table VI-2 (cont.)

Reference	Study Description	Observation
Waller et al. (2001)	Reanalysis of total trihalomethane exposure data reported in Waller et al. (1998).	The study authors reported no apparent advantage in using a closest-site (vs. utility-wide) measurement approach for estimation of exposure to total trihalomethanes.
Windham et al. (2003)	Prospective study of association between total and individual THM exposure and menstrual cycle function. Concurrent THM data obtained from public water supplies.	Exposure to dibromochloromethane and sum of brominated trihalomethanes was associated with a reductions in length of the menstrual cycle and follicular phase of the menstrual cycle, suggesting possible effects on ovarian function. Concentrations of ≥20 µg/L for dibromochloromethane and ≥45 µg/L for total brominated trihalomethanes were associated with reductions in cycle and follicular phase lengths of approximately one day. No effect was noted on length of luteal phase or duration of menses.
Fenster et al. (2003)	Prospective study of association between total and individual THM exposure and sperm quality in healthy men. Concurrent data obtained from public water supplies.	Exposure to TTHM was not associated with decrements in semen quality. Individual THM levels not strongly associated with any semen parameter. Inverse relationship between exposure to bromodichloromethane and sperm linearity (linearity decreased by -0.09 ± 0.04 per unit increase in bromodichloromethane).
Shaw et al. (2003)	Case control study of association between total and individual THM exposure and birth defects. Exposure data were obtained from municipal water supplies. Effects were estimated using both continuous and categorical measures of THM exposure.	No association was found between THM as a continuous variable and neural tube defects or other malformations. When exposure to brominated THMs was evaluated, either no effect or reduced odds ratios were observed for all of the malformations considered in the study (ORs ranged from 0.59 to 1.2).

Table VI-2 (cont.)

Reference	Study Description	Observation
King et al. (2004)	An exposure assessment was performed as part of a case-control study of stillbirth and abruptio placentae in Nova Scotia and Eastern Ontario. Residential water samples were analyzed for specific and total THMs and haloacetic acids. Temporal and spatial variation within the water distribution systems was examined and the impact of water use behaviors on the total exposure metric was determined	There was variability in the composition of THM in the two geographic areas under study. Significant spatial variation was observed in large water distribution systems and water use behaviors were shown to significantly affect the total exposure metric with showering accounting for approximately 60% of the total THM exposure. Recommendations include the direct measurement of different species of byproducts, the sampling of individual households rather than distribution systems and incorporation of water use behaviors in estimating the exposure of subjects in epidemiological investigations.
Dodds et al. (2004)	Case-control study conducted in Nova Scotia and Eastern Ontario, Canada , to evaluate the relationship between exposure to chloroform and BDCM and stillbirth. Stillbirths occurring between July 1999 and December 2001 were identified through a population-based perinatal database (112 stillbirth cases and 398 live birth controls).	Women with a residential THM concentration >80 ug/L had elevated risk of stillbirth as compared to women with no exposure (OR = 2.2, 95% C.I. 1.1-4.4). Similar results were seen for chloroform and BDCM concentrations.
Infante-Rivard (2004)	Case-control study to evaluate exposure to total and individual THMs and potential effects on fetal growth. Cases include newborns with low birth weight born at a medical center in Montreal between May 1998 and June 2000. Distribution system data was used to calculate THM concentrations. Mothers and newborns were evaluated for genetic polymorphisms in metabolic enzyme systems.	Exposure to >30 ug/L THM was shown to affect fetal growth, but only in newborns with a genetic polymorphism in the CYP2E1 gene (OR = 13.2, 95% C.I. 1.19-146.72). Exposure information on showering and water consumption were used to derive risk estimates.

Table VI-2 (cont.)

Reference	Study Description	Observation
Wright et al. (2004)	Retrospective cohort study which linked birth certificate data from 1995-1998 with measurement of THM and mutagenicity for all towns in Massachusetts with a population >10,000. Studied the effect of 3rd trimester exposure on birth weight, mean gestational age, SGA, and preterm delivery.	Reductions in mean birth weight were associated with elevated exposure to individual THMs, MX, and mutagenic activity. Dose-response trends were observed for THM concentrations and risk of small for gestational age (SGA), which is defined as a birth weight below the 10th percentile of birth weight per gestational age, sex, and race. Increased risks of SGA were observed for total THM at >40 ug/L (OR range 1.02 to 1.2), chloroform at >20 ug/L (OR range 1.02 to 1.17), and BDCM at >5 ug/L (OR range 1.07 to 1.22). Exposure to BDCM and total THM was associated with an increase in mean gestational age and a decrease in pre-term delivery.
Savitz et al. (2005)	Population-based, prospective cohort of 2,413 pregnant women from 3 water systems in the U.S., 2000-2004. Estimated TTHM, HAA9, and TOX (total organic halide) exposures during pregnancy were considered. Individual brominated THMs and HAA species were examined. Weekly or biweekly distribution system DBP concentration data were collected and linked with maternal residence and water consumption data (during first and second trimesters). Outcomes examined were early (<12 wks) and late (>= 12 wks) pregnancy fetal loss, preterm birth, small-for-gestational-age birth, and term birth weight.	No association with pregnancy loss was seen when high TTHM exposures were compared to low exposures. An association was found between bromodichloromethane and pregnancy loss. Some increased risks were seen for losses at greater than 12 weeks' gestation for TTHM, bromodichloromethane, and TOX, but most results generally did not provide support for an association. TTHM exposure of 80 ug/L was significantly associated with twice the risk for small-for-gestational-age (SGA) births during the third trimester.

Table VI-2 (cont.)

Reference	Study Description	Observation
Toledano et al. (2005)	National registries were used to identify stillbirths and low or very low birth weight deliveries between 1992 and 1998. Postal code from the registry was used to link each birth with a location in each water zone. Concentration data were derived from water supply company sampling programs.	The adjusted odds ratios for stillbirths and low and very low birthweight increased slightly with exposure to total THM in one of three water zones. Odds ratios for this zone ranged from 1.09 (for low exposure) to 1.21 (for high exposure). When the three zones were considered together, the odds ratio for stillbirths was increased with high exposure to total THM (OR = 1.11, 95% CI = 1.00 -1.23). Odds ratios for low or very low birth weight were not increased. The authors reported that concentrations of bromodichloromethane and total brominated THM were not associated with an increased risk of stillbirths or low or very low birthweight, but did not provide the data from this analysis.

A number of studies have examined the association between consumption of chlorinated water and incidence of cancer in the intestine, rectum, bladder, brain, and/or pancreas. Based on the evaluation of the entire cancer epidemiology database, U.S. EPA has concluded that bladder cancer studies provide the strongest evidence for an association between exposure to chlorinated surface water and cancer (U.S. EPA, 1998d). The association between exposure to chlorinated surface water and cancer at other sites cannot be determined at this time because the available data are limited.

A number of epidemiological studies have examined the potential association of reproductive or developmental outcomes with consumption of tap water containing trihalomethanes (reviewed in Bove et al., 1995, 2002; Reif et al., 1996; Mills et al. 1998; Nieuwenhuijsen et al. 2000). These studies have examined three general categories of reproductive and developmental outcomes: 1) spontaneous abortion, stillbirth, and pre-term delivery; 2) low birth weight and growth retardation; and 3) birth defects (neural tube defects, oral cleft, and cardiac effects). In addition, recent studies have examined the potential association between tap water consumption and reproductive toxicity manifested as alterations in menstrual cycle function or semen quality. Studies which found an association with one or more individual brominated trihalomethanes are discussed by chemical below.

In their assessment of available data on the available data for reproductive and developmental effects of disinfection byproducts, Reif et al. (1996) stress that interpretation of epidemiologic findings for these contaminants are potentially complicated by unmeasured confounding variables and misclassification errors. Smoking, socioeconomic status, alcohol consumption, other environmental exposures, and reproductive history are examples of confounding variables that have the potential to bias estimates of risk in studies of disinfection byproducts if not measured. Misclassification errors can arise from failure to account for spatial and temporal variability in contaminant measurements, migration of study participants, incorrect assumptions related to water source or use, and use of water treatment data as a surrogate for tap water concentrations. These factors may result in under- or over-classification of health risks associated with the consumption of disinfected water. Of greatest concern are variables or errors which might lead to underestimation of the true public health risks associated with exposure to tap water containing brominated trihalomethanes. The positive findings in studies of brominated trihalomethanes thus form a foundation for further studies, but should be interpreted cautiously.

It is also important to recognize that detection of an association between an increased incidence of a reproductive effect and the concentration of an individual compound in chlorinated water can not be interpreted as proof that the compound caused the effect. This is because chlorinated drinking water contains many different disinfection byproducts in addition to brominated trihalomethanes, and the occurrence and concentration of many of these products tend to be correlated.

1. Bromodichloromethane

a. Cancer

No workplace or other epidemiological cancer studies were located in which humans were exposed exclusively or primarily to bromodichloromethane.

b. Pregnancy, Birth Defects, and Reproductive Function

Ten studies were located that reported examination of associations between bromodichloromethane and reproductive or developmental endpoints (Kramer et al., 1992; Waller et al., 1998; King et al., 2000; Dodds and King, 2001; Fenster et al., 2003, Shaw et al., 2003; Windham et al., 2003; Dodds et al., 2004; Savitz et al., 2005; Toledano et al., 2005).

Kramer et al. (1992) conducted a population-based case-control study to determine if exposure to trihalomethanes in drinking water is associated with low birthweight (159 cases, 795 controls), prematurity (342 cases, 1710 controls), or intrauterine growth retardation (defined as being lower than the 5th percentile of weight for gestational age; 187 cases, 935 controls). A separate analysis was conducted for each endpoint, using five randomly selected controls for each affected newborn. Data were collected from Iowa birth certificates from January 1, 1989, to June 30, 1990; the study population was restricted to residents of small towns where all of the drinking water was derived from a single source (surface water, shallow wells, or deep wells). Exposure data were based on a 1987 municipal water survey; birth certificate data from 1987 were not used because data on maternal smoking status first became available in 1989. The study authors adjusted for maternal age, number of previous children, marital status, education, adequacy of prenatal care, and maternal smoking. Prematurity (OR = 1.0, 95% C.I. 0.6, 1.5) and low birth weight (OR = 1.0, 95% C.I. 0.7, 1.5) did not show an association with exposure to drinking water containing bromodichloromethane when compared to sources without detectable levels of the compound. A possible association was noted between exposure to drinking water concentrations of bromodichloromethane $\geq$10 µg/L and intrauterine growth retardation (OR = 1.7; 95% C.I. 0.9, 2.9) when compared to drinking water sources without detectable levels. However, the confidence interval included 1, indicating that the increase was not statistically significant.

Waller et al. (1998) conducted a prospective study in pregnant women to examine the association between trihalomethanes in drinking water and spontaneous abortion (pregnancy loss at 20 or less completed weeks of gestation). The study participants were recruited from three facilities of a large managed health care organization which were located in regions of California that primarily received either mixed, surface, or groundwater. Recruitment occurred when the women scheduled their first prenatal exam after confirmation of pregnancy. A group of 5,342 subjects completed a telephone interview that obtained information on demographics, previous pregnancy history, employment status, consumption of tap and bottled water, use of alcohol, tobacco, and caffeine, and other factors. At the time of enrollment in the study, each woman was at least 18 years of age, at 13 or less weeks of gestation, spoke English or Spanish, and could provide with certainty the date of her last menstrual period. Following adjustment for elective

termination of pregnancy, ectopic or molar pregnancies, and multiple gestations, a total of 5,144 pregnancies remained for analysis.

Waller et al. (1998) quantified exposure to trihalomethanes by estimating the subject's daily tap water intake at 8 weeks gestation. Concentration of total trihalomethanes and any available data on individual trihalomethanes were obtained directly from the utility supplying drinking water to a subject's address or zip code. Total trihalomethane levels were calculated by averaging all measurements taken by the utility supplying a participant's home. Each participant was assigned a personal exposure classification (high or low) to total trihalomethanes (TTHM) and individual trihalomethanes (THM) based on the following criteria. A high personal exposure to TTHM was defined as drinking 5 or more glasses of cold tap water per day and having a TTHM level of 75 μg/L or higher. Low personal exposure to TTHM was defined as either 1) drinking less than 5 glasses of cold tap water per day, 2) having a TTHM level of less than 75 μg/L, or 3) receiving water from a utility that provided 95% or greater groundwater. Personal exposures to the individual THMs (bromoform, bromodichloromethane and dibromochloromethane) were defined in a similar manner, with a high personal exposure being defined as drinking 5 or more glasses of cold tap water per day with an individual brominated THM level of 16 μg/L or higher for bromoform, 18 μg/L or higher for bromodichloromethane, or 31 μg/L or higher for dibromochloromethane. Low personal exposures to the individual THMs were defined as either 1) drinking less than 5 glasses of cold tap water per day, 2) having an individual THM level below the cutoff, or 3) having a TTHM level less than 72 μg/L if individual THM levels were not reported.

The authors found a modest association between consumption of trihalomethane-containing water and incidence of spontaneous abortion. Increased risk of spontaneous abortion was noted starting at approximately 75 μg/L. The adjusted odds ratio (OR) for women who drank 5 or more glasses of cold tap water per day containing an average TTHM level of 75 μg/L or higher during their first trimester was 1.8 (95% C.I. 1.1, 3.0). An estimated 18.4% of the study participants were exposed to TTHM at or above this level. Because heating can volatilize and thus reduce TTHM levels in water, the study authors recalculated personal TTHM consumption using total tap water consumption (i.e., hot plus cold). This recalculation resulted in an OR of 1.2 (C.I. 0.8, 1.9) for high personal exposure that was substantially lower than the OR of 1.8 for high personal exposure based on consumption of cold tap water alone. This result implicates volatile agents in the association between tap water consumption and risk of spontaneous abortion.

Of the four individual THMs, only high bromodichloromethane exposure was associated with spontaneous abortion alone (adjusted OR = 2.0, 95% C.I. 1.2, 3.5) and after adjustment for other THMs in a logistic regression model (adjusted OR = 3.0, C.I. 1.4, 6.6). Waller et al. (1998) noted that there was no additive or other effect from showering or swimming. Therefore, no adjustment was required for these variables. Potential misclassification of exposure was identified as the primary limitation of this study. Concentration levels for most subjects were based on test results for a single day, and thus do not reflect potential variation in trihalomethane levels over time. In addition, the exposure to THMs from sources other than ingestion could not be fully characterized.

Because exposure misclassification appeared to be a limitation of the Waller et al. (1998) study, Waller et al. (2001) reported a reanalysis of exposure data from that study. The objective of the new analysis was to examine how use of alternative methods for estimation of exposure would affect associations between TTHM exposure and risk of spontaneous abortion. This reanalysis did not address dose-response relationships between individual brominated trihalomethanes and occurrence of spontaneous abortion. Two exposure analyses were tested. The first method used the utility-wide average concentration (the metric used in Waller et al., 1998). The second method used THM measurements taken from the water system sampling site nearest the subject's home. For each method, the authors performed 1) an unweighted analysis; 2) an analysis weighted by a factor intended to reduce exposure misclassification (see below); and 3) an analysis within a subset of the cohort that possibly had less exposure misclassification than the entire cohort. The weighted and subset analyses were performed in an effort to reduce exposure misclassification.

The utility-wide average method estimated the concentration of total trihalomethanes by averaging all distribution measurements taken by the subject's utility during the first trimester of pregnancy. In contrast to the method used in Waller et al. (1998) the time interval was not expanded in order to capture a measurement and thus reduce missing data in cases where no measurements were available in the first trimester. For weighted analyses of the utility-wide average data, the study authors calculated a weighting factor that reflected the variance of the utility-wide average. This factor approached 1 if the variance (as estimated using the standard deviation, SD) was small and approached 0 if the variance was large:

$$\text{weight}_{\text{utility-wide average}} = 1 - (\text{SD}_{\text{utility-wide average}}/\text{mean TTHM level across sampling data base})$$

The mean TTHM level across the sampling database was 50 μg/L for the years 1990-1991. The weighting factor was set to 0 for women whose SD was greater than 50 μg/L. The weighting factor for women served by groundwater utilities was set to 1, because (at the time that the study was conducted) groundwater was often not chlorinated, trihalomethane levels in these utilities were assumed to be negligible, and the utilities were exempt from quarterly TTHM measurements.

The closest-site method took the average of all measurements taken during the first trimester of pregnancy at the water distribution site nearest to the subject's home. The closest-site approach used TTHM measurements taken from the water system sampling site nearest the subject's home and adjusted for distance between the subject's home and the sampling site. For the weighted analyses, a factor was created that would give a high weight to women living near a water utility sampling site and low weight to women living at a distance from a sampling site:

$$\text{weight}_{\text{closest-site}} = 1 - [(\text{miles to closest sampling site})^2/10]$$

If a subject lived more than 3.16 miles (the square root of 10) from a sampling site, the weight was set to 0. Weighting factors for women served by groundwater utilities were set to 1 as previously described.

For the subset approach, analyses were restricted to groups of women for whom the exposure assessment was likely to be more accurate. Subset analyses using the utility-wide average TTHM concentration included women whose utility measurements were all within 20 μg/L of each other and women served by groundwater utilities. Subset analyses using the closest-site average concentrations used women who lived within 0.5 miles of the utility sampling site and all women served by groundwater utilities. An ingestion metric was calculated using individual daily cold tap water intake at eight weeks gestation as determined in Waller et al. (1998). A categorical ingestion exposure metric was created using the first trimester THM concentration dichotomized at 75 μg/L and cold tap water ingestion dichotomized at 5 glasses per day. Ingestion was also estimated by multiplying the TTHM concentration by cold tap water consumption. A metric to capture exposure to trihalomethanes during showering was created by multiplying THM concentration by typical shower duration and the frequency of showering.

Use of the utility-wide approach generally resulted in odds ratios equivalent to or slightly higher than the closest-site approach. Odds ratios obtained using the utility-wide average method for estimating TTHM (but not the closest-site method) became progressively stronger in the weighted and subset analyses. The study authors reported a positive, monotonic dose-response relationship between spontaneous abortion rate and an exposure metric incorporating TTHM and personal ingestion. The study authors noted that a major limitation of this reanalysis is the lack of a "gold standard" with which to compare the estimated TTHM ingestion of subjects in the study. In the absence of such a standard, it is not possible to determine whether the reanalysis actually reduced exposure misclassification. The conclusions reached by the study authors were 1) there was no advantage in using the closest-site method over the utility-wide method for exposure analysis; 2) use of variance-based weighting factors and subset analyses is defensible and resulted in some increases of odds ratio, but resulting loss of sample size may limit the utility of these techniques; and 3) use of a variety of exposure assessment techniques may lessen the impact of bias resulting from utility-specific factors such as inconsistencies in sampling density or unrecognized contamination problems.

The reanalysis conducted by Waller et al. (2001) identified evidence for differential misclassification in the prior analysis of an area predominantly served by ground water ("Zone A") reported in Waller et al. (1998). The effect of this misclassification was to bias the original estimate of the relationship between TTHM ingestion and spontaneous abortion away from the null. Over 400 of the women in the study cohort resided in Zone A, an area within a large mixed-source utility that received predominantly groundwater. Zone A was not sampled for THMs during the study period. Because other areas within the utility frequently had high TTHM concentrations, use of a utility-wide approach for estimating TTHM concentration probably resulted in an overestimation of exposure for Zone A residents. An investigation by the study authors revealed that although the spontaneous abortion rate of women in Zone A was low overall, women who drank at least 5 glasses of water per day had a spontaneous abortion rate of 14.6%. The reason for the high spontaneous abortion rate among women consuming large amounts of Zone A water was unclear, but was reported to be consistent with other epidemiological studies that found high rates of spontaneous abortion among women ingesting large amounts of unchlorinated water in Region 1 of the original study. Exclusion of Zone A residents or recoding them to a level determined by later testing within the zone decreased the

adjusted OR for high exposure to TTHM (TTHM ≥ 75 μg/L and intake ≥ 5 glasses per day) to 1.5 (95% C.I. 0.8, 2.8) as compared to the adjusted OR of 1.8 (95% C.I. 1.1, 3.0) identified in the original analysis (Waller et al., 1998). The impact of this finding on the adjusted OR calculated for individual brominated THMs is currently unknown, but is expected to be addressed in a future publication by Waller et al.

King et al. (2000) conducted a population-based retrospective cohort study to examine the relationship between TTHM or individual THMs and risk for stillbirth of fetuses greater than 500 grams. The study cohort was assembled from a perinatal database in Nova Scotia, Canada and consisted of 49,756 singleton births that occurred between 1988 and 1995. Exposure was assigned by relating the mother's residence at the time of delivery to the levels of total and individual THMs measured in public water supplies. Maternal age, parity, smoking during pregnancy, infant's sex, and neighborhood family income were evaluated as potential confounders. Relative risks (RR) were adjusted for smoking and maternal age. Exposure to TTHMs, chloroform, and bromodichloromethane were associated with increased risk of stillbirth. Analysis of the results suggested that exposure to bromodichloromethane was a stronger predictor of risk than exposure to chloroform. Risk doubled for women exposed to a bromodichloromethane level of greater than or equal to 20 μg/L when compared to women consuming concentrations of less than 5 μg/L (adjusted RR = 1.98, 95% C.I. 1.23, 3.49). When subcategories of stillbirth (unexplained deaths and asphyxia-related deaths) were examined, relative risk estimates for asphyxia-related deaths increased by 32% for each 10 μg/L increase in concentration of bromodichloromethane. As noted by the authors, a potential limitation of this study was misclassification of exposure as a result of mobility within the study population (estimated to affect less than 10% of study subjects). This study did not examine early fetal death (e.g. spontaneous abortion) because the perinatal database employed in this investigation contained information only on fetuses that weighed 500 grams or more.

Dodds and King (2001) conducted a retrospective cohort study of singleton births among 49,842 residents of Nova Scotia, Canada between 1988 and 1995 to assess the relationship between exposure to THMs and birth defects. Information on exposure concentrations consisted of routine water monitoring data obtained from the Nova Scotia Department of the Environment and reflected samples collected from within the water distribution system. The birth defects examined had previously been reported in other epidemiological studies, and included neural tube defects, cardiovascular defects, oral clefts, and chromosomal abnormalities. The perinatal information used in the study was obtained from the Nova Scotia Atlee perinatal database. This database contains information abstracted from medical records and includes infant diagnoses among stillborn and live born infants up to the time of discharge from the hospital after birth. In addition, information on prenatally diagnosed congential anomalies was obtained from pregnancy terminations. Inclusion of these data was deemed important because, in Nova Scotia, approximately 80% of neural tube defects are detected antenatally and the pregnancy is electively terminated. Exposure windows were selected to target the period before or during gestation when exposure to a potential developmental toxicant or mutagen might have the most profound effect on a particular developmental or genotoxic endpoint. The selected windows were as follows: average bromodichloromethane concentrations from one month before and one month after conception were used for analysis of neural tube defects; concentrations during the first two

months of pregnancy were used for analysis of cardiac defects and oral clefts; and the average concentrations three months before pregnancy were used for the analysis of chromosomal abnormalities. Estimates of relative risks and 95% confidence intervals were obtained from Poisson regression models. Maternal age, parity, maternal smoking, and neighborhood family income were assessed as potential confounders. The categories used for bromodichloromethane concentration were <5 μg/L; 5 - 9 μg/L; 10 - 19 μg/L; and ≥ 20 μg/L.

Exposure to bromodichloromethane at concentrations ≥ 20 μg/L was associated with increased risk of neural tube defects (adjusted RR = 2.5; 95% C.I. 1.2, 5.1) when adjusted for maternal age and income level. However, there was no evidence of a dose-response trend with increasing concentration of bromodichloromethane. In addition, the study authors noted that this point estimate was "fairly unstable" as a result of the low number of cases (n=10) in the ≥20 μg/L exposure category. For cardiac defects, a significant reduction in risk was associated with exposure to concentrations of ≥20 μg/L (RR = 0.3; 95% C.I. 0.2, 0.7) and there was a trend of decreasing risk with increasing exposure. The study authors considered it unlikely that exposure above ≥20 μg/L was actually protective. They suggested that this may be a chance finding or a reflection of a negative association of bromodichloromethane with other disinfection byproducts in this region which may increase cardiac risks. There was no apparent trend or significant association for exposure to bromodichloromethane and occurrence of cleft defects or chromosomal aberrations.

Fenster et al. (2003) examined the relationship between semen quality and exposure to trihalomethanes in home tap water, using data from the California Men's Reproductive Health Study. The participants were 157 healthy men from couples without known factors for infertility, recruited from among 324 men after their wives met eligibility criteria for a prospective study of menstrual function and fecundity. All participants completed an extensive baseline interview that included questions on demographic factors, reproductive history, medical history related to risk of infertility, occupational exposures, consumption of alcohol, caffeine and tobacco, and daily consumption of cold tap water (or beverages made from cold tap water) at home. Cotinine was measured in saliva samples as an indicator of exposure to tobacco smoke. Semen specimens (two samples from each participant in most cases) were obtained between May 1990 and September 1991. Semen parameters assessed included semen volume, sperm concentration, and percent sperm motility. The percentage of sperm with normal morphology was estimated by two methods (the strict method and a modified form of the World Health Organization method of 1987). Motile sperm were analyzed for straight line velocity, curvilinear velocity, linearity (the ratio of straight line velocity to curvilinear velocity), amplitude of head displacement, and percentage of progressively motile sperm. Total trihalomethane levels were assigned based on water utility measurements taken during the 90 days preceding semen collection (an interval corresponding to the period of spermatogenesis). Semen parameters were analyzed as continuous variables with statistics accounting for repeated measures from the same man. Analyses were performed using the following exposure variables: 1) utility-wide TTHM levels; 2) utility-wide individual THM levels; 3) a TTHM ingestion metric obtained by multiplying TTHM concentration (μg/L) by cold home tap water consumption (glasses/day); and 4) self-reported usual daily consumption of cold water at home (glasses/day). All final models were adjusted for duration of abstinence before

sample collection, age, smoking status as determined by cotinine level, work in temperatures greater than 80°F, education, income, and race.

TTHM level was not associated with decrements in semen quality. Data on individual THMs were not included in the report; however, the authors noted that individual THM levels were not strongly associated with any semen parameter, with the exception of an inverse association between exposure to bromodichloromethane and linearity of sperm motion. Use of an adjusted continuous model resulted in a decrease in linearity of - 0.09 ± 0.04 per unit increase in bromodichloromethane concentration. Monotonic, dose-related trends were not evident for the various measures of TTHM exposure and sperm motility, concentration, or count. The highest level of ingestion metric (concentration multiplied by cold tap water ingestion) was associated with a strong decrease in percent normal morphology and an increase in head defects. A difference of -7.1 (95% C.I. -12.7, -1.6) was observed for percent morphologically normal sperm as determined by the modified WHO method at the highest level (>160 μg/L x glasses/day) when compared to the lowest level (≤40 μg/L x glasses/day). However, there was no appreciable decrement in percent normal morphology when assessed using the strict criteria. Consumption of cold home tap water appeared to be weakly related to decreases in percent normal morphology. For every cup of cold home tap water consumed, percent normal morphology (modified WHO method) decreased -0.48 (standard error = 0.43) and percent normal (strict method) decreased by0.31 (standard error = 0.21). Consumption of cold home tap water was not associated with any other semen parameter.

The study authors noted the following strengths and weaknesses of this investigation. Strengths of the study included a prospective study design, a healthy population, data on potential confounders, and state-of-the-art semen analysis techniques. Potential limitations include 1) possible exposure misclassification resulting from use of quarterly utility measurements for estimation of exposure in the home; 2) incomplete characterization of personal exposure (e.g., no information was collected on potential inhalation and dermal exposure via showering, bathing, or swimming, or on factors such as storage or filtration of tap water that might modify THM levels); and 3) lack of data on exposure to other disinfection byproducts in tap water that are known spermatotoxicants in animals, such as halogenated acetic acids. An additional limitation may be the lack of consistency in sperm morphology results for the two methods employed. However, as noted by the study authors, there was no information available that directly compared the methods utilized in this study. Because the selection criteria used in this investigation resulted in a study population more representative of fertile men, the results can not necessarily be generalized to a larger population. The results of this study suggest the need for further study of the effects of disinfection byproducts (including THMs and haloacetic acids) on semen quality.

Shaw et al. (2003) evaluated the relationship between congenital malformations and trihalomethane exposure using data from two previous case-control studies. In the first study, the study population consisted of all livebirths and fetal deaths (after 20 weeks of gestation) occurring among residents of 55 California counties between June 1989 and May 1991. Cases of neural tube defects (anencephaly, spina bifida cystica, craniorachischisis, or iniencephaly) among live births, fetal deaths, or electively terminated fetuses were compared with control liveborn singleton infants randomly selected from each hospital. In the second study, the study population

consisted of all deliveries (live or stillborn) between January 1987 and December 1988 among California residents. Cases were defined as infants or fetuses with orofacial clefts, conotruncal heart defects, or neural tube defects that had not been included in the first study. Controls were randomly selected from births in the same area and time period.

In both studies, mothers were interviewed to obtain medical and reproductive history and to determine all locations where they had resided for at least 2 weeks during the periconceptional period. Exposure to THM was estimated by matching each address to a municipal water supply and requesting data from the company on measured or estimated concentrations of total THM, chloroform, bromodichloromethane, dibromochloromethane, and bromoform associated with each address and time period. Effects were estimated using a continuous measure of THM and also using categorical measures (0, 1-24 ppb, 25-49 ppb, 50-74 ppb, $\geq$75 ppb). In addition, effects associated with exposure to chloroform, bromodichloromethane, and dibromochloromethane (data on bromoform were inadequate for the assessment) above or below the 80th percentile concentration were estimated. For study 1, neural tube defects were inversely associated with total THM both as a continuous and a categorical variable (adjusted odds ratios were from 0.16 to 0.9). For study 2, there was no association between THM as a continuous variable and neural tube defects or other malformations. An increased incidence of neural tube defects was observed for the lowest category of THM (adjusted OR of 2.2), but an exposure-response relationship was not observed. When exposure to brominated THMs was evaluated, either no effect or reduced odds ratios were observed for all of the malformations considered in the study (ORs ranged from 0.59 to 1.2). The authors concluded that these results did not provide evidence of an association between exposure to THM and the occurrences of congenital malformations, but noted that the potential for exposure misclassification may have caused associations to be underestimated

Windham et al. (2003) examined menstrual cycle characteristics in relation to the presence of brominated trihalomethanes in tap water in a prospective study of women living in northern California. Data were also reported for chloroform and total trihalomethanes. The relationships examined included: 1) cycle characteristics and concentration of individual trihalomethanes, total trihalomethanes, and total brominated trihalomethanes in tap water; 2) cycle characteristics and estimated water consumption (total trihalomethanes); and 3) cycle characteristics and duration of showering (total trihalomethanes). The target population was married women of reproductive age (18-39 years old) who were members of the Kaiser Permanente Medical Care Program. Participants in the study were enlisted between May 1990 and June 1991. Participants were selected from among nearly 6500 women using a short screening interview to identify women who were more likely to become pregnant (i.e., those who reported a menstrual period within the last six weeks, were not surgically sterilized, did not use birth control pills or IUDs, and were non-contracepting for less than 3 months). Out of 1092 eligible women, a total of 403 women collected first morning urine samples daily for 2-9 menstrual cycles (average 5.6 cycles) for measurement of steroid metabolites. The participants filled out a daily diary during the urine collection phase and recorded vaginal bleeding as number of pads or tampons. These data (diary and urinary hormone metabolites) were used to estimate menstrual parameters such as cycle and phase length. Cycle length was calculated from the first day of menses to the day before onset of the next menses. When the available data permitted, the

cycle was divided into the follicular phase (first day of menses through estimated day of ovulation) and the subsequent luteal phase. Between 1424 and 1714 cycles were available for evaluation of each parameter.

Information on water consumption (as unheated tap water or drinks made from unheated tap water at home, drinks made from heated tap water at home, and bottled water) and other variables (age, race, education, employment, income, pregnancy history, exercise type and frequency, smoking, alcohol and caffeine consumption) was collected in a baseline telephone interview prior to urine collection. Information on the number of showers taken at home per week and their duration was also collected. Showering was examined as minutes per week and by combining the duration and cycle-specific trihalomethane level to create combinations of high and low exposure. Exposure to trihalomethanes was estimated from quarterly utility monitoring data and information on drinking water and other tap water use collected during the baseline interview. A 90-day exposure time period was assigned for each cycle because trihalomethane monitoring was conducted by the utilities on a quarterly (i.e., about 90 days) basis. A period of 60 days before and 30 days after each cycle start date was selected for the 90-day window. Cycle-specific exposures to total trihalomethanes and individual trihalomethanes were calculated by averaging all trihalomethane measurements taken by a participant's utility at various points in the distribution system (i.e., the "utility-wide average" described by Waller et al., 1998, 2001) during the that 90-day period. Because the brominated trihalomethanes were highly correlated and thus difficult to examine independently, the study authors also examined the sum of the levels of the three brominated compounds. Exposure levels for the brominated trihalomethanes were examined as categorical variables (quartiles). The cycle-specific total trihalomethane concentration was examined as a categorical and continuous variable, as well as combined with amount of unheated tap water and the sum of heated and unheated tap water consumed, to calculate ingestion metrics. Statistical analyses were conducted using the menstrual cycle as the unit of observation. Menstrual parameters were analyzed as continuous or categorical variables in relation to primarily categorical exposure indices and the methods used accounted for repeated measures from the same individual. Numerous covariates reflecting demographic, reproductive history, and lifestyle factors were examined in relation to categorical trihalomethane levels and ingestion to identify potential confounders. Age, pregnancy history, body mass index, caffeine consumption, and alcohol consumption, as well as race and smoking, were included as variables in all adjusted models.

Increased intake of individual brominated compounds or total brominated trihalomethanes was associated with significantly shorter cycles when examined by quartile (Table VI-3). Similar decrements were observed in follicular, but not luteal, phase length. Dose-response patterns were evident for both individual and total brominated trihalomethanes. The strongest association for an individual compound was observed for dibromochloromethane, with adjusted decrements of 1.2 days (95% C.I. -2.0, -0.38) for mean cycle length and 1.1 days (95% C.I. -1.9, -0.25) for mean follicular phase length at the highest quartile ($\geq$20 μg/L). Adjusted decrements for bromodichloromethane at the highest quartile of exposure ($\geq$16 μg/L) were -0.74 (95% C.I. -1.5, -0.02) for cycle length and -0.80 (95% C.I. -1.5, -0.08) for follicular phase length. In contrast to the brominated trihalomethanes, a clear association with reduced cycle length was not observed for chloroform even at the highest quartile ($\geq$17 μg/L) (difference -0.3 days; 95% C.I. -1.0, 0.40).

Menses length was slightly increased at the highest quartile for bromodichloromethane exposure. For categorical variables, the odds of having a long cycle (OR = -1.2; 95% C.I. -2.0, -0.40) or long follicular phase (OR = -1.1; 95% C.I. -1.9, -0.29) were significantly reduced at the highest quartile for summed brominated trihalomethane concentration (≥45 μg/L). These data suggest that exposure to brominated trihalomethanes (or other disinfection by-products that are associated with brominated trihalomethanes in these waters) may affect ovarian function.

Windham et al. (2003) also examined relationships between total trihalomethane (brominated compounds plus chloroform) exposure and menstrual parameters. A monotonic decrease in mean cycle length was observed with increasing total trihalomethane exposure category. At concentrations greater than 60 μg/L, the adjusted decrement was 1.1 day (95% C.I. -1.8, -0.40) when compared to concentrations of 40 μg/L or less. The decrease in follicular phase length was similar (-0.94 day; 95% C.I. -1.6, -0.24). Cycles with total trihalomethane concentrations above the current MCL of 80 μg/L were shorter by about one day as well (0.99 days; 95% C.I. -2.2, 0.17). A unit decrement in mean cycle and follicular phase length of 0.18 days per 10 μg/L increase in total trihalomethane concentration (95% C.I. -0.29, -0.07) was determined when the cycle-specific total trihalomethane level was examined as a continuous variable. When ingestion patterns were examined, mean cycle and phase lengths showed little variation in relation to consumption of unheated tap water at home. In contrast, increased consumption of heated tap water was associated with significantly decreased cycle and follicular phase lengths. The observed decrements were greater than one day with daily consumption of three or more drinks made from heated tap water. These decrements were reduced by adjustment, particularly when caffeine was included in the model; the adjusted decrement in cycle length was 0.68 days (95% C.I. -2.1, 0.72).

Table VI-3 Means and Adjusted Differences in Menstrual Cycle and Follicular Phase Length by Quartile of Individual and Summed Brominated Trihalomethanes

Compound	Quartile of Exposure[a]		
	1[b]	2-3	4
	Mean ± SE (days)	Adjusted Difference[c] (95% CI)	Adjusted Difference[c] (95% CI)
Cycle Length			
Bromodichloromethane	29.8 (0.30)	-0.59 (-1.2, -0.02)	-0.74 (-1.5, -0.02)
Dibromochloromethane	30.0 (0.33)	-0.69 (-1.4, -0.02)	-1.2 (-2.0, -0.38)
Bromoform	29.7 (0.26)	-0.42 (-0.96, 0.13)	-0.79 (-1.4, -0.14)
Sum of Brominated Compounds	30.0 (0.34)	-0.72 (-1.4, -0.04)	-1.2 (-2.0, -0.40)
Follicular Phase			
Bromodichloromethane	17.0 (0.31)	-0.54 (-1.1, 0.01)	-0.80 (-1.5, -0.08)
Dibromochloromethane	17.1 (0.34)	-0.62 (-1.3, 0.05)	-1.1 (-1.9, -0.25)
Bromoform	16.9 (0.27)	-0.30 (-0.83, 0.23)	-0.78 (-1.4, -0.14)
Sum of Brominated Compounds	17.2 (0.35)	-0.66 (-1.3, 0.02)	-1.1 (-1.9, -0.29)

Source: Windham et al. (2003)

[a] Top quartiles for bromodichloromethane, dibromochloromethane, bromoform, and the summed brominated compounds are ≥16, ≥20, ≥12, and ≥45 µg/L, respectively.

[b] Difference from reference group; the mean provided is unadjusted with standard error (SE)

[c] Adjusted for age, race, body mass index, income, pregnancy history, caffeine and alcohol consumption, and smoking.

A non-monotonic relationship was observed for mean cycle length and an ingestion metric combining total trihalomethane concentration and consumption of unheated tap water, with the highest category (>60 µg/day) showing a decrement of 0.4 days and the third category (>40-60 µg/day) showing a decrement of one day. Use of an ingestion metric based on total home tap water consumption (i.e., heated and unheated tap water) revealed a more consistent pattern of reduced cycle length, with adjusted decrements of greater than one day for cycle (-1.1 day; 95% C.I. -2.2, -0.06) and follicular phase (-1.1 day; 95% C.I. -2.2, 0.03) lengths at >40 µg/day. Examination of time spent showering did not reveal additional risks with longer showers. The unadjusted mean cycle length varied little by time spent showering. Following adjustment, there was a tendency toward decreased length with any category of showering above 35 minutes/week. This relationship was stronger for follicular phase duration than for cycle length. For example,

the adjusted mean decrements at the longest duration (≥ 105 minutes) were -0.68 days (95% C.I. -2.0, 0.68) for cycle length and -1.2 days (95% C.I. -2.6, 0.26) for follicular phase length. When combined with TTHM concentration, decrements in cycle and follicular phase length were seen at the higher TTHM (>60 μg/L) and longer showers (≥ 70 minutes) categories (-1.2 and -1.6 days respectively). However, the confidence intervals were wide for all duration categories and a clear dose response pattern (i.e., shorter lengths at higher durations) was not evident.

The study authors noted several strengths and potential limitations of this study. Strengths include the use of a prospective study design, use of biologic measures to determine menstrual parameters, and consideration of many potential confounders. Potential limitations include a study sample that may not be representative of the general population, and exposure misclassification due to a lack of information on other sources of exposure such as washing, cooking and cleaning, as well as exposures outside the home.

There are two observations in this study that might suggest involvement of compounds other than trihalomethanes in the reduction of cycle length. First, the more consistent association of decreased cycle length reported for heated compared to unheated tap water is unexpected if trihalomethanes alone are the causative agent. This is because trihalomethanes volatilize from heated water and exposure to these compounds should therefore be lower for heated tap water, unless the volatilized compound is inhaled. It is not known if the women stayed in the room while heating water and inhaled the trihalomethanes. Second, examination of time spent showering did not reveal additional risks with longer showers. This is also counter to the expected trend, as elevated blood levels of trihalomethanes have been documented after showering (Backer et al., 2000; Miles et al., 2002) as a result of dermal and inhalation exposure. However, information on shower duration was collected by interview and the reported lengths may not have accurately reflected actual shower duration. Other factors that influence exposure could explain the failure to observe an increased risk with longer showers (e.g., shower water temperature, ventilation, etc.), but these data were not available.

Because the study by Windham et al. (2003) is the first to examine changes in menstrual cycle function in relation to tap water exposure, there are no supporting data on the association of disinfection by-products other than the trihalomethanes with changes in menstrual cycle function. Although this study suggests that bromodichloromethane and other brominated trihalomethanes (or other disinfection byproducts associated with them) have effects on ovarian function, no definitive conclusions can be drawn regarding the identity of the compounds responsible for these effects based on the available data.

Dodds et al., (2004) performed a population-based case-control study to examine the relationship between THM exposure during pregnancy and stillbirth in Nova Scotia and Eastern Ontario, Canada. Population-based perinatal databases were used to identify all stillbirths occurring between July 1999 and December 2001. Cases included women who had stillborn infants (i.e., a fetus weighing more than 500 g at delivery, not including pregnancy termination for fetal anomaly) and controls were women who delivered a live born infant during the same time period. A large number of controls was selected to maximize the statistical power of the study. The water use behavior of study participants was evaluated through use of a telephone

interview and residential tap water samples were collected. A total exposure metric was calculated from various exposure sources including bathing and showering. Odds ratios were adjusted for potential confounding factors (i.e., age, province of residence, household income) and multi-variate analyses were conducted separately for total THM, chloroform and bromodichloromethane and for subgroups of cause of death. Asphyxia-related deaths and unexplained deaths were considered of primary interest in this study.

The study results were based on 112 cases and 398 controls. The mean total THM level was 57 ug/L among cases and 55 ug/L among controls. The maximum concentration of total THM, chloroform, and bromodichloromethane among study participants was 318, 315, and 21 ug/L, respectively. Subjects with a total THM level of greater than 80 ug/L had a 2-fold higher risk of stillbirth (OR = 2.2, 95% C.I. = 1.1-4.4) relative to women that were not exposed to THM. Similar results were seen for chloroform (OR = 2.2, 95% CI = 1.0-4.8) and bromodichloromethane (OR = 2.2, 95% CI = 1.0-4.5). When considering the total dose metric, women with the highest exposure to bromodichloromethane had 2.5 times the risk of stillbirth compared with those with no exposure (OR = 2.5, 95% C.I. = 1.3-4.9). The largest odds ratio (OR = 4.0, 95% C.I. = 1.4-11) was observed in women consuming more than 5 glasses of water each day at a total THM concentration of greater than 50 ug/L. Bathing and showering were considered to contribute to increased risk of stillbirth at high concentrations of THM. Although the highest risks for stillbirth were consistently observed in the highest exposure category, no dose-response relationship could be demonstrated for risks related to either the total THM concentration or the total THM exposure metric.

King et al. (2004) performed an exposure assessment as part of the case-control study of stillbirth and abruptio placentae conducted in Nova Scotia and Eastern Ontario (Dodds et al., 2004). Residential water samples were analyzed for specific and total THMs and haloacetic acids. Temporal and spatial variation within the water distribution systems was examined and the impact of water use behaviors on the total exposure metric was determined. A similar level of total THM in the two provinces was associated with a different mixture of specific disinfection byproducts. The correlation between total THMs and bromodichloromethane was high in Nova Scotia (r=0.63), but low in Ontario (r=0.26). Significant spatial variation was observed in large water distribution systems and water use behaviors were shown to significantly affect the total exposure metric with showering accounting for approximately 60% of the total THM exposure. This study highlighted the importance of the direct measurement of different species of byproducts, the sampling of individual households rather than distribution systems and incorporation of water use behaviors in estimating the exposure of subjects in epidemiological investigations.

Infante-Rivard (2004) performed a hospital-based case control study in Canada to evaluate the relationship between THM exposure, gene polymorphism, and intrauterine growth restriction. Cases were defined as newborns whose birth weight was below the 10^{th} percentile for gestational age and sex, based on Canadian standards. Controls were newborns born at the same hospital with a birth weight above the 10^{th} percentile. Participants in the study included 493 cases and 472 controls born at the same medical center between May 1998 and June 2000. Mothers of newborns were interviewed to gather information on demographics, water use behavior, and

medical history. Water concentrations of total THM, chloroform, bromoform, bromodichloromethane, and dibromochloromethane were derived from regulatory data for 189 distribution systems. Exposure estimates were determined for both drinking water and showering. Mothers and newborns were characterized for genetic polymorphism in the CYP2E1 gene and in the 5,10-methylenetetrahydrofolate reductase gene.

For the overall study population, no association was demonstrated between THM exposure (total THM or constituent compounds) and intrauterine growth restriction (odds ratios ranged from 0.62 to 2.44). Both average and cumulative exposure via drinking water and showering were considered. Odds ratios were adjusted to account for confounding factors including, gestational age, sex, race, maternal weight gain, prepregnancy body mass index, smoking during the third trimester, primiparity, preeclampsia and previous intrauterine growth restriction. An association was observed between intrauterine growth restriction and exposure to total THM concentrations above the 90^{th} percentile (29.4 ug/L) in those newborns with 1 or 2 variant alleles in the gene for CYP2E1 (OR = 13.2, 95% C.I. 1.19-146.72). This finding suggests that exposure to THM may affect fetal growth in genetically susceptible newborns.

In a retrospective cohort study, Wright et al. (2004) used birth certificate data on 196,000 infants to evaluate the effect of total THM exposure on various indices of fetal development. Birth certificate data from 1995 to 1998 were derived from towns in Massachusetts with populations greater than 10,000. Information on birth weight and gestational age was linked with town-specific aggregate data on THM concentrations. Concentration data included water concentrations of total THM, chloroform, bromodichloromethane, total haloacetic acids, dichloroacetic acid, trichloroacetic acid, and 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX). Mutagenicity of water samples was also considered in the analysis.

Reductions in mean birth weight were associated with elevated exposure to individual THMs, MX, and mutagenic activity. For total THM, exposures in the 50th-90th percentile (33 to 74 ug/L) were associated with a 12 g reduction in mean birth weight (95% C.I. = -7 to -16 grams), while exposure to concentrations greater than 74 ug/L (>90^{th} percentile) was associated with an 18 g reduction in mean birth weight (95% C.I. = -10 to -26 g). Similar associations were observed for chloroform and bromodichloromethane. Exposure to haloacetic acids was not associated with decreases in mean birth weight. An association was observed between increased THM exposure, mutagenicity and increased gestational age/reduced risk of preterm delivery. Dose-response trends were observed for THM concentrations and risk of small for gestational age (SGA), which is defined as a birth weight below the 10^{th} percentile of birth weight matched on gestational age, sex, and race. Statistically significant increased risks of SGA were observed for total THM at >33 ug/L (ORs of 1.06 to 1.13 for mid and high concentration categories), chloroform at >26 ug/L (ORs of 1.05 to 1.11 for mid and high concentration categories), and bromodichloromethane at >5 ug/L (ORs of 1.1 to 1.15 for mid and high concentration categories). The primary limitation of this study was the potential for exposure misclassification due to the use of town average data for exposure estimates.

Savitz et al. (2005) was a population-based, prospective cohort of 2,413 pregnant women from 3 water systems in the U.S., 2000-2004. Estimated TTHM, HAA9, and TOX (total organic

halide) exposures during pregnancy were considered. Individual brominated THMs and HAA species were examined. Indices examined included concentration, ingested amount, exposure from showering and bathing, and an integration of all exposures combined. Weekly or biweekly distribution system DBP concentration data were collected and linked with maternal residence and water consumption data (during first and second trimesters). Periconceptual, early and late gestational exposure windows were examined. Outcomes examined were early (<12 wks) and late (>= 12 wks) pregnancy fetal loss, preterm birth, small for gestational age, and term birth weight. Potential confounding factors considered were maternal age, tobacco use, race, ethnicity, education, marital status, income, alcohol use, caffeine consumption, body mass index, age at menarche, employment, diabetes, pregnancy history, prior fetal loss, induced abortion history, vitamin use.

No association with pregnancy loss was seen when high TTHM exposures were compared to low exposures. When examining individual THMs, a statistically significant association was found between bromodichloromethane and pregnancy loss. Although non-statistically significant, an increased risk similar in magnitude was seen between dibromochloromethane and pregnancy loss. Some increased risks were seen for losses at greater than 12 weeks' gestation for TTHM, bromodichloromethane, and TOX, but most results generally did not provide support for an association. Preterm birth showed a small inverse relationship with DBP exposure (i.e. higher exposures were less likely to have a preterm birth), but this association was weak.TTHM exposure of 80 ug/L was significantly associated with twice the risk for small-for-gestational-age (SGA) births during the third trimester.

Toledano et al. (2005) evaluated the association between exposure to total THM in public water supplies and stillbirths and birthweight. Three water zones in the UK were selected for study. National registries were used to identify stillbirths and low or very low birth weight deliveries between 1992 and 1998. Postal code from the registry was used to link each birth with a location in each water zone. The weighted average total THM concentrations for the 3-month period prior to birth (corresponding to the last trimester for full-term births) was estimated using data from mandatory sampling conducted by the water supply companies and modeling to estimate missing data. Exposure to total THM was categorized as low, medium, or high; ranges in each category varied between water zones. Odds ratios for stillbirth were adjusted for maternal age and an index of socioeconomic deprivation (the Carstairs index). Odds ratios for low and very low birth weight were adjusted for these variables as well as for sex of infant (low birth weight only) and year of study. The adjusted odds ratios for stillbirths and low and very low birthweight increased slightly with exposure to total THM in one of the three water zones (United Utilities) but not the others. Odds ratios for this zone ranged from 1.09 (for low exposure) to 1.21 (for high exposure). Lower 95% confidence limits for all of the odds ratios were close to unity (values were from 0.98 to 1.14). When the three zones were considered together, the odds ratio for stillbirths was increased with high exposure to total THM (OR = 1.11, 95% CI = 1.00 -1.23). Odds ratios for other comparisons were not increased. The authors reported that concentrations of bromodichloromethane and total brominated THM were not associated with an increased risk of stillbirths or low or very low birthweight, but did not provide the data from this analysis.

2. Dibromochloromethane

a. Cancer

No workplace or other epidemiological cancer studies were located in which humans were exposed exclusively or primarily to dibromochloromethane.

b. Pregnancy, Birth Defects, and Reproductive Function

Nine studies were located that specifically sought to investigate associations between dibromochloromethane and reproductive or developmental endpoints (Kramer et al., 1992; Waller et al., 1998; King et al., 2000; Dodds and King, 2001; Fenster et al., 2003, Shaw et al., 2003; Windham et al., 2003; Infante-Rivard et al., 2004; Savitz et al., 2005). The studies of Kramer et al. (1992), Waller et al. (1998), Fenster et al. (2003), Shaw et al. (2003); Windham et al. (2003); Infante-Rivard et al. (2004), and Savitz et al., (2005) are summarized below. The studies by King et al. (2000) and Dodds and King (2001) are not presented because dibromochloromethane concentrations in water were too low to allow quantitative assessment of exposure-response relationships.

Kramer et al. (1992) reported the results of a population-based case-control study that examined potential associations between exposure to trihalomethanes in tap water and occurrence of low birth weight (159 cases, 795 controls), preterm delivery (342 cases, 1710 controls), and intrauterine growth retardation (187 cases, 935 controls). Data on pregnancy outcomes for cases and controls were collected from Iowa birth certificates for non-Hispanic white singleton births during the period January 1, 1989 to June 30, 1990. Pregnancy outcomes were defined as follows: low birth weight was defined as body weight less than 2,500 grams; prematurity was defined as delivery at less than 37 weeks of gestation; and intrauterine growth retardation was defined as body weight less than the 5th percentile of weight for gestational age. The study population was restricted to residents of small towns (population 1,000 to 5,000) where all tap water was derived from a single source (surface water, shallow wells, or deep wells). The concentration of dibromochloromethane in tap water was estimated from a municipal water survey of tap water conducted in 1986 and 1987. Exposure was assigned based on the mother's town of residence at delivery, as reported on the baby's birth certificate. A separate analysis was conducted for each of the three endpoints, with five randomly selected controls used for each affected newborn. The study data were adjusted for maternal age, number of previous children, marital status, education, number of prenatal visits, and maternal smoking. This study did not identify any association between dibromochloromethane concentration ($\geq$4 μg/L in the highest exposure category) in tap water and occurrence of low birth weight, prematurity, or intrauterine growth retardation (OR=0.8, 1.1, and 0.9, respectively).

Waller et al. (1998) conducted a prospective study in pregnant women to examine the association between consumption of trihalomethanes in drinking water and spontaneous abortion (defined as pregnancy loss at 20 or less weeks of gestation). The study participants were recruited from three branches of a large managed health care organization that were located in regions of California that received either mixed, surface, or groundwater. The protocol used for

this study is provided in the summary for bromodichloromethane in section VI.B.1.b. Exposure to dibromochloromethane exposure was quantified by estimating the participant's daily tap water intake at eight weeks gestation. Any available data on dibromochloromethane concentration were obtained directly from the utility supplying drinking water to a subject's address or zip code. Exposure classifications (high or low) for ingestion of dibromochloromethane were assigned to each participant based on the following criteria. A high personal exposure to dibromochloromethane was defined as drinking five or more glasses of cold tap water per day and having a dibromochloromethane concentration of 31 µg/L or higher. Low personal exposure to dibromochloromethane was defined as either 1) drinking less than five glasses of cold tap water per day, 2) having a dibromochloromethane level below the cutoff, or 3) having a total trihalomethanes level of less than 72 µg/L if dibromochloromethane concentration was not reported.

High personal exposure to dibromochloromethane was not associated with increased risk of spontaneous abortion, when data were adjusted for gestational or maternal age at interview, smoking, history of pregnancy loss, maternal race, and employment during pregnancy (adjusted OR for all regions = 1.3, 95 percent C.I. = 0.7 – 2.4) or when other trihalomethanes were included as covariates (adjusted OR for all regions = 0.8, C.I. = 0.2 – 2.8). The primary limitation of this study is potential misclassification of exposure. Concentration levels for most subjects were based on test results for a single day, and thus do not reflect potential variation in trihalomethane levels over time. In addition, the exposure to trihalomethanes from sources other than ingestion could not be fully characterized.

Windham et al. (2003) examined menstrual cycle characteristics in relation to the presence of brominated trihalomethanes in tap water in a prospective study of women living in northern California. Data were also reported for chloroform and total trihalomethanes. The relationships examined included: 1) cycle characteristics and concentration of individual trihalomethanes, total trihalomethanes, and total brominated trihalomethanes in tap water; 2) cycle characteristics and estimated water consumption (total trihalomethanes); and 3) cycle characteristics and duration of showering (total trihalomethanes). The target population was married women of reproductive age (18-39 years old) who were members of the Kaiser Permanente Medical Care Program. Details of the protocol used for this study and a complete review of the results for total brominated compounds are provided in the summary of results for bromodichloromethane in Section VI.B.1.b.

Increasing levels of individual brominated compounds or total brominated trihalomethanes were associated with significantly shorter menstrual cycles when examined by quartile (Table VI-2 above). Similar decrements were observed in follicular, but not luteal, phase length. Dose-response patterns were evident for both individual and total brominated trihalomethanes. Dibromochloromethane showed the strongest association for an individual compound with adjusted decrements of 1.2 days (95% C.I. -2.0, -0.38) for mean cycle length and 1.1 days (95% C.I. -1.9, -0.25) for mean follicular phase length at the highest quartile ($\geq$20 µg/L). These data suggest that exposure to dibromochloromethane and other brominated trihalomethanes (or other disinfection by-products that are associated with brominated trihalomethanes in these waters) could affect ovarian function. However, the physiological impacts of the relatively small

(i.e., approximately one day) decreases in menstrual cycle and follicular phase lengths, respectively, associated with ingestion of tap water are currently unknown.

Fenster et al. (2003) examined the relationship between semen quality and exposure to trihalomethanes in home tap water, using data from the California Men's Reproductive Health Study. Details of the protocol used for this study and a complete review of the results for total brominated compounds are provided in the summary of results for bromodichloromethane in Section VI.B.1.b. The level of dibromochloromethane in home tap water was not strongly associated with any semen parameter.

Shaw et al. (2003) evaluated the relationship between congenital malformations and trihalomethane exposure using data from two previous case-control studies. In the first study, the study population consisted of all livebirths and fetal deaths (after 20 weeks of gestation) occurring among residents of 55 California counties between June 1989 and May 1991. Cases of neural tube defects (anencephaly, spina bifida cystica, craniorachischisis, or iniencephaly) among live births, fetal deaths, or electively terminated fetuses were compared with 644 control liveborn singleton infants randomly selected from each hospital. In the second study, the study population consisted of all deliveries (live or stillborn) between January 1987 and December 1988 among California residents. Cases were defined as infants or fetuses with orofacial clefts, conotruncal heart defects, or neural tube defects that had not been included in the first study. Controls were randomly selected from births in the same area and time period. Details of the protocol used for this study and a complete review of the results for total brominated compounds are provided in the summary of results for bromodichloromethane in Section VI.B.1.b. Exposure to dibromochloromethane was not associated with increases in the incidence of any measure of congenital malformations in either study.

Infante-Rivard (2004) performed a hospital-based case control study in Canada to evaluate the relationship between THM exposure, gene polymorphism, and intrauterine growth restriction. Cases were defined as newborns whose birth weight was below the 10^{th} percentile for gestational age and sex, based on Canadian standards. Controls were newborns born at the same hospital with a birth weight above the 10^{th} percentile. Participants in the study included 493 cases and 472 controls born at the same medical center between may 1998 and June 2000. Details of the protocol used for this study and a complete review of the results for total brominated compounds are provided in the summary of results for bromodichloromethane in Section VI.B.1.b. No association was demonstrated between exposure to dibromochloromethane and intrauterine growth restriction (OR = 0.62, 95% CI = 0.27-1.44).

Savitz et al. (2005) was a population-based, prospective cohort of 2,413 pregnant women from 3 water systems in the U.S., 2000-2004. Estimated TTHM, HAA9, and TOX (total organic halide) exposures during pregnancy were considered. Individual brominated THMs and HAA species were examined. Details of the protocol used for this study and a complete review of the results for total brominated compounds are provided in the summary of results for bromodichloromethane in Section VI.B.1.b. Although non-statistically significant, an increased risk similar in magnitude was seen between dibromochloromethane and pregnancy loss.

3. Bromoform

a. Cancer

No workplace or other epidemiological cancer studies were located in which humans were exposed exclusively or primarily to bromodichloromethane.

b. Pregnancy, Birth Defects, and Reproductive Function

Four studies were located that examined associations between bromoform consumption or concentration in tap water and reproductive endpoints (Waller et al., 1998; Windham et al., 2003; Fenster et al., 2003; Infante-Rivard et al., 2004).

Waller et al. (1998) conducted a prospective study in pregnant women to examine the association between trihalomethanes in drinking water and spontaneous abortion (pregnancy loss before reaching the 21st week of gestation). The study participants were recruited from three branches of a large managed health care organization that were located in regions of California that received either mixed, surface, or groundwater. A detailed description of the study protocol is provided in the summary for bromodichloromethane in section VI.B.1.b.

The authors quantified exposure to trihalomethanes by estimating the participant's daily tap water intake at eight weeks gestation. Data on concentration of total trihalomethanes and any available data on bromoform were obtained directly from the utility supplying drinking water to a subject's address or zip code. Each participant was assigned a personal exposure classification (high or low) to bromoform based on the following criteria. High personal exposure was defined as drinking five or more glasses of cold tap water per day containing a bromoform concentration of 16 μg/L or higher. Low personal exposure to bromoform was defined as either 1) drinking less than five glasses of cold tap water per day, 2) having a bromoform level below the cutoff, or 3) having a total trihalomethanes level of less than 72 μg/L if bromoform concentration was not reported. No association with incidence of spontaneous abortion was observed for bromoform when data were adjusted for gestation or maternal age at interview, smoking, history of pregnancy loss, maternal race and employment during pregnancy (AOR for all regions = 1.0, 95% C.I. = 0.5 - 2.0) or when other trihalomethanes were included as covariates (AOR for all regions = 0.7, 95% C.I. = 0.2 - 2.1). The primary limitation of this study is potential misclassification of exposure. Bromoform concentrations for most subjects were based on test results for a single day, and thus do not reflect potential variation in tap water levels over time. In addition, the exposure to bromoform from sources other than ingestion could not be fully characterized.

Windham et al. (2003) examined menstrual cycle characteristics in relation to the presence of trihalomethanes in home tap water in a prospective study of women living in northern California. The relationships examined included: 1) cycle characteristics and concentration of individual trihalomethanes, total trihalomethanes, and total brominated trihalomethanes in tap water; 2) cycle characteristics and estimated water consumption (total trihalomethanes); and 3) cycle characteristics and duration of showering (total trihalomethanes). The target population was married women of reproductive age (18-39 years old) who were members of the Kaiser

Permanente Medical Care Program. A detailed description of this study is provided in the summary for bromodichloromethane in section VI.B.1.b.

Increasing levels of individual brominated compounds or total brominated trihalomethanes in home tap water were associated with significantly shorter cycles when examined by quartile (Table VI-3 above). Similar decrements were observed in follicular, but not luteal, phase length. Dose-response patterns were evident for both individual and total brominated trihalomethanes. Bromoform had adjusted decrements of 0.79 days (95% C.I. -1.4, -0.14) for mean cycle length and 0.78 days (95% C.I. -1.4, -0.14) for mean follicular phase length at the highest quartile ($\geq$12 μg/L). These data suggest that bromoform and other brominated trihalomethanes (or other disinfection by-products that are associated with brominated trihalomethanes in these waters) may affect ovarian function. Because the study by Windham et al. (2003) is the first to examine changes in menstrual cycle function in relation to tap water exposure, there are no supporting data on the association of disinfection by-products other than the trihalomethanes with changes in menstrual cycle function. Thus, although this study indicates an association of bromoform exposure with effects on ovarian function, no definitive conclusions can be drawn from the current data regarding the identity of the compounds in tap water responsible for the observed effects. The physiological impacts of the relatively small (i.e., less than one day) decreases in menstrual cycle and follicular phase lengths observed in this study are unknown.

Fenster et al. (2003) examined the relationship between semen quality and exposure to trihalomethanes in home tap water, using data from the California Men's Reproductive Health Study. The participants were 157 healthy men from couples without known risk factors for infertility, recruited from among 324 men after their wives met eligibility criteria for a prospective study of menstrual function and fecundity. A detailed description of this study is provided in the summary for bromodichloromethane in section VI.B.1.b. Bromoform level in home tap water was not strongly associated with decrements in any semen parameter. Details of the analysis of individual THMs were not included in the report.

Infante-Rivard (2004) performed a hospital-based case control study in Canada to evaluate the relationship between THM exposure, gene polymorphism, and intrauterine growth restriction. Cases were defined as newborns whose birth weight was below the 10th percentile for gestational age and sex, based on Canadian standards. Controls were newborns born at the same hospital with a birth weight above the 10th percentile. Participants in the study included 493 cases and 472 controls born at the same medical center between may 1998 and June 2000. Details of the protocol used for this study and a complete review of the results for total brominated compounds are provided in the summary of results for bromodichloromethane in Section VI.B.1.b. No association was demonstrated between exposure to bromoform and intrauterine growth restriction.

C. <u>High Risk Populations</u>

High risk (or susceptible) populations are those which experience more adverse effects at comparable levels of exposure, which experience adverse effects at lower exposure levels than

the general population, or which experience a higher than average exposure because they live or work in settings with elevated environmental concentrations of the chemical of interest. The enhanced response of these susceptible subpopulations may result from intrinsic or extrinsic factors. Factors that may be important include, but are not limited to: impaired function of detoxification, excretory, or compensatory processes that protect against or reduce toxicity; differences in physiological protective mechanisms; genetic differences in metabolism; developmental stage; health status; gender; or age of the individual. For brominated trihalomethanes, high risk populations may potentially include those with elevated levels of CYP2E1 (via exposure to inducing substances or because of altered physiological or health states) or elevated levels of glutathione-*S*-transferase theta. These factors are discussed in greater detail in Section VII.D.3 of this document.

A growing body of scientific evidence indicates that children may suffer disproportionately from some environmental health risks. These risks may arise because the neurological, immunological, and digestive systems of children are still developing (U.S. EPA, (1998a). In addition, children may incur greater exposure because they eat more food, consume more fluids, and breathe more air in proportion to their body weight when compared to adults (U.S. EPA, 1998a). Factors contributing to potentially greater risk in children are discussed in Section VII.D.2 of this document.

D. Summary

Limited human health data are available for the brominated trihalomethanes. In the past, bromoform was used as a sedative for children with whooping cough. Doses of 50 to 100 mg/kg-day usually produced sedation without apparent adverse effects. Some rare instances of death or near-death were reported, although these cases were generally attributed to accidental overdoses. No human toxicological data were available for bromodichloromethane or dibromochloromethane.

Numerous epidemiological studies have examined the association between water chlorination and increased cancer mortality rates. Very few studies have examined the association between cancer and exposure to brominated trihalomethanes, and possible increased cancer incidence in bladder was have been suggested. Recent studies have examined the association of chlorinated water use with various pregnancy outcomes, including low birth weight, premature birth, intrauterine growth retardation, spontaneous abortion, stillbirth , and birth defects. An association has been reported for exposure to bromodichloromethane (or a closely associated compound) and a moderately increased risk of spontaneous abortion during the first trimester. An association has also been reported for exposure to bromodichloromethane (or a closely associated compound) and 1) stillbirth of fetuses weighing more than 500 g, 2) reduction in birth weight (small for gestational age), and 3) increased risk of neural tube defects in women exposed to $\geq$20 µg/L of bromodichloromethane prior to conception through the first month of pregnancy. An association has been reported for total brominated trihalomethanes and reduced menstrual cycle and follicular phase length in women of child-bearing age. Among the individual brominated trihalomethanes, dibromochloromethane displayed the strongest association with altered menstrual function. A study of semen quality in healthy men found an association

between increased exposure to bromodichloromethane in residential tap water and decreased sperm linearity; exposure to dibromochloromethane or bromoform was not associated with decrements in semen quality.

To directly conclude from these studies that bromodichloromethane and dibromochloromethane are developmental or reproductive toxicants in humans can be complicated by the fact that there are many disinfection byproducts in chlorinated water. Nevertheless, these studies raise significant concern for possible human health effects. The methodology used to estimate exposure to brominated trihalomethanes in tap water has been examined with the goal of refining estimates of intake of these compounds in epidemiological studies.

For the brominated trihalomethanes, populations at high risk may potentially include those with elevated levels of CYP2E1 (via exposure to inducing substances or because of altered physiological or health states) or elevated levels of glutathione-*S*-transferase theta. In addition, users of hot tubs and swimming pools may experience additional exposure to brominated trihalomethanes.

VII. MECHANISM OF TOXICITY

A. Role of Metabolism

The toxicity of the brominated trihalomethanes is related to their metabolism. This conclusion is based largely on the observation that liver and kidney, the chief target tissues for these compounds, are also the primary sites of their metabolism. In addition, treatments which increase or decrease metabolism also tend to increase or decrease trihalomethane-induced toxicity in parallel. Pankow et al. (1997), for example, examined the relationship between metabolism of dibromochloromethane and hepatotoxicity. Serum leucine aminopeptidase (LAP) activity (an indicator of hepatotoxicity) increased in a dose-dependant fashion with any treatment that increased the metabolism of dibromochloromethane (e.g. pretreatment with isoniazid or m-xylene).

B. Biochemical Basis of Toxicity

The precise biochemical mechanisms which link brominated trihalomethane metabolism to toxicity are not certain, but many researchers have proposed that toxicity results from the production of reactive intermediates. These reactive intermediates are believed to form covalent adducts with various cellular molecules and to impair the function of those molecules, resulting in cell injury. Reactive intermediates may arise from the oxidative (dihalocarbonyls) or the reductive (free radicals) pathways of metabolism as discussed in Section III.C. Support for this mode of action has been obtained from *in vitro* studies of bromodichloromethane. Under both aerobic and anoxic conditions, bromodichloromethane is metabolized to intermediates that covalently bind to rat microsome proteins and lipids. Direct evidence showing a relationship between the levels of covalent binding intermediates generated by the oxidative or reductive pathways and the extent of toxicity is not currently available for brominated trihalomethanes.

Free radical generation by the reductive pathway for brominated trihalomethane metabolism may result in lipid peroxidation. Although evidence demonstrating that lipid peroxidation actually accounts for the observed cellular toxicity associated with brominated trihalomethanes is lacking, at least one study has established that lipid peroxidation does occur in conjunction with brominated trihalomethane metabolism. De Groot and Noll (1989) reported that all three brominated trihalomethanes induced lipid peroxidation in rat liver microsomes *in vitro*, and that this was maximal at low oxygen levels (between 1 and 10 mm Hg of O_2). The authors interpreted these data to support the concept that lipid peroxidation is caused by free radical metabolites generated by the reductive metabolism of trihalomethanes.

Glutathione has been implicated in both defense against toxicity induced by brominated trihalomethanes and in generation of mutagenic metabolites. Gao et al. (1996) examined the effect of glutathione on the toxicity of bromodichloromethane *in vivo* and *in vitro*. Depletion of glutathione by pretreatment of male F344 rats with the glutathione synthesis inhibitor buthionine sulfoximine increased the hepatotoxicity of a single gavage dose of 400 mg/kg bromodichloromethane administered in 10% Emulphor®. Biochemical indicators of hepatotoxicity (e.g. AST, ALT, LDH) were increased approximately 11-fold and the severity of

morphological changes (centrilobular vacuolar degeneration and hepatocellular necrosis) was greater in the glutathione-depleted animals. Serum and urinary markers of renal damage were also significantly increased by glutathione depletion. Renal tubule necrosis was observed only in the glutathione-depleted group. Overall, glutathione depletion appeared to enhance hepatotoxicity more than nephrotoxicity, an effect that was attributed to organ-specific differences in bromodichloromethane metabolism. The addition of glutathione to reaction mixtures of rat hepatic or renal microsomal fraction and radiolabeled bromodichloromethane resulted in 92% and 20% reductions in protein binding to bromodichloromethane, respectively. The difference in response to glutathione addition was interpreted as evidence for existence of different metabolic pathways in liver and kidney. Bromodichloromethane binding to lipid in liver microsomes under anaerobic conditions was decreased in the presence of glutathione, suggesting that glutathione can react with the dihalomethyl radical.

In contrast to the apparent protective role of glutathione described above, studies in strains of *S. typhimurium* that express rat theta class glutathione S-transferase suggest that conjugation with glutathione leads to formation of mutagenic metabolites (Pegram et al., 1997; DeMarini et al., 1997, Landi et al., 1999b). These studies are described in greater detail in Section V.F.1. Proposed pathways for generation of the mutagenic species are outlined in Figure III-2, also located in Section III.C.1. Briefly, similar mutational specificity, site specificity, and mutation spectra for the three brominated trihalomethanes support the conclusion that they are activated by one or more common pathways. In contrast, the data do not support a glutathione S-transferase mediated pathway for the structurally-related trihalomethane chloroform. This finding suggests that chloroform and the brominated trihalomethanes may in some instances be metabolized by different pathways.

C. Mode of Action of Carcinogenesis

Administration of individual brominated trihalomethanes has been associated with formation of liver tumors (bromodichloromethane, dibromochloromethane), kidney tumors (bromodichloromethane), and tumors of the large intestine (bromodichloromethane, bromoform) in some experimental animals. The mode of action by which brominated trihalomethanes induce tumors in laboratory animals is not known. However, two general modes of action have been proposed: 1) formation of DNA adducts resulting from interaction with one or more classes of reactive metabolites and 2) production of cytotoxicity coupled with regenerative hyperplasia.

The production of reactive metabolites from trihalomethanes is well-established. Classes of reactive metabolites produced include dihalocarbonyls produced by oxidative metabolism and and dihalomethyl radicals produced by reductive metabolism. Additional evidence suggests that reactive species can be also formed via glutathione conjugation (DeMarini et al., 1997; Pegram et al., 1997). Detection of adduct formation and consistent evidence of DNA reactivity in standard assays are two lines of experimental evidence that would strongly support the adduct formation hypothesis. At present there are no *in vivo* data available on DNA adducts resulting from metabolism of brominated trihalomethanes. DNA reactivity can be inferred from test results of mutagenic and genotoxic potential. As noted previously (U.S. EPA, 1994b), synthesis of the

overall weight of evidence for genotoxicity of individual brominated trihalomethanes is complicated by the use of a variety of testing protocols, different strains of test organisms, different activating systems, different dose levels, different exposure methods (gas versus liquid), and in some cases, problems due to evaporation of the test chemical. Overall, a majority of studies yielded more positive results for bromoform and bromodichloromethane, and evidence of mutagenicity is considered adequate for these chemicals. Study results for the mutagenicity of dibromochloromethane are mixed, and the overall evidence for mutagenicity of this chemical is judged to be inconclusive (U.S. EPA, 1994b).

Alternatively, the induction of tumors by individual brominated trihalomethanes could involve an epigenetic mode of action. Induction of tumors in animal studies has been noted to occur primarily at sites where cytotoxicity was observed (i.e., liver and kidney), and there appears to be a correlation between hepatotoxicity and liver tumorigenicity of brominated trihalomethanes in mice (bromodichloromethane > dibromochloromethane > bromoform) (U.S. EPA, 1994b). This raises the possibility that regenerative hyperplasia caused by the cytotoxic effects of brominated trihalomethanes may contribute to the tumorigenic potential of these chemicals. A brief review of studies that have evaluated regenerative hyperplasia following exposure to brominated trihalomethanes is provided below.

A number of studies have measured cell proliferation in liver and/or kidney following exposure to brominated trihalomethanes. Miyagawa et al. (1995) observed evidence for the induction of hepatocyte proliferation in male $B6C3F_1$ mice following a single oral gavage dose of dibromochloromethane in corn oil at the maximum tolerated dose (MTD) or at one half the MTD (200 or 400 mg/kg). Proliferation was assessed by incorporation of [^{3}H]- thymidine using the *in vivo-in vitro* replicative DNA synthesis assay at 24, 39, and 48 hours postexposure.

Potter et al. (1996) investigated cell proliferation in the kidney of male F344 rats. Test animals received 0.75 or 1.5 mmol/kg of bromodichloromethane in 4% Emulphor® by gavage for 1, 3, or 7 days. The administered doses corresponded to 123 or 246 mg/kg-day for bromodichloromethane, 156 or 312 mg/kg-day for dibromochloromethane, and 190 or 379 mg/kg-day for bromoform. Cell proliferation in the kidney was assessed *in vivo* by [^{3}H]-thymidine incorporation. No statistically significant effect of bromodichloromethane on tubular cell proliferation was observed following exposures of up to 7 days, although high labeling levels were observed in 3 of 4 rats at the 246 mg/kg-day dose of bromodichloromethane.

NTP (1998) evaluated cell proliferation in the kidney and liver of Sprague-Dawley rats as part of a short-term reproductive and developmental toxicity screen of bromodichloromethane. The compound was administered in drinking water for 35 days. Groups of male and female rats were exposed to drinking water concentrations of 0, 100, 700 and 1300 ppm bromodichloromethane using the study design described in Table V-6 (Section V.D.1). BrdU labeling index (LI) was unchanged in the livers and kidneys of Group B males at doses up to 69 mg/kg-day. A small but statistically significant increase in the LI was noted in the livers and kidneys of Group C females in the 1300 ppm dose group (126 mg/kg-day). The study authors noted that the result in females was probably biologically insignificant.

Melnick et al. (1998) exposed female B6C3F$_1$ mice (10/dose) to bromodichloromethane, dibromochloromethane, or bromoform in corn oil via gavage for 3 weeks (5 days/week). BrdU was administered to the animals during the last 6 days of the study, and hepatocyte labeling index (LI) analysis was conducted. Time-adjusted doses of 107, 336, and 357 mg/kg of bromodichloromethane, dibromochloromethane, and bromoform, respectively, resulted in significantly elevated hepatocyte proliferation as measured by the LI. The authors compared the dose response for liver toxicity (including hepatic enzyme activity and LI data) and tumorigenicity (using data from previously published NTP bioassays) for the brominated trihalomethanes using the Hill equation model. This analysis indicated that the shape of the dose response as well as the Hill exponents were different for liver toxicity and tumorigenicity. The authors concluded that these data do not support a causal relationship between liver toxicity/reparative hyperplasia and tumor development.

Coffin et al. (2000) examined the effect of bromodichloromethane, dibromochloromethane, or bromoform administered by corn oil gavage or in drinking water on cell proliferation and DNA methylation in the liver of female B6C3F1 mice. Administration of all three brominated trihalomethanes by gavage or in drinking water decreased methylation of the *c-myc* gene. The LOAELs identified for liver toxicity and increased cell proliferation in animals administered in corn oil were 150-,, 100-, and 200 mg/kg , all the lowest doses tested, for bromodichloromethane, dibromochloromethane, and bromoform, respectively. The histopathology findings for animals receiving bromoform in the drinking water were similar to those observed in the lowest-dose gavage group. The results of the single-dose drinking water experiment suggest slightly lower LOAELs for liver toxicity of 138, 171- and 301 mg/kg-day for bromodichloromethane, dibromochloromethane, and bromoform, respectively. It had previously been shown that bromodichloromethane and dibrmomchloromethane administered by corn oil gavage at 75- or 150 mg/kg-day and 50- and 100 mg/kg-day, respectively, caused significant dose-dependent increases of helpatocellular adenoma and adenocarcinoma female mice,(NTP 1987, NTP 1985). Bromoform administered in corn oil at 100- or 200 mg/kg-day is not carcinogenic to female B6C3F1 mice (NTP 1989a).

Torti et al. (2001) conducted 1-week and 3-week inhalation exposure studies of bromodichloromethane in wild type and transgenic mice. Bromodichloromethane toxicity was transient. Regenerative lesions and increased labeling index were evident in the kidney cortex of mice exposed to concentrations of 10 ppm and above for one week. After three weeks of bromodichloromethane exposure, damaged areas of kidney cortex were entirely regenerated (residual scarring was present) and labeling index measurements had returned to near baseline levels. The study authors noted that these results are in contrast to those observed in similar experiments performed with chloroform, where treatment of F344 rats and B6C3F$_1$ mice resulted in continued cytotoxicity and elevated cell turnover for up to 90 days (Larson et al., 1996; Templin et al., 1996). The mechanistic basis for these different responses to structurally similar compound is unclear, but may reflect an induced change in metabolism or emergence of a resistant cell population in animals treated with bromodichloromethane.

George et al. (2002) reported that exposure of male F344/N rats to bromodichloromethane in drinking water for two years at a level that significantly enhanced the prevalence and multiplicity of hepatocellular adenomas and carcinomas had no effect on hepatocellular proliferation. In the same study, the prevalence of renal tubular hyperplasia, but not tumor incidence, was significantly increased at the high dose.

Lock et al. (2004) administered bromodichloromethane by gavage in corn oil to male F344 rats (5/group) at doses of 0, 50, or 100 mg/kg and male B6C3F1 mice (6/group) at doses of 0, 25, or 50 mg/kg-day for 5 days per week over a 28-day period. No change was observed in body weight or clinical chemistry markers of liver or kidney injury in exposed rats and mice. Kidney histopathology was not altered in mice, but mild renal tubule injury was observed in 2 of 5 rats exposed to the highest dose of bromodichloromethane (100 mg/kg-day). Increased cell proliferation was observed in all rats exposed to the highest dose, but was not seen in low-dose rats or in mice at either dose level. It had previously been shown does-dependent increase in incidences of large intestine and kidney tumors in male rats exposed at 50 or 100 mg/kg-day in dose-dependent manner (NTP 1987). It also had previously been shown does-dependent increase in incidences of kidney tumors in male mice exposed at 25 or 50 mg/kg-day in dose-dependent manner (NTP 1987).

Based on an extensive evaluation of carcinogenicity data, cytotoxicity coupled with regenerative hyperplasia is considered the primary mode of action for tumor formation following exposure to high concentrations of chloroform, a structurally-related trihalomethane which has low genotoxic potential (U.S. EPA, 2000c). However, two lines of evidence suggest that chloroform is not a prototypical trihalomethane. First, the weight-of-evidence for at least two of the brominated trihalomethanes indicates that they are genotoxic. This contrasts with the negative weight of evidence evaluation for chloroform. Second, there is evidence that the brominated trihalomethanes are readily bioactivated to mutagenic products via a glutathione *S*-transferase mediated pathway, while chloroform is bioactivated only at very high concentrations. Therefore, a common mode of action for carcinogenicity of chloroform and brominated trihalomethanes cannot be assumed on the basis of current experimental evidence. Data to support a nonlinear primary mode of action for tumor development in liver, kidney, and large intestine are currently lacking for the brominated trihalomethanes. In the absence of such information, combined with a positive weight-of-evidence evaluation for genotoxicity, the mode of action for tumor development is assumed to be a linear process.

D. Interactions and Susceptibilities

1. Potential Interactions

The toxicity of the brominated trihalomethanes appears to result from cytochrome P450-mediated metabolism to reactive metabolites (U.S. EPA, 1994b). Therefore, agents which increase or decrease the activity of enzymes responsible for metabolism of brominated trihalomethanes may modify toxicity. Pankow et al. (1997) observed that pretreatment with isoniazid or m-xylene (inducers of CYP2E1 and CYP2B1/CYP2B2, respectively) increased the

hepatotoxicity of dibromochloromethane in male rats, as measured by elevated serum leucine aminopeptidase activity. Hewitt et al. (1983) observed that pretreatment with acetone, a CYP2E1 inducer, potentiated the acute toxicity of bromodichloromethane and dibromochloromethane in male rats. Thornton-Manning et al. (1993) also found that pretreatment with acetone potentiated the acute hepatotoxicity of bromodichloromethane in male rats. Conversely, the cytochrome P450 inhibitor 1-aminobenzotriazole prevented bromodichloromethane-induced hepatotoxicity in rats (Thornton-Manning et al. 1993). Current findings regarding the existence of glutathione-mediated pathways for brominated trihalomethane metabolism (see sectionV.E.1) suggest that treatments or agents which alter glutathione-*S*-transferase activity may potentially modify the toxicity of brominated trihalomethanes.

The severity of brominated trihalomethane toxicity is potentially affected by the vehicle of administration. Vehicle effects are well-documented in the toxicity of chloroform (e.g., Bull et al. 1986; Jorgenson et al. 1985) and there is some evidence that similar effects occur with brominated trihalomethanes. In a study of vehicle effects on the acute toxicity of bromodichloromethane, a high dose (400 mg/kg) of the chemical was more hepato- and nephrotoxic when given in corn oil compared to aqueous administration, but this difference was not evident at a lower dose (200 mg/kg) (Lilly et al. 1994).

2. Greater Childhood Susceptibility

A growing body of scientific evidence indicates that children may suffer disproportionately from some environmental health risks. These risks may arise because the neurological, immunological, and digestive systems of children are still developing (U.S. EPA, (1998a). In addition, children may incur greater exposure because they eat more food, consume more fluids, and breathe more air in proportion to their body weight when compared to adults (U.S. EPA, 1998a).

U.S. EPA (1998a) recently identified three key questions to consider when evaluating health risks in children from exposure to drinking water disinfection byproducts (DBP) such as the brominated trihalomethanes:

- Is there information which shows that the disinfectant or DBP causes effects in the developing fetus or impairs ability to conceive and bear children? If it causes this type of problem will it occur at a lower dose than that which will cause other types of effects?

- If the disinfectant or DBP causes cancer, are children more likely to be affected by it than are adults?

- If the disinfectant or DBP causes some noncancer toxic effect, are children more likely to be affected by it than are adults?

The data available for evaluation of these issues as they relate to brominated trihalomethanes are addressed below.

a. Effects on the fetus and ability to conceive and bear children

General Results from Animal Studies

Animal studies on the reproductive and developmental effects of brominated trihalomethanes are summarized in Section V.E. At the present time, there are limited studies on dibromochloromethane with one reported developmental effects at doses that were comparable to those of maternal toxicity. There are two studies reported developmental toxicity of bromoform. One study reported developmental effects at dose lower than the level that caused maternal toxicity. There is no data that indicates that either of these chemicals impairs ability to conceive and bear offsprings. Studies of these chemicals in animals indicate that reproductive and developmental effects occur at doses slightly higher than those observed to cause liver and renal effects.

Bromodichloromethane has the most extensive database for developmental and reproductive effect among the brominated trihalomethanes. Study results for the reproductive and developmental effects of bromodichloromethane are mixed. No reproductive or developmental effects were observed at doses up to approximately 116 mg/kg-day in females or 68 mg/kg-day in males in studies conducted in Sprague-Dawley rats (NTP, 1998). Adverse reproductive or developmental effects were not observed in rabbits exposed to doses as high as 55 or 76 mg/kg-day in drinking water on gestation days 6 to 29 (CCC, 2000c,d; Christian et al., 2001a, b). Increased incidences of sternebral aberrations (Ruddick et. al., 1983) and decreased ossification sites in the forelimb and hindlimb (CCC, 2000b; Christian et al., 2001a) have been observed in Sprague-Dawley rats administered bromodichloromethane in corn oil and drinking water, respectively, at doses which induced maternal toxicity.

Reproductive effects of bromodichloromethane have been noted in rodent assays. Klinefelter et al. (1995) observed effects on sperm motility in rats administered 39 mg/kg-day in drinking water for 52 weeks, but these effects were not accompanied by histopathological changes in male reproductive tissues. Narotsky et al. (1997) observed a significantly increased incidence of full litter resorption (FLR) in F344 rats treated with 50 or 75 mg/kg-day bromodichloromethane by aqueous gavage throughout the period of organogenesis. This effect was described as an all-or-nothing phenomenon, in that the litter was either fully resorbed or appeared normal at parturition. This pattern was interpreted by the study authors as evidence for a maternally-mediated mechanism, rather than a direct effect of bromodichloromethane on the developing embryo. Bielmeier et al. (2001) observed increased incidence of FLR in F344 rats treated with 75 or 100 mg/kg-day bromodichloromethane by aqueous gavage on one or more days during the interval from gestation day 6 to 10. This response was strain-specific and FLR was not observed in Sprague-Dawley rats treated with up to 100 mg/kg-day on gestation days 6 to10. Analysis of hormone profiles reported by Bielmeier et al. (2001, 2004) suggest that

bromodichloromethane disrupts normal endocrine function in F344 rats, either by inhibiting LH secretion or by decreasing luteal responsiveness to LH.

Data from Epidemiological Studies

No information on developmental and reproductive effects is available from human studies that examined populations exposed exclusively or primarily to brominated trihalomethanes. Several studies of exposure to chlorinated water have identified potential associations between bromodichloromethane intake or concentration in tap water and adverse health effects. These studies have examined associations between exposure to bromodichloromethane in drinking water and adverse pregnancy outcomes (low birth weight, prematurity, intrauterine growth retardation, spontaneous abortion, still birth) and reproductive function (sperm quality, menstrual cycle function). Summaries of these studies are provided in Section VI.B.1. Weak, but significant, associations have been noted for 1) ingestion of $\geq$18 µg/L during the first trimester of pregnancy and increased risk spontaneous abortion (adjusted OR 2.0, 95% C.I. 1.2, 3.5; adjusted OR with adjustment for other trihalomethanes 3.0, 95% C.I. 1.4, 6.6) (Waller et al., 1998); 2) exposure to concentrations$\geq$20 µg/L and increased risk of stillbirth (adjusted OR 1.98, 95% C.I. 01.23, 3.49) (King et al., 2000); and 3) exposure to concentrations$\geq$20 µg/L and increased risk of neural tube defects (Dodds and King, 2001).

In epidemiological studies of reproductive function, exposure to bromodichloromethane in tap water was associated with decreased menstrual cycle length (Windham et al., 2003). The observed decrease at the highest quartile of exposure ($\geq$16 µg/L) was less than one day (-0.74 days, 95% C.I. -1.5, -0.02). The decrease in overall cycle length appeared to result from decreased follicular phase length (-0.80, 95% C.I. -1.5, -0.08), suggesting that the bromodichloromethane has an effect on ovarian function. In a study of semen quality, exposure to bromodichloromethane was associated with significantly decreased sperm linearity (- 0.09 ± 0.04 per unit increase in bromodichloromethane concentration), but associations with other semen quality or sperm motility parameters were not observed (Fenster et al, 2003).

Modes of action have not been proposed to explain the responses attributed to bromodichloromethane in the epidemiological studies described in Section VI.B.1. The results of *in vitro* studies (see Section V.H.2) using differentiated human placental trophoblasts in primary culture suggest that the placenta is a target tissue for adverse effects of bromodichloromethane in humans and that this could be related to adverse effects on human pregnancy (Chen et al., 2003, 2004). Exposure of primary cultured trophoblasts to 0.02-2000 µM bromodichloromethane in the culture medium significantly decreased secretion of immunoreactive and bioactive chorionic gonadotropin (CG), a glycoprotein that plays a major role in maintenance of the human conceptus during early pregnancy. The events at the molecular and biochemical level that cause decreased secretion are unknown, but could include direct effects on synthesis (e.g., glycosylation or dimerization) or indirect effects via disruption of gonadotropin releasing hormone (GnRH) activity (a putative major regulator of placental CG synthesis and secretion). If substantiated, an effect on GnRH would parallel one of the proposed mechanisms for pregnancy loss in F344 rats (Bielmeier et al. 2001; 2004).

Full Litter Resorption in F344 Rats

The results of the Narotsky et al. (1997) and Bielmeier et al. (2001) studies identify FLR as the most sensitive female reproductive response among the currently available animal studies. Strain-specific FLR observed in F344 rats exposed to bromodichloromethane is of concern because associations have been noted between exposure to bromodichloromethane in tap water and adverse pregnancy outcomes (spontaneous abortion, stillbirth) in epidemiological studies. There are two issues to consider regarding the relevance of FLR observed in F344 rats to human health. The first issue is whether the mechanism responsible for this effect in rats is applicable in humans. Although the exact mechanism of FLR in F344 rats is unknown, the high incidence of FLR during the LH-dependent period of pregnancy and the lack of response thereafter suggests an LH-mediated mechanism. Serum progesterone levels were reduced at 12 and 24 hours after dosing in all rats that experienced FLR. Because LH is required to stimulate progesterone secretion by the corpus luteum during the period of pregnancy examined by Bielmeier et al. (2001), these data are consistent with an effect of bromodichloromethane on LH secretion and/or single transduction. Although the hormone profile data collected by Bielmeier et al. (2001) did not detect an effect on LH levels, subsequent work with a more sensitive assay demonstrated reduced LH levels following bromodichloromethane exposure (Bielmeier et al., 2004). This result supports an effect on LH secretion. Although species differences exist, rats and humans are similar in that either LH (in rats) or chorionic gonadotropin (hCG; in humans) is required at specific periods for maintenance of pregnancy (Bielmeier et al., 2001; 2004). LH and hCG act via the same receptor in rats and humans. Thus, if bromodichloromethane reduces luteal response to LH in F344 rats by inhibition or down-regulation of the LH/hCG receptor, then it might also be expected to have effects on hCG signal transduction in humans. If this were the case, bromodichloromethane would be expected to increase the risk of early termination of pregnancy in humans.

An alternative hypothesis is that bromodichloromethane causes FLR by a mechanism that involves alteration of maternal LH secretion. Bielmeier et al. (2004) demonstrated reduced LH serum levels in pregnant F344 rats treated with bromodichloromethane, indicating an effect on LH secretion. Gonadotropin releasing hormone (GnRH) secreted by the hypothalamus regulates LH secretion in the rat. GnRH from the cytotrophoblast is thought to regulate hCG secretion in the human placenta. An effect of bromodichloromethane on hCG secretion by human cytotrophoblast cells has been demonstrated *in vitro* (Chen et al., 2003, 2004). Although the mode of action underlying this in vitro effect is unknown, it is possible that bromodichloromethane could inhibit hCG secretion in a manner analogous to LH secretion. If so, bromodichloromethane would be expected to increase the risk of early termination of pregnancy. Even if this were not the case, interference of bromodichloromethane with maternal LH secretion in humans would be expected to have other adverse effects on reproductive function in the female, whether or not it was able to cause an event similar to full-litter resorption. It should be noted that a possible contributing effect of bromodichloromethane on luteal responsiveness to LH has not been ruled out.

The second issue to consider in evaluating these data concerns the use of the F344 strain as a model for reproductive effects in humans. The Sprague-Dawley rat is often identified as the preferred rodent model in reproductive and developmental toxicity testing. However, this preference is based on pragmatic considerations (high fecundity, favorable maternal behavior, availability of extensive historical control data for reproductive and developmental endpoints), and is not based on any evidence that there are important physiological or biochemical differences between young adults of these strains or that one is strain is inherently a more relevant biological model for human reproductive health than the other. Thus, the fact that this response has been observed in F344 rats but not in Sprague Dawley rats is not a reason to consider that the effect may not be applicable to humans. Based on these considerations, in accordance with U.S. EPA policy, the results of this study are considered relevant to human health in the absence of data that demonstrate otherwise.

b. Childhood Cancer and Noncancer Effects

Bioactivation to reactive metabolites is an apparent prerequisite for toxicity and carcinogenicity of the brominated trihalomethanes. Therefore, an important issue in the assessment of childhood risk of cancer and other adverse effects is whether the enzymes responsible for metabolism are more active in fetuses, neonates, and or children than in adults. This section evaluates the available data for developmental expression and/or activity of three key metabolizing enzymes that are known or anticipated to bioactivate the brominated trihalomethanes: CYP2E1, CYP2B1/2 (in rodents only), and glutathione-*S*-transferase theta.

CYP2E1

Carcinogenicity of brominated trihalomethanes has been shown to be at least partly related to bioactivation by the cytochrome P450 isoform CYP2E1 (U.S. EPA, 1994b). Thus, a higher level of CYP2E1 activity in children relative to adults might predispose children to greater toxicity. Studies of human fetal liver have produced contradictory results, but suggest that CYP2E1 protein is either not expressed or is expressed at levels lower than in adults (Hakkola et al., 1998). Carpenter et al. (1996) detected immunoreactive CYP2E1 protein in liver samples from fetuses ranging from 16 to 24 weeks in gestational age. The samples obtained were from fetuses whose mothers did not have a history of alcohol use. The immunoreactive protein exhibited a slightly lower molecular weight than observed for CYP2E1 from adult liver samples. Expression of the corresponding mRNA was confirmed in a fetal liver sample of 19 weeks gestational age by reverse transcriptase-polymerase chain reaction (RT-PCR). However, CYP2E1 mRNA was not detectable in a fetal liver sample of 10 weeks gestational age, suggesting (in the opinion of the study authors) that CYP2E1 expression may be related to specific stages of fetal development. The catalytic capability of CYP2E1 protein in human fetal microsomes was demonstrated by measuring the rate of ethanol oxidation to acetaldehyde. The rate of conversion varied from 12 to 27% of that measured in adult microsomes. Treatment of fetal hepatocytes in primary culture with ethanol or clofibrate indicated that fetal CYP2E1 protein is inducible (approximately two-fold compared to untreated cells).

Vieira et al. (1996) detected small amounts of CYP2E1 mRNA in fetal liver samples (approximately 5 to 10% of the levels in adult liver) collected from fetuses aged 14 to 40 weeks. However, these authors could not detect immunoreactive CYP2E1 protein in any of 27 fetal liver samples Other studies have failed to detect either CYP2E1 protein or mRNA in fetal liver samples. Cresteil et al. (1985) and Komori et al. (1989) did not detect immunoreactive protein or mRNA in fetal liver samples of less than 16 weeks gestational age. Jones et al. (1992) did not detect CYP2E1 mRNA or protein in liver samples that were of similar gestational age (16 to 18 weeks) to the samples examined by Carpenter et al. (1996). Juchau and Yang (1996) did not detect CYP2E1 mRNA by RT-PCR in human embryonic tissues between days 45 and 60 of gestation. The factors contributing to the different results are unknown, but may include inter-individual variability, gestational age of the tissue examined (for the samples less than 16 weeks gestational age), or the existence of factors other than developmental stage that control expression.

Information on the presence of CYP2E1 in human fetal tissues other than the liver is limited. Vieira et al. (1998) examined the mRNA content of human fetal lung and kidney. CYP2E1 mRNA was expressed at a very low level in both tissues and the levels remained stable after birth. Studies of human fetal brain tissue indicate that CYP2E1 is expressed in human embryonic brain tissue (see Juchau et al., 1998) and that relatively low levels of CYP2E1 mRNA, immunoreactive protein, and catalytically active protein are present during the early fetal period of development (Brzezinski et al., 1999). In one study, a dramatic increase in CYP2E1 was observed at approximately gestation day 50, and a fairly constant level was maintained until at least day 113 (Brzezinski et al., 1999). The relevance of the data for lung and brain is uncertain, since these organs are not known to be targets for brominated trihalomethane toxicity.

Vieira et al. (1996) investigated age-related variations in human CYP2E1 protein levels and catalytic activity from birth through adulthood. These authors observed a rapid increase in the immunoreactive CYP2E1 microsomal content within 24 hours after birth that was independent of the gestational age of the newborn. This activation was accompanied by a demethylation of cytosine residues in the 5'-regulatory region of the gene, suggesting that methylation of specific residues prevents transcription in the fetal liver. The CYP2E1 protein level gradually increased during the first year and reached the adult level in children aged 1 to 10 years. CYP2E1 catalytic activity was assessed by determination of *in vitro* hydroxylation of chlorzoxazone in 89 microsomal preparations. Chlorzoxazone hydroxylation activity increased within 24 hours after birth and steadily increased during the first year. Catalytic activity reached adult levels at age 1 to 10 years.

Animal studies of CYP2E1 expression during development have given variable results. One study indicated that CYP2E1 is expressed in the fetal rat liver and placenta and that levels are increased in rat pups exposed to ethanol in utero or via lactation (Carpenter et al., 1997). Liver samples from rat fetuses exposed to ethanol in utero showed a 2.4-fold increase in protein levels and 1.5-fold increase in catalytic activity (Carpenter et al., 1997). Other authors have reported that hepatic CYP2E1 gene transcription in rats is activated at birth and that the amount

of CYP2E1 reaches a peak prior to weaning (see Ronis et al., 1996). The protein level then falls to approximately 25% of the peak level and remains stable into adulthood (Ronis et al., 1996).

The regulation of CYP2E1 is complex when examined at both the molecular (Lieber 1997) and physiological (Ronis et al. 1996) levels. The factors and processes responsible for the increase in CYP2E1 protein levels and activity at birth have not been clearly identified. At the physiological level, there is some evidence from rodent studies to suggest that growth hormone regulates the constitutive expression of CYP2E1 (Ronis et al., 1996). The reduction of CYP2E1 from peak levels before weaning is reported to coincide with the increased levels of growth hormone and with development of adult levels of growth hormone receptors (Ronis et al., 1996). The occurrence of peak expression after birth has been attributed to a role of CYP2E1 in gluconeogenesis, since there is a very high demand for energy production from glucose at this developmental stage (see Ronis et al. 1996; Vieira et al. 1996).

CYP2B1/2 (Rodents)

Research conducted by Pankow et al. (1997) suggests that the closely-related CYP isoforms 2B1 and 2B2 participate in the catabolism of dibromochloromethane in rats. These isoforms show greater than 97% homology of amino acid sequence and have highly similar genomic organization. To date, these isoforms have not been reported in adult or fetal human tissues (Nelson et al., 1996; Juchau et al., 1998). Omiecinski et al. (1990) detected low levels of CYP2B isoform mRNA in fetal rat liver on gestation day 15 (the earliest day in development when the authors were able to macroscopically recognize and dissect the fetal liver) using the polymerase chain reaction (PCR). Although the levels of mRNA expression were "substantially lower" lower at day 15 than observed later in development, expression was clearly inducible by pretreatment of pregnant rats with phenobarbital. Both constitutive and phenobarbital-induced levels of mRNA increased with developmental age, reaching maximal levels at approximately three weeks postpartum. No measurements of CYP2B activity were made in this study, so it is not known whether changes in mRNA levels were paralleled by changes in catalytic activity.

Juchau et al. (1998) reviewed a series of experiments that employed the selective substrate probe pentoxyresorufin to test for CYP2B1/2 catalytic activity in fetal rat tissues. The overall conclusion upon examination of all results was that if CYP2B isoforms are expressed in fetal rats, they occur at biologically insignificant levels. Asoh et al. (1999) examined the catalytic activity of CYP2B isoforms in fetal rat liver and found very low activity, a finding consistent with the conclusion of Juchau et al. (1998).

Gebremichael et al. (1995) investigated the postnatal developmental profile of CYP2B1 in Sprague-Dawley rats. CYP2B1 activity was detectable as early as seven days postnatally and exhibited a variable pattern of expression (no clear trend evident) when assayed at days 14, 21, 50, and 100. Asoh et al. (1999) examined the induction of CYP2B isoforms in neonatal rats. The level of CYP2B catalytic activity was markedly higher at five days after birth relative to levels observed in fetal hepatic tissue. Oral or intraperitoneal administration of phenobarbital to pregnant rats increased the level of CYP2B expression and activity in neonates. Overall, these

findings suggest that CYP2B isoform activity is likely to be lower in fetuses than in neonates or adults and that increased levels of activity may be observed in fetuses and neonates exposed to inducing xenobiotics. The significance of this information for risk of cancer in human fetuses, neonates, and children is uncertain since, as noted above, the CYP2B1/2 isoforms have not been identified in humans.

Glutathione S-Transferase Theta

Recent mutagenicity studies suggest that brominated trihalomethanes can also be activated to mutagens by the product of the glutathione *S*-transferase (GST) theta gene *GSTT1-1* (DeMarini et al.,1997; Landi et al., 1999b). Children and the fetus could potentially experience increased risk of adverse effects if the activity of this enzyme was higher at these life stages than in adults. Information on the developmental expression of GST genes is currently limited. Although other classes of GSTs (alpha, mu, and pi) are expressed in fetal liver, Mera et al. (1994) reported that theta-class GSTs were expressed in only adult liver. This finding suggests that the fetus does not experience increased risk as a result of GST theta-mediated mutagenicity. The occurrence of increased risk in children cannot be evaluated, since postnatal developmental pattern of GST theta is unknown.

c. Childhood Cancers: Other Considerations

There are no studies that have examined the carcinogenicity of brominated trihalomethanes in immature animals. Examination of childhood cancer data compiled by the National Cancer Institute (Ries et al. 1999) indicates that the incidence of hepatic, renal, and intestinal cancer (the types of cancer observed in animal studies of bromodichloromethane) from causes other than genetic predisposition are low. Primary neoplasms of the liver are rare in children younger than 15 years of age. The incidence of hepatocellular carcinoma (the type of neoplasm observed in mice treated with bromodichloromethane or dibromochloromethane) decreased in children younger than 15 years of age during the period 1975 to 1995. The incidence rate of renal carcinoma remained very low (well under one case per million) in children younger than 15 years during the period 1975 to 1995. Trend data were not available in Ries et al. (1999) for intestinal cancer. These data do not address cancers that may be initiated in childhood and manifested in adults.

d. Conclusion

The available evidence for developmental expression of enzymes known to metabolize brominated trihalomethanes supports the conclusion that children do not experience greater risk from exposure to these compounds than do adults. At present there are no cancer incidence data from humans to suggest that brominated trihalomethanes contribute to increased risk of cancer in children.

3. Other Potentially Susceptible Populations

a. Subpopulations with altered levels of CYP2E1

CYP2E1 catalyzes the metabolism of brominated trihalomethanes to reactive intermediates that mediate toxicity. Individuals with higher levels of CYP2E1 activity may therefore be at greater risk for adverse health effects. This section describes factors associated with increased levels of CYP2E1 activity and subpopulations who may be at increased risk as a result of these factors.

Genetic Polymorphisms

Significant inter-ethnic differences exist in CYP2E1 polymorphism (Ronis et al., 1996; Lieber, 1997) and it is possible that these differences could influence susceptibility to toxic effects. The CYP2E1 polymorphisms currently reported in the literature are located in the 5'-flanking (noncoding) regions of the gene, while the coding regions of the gene which specify sequence appear to be well conserved among various ethnic groups (Ronis et al., 1996). Mutations in the 5'-region of a gene can affect the regulation of gene expression. The rare mutant *c2* polymorphism of CYP2E1 is reported to be associated with higher transcriptional activity, protein levels, and catalytic activity than the more common wild type allele (Lieber et al., 1997). As reported by Lieber et al. (1997), the highest frequency of the *c2* allele occurs in the Taiwanese (0.28) and Japanese (0.19 to 0.27) populations. The frequencies in African-Americans, European-Americans, and Scandinavians are much lower, generally in the range 0.01 and 0.05. Efforts to link the occurrence of the c2 allele to higher rates of CYP2E1-mediated liver disease have yielded inconsistent results. Thus, the functional significance of CYP2E1 polymorphism is presently uncertain, and no conclusion can as yet be drawn about the relative risk for different ethnic populations exposed to brominated trihalomethanes.

Altered Physiological or Health States

The physiological functions of CYP2E1 include lipid metabolism and ketone utilization (Lieber, 1997). Induction of CYP2E1 is observed in many conditions that elevate circulating levels of lipids, including consumption of a high-fat or low-carbohydrate diet, starvation, obesity, and insulin-dependent diabetes. Among the individuals likely to be affected by such conditions, diabetics constitute the most clearly-defined susceptible population. Induction of CYP2E1 in uncontrolled insulin-dependent diabetes is well-studied. In animals, this induction results in elevated levels of CYP2E1 in the liver, kidney, and lung (Ioannides et al., 1996). Acetone (a substrate of CYP2E1) is thought to be the inducing compound (Ronis et al., 1996). As a result of induction, diabetic animals are more susceptible to the toxicity of some chemicals metabolized by CYP2E1. While there are no specific data for the brominated trihalomethanes, this phenomenon has been demonstrated for other halogenated compounds including chloroform, carbon tetrachloride, trichloroethylene, and bromobenzene (Ioannides et al., 1996). Because the animal and human orthologues of CYP2E1 show similar substrate specificity and bioactivation potential, it is possible that diabetic humans may also be more susceptible to CYP2E1-mediated toxicity.

As CYP2E1 levels are reduced by insulin treatment, increased toxicity would be anticipated only in poorly controlled or uncontrolled diabetics (Ioannides et al., 1996).

Alcohol consumption

CYP2E1 contributes to the metabolism of ethanol in humans and animals. Consumption of ethanol induces CYP2E1 and chronic alcohol consumption is reported to result in as much as a 10-fold induction (Lieber, 1997). Hence, concurrent exposure to ethanol and brominated trihalomethanes may increase susceptibility to adverse health effects. This interaction is of concern because concurrent exposure to brominated trihalomethanes and ethanol is likely to occur in a significant number of people. At present, there are no human or animal studies which examine this interaction for brominated trihalomethanes. However, Wang et al. (1994) reported that a single 100 mg/kg oral dose of ethanol administered to rats significantly increased the toxicity of the structurally-related trihalomethane chloroform (also metabolized by CYP2E1). Lieber (1997) noted that the hepatotoxicity of commonly used industrial solvents (e.g. carbon tetrachloride, bromobenzene, and vinylidene chloride) and anesthetics (enflurane and halothane) was increased in heavy drinkers, with a pattern of damage that was consistent with the selective expression and induction of CYP2E1 in certain regions of the liver.

Concurrent exposure to other CYP2E1 inducers including pharmaceuticals

Because CYP2E1 is highly inducible by a wide range of xenobiotic compounds, prior exposure to such inducers may potentially play a significant role in brominated trihalomethane toxicity. Known inducers of CYP2E1 include certain therapeutic agents (acetaminophen, isoniazid), volatile anaesthetics (halothane, isoflurane), and solvents (acetone, benzene, carbon tetrachloride, trichloroethylene) (Raucy, 1995). Individuals exposed to or consuming these inducers on a regular basis may therefore be at greater risk for brominated trihalomethane toxicity.

b. Subpopulations with altered levels of glutathione *S*-transferase theta

Individuals with Genetic Polymorphisms

Genotoxicity studies in bacteria (discussed in section V.F.1) indicate that brominated trihalomethanes can be activated to mutagens by the product of the glutathione *S*-transferase theta gene *GSTT1-1* (DeMarini et al.,1997; Landi et al., 1999b). If similar pathways for bioactivation exist in humans, *GSTT1-1* polymorphism may influence susceptibility to brominated trihalomethane-mediated toxicity. *GSTT1-1* is characterized by a deletion polymorphism which results in total loss of glutathione *S*-transferase-θ activity in individuals (10 to 60% of the population depending upon ethnicity and race) homozygous for the null genotype ($GSTT1\text{-}1^{-/-}$). Individuals who are heterozygous for GSTT-1 ($GSTT1\text{-}1^{+/-}$) have intermediate levels of enzyme activity, while individuals homozygous for *GSTT-1* ($GSTT1\text{-}1^{+/+}$) have the highest levels. Landi et al. (1999b) have suggested that $GSTT1\text{-}1^{+/+}$ individuals may experience excess genotoxic risk when exposed to brominated trihalomethanes, particularly in organs which express glutathione-*S*-

transferase-theta and come in direct contact with brominated trihalomethanes. Potential target sites would include the gastrointestinal tract and the bladder.

Concurrent Exposure to Inducers

If *GSTT-1*-mediated pathways for bioactivation of brominated trihalomethanes exist in humans, factors which induce this enzyme may increase the risk of adverse health effects from exposure. Although *GSTT-1* is constitutively expressed, the level of its expression can be altered by exposure to exogenous chemicals. Landi (2000) has summarized information on factors which increase expression of the enzyme. In rats, aspirin increased *GSTT-1* levels in the colon. Alpha-tocopherol, coumarin; and other anticarcinogenic drugs increased gastric and esophageal levels; and indole-3-carbinol and coumarin increased *GSTT-1* levels in the liver. In mice, phenobarbital induced hepatic *GSTT-1* levels. Data for humans are limited, but there are indications that the dietary intake of cruciferous vegetables enhances the expression of *GSTT-1*. It is possible that consumption of these substances by *GSTT-1* positive individuals could result in increased risk of adverse effects. However, there are presently no data available for evaluation of this hypothesis.

c. Subpopulations with altered levels of putative protective compounds

Glutathione depletion has been observed to increase the hepatotoxicity of bromodichloromethane (Gao et al., 1996). On the basis of these data, Gao et al. (1996) proposed that populations with low baseline levels of glutathione (e.g., due to dietary deficiencies of glutathione precursors such as cysteine and selenium) may be more sensitive to bromodichloromethane-induced toxicity.

d. Possible gender differences

Apparent gender-related differences in the toxicity of brominated trihalomethanes have been noted in studies where male and female animals were exposed concurrently (e.g. Aida, 1992a; Daniel, 1990; NTP,1987, 1989a; Tobe et al., 1982). In general, male rats and mice appear to be somewhat more sensitive to the hepatic and renal toxicity induced by brominated trihalomethanes than are females, although there are exceptions to this pattern (eg. the chronic oral exposure study of bromoform conducted by NTP, 1989a in mice and the short-term study of bromoform conducted by Aida et al., 1992a). While the basis for the apparent greater sensitivity of males is unknown, the difference may be related to gender-specific differences in the level of enzymes responsible for bioactivation of brominated trihalomethanes to toxic metabolites, or to gender-specific differences in cellular protective mechanisms. It is important to note that at present there is no evidence for gender-related differences in the activity levels of CYP2E1 or *GSTT-1* in humans.

E. Summary

It is generally believed that the toxicity of the brominated trihalomethanes is related to their metabolism. This conclusion is based largely on the observation that liver and kidney, the

chief target tissues for these compounds, are also the primary sites of their metabolism. In addition, treatments which increase or decrease metabolism also tend to increase or decrease trihalomethane-induced toxicity in parallel.

Metabolism of brominated trihalomethanes is believed to occur via oxidative and reductive pathways. Limited structure-activity data for brominated trihalomethanes and the structurally-related trihalomethane chloroform suggest that bromination may influence the proportion of compound metabolized via the oxidative and reductive pathways, with brominated compounds being more extensively metabolized by the reductive pathway. Additional evidence suggests that a GSH-mediated pathway may play an important role in metabolism of brominated trihalomethanes. These data raise the possibility that brominated trihalomethanes may induce adverse effects (toxicity and carcinogenicity) via several different pathways.

The precise biochemical mechanisms which link brominated trihalomethane metabolism to toxicity have not been characterized, but many researchers have proposed that toxicity results from the production of reactive intermediates. Reactive intermediates may arise from either the oxidative (dihalocarbonyls) or the reductive (free radicals) pathways of metabolism. Such reactive intermediates are known to form covalent adducts with various cellular molecules, and may impair the function of those molecules and cause cell injury. Free radical production may also lead to cell injury by inducing lipid peroxidation in cellular membranes. Direct evidence showing a relationship between the level of covalent binding intermediates generated by either pathway and the extent of toxicity is not available for the brominated trihalomethanes. Manipulation of cellular glutathione levels suggests that this compound may play a protective role in brominated trihalomethane-induced toxicity.

Individual brominated trihalomethanes have been shown to induce tumors in laboratory animals. The mechanism by which brominated trihalomethanes induce tumors in target tissues has not been fully characterized. DNA adducts can be formed by interaction of reactive metabolites (resulting from oxidative and reductive metabolism) with DNA. In addition, preliminary evidence suggested that DNA adducts can be formed through conjugation with glutathione and bioactivation of the resulting conjugates. Comparison of dose-response data for liver and kidney toxicity (including cell proliferation) and tumorigenicity in mice and rats suggests that tumor formation occurs at concentrations lower than those which stimulate cell proliferation.

Interaction with agents which increase or decrease the activity of enzymes responsible for metabolism of brominated trihalomethanes may modify carcinogenicity/toxicity. Pretreatment with inducers of CYP2E1 has been observed to increase the hepatotoxicity of bromodichloromethane and dibromochloromethane in male rats. Pretreatment with m-xylene, an inducer of the CYP2B1/CYP2B2 isoforms, increased the hepatotoxicity of dibromochloromethane in male rats. Conversely, administration of the cytochrome P450 inhibitor 1-aminobenzotriazole prevented bromodichloromethane-induced hepatotoxicity in rats. Recent findings indicating possible glutathione-mediated metabolism of brominated

trihalomethanes suggest that treatments or agents which alter glutathione-*S*-transferase activity could potentially modify the toxicity of brominated trihalomethanes.

The severity of brominated trihalomethane toxicity is potentially affected by the vehicle of administration. In a study of vehicle effects on the acute toxicity of bromodichloromethane, a high dose (400 mg/kg) of the chemical was more hepato- and nephrotoxic when given in corn oil compared to aqueous administration, but this difference was not evident at a lower dose (200 mg/kg).

A number of potentially sensitive subpopulations have been identified for health effects of brominated trihalomethanes. A growing body of scientific evidence indicates that children may suffer disproportionately from some environmental health risks. These risks may arise because the neurological, immunological, and digestive systems of children are still developing. In addition, children may incur greater exposure because they eat more food, consume more fluids, and breathe more air in proportion to their body weight when compared to adults. U.S. EPA has identified three key questions to consider when evaluating health risks to children from drinking water disinfection byproducts (DBP), including the brominated trihalomethanes: 1) Is there information which shows that the DBP causes effects in the developing fetus or impairs ability to conceive and bear children? 2) If the DBP causes cancer, are children more likely to be affected by it than are adults? and 3) If the DBP causes a noncancer toxic effect, are children more likely to be affected by it than are adults? There are limited available animal studies reported developmental effects from oral exposure to dibromochloromethane and bromoform. These developmental effects in animals occurred at doses slightly higher than those which induce histopathological effects in the liver and kidney. There is no evidence that these compounds impair the ability to conceive or have offsprings.

Epidemiological studies have found an association between exposure to bromodichloromethane in drinking water and increased spontaneous abortion and increased stillbirth. These studies raise concern for human health effects, although the occurrence of multiple disinfection byproducts in drinking water is a significant source of uncertainty with resect to the causative agent. The results of *in vitro* studies using cultured human placental trophoblasts show that bromodichloromethane can affect hormone secretion and affect the morphological differentiation, suggesting that the placenta is a possible target of bromodichloromethane toxicity in humans.

Exposure to bromodichloromethane has been linked to reproductive effects in animals. In rats, exposure to bromodichloromethane resulted in reduced sperm motility; this effect was not accompanied by histopathologic changes in the male reproductive system. Exposure of pregnant F344 rats to bromodichloromethane on one or more days during the luteinizing hormone-dependent period of gestation causes full litter resorption. This response is not observed in similarly exposed pregnant Sprague-Dawley rats. The pregnancy loss observed in F344 rats may result from perturbation of LH secretion or signaling processes.

At present, there are no cancer data which indicate that brominated trihalomethanes contribute to increased risk of cancer in children. No studies were located which examined pre- or post-pubertal cancer rates in humans in relation to brominated trihalomethane exposure. Cancer bioassays of brominated trihalomethanes conducted in mice and rats have not used study designs that included perinatal exposure.

The available evidence suggests that the toxic effects of brominated trihalomethanes are mediated by the enzymes CYP2E1, CYP2B1/2 (in rodents), and glutathione S-transferase theta (*GSTT-1*). The weight of evidence from studies of the developmental expression of these enzymes supports the conclusion that children do not experience greater risk from brominated trihalomethane exposure as a result of higher metabolic activity.

In addition to children, other potentially sensitive populations include those with altered levels or activity of CYP2E1 or *GSTT-1* and those with altered levels of glutathione. Factors contributing to increases in CYP2E1 activity potentially include genetic polymorphisms; altered physiological or health states; alcohol consumption; and concurrent exposure to other inducers, including some pharmaceuticals and solvents. Factors contributing to increased *GSTT-1* activity include genetic polymorphisms and concurrent exposure to inducers. Based on observations in animals, human populations with reduced levels of glutathione as a result of dietary deficiency or other factors may experience increased sensitivity to the toxic effects of bromodichloromethane.

Apparent gender-related differences in the toxicity of brominated trihalomethanes have been noted in studies where male and female animals were exposed concurrently. In general, male rats and mice appeared to be more sensitive than females to liver and renal toxicity, although some exceptions to this pattern have been noted. There is no evidence for a similar pattern of gender response in humans.

VIII. QUANTIFICATION OF TOXICOLOGICAL EFFECTS

This section quantifies the toxicological effects of brominated trihalomethanes based on health effects information presented in Sections V and VI. At present, there are two basic approaches to quantification of toxicological effects: the conventional NOAEL/LOAEL approach and benchmark dose modeling. Benchmark dose (BMD) modeling (U.S. EPA, 1995; 2000b) was chosen as the preferred approach for quantifying toxicological effects of the brominated trihalomethanes. BMD modeling avoids several limitations of the NOAEL/LOAEL approach, including: 1) the slope of the dose-response plays little role in determining the NOAEL; 2) the NOAEL (or LOAEL) is limited to the doses tested experimentally; 3) the determination of the NOAEL is based on scientific judgement, and is subject to inconsistency; and 4) experiments using fewer animals tend to produce larger NOAELs, and as a result may produce larger health advisories (HAs) or reference doses (RfDs) (U.S. EPA, 1995) that may not be sufficiently protective of human health. In contrast, benchmark doses (BMDs) are not limited to the experimental doses, appropriately reflect the sample size, and can be defined in a statistically consistent manner. The BMD approach was therefore selected for quantification of the toxicological effects of the brominated trihalomethanes. Values for HAs and RfDs derived using the conventional NOAEL/LOAEL approach are presented in the text for comparison with those obtained using the BMD approach.

The methods employed for BMD modeling are described in Appendix A. The modeling was performed using the BMDS software (Version 1.2) developed by the U.S. EPA National Center for Environmental Assessment. The BMDs and BMDLs were calculated based on a BMR of 10% extra risk for all quantal endpoints analyzed. For continuous data, the BMR was defined as 1.1 standard deviations, which corresponds to an additional risk of approximately 10% when the background response rate is assumed to be 1% with normal variation around the mean and constant standard deviation (Crump, 1995). The $BMDL_{10}$ was defined as the 95% lower bound on the corresponding BMD estimate. Confidence bounds were automatically calculated by the BMDS software using a likelihood profile method.

A. Bromodichloromethane

1. Noncarcinogenic effects

a. One-day Health Advisory

Studies of the acute toxicity of bromodichloromethane are summarized in Table VIII-1. Lilly et al. (1994) administered single doses of bromodichloromethane by either oil or aqueous gavage to male F344 rats at dose levels of 200 or 400 mg/kg. This study identified a LOAEL of 200 mg/kg-day based on histologic lesions in the kidney and changes in urinary parameters. A NOAEL value was not identified for either vehicle. Data for hepatic vacuolar degeneration and renal tubular degeneration obtained using the aqueous vehicle were modeled using the BMDS software. BMD and $BMDL_{10}$ values of 263 and 182 mg/kg-day, respectively, were calculated using the hepatic data. BMD and $BMDL_{10}$ values of 131 and 8.9 mg/kg-day, respectively, were

obtained using the renal data. The $BMDL_{10}$ for renal tubular degeneration is the lowest calculated across studies, but is not considered a reliable estimate because there is insufficient information to accurately characterize the shape of the dose-response curve in the region of interest.

Thornton-Manning et al. (1994) administered bromodichloromethane to female F344 rats by aqueous gavage for five consecutive days at dose levels ranging from 75 to 300 mg/kg-day. This study identified a NOAEL of 75 mg/kg-day and a LOAEL of 150 mg/kg-day based on increased liver and kidney weights and histologic lesions in the liver (mild centrilobular hepatocellular vacuolar degeneration) and in the kidney (mild renal tubule vacuolar degeneration). An analogous study (Thornton-Manning et al., 1994) conducted in female C57BL/6J mice indicated that the mice were less sensitive to bromodichloromethane than the rats, as no treatment-related histologic lesions were observed in the liver or kidney. However, similar NOAEL and LOAEL values were identified based on increased liver weight and changes in serum chemistry parameters. Data for renal tubular degeneration in rats were analyzed using the BMD approach. BMD and $BMDL_{10}$ values of 133 and 65 mg/kg-day, respectively, were calculated for this endpoint. The BMD is in close agreement with the BMD value calculated for the same endpoint using the data of Lilly et al. (1994).

Two reproductive studies which examined full litter resorption were also considered for derivation of the One-day HA. Bielmeier et al. (2001) examined the occurrence of full litter resorption in F344 rats treated with 0, 75 or 100 mg/kg-day bromodichloromethane by aqueous gavage on gestation day 9. The LOAEL for this effect was 75 mg/kg-day. Narotsky et al. (1997) evaluated the same endpoint in F344 rats administered 0, 25, 50, or 75 mg/kg-day on gestation days 6 through 15. Full litter resorption was observed at 50 and 75 mg/kg-day. The NOAEL and LOAEL in this study were thus identified as 25 and 50 mg/kg-day, respectively. When data from these studies were analyzed using the BMD approach, BMD values of 48 and 23 mg/kg-day were obtained for the Narotsky et al. (1997) and Bielmeier et al. (2001) studies, respectively. The higher value from the Narotsky et al. (1997) study was considered the more reliable estimate of the BMD because it was based on response data that included lower doses, one of which was an apparent NOAEL. The $BMDL_{10}$ calculated from the Narotsky et al. (1997) data was 30 mg/kg-day.

Three additional studies were considered as candidates for derivation of the One-day HA. Lilly et al. (1997) administered single doses of bromodichloromethane by aqueous gavage to male F344 rats at dose levels ranging from 123 to 492 mg/kg. Based on changes in urinary parameters, this study identified a NOAEL of 164 mg/kg-day and a LOAEL of 246 mg/kg-day. No histopathological examination was conducted in this study. The study by French et al. (1999), which investigated immune system response, identified a similar NOAEL value. However, the database for immune response to bromodichloromethane is limited when compared to information on hepatic and renal toxicity. Adverse effects were noted only at the highest dose and frank effect level, and evidence for vehicle effects on immunotoxicity endpoints was observed. Keegan et al. (1998) administered single doses of bromodichloromethane to male F344 rats by gavage at dose levels ranging from 21 to 246 mg/kg. The study authors identified a NOAEL of 41.0 mg/kg-day and a LOAEL of 81.9 mg/kg-day based on elevations in serum markers of hepatotoxicity (ALT, AST, and SDH). Histopathological examination was not

conducted and this was considered to be a limitation of the investigation. These three studies were not considered further for derivation of the One-day Health Advisory (HA), and thus data reported in them were not analyzed using the BMD approach.

The study conducted by Narotsky et al. (1997) was selected for derivation of the One-day HA. The critical effect in this study was full litter resorption observed in pregnant F344 rats treated with bromodichloromethane. The $BMDL_{10}$ value calculated for this endpoint was 30 mg/kg-day, which is roughly half of the most reliable $BMDL_{10}$ value calculated for histopathological changes in kidney (Thornton-Manning et al., 1994). Although dosing in the Narotsky et al. (1997) study lasted from gestation days 6 through 15, a subsequent study by Bielmeier et al. (2001) indicated that a single dose (75 mg/kg) of bromodichloromethane on gestation day 9 was sufficient to elicit full litter resorption in the same strain of rats. Since there is presently insufficient information available to fully assess the occurrence of reproductive effects in humans exposed to bromodichloromethane, use of data for full litter resorption was adopted as a conservative approach to derivation of the One-day HA. The One-day HA for a 10-kg child is calculated using the following equation:

$$\text{One-day HA} = \frac{(30 \text{ mg/kg-day})\ (10 \text{ kg})}{(300)\ (1 \text{ L/day})} = 1.0 \text{ mg/L}$$

where:

30 mg/kg-day	=	$BMDL_{10}$ based on incidence of full litter resorption in F344 rats treated with bromodichloromethane on gestation days 6 to 15.
10 kg	=	Assumed body weight of a child
300	=	Uncertainty factor based on NAS/OW guidelines. This value includes a factor of 10 to protect sensitive human populations; a factor of 10 for extrapolation from animals to humans; and a factor of 3 to account for database limitations and uncertainty regarding possible reproductive effects of bromodichloromethane in humans
1 L/day	=	Assumed water consumption of a 10-kg child

For comparative purposes, the One-day HA was derived using the conventional NOAEL/LOAEL approach would also be based on data from the Narotsky et al. (1997) study. This study identified a NOAEL of 25 mg/kg-day based on FLR, which was the lowest value among the candidate studies. Using this NOAEL and an uncertainty factor of 300 as described

Table VIII-1 Summary of Candidate Studies for Derivation of the One-day HA for Bromodichloromethane

Reference	Species Sex	n	Dose	Route	Exposure Duration	Endpoints	NOAEL mg/kg-day	LOAEL mg/kg-day	BMD mg/kg-day	$BMDL_{10}$ mg/kg-day
Lilly et al. (1994)	Rat F344 M	6	0 200 400	Gavage (oil)	Single Dose	Body, liver, and kidney weights, serum and urine chemistry, liver and kidney histology	--	200 (minimal renal tubule degeneration and necrosis, changes in urinary parameters)	Not modeled	--
Lilly et al. (1994)	Rat F344 M	6	0 200 400	Gavage (aqueous)	Single Dose	Body, liver, and kidney weights, serum and urine chemistry, liver and kidney histology	--	200 (minimal renal tubule degeneration, changes in urinary parameters)	263	182 (Hepatic vacuolar degeneration in males)
									131	8.9 (Renal tubule degeneration in males)
Lilly et al. (1997)	Rat F344 M	5	0 123 164 246 328 492	Gavage (aqueous)	Single Dose	Body, liver, and kidney weights, serum and urine chemistry	164	246 (changes in urinary parameters)	Not modeled	--
Keegan et al. (1998)	Rat F344 M	6	0 21 31 41 82 123 164 246	Gavage (aqueous)	Single Dose	Body, liver, and kidney weights, serum chemistry	41	82 (elevated ALT, AST, and SDH activities)	Not modeled	–

Table VIII-1 (cont.)

Reference	Species Sex	n	Dose	Route	Exposure Duration	Endpoints	NOAEL mg/kg-day	LOAEL mg/kg-day	BMD mg/kg-day	$BMDL_{10}$ mg/kg-day
French et al. (1999)	Rat C57BL/6 F	6	0 75 150 300	Gavage (aqueous)	5 days	Body, spleen, and thymus weights, immune function	150	300 (FEL) (mortality, decreased body weight, altered immune response)	Not modeled	--
Thornton-Manning et al. (1994)	Rat F344 F	6	0 75 150 300	Gavage (aqueous)	5 days	Body, liver, and kidney weights, serum chemistry, liver and kidney histology	75	150 (increased liver and kidney weights, mild centrilobular hepatocellular vacuolar degeneration, mild renal tubule vacuolar degeneration)	133	65 (renal tubular degeneration)
Thornton-Manning et al. (1994)	Mouse C57BL/6J F	6	0 75 100	Gavage (aqueous)	5 days	Body, liver, and kidney weights, serum chemistry, liver and kidney histology	75	150 (increased liver weight, elevated ALT and SDH activities)	Not modeled	–
Narotsky et al. (1997) *	Rat F344 F	12-14	0 25 50 75	Gavage (oil) (water)**	Gestation days 6-15	Body weight, clinical signs, developmental parameters	25	50 (full-litter resorption)	48	30 (full-litter resorption)
Bielmeier et al. (2001)*	Rat F344 F	8-11	0 75 100	Gavage (aq)	Gestation day 9	Full litter resorption; hormone profiles	--	75 (full-litter resorption)	23	4.2 (full-litter resorption)

* The NOAEL and LOAEL values listed are for reproductive or developmental effects.

** The NOAEL and LOAEL values were the same in either vehicle. BMD modeling was performed on aqueous vehicle data only.

above, the One-day HA for a 10-kg child calculated using the conventional approach would be 0.8 mg/L (rounded from 0.83 mg/L).

b. Ten-day Health Advisory

Sixteen studies were considered for derivation of the Ten-day HA for bromodichloromethane. These studies are summarized in Table VIII-2 below. Aida et al. (1992a) administered microencapsulated bromodichloromethane in the diet to Wistar rats for one month at dose levels ranging from 20.6 to 203.8 mg/kg-day. This study identified a NOAEL of 61.7 mg/kg-day and a LOAEL of 189.0 mg/kg-day in male rats based on histologic changes in the liver (swelling of hepatocytes, single cell necrosis, hepatic cord irregularity, and bile duct proliferation). Analysis using the BMD approach calculated BMD and $BMDL_{10}$ values of 34 and 17 mg/kg-day, respectively, based on data for liver cell vacuolization in females.

Data from four of the other candidate studies are consistent with the histopathological results obtained by Aida et al. (1992a). Melnick et al. (1998) administered bromodichloromethane by gavage to female $B6C3F_1$ mice for 5 days/week for 3 weeks and identified a NOAEL of 75 mg/kg-day (duration-adjusted NOAEL of 54 mg/kg-day) and a LOAEL of 150 mg/kg-day (duration-adjusted LOAEL of 107 mg/kg-day) based on histologic changes in the liver (hepatocyte hydropic degeneration). Analysis using the BMD approach calculated duration-adjusted BMD and $BMDL_{10}$ values of 31 and 8.4 mg/kg-day, respectively, for this endpoint. Condie et al. (1983) administered bromodichloromethane by gavage to male CD-1 mice for 14 days and identified a NOAEL of 74 mg/kg-day and a LOAEL of 148 mg/kg-day based on minimal to moderate liver and kidney lesions. Analysis using the BMD approach calculated BMD and $BMDL_{10}$ values of 24 and 7.5 mg/kg-day, respectively, based on data for histopathological changes in the liver. NTP (1998) conducted histopathologic examinations in conjunction with a study of reproductive and developmental toxicity in Sprague-Dawley rats. Although no reproductive or developmental toxicity was observed at the dose levels investigated, histopathological changes were noted in the liver of males rats treated with the compound for 35 days. The NOAEL and LOAEL for this effect were 9 and 38 mg/kg-day, respectively. Analysis of data for single cell hepatic necrosis using the BMD approach calculated BMD and $BMDL_{10}$ values of 35 and 18 mg/kg-day, respectively, which are virtually identical to the values calculated using the liver cell vacuolization data for females from the Aida et al. (1992b) study. Coffin et al. (2000) observed hydropic degeneration in female mice treated with 150 mg/kg-day bromodichloromethane in corn oil for 11 days. These data were not modeled because other studies utilized doses lower than 150 mg/kg-day, which allowed better characterization of response in the low-dose region of the dose-response curve.

In contrast to the studies described above, Chu et al. (1982a) did not observe any microscopic lesions in the liver when Sprague-Dawley rats were administered bromodichloromethane at doses up to 68 mg/kg-day in the drinking water for 28 days. The studies of Munson et al. (1982) and NTP (1987) did not conduct histopathological examinations. These studies identified NOAELs ranging from 50 to 150 mg/kg-day and LOAELs ranging from 125 to 300 mg/kg-day for other endpoints, including depressed humoral immunity (Munson et al.,

1982), decreased weight gain (NTP, 1987; rats), and increased mortality and gross renal pathology (NTP, 1987; mice). These data were not analyzed by the BMD approach.

Seven studies that examined developmental and/or reproductive endpoints were evaluated. Two studies reported the incidence of full litter resorption in F344 rats following treatment with bromodichloromethane. Bielmeier et al. (2001) examined the occurrence of full litter resorption in F344 rats treated with 0, 75 or 100 mg/kg-day bromodichloromethane by aqueous gavage on gestation day 9. The LOAEL for this effect was 75 mg/kg-day. Narotsky et al. (1997) evaluated the same endpoint in F344 rats administered 0, 25, 50, or 75 mg/kg-day on gestation days 6 through 15. Full litter resorption was observed at 50 and 75 mg/kg-day. The NOAEL and LOAEL in this study were thus identified as 25 and 50 mg/kg-day, respectively. When data from these studies were analyzed using the BMD approach, BMD values of 48 and 23 mg/kg-day were obtained for the Narotsky et al. (1997) and Bielmeier et al. (2001) studies, respectively. The higher value from the Narotsky et al. (1997) study was considered the more reliable estimate of the BMD because it was based on response data that included lower doses, one of which was an apparent NOAEL. The $BMDL_{10}$ calculated from the Narotsky et al. (1997) data was 30 mg/kg-day. Ruddick et al. (1983) observed an increased incidence of sternebral aberrations in the pups of Sprague-Dawley rats administered bromodichloromethane in corn oil by gavage. Statistical analysis of the published data indicated that the NOAEL and LOAEL for this effect were 100 mg/kg-day and 200 mg/kg-day, respectively. The BMD and $BMDL_{10}$ obtained for this study were 27 and 15 mg/kg-day, respectively.

The remaining reproductive/developmental studies sponsored by the Chlorine Chemistry Council (CCC) were also evaluated. These studies were summarized by Christian et al. (2001a, b). CCC (2000a, b) examined developmental toxicity in New Zealand rabbits and identified developmental NOAELs of 76 and 55 mg/kg-day (the highest doses tested in each study). The CCC (CCC, 2000c,d) also examined reproductive and developmental toxicity in Sprague Dawley rats. In a range-finding study (CCC, 2000c), F_1 generation pups exposed to bromodichloromethane via lactation and possibly by consumption of water supplied to the dams exhibited reduced body weights and body weight gains. These effects occurred at exposure levels which also resulted in maternal toxicity. Biologically meaningful average daily doses could not be established in this experiment; therefore, the concentration-based NOAEL and LOAEL values for developmental effects were 50 and 150 ppm based on changes in F_1 pup body weight and body weight gain. In a subsequent developmental study, CCC (2000d) identified NOAEL and LOAEL values of 45 and 82 mg/kg-day, respectively, based on decreased number of ossification sites per fetus for the forelimb phalanges and hindlimb metatarsals and phalanges. This effect was observed at doses associated with maternal toxicity. Endpoints from these studies were not modeled because other studies identified adverse effects at lower doses.

Data for maternal toxicity from three reproductive/developmental studies were also considered for derivation of the 10-day HA for bromodichloromethane. Narotsky et al. (1997) observed decreased maternal body weight gain on gestation days 6 to 8 in female rats administered 25 mg/kg-day (the lowest dose tested) by aqueous gavage in 10% Emulphor. BMD modeling identified BMD and $BMDL_{10}$ values of 18 and 10 mg/kg-day, respectively, for this

endpoint. The data reported in this study did not permit evaluation of body weight or body weight gain at other time points during the treatment period. CCC (2000d) reported decreased maternal body weight gain at several time points in pregnant rats administered bromodichloromethane in drinking water, with the most severe effect observed immediately after initiation of treatment on gestation days 6 to 7. The NOAEL and LOAEL for decreased body weight on gestation days 6 to 7 were 18.4 and 45 mg/kg-day, respectively. When body weight gain for this interval was modeled, the resulting BMD and $BMDL_{10}$ values were approximately 18 and 15 mg/kg-day, respectively. However, the modeled fits to the data were poor, and the results were not considered sufficiently reliable for derivation of a health advisory. To address this problem, body weight gain data for gestation days 6 to 9 were also modeled. Reliable values of 23 and 18 mg/kg-day were obtained for the BMD and $BMDL_{10}$, respectively. The CCC (2000b) study observed decreased maternal body weight gain at several time points in pregnant rabbits administered bromodichloromethane in the drinking water on gestation days 6 to 29. The NOAEL and LOAEL for decreased maternal body weight gain (corrected for gravid uterine weight) on gestation days 6 to 21 were 13.4 and 35.3 mg/kg-day, respectively. A BMD value of 50 mg/kg-day was obtained for this data set, but the BMDS software failed to identify the corresponding $BMDL_{10}$. No further modeling was attempted since this value was well above the lowest BMDs obtained in some other candidate studies.

As evident from the data in Table VIII-2, the four studies that examined histopathological changes in the liver are in close agreement, having identified BMD values ranging from 24 to 35 mg/kg-day. The corresponding $BMDL_{10}$ values ranged from 7.5 to 18 mg/kg-day. Maternal toxicity occurred in rats at similar levels in two developmental studies. These studies identified BMD values of 18 and 23 mg/kg-day, with corresponding $BMDL_{10}$ values of 10 and 18 mg/kg-day. The NTP (1998) and CCC (2000d) drinking water studies were selected to derive the Ten-day HA. Selection of these studies was based on the administration of bromodichloromethane in drinking water, the most relevant route of exposure. In addition, these studies utilized a lower range of doses, which provided information on the shape of the dose-response curve in the region of interest. The Ten-day HA is calculated according to the following equation:

$$\text{Ten-day HA} = \frac{(18 \text{ mg/kg-day})\ (10 \text{ kg})}{(300)\ (1 \text{ L/day})} = 0.60 \text{ mg/L (rounded to 0.6 mg/L)}$$

where:

18 mg/kg-day = $BMDL_{10}$ based on single cell hepatic necrosis in rats administered bromodichloromethane in the drinking water for 35 days or decreased maternal body weight gain on gestation days 6-9 in pregnant female rats administered bromodichloromethane in the drinking water.

10 kg = Assumed body weight of a child

300	=	Uncertainty factor based on NAS/OW guidelines. This value includes a factor of 10 to protect sensitive human populations and a factor of 10 for extrapolation from animals to humans, and a factor of 3 to account for database limitations and uncertainty regarding possible reproductive effects of bromodichloromethane in humans
1 L/day	=	Assumed water consumption of a 10-kg child

For comparative purposes, the Ten-day HA derived using the conventional NOAEL/LOAEL approach would be based on data from the CCC (2000d) study. This study identified a NOAEL of 18 mg/kg-day based on reduced maternal body weight gain in pregnant rabbits. Using this NOAEL and an uncertainty factor of 300 as described above, the Ten-day HA for a 10-kg child calculated using the conventional approach would be 0.60 mg/L (rounded to 0.6 mg/L).

Table VIII-2 Summary of Candidate Studies for Derivation of the Ten-day HA for Bromodichloromethane

Reference	Species Sex	n	Dose	Route	Exposure Duration	Endpoints	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	BMD (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Aida et al. (1992a)	Rat Wistar M, F	7	Male 0 21 62 189 Females 0 21 66 204	Feed	1 month	Clinical signs, body weight, serum chemistry, hematology, histology	62	189 (liver histopathology in males)	34	17 (liver cell vacuolation in females)
Chu et al. (1982a)	Rat SD M	10	0 0.8 8 68	Drinking water	28 days	Clinical signs, serum chemistry, histology	68	--	No data to model	--
Condie et al. (1983)	Mouse CD-1 M	8-16	0 37 74 148	Gavage (oil)	14 days	Serum enzymes, PAH uptake *in vitro*, histology	74	148 (elevated ALT, decreased PAH uptake, liver and kidney histopathology)	24	7.5 (hepatic centrilobular pallor)
									125	53 (Renal epithelial hyperplasia)
Melnick et al. (1998)	Mouse $B6C3F_1$ F	10	0 75 150 326	Gavage (oil)	3 weeks (5 d/wk)	Body and liver weights, serum chemistry, liver histology	75	150 (liver histopathology)	31 †	8.4 (Hepatocyte hydropic degeneration)
Munson et al. (1982)	Mouse CD-1 M, F	8-12	Males 0 50 125 250	Gavage (aq.)	14 days	Body and organ weights, serum chemistry, hematology, and immune function	50	125 (depressed humoral immunity)	Not modeled	–

Table VIII-2 (cont.)

Reference	Species Sex	n	Dose	Route	Exposure Duration	Endpoints	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	BMD (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
NTP (1987)	Rat F344/N M, F	5	0 38 75 150 300 600	Gavage (oil)	14 days	Body weight, clinical signs, gross necropsy	150	300 (decreased weight gain)	Not modeled	--
NTP (1987)	Mouse $B6C3F_1$ M, F	5	0 19 38 75 150 300	Gavage (oil)	14 days	Body weight, clinical signs, gross necropsy	75	150 (FEL)‡ (mortality, lethargy, gross renal pathology)	Not modeled	--
Coffin et al. (2000)	Mouse $B6C3F_1$ F	10	0 150 300	Gavage (oil)	11 days	Relative liver wt., liver histopathology; labeling index	--	150	Not modeled	--
NTP (1998)	Rat SD M (group A)	5-13	0 9 38 67	Drinking water	35 days	Body and organ weights, serum chemistry, hematology, gross necropsy, histology, sperm evaluation	9	38 (liver histopathology)	35	18 (liver cell necrosis)
Ruddick et al. (1983)*	Rat SD F	9-14	0 50 100 200	Gavage (oil)	Gestation days 6-15	Body and organ weights; maternal serum chemistry; hematology, and histopathology; developmental parameters	100	200 (sternebral aberrations)	27	15 (sternebral aberrations)

Table VIII-2 (cont.)

Reference	Species Sex	n	Dose	Route	Exposure Duration	Endpoints	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	BMD (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Narotsky et al. (1997)*	Rat F344 F	12-14	0 25 50 75	Gavage (oil) (Emul-phor)**	Gestation days 6-15	Body weight, clinical signs, developmental parameters	25 (developmental)	50 (full-litter resorption)	48	30 (full-litter resorption)
							- (maternal)	25 (reduced maternal body weight gain gestation days 6-8, aqueous vehicle only)	18	10 (reduced maternal body weight gain gestation days 6-8, aqueous vehicle only)
Bielmeier et al. (2001)*	Rat F344 F	8-11	0 75 100	Gavage (aq)	Gestation day 9	Full litter resorption; hormone profiles	--	75 (full-litter resorption)	23	4.2 (full-litter resorption)
CCC (2000c)*	Rat SD M, F	10	0 ppm 50 ppm 150 ppm 450 ppm 1350 ppm	Drinking water	Males 64 days Females 74 days	Reproductive and developmental parameters	50 ppm	150 ppm (reduced F_1 pup weight and weight gain)	Not modeled	--
CCC (2000d)*	Rat SD F	25	0.0 2.2 18.4 45.0 82.0	Drinking water	Gestation days 6-21	Reproductive and developmental parameters	45.3 (developmental)	82.0 (reduced number of ossification sites in phalanges or metatarsals occurring with maternal toxicity)	Not modeled	--
							18.4 (maternal)	45 (reduced maternal body weight gain gestation days 6-7)	23	18 (reduced maternal body weight gain gestation days 6-9; see text for comment)

Table VIII-2 (cont.)

Reference	Species Sex	n	Dose	Route	Exposure Duration	Endpoints	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	BMD (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
CCC (2000a)*	Rabbit NZW F	5	0.0 13.9 32.3 76.3	Drinking water	Gestation days 6-29	Reproductive and developmental parameters	76.3 (developmental)	-	Not modeled	--
CCC (2000b)*	Rabbit NZW F	25	0 1.4 13.4 35.6 55.3	Drinking Water	Gestation days 6-29	Clinical sign, gross lesions, reproductive and developmental endpoints	55 (developmental)	-	Not modeled	--
							13.4 (maternal)	35.3 (reduced corrected maternal body weight gain gestation days 6-29)	50	BMDS software failed

* The NOAEL and LOAEL values listed are for reproductive or developmental effects.

** The NOAEL and LOAEL values were the same for developmental effects in either vehicle. The LOAEL for maternal toxicity was 25 mg/kg-day for the aqueous vehicle (10% Emulphor). The NOAEL and LOAEL for maternal toxicity using the corn oil vehicle were 25 mg/kg-day and 50 mg/kg-day, respectively. BMD modeling was performed on aqueous vehicle data only.

† BMD and $BMDL_{10}$ calculated using duration adjusted doses

Abbreviations: FEL, Frank effect level; SD, Sprague-Dawley; NZW, New Zealand White

c. Longer-term Health Advisory

Two rodent oral exposure studies conducted by NTP (1987) were considered for derivation of the Longer-term HA for bromodichloromethane. In addition, eight reproductive studies were considered. These studies are summarized in Table VIII-3 below.

NTP (1987) administered bromodichloromethane by gavage to F344/N rats for 5 days/week for 13 weeks at dose levels ranging from 19 to 300 mg/kg-day. Based on decreased weight gain, this study identified a NOAEL of 75 mg/kg-day and a LOAEL of 150 mg/kg-day. Treatment-related hepatic and renal lesions were observed only at the high dose. In a similar study, NTP (1987) administered bromodichloromethane by gavage to B6C3F_1 mice for 5 days/week for 13 weeks at dose levels ranging from 6.25 to 100 mg/kg-day for males and from 25 to 400 mg/kg-day for females. This study identified a NOAEL of 50 mg/kg-day and a LOAEL of 100 mg/kg-day based on histologic alterations in the kidney (focal necrosis of the proximal renal tubular epithelium and nephrosis) of male mice. BMD analysis using the BMDS program identified duration-adjusted BMD values of 63 and 75 mg/kg-day for focal necrosis of renal tubular epithelium in males and vacuolated cytoplasm in the liver of females, respectively. The corresponding $BMDL_{10}$ values for these renal and hepatic effects were 35 and 47 mg/kg-day, respectively.

Eight reproductive or developmental studies (Ruddick et al., 1983; Narotsky et al., 1997; Bielmeier et al. 2001; CCC, 2000a,b,c,d; CCC, 2002) were also considered for derivation of the Longer-term HA. CCC (2002) identified a LOAEL of 150 ppm (approximately 11.6 to 40.2 mg/kg-day) for delayed sexual maturation in F_1 male rats in a two-generation study of bromodichloromethane administered in drinking water. The LOAEL for parental effects in the study was also 150 ppm, based on decreased body weight and body weight gain in F_0 females and F_1 males and females. Bielmeier et al. (2001) examined the occurrence of full litter resorption in F344 rats treated with 0, 75 or 100 mg/kg-day bromodichloromethane by aqueous gavage on gestation day 9. The LOAEL for this effect was 75 mg/kg-day. Narotsky et al. (1997) evaluated the same endpoint in F344 rats administered 0, 25, 50, or 75 mg/kg-day on gestation days 6 through 15. Full litter resorption was observed at 50 and 75 mg/kg-day. The NOAEL and LOAEL in this study were thus identified as 25 and 50 mg/kg-day, respectively. When data from these studies were analyzed using the BMD approach, BMD values of 48 and 23 mg/kg-day were obtained for the Narotsky et al. (1997) and Bielmeier et al. (2001) studies, respectively. The higher value from the Narotsky et al. (1997) study was considered the more reliable estimate of the BMD because it was based on response data that included lower doses, one of which was an apparent NOAEL. The $BMDL_{10}$ calculated from the Narotsky et al. (1997) data was 30 mg/kg-day. Studies conducted by NTP (1998) did not detect reproductive or developmental toxicity at doses up to 116 mg/kg-day. Two studies conducted in New Zealand White rabbits did not detect developmental effects at doses up to 55 and 76 mg/kg-day, respectively (CCC, 2000a,b).

Three additional studies in rats identified developmental effects that occurred at dose levels that also resulted in maternal toxicity. In a range-finding study (CCC, 2000c), F_1 generation pups exposed to bromodichloromethane via lactation and possibly by consumption of

water supplied to the dams exhibited reduced body weights and body weight gains. Biologically meaningful daily doses could not be established in this experiment; therefore, the concentration-based NOAEL and LOAEL values are 50 ppm and 150 ppm. based on reduced body weight and body weight gain in the F_1 pups. In a subsequent developmental study, CCC (2000d) identified NOAEL and LOAEL values of 45 and 82 mg/kg-day, respectively, based on decreased number of ossification sites per fetus for the forelimb phalanges and hindlimb metatarsals and phalanges. These reversible developmental delays occurred at doses which also resulted in maternal. Endpoints from these studies were not modeled because other effects were observed at lower doses. Ruddick et al. (1983) observed an increased incidence of sternebral aberrations in the pups of Sprague-Dawley rats administered bromodichloromethane in corn oil by gavage. Statistical analysis of the published data indicated that the NOAEL and LOAEL for this effect were 100 mg/kg-day and 200 mg/kg-day, respectively. The lowest dose tested was 50 mg/kg-day. The BMD and $BMDL_{10}$ obtained for this study were 27 and 15 mg/kg-day, respectively. However, examination of the modeling output indicated that none of the available models fit the data well in the low-dose region of the curve. Therefore, the reliability of these values is questionable.

The Narotsky et al. (1997) and CCC (2000d) studies identified $BMDL_{10}$ values of 10 and 18 mg/kg-day based on reduced maternal body weight gain. The CCC data were considered the most relevant since they were obtained from a drinking water study which utilized concentrations that resulted in daily doses well below those used in the Narotsky study. The NTP (1987) and the Narotsky et al. (1997) studies provided similar, but higher, $BMDL_{10}$ values, based on reproductive and histopathological endpoints. The NTP (1987) study utilized bromodichloromethane doses as low as 6.3 mg/kg-day, in contrast to the reproductive study conducted by Narotsky et al. (1997) in which the lowest dose was 25 mg/kg-day. The NTP (1987) data thus provide more information about the shape of the dose-response curve in the region of interest. The BMD data for focal necrosis of renal tubular epithelium and reduced body weight gain in pregnant female rats were thus selected as the most reliable basis for determining the Longer-term HA. Using the lower of the two values, the duration-adjusted $BMDL_{10}$ of 18 mg/kg-day for reduced maternal body weight gain, the Longer-term HA for a 10 kg child is calculated according to the following equation:

$$\text{Longer-term HA} = \frac{(18 \text{ mg/kg-day})\ (10 \text{ kg})}{(300)\ (1 \text{ L/day})} = 0.60 \text{ mg/L (rounded to 0.6 mg/L)}$$

where:

18 mg/kg-day = $BMDL_{10}$ based on decreased maternal body weight gain on gestation days 6-9 in pregnant female rats administered bromodichloromethane in the drinking water.

10 kg = Assumed body weight of a child

300	=	Uncertainty factor based on NAS/OW guidelines. This value includes a factor of 10 to protect sensitive human populations and a factor of 10 for extrapolation from animals to humans, and a factor of 3 to account for uncertainty regarding possible reproductive effects of bromodichloromethane in humans
1 L/day	=	Assumed water consumption of a 10-kg child

The Longer-term HA for adults is calculated as follows:

$$\text{Longer-term HA} = \frac{(18 \text{ mg/kg-day})\ (70 \text{ kg})}{(300)\ (2 \text{ L/day})} = 2.1 \text{ mg/L (rounded to 2 mg/L)}$$

where:

18 mg/kg-day	=	$BMDL_{10}$ based on reduced body weight gain in female rats administered bromodichloromethane in the drinking water
70 kg	=	Assumed body weight of an adult
300	=	Uncertainty factor based on NAS/OW guidelines. This value includes a factor of 10 to protect sensitive human populations and a factor of 10 for extrapolation from animals to humans, and a factor of 3 to account for database limitations and uncertainty related to potential reproductive effects of bromodichloromethane in humans
2 L/day	=	Assumed water consumption of a 70-kg adult

For purposes of comparison , a Longer-term HA derived using the conventional NOAEL/LOAEL approach would be based on the study conducted by CCC (2000d). This study identified a NOAEL of 18.4 mg/kg-day and a LOAEL of 45 mg/kg-day based on reduced body weight gain on gestation days 6 to 7. Using the NOAEL of 18.4 mg/kg-day, and assuming drinking water ingestion of 1 L/day and an uncertainty factor of 300 (including factors of 10 for interspecies extrapolation and protection of susceptible populations and a factor of 3 for database limitations and uncertainty regarding potential reproductive effects in humans), the Longer-term HA for a 10 kg child would be 0.6 mg/L. The Longer-term HA for a 70 kg adult consuming 2 L/day would be 2 mg/kg-day.

Table VIII-3 Summary of Candidate Studies for Derivation of the Longer-term HA for Bromodichloromethane

Reference	Species Sex	n	Dose	Route	Exposure Duration	Endpoints	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	BMD (mg/kg-day)	$BMDL_{10}$ (mg/kg-day) ‡
NTP (1987)	Rat F344/N M, F	10	0 19 38 75 150 300	Gavage (oil)	13 weeks (5 d/wk)	Body weight, clinical signs, histology	75	150 (decreased weight gain) (hepatic and renal lesions at 300)	Not modeled	--
NTP (1987)	Mouse $B6C3F_1$ M, F	10	Male 0 6.3 13 25 50 100 Female 0 25 50 100 200 400	Gavage (oil)	13 weeks (5 d/wk)	Body weight, clinical signs, histology	50	100 (renal lesions)	63	35 (focal necrosis of renal tubular epithelium in males)
									75	47 (Hepatic vacuolated cytoplasm in females)
Ruddick et al. (1983)*	Rat SD F	9-14	0 50 100 200	Gavage (oil)	Gestation days 6-15	Body and organ weights; maternal serum chemistry; hematology, and histopathology; developmental parameters	100	200 (increased incidence of sternebral variations)	27	15 (increased incidence of sternebral variations)
Narotsky et al. (1997) *	Rat F344 F	12-14	0 25 50 75	Gavage (oil) (aq)**	Gestation days 6-15	Body weight, clinical signs, developmental parameters	25 (developmental)	50 (full-litter resorption)	48	30 (full-litter resorption)
							- (maternal)	25 (reduced maternal body weight gain gestation days 6-8, aq. vehicle)	18	10 (reduced maternal body weight gain, gestation days 6-8, aq. vehicle)

Table VIII-3 (cont.)

Reference	Species Sex	n	Dose	Route	Exposure Duration	Endpoints	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	BMD (mg/kg-day)	$BMDL_{10}$ (mg/kg-day) ‡
Bielmeier et al. (2001)*	Rat F344 F	8-11	0 75 100	Gavage (aq)	Gestation day 9	Full litter resorption; hormone profiles	--	75 (full-litter resorption)	23	4.2 (full-litter resorption)
CCC (2000c)*	Rat SD M, F	10	0 ppm 50 ppm 150 ppm 450 ppm 1350 ppm	Drinking water	Males 64 days Females 74 days	Reproductive/ developmental parameters	Developmental 50 ppm Parental 50 ppm	Developmental 150 ppm- Parental 50 ppm	Not modeled	--
CCC (2000d)*	Rat SD F	25	0.0 2.2 18.4 45.0 82.0	Drinking water	Gestation days 6-21	Reproductive/ developmental parameters	45.3 (developmental)	82.0 (reduced number of ossification sites in phalanges or metatarsals occurring with maternal toxicity)	Not modeled	--
							18.4 (maternal)	45 (reduced maternal body weight gain gestation days 6-7)	23	18 (reduced maternal body weight gain gestation days 6-9; see comments in text)
CCC (2002)*	Rat SD M, F	30	0 ppm 50 ppm 150 ppm 450 ppm	Drinking water	Two generations	Reproductive parameters	50 ppm (offspring)	150 ppm (delayed sexual maturation in F_1 males)	Not modeled	–
							50 ppm (parental)	150 ppm (Reduced body wt and body wt gain in F_0 females and F_1 males and females)		

Table VIII-3 (cont.)

Reference	Species Sex	n	Dose	Route	Exposure Duration	Endpoints	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	BMD (mg/kg-day)	$BMDL_{10}$ (mg/kg-day) ‡
CCC (2000a)*	Rabbit NZW F	5	0 4.9 13.9 32.3 76.3	Drinking Water	Gestation day 6 to 29	Body weight, clinical signs, reproductive and developmental parameters	76.3 (developmental)	-	Not modeled	--
CCC (2000b)*	Rabbit NZW F	25	0 1.4 13.4 35.6 55.3	Drinking Water	Gestation days 6-29	Clinical sign, gross lesions, reproductive and developmental endpoints	55.3 (developmental)	-	Not modeled	--
							13.4 (maternal)	35.3 (reduced maternal body weight gain	50 (developmental)	BMD software failed

‡ $BMDL_{10}$ value was derived using duration-adjusted doses.
† Modeled using Crump Benchmark Dose Software
* The NOAEL and LOAEL values listed are for reproductive/developmental effects.
** The developmental NOAEL and LOAEL values were the same in either vehicle. The LOAELs for maternal toxicity were 25 mg/kg-day and 50 mg/kg-day for the aqueous and corn oil vehicles respectively. BMD modeling was performed on aqueous vehicle data only.
Abbreviations: NA, Not available; SD, Sprague-Dawley; NZW, New Zealand White

d. Reference Dose, Drinking Water Equivalent Level and Lifetime Health Advisory

This section reports the existing RfD value for bromodichloromethane and describes the derivation of the RfD for this compound. This section also describes the calculation of Drinking Water Equivalent Level and Lifetime Health Advisory values which require the RfD as input. For this document, new and existing studies were reviewed and appropriate candidate data were selected for benchmark dose (BMD) modeling. The results of BMD modeling were used in conjunction with appropriate uncertainty factors to calculate the RfD. A comparison of the RfD derived using the BMD approach to the results obtained using the conventional NOAEL/LOAEL approach is also provided.

Description of the Existing RfD

The existing RfD for bromodichloromethane is 0.02 mg/kg-day (IRIS, 1993a). This value was derived using a duration-adjusted LOAEL of 17.9 mg/kg-day identified for renal cytomegaly in $B6C3F_1$ mice administered bromodichloromethane by corn oil gavage for 5 days/week for 102 weeks (NTP, 1987). An uncertainty factor of 100 was used to account for extrapolation from animal data and for protection of sensitive human subpopulations. An additional factor of 10 was used because the RfD was based on a LOAEL (although it was considered minimally adverse) and to account for lack of reproductive data.

Identification of Candidate Studies for Derivation of the RfD

Several studies of chronic duration were considered for derivation of the RfD for bromodichloromethane. These studies are summarized in Table VIII-4 below. NTP (1987) administered bromodichloromethane to F344/N rats by gavage in corn oil at doses of 50 or 100 mg/kg-day for 5 day/week for 102 weeks. This study identified a LOAEL of 50 mg/kg-day based on histologic alterations in the liver and kidney. In a similar study, NTP (1987) administered bromodichloromethane by gavage in corn oil to $B6C3F_1$ mice for 5 days/week for 102 weeks at dose levels of 25 or 50 mg/kg-day for males and 75 or 150 mg/kg-day for females. Based on histologic alterations in the liver, kidney, and thyroid of male mice, this study identified a LOAEL of 25 mg/kg-day, which is consistent with the value identified in the rat study. In a third study, Tobe et al. (1982) administered microencapsulated bromodichloromethane to Wistar rats in the diet at dose levels ranging from 6 to 168 mg/kg-day. Histologic data for the animals exposed to bromodichloromethane were reported by Aida et al. (1992b). This study identified a LOAEL for male rats of 6 mg/kg-day on the basis of histopathologic changes in the liver.

Ten reproductive and/or developmental toxicity studies (Ruddick et al., 1983; Klinefelter et al., 1995; Narotsky et al., 1997; NTP, 1998; Bielmeier et al. 2001; CCC, 2000a,b,c,d; CCC, 2002) were considered for derivation of the RfD in addition to the chronic studies. The investigations of Ruddick et al. (1983), Klinefelter et al. (1995), and Narotsky et al. (1997) identified NOAELs or LOAELs in rats that were substantially higher than the LOAEL identified by Aida et al. (1992b) (Table VIII-4 below). The studies conducted by NTP (1998) did not observe developmental or reproductive effects at doses up to 116 mg/kg-day. The study by Bielmeier et al. (2001) identified a free-standing LOAEL of 75 mg/kg-day in F344 rats. The studies conducted by CCC (2000a,b) identified NOAELs of 55 and 76 mg/kg-day, respectively,

for developmental effects in New Zealand White rabbits. The study conducted in rats by CCC (2000c) identified concentration-based NOAEL and LOAEL values of 50 ppm and 150 ppm for reduced body weight and body weight gain in F_1 pups. The study conducted in rats by CCC (2000d) identified NOAEL and LOAEL values of 45 mg/kg-day and 82 mg/kg-day, respectively on the basis of decreased ossification sites per fetus per litter in the forelimb and hindlimb. Low-range LOAEL values for maternal toxicity ranged from 13 to 25 mg/kg-day (CCC, 2000b; CCC 2000d; Narotsky et al., 1997). CCC (2002) identified a LOAEL of 150 ppm (approximately 11.6 to 40.2 mg/kg-day) for delayed sexual maturation in F_1 male rats in a two-generation study of bromodichloromethane administered in drinking water. The LOAEL for parental effects in the study was also 150 ppm, based on decreased body weight and body weight gain in F_0 females and F_1 males and females. Since these studies identified NOAEL and/or LOAEL values substantially higher than that identified by Aida et al. (1992b), they were not further considered for derivation of the RfD.

Method of Analysis

Selected data from the candidate studies were analyzed using the benchmark dose (BMD) modeling approach. Initially, data sets for potentially sensitive endpoints were selected as described in U.S. EPA (1998b) and analyzed using the Crump Benchmark Dose Modeling Software (K. S. Crump, Inc.). Results of this analysis are summarized in Table VIII-5. Following the release of Version 1.2 of the BMDS program (U.S. EPA, 2000a), a subset of the most sensitive endpoints identified using the Crump software was reanalyzed in accordance with proposed U.S. EPA (2000b) recommendations. An advantage of analysis with the BMDS software is that several additional models are available to fit the data. The results of the analysis with the BMDS software are included in Table VIII-4.

Choice of Principal Study and Critical Effect for the RfD

Three data sets for histopathological effects in liver were analyzed using the BMDS software (Table VIII-4). BMD modeling identified several endpoints with BMD values lower than the conventionally determined LOAEL of 6 mg/kg-day (Aida, 1992b). The lowest BMD values were obtained for fatty degeneration (1.9 mg/kg-day) in male rats (Aida et al., 1992b) and for kidney cytomegaly (2.0 mg/kg-day) in male mice (NTP, 1987). Comparably low BMD values were also obtained for granulomas observed in the liver of male rats (2.1 mg/kg-day) and for fatty degeneration in the liver of female rats (3.1 mg/kg-day) in the study conducted by Aida et al. (1992b). In contrast, the BMD values calculated for endpoints examined in other studies were approximately 10- to 20-fold higher.

Table VIII-4 Summary of Candidate Studies for Derivation of the RfD for Bromodichloromethane

Reference	Species Sex	n	Dose	Route	Exposure Duration	Endpoints	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	BMD (mg/kg-day)	$BMDL_{10}$ (mg/kg-day) [a]
NTP (1987)	Rat F344/N M, F	50	0 50 100	Gavage (oil)	102 weeks (5 d/wk)	Body weight, clinical signs, gross necropsy, histology	--	50 (lesions of kidney and liver)	--	36.5[c] (liver necrosis in male rats)
NTP (1987)	Mouse $B6C3F_1$ M, F	50	0 25 50	Gavage (oil)	102 weeks (5 d/wk)	Body weight, clinical signs, gross necropsy, histology	--	25 (lesions of liver, kidney, and thyroid)	2.0	1.5 (kidney cytomegaly in male mice)
Aida et al. (1992b)	Rat Wistar M, F	40	Male 0 6 26 138 Female 0 8 32 168	Diet	24 months	Body weight, clinical signs, serum biochemistry, gross necropsy, histology	--	6 (liver fatty degeneration and granuloma)	3.1	2.1 (fatty degeneration in liver of females)
									1.9	0.8 (fatty degeneration in liver of males)
									2.1	1.4 (Granulomas in livers of males)
Ruddick et al. (1983)[b]	Rat SD F	9-14	0 ppm 50 ppm 150 ppm 450 ppm 1350 ppm	Gavage (oil)	Gestation days 6-15	Body and organ weights; maternal serum chemistry; hematology, and histopathology; developmental parameters	100	200 (sternebral variations)	27	15 (sternebral variations)
Narotsky et al. (1997) [b]	Rat F344	13-14	0 75 100	Gavage (oil) (water)	Gestation days 6-15	Body weight, clinical signs, developmental parameters	25 (developmental)	50 (full-litter resorption)	48	30 (full litter resorption)

Table VIII-4 (cont.)

Klinefelter et al. (1995)[b]	Rat F344 M	7	0 22 39	Drinking water	52 weeks	Body and organ weights, gross necropsy, histology, sperm motion parameters	22	39 (decreased sperm velocities)	--	--[d]
Bielmeier et al. (2001)[b]	Rat F344 F	8-11	0 75 100	Gavage (aq)	Gestation day 9	Full litter resorption, hormone profiles, body weight	--	75 (full-litter resorption)	23	4.2 (full-litter resorption)
CCC (2000a)[b]	Rabbit NZW F	5	0 4.9 13.9 32.3 76.3	Drinking Water	Gestation days 6-29	Body wt., clinical signs, reproductive developmental parameters	76	--	--	-- [e]
CCC (2000b)[b]	Rabbit NZW F	25	0 1.4 13 36 55	Drinking water	Gestation days 6-29	Maternal feed and water intake, body wt.; gross lesions; uterine weight, no. implantation sites, uterine contents, and no. corpora; Fetal wt., gross ext. alterations, skel. alterations, sex, visceral alterations	55 (developmental)	--	--	-- [e]
							13.4 (maternal)	35.3 (reduced maternal body weight gain)	50 (maternal)	BMDS software failed
CCC (2000c)[b]	Rat SD M,F	10	0 ppm 50 ppm 150 ppm 450 ppm 1350 ppm	Drinking water	Males 64 days Females 74 days	Reproductive/ developmental parameters	50 ppm	150 ppm	--	–

Table VIII-4 (cont.)

CCC (2000d)[b]	Rat SD F	25	0.0 2.2 18.4 45.0 82.0	Drinking water	Gestation days 6-21	Reproductive/ developmental parameters	45 (developmental)	82 (reduced no. of ossification sites in phalanges or metatarsals occurring with maternal toxicity)	– (developmental)	–[e]
							18.4 (maternal)	45 (reduced maternal body weight gain)	23 (maternal)	18 (reduced maternal body weight gain)
CCC (2002)	Rat SD M, F	30	0 ppm 50 ppm 150 ppm 450 ppm	Drinking water	Two generations	Reproductive/ developmental parameters	50 (offspring)	150 (delayed sexual maturation in F_1 males)	Not modeled	--
							50 (parental)	150 (decrecreased body weight and body weight gain in F_0 females and F_1 males and females)	Not modeled	--

[a] $BMDL_{10}$ values were derived using duration-adjusted doses.
[b] Ruddick et al. (1983); Klinefelter et al. (1995), Narotsky et al. (1997), Bielmeier et al. (2001), and CCC (2000a-d)are included in this table because they are reproductive and/or developmental studies. The NOAEL, LOAEL, BMD, and $BMDL_{10}$ values listed are for reproductive and/or developmental endpoints.
[c] Data modeled using Crump BMD software
[d] No histopathological abnormalities were noted in this study, and similar effects on sperm velocity were not observed in NTP (1998); therefore, data for sperm velocity were not modeled
[e] Data were not modeled since effects occurred at higher doses than other candidate endpoints
– Indicates that data were not modeled
Abbreviations: NZW, New Zealand White; SD, Sprague-Dawley

NOTE: The short-term reproductive and developmental toxicity study conducted by NTP (1998) was not included in this table because no developmental or reproductive effects were noted at dose levels ranging from 67 to 126 mg/kg-day.

Table VIII-5 Summary of Preliminary BMD Modeling Results for the Bromodichloromethane RfD

Study	Endpoint Modeled	$BMDL_{10}$ (mg/kg-day) *
Subchronic NTP (1987) mouse study	Focal necrosis of renal tubular epithelium in males	34
	Vacuolated hepatocytes in females	64
Chronic NTP (1987) rat study	Kidney cytomegaly in males	No acceptable fit
	Liver necrosis in males	36.5
	Liver fatty metamorphosis in males	No acceptable fit
	Clear cell changes in liver of females	No acceptable fit
Chronic NTP (1987) mouse study	Kidney cytomegaly in males	0.96
	Liver fatty metamorphosis in males	7.5
	Thyroid follicular cell hyperplasia in females	15
Chronic Aida et al. (1992b) rat study	Fatty degeneration in liver of males	2.38
	Granulomas in liver of males	4.5
	Fatty degeneration in liver of females	1.20
	Granulomas in liver of females	No acceptable fit

* BMD modeling conducted on duration-adjusted doses using the Crump BMD Software (K. S. Crump, Inc.).

The chronic study conducted by Aida et al. (1992b) was selected for derivation of the RfD. The lowest dose utilized in this study was 6 mg/kg-day (in contrast to low doses of 22 to 75 mg/kg-day utilized in other candidate studies), which provides some information on the shape of the dose-response curve in the region of interest. The lowest BMD (1.9 mg/kg-day) was obtained for fatty degeneration in the liver of male mice. The corresponding $BMDL_{10}$ for this endpoint was 0.8 mg/kg-day. The incidence of this lesion was strongly dose-dependent, with incidences of 0/24, 5/14, 12/13, and 19/19 observed at the doses of 0, 6, 25, and 138 mg/kg-day, respectively. The occurrence of this lesion in rats treated with bromodichloromethane is consistent with current understanding of the mode of action of brominated trihalomethanes. This endpoint was therefore selected to derive the RfD for bromodichloromethane.

Derivation of the RfD

The $BMDL_{10}$ calculated for fatty degeneration in the liver of male rats in the chronic rat study conducted by Aida et al. (1992b) was selected as the most appropriate basis for derivation of the RfD for bromodichloromethane. The RfD is calculated according to the following equation:

$$\text{RfD} = \frac{(0.8 \text{ mg/kg-day})}{(300)} = 0.003 \text{ mg/kg-day } (3 \text{ µg/L})$$

where:

0.8 mg/kg-day	=	Duration-adjusted $BMDL_{10}$ based on fatty degeneration of the liver in male rats
300	=	Uncertainty factor based on NAS/OW guidelines. This value includes a factor of 10 to account for intrahuman variability, and a factor of 10 for interspecies variability, and a factor of 3 to account for uncertainty related to possible human reproductive effects suggested (causality can not be established from available data) by epidemiological studies.

A composite UF of 300 was used. The standard factors of 10 were used for interspecies extrapolation and for protection of sensitive subpopulations. An additional factor of 3 was used to account for database deficiencies related to possible reproductive or developmental effects in humans. Use of an additional uncertainty factor of 3 is supported by findings in epidemiological studies (Waller et al., 1998; King et al., 2000) which suggest potential associations between bromodichloromethane exposure via drinking water and adverse pregnancy outcomes and changes in semen quality. Although the results of the epidemiological studies can not establish that bromodichloromethane caused the observed effects, they do raise significant concern for potential reproductive effects in exposed human populations, and the inclusion of an additional uncertainty factor is thus considered appropriate for protection of human health.

The DWEL for bromodichloromethane is calculated as follows:

$$\text{DWEL} = \frac{(0.003 \text{ mg/kg-day}) (70 \text{ kg})}{2 \text{ L/day}} = 0.100 \text{ mg/L } (100 \text{ µg/L})$$

where:

0.003 mg/kg-day	=	RfD for bromodichloromethane
70 kg	=	Assumed weight of an adult
2 L/day	=	Assumed water consumption by a 70-kg adult

Lifetime Health Advisory

The Lifetime Health Advisory (HA) represents that portion of an individual's total exposure that is attributed to drinking water and is considered protective of noncarcinogenic health effects over a lifetime of exposure. Bromodichloromethane has been categorized with respect to carcinogenic potential as Group B2: Probable human carcinogen (IRIS, 1993a). Therefore, in accordance with U.S. EPA Policy, a Lifetime HA is not recommended.

Alternative Approach for Derivation of the RfD

Use of the conventional NOAEL/LOAEL approach represents an alternative means for deriving the RfD and DWEL. Aida et al. (1992b) identified a LOAEL of 6 mg/kg-day in male rats on the basis of histopathological changes in the liver. Using this value and a composite uncertainty factor of 3,000 (including factors of 10 for interspecies extrapolation, protection of sensitive subpopulations, and use of a LOAEL, and a factor of 3 for database limitations and uncertainty regarding potential reproductive effects in humans), the RfD derived using the conventional approach is 0.002 mg/kg-day. Assuming a body weight of 70 kg and drinking water ingestion of 2 L/day, the corresponding DWEL is 0.07 mg/L (70 μg/L).

2. Carcinogenic Effects

a. Categorization of Carcinogenic Potential

Previous Evaluations

The Carcinogenic Risk Assessment Verification Endeavor (CRAVE) group of the U.S. EPA reviewed the available evidence on the carcinogenicity of bromodichloromethane and assigned it to Group B2: probable human carcinogen (IRIS, 1993a). Assignment to this category is appropriate for chemicals where there are no or inadequate human data, but which have sufficient animal data to indicate carcinogenic potential.

IARC has recently re-evaluated the carcinogenic potential of bromodichloromethane (IARC 1999a). IARC concluded that there is sufficient evidence of carcinogenicity for bromodichloromethane in experimental animals, but inadequate evidence in humans. On this basis, IARC classified bromodichloromethane as a Group 2B carcinogen: possibly carcinogenic to humans.

Categorization of Carcinogenic Potential Under the Proposed 1999 Cancer Guidelines

Cancer Hazard Summary

Under the proposed guidelines for Carcinogen Risk Assessment (U.S. EPA, 1999) bromodichloromethane is *likely to be carcinogenic to humans* by all routes. This descriptor is appropriate when the available tumor data and other key data are adequate to demonstrate carcinogenic potential to humans. This finding is based on the weight of experimental evidence in animal models which shows carcinogenicity by modes of action that are relevant to humans.

Supporting Information for Cancer Hazard Assessment

Human Data

The information on the carcinogenicity of bromodichloromethane from human studies is inadequate. There are no epidemiological data specifically relating increased incidence of cancer to exposure to bromodichloromethane. There are equivocal epidemiological data describing a weak association of chlorinated drinking water exposures with increased incidences of bladder, rectal, and colon cancer. U.S. EPA has determined that these studies cannot attribute the observed effects to a single compound, as chlorinated water contains numerous other disinfection byproducts that are potentially carcinogenic.

Animal Data

The carcinogenicity of bromodichloromethane in male and female animals has been investigated in a well-designed and conducted corn oil gavage study conducted in rats and mice, a dietary exposure study in rats, and two drinking water studies in rats. Additional data are available from a study in which male Strain A mice were exposed to bromodichloromethane by intraperitoneal injection. No data are available on the carcinogenic potential of bromodichloromethane administered via the inhalation or dermal routes.

In the corn oil gavage study (NTP, 1987), statistically significant increases were observed in the incidences of neoplasms of the large intestine and kidney in male and female rats, the kidney in male mice, and liver in female mice. The neoplasms observed in the large intestine and kidney are considered rare neoplasms based on historical control data for the tested strains. In the feeding study (Aida et al., 1992b), exposure to microencapsulated bromodichloromethane did not result in statistically significant increases in any tumor type. Observed neoplastic lesions included three cholangiocarcinomas and two hepatocellular adenomas in high-dose females, one hepatocellular adenoma in a control female, one cholangiosarcoma in a high-dose male, and one hepatocellular carcinoma each in a low- and a high-dose male. In the drinking water study conducted by Tumasonis et al. (1985), hepatic neoplastic nodules, hepatic adenofibrosis, and lymphosarcoma were significantly increased in female rats. No significant increase in the occurrence of any tumor type was observed in male rats. Renal adenoma or adenocarcinoma were noted in two males and one female treated with bromodichloromethane, while neither tumor type was reported in the control group. In the drinking water study conducted by George et al. (2002), the prevalence of neoplastic lesions in the liver was significantly increased only at the lowest administered dose. Intraperitoneal injection of Strain A mice with bromodichloromethane resulted in an apparent increase in the number of pulmonary adenomas per animals, although the response did not reach statistical significance in any dose group.

Structural Analogue Data

Trihalomethanes structurally related to bromodichloromethane have shown varying degrees of carcinogenic potential in rodents. Chloroform, the most extensively characterized trihalomethane, is reported to be carcinogenic at high doses in several chronic animal bioassays, with significant increases in the incidence of liver tumors in male and female mice and significant

increases in the incidence of kidney tumors in male rats and mice (U.S. EPA, 2001a). The occurrence of tumors in animals exposed to chloroform is demonstrably species-, strain-, and gender-specific, and has only been observed under dose conditions that caused cytotoxicity and regenerative cell proliferation in the target organ. The cancer database for structurally-related brominated trihalomethanes is more limited, but includes well-conducted studies performed by the National Toxicology Program. In a two-year corn oil gavage study of bromoform, NTP (1989a) found clear evidence for carcinogenicity in female rats and some evidence of carcinogenicity in male rats based on occurrence of tumors of the large intestine (adenomatous polyps or adenocarcinoma). In a two-year corn oil gavage study of dibromochloromethane, NTP (1985) determined that there was some evidence of carcinogenicity in female mice and equivocal evidence of carcinogenicity in male mice, based on the occurrence of hepatocellular adenomas and carcinomas. Other, less well-documented, oral exposure studies (Tobe et al., 1982; Kurokawa, 1987; Voronin et al., 1987) found no evidence for carcinogenicity of bromoform or dibromochloromethane.

Other Key Data

Bromodichloromethane is formed as a byproduct of drinking water disinfection with chlorine. Exposure to bromodichloromethane may occur via ingestion of tap water, via dermal contact during showering or bathing, or by inhalation of bromodichloromethane volatilized during household activities. Absorption of single oral doses appears to be extensive. Bromodichloromethane is rapidly metabolized and eliminated predominately as expired volatiles, carbon dioxide, or carbon monoxide. Only a small amount (less than 10%) is eliminated in urine or in feces. No comprehensive tissue data are available regarding the bioaccumulation or retention of bromodichloromethane following repeated exposure. However, because of the rapid metabolism and excretion of bromodichloromethane, marked accumulation and retention is not expected.

Bromodichloromethane itself is not directly reactive with DNA. Metabolism to reactive species is a prerequisite for toxicity, as inferred from metabolic induction and inhibition studies. *In vitro* and *in vivo* studies of the mutagenic and genotoxic potential of bromodichloromethane have yielded both positive and negative results. Synthesis of the overall weight of evidence from these studies is complicated by the use of a variety of testing protocols, different strains of test organisms, different activating systems, different dose levels, different exposure methods (gas versus liquid) and, in some cases, problems due to evaporation of the test chemical. However, because a majority of studies yielded positive results, bromodichloromethane is considered to be at least weakly mutagenic and genotoxic. Recent studies conducted with strains of *Salmonella* that express rat theta-class glutathione *S*-transferase suggest that mutagenicity of the brominated trihalomethanes may be mediated by glutathione conjugation.

Mode of Action

The mode of action for tumor induction by bromodichloromethane has not been clearly elucidated and may involve contributions from multiple bioactivation pathways. In each case, toxicity is believed to result from interaction of reactive metabolites with cellular macromolecules. Proposed bioactivation pathways for bromodichloromethane include: 1)

production of reactive dihalocarbonyls by oxidative metabolism; 2) production of reactive dihalomethyl radicals by oxidative metabolism; and 3) formation of DNA-reactive species via a glutathione-dependent pathway. The relative contribution of each pathway to tumor induction by bromodichloromethane has not been characterized. It is possible that only the latter two processes lead to DNA damage *in vivo*, because the highly reactive dihalocarbonyl intermediate may not survive long enough to enter the nucleus and react with DNA. For this reason, cytotoxicity may be the primary consequence of the oxidative pathway. Cytotoxicity coupled with regenerative hyperplasia is considered the primary mode of action for tumor formation following exposure to high concentrations of chloroform, a structurally-related trihalomethane which has low genotoxic potential. Data to support a similar primary mode of action for tumor development in liver, kidney, and large intestine are currently lacking for bromodichloromethane. In the absence of such information, combined with a positive weight-of-evidence evaluation for genotoxicity, the mode of action for tumor development is assumed to be a linear process. The processes leading to tumor formation in animals are expected to be relevant to humans.

Conclusion

Under the proposed guidelines for Carcinogen Risk Assessment (U.S. EPA, 1999) bromodichloromethane is *likely to be carcinogenic to humans* by the oral route. This weight-of-evidence evaluation is based on 1) observations of tumors in animals treated by oral pathways; 2) lack of epidemiological data specific to bromodichloromethane and equivocal data for drinking water drinking water exposures that cannot reliably be attributed to bromodichloromethane among multiple other disinfection byproducts; 3) positive results for a majority of the available genotoxicity and mutagenicity tests; and 4) metabolism and mode of action that are reasonably expected to be comparable across species. Although no cancer data exist for exposures via the dermal or inhalation pathways, the weight-of-evidence conclusion is considered to be applicable to these pathways as well. The finding for inhalation is based on the observation that patterns of metabolizing enzyme activity in male rats are similar for exposure via the inhalation and gavage routes. Bromodichloromethane absorbed through the skin is expected to be metabolized and cause toxicity in much the same way as bromodichloromethane absorbed by the oral and inhalation routes.

b. Choice of Study for Quantification of Carcinogenic Risk

In accordance with the Proposed 1999 Cancer Guidelines (U.S. EPA, 1999), quantification of cancer risk is appropriate for compounds categorized *as likely to be carcinogenic to humans*. Five oral exposure studies were available for quantification of the carcinogenic risk associated with exposure to bromodichloromethane. Detailed summaries of these studies are provided in Section V.G.1. The two-year study conducted by NTP (1987) in rats and mice was selected for quantification of carcinogenic effects associated with exposure to bromodichloromethane. Selection of this study was based on significantly increased incidence of several tumor types, monotonic dose response curves, and comprehensive documentation of study design and results.

In the NTP (1987) study, groups of male and female F344/N rats (50/sex/dose) received 0, 50, or 100 mg/kg-day gavage doses of bromodichloromethane in corn oil. The doses were

administered 5 days/week for 102 weeks. In a similar experiment, groups of male and female $B6C3F_1$ mice (50/sex/dose) were administered doses of 0, 25, or 50 mg/kg-day (males) or 0, 75, or 150 mg/kg-day (females) for 5 days/week for 102 weeks. All animals were subjected to gross and microscopic examinations for neoplastic lesions. Survival of all dosed animals was comparable to or greater than the corresponding control group. Statistically significant increases were observed in the incidences of neoplasms of the large intestine and kidney in male and female rats, the kidney in male mice, and the liver in female mice (Table VIII-6). The authors noted that neoplasms of the large intestine and kidney are uncommon tumors in F344/N rats and $B6C3F_1$ mice. They concluded that under the conditions of these 2-year gavage studies, clear evidence of carcinogenic activity existed for male and female rats and mice.

Table VIII-6 Tumor Frequencies in Rats and Mice Exposed to Bromodichloromethane in Corn Oil for 2 Years - Adapted from NTP (1987)

Animal		Tissue/Tumor	Tumor Frequency		
Male rat			**Control**	**50 mg/kg**	**100 mg/kg**
	Large intestine[a]	Adenomatous polyp	0/50	3/49	33/50[b]
		Adenocarcinoma	0/50	11/49[b]	38/50[b]
		Combined	0/50	13/49[b]	45/50[b]
	Kidney [a]	Tubular cell adenoma	0/50	1/49	3/50
		Tubular cell adenocarcinoma	0/50	0/49	10/50[b]
		Combined	0/50	1/49	13/50[b]
Female rat			**Control**	**50 mg/kg**	**100 mg/kg**
	Large intestine [c]	Adenomatous polyp	0/46	0/50	7/47[b]
		Adenocarcinoma	0/46	0/50	6/47[b]
		Combined	0/46	0/50	12/47[b]
	Kidney	Tubular cell adenoma	0/50	1/50	6/50[b]
		Tubular cell adenocarcinoma	0/50	0/50	9/50[b]
		Combined	0/50	1/50	15/50[b]
Male mouse			**Control**	**25 mg/kg**	**50 mg/kg**
	Kidney [d]	Tubular cell adenoma	1/46	2/49	6/50
		Tubular cell adenocarcinoma	0/46	0/49	4/50
		Combined	1/46	2/49	9/50[b]
Female mouse			**Control**	**75 mg/kg**	**150 mg/kg**
	Liver	Hepatocellular adenoma	1/50	13/48[b]	23/50[b]
		Hepatocellular carcinoma	2/50	5/48	10/50[b]
		Combined	3/50	18/48[b]	29/50[b]

[a] One rat died at week 33 in the low-dose group and was eliminated from the cancer risk calculation.
[b] Statistically significant at $p<0.05$, compared to controls.
[c] Intestine not examined in four rats from control group and three rats from high-dose group.
[d] In the control group, two mice died during the first week, one mouse died during week, nine and one escaped in week 79. One mouse in the low-dose group died in the first week. All of these mice were eliminated from the cancer risk calculations.

Use of the NTP rodent studies (NTP,1987) for derivation of cancer risk estimates for bromodichloromethane is complicated by the use of corn oil as a dosing vehicle. Although a vehicle effect has not been reported for brominated trihalomethanes, it can be inferred from studies of chloroform carcinogenicity that such an effect might exist, at least for hepatic tumors in mice. Therefore, in the case of bromodichloromethane, the U.S. EPA believes that the most appropriate basis of the cancer risk estimate is the incidence of renal tumors in male mice. Renal tumors are considered to be appropriate because these tumors were increased in a dose-dependent manner in both mice (male) and rats (both sexes).

c. Choice of Approach and Rationale

The LMS model (U.S. EPA, 1986) and the default linear approach described by the Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996; 1999) were used to quantify the cancer risk associated with exposure to bromodichloromethane. Although data are mixed, the weight of evidence indicates that bromodichloromethane is mutagenic (see Section V.F.1). Furthermore, recent studies (Melnick, et al. 1998; George et al., 2002) suggest that induction of hepatic tumors occurs at doses of bromodichloromethane that have marginal or no effect on hepatocyte labeling index, indicating that regenerative hyperplasia is not required for tumor induction. Thus, use of a linear approach was considered appropriate for quantification of cancer risk associated with exposure to bromodichloromethane.

d. Cancer Potency and Risk Estimates

The available estimates for cancer risk associated with bromodichloromethane are summarized in Table VIII-7. U.S. EPA (1994b) recommended use of a cancer potency estimate of 6.2×10^{-2} $(mg/kg\text{-}day)^{-1}$ as reported in IRIS (1993a). This value was derived in accordance with the 1986 Guidelines for Carcinogenic Risk Assessment (U.S. EPA, 1986), based on the occurrence of renal tumors in male mice. A unit risk of 1.8×10^{-6} was estimated using an assumed body weight of 70 kg and a drinking water ingestion rate of 2 L. This estimate was used to calculate a drinking water concentration of approximately 6 μg/L associated with a 10^{-5} risk (0.6 μg/L for 10^{-6} risk).

A cancer potency value of 3.5×10^{-2} $(mg/kg\text{-}day)^{-1}$ (U.S. EPA, 1998b) was derived using the LMS method and an animal-to-human conversion factor of body weight$^{3/4}$ (Table VIII-7). The use of body weight$^{3/4}$ is consistent with recommendations in U.S. EPA (1992b). This potency factor is also based on the occurrence of renal tumors in male mice. A unit risk of 1×10^{-6} $(\mu g/L)^{-1}$ was estimated for bromodichloromethane using an assumed body weight of 70 kg and a drinking water ingestion rate of 2 L. This estimate was used to calculate a drinking water concentration of approximately 10 μg/L associated with a 10^{-5} risk (1 μg/L for 10^{-6} risk).

Cancer risk estimates were also obtained using the LED_{10} (the lower 95% confidence limit on a dose associated with 10% extra risk) of 3.0×10^{3} μg/kg-day for renal tumors in mice and assuming a linear mode of action for the carcinogenicity of bromodichloromethane (Table VIII-7). A cancer potency value of 3.4×10^{-2} $(mg/kg\text{-}day)^{-1}$ was derived using this approach. A unit risk of 9.6×10^{-7} $(\mu g/L)^{-1}$ was estimated for bromodichloromethane using an assumed body weight of 70 kg and a drinking water ingestion rate of 2 L. This estimate was used to calculate a

drinking water concentration of approximately 10 μg/L associated with a 10^{-5} risk (1 μg/L for 10^{-6} risk). These values are closely similar to corresponding values derived using the LMS approach with body weight scaling to the 3/4 power.

Table VIII-7 Summary of Cancer Risk Estimates for Bromodichloromethane

Method of Estimation	Tumor Site	Species	Sex	Slope Factor $(mg/kg\text{-}day)^{-1}$	Unit Risk $(\mu g/L)^{-1}$	LED_{10} (μg/kg-day)	10^{-5} Risk Concentration (μg/L)
LMS Method Using $BW^{3/4}$ Conversion (U.S. EPA, 1998b)	Liver	Mouse	F	6.9×10^{-2}	2.0×10^{-6}	-	5
	Kidney	Rat	M F	5.5×10^{-3} 6.1×10^{-3}	1.6×10^{-7} 1.7×10^{-7}	-	64 57
		Mouse	M	3.5×10^{-2}	1.0×10^{-6}	-	10
	Large intestine	Rat	M F	1.7×10^{-2} 6.1×10^{-3}	4.9×10^{-7} 1.7×10^{-7}	-	20 57
LMS Method Using $BW^{2/3}$ Conversion U.S. EPA (1994b)*	Liver	Mouse	F	1.3×10^{-1}	3.7×10^{-6}	-	3
	Kidney	Rat	M F	8.7×10^{-3} 9.5×10^{-3}	2.5×10^{-7} 2.7×10^{-7}	-	40 37
		Mouse	M	6.2×10^{-2}	1.8×10^{-6}	-	6
	Large intestine	Rat	M F	2.5×10^{-2} 4.9×10^{-3}	7.1×10^{-7} 1.4×10^{-7}	-	14 72
LED_{10}/Linear Method (U.S. EPA, 1998b)	Liver	Mouse	F	6.5×10^{-2}	1.9×10^{-6}	1.5×10^{3}	5
	Kidney	Rat	M F	8.1×10^{-3} 8.8×10^{-3}	2.3×10^{-7} 2.5×10^{-7}	1.2×10^{4} 1.1×10^{4}	43 40
		Mouse	M	3.4×10^{-2}	9.6×10^{-7}	3.0×10^{3}	10
	Large intestine	Rat	M F	2.8×10^{-2} 1.0×10^{-2}	8×10^{-7} 3×10^{-7}	3.5×10^{3} 9.6×10^{3}	12 34

* Based on information adapted from IRIS (1993a)

B. Dibromochloromethane

1. Noncarcinogenic effects

a. One-day Health Advisory

Four candidate studies that investigated the acute oral toxicity of dibromochloromethane were available. These studies are summarized in Table VIII-8 (below). Bowman et al. (1978) administered dibromochloromethane by gavage to ICR Swiss mice at doses ranging from 500 to 4,000 mg/kg and found that sedation and anesthesia resulted at doses of 500 mg/kg or higher. NTP (1985) conducted a preliminary range-finding study in which F344/N rats and $B6C3F_1$ mice were administered dibromochloromethane by gavage at doses ranging from 160 to 2,500 mg/kg and found that death may result from doses at 310 mg/kg or higher in mice or rats. More recently, Müller et al. (1997) investigated the cardiotoxic effects of acute oral

dibromochloromethane exposure in male Wistar rats. In this study, rats administered doses of 83 or 167 mg/kg exhibited transient changes in cardiovascular parameters, while rats administered doses of 333 or 667 mg/kg exhibited persistent alterations in at least one of the cardiovascular parameters that lasted throughout the postexposure observation period. Finally, Korz and Gatterman (1997) investigated the behavioral toxicity of acute oral dibromochloromethane exposure in male golden hamsters and observed only transient effects on the behavioral parameters investigated.

These studies were not considered adequate for deriving the One-day HA, since more sensitive endpoints such as histopathology were not evaluated. Therefore, the Ten-day HA for dibromochloromethane (0.6 mg/L) calculated below is recommended for use as the One-day HA.

b. Ten-day Health Advisory

Studies considered for derivation of the Ten-day HA for dibromochloromethane are summarized in Table VIII-9 below. The key studies in this group are those of Aida et al. (1992a), Condie et al. (1983), and Melnick et al. (1998). These studies reported effects on sensitive endpoints and had data suitable for BMD analysis. Use of the remaining studies was limited by a variety of considerations, including lack of data suitable for BMD analysis (Chu et al., 1982a; NTP, 1996; Coffin et al., 2000), toxicological relevance or difficulty in interpretation of the most sensitive endpoint (NTP, 1985; Munson et al. 1982), and occurrence of effects only at the frank toxicity level (NTP, 1985).

Melnick et al. (1998) administered dibromochloromethane to female $B6C3F_1$ mice by gavage for 5 days/week for 3 weeks and identified a NOAEL of 100 mg/kg-day (duration-adjusted NOAEL of 71 mg/kg-day) and a LOAEL of 192 mg/kg-day (duration-adjusted LOAEL of 137 mg/kg-day) based on histologic changes in the liver (hepatocyte hydropic degeneration). BMD analysis calculated BMD and $BMDL_{10}$ values of 112 and 68 mg/kg-day, respectively.

Table VIII-8 Summary of Candidate Studies for Derivation of the One-day HA for Dibromochloromethane

Reference	Species	Route	Exposure Duration	Dose (mg/kg-day)	Result
Bowman et al. (1978)	Mouse ICR Swiss	Gavage (aqueous)	Single dose	500 - 4000	Sedation; anesthesia; increased mortality
NTP (1985)	Rat F344/N Mouse $B6C3F_1$	Gavage (corn oil)	Single dose	160 - 2500	Lethargy; death
Müller et al. (1997)	Rat Wistar	Gavage (olive oil)	Single dose	83 - 667	Transient changes in blood pressure; effects on cardiac muscle contractility
Korz and Gatterman (1997)	Hamster	Gavage (olive oil)	Single dose	50	Transient changes in post-treatment behavior

Table VIII-9 Summary of Candidate Studies for Derivation of the Ten-day HA for Dibromochloromethane

Reference	Species Sex	n	Dose	Route	Exposure Duration	Endpoints	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	BMD (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Aida et al. (1992a)	Rat Wistar M, F	7	Males 0 18 56 173 Females 0 34 101 332	Feed	1 month	Body weight, clinical signs, serum biochemistry, hematology, histology	18.3	56 (liver histopathology)	29 14	6.7 (Liver cell vacuolation in females) 5.5 (Liver cell vacuolation in males)
Chu et al. (1982a)	Rat SD M	10	0 0.7 8.5 68	Drinking water	28 days	Clinical signs, serum biochemistry, histology	68	--	Not modeled	--
Condie et al. (1983)	Mouse CD-1 M	8-16	0 37 74 147	Gavage (oil)	14 days	Serum enzymes, PAH uptake *in vitro*, histology	74	147 (elevated ALT, decreased PAH, liver and kidney histopathology)	3.5 11	1.6 (Renal mesangial hypertrophy) 6.9 (hepatic cytoplasmic vacuolation)
Melnick et al. (1998)	Mouse $B6C3F_1$ F	10	0 50/37 100/71 192/137 417/298	Gavage (oil)	3 weeks (5 d/wk)	Body and liver weights, serum chemistry, liver histology	100 (marginal)	192 (liver histopathology)	112*	68 (hepatic hydropic degeneration)
Munson et al. (1982)	Mouse CD-1 M, F	8-12	0 50 125 250	Gavage (aqueous)	14 days	Body and organ weights, serum chemistry, hematology, immune function	50	125 (decreased humoral immunity)	Not modeled	–

Table VIII-9 (cont.)

Reference	Species Sex	n	Dose	Route	Exposure Duration	Endpoints	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	BMD (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
NTP (1985)	Rat F344/N M, F	5	0 60 125 250 500 1000	Gavage (oil)	14 days	Body weight, clinical signs, gross necropsy	250	500 (FEL) (mortality, lethargy, gross pathology)	Not modeled	--
NTP (1985)	Mouse $B6C3F_1$ M, F	5	0 30 60 125 250 500	Gavage (oil)	14 days	Body weight, clinical signs, gross necropsy	60	125 (stomach lesions)	143 218	54 (stomach nodules - males) 77 (stomach nodules - females)
NTP (1996)	Rat F344/N M, F	10	Males 0 4 12 28 Females 0 6-7 17-20 48-48	Drinking water	29 days	Body weight, serum chemistry, hematology, gross necropsy, histology, sperm evaluation	28	--	Not modeled	–
Coffin et al. (2000)	Mouse $B6C3F_1$ F	10	0 100 300	Gavage (oil)	11 days	Relative liver wt.; liver histopathology; labeling index	--	100	Not modeled	--

*BMD and $BMDL_{10}$ calculated using duration-adjusted doses
Abbreviations :FEL, Frank Effect Level; SD, Sprague-Dawley
– No data

These values are considerably higher (approximately 5- to 10-fold) than BMD and $BMDL_{10}$ values calculated for hepatic effects using data from Condie et al. (1983) or Aida et al. (1992a).

Condie et al. (1983) administered dibromochloromethane by gavage to male CD-1 mice for 14 days and identified a NOAEL of 74 mg/kg-day and a LOAEL of 147 mg/kg-day based on minimal to moderate liver and kidney injury. Histologic changes in the liver included focal inflammation and cytoplasmic vacuolization similar to that observed in the study by Aida et al. (1992a). Effects in the kidney included minimal to slight epithelial hyperplasia at the high dose and minimal to slight mesangial hypertrophy at all (non-control) doses. Data for cytoplasmic vacuolization and renal mesangial hypertrophy were analyzed by BMD modeling. The lowest BMD and $BMDL_{10}$ (3.5 and 1.6 mg/kg-day, respectively) among all candidate studies were identified for mesangial hypertrophy. However, the pattern of dose-response for this endpoint (0/16, 4/5, 7/10, 7/10 at doses of 0, 37, 74, and 147 mg/kg-day, respectively) resulted in generally poor curve fits (0.1<goodness of fit p value>0.49) and a high degree of model-dependence (See summary of modeling results in Appendix A). Thus, confidence in the reliability of the BMD for renal effects was low. The BMD and $BMDL_{10}$ values for hepatic cytoplasmic vacuolization were higher (11 and 6.9 mg/kg-day, respectively). These results were based on incidences of 1/16, 3/5, 4/10, and 8/10 at doses of 0, 37, 74, and 147 mg/kg-day, respectively.

The study by Aida et al. (1992a) was selected as the basis for derivation of the Ten-day HA. In this study, Wistar rats of both sexes were administered microencapsulated dibromochloromethane in the diet for one month. The dose levels ranged from 18.3 to 173.3 mg/kg-day for males and from 34.0 to 332.5 mg/kg-day for females. A NOAEL of 18.3 mg/kg-day and a LOAEL of 56.2 mg/kg-day were identified based on histologic changes (cell vacuolization, swelling, and single cell necrosis) in the livers of male rats. BMD analysis of data for hepatic cell vacuolization calculated BMD and $BMDL_{10}$ values of 29 and 6.7 mg/kg-day in females and 14 and 5.5 mg/kg-day in males, respectively. The $BDML_{10}$ for hepatic cell vacuolization in male rats was selected for calculation of the 10-day HA because it was considered the lowest reliable value based on examination of the raw data and modeling results. The incidence of this lesion was 0/7, 1/7, 3/7, and 7/7 at doses of 0, 18, 56, and 173 mg/kg-day, respectively.

Based on the $BMDL_{10}$ calculated from the data of Aida et al. (1992a), the Ten-day HA is derived as follows:

$$\text{Ten-day HA} = \frac{(5.5 \text{ mg/kg-day})\ (10 \text{ kg})}{(100)\ (1 \text{ L/day})} = 0.55 \text{ mg/L (rounded to 0.6 mg/L)}$$

5.5 mg/kg-day = $BMDL_{10}$ based on hepatic cell vacuolization in rats fed dibromochloromethane for one month

10 kg = Assumed body weight of a child

100	=	Composite uncertainty factor based on NAS/OW guidelines; includes a factor of 10 for interspecies variation and a factor of 10 for protection of sensitive human populations
1 L/day	=	Assumed water consumption of a 10-kg child

The Ten-day HA was calculated using the conventional NOAEL/LOAEL approach for comparison with the value obtained using the BMD approach. The Aida et al. (1992a) study identified a NOAEL of 18.3 mg/kg-day based on the absence of hepatic effects in rats. Using this value and the assumptions described above, the Ten-day HA would be 1.8 mg/L (rounded to 2 mg/L).

c. Longer-term Health Advisory

Four candidate studies were considered for derivation of the Longer-term HA for dibromochloromethane. These studies are summarized in Table VIII-10 (below). Selected data from three of these studies were modeled using the BMD approach. The results of BMD analysis are included in Table VIII-11.

Chu et al. (1982b) administered dibromochloromethane to Sprague-Dawley rats in the drinking water at doses ranging from 0.57 to 236 mg/kg-day. This study identified a NOAEL of 49 mg/kg-day, and a LOAEL of 224 mg/kg-day based on mild hepatic lesions (increased cytoplasmic volume and vacuolation due to fatty infiltration) observed in males. BMD and $BMDL_{10}$ values of 18 and 5.3 mg/kg-day, respectively, were calculated for hepatic vacuolization using the BMDS software. Daniel et al. (1990) identified a LOAEL of 50 mg/kg-day based on hepatic lesions (centrilobular lipidosis) observed in male Sprague-Dawley rats and on kidney lesions (tubular degeneration) observed in female Sprague-Dawley rats administered dibromochloromethane by gavage for 90 consecutive days. BMD and $BMDL_{10}$ values of 20 and 4.2 mg/kg-day, respectively, were calculated for renal tubular degeneration using the Crump BMD software (K. S. Crump, Inc.). NTP (1985) administered doses of dibromochloromethane ranging from 15 to 250 mg/kg-day to male and female mice. The compound was administered by gavage in corn oil, five days per week for 13 weeks. NOAEL and LOAEL values of 125 and 250 mg/kg-day were identified on the basis of renal and hepatic lesions. BMD and $BMDL_{10}$ values were not calculated since lesions occurred only at the high dose of 250 mg/kg-day.

The NTP (1985) study conducted in rats was selected as the basis for derivation of the Longer-term HA. In this study, F344/N rats were administered dibromochloromethane by gavage at dose levels ranging from 15 to 250 mg/kg for 5 days/week for 13 weeks. Severe lesions and necrosis of the kidney, liver, and salivary glands were observed primarily at the high dose. However, males exhibited a dose-dependent increase in the frequency of clear cytoplasmic vacuoles indicative of fatty metamorphosis in the liver. This effect reached statistical

Table VIII-10 Summary of Candidate Studies for Derivation of the Longer-term HA for Dibromochloromethane

Reference	Species	n	Dose	Route	Exposure Duration	Endpoints	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	BMD (mg/kg-day)*	$BMDL_{10}$ (mg/kg-day) *
Chu et al. (1982b)	Rat SD M, F	20	Males 0 0.57 6.1 49 224 Females 0 0.64 6.9 55 236	Drinking water	90 days	Body weight, serum chemistry, histology	49	224 (decreased weight gain, mild hepatic lesions)	18	5.3 (Liver lesions in males)
Daniel et al. (1990)	Rat SD M, F	10	0 50 100 200	Gavage (oil)	90 days	Body weight, clinical signs, serum biochemistry, gross necropsy, histology	--	50 (hepatic and renal lesions)	20†	4.2† (kidney cortex degeneration in females)
NTP (1985)	Rat F344/N M, F	10	0 15 30 60 125 250	Gavage (oil)	13 weeks (5 d/wk)	Body weight, clinical signs, histology	30	60 (hepatic lesions)	2.5	1.7 (liver fatty metamorphosis in males)
NTP (1985)	Mouse $B6C3F_1$ M, F	10	0 15 30 60 125 250	Gavage (oil)	13 weeks (5 d/wk)	Body weight, clinical signs, histology	125	250 (renal and hepatic lesions)	Not modeled	--

* $BMDL_{10}$ values were derived using duration-adjusted doses.
† Modeled using Crump benchmark dose software
Abbreviations: SD, Sprague-Dawley

significance at 60 mg/kg-day, and this dose was designated the LOAEL. The next lower dose (30 mg/kg-day) was designated as the NOAEL. BMD analysis using the BMDS program obtained duration-adjusted BMD and $BMDL_{10}$ values of 2.5 and 1.7 mg/kg-day, respectively. These values were the lowest calculated among the three studies for which BMD analysis was conducted.

Using the NTP (1985) rat study, the Longer-term HA for the 10-kg child is calculated as follows:

$$\text{Longer-term HA} = \frac{(1.7 \text{ mg/kg-day})(10 \text{ kg})}{(100)\ (1 \text{ L/day})} = 0.17 \text{ mg/L (rounded to 0.2 mg/L)}$$

where:

1.7 mg/kg-day	=	Duration-adjusted $BMDL_{10}$ based on hepatic cell vacuolization in rats exposed to dibromochloromethane by oil gavage for 13 weeks
10 kg	=	Assumed body weight of a child
100	=	Composite uncertainty factor based on NAS/OW guidelines; includes a factor of 10 for interspecies variation and a factor of 10 for protection of sensitive human populations
1 L/day	=	Assumed water consumption of a 10-kg child

The Longer-term HA for a 70-kg adult consuming 2 liters of water per day is calculated according to the following equation:

$$\text{Longer-term HA} = \frac{(1.7 \text{ mg/kg-day})(70 \text{ kg})}{(100)\ (2 \text{ L/day})} = 0.60 \text{ mg/L (rounded to 0.6 mg/L)}$$

For purposes of comparison, the Longer-term HAs were also calculated using the conventional NOAEL/LOAEL approach. NTP (1985) identified a NOAEL of 30 mg/kg-day by based on the absence of clinical signs or histologic alterations in rats exposed to dibromochloromethane by corn oil gavage for 13 weeks. Using a duration adjusted dose of 21 mg/kg-day (obtained by multiplying the nominal dose by 5/7) and the assumptions for body weight and drinking water ingestion described above, the Longer-term HAs would be 2.1 and 7.5 mg/kg-day for a 10 kg child and 70 kg adult, respectively.

d. Reference Dose, Drinking Water Equivalent Level and Lifetime Health Advisory

This section reports the existing RfD value for dibromochloromethane and describes the derivation of the RfD for this compound. This section also describes the calculation of Drinking Water Equivalent Level and Lifetime Health Advisory values which require the RfD as input. For this document, new and existing studies were reviewed and appropriate candidate data were selected for benchmark dose (BMD) modeling. The results of BMD modeling were used in conjunction with appropriate uncertainty factors to calculate the RfD. A comparison of the RfD derived using the BMD approach to the results obtained using the conventional NOAEL/LOAEL approach is also provided.

Description of the Existing RfD

The existing RfD for dibromochloromethane is 0.02 mg/kg-day (IRIS, 1992). This value was derived using a duration-adjusted NOAEL of 21.4 mg/kg-day identified for the occurrence of hepatic lesions in F344/N rats administered dibromochloromethane by corn oil gavage, 5 days/week for 13 weeks . An uncertainty factor of 1000 was used to account for extrapolation from animal data, for protection of sensitive human subpopulations, and for use of a subchronic study.

Identification of Candidate Studies for Derivation of the RfD

Candidate studies considered for derivation of the RfD for dibromochloromethane are summarized in Table VIII-11 (below). Tobe et al. (1982) administered microencapsulated dibromochloromethane in the diet to Wistar rats for 24 months at dose levels that ranged from 12 to 278 mg/kg-day. Although the study identified a NOAEL and a LOAEL of 12 and 49 mg/kg-day, respectively, based on decreased body weight, changes in clinical chemistry parameters, and gross liver appearance in males, a histopathological examination was not conducted. NTP (1985) investigated the chronic oral toxicity of dibromochloromethane in F344/N rats and B6C3F$_1$ mice. Only LOAEL values were identified in these studies. Specifically, the rat study identified a LOAEL of 40 mg/kg-day based on histologic lesions in both male and female rats (e.g., fatty change), and the mouse study identified a LOAEL of 50 mg/kg-day based on lesions in the liver (fatty metamorphosis) and the thyroid (follicular cell hyperplasia) in the female mice. A thirteen-week oral exposure study in rats (NTP, 1985) examined toxicity at a wider range of doses than the chronic studies and identified NOAEL and LOAEL values of 30 and 60 mg/kg-day, respectively, for histopathological changes in the liver.

Method of Analysis

Selected data from the candidate studies were analyzed using the benchmark dose (BMD) modeling approach. Initially, data sets for potentially sensitive endpoints were selected as described in U.S. EPA (1998b) and analyzed using the Crump Benchmark Dose Modeling Software (K. S. Crump, Inc.). Results of this preliminary analysis are summarized in Table VIII-12. Following the release of Version 1.2 of the BMDS program (U.S. EPA, 2000a), a subset of the most sensitive endpoints identified using the Crump software was reanalyzed in accordance with proposed U.S. EPA (2000b) recommendations. An advantage of analysis with the BMDS

software is that several additional models are available to fit the data. The results of the analysis using the BMDS software are included in Table VIII-11.

Choice of Principal Study and Critical Effect for the RfD

Two studies were identified as strong candidates for selection as the principal study. The NTP (1985) subchronic study evaluated toxicological effects in male and female rats at five concentrations of dibromochloromethane (15, 30, 60, 125, and 250 mg/kg-day) in addition to the control. The chemical was administered by gavage in corn oil on five days per week for 13 weeks. A relatively small sample size of 10 animals/treatment group was utilized. The endpoints evaluated included clinical signs, body weight, serum biochemistry, and histopathological changes in organs. NOAEL and LOAEL values of 30 and 60 mg/kg-day, respectively, were identified on the basis of hepatic lesions using the conventional approach. Analysis of the data for fatty metamorphosis in the liver using the BMDS program resulted in a duration-adjusted BMD of 2.7 mg/kg-day for this endpoint, with a corresponding duration-adjusted $BMDL_{10}$ of 1.7 mg/kg-day. A strength of this study with respect to BMD modeling was the use of additional doses at the lower end of the dose-response range. Inclusion of these doses permits more accurate characterization of the shape of the dose-response curve and thus less uncertainty in the range of interest.

The second candidate for selection as the principal study for derivation of the RfD was the chronic study conducted by NTP (1985). This study evaluated dibromochloromethane effects at administered doses of 0, 40 and 80 mg/kg-day. The chemical was administered by gavage in oil on five days per week for 104 weeks. The endpoints evaluated included clinical signs, body weight, serum biochemistry, and histopathological changes in organs, and a LOAEL of 40 mg/kg-day based on hepatic lesions was identified using the conventional approach. Analysis of the data for fatty metamorphosis in the liver using the BMDS program resulted in a duration-adjusted BMD of 2.5 mg/kg-day for this endpoint, with a corresponding duration-adjusted $BMDL_{10}$ of 1.6 mg/kg-day based on duration adjusted doses.

A potential weakness of the NTP (1985) chronic study is the lack of dose-response information at administered doses less than 40 mg/kg-day. *A priori*, the lack of information regarding the shape of the curve at low doses would be expected to result in greater uncertainty (and thus wider confidence limits) in the estimate of the chronic BMD. However, the BMD and $BMDL_{10}$ values calculated for fatty metamorphosis in the subchronic and chronic studies are closely similar. This observation suggests that there is little potential for cumulative effects on the occurrence of this lesion. The slight differences in the values may reflect both experimental uncertainty and uncertainty in modeling.

Table VIII-11 Summary of Candidate Studies for Derivation of the RfD for Dibromochloromethane

Reference	Species Sex	n	Dose (mg/kg-day)	Route	Exposure Duration	Endpoints	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	BMD (mg/kg-day)	$BMDL_{10}$ (mg/kg-day) *
Tobe et al. (1982)	Rat Wistar M, F	40	Males 0 12 49 196 Females 0 17 70 278	Diet	24 months	Body weight, serum biochemistry, gross pathology	12	49 (serum enzyme changes and altered liver appearance)	Not modeled	--
NTP (1985)	Rat F344/N M, F	10	0 15 30 60 125 250	Gavage (oil)	13 weeks (5 d/wk)	Body weight, clinical signs, serum biochemistry, gross necropsy, histology	30	60 (hepatic lesions)	2.5	1.7 (fatty metamorphosis in liver of males)
NTP (1985)	Rat F344/N M, F	50	0 40 80	Gavage (oil)	104 weeks (5 d/wk)	Body weight, clinical signs, gross necropsy, histology	--	40 (hepatic lesions)	2.7	1.6 (fatty changes in liver of males)
NTP (1985)	Mouse $B6C3F_1$ M, F	50	0 50 100	Gavage (oil)	105 weeks (5 d/wk)	Body weight, clinical signs, gross necropsy, histology	--	50 (hepatic lesions)	$9.1^{\dagger}$	$7.1^{\dagger}$ (thyroid follicular cell hyperplasia in females)
Borzelleca and Carchman (1982) **	Mouse ICR Swiss -	10M 30F	0 17 171 685	Drinking water	27 weeks	Maternal body weight, gross pathology, fetal weight, survival, teratogenicity	17 (marginal)	171 (maternal toxicity, possible fetotoxicity)	Not modeled (insuff. data provided in publication)	--

Table VIII-11 (cont.)

Reference	Species Sex	n	Dose (mg/kg-day)	Route	Exposure Duration	Endpoints	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	BMD (mg/kg-day)	$BMDL_{10}$ (mg/kg-day) *
NTP (1996) **	Rat SD M, F	10	males 0 4.2 12.4 28.2 Group A females 0 6.3 17.4 46.0 Group B females 0 7.1 20.0 47.8	Drinking water	29 days	Body weight, serum chemistry, hematology, gross necropsy, histology, sperm evaluation	28	--	Not modeled	--

* $BMDL_{10}$ values were derived using duration-adjusted doses.

** These studies have been included in this table because they are reproductive/developmental studies and would be considered relevant for derivation of the RfD. However, Borzelleca and Carchman (1982) found only marginal evidence for developmental toxicity at the low-dose level and the NTP (1996) study did not observe any reproductive or developmental effects at the dose levels evaluated.

† Modeled using Crump BMD software

Abbreviations: SD, Sprague-Dawley

Table VIII-12 Results of Preliminary BMD Modeling of Selected Data from NTP (1985) Studies

Study	Endpoint Modeled	$BMDL_{10}$ (mg/kg-day) *
Subchronic NTP (1985) rat study	Fatty metamorphosis in liver of male rats	0.93
Chronic NTP (1985) rat study	Fatty metamorphosis in liver of male rats	1.16
	Fatty metamorphosis in liver of female rats	No acceptable fit
	"Ground glass" cytoplasm in liver of male rats	4.93
	Nephrosis in liver of female rats	17
Chronic NTP (1985) mouse study	Fatty metamorphosis in liver of female mice	7.68
	Thyroid follicular cell hyperplasia in female mice	7.09

* BMD modeling was conducted on duration-adjusted values using the Crump BMD software.

Both studies were considered appropriate for derivation of the RfD. The NTP (1985) investigation of chronic toxicity in rats was selected as the principal study on the basis of its longer duration. The critical endpoint is hepatotoxicity, as evidenced by the occurrence of fatty metamorphosis in the livers of dibromochloromethane-treated animals. This effect was dose-dependent, with incidences of 27/50, 47/50, and 49/50 at the duration-adjusted doses of 0, 29, and 57 mg/kg-day. Selection of this study is strongly supported by the similar BMD calculated for the same effect in the NTP (1985) subchronic study.

Derivation of the RfD

The duration-adjusted $BMDL_{10}$ value from the chronic NTP (1985) rat study was selected as the most appropriate basis for derivation of the RfD for dibromochloromethane. The RfD is calculated using the following equation:

$$RfD = \frac{(1.6 \text{ mg/kg-day})}{(100)} = 0.016 \text{ mg/kg-day (rounded to 0.02 mg/kg-day)}$$

where:

1.6 mg/kg-day = Duration-adjusted $BMDL_{10}$ based on fatty changes in the liver of male rats

100 = Composite uncertainty factor based on NAS/OW guidelines; includes a factor of 10 interspecies extrapolation and a factor of 10 for protection of sensitive human populations

A composite UF of 100 was used. The standard factors of 10 were used for interspecies extrapolation and for protection of sensitive subpopulations. Furthermore, no additional uncertainty factor was needed to account for an incomplete database. The database for dibromochloromethane includes a two-generation study in ICR Swiss mice (Borzelleca and Carchman 1982), a developmental toxicity study in Sprague-Dawley rats (Ruddick et al., 1983), and a short-term reproductive and developmental toxicity study in rats (NTP, 1996). Therefore, the database is considered nearly complete despite the lack of a developmental toxicity study in a second species.

The DWEL for dibromochloromethane is calculated as follows:

$$\text{DWEL} = \frac{(0.02 \text{ mg/kg-day})\ (70 \text{ kg})}{2 \text{ L/day}} = 0.7 \text{ mg/L } (700 \text{ µg/L})$$

where:

0.02 mg/kg-day = RfD

70 kg = Assumed weight of an adult

2 L/day = Assumed water consumption by a 70-kg adult

Lifetime Health Advisory

The Lifetime Health Advisory (HA) represents that portion of an individual's total exposure that is attributed to drinking water and is considered protective of noncarcinogenic health effects over a lifetime of exposure. Dibromochloromethane is classified with respect to carcinogenic potential as Group C: Possible human carcinogen. The Lifetime Health Advisory (HA) is therefore calculated as follows:

$$\text{Lifetime HA} = \frac{(0.7 \text{ mg/L})\ (0.8)}{10} = 0.06 \text{ mg/L } (60 \text{ µg/L})$$

where:

0.7 mg/kg-day = DWEL

0.8 = Relative Source Contribution (RSC), the proportion of the total daily exposure contributed by the dibromochloromethane in drinking water

10 = Uncertainty factor used in accordance with U.S. EPA policy for Group C contaminants to account for possible carcinogenicity

Alternative Approach for Derivation of the RfD

An alternative approach to the derivation of the RfD is use of the conventional NOAEL/LOAEL method. The subchronic oral exposure study conducted by NTP (1985) identified a NOAEL of 30 mg/kg-day. Using this value, a duration adjustment factor of 5/7, and a composite uncertainty factor of 1000 (includes factors of 10 for interspecies extrapolation, protection of sensitive subpopulations, and use of a subchronic study), the resulting RfD is 0.02 mg/kg-day (the same value as derived using the BMD approach). The corresponding DWEL is 0.7 mg/L, assuming an adult body weight of 70 kg and a drinking water ingestion rate of 2 L/day.

2. Carcinogenic Effects

a. Categorization of Carcinogenic Potential

Previous Evaluations of Carcinogenic Potential

The Carcinogenic Risk Assessment Verification Endeavor (CRAVE) group of the U.S. EPA reviewed the available evidence on the carcinogenicity of the brominated trihalomethanes and assigned dibromochloromethane to Group C: possible human carcinogen (IRIS, 1992). This classification reflects inadequate human data and limited evidence of carcinogenicity in animals.

Based on the 1996 Proposed Guidelines for Carcinogen Risk Assessment published in 1996 (U.S. EPA, 1996), dibromochloromethane is classified *as cannot be determined*. This descriptor is considered appropriate when there are no or inadequate data in humans, and limited evidence for carcinogenicity in animals.

IARC (1999c) has recently re-evaluated the carcinogenic potential of dibromochloromethane. IARC concluded that there is limited evidence of carcinogenicity in experimental animals and inadequate evidence in humans for dibromochloromethane. Dibromochloromethane is therefore classified as Group 3: not classifiable as to carcinogenicity in humans.

Categorization of Carcinogenic Potential Under the Proposed 1999 Cancer Guidelines

Cancer Hazard Summary

Under the proposed guidelines for Carcinogen Risk Assessment (U.S. EPA, 1999) dibromochloromethane shows *suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential*. This descriptor is appropriate when the evidence from human or animal data is suggestive of carcinogenicity, which raises a concern for carcinogenic effects but is not judged sufficient for a conclusion as to human carcinogenic potential. This finding is based on the weight of experimental evidence in animal models which indicate limited or equivocal evidence of carcinogenicity.

Supporting Information for Cancer Hazard Assessment

Human Data

The information on the carcinogenicity of dibromochloromethane from human studies is inadequate. There are no epidemiological data specifically relating increased incidence of cancer to exposure to dibromochloromethane. There are equivocal epidemiological data describing a weak association of chlorinated drinking water exposures with increased incidences of bladder, rectal, and colon cancer. U.S. EPA has determined that these studies cannot attribute the observed effects to a single compound, as chlorinated water contains numerous other disinfection byproducts that are potentially carcinogenic.

Animal Data

The carcinogenicity of dibromochloromethane in male and female animals has been investigated in a well-designed and conducted corn oil gavage study conducted in rats and mice, a dietary exposure study in rats, and a drinking water study in mice. No data are available on the carcinogenic potential of dibromochloromethane administered via the inhalation or dermal routes.

In the corn oil gavage study (NTP, 1985), the incidence of hepatocellular adenomas and carcinomas and combined adenomas and carcinomas was significantly increased in high-dose female mice and the incidence of hepatocellular adenomas was significantly increased in high-dose male mice. No evidence was observed for carcinogenicity in male or female rats under the experimental conditions employed. Voronin (1987) did not observe significant increases in mice treated with dibromochloromethane in drinking water for 104 weeks. Tobe et al. (1982) reported no increase in gross tumors in rats treated exposed to dibromochloromethane in the diet for two years.

Structural Analogue Data

Dibromochloromethane is structurally related to trihalomethanes that have shown varying degrees of carcinogenic potential in rodents. Chloroform, the most extensively characterized trihalomethane, is reported to be carcinogenic at high doses in several chronic

animal bioassays, with significant increases in the incidence of liver tumors in male and female mice and significant increases in the incidence of kidney tumors in male rats and mice (U.S. EPA, 2001a). The occurrence of tumors in animals exposed to chloroform is demonstrably species-, strain-, and gender-specific, and has only been observed under dose conditions that caused cytotoxicity and regenerative cell proliferation in the target organ. The cancer database for structurally-related brominated trihalomethanes is more limited, but includes well-conducted studies performed by the National Toxicology Program. In a two-year corn oil gavage study of bromoform, NTP (1989a) found clear evidence for carcinogenicity in female rats and some evidence of carcinogenicity based on occurrence of tumors of the large intestine. In a two-year corn oil gavage study of bromodichloromethane, NTP (1987) found clear evidence of carcinogenicity in male and female rats (tumors of the large intestine), male mice (kidney tumors), and female mice (liver tumors). In other bioassays, George et al. (2002) observed a significantly increased prevalence of neoplastic lesions in the liver of male rats at the lowest dose of bromodichloromethane administered in drinking water, but not at higher doses. Tumasonis et al. (1985) reported significantly increased incidences of hepatic neoplastic nodules, hepatic adenofibrosis, and lymphosarcoma in female rats exposed to bromodichloromethane in drinking water.

Other Key Data

Dibromochloromethane is formed as a byproduct of drinking water disinfection with chlorine. Exposure to dibromochloromethane may occur via ingestion of tap water, via dermal contact during showering or bathing, or by inhalation of dibromochloromethane volatilized during household activities. Absorption of single oral doses appears to be extensive. Dibromochloromethane is rapidly metabolized and eliminated predominately as expired volatiles, carbon dioxide, or carbon monoxide. Only a small amount (less than 10%) is eliminated in urine or in feces. No comprehensive tissue data are available regarding the bioaccumulation or retention of dibromochloromethane following repeated exposure. However, because of the rapid metabolism and excretion of dibromochloromethane, marked accumulation and retention is not expected.

Dibromochloromethane itself is not directly reactive with DNA. Metabolism to reactive species is a prerequisite for toxicity, as inferred from metabolic induction and inhibition studies. *In vitro* and *in vivo* studies of the mutagenic and genotoxic potential of bromodichloromethane have yielded both positive and negative results. Synthesis of the overall weight of evidence from these studies is complicated by the use of a variety of testing protocols, different strains of test organisms, different activating systems, different dose levels, different exposure methods (gas versus liquid) and, in some cases, problems due to evaporation of the test chemical. Study results for the mutagenicity of dibromochloromethane are mixed, and the overall evidence for mutagenicity of this chemical is judged to be inconclusive (U.S. EPA, 1994b). Recent studies conducted with strains of *Salmonella* that express rat theta-class glutathione *S*-transferase indicate that dibromochloromethane is mutagenic in this test system and suggest that mutagenicity is mediated by formation of a reactive glutathione conjugate.

Mode of Action

Limited or equivocal evidence has been obtained for the carcinogenic potential of dibromochloromethane. Data to support a primary mode of action for tumor development in the liver of mice exposed to dibromochloromethane are lacking. In the absence of such information, combined with an inconclusive weight-of-evidence evaluation for genotoxicity, the mode of action for tumor development is assumed to be a linear process. The processes leading to tumor formation in animals are expected to be relevant to humans.

Conclusion

Under the proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1999) dibromochloromethane shows *suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential* by the oral route. This weight-of-evidence evaluation is based on 1) limited or equivocal evidence of carcinogenicity in mice, but not rats, treated by oral pathways; 2) lack of epidemiological data specific to dibromochloromethane and equivocal data for drinking water drinking water exposures that cannot reliably be attributed to dibromochloromethane among multiple other disinfection byproducts; 3) inconclusive results for many of the available genotoxicity and mutagenicity tests; and 4) metabolism and mode of action that are reasonably expected to be similar to those of structurally-related compounds that induce tumors in experimental animals. Although no cancer data exist for exposures via the dermal or inhalation pathways, the weight-of-evidence conclusion is considered to be applicable to these pathways as well. The finding for inhalation is based on the observation that patterns of metabolizing enzyme activity in male rats for the related trihalomethane bromodichloromethane are similar for exposure via the inhalation and gavage routes. Dibromochloromethane absorbed through the skin is expected to be metabolized and cause toxicity in much the same way as dibromochloromethane absorbed by the oral and inhalation routes.

b. Choice of Study for Quantification of Carcinogenic Risk

The proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1999) do not indicate dose-response assessment for chemicals for which there is *suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential*. However, the single oral exposure study with positive tumor data for dibromochloromethane suggests significant cancer potency for this compound in mice. A quantitative assessment of potency was therefore considered appropriate.

In the absence of other carcinogenicity data, hepatic tumor incidence in female mice was selected for estimation of carcinogenic risks associated with dibromochloromethane. These data were obtained in an NTP (1985) study in which dibromochloromethane was administered in corn oil to male and female $B6C3F_1$ mice (50 mice/sex/dose) by gavage 5 times/week for 104 to 105 weeks. The administered doses were 0, 50, or 100 mg/kg-day. Survival of dosed female mice was comparable to that of the corresponding vehicle-control groups. High-dose male mice had lower survival rates than the vehicle controls. At week 82, nine high-dose male mice died of an unknown cause. An inadvertent overdose of dibromochloromethane given to low-dose male and female mice at week 58 killed 35 male mice, but apparently did not affect the female mice. The

low-dose male mouse group was, therefore, considered to be unsuitable for analysis of neoplasms. Compound-related nonneoplastic lesions were found in primarily in the livers of males (hepatocytomegaly, necrosis, fatty metamorphosis) and females (calcification and fatty metamorphosis). Nephrosis was also observed in male mice. Statistically significant increases in the incidence of hepatocellular adenomas and in the combined incidence of adenomas and carcinomas were observed in high-dose female mice. In male mice, a statistically significant increase in the incidence of hepatocellular carcinomas and combined adenomas and carcinomas was observed in the high-dose group; however, due to the overdose of dibromochloromethane in the mid-dose group, the authors considered the tumor incidence data inadequate for tumor analysis. Tumor incidence data from this study are presented in Table VIII-13.

c. Extrapolation model

The LMS model (U.S. EPA, 1986) and the default linear approach described by Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996; 1999) were used to quantify the risk associated with exposure to dibromochloromethane. Data for the mutagenicity and genotoxicity of dibromochloromethane are mixed (see Section V.F.2). U.S. EPA (1994b) has previously determined that the weight of evidence for dibromochloromethane mutagenicity and genotoxicity is inconclusive. At the present time there is insufficient evidence to establish with certainty that dibromochloromethane exerts its carcinogenic effects via a non-genotoxic mechanism. Thus, use of linear approaches was considered appropriate for quantification of cancer risk associated with exposure to this compound.

Table VIII-13 Frequencies of Liver Tumors in Mice Administered Dibromochloromethane in Corn Oil for 105 Weeks - Adapted from NTP (1985)

Treatment (mg/kg-day)	Sex	Adenoma	Carcinoma	Adenoma or Carcinoma (combined)
Vehicle Control	M F	14/50 2/50	10/50 4/50	23/50 6/50
50	M F	--[a] 4/49	-- 6/49	-- 10/49
100	M F	10/50 11/50[b]	19/50[b] 8/50	27/50[c] 19/50[d]

[a] Male low-dose group was inadequate for statistical analysis.
[b] $p < 0.05$ relative to controls.
[c] $p < 0.01$ (life table analysis); $p = 0.065$ (incidental tumor test) relative to controls.
[d] $p < 0.01$ relative to controls.

d. Cancer Potency and Unit Risk

The only tumor data available for dibromochloromethane are for liver tumors in female $B6C3F_1$ mice (NTP, 1985). NAS (1987) previously utilized the tumor frequency data reported by NTP (1985) to calculate an excess lifetime cancer unit risk of 8.3×10^{-7}. The linearized

multistage model was utilized, with the assumption that 1 L of water per day containing 1 μg/L of dibromochloromethane was ingested. Based on this calculation, the concentration associated with a risk of 10^{-6} is 0.6 μg/L, assuming consumption of 2 L of water per day.

Other available estimates of cancer risks are summarized in Table VIII-14. U.S. EPA (1994b) reported a slope factor of 8.4 x 10^{-2} $(mg/kg\text{-}day)^{-1}$ calculated from the NTP (1985) data in the absence of other appropriate tumorigenicity data for dibromochloromethane (IRIS, 1992). This value was derived using the LMS model (extra risk) and a scaling factor of body $weight^{2/3}$, as specified in the 1986 Guidelines for Carcinogenic Risk Assessment (U.S. EPA, 1986). The reported unit risk and 10^{-5} risk concentration were 2.4 x 10^{-6} $(\mu g/L)^{-1}$ and 4 μg/L, respectively.

A slope factor of 4.3 x 10^{-2} $(mg/kg\text{-}day)^{-1}$ (U.S. EPA, 1998b) was derived using the LMS model and a scaling factor of body $weight^{3/4}$. The use of body $weight^{3/4}$ as the scaling factor is consistent with recommendations in U.S. EPA. (1992b). A unit risk value of 1.2 × 10^{-6} $(\mu g/L)^{-1}$ was estimated using an assumed body weight of 70 kg and a drinking water ingestion rate of 2 L. This estimate was used to calculate a drinking water 10^{-5} risk concentration of 8 μg/L (0.8 μg/L at 10^{-6} risk).

Table VIII-14 Carcinogenic Risk Estimates for Dibromochloromethane

Method of Estimation	Tumor Site	Species	Sex	Slope Factor $(mg/kg\text{-}day)^{-1}$	Unit Risk $(\mu g/L)^{-1}$	10^{-5} Risk Conc. (μg/L)	LED_{10} (μg/kg-day)
LMS Method Using $BW^{3/4}$ Conversion U.S. EPA (1998b)	Liver	Mouse	F	4.3×10^{-2}	1.2×10^{-6}	8	-
LMS Method Using $BW^{2/3}$ Conversion U.S. EPA (1994b)*	Liver	Mouse	F	8.4×10^{-2}	2.4×10^{-6}	4	-
LED_{10}/Linear Method U.S. EPA (1998b)	Liver	Mouse	F	4.0×10^{-2}	$1.2x10^{-6}$	9	2.5×10^{3}

*Adapted from IRIS (1992)

Cancer risk estimates were also obtained using the LED_{10} (the lower 95% confidence limit on a dose associated with 10% extra risk) of 2.5 x 10^{3} μg/kg-day for hepatic tumors and assuming a linear mode of action for the carcinogenicity of dibromochloromethane (Table VIII-14). A cancer potency value of 4.0 x 10^{-2} $(mg/kg\text{-}day)^{-1}$ was derived using this approach. A unit risk of 1.2 x 10^{-6} $(\mu g/L)^{-1}$ was calculated using an assumed body weight of 70 kg and a drinking water ingestion rate of 2 L. This estimate was used to calculate a drinking water concentration of 9 μg/L associated with a 10^{-5} risk (0.9 μg/L for 10^{-6} risk). These values are similar to values derived using the LMS approach with body weight scaling to the 3/4 power.

The use of a corn oil vehicle in the NTP (1985) study from which these data are derived contributes uncertainty regarding the relevance of this value to exposure via drinking water. The U.S. EPA plans to seek data on the tumorigenicity of dibromochloromethane in water in order to clarify this issue.

C. Bromoform

1. Noncarcinogenic effects

a. One-day Health Advisory

Acute toxicity information on bromoform is limited and no data suitable for BMD modeling were identified. Some information is available on the former medicinal use of bromoform in humans. In the past, oral doses of bromoform were used as a sedative for children with whooping cough. Doses were typically one drop (approximately 180 mg) given three to six times per day (Burton-Fanning, 1901). This treatment usually resulted in mild sedation in children, although a few rare cases of death or near-death (believed to be due to accidental overdoses) have been reported (e.g., Dwelle, 1903; Benson, 1907). Based on a dose of 540 mg/day given to a 10-kg child, the LOAEL for mild sedation is about 54 mg/kg-day. Using these data, the one day-HA for bromoform is calculated according to the following equation:

$$\text{One-day HA} = \frac{(54\ \text{mg/kg-day})(10\ \text{kg})}{(100)\ (1\ \text{L/day})} = 5.4\ \text{mg/L (rounded to 5 mg/L)}$$

where:

54 mg/kg-day	=	LOAEL based on sedation in children given oral doses of bromoform
10 kg	=	Assumed weight of a child
100	=	Composite uncertainty factor based on NAS/OW guidelines. Includes a factor of 10 for interspecies variation and a factor of 10 for protection of sensitive human populations
1 L/day	=	Assumed water consumption of a 10-kg child

b. Ten-day health Advisory

Candidate studies considered for derivation of the Ten-day HA are summarized in Table VIII-15 (below). Condie et al. (1983) administered bromoform by gavage to male CD-1 mice at doses ranging from 72 to 289 mg/kg-day for 14 days and identified a NOAEL of 145 mg/kg-day and a LOAEL of 289 mg/kg-day. The LOAEL is based on changes in clinical chemistry and on minimal to moderate histologic changes in the kidney (intratubular mineralization, epithelial hyperplasia, and mesangial hypertrophy and nephrosis) and in the liver (centrilobular pallor, mitotic figures, focal inflammation, and cytoplasmic vacuolization). BMD modeling of data for renal mesangial hypertrophy calculated BMD and $BMDL_{10}$ values of 73 and 34 mg/kg-day, respectively.

Melnick et al. (1998) administered bromoform to female $B6C3F_1$ mice by gavage 5 days/week for 3 weeks and identified a NOAEL of 200 mg/kg-day (the lowest dose tested) and a LOAEL of 500 mg/kg-day based on histologic changes in the liver (hepatocyte hydropic degeneration). The duration-adjusted BMD and $BMDL_{10}$ values for this endpoint were 146 and 104 mg/kg-day, respectively.

Munson et al. (1982) identified NOAEL and LOAEL values of 125 and 250 mg/kg-day, respectively, based on elevated serum enzyme activity in mice. BMD modeling was not conducted for this endpoint, since it was not considered a reliable basis for the Ten-day HA in the absence of histopathological data. NTP (1989a) identified NOAEL and LOAEL values of 200 and 400 mg/kg-day, respectively, based on the occurrence of stomach nodules in rats and mice. A BMD of 167 mg/kg-day was calculated for this endpoint in mice, with a corresponding $BMDL_{10}$ of 66 mg/kg-day. However, occurrence of these nodules may represent a portal of entry effect. Chu et al. (1982a) identified a freestanding NOAEL of 80 mg/kg-day in a drinking water study conducted in rats. Coffin et al. (2000) identified a LOAEL of 200 mg/kg-day based on the occurrence of liver histopathology and increased labeling index. The data of Coffin et al. were not modeled because other studies used lower doses and were thus able to better characterize the low-dose portion of the dose response curve.

The study conducted by Aida et al. (1992a) assessed toxicity in Wistar rats administered bromoform microencapsulated in the diet at doses ranging from 56 to 728 mg/kg-day. The duration of the study was one month. This study identified a NOAEL of 56 mg/kg-day and a LOAEL of 208 mg/kg-day based on clinical chemistry changes and histologic changes in the liver (cell vacuolization and swelling) of females. BMD modeling of results for liver cell vacuolization in female rats calculated BMD and $BMDL_{10}$ values of 16 and 2.3 mg/kg-day, respectively. These were the lowest values observed among modeling results for candidate studies for the 10-day HA. On this basis, and because histopathological changes in the liver are considered a sensitive indicator of brominated trihalomethane toxicity, the study conducted by Aida et al. (1992a) was considered the best choice for derivation of the Ten-day HA.

Table VIII-15 Summary of Candidate Studies for Derivation of the Ten-day HA for Bromoform

Reference	Species Sex	n	Route	Dose	Exposure Duration	Endpoints	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	BMD (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Aida et al. (1992a)	Rat Wistar M, F	7	Feed	Males 0 62 187 618 Females 0 56 208 728	1 month	Body weight, clinical signs, serum biochemistry, hematology, histology	62 males 56 females	187 males 208 females (serum chemistry changes, liver histopathology)	Males 140 Females 16	Males 51 (Liver cell vacuolization) Females 2.3 (Liver cell vacuolization)
Chu et al. (1982a)	Rat SD M	10	Drinking water	0.7 8.5 80	28 days	Clinical signs, serum biochemistry, histology	80	--	Not modeled	--
Condie et al. (1983)	Mouse CD-1 M	8-16	Gavage (oil)	0 72 145 289	14 days	Serum enzymes, PAH uptake *in vitro*, histology	145	289 (elevated ALT, decreased PAH, liver and kidney histopathology)	73	34 (Renal mesangial nephrosis)
Melnick et al. (1998)	Mouse $B6C3F_1$ F	10	Gavage (oil)	0 200 500	3 weeks (5 d/wk)	Body and liver weights, serum chemistry, liver histology	200	500 (liver histopathology)	146*	104* (Liver hydropic degeneration)
Munson et al. (1982)	Mouse CD-1 M, F	6-12	Gavage (aqueous)	0 50 125 250	14 days	Body and organ weights, serum chemistry, hematology, immune function	125	250 (elevated serum enzymes)	Not modeled	--
NTP (1989a)	Mouse $B6C3F_1$ M	5	Gavage (oil)	0 50 100 200 400 600	14 days	Body weight, clinical signs, gross pathology	200	400 (stomach nodules)	167	66 (stomach nodules)

Table VIII-15 (cont.)

Reference	Species Sex	n	Route	Dose	Exposure Duration	Endpoints	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	BMD (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Coffin et al. (2000)	Mouse $B6C3F_1$ F	10	Gavage (oil)	0 200 500	11 days	Relative liver weight; liver histopathology; labeling index	--	200 (liver histopathology; labeling index)	Not modeled	--
NTP (1989a)	Rat F344/N M, F	5	Gavage (oil)	0 100 200 400 600 800	14 days	Body weight, clinical signs, gross pathology	200	400 (decreased body weight)	Not modeled	--
Ruddick et al. (1983)**	Rat SD F	14-15	Gavage (oil)	0 50 100 200	Gestation days 6-15	Body and organ weights; maternal serum chemistry; hematology, and histopathology; developmental parameters	50	100 (sternebral aberrations)	50	33 (sternebral aberrations)

* Duration-adjusted dose used to calculate BMD and $BMDL_{10}$

** Ruddick et al (1983) is included because it is a reproductive study.

Abbreviations: SD, Sprague-Dawley

Based on the $BMDL_{10}$ identified in the Aida et al. (1992a) study, the Ten-day HA for a 10-kg child is calculated according to the following equation:

$$\text{Ten-day HA} = \frac{(2.3 \text{ mg/kg-day})(10 \text{ kg})}{(100)\ (1 \text{ L/day})} = 0.23 \text{ mg/L (rounded to 0.2 mg/L)}$$

where:

2.3 mg/kg-day = $BMDL_{10}$ based on the occurrence of hepatic vacuolization in female rats exposed to bromoform in the diet for one month

10 kg = Assumed body weight of a child

100 = Uncertainty factor based on NAS/OW guidelines. Includes a factor of 10 for interspecies variation and a factor of 10 for protection of sensitive human populations

1 L/day = Assumed water consumption of a 10-kg child

When the $BMDL_{10}$ value for liver cell vacuolization in the Aida et al. (1992a) study is used, the Ten-day HA for a 10-kg child is calculated to be 0.2 mg/L, assuming a drinking water ingestion rate of 1 L/day and use of a composite uncertainty factor of 100. This value is slightly lower than the Longer-term HA for a 10 kg child of 0.3 mg/L derived using subchronic data for the same histopathological endpoint. This small difference may reflect experimental or BMD modeling uncertainty.

For purposes of comparison, the Ten-day HA may also be derived using the conventional NOAEL/LOAEL approach. The lowest LOAEL among the candidate studies was 100 mg/kg-day for developmental effects in rats (Ruddick et al., 1983). The NOAEL in this study was 50 mg/kg-day. Aida et al. (1992a) identified NOAEL values of 56 and 62 mg/kg-day and LOAEL values of 187 and 208 mg/kg-day for histopathological changes in male and female rats administered bromoform in the diet. Chu et al. (1982a) identified a freestanding NOAEL of 80 mg/kg-day in rats. The data of Aida et al. (1992a) were selected for calculation of the Ten-day HA because the study tested both male and female rats, incorporated more dose levels, and identified both NOAEL and LOAEL values and because the NOAEL identified by Chu et al. (1982a) is close to the lowest LOAEL of 100 mg/kg-day. Using the NOAEL of 62 mg/kg-day for male rats and assuming the default body weight for a child (10 kg), the default drinking water intake for a child (1 L/day), and a composite uncertainty factor of 100, the Ten-day HA would be 6.2 mg/L (rounded to 6 mg/L).

c. Longer-term Health Advisory

Candidate studies for derivation of the Longer-term HA are summarized in Table VIII-16 below. All studies identified histopathological changes in liver tissue as the critical toxicological effect. In one NTP (1989a) study, F344/N rats were administered bromoform by gavage at doses ranging from 12 to 200 mg/kg-day for 5 days/week for 13 weeks. This study identified a NOAEL of 25 mg/kg-day and a LOAEL of 50 mg/kg-day based on hepatic vacuolization observed in male rats. BMD modeling with the BMDS program calculated a duration-adjusted BMD of 4.4 mg/kg-day (based on duration-adjusted doses), with a corresponding $BMDL_{10}$ of 2.6 mg/kg-day. These values were the lowest among the candidate studies.

In an analogous subchronic oral exposure study, NTP (1989a) exposed mice of both sexes to doses of bromoform ranging from 25 to 400 mg/kg-day in addition to the control. This study identified NOAEL and LOAEL values of 100 and 200 mg/kg-day, respectively, based on hepatic vacuolization. BMD modeling with the BMDS program calculated a BMD of 88 mg/kg-day (based on duration-adjusted doses), with a corresponding $BMDL_{10}$ of 55 mg/kg-day. These values were approximately 20-fold higher than the BMD and $BMDL_{10}$ calculated for the NTP (1989a) oral exposure study in rats.

Chu et al. (1982b) exposed rats of both sexes to bromoform in the drinking water for 90 days. The doses of bromoform ranged from 0.64 to 283 mg/kg-day in addition to the control. This study identified NOAEL and LOAEL values of 57 and 218 mg/kg-day , respectively, based on decreased weight gain and mild hepatic lesions in male mice. BMD modeling identified BMD and $BMDL_{10}$ values of 10 and 5.9 mg/kg-day using data for occurrence of hepatic lesions in male mice. These values were approximately two-fold higher than the BMD and $BMDL_{10}$ values derived using data from the NTP (1989a) oral exposure study in rats. Strengths of this study include exposure via drinking water, larger sample size (20 animals/treatment group), and the administration of lower doses than used in the NTP (1989a) subchronic studies. Liver histopathology data from this study were reported as combined lesions, with the types of lesions described in the text.

The NTP (1989a) oral exposure study conducted in rats was selected for the derivation of the Longer-term HA on the basis of the low values obtained for the BMD and $BMDL_{10}$. Selection of this study is strongly supported by the results of Chu et al. (1982b), which identified slightly higher values in a drinking water study.

Table VIII-16 Summary of Candidate Studies for Derivation of the Longer-term HA for Bromoform

Reference	Species Sex	n	Route	Dose	Exposure duration	Endpoints	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	BMD (mg/kg-day)*	$BMDL_{10}$ (mg/kg-day) *
Chu et al. (1982b)	Rat SD M, F	20	Drinking water	Male 0 0.65 6.1 57 218 Females 0 0.64 6.9 55 283	90 days	Body weight, serum chemistry, histology	57	218 (decreased weight gain, mild hepatic lesions)	Male 10 Females No fit	Male 5.9 (Hepatic lesions) Females --
NTP (1989a)	Rat F344/N M, F	10	Gavage (corn oil)	0 12 25 50 100 200	13 weeks (5 d/wk)	Body weight, clinical signs, gross necropsy, histology	25	50 (hepatic vacuolization)	4.4	2.6 (hepatic vacuolization in male rats)
NTP (1989a)	Mouse $B6C3F_1$ M, F	10	Gavage (corn oil)	0 25 50 100 200 400	13 weeks (5 d/wk)	Body weight, clinical signs, gross necropsy, histology	100	200 (hepatic vacuolization)	88	55 (hepatic vacuolization in male mice)
Ruddick et al. (1983)**	Rat SD F	9-14	Gavage (corn oil)	0 50 100 200	Gestation days 6-15	Body and organ weights; maternal serum chemistry; hematology, and histopathology; developmental parameters	50	100 (sternebral aberrations)	50	33 (sternebral aberrations)
NTP (1989b)	Mouse ICR Swiss M, F	20	Gavage (corn oil)	0 50 100 200	105 days	Continuous breeding reprod. study. Body and organ weights, histopathology, reproductive parameters	100	200 (decreased maternal body weight)	- (not modeled)	-

* BMD and $BMDL_{10}$ calculated using duration-adjusted doses

** Ruddick et al (1983) is included because it is a reproductive study.

– Not modeled

Abbreviations: SD, Sprague-Dawley

Based on the $BMDL_{10}$ identified in the NTP (1989a) rat study, the Longer-term HA for a 10-kg child is calculated according to the following equation:

$$\text{Longer-term HA} = \frac{(2.6 \text{ mg/kg-day})(10 \text{ kg})}{(100)\ (1 \text{ L/day})} = 0.26 \text{ mg/L (rounded to 0.3 mg/L)}$$

where:

2.6 mg/kg-day = Duration-adjusted $BMDL_{10}$ based on the occurrence of hepatic vacuolization in male rats exposed to bromoform by gavage for 13 weeks

10 kg = Assumed body weight of a child

100 = Uncertainty factor based on NAS/OW guidelines; includes a factor of 10 for interspecies variation and a factor of 10 for protection of sensitive human populations

1 L/day = Assumed water consumption of a 10-kg child

The Longer-term HA for an adult consuming 2 liters of water per day is calculated according to the following equation:

$$\text{Longer-term HA} = \frac{(2.6 \text{ mg/kg-day})(70\text{kg})}{(100)\ (2 \text{ L/day})} = 0.91 \text{ mg/L (rounded to 0.9 mg/L)}$$

where:

2.6 mg/kg-day = Duration-adjusted $BMDL_{10}$ based on the occurrence of hepatic vacuolization in male rats exposed to bromoform by gavage for 13 weeks

70 kg = Assumed body weight of an adult

100 = Composite uncertainty factor based on NAS/OW guidelines; includes a factor of 10 for interspecies variation and a factor of 10 for protection of sensitive human populations

2 L/day = Assumed water consumption of a 70-kg adult

For purposes of comparison, the Longer-term Health Advisories may also be derived using the conventional NOAEL/LOAEL approach. The chronic oral exposure study conducted by NTP (1989a) identified a NOAEL of 25 mg/kg-day based on the absence of clinical signs or

histological alterations in rats exposed to bromoform for 13 weeks. Using this value and assuming default body weights (10 and 70 mg/kg-day, for adults and children, respectively), default drinking water intake rates (1 and 10 L/day for children and adults, respectively), a composite uncertainty factor of 100, and an exposure duration factor of 5/7, the Longer-term HAs for a child and an adult would be 2 mg/L and 6 mg/L, respectively.

d. Reference Dose, Drinking Water Equivalent Level and Lifetime Health Advisory

This section reports the existing RfD value for bromoform and describes the derivation of the RfD for this compound. This section also describes the calculation of Drinking Water Equivalent Level and Lifetime Health Advisory values which require the RfD as input. For this document, new and existing studies were reviewed and appropriate candidate data were selected for benchmark (BMD) dose modeling. The results of BMD modeling were used in conjunction with appropriate uncertainty factors to calculate the RfD. A comparison of the RfD derived using the BMD approach to the results obtained using the conventional NOAEL/LOAEL approach is also provided.

Description of the Existing RfD

The existing RfD for bromoform is 0.02 mg/kg-day (IRIS, 1993b). This value was derived using a duration-adjusted NOAEL of 17.9 mg/kg-day identified for the occurrence of hepatic lesions in F344 rats administered bromoform by corn oil gavage 5 days/week for 13 weeks (NTP, 1989a). An uncertainty factor of 1000 was used to account for extrapolation from animal data, for protection of sensitive human subpopulations, and for use of a subchronic study.

Identification of Candidate Studies for the Derivation of the RfD

Three chronic exposure studies, a subchronic exposure study, a prenatal developmental toxicity study, and a reproductive toxicity study were considered for derivation of the RfD for bromoform. These studies are summarized in Table VIII-17 (below).

Tobe et al. (1982) administered bromoform microencapsulated in the diet to Wistar rats at dose levels ranging from 22 to 619 mg/kg-day for 24 months. A NOAEL of 22 mg/kg-day and a LOAEL of 90 mg/kg-day were identified based on gross liver lesions and changes in clinical chemistry parameters in male rats.

NTP (1989a) conducted chronic oral exposure studies in rats and mice. In the rat study, animals were administered bromoform by gavage in oil for 5 days/week for 103 weeks at doses of 100 or 200 mg/kg-day. This study identified the low dose as the LOAEL based on histologic lesions in the liver (fatty change and chronic inflammation). In the mouse study, animals were administered bromoform by gavage in corn oil, 5 days/week for 103 weeks at doses of 50 or 100 mg/kg-day for male mice and 100 or 200 mg/kg-day for female mice. Although no treatment-related effects were observed in the male mice at the dose levels tested, treatment-related histologic lesions in the liver (fatty changes) were observed in both low- and high-dose females. Accordingly, this study identified a LOAEL of 100 mg/kg-day in female mice.

A subchronic oral exposure study conducted in rats (NTP, 1989a) was also considered as a candidate for derivation of the RfD. This study utilized five doses of bromoform ranging from 12 to 200 mg/kg-day in addition to the control. The compound was administered to ten animals per treatment group by gavage in corn oil, 5 days per week for 13 weeks. The endpoints evaluated included body weight, clinical signs, gross necropsy, and histological changes. This study identified a NOAEL and LOAEL of 25 and 50 mg/kg-day, respectively, on the basis of histopathological changes (vacuolization) in the liver. The LOAEL identified in this study was the lowest among all candidate studies.

The developmental study conducted by Ruddick et al. (1983) identified NOAEL and LOAEL values of 50 and 100 mg/kg-day, respectively, for sternebral variations in the offspring of female rats dosed with bromoform on gestation days 6-15. The reproductive toxicity study reported by NTP (1989b) identified NOAEL and LOAEL values of 100 and 200 mg/kg-day for reduced maternal body weight and decreased postnatal survival and liver histopathology in F_1 mice of both sexes.

Table VIII-17 Summary of Candidate Studies for Derivation of the RfD for Bromoform

Reference	Species Sex	n	Dose	Route	Exposure Duration	Endpoints	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	BMD (mg/kg-day)	$BMDL_{10}$ (mg/kg-day) *
Tobe et al. (1982)	Rat Wistar M, F	40	Male 0 22 90 364 Female 0 38 152 619	Diet	24 months	Body weight, serum chemistry, gross pathology	22	90 (serum chemistry changes, gross liver lesions)	Not modeled	--
NTP (1989a)	Rat F344/N M, F	10	0 12 25 50 100 200	Gavage (corn oil)	13 weeks (5 days/wk)	Body weight, clinical signs, gross necropsy, histology	25	50 (hepatic vacuolization)	4.4	2.6 (Hepatic vacuolization in male rats)
NTP (1989a)	Rat F344/N M, F	50	0 100 200	Gavage (corn oil)	103 weeks (5 d/wk)	Body weight, clinical signs, gross necropsy, histology	--	100 (decreased body weight, lethargy, mild liver histopathology)	13	1.4 (fatty changes in liver of males)
NTP (1989a)	Mouse $B6C3F_1$ M, F	50	Male 0 50 100 Female 0 100 200	Gavage (corn oil)	103 weeks (5 d/wk)	Body weight, clinical signs, gross necropsy, histology	100 (male)	100 (female) (decreased body weight, mild liver histopathology)	14.2†	10.6† (fatty changes in liver of females)
Ruddick et al. (1983)‡	Rat SD F	9-14	0 50 100 200	Gavage (con oil)	Gestation days 6-15	Developmental toxicity study; body and organ weights	50 (developmental) 200 (maternal)	100 (sternebral variations)	50	33 (sternebral variations)

Reference	Species Sex	n	Dose	Route	Exposure Duration	Endpoints	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	BMD (mg/kg-day)	$BMDL_{10}$ (mg/kg-day) *
NTP (1989b)‡	Mouse $B6C3F_1$ M, F	50	0 50 100 200	Gavage (corn oil)	105 days	Continuous breeding reprod. study. Body and organ weights, histopathology, reproductive parameters	100	200 (decreased maternal body weight; reduced postnatal survival and liver histopathology in F_1 generation males and females)	- (not modeled)	-

* BMD and $BMDL_{10}$ values were derived using duration-adjusted doses.
† BMD modeled using the Crump BMD software (K. S. Crump, Inc.)
‡ These studies are included because they are reproductive/developmental studies.
Abbreviations: SD, Sprague-Dawley

Method of Analysis

Selected data from the candidate studies were analyzed using the benchmark dose (BMD) modeling approach. Initially, data sets for potentially sensitive endpoints were selected as described in U.S. EPA (1998b) and analyzed using the Crump Benchmark Dose Modeling Software (K. S. Crump, Inc.). Following the release of Version 1.2 of the BMDS program (U.S. EPA, 2000a), data from the NTP (1989a) subchronic and chronic studies conducted in rats were reanalyzed in accordance with proposed U.S. EPA (2000b) recommendations. An advantage of analysis with the BMDS software is that several additional models are available to fit the data. The results of the analysis using the BMDS software are included in Table VIII-17.

Choice of Principal Study and Critical Effect for the RfD

The subchronic study conducted by NTP (1989a) was selected for derivation of the RfD. Two factors supported selection of this study. First, the critical effect identified in the subchronic study was consistent with the critical effects identified in the chronic NTP studies (fatty changes in liver of male rats and female mice). Second, BMD modeling of both data sets supported selection of the subchronic study. BMD analysis using the BMDS program calculated a BMD of 13 mg/kg-day for fatty changes in the liver of males in the chronic study, with a corresponding $BMDL_{10}$ of 1.4 mg/kg-day. The lowest duration-adjusted dose in this study was 71 mg/kg-day and the response at this dose was high (49/50). The magnitude of the difference between the BMD and $BMDL_{10}$ values thus reflects considerable uncertainty about the shape of the curve in the low-dose region.

Duration-adjusted BMD and $BMDL_{10}$ values for hepatic vacuolization in male rats of 4.4 and 2.6 mg/kg-day, respectively, were obtained using data for the subchronic study. This BMD is approximately three-fold less than the BMD calculated from chronic data (above). The availability of response data for three doses below 71 mg/kg-day (duration-adjusted dose) in the subchronic study provided additional information about the shape of the dose-response curve in the region of interest, and thus a more reliable estimate of the BMD. Although the $BMDL_{10}$ value for the subchronic study is higher than the value for the chronic study, this observation reflects less uncertainty (smaller confidence interval) in the estimate of the subchronic BMD when the results for the two studies are compared. The $BMDL_{10}$ value calculated from the subchronic NTP (1987) data was therefore selected for derivation of the RfD for bromoform.

The remaining studies were eliminated from consideration for the following reasons. The study conducted by Tobe et al. (1982) did not identify a suitably sensitive endpoint (histopathological examination was not conducted) and the data were never formally published or submitted for peer review. The chronic study conducted by NTP (1989a) in mice reported mild histopathological changes in female mice at a duration-adjusted dose of 71 mg/kg-day, the lowest tested in this gender. However, both the BMD and $BMDL_{10}$ were higher than those identified in the subchronic study in rats, and these values were considered less reliable in the absence of data at lower doses. The LOAELs identified in the developmental (Ruddick et al., 1983) and reproductive (NTP, 1989a) studies were higher than the LOAEL values observed for histopathology effects in the NTP subchronic study. The BMD and $BMDL_{10}$ calculated for sternebral variations in the Ruddick et al. (1983) were approximately 10-fold higher than those identified in the subchronic study.

Derivation of the RfD

The duration-adjusted $BMDL_{10}$ from the subchronic NTP (1989a) rat study was selected for derivation of the RfD for bromoform. The RfD is calculated using the following equation:

$$RfD = \frac{(2.6 \text{ mg/kg-day})}{100} = 0.03 \text{ mg/kg-day}$$

where:

2.6 mg/kg-day = Duration-adjusted $BMDL_{10}$ based on hepatocellular vacuolization in the liver of male rats

100 = Composite uncertainty factor based on NAS/OW guidelines; includes a factor of 10 for interspecies variation, a factor of 10 for protection of sensitive human populations

A composite uncertainty factor of 100 was used in the calculation of the bromoform RfD. The standard factors of 10 were used for interspecies extrapolation and for protection of sensitive subpopulations. No uncertainty factor was added for extrapolation from a subchronic to a chronic study because the BMD and $BMDL_{10}$ for the subchronic study was either comparable to or lower than the corresponding values from the chronic study. This observation suggests that a cumulative effect on the liver does not occur for the endpoints examined. The database for bromoform includes subchronic and chronic bioassays conducted in rats and mice (e.g. NTP 1989a), a two-generation reproductive toxicity study in mice (NTP 1989b), and a developmental toxicity study in rats (Ruddick et al. 1983). Therefore, the database for bromoform was considered sufficient and an uncertainty factor for database deficiencies was not included in the calculation.

The DWEL for bromoform is calculated as follows:

$$DWEL = \frac{(0.03 \text{ mg/kg-day}) (70 \text{ kg})}{2 \text{ L/day}} = 1.0 \text{ mg/L } (1000 \text{ } \mu\text{g/L})$$

where:

0.03 mg/kg-day = RfD

70 kg = assumed weight of an adult

2 L/day = assumed water consumption by a 70-kg adult

Lifetime Health Advisory

The Lifetime Health Advisory (HA) represents that portion of an individual's total exposure that is attributed to drinking water and is considered protective of noncarcinogenic health effects over a lifetime of exposure. Bromoform has been categorized with respect to carcinogenic potential as Group B2: Probable human carcinogen (IRIS, 1993b). Therefore, in accordance with U.S. EPA Policy, a Lifetime Health Advisory is not recommended.

Alternative Approach for Derivation of the RfD

For comparison, the RfD can be calculated using the conventional NOAEL/LOAEL approach. The subchronic NTP (1989a) oral exposure study identified a NOAEL of 25 mg/kg-day based on absence of histopathological effects in rats exposed to bromoform by gavage in corn oil for 13 weeks. Using this value, a duration adjustment factor of 5/7, and an uncertainty factor of 1000 (including factors of 10 for interspecies extrapolation, protection of sensitive subpopulations, and use of a subchronic study), the RfD would be 0.02 mg/kg-day. The corresponding DWEL would be 0.7 mg/L assuming an adult body weight of 70 kg and a drinking water ingestion rate of 2 L/day.

2. Carcinogenic Effects

a. Categorization of Carcinogenic Potential

Previous Evaluations of Carcinogenic Potential

The Carcinogenic Risk Assessment Verification Endeavor (CRAVE) group of the U.S. EPA has reviewed the available evidence on the carcinogenicity of bromoform and has assigned it to Group B2: probable human carcinogen (IRIS, 1993b). Assignment to this category is appropriate for chemicals where there are no or inadequate human data, but which have sufficient animal data to indicate carcinogenic potential.

IARC (1999b) has recently re-evaluated the carcinogenic potential of bromoform. IARC concluded that there is limited evidence of carcinogenicity in experimental animals and inadequate evidence in humans for bromoform. Bromoform is therefore categorized as Group 3: not classifiable as to carcinogenicity in humans.

Categorization of Carcinogenic Potential Under the Proposed 1999 Cancer Guidelines

Cancer Hazard Summary

Under the proposed guidelines for Carcinogen Risk Assessment (U.S. EPA, 1999) bromoform is *likely to be carcinogenic to humans* by all routes of exposure. This descriptor is appropriate when the available tumor data and other key data are adequate to demonstrate carcinogenic potential to humans. This finding is based on the weight of experimental evidence in animal models which shows carcinogenicity by modes of action that are relevant to humans.

Supporting Information for Cancer Hazard Assessment

Human Data

The information on the carcinogenicity of bromoform from human studies is inadequate. There are no epidemiological data specifically relating increased incidence of cancer to exposure to bromoform. There are equivocal epidemiological data describing a weak association of chlorinated drinking water exposures with increased incidences of bladder, rectal, and colon cancer. U.S. EPA has determined that these studies cannot attribute the observed effects to a single compound, as chlorinated water contains numerous other disinfection byproducts that are potentially carcinogenic.

Animal Data

The carcinogenicity of bromoform has been investigated in two species. These studies include a well-designed and conducted corn oil gavage study conducted in rats and mice and a study in which male Strain A mice were administered bromoform by intraperitoneal injection. No data are available on the carcinogenic potential of bromoform administered via the inhalation or dermal routes.

In the corn oil gavage study (NTP, 1989a), neoplasms of the large intestine (adenomatous polyps or adenocarcinoma) were observed in male and female rats. The response for combined adenoma and carcinoma reached statistical significance in female rats. The occurrence of tumors of the large intestine in this study was considered biologically significant because they are historically rare in rats. NTP (1989a) concluded that there was clear evidence for carcinogenicity in females and some evidence of carcinogenicity in males. No evidence of bromoform carcinogenicity was observed in male or female mice. Intraperitoneal injection of Strain A mice with three concentrations of bromoform resulted in significantly increased tumor incidence only at the middle dose tested.

Structural Analogue Data

Trihalomethanes structurally related to bromoform have shown varying degrees of carcinogenic potential in rodents. Chloroform, the most extensively characterized trihalomethane, is reported to be carcinogenic at high doses in several chronic animal bioassays, with significant increases in the incidence of liver tumors in male and female mice and significant increases in the incidence of kidney tumors in male rats and mice (U.S. EPA, 2001). The occurrence of tumors in animals exposed to chloroform is demonstrably species-, strain-, and gender-specific, and has only been observed under dose conditions that caused cytotoxicity and regenerative cell proliferation in the target organ. The cancer database for structurally-related brominated trihalomethanes is more limited, but includes well-conducted studies performed by the National Toxicology Program. In a two-year corn oil gavage study of bromodichloromethane, NTP (1987) found clear evidence for carcinogenicity in male and female rats (large intestine and kidney) and male (kidney) and female (liver) mice. Tumasonis et al. (1987) reported a statistically significant increase in the incidence of hepatic neoplastic nodules in rats exposed to bromodichloromethane in the drinking water. In a two-year corn oil gavage study of dibromochloromethane, NTP (1985) determined that there was some evidence

of carcinogenicity in female mice and equivocal evidence of carcinogenicity in male mice, based on the occurrence of hepatocellular adenomas and carcinomas. George et al. (2002) observed a significantly increased prevalence of hepatocellular tumors only at the lowest dose administered in a drinking water study. Other oral exposure studies found no evidence for carcinogenicity of bromodichloromethane (Aida et al., 1992b) or dibromochloromethane (Tobe et al., 1982; Voronin et al., 1987).

Other Key Data

Bromoform is formed as a byproduct of drinking water disinfection with chlorine. Exposure to bromoform may occur via ingestion of tap water, via dermal contact during showering or bathing, or by inhalation of bromoform volatilized during household activities. Absorption of single oral doses appears to be extensive. Bromoform is rapidly metabolized and eliminated predominately as expired volatiles, carbon monoxide, or carbon dioxide. Only a small amount (less than 10%) is eliminated in urine or in feces. No comprehensive tissue data are available regarding the bioaccumulation or retention of bromoform following repeated exposure. However, because of the rapid metabolism and excretion of bromoform, marked accumulation and retention is not expected.

Bromoform itself is not directly reactive with DNA. Metabolism to reactive species is a prerequisite for toxicity, as inferred from metabolic induction and inhibition studies. *In vitro* and *in vivo* studies of the mutagenic and genotoxic potential of bromoform have yielded both positive and negative results. Synthesis of the overall weight of evidence from these studies is complicated by the use of a variety of testing protocols, different strains of test organisms, different activating systems, different dose levels, different exposure methods (gas versus liquid) and, in some cases, problems due to evaporation of the test chemical. However, because a majority of studies yielded positive results, bromoform is considered to be at least weakly mutagenic and genotoxic. Recent studies conducted with strains of *Salmonella* thatexpress rat theta-class glutathione *S*-transferase suggest that mutagenicity of the brominated trihalomethanes may be mediated by glutathione conjugation.

Mode of Action

The mode of action for tumor induction by bromoform has not been clearly elucidated and may involve contributions from multiple bioactivation pathways. In each case, toxicity is believed to result from interaction of reactive metabolites with cellular macromolecules. Proposed bioactivation pathways for bromoform include: 1) production of reactive dihalocarbonyls by oxidative metabolism; 2) production of reactive dihalomethyl radicals by oxidative metabolism; and 3) formation of DNA-reactive species via a glutathione-dependent pathway. The relative contribution of each pathway to tumor induction by bromoform has not been characterized. It is possible that only the latter two processes lead to DNA damage *in vivo*, because the highly reactive dihalocarbonyl intermediate may not survive long enough to enter the nucleus and react with DNA. For this reason, cytotoxicity may be the primary consequence of the oxidative pathway. Cytotoxicity coupled with regenerative hyperplasia is considered the primary mode of action for tumor formation following exposure to high concentrations of chloroform, a structurally-related trihalomethane which has low genotoxic potential. Data to support a similar primary mode of action for tumor development in liver, kidney, and large

intestine are currently lacking for bromoform. In the absence of such information, combined with a positive weight-of-evidence evaluation for genotoxicity, the mode of action for tumor development is assumed to be a linear process. The processes leading to tumor formation in animals are expected to be relevant to humans.

Conclusion

Under the proposed guidelines for Carcinogen Risk Assessment (U.S. EPA, 1999) bromoform is *likely to be carcinogenic to humans* by the oral route. This weight-of-evidence evaluation is based on 1) observations of tumors in rats treated by oral pathways; 2) lack of epidemiological data specific to bromoform and equivocal data for drinking water drinking water exposures that cannot reliably be attributed to bromoform among multiple other disinfection byproducts; 3) positive results for a majority of the available genotoxicity and mutagenicity tests; and 4) metabolism and mode of action that are reasonably expected to be comparable across species. Although no cancer data exist for exposures via the dermal or inhalation pathways, the weight-of-evidence conclusion is considered to be applicable to these pathways as well. The finding for inhalation is based on the observation that patterns of metabolizing enzyme activity in male rats are similar following exposure to a structurally-related compound (bromodichloromethane) via the inhalation and gavage routes. Bromoform absorbed through the skin is expected to be metabolized and cause toxicity in much the same way as bromoform absorbed by the oral and inhalation routes.

b. Choice of Study for Quantification of Carcinogenic Risk

A single oral exposure study was available for the quantification of carcinogenic risk associated with oral exposure to bromoform. NTP (1989a) conducted an oral exposure study in $B6C3F_1$ mice and F344/N rats. No evidence of carcinogenicity was observed in male $B6C3F_1$ mice exposed to bromoform via gavage (corn oil) at doses up to 100 mg/kg-day, or in female mice exposed at doses up to 200 mg/kg-day for 5 days/week. Male and female F344/N rats (50 rats/sex/dose) were administered bromoform via gavage at doses of 0, 100, or 200 mg/kg-day for 5 days/week for 103 weeks (NTP 1989a). At termination, all animals were necropsied, and a thorough histological examination of tissues was performed. Adenomatous polyps or adenocarcinomas of the large intestine were noted in three high-dose male rats, eight high-dose female rats, and one low-dose female rat (Table VIII-18). Despite the small number of tumors found, the increase was considered biologically significant because these tumors are historically rare in the rat. The study authors concluded that there was some evidence for carcinogenic activity in male rats and clear evidence in female rats.

c. Extrapolation model

The LMS model (U.S. EPA, 1986) and the default linear approach described by the Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996; 1999) were used to quantify the risk associated with exposure to bromoform. Although data are mixed , U.S. EPA has previously concluded that the weight of evidence suggests that bromoform is mutagenic (see Section V.F.3). At the present time, there are no data which indicate that bromoform-induced

tumorigenesis occurs as a consequence of cytotoxicity followed by regenerative hyperplasia. Thus, use of a linear approach was considered appropriate for quantification of cancer risk associated with exposure to bromodichloromethane.

Table VIII-18 Tumor Frequencies in Rats Exposed to Bromoform in Corn Oil for 2 Years - Adapted from NTP (1989a)

Animal	Tissue/Tumor		Tumor Frequency		
			Control	100 mg/kg	200 mg/kg
Male rat	Large intestine	Adenocarcinoma	0/50	0/50	1/50
		Polyp (adenomatous)	0/50	0/50	2/50
Female rat	Large intestine	Adenocarcinoma	0/48	0/50	2/50
		Polyp (adenomatous)	0/48	1/50	6/50

d. Cancer Potency and Unit Risk

Estimates of cancer risk associated with exposure to bromoform are summarized in Table VIII-19. U.S. EPA (1994b) reported a cancer potency estimate of 7.9×10^{-3} $(mg/kg\text{-}day)^{-1}$ for bromoform based on the incidence of intestinal tumors in rats and derived using recommendations in the 1986 Cancer Guidelines (U.S. EPA, 1986). The calculated value for unit risk is 2.3×10^{-7} $(\mu g/L)^{-1}$. This estimate was used to calculate a drinking water concentration of 40 μg/L associated with a 10^{-5} risk.

A cancer potency estimate of 4.6×10^{-3} $(mg/kg\text{-}day)^{-1}$ (U.S. EPA, 1998b) based on the incidence of intestinal tumors in rats was calculated using the LMS model and a scaling factor of body weight$^{3/4}$. Use of this scaling factor is consistent with recommendations in U.S. EPA (1992b). Unit risk was estimated for bromoform using an assumed body weight of 70 kg and a drinking water ingestion rate of 2 L. The calculated value for unit risk is 1.30×10^{-7} $(\mu g/L)^{-1}$. This estimate was used to calculate a drinking water concentration of 77μg/L associated with a 10^{-5} risk (8 μg/L for a risk of 10^{-6}).

Cancer risk estimates were also obtained using the LED_{10} (the lower 95% confidence limit on a dose associated with 10% extra risk) of 2.2×10^{4} μg/kg-day for intestinal tumors and assuming a linear mode of action for the carcinogenicity of bromoform (Table VIII-19). A cancer potency value of 4.5×10^{-3} $(mg/kg\text{-}day)^{-1}$ was derived using this approach. A unit risk of 1.3×10^{-7} $(\mu g/L)^{-1}$ was calculated using an assumed body weight of 70 kg and a drinking water ingestion rate of 2 L. This estimate was used to calculate a drinking water concentration of 78 μg/L associated with a 10^{-5} risk (8 μg/L for 10^{-6} risk). These values are similar to values derived using the LMS approach with body weight scaling to the 3/4 power.

There are no data to suggest that tumor incidence in the large intestine is influenced by the use of an oil vehicle. Therefore, the risk estimates reported above are believed to be applicable to drinking water exposures.

Table VIII-19 Carcinogenic Risk Estimates for Bromoform

Method of Estimation	Tumor Site	Species	Sex	Slope Factor $(mg/kg\text{-}day)^{-1}$	Unit Risk $(\mu g/L)^{-1}$	LED_{10} (µg/kg-day)	10^{-5} Risk Concentration (µg/L)
LMS Method Using $BW^{3/4}$ Conversion U.S. EPA (1998b)	Large intestine	Rat	F	4.6×10^{-3}	1.3×10^{-7}	-	77
U.S. EPA (1994b)*	Large intestine	Rat	F	7.9×10^{-3}	2.3×10^{-7}	-	40
LED_{10}/Linear Method U.S. EPA (1998b)	Large intestine	Rat	F	4.5×10^{-3}	1.3×10^{-7}	2.2×10^{4}	78

* Adapted from IRIS (1993b)

D. Summary

Table VIII-20 Summary of Advisory Values for Bromodichloromethane, Dibromochloromethane, and Bromoform

Advisory	Value	Reference
Bromodichloromethane		
One-day HA for 10-kg child	1 mg/L	Narotsky et al. (1997)
Ten-day HA for 10-kg child	0.6 mg/L	NTP (1998)
Longer-term HA for 10-kg child	0.6 mg/L	CCC (2000d)
Longer-term HA for 70-kg adult	2 mg/L	CCC (2000d)
RfD	0.003 mg/kg-day	Aida et al. (1992b)
DWEL	100 µg/L	Aida et al. (1992b)
Lifetime HA	Not applicable	--
Oral Slope Factor [c]	3.5×10^{-2} $(mg/kg\text{-}day)^{-1}$	NTP (1987)
Concentration for excess cancer risk (10^{-6})	1.0 µg/L	NTP (1987)
Unit Risk	1.0×10^{-6} $(\mu g/L)^{-1}$	NTP (1987)
Dibromochloromethane		
One-day HA for 10-kg child [b]	0.6 mg/L	Aida et al. (1992a)
Ten-day HA for 10-kg child	0.6 mg/L	Aida et al. (1992a)
Longer-term HA for 10-kg child	0.2 mg/L	NTP (1985)
Longer-term HA for 70-kg adult	0.6 mg/L	NTP (1985)
RfD	0.02 mg/kg-day	NTP (1985)
DWEL	700 µg/L	NTP (1985)
Lifetime HA	60 µg/L	NTP (1985)
Oral Slope Factor [c]	4.3×10^{-2} $(mg/kg\text{-}day)^{-1}$	NTP (1985)
Concentration for Excess cancer risk (10^{-6})	0.8 µg/L	NTP (1985)
Unit Risk	1.2×10^{-6} $(\mu g/L)^{-1}$	NTP (1985)
Bromoform		
One-day HA for 10-kg child	5 mg/L	Burton-Fanning (1901)
Ten-day HA for 10-kg child	0.2 mg/L	NTP (1989a)
Longer-term HA for 10-kg child [a]	0.2 mg/L	NTP (1989a)
Longer-term HA for 70-kg adult	0.9 mg/L	NTP (1989a)

Advisory	Value	Reference
RfD	0.03 mg/kg-day	NTP (1989a)
DWEL	1000 µg/L	NTP (1989a)
Lifetime HA	Not applicable	--
Oral Slope Factor [c]	4.56×10^{-3} $(mg/kg\text{-}day)^{-1}$	NTP (1989a)
Concentration for Excess cancer risk (10^{-6})	8 µg/L	NTP (1989a)
Unit Risk	1.3×10^{-7} $(\mu g/L)^{-1}$	NTP (1989a)

[a] The calculated value for the Longer-term HA was slightly higher than the values for the Ten-day HA. Therefore, use of the Ten-day HA for a 10-kg child is recommended as an estimate of the Longer-term HA for a 10-kg child.

[b] Use of the Ten-day HA recommended as a conservative estimate of the One-day HA for a 10-kg child.

[c] Use of the Longer-term HA recommended as a conservative estimate of the Ten-day HA for a 10-kg child.

[d] The oral slope factor was calculated using the Linearized Multistage model and an animal-to-human scaling factor of body weight$^{3/4}$

Abbreviations: BW = Body weight; DWEL = Drinking water exposure limit; HA = Health advisory; LMS = Linearized Multistage Model

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APPENDIX A

BENCHMARK DOSE MODELING OF HEALTH EFFECTS ENDPOINTS FOR THE BROMINATED TRIHALOMETHANES: BROMODICHLOROMETHANE, DIBROMOCHLOROMETHANE, AND BROMOFORM

A. INTRODUCTION

The limitations of the NOAEL/LOAEL approach as the basis for estimating thresholds of toxic effect are well-documented (e.g., U.S. EPA, 1995, 2001b). These limitations include:

1) the slope of the dose-response plays little role in determining the NOAEL;

2) the NOAEL (or LOAEL) is limited to the doses tested experimentally;

3) the determination of the NOAEL is based on scientific judgement, and is subject to inconsistency;

4) experiments using fewer animals tend to produce larger NOAELs, and as a result may produce larger health advisories (HAs) or reference doses (RfDs) (U.S. EPA, 1995, 2001b) that may not be sufficiently protective of human health.

In contrast, benchmark doses (BMDs) are not limited to the experimental doses, appropriately reflect the sample size, and can be defined in a statistically consistent manner. In light of these considerations, it is becoming common practice to conduct assessments by performing BMD modeling for key endpoints, in addition to identification of NOAELs and LOAELs.

This document describes the analysis of the data relevant to the development of the One-day, Ten-day, and Longer-term Health Advisories (HAs) for bromodichloromethane (BDCM), dibromochloromethane (DBCM), and bromoform. Available data of appropriate duration were analyzed and the implications of the calculated benchmark doses for the development of HAs were considered. Comparisons of the resulting health advisories with existing values are also made. Developmental and reproductive toxicity studies were also considered when effects were seen at doses comparable to or lower than those causing systemic toxicity in subchronic or chronic studies. The data modeled in support of HA development were also used in derivation of the reference doses for the three brominated trihalomethanes.

B. SELECTION OF STUDIES AND ENDPOINTS FOR MODELING

The large number of candidate data sets for BMD modeling -required development of a data prioritization system. The available studies were first reviewed for endpoints and data sets appropriate for BMD modeling. Priority for modeling was given to those endpoints that showed the greatest toxicological relevance (e.g., developmental/reproductive endpoints, target organ histopathology) and ease of interpretation. Ease of interpretation refers to the ability to characterize the response as adverse and to translate this into an appropriate response level for input into the BMDS program. In addition, endpoints for which the LOAEL was less than ten times the lowest LOAEL observed in the category (e.g., 1-day, 10-day, and Longer term HAs, RfD etc.) were given priority for modeling. Data considered for BMD and general criteria for selection are listed in Tables A-1, A-2, and A-3.

Table A-1 Candidate Studies and Data for BMD Modeling - Bromodichloromethane

Reference	Species Sex	Route	n	Doses	Exposure Duration	Candidate Endpoints	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	L<10*LL†	Ease of Interp/Toxicologic Relevance	BMD model ?	Comments
Candidate Studies for Derivation of the One-day HA												
Lilly et al. (1994)	Rat M	Gavage (oil)	6	0 200 400	Single Dose	Kidney wt	200	400	Yes	Low	No	Model data for aqueous vehicle from this study (see below)
						Rel. kidney wt.	200	400	Yes	Low	No	
						Serum & urine chem : Serum AST, LDH, ALT, Creatinine, BUN Urine pH, osmolality	200	400	Yes	Generally low	No	
						Kidney histopath minimal renal tubule degeneration and necrosis	--	200	Yes	High	No	
						Liver histopath minimal vacuolar degeneration	200	400	Yes	High	No	

Table A-1 Candidate Studies and Data for BMD Modeling - Bromodichloromethane (cont.)

Reference	Species Sex	Route	n	Doses	Exposure Duration	Candidate Endpoints	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	L<10*LL†	Ease of Interp/Toxicologic Relevance	BMD model ?	Comments
Lilly et al. (1994)	Rat M	Gavage (aqueous)	6	0 200 400	Single Dose	Body wt.	200	400	Yes	Moderate	No	Model midzonal vacuolar degeneration (48 hr) and renal tubule degeneration (48 hr) Table 2, p.135 Model SDH as test
						Liver wt	–	200	Yes	Low	No	
						Rel. liver wt.	–	200	Yes	Low	No	
						Kidney weights	200	400	Yes	Low	No	
						Rel kidney wt	–	400 NS	Yes	Low	No	
						Serum & urine chem Serum AST, LDH, ALT, Creatinine, BUN, urine pH, osmolality	200	400	Yes	Generally low	No	
						Kidney histopath	--	200	Yes	High	Yes	
						Liver histopath	200	400	Yes	High	Yes	

Table A-1 Candidate Studies and Data for BMD Modeling - Bromodichloromethane (cont.)

Reference	Species Sex	Route	n	Doses	Exposure Duration	Candidate Endpoints	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	L<10*LL†	Ease of Interp/Toxicologic Relevance	BMD model ?	Comments
Lilly et al. (1997)	Rat M	Gavage (aqueous)	5	0 123 164 246 328 492	Single Dose	Body wt 48 hr post	328	492 (for ≥10%)	Yes	Low	No	Model SDH 24 hr post as test
						Liver wt 48 hr post	164	246	Yes	Low	No	
						Rel liver wt 48 hr post	328	492	Yes	Low	No	
						Kidney wt 24 hr post	246	328	Yes	Low	No	
						Rel kidney 24 hr post	164	246	Yes	Low	No	
						Rel kidney 48 hr post	328	492	Yes	Low	No	
						Serum / urine chemist SDH 24 hr post	--	123	Yes	Moderate	No	
Keegan et al. (1998)	Rat	Gavage (aqueous)		0 21 31 41 82 123 164 246	Single Dose	Body wt	82	123	Yes	Moderate	No	Test model SDH-24 hrs control group means in Table 2 as test
						Liver wt.	41	82	Yes	Low	No	
						Rel kidney wt	123	164	Yes	Low	No	
						Serum chemistry (elevated ALT, AST, and SDH activities)	41	82	Yes	Moderate	No	

Table A-1 Candidate Studies and Data for BMD Modeling - Bromodichloromethane (cont.)

Reference	Species Sex	Route	n	Doses	Exposure Duration	Candidate Endpoints	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	L<10*LL†	Ease of Interp/Toxicologic Relevance	BMD model ?	Comments
French et al. (1999)	Rat F	Gavage (aqueous)	3-6	0 (water) 0 (emulph) 75 150 300	5 days	Body wt.	150	300 (FEL)	Yes	Low (at FEL)	No	Most effects occurred at FEL PHA is sig. effect at dose below FEL. however, administration of vehicle alone caused significant increase in this endpoint
						Spleen wt.	150	300	Yes	Low	No	
						Thymus wt.	150	300	Yes	Low	No	
						Rel. thymus wt.	150	300	Yes	Low	No	
						MLNC prolif ConA	150	300	Yes	Low	No	
						MLNC prolif PHA	75	150	Yes	?	No	
Thornton-Manning et al. (1994)	Rat F	Gavage (aqueous)	4-6	0 75 150 300	5 days	Body wt	150	300	Yes	Moderate	No	Model liver histo path (centrilobul. vacuolar degener.) Table 4; p. 11 Model kidney histopath (renal tubule vacuolar degeneration and regeneration) Table 5, p.13 model SDH as test
						Liver wt	75	150	Yes	Low	No	
						Rel. liver wt.	75	150	Yes	Low	No	
						Kidney wt	75	150	Yes	Low	No	
						Rel. kidney wt.	75	150	Yes	Low	No	
						Serum chemistry (hepatotoxicity)	75	150	Yes	Moderate	No	
						Serum chemistry (renal toxicity)	150	300	Yes	Low	No	

Table A-1 Candidate Studies and Data for BMD Modeling - Bromodichloromethane (cont.)

Reference	Species Sex	Route	n	Doses	Exposure Duration	Candidate Endpoints	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	L<10*LL†	Ease of Interp/Toxicologic Relevance	BMD model ?	Comments
						Liver/kidney histopath (mild to moderate centrilobular hepatocell. vacuolar degeneration, mild renal tubule vacuolar degener.)	75	150	Yes	High	Yes	
Thornton-Manning et al. (1994)	Mouse F	Gavage (aqueous)	5-6	0 75 150	5 days	Liver wt	75	150	Yes	Low	No	
						Serum chemistry SDH	--	75	Yes	Moderate	No	

Table A-1 Candidate Studies and Data for BMD Modeling - Bromodichloromethane (cont.)

Reference	Species Sex	Route	n	Doses	Exposure Duration	Candidate Endpoints	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	L<10*LL†	Ease of Interp/Toxicologic Relevance	BMD model ?	Comments
Candidate Studies for Derivation of the Ten-day HA												
Aida et al. (1992a)	Rat M F	Feed	7 7	0 21 62 189 M 204 F	1 month	Body wt (M)	62	189	Yes	Moderate	No	Model Liver cell vacuol. in females Table 8 p. 129
						Liver wt (M)	62	189	Yes	Low	No	
						Kidney wt (M)	62	189	Yes	Low	No	
						Body wt. (F)	62	204	Yes	Moderate	No	
						Rel. liver wt. (F)	62	204	Yes	Low	No	
						Liver histopath (M)	62	189	Yes	High	No	
						Liver histopath (F)	21	62	Yes	High	Yes	
Chu et al. (1982a)	Rat M	Drinking water	10	0 0.8 8.0 68	28 days	Clinical signs serum chemistry histology	68	--	Yes	Low	No	Lack of effect; No data selected for modelling
Condie et al. (1983)	Mouse	Gavage (oil)	9-10	0 37 74 148	14 days	Serum enzymes [elevated SPGT/ALT]	74	148	Yes	Moderate	No	Model kidney histopath (Epithelial hyperplas.); Table 4, p. 571 Liver histopath (centrilob. pallor) Table 5, p. 572
						Decreased PAH uptake *in vitro*	37	74	Yes	Low	No	
						Liver Histopath	37?	74	Yes	Mod. - High	Yes	
						Kidney Histopath	74	148	Yes	High	Yes	

Table A-1 Candidate Studies and Data for BMD Modeling - Bromodichloromethane (cont.)

Reference	Species Sex	Route	n	Doses	Exposure Duration	Candidate Endpoints	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	L<10*LL†	Ease of Interp/Toxicologic Relevance	BMD model ?	Comments
Melnick et al. (1998)	Mouse	Gavage (oil)	10	0 75/54 150/107 326/233	3 weeks (5 d/wk)	Liver wt	75	150	Yes	Low	No	Model hepatocyte hydropic degener. Fig. 4, p. 142
						Serum chem	-	75	Yes	Moderate	No	
						Liver histopath	75	150	Yes	High	Yes	
						Labeling index	75	150	Yes	Moderate	No	
Munson et al. (1982)	Mouse M F	Gavage (aq)	8-12	0 50 125 250	14 days	Body wt (M)	125	250	Yes	Moderate	No	
						Rel liver wt (M)	50	125	Yes	Low	No	
						Spleen wt (M)	125	250	Yes	Low	No	
						Serum chem (M) SPGT SGOT	125	250	Yes	Low - moderate	No	
						Hematology (M)	125	250	Yes	Low	No	
						Hemagglut (M)	50	125	Yes	Low	No	
						Body wt (F)	125	250	Yes	Moderate	No	
						Rel liver wt (F)	50	125	Yes	Low	No	
						Spleen wt (F)	50	125	Yes	Low	No	
						Rel Spleen wt (F)	50	125	Yes	Low	No	
						Serum chem (F) SPGT SGOT	125	250	yes	Low	No	

Table A-1 Candidate Studies and Data for BMD Modeling - Bromodichloromethane (cont.)

Reference	Species Sex	Route	n	Doses	Exposure Duration	Candidate Endpoints	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	L<10*LL†	Ease of Interp/Toxicologic Relevance	BMD model ?	Comments
						Hematology (F)	125	250	Yes	Low	No	
						AFC/spleen (F)	50	125	Yes	Low-Moderate?	No	
						Hemagglutin (F)	125	250	Yes	Low	No	
NTP (1987)	Rat M F	Gavage (oil)	5M 4-5F	0 38 75 150 300 600	14 days	Body wt (M)	150	300	Yes	Moderate	No	LOAEL for effect higher than other for other endpoints
NTP (1987)	Mouse M F	Gavage (oil)	5M 4-5F	0 19 38 75 150 300	14 days	Mortality, lethargy, gross renal pathology	75	150 (FEL)	Yes	Low	No	No suitably sensitive endpoint
NTP (1998)	Rat M F	Drinking water	6	0 9 38 67 (Grp A males)	30 days	Liver histopath	9	38	Yes	High	Yes	Hepatocyte indiv. cell necrosis Table 2, p.36
Narotsky et al. (1997) *	Rat F	Gavage (oil) (aq.)	12-14	0 25 50 75	Gestation days 6-15	Full-litter resorption	25	50	Yes	High	Yes	Model full litter resorption (aqueous vehicle) Fig. 2, incidence reported in text above table

Table A-1 Candidate Studies and Data for BMD Modeling - Bromodichloromethane (cont.)

Reference	Species Sex	Route	n	Doses	Exposure Duration	Candidate Endpoints	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	L<10*LL†	Ease of Interp/Toxicologic Relevance	BMD model ?	Comments
Bielmeier et al. (2001)	Rat F	Gavage (aq.)	8-11	0 75 100	Gestation day 9	Full-litter resorption	--	75	Yes	High	Yes	Model full litter resorption Table p. 23 of manuscript ("Hormone profile II")
Coffin et al. (2000)	Mouse	Gavage (corn oil)	10	0 150 300	11 days	Liver histopathology	-	150	Yes	High	No	Aida et al. (1992a) used an additional, lower dose which provides more information about shape of curve in low-dose region for histopath. effects. No incidence data for histopathology.
						Increased labeling index	-	200	Yes	Moderate	No	
						Increased relative liver wt.	-	200	Yes	Low	No	
Candidate Studies for Derivation of the Longer-term HA												
NTP (1987)	Rat M F	Gavage (oil)	9-10	0/0 19/14 38/27 75/54 150/107 300/214	13 weeks (5 d/wk)	Body weight	75/54 (M) 150/107 (F)	150/107 (M) 300/ 214 (F)	Yes	Moderate	No	Data in text on p. 35-36 Histopath effects occurred only at FEL
						Hepatic and renal histopath (M)	150/107	300/ 214	Yes	High	No	

Table A-1 Candidate Studies and Data for BMD Modeling - Bromodichloromethane (cont.)

Reference	Species Sex	Route	n	Doses	Exposure Duration	Candidate Endpoints	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	L<10*LL†	Ease of Interp/Toxicologic Relevance	BMD model ?	Comments
NTP (1987)	Mouse M	Gavage (oil)	10	0 6.25/4.5 12.5/9 25/18 50/36 100/71	13 weeks (5 d/wk)	Renal histopath	50/36	100/71	Yes	High	Yes	BMD modelling conducted by ICF on data for focal necrosis of renal tubular epithelium in males
NTP (1987)	Mouse F	Gavage (oil)	10	0 25/18 50/36 100/71 200/142 400/284	13 weeks (5 d/wk)	Liver histopath Vacuolated cytoplasm	50/36	100/71	Yes	High	Yes	Data in text on p. 49 Model vacuolated cytoplasm
Reproductive and Developmental Studies												
Ruddick et al. (1983)	Rat	Gavage (oil)	9-14	0 50 100 200	GD 6-15	Sternebral aberrations	100	200	Yes	High	Yes	Model sternebra variations
Narotsky et al. (1997)	Rat	Gavage (oil)	12-14	0 25 50 75	GD 6-15	Developmental Full litter resorption	25	50	Yes	Moderate (vehicle)	No	Model aq. data. from same study
Narotsky et al. (1997)	Rat	Gavage (aq)	12-14	0 25 50 75	GD 6-15	Developmental Full litter resorption	25	50	Yes	High	Yes	This study listed in table for Longer term HA.
						Maternal Reduced body weight gain	-	25	Yes	Moderate to high	Yes	
CCC (2000a)	Rabbit	Drinking water	5	0 4.9 13.9 32.3 76.3	Gestation days 6-29	Reproductive developmental endpoints	76	--	No	Potentially High	No	No adverse effects; small sample size

Table A-1 Candidate Studies and Data for BMD Modeling - Bromodichloromethane (cont.)

Reference	Species Sex	Route	n	Doses	Exposure Duration	Candidate Endpoints	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	L<10*LL†	Ease of Interp/Toxicologic Relevance	BMD model ?	Comments
CCC (2000b)	Rabbit	Drinking water	25	0 1.4 13.4 35.6 55.3	Gestation days 6-29	Reproductive/ developmental	55	--	--	Potentially High	No	No adverse repro. or develop. effects. Model corrected maternal wt. gain gd 6-29 as maternal effect.
						Maternal Reduced body weight gain	13.4	36	Yes	Moderate to high	Yes	
CCC (2000c)	Rat	Drinking water	10	Females 0 ppm 50 ppm 150 ppm 450 ppm 1350 ppm	Gestation days 0-21	Reproductive/ developmental	50 ppm	150	--	Potentially High	No	Decreased pup wt. and wt. gain at doses that caused parental toxicity; reliable mg/kg-day dose could not be estimated.
CCC (2000d)	Rat	Drinking water	25	0.0 2.2 18.4 45.0 82.0	Gestation days 6-21	Developmental Reduced number of ossification sites in phalanges and metatarsals	45	82	Yes	Moderate	No	Reversible variation occurring at doses that cause maternal toxicity
						Maternal Reduced body weight gain	18.4	45	Yes	Moderate to high	Yes	Model body weight gain for gestation days 6-7 and 6-9.

Table A-1 Candidate Studies and Data for BMD Modeling - Bromodichloromethane (cont.)

Reference	Species Sex	Route	n	Doses	Exposure Duration	Candidate Endpoints	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	L<10*LL†	Ease of Interp/Toxicologic Relevance	BMD model ?	Comments
Bielmeier et al. (2001)	Rat	Gavage (aq.)	8-11	0 75 100	Gestation day 9	Full-litter resorption	--	75	Yes	High	Yes	Model full litter resorption Table p. 23 of manuscript ("Hormone profile II") Study also listed under Longer-term HA

† L<LL*10 : LOAEL for endpoint less than 10 times the lowest LOAEL observed across all studies in category

Table A-2 Candidate Studies and Data for BMD Modeling -Dibromochloromethane

Reference	Species Sex	Route	n	Doses	Exposure Duration	Candidate Endpoints	NOAEL	LOAEL	L<10*LL†	Ease of Interpretation/ Toxicologic Relevance	BMD model ?	Comments
Candidate Studies for Derivation of the One-day HA - none												
Candidate Studies for Derivation of the Ten-day HA												
Aida et al. (1992a)	Rat M F	Feed	7	Males 0 18 56 173 Females 0 34 101 332	1 month	Liver wt (M)	56	173	Yes	Moderate	No	Model: liver histopath (liver cell vacuolization) in M and F Table 8, p.129
						Rel liver wt (M)	56	173	Yes	Moderate	No	
						Liver histopath (M)	56	173	Yes	High	Yes	
						Body wt (F)	101	332	Yes	Moderate	No	
						Liver wt (F)	34	101	Yes	Low	No	
						Rel liver wt (F)	--	34	Yes	Low	No	
						Rel kidney wt (F)	101	332	Yes	Low	No	
						Liver histopath (F)	101	332	Yes	High	Yes	
Chu et al. (1982a)	Rat M	Drinking water	10	0 .7 8.5 68	28 days	--	68	--	--	--	--	No adverse effects observed

Table A-2 Candidate Studies and Data for BMD Modeling -Dichlorobromomethane (cont.)

Reference	Species Sex	Route	n	Doses	Exposure Duration	Candidate Endpoints	NOAEL	LOAEL	L<10*LL†	Ease of Interpretation/ Toxicologic Relevance	BMD model ?	Comments
Condie et al. (1983)	Mouse M	Gavage (oil)	8-16	0 37 74 147	14 days	Serum SPGT	74	147	Yes	Moderate	No	Model renal mesangial hypertrophy and hepatic cytoplasmic vacuolation Table 5, p.572
						Liver histopath	74	147	Yes	High	Yes	
						Renal histopath	74	147	Yes	High	Yes	
Melnick et al. (1998)	Mouse F	Gavage (oil)	10	0 50/37 100/71 192/137 417/298	3 weeks (5 d/wk)	Relative Liver wt	--	50	Yes	Low	No	Model Incidence of hepatocyte hydropic degeneration Table 4, p.142
						Serum ALT	100	192	Yes	Moderate	No	
						Serum SDH	--	50	Yes	Moderate	No	
						Liver histopath	100	192	Yes	High	Yes	
						Inc. labeling index	192	417	Yes	High	No	
Munson et al. (1982)	Mouse M F	Gavage (aq)	8-12	0 50 125 250	14 days	Body wt. (M)	125	150	Yes	Moderate	No	
						Rel liver wt (M)	50	125	Yes	Low	No	
						Spleen wt (M)	125	250	Yes	Moderate?	No	
						Rel spleen wt (M)	125	250	Yes	Moderate?	No	
						Hematology - Fibr (M)	125	250	Yes	Low	No	
						Serum chem SGPT (M)	125	250	Yes	Low	No	

Table A-2 Candidate Studies and Data for BMD Modeling -Dichlorobromomethane (cont.)

Reference	Species Sex	Route	n	Doses	Exposure Duration	Candidate Endpoints	NOAEL	LOAEL	L<10*LL†	Ease of Interpretation/ Toxicologic Relevance	BMD model ?	Comments
						AFC/Spleen (M)	125	250	Yes	Moderate	No	
						*AFC/10^6 (M)	50	125	Yes	Moderate	No	
						Liver wt (F)	125	250	Yes	Low	No	
						Rel liver wt (F)	50	125	Yes	Low	No	
						Hematology - Fibr (F)	125	250	Yes	Low	No	
						Serum SGPT (F)	125	250	Yes	?	No	
						AFC/spleen (F)	125	250	Yes	Moderate	No	
						AFC/10^6 (F)	50	125	Yes	Moderate	No	
NTP (1985)	Rat M F	Gavage (oil)	5	0 60 125 250 500 1000	14 days	Body wt (M)	250	500 (FEL)	No	Moderate	No	Tables 3 and 4 p.33
						Dark'd kid medulla (M)	250	500	No	Low	No	Effects observed only at levels where reduced survival occurred: survival(2/5 and 0/5 for M and F, respectively at the 250 mg/kg-day LOAEL
						Mottled liver (M)	500	1000	No	Low	No	
						Dark'd kid medulla (F)	250	500	No	Low	No	
						Mottled liver (F)	250	500	No	Low	No	

Table A-2 Candidate Studies and Data for BMD Modeling -Dichlorobromomethane (cont.)

Reference	Species Sex	Route	n	Doses	Exposure Duration	Candidate Endpoints	NOAEL	LOAEL	L<10*LL†	Ease of Interpretation/ Toxicologic Relevance	BMD model ?	Comments
NTP (1985)	Mouse M F	Gavage (oil)	5	0 30 60 125 250 500	14 days	Stomach nodules (F)	125	250	Yes	Moderate	No	Model stomach nodules in M and F Table 13 p.44 Renal and hepatic effects observed only at levels where reduced survival observed: Survival at 500 mg/kg-day 1/5 and 2/5 for M and F respectively
						Stomach nodules (M)	60	125	Yes	Moderate	Yes	
						Red'd kid medulla (F)	250	500	No	Low	No	
						Red'd kid medulla (M)	250	500	No	Low	No	
						Mottled liver (M)	125	250	Yes	Low	No	
						Mottled liver (F)	125	250	Yes	Low	No	
Coffin et al. (2000)	Mouse F	Gavage (oil)	10	0 100 300	11 days	Liver histopathology	-	100	Yes	High	No	Other studies showing histopath. effects used lower range of doses. No incidence data for histopathology.
						Increased labeling index	-	100	Yes	Moderate	No	
						Increased relative liver wt.	-	100	Yes	Low	No	

Table A-2 Candidate Studies and Data for BMD Modeling -Dichlorobromomethane (cont.)

Reference	Species Sex	Route	n	Doses	Exposure Duration	Candidate Endpoints	NOAEL	LOAEL	L<10*LL†	Ease of Interpretation/ Toxicologic Relevance	BMD model ?	Comments
NTP (1996)	Rat	Drinking water	10	Males 0 4.2 12.4 28.2 Group A Females 0 6.3 17.4 46.0 Group B Females 0 7.1 20 47.8	29 days	No clearly treatment-related adverse effects observed	28	--	--	--	--	Decreased wt gain observed in some groups, but effect did not reach statistical significance

Table A-2 Candidate Studies and Data for BMD Modeling -Dichlorobromomethane (cont.)

Reference	Species Sex	Route	n	Doses	Exposure Duration	Candidate Endpoints	NOAEL	LOAEL	L<10*LL†	Ease of Interpretation/ Toxicologic Relevance	BMD model ?	Comments
Candidate Studies for Derivation of the Longer-term HA												
Chu et al. (1982b)	Rat M F	Drinking water	20	Males 0 0.57 6.1 49 224 Females 0 0.64 6.9 55 236	90 days	Liver histopath - prevalence (M)	?	?	Yes	High	Yes	Model incidence data Tables 5 and 6 "treatment" results
						Liver histopath - prevalence (M)	49	224	Yes	High	Yes	
Daniel et al. (1990)	Rat M F	Gavage (oil)	10	0 50 100 200	90 days	Hepatic and renal lesions Modeled previously by ICF	--	50	Yes	High	-	Crump $BMDL_{10}$ = 4.2 (kidney cortex degeneration in females)
NTP (1985)	Rat	Gavage (oil)	10	0 15 30 60 125 250	13 weeks (5 d/wk)	hepatic lesions Modeled previously by ICF	30	60	Yes	High	-	Crump $BMDL_{10}$ = 0.93 (liver fatty metamorphosis in males)

Table A-2 Candidate Studies and Data for BMD Modeling -Dichlorobromomethane (cont.)

Reference	Species Sex	Route	n	Doses	Exposure Duration	Candidate Endpoints	NOAEL	LOAEL	L<10*LL†	Ease of Interpretation/ Toxicologic Relevance	BMD model ?	Comments
NTP (1985)	Mouse M F	Gavage (oil)	10	0 15 30 60 125 250	13 weeks (5 d/wk)	Liver histopath (M) Kidney histopath (F)	125	250	Yes	High	No	Occurred only at highest dose; data provided only for 0, 125, and 250 mg/kg doses; Incidence at 125 0/10 for all endpoints; 5/10 for hepatic vac. change and nephropathy in males; incidence at 15, 30, and 60 not examined.

Table A-2 Candidate Studies and Data for BMD Modeling -Dichlorobromomethane (cont.)

Reference	Species Sex	Route	n	Doses	Exposure Duration	Candidate Endpoints	NOAEL	LOAEL	L<10*LL†	Ease of Interpretation/ Toxicologic Relevance	BMD model ?	Comments
Reproductive and Developmental Studies												
Borzelleca and Carchman (1982)	Mouse M F	Drinking Water	10 M 30 F	0 17 171 685	25-27 weeks	Postnatal body wt. (cannot be modeled due to insufficient data on number of litters evaluated)	-	17 (marginal)	Yes	High	No	Marginal LOAEL for parental toxicity is 17 mg/kg-day
Ruddick et al. (1983)	Rat F	Gavage (Corn oil)	9-14	0 50 100 200	g.d. 6-15	-	None identified	None identified	--	--	No	No clearly adverse effect
NTP (1996)	Rat M	Drinking Water	10	4.2 12.4 28.2	29 days	-	28.2	--	--	--	No	No clearly adverse effect on any reproductive endpoint at tested doses
NTP (1996)	Rat F	Drinking Water	10	6.3 17.4 46.0	35 days	-	46.0	--	--	--	No	No clearly adverse effect on any reproductive or developmental endpoint at tested doses

Table A-2 Candidate Studies and Data for BMD Modeling -Dichlorobromomethane (cont.)

Reference	Species Sex	Route	n	Doses	Exposure Duration	Candidate Endpoints	NOAEL	LOAEL	L<10*LL†	Ease of Interpreta-tion/ Toxicologic Relevance	BMD model ?	Comments
NTP (1996)	Rat F	Drinking Water	7.1 20.0 47.8	13	6 days	-	47.8	--	--	--	-	No clearly adverse effect on any reprod or develop endpoint at tested doses

† L<LL*10 : LOAEL for endpoint less than 10 times the lowest LOAEL observed across all studies in category

FEL, Frank effect level

Table A-3 Candidate Studies and Data for BMD Modeling - Bromoform

Reference	Species Sex	Route	n	Doses	Exposure Duration	Candidate Endpoints	NOAEL	LOAEL	L<10*LL†	Ease of Interpretation/ Toxicologic Relevance	BMD model ?	Comments
Candidate Studies for Derivation of the One-day HA for Bromoform - No suitable studies												
Candidate Studies for Derivation of the Ten-day HA for Bromoform												
Aida et al. (1992a)	Rat M F	Feed	7	Males 0 62 187 618 Females 0 56 208 728	1 month	Liver histopath (M)	62	187	Yes	High	Yes	Model liver cell vacuolization in M and F Table 7, p.128
						Serum LDH	56	208	Yes	Low	No	
						BUN (F)	56	208	Yes	Low	No	
						Liver histopath. (F)	56	208	Yes	High	Yes	
Chu et al. (1982a)	Rat M	Drinking water	20	0 0.7 8.5 80	28 days	None	80	--	Yes	--	No	No adverse effects
Condie et al. (1983)	Mouse M	Gavage (oil)	5-16	0 72 145 289	14 days	Renal slice uptake PAH	145	289	Yes	Low	No	Model Liver histopath: centrilobular pallor Model kidney histopath: mesangial nephrosis Table 4, p.571
						Renal Histopath	145	289	Yes	High	Yes	
						Liver histopath	145	289	Yes	High	Yes	
						SGPT activity	145	289	Yes	Low	No	

Table A-3 Candidate Studies and Data for BMD Modeling - Bromoform (cont.)

Reference	Species Sex	Route	n	Doses	Exposure Duration	Candidate Endpoints	NOAEL	LOAEL	L<10*LL†	Ease of Interpretation/ Toxicologic Relevance	BMD model ?	Comments
Melnick et al. (1998)	Mouse F	Gavage (oil)	10	0 200 500	3 weeks (5 d/wk)	Rel Liver wt	200	500	Yes	Low	No	Stat sign increase in rel liver wt at 200 - reported to be about 17% in text. Value of 200 for NOAEL based on consistency of effects at higher dose per Mantus Model: liver hydropic degeneration Graph p.140
						Serum chemistry ALT	200	500	Yes	?	No	
						Serum Chemistry SDH	200	500	Yes	?	?	
						Liver histopath	200	500	Yes	High	Yes	
						Labeling index	200	500	Yes	Moderate	No	
Coffin et al. (2000)	Mouse F	Gavage (oil)	10	0 200 500	11 days	Liver histopath.	-	200	Yes	High	No	Other studies with lower range of dose. No incidence data for histopathology.
						Labeling index	-	200	Yes	Moderate	No	

Table A-3 Candidate Studies and Data for BMD Modeling - Bromoform (cont.)

Reference	Species Sex	Route	n	Doses	Exposure Duration	Candidate Endpoints	NOAEL	LOAEL	L<10*LL†	Ease of Interpretation/ Toxicologic Relevance	BMD model ?	Comments
Munson et al. (1982)	Mouse M F	Gavage (aq.)	7-12	0 50 125 250	14 days	Liver wt (M)	50	125	Yes	Low	No	
						Rel liver wt (M)	50	125	Yes	Low	No	
						Hematology - Fibr (M)	125	250	Yes	Low	No	
						*Serum SGOT (M)	125	250	Yes	Low	No	
NTP (1989a)	Mouse M F	Gavage (oil)	5	Male 0 50 100 200 400 600	14 days	Stomach nodules (M)	200	400	Yes	Moderate	Yes	Model incidence of stomach nodules in males p. 45 Males: 400 4/5 600 3/5 Females: 600 2/5 800 1/5
				Female 0 100 200 400 600 800		Stomach nodules (F)	400	600	Yes	Moderate	No	
NTP (1989a)	Rat M F	Gavage (oil)	5	0 100 200 400 600 800	14 days	Body wt (M)	200	400	Yes	Moderate	No	Possibly model body weight p. 36

Table A-3 Candidate Studies and Data for BMD Modeling - Bromoform (cont.)

Reference	Species Sex	Route	n	Doses	Exposure Duration	Candidate Endpoints	NOAEL	LOAEL	L<10*LL†	Ease of Interpretation/ Toxicologic Relevance	BMD model ?	Comments
Candidate Studies for Derivation of the Longer-term HA												
Chu et al. (1982b)	Rat M F	Drinking water	9-10	Males 0 0.65 6.1 57 218 Females 0 0.64 6.9 55 283	90 days	Liver Histopath (M)	57	218	Yes	High	Yes	Incidence and mean severity score provided for combined hepatic lesions. Model 'treatment' prevalence for liver lesions in M and F Serum chem data (LDH) presented only for high dose (Insuff data for modeling)
						Liver Histopath (F)	55	283	Yes	High	Yes	
NTP (1989a)	Rat M F	Gavage (corn oil)	10	0 12 25 50 100 200	13 weeks (5 d/wk)	Liver Histopath.	25	50 (hepatic vacuolization)	Yes	High	-	Previously calculated Crump $BMDL_{10}$ 2.65 (hepatic vacuolization in male rats)

Table A-3 Candidate Studies and Data for BMD Modeling - Bromoform (cont.)

Reference	Species Sex	Route	n	Doses	Exposure Duration	Candidate Endpoints	NOAEL	LOAEL	L<10*LL†	Ease of Interpretation/ Toxicologic Relevance	BMD model ?	Comments
NTP (1989a)	Mouse	Gavage (corn oil)	10	0 25 50 100 200 400	13 weeks (5 d/wk)	Liver Histopath	100	200	Yes	High	Yes	Model hepatic vacuolization
Reproductive and Developmental Studies												
Ruddick et al. (1983)	F	Gavage (Corn oil)	14-15	0 50 100 200	gd 6-15	Sternebra aberrations	50	100	Yes	High	Yes	Dose-dependent increase in sternebra aberrations; intraparietal deviations at mid- and high doses.
						Intraparietal variations	-	-	-	High	No	
NTP (1989b)	Mouse M F	Gavage (oil)	20 20	0 50 100 200 (NOAEL)	105 days	No adverse effects at doses tested	200	-	-	High	No	No detectable effect on fertility, litters/pair, live pups/litter; proportion of live births, sex of live pups, or pup body weight.

† L<LL*10 : LOAEL for endpoint less than 10 times the lowest LOAEL observed across all studies in category

C. METHODS

Benchmark Dose

The brominated trihalomethane data sets considered for dose-response modeling include both quantal and continuous endpoints. EPA's Benchmark Dose Software (BMDS) (U.S. EPA, 2000a) was used to accomplish all of the model fitting and estimation of the BMD and lower 95% confidence limit (BMDL). The methods and models applied to both quantal and continuous endpoints are presented here.

Quantal Models

Seven of the nine quantal models implemented in the BMDS package were used to represent the dose-response behavior of the quantal endpoints. Specifically, the models used were the gamma model, the logistic and log-logistic models, the probit and log-probit models, the multistage model, and the Weibull model. Two other models, the linear and the quadratic models, were not fit to the data because they are special cases of both the multistage and the Weibull models. If the fitting of the multistage or Weibull models resulted in a linear or a quadratic form, then those result were used; otherwise, the linear or quadratic models would not provide a fit as good as the multistage or Weibull model and so were not separately obtained.

The equations defining each of these models are presented here (U.S. EPA, 2000a). In all of the following, P(d) represents the probability of response (i.e., adverse effect) following exposure to "dose" d. In all of these models, α, β, and γ are model parameters estimated using maximum likelihood techniques, as described below.

Table A-4 Model Equations used in BMD Calculations for Health Advisories

Model	Equation	Conditions
gamma	$P(d) = \gamma + (1 - \gamma)\cdot(1/\Gamma(a))\cdot \int t\alpha\text{-}1e^{-t}dt$	$0 \le \gamma < 1$, $\beta \ge 0$, and $\alpha \ge 1$. $\Gamma(x)$ is the gamma function, and the integral runs from 0 to βd.
logistic	$P(d) = [1 + \exp\{-(\alpha + \beta d)\}]^{-1}$	$\beta \ge 0$
log-logistic	$P(d) = \gamma + (1 - \gamma)\cdot[1 + \exp\{-(\alpha + \beta \ln(d))\}]^{-1}$	The log-logistic model has much the same form as the logistic model except when d = 0, in which case P(d) = g. In this case $b \ge 0$, and for the background parameter γ, $0 \le \gamma < 1$.
probit	$P(d) = \Phi(\alpha + \beta d)$	$\Phi(x)$ is the standard normal cumulative distribution function and $\beta \ge 0$.

log-probit	$P(d) = \gamma + (1 - \gamma)\cdot\Phi(\alpha + \beta\cdot\ln(d))$	The log-probit model has a form similar to the probit model except when d = 0, in which case $P(d) = \gamma$. Here $0 \leq \gamma < 1$, and $\beta \geq 1$
multistage model	$P(d) = \gamma + (1 - \gamma)\cdot(1 - \exp\{-(\beta_1 d + \beta_2 d^2 + ... + \beta_n d^n)\})$	all the β parameters are restricted to be nonnegative and $0 \leq \gamma < 1$. When applied to the brominated trihalomethane data sets in these analyses, the degree of the multistage model (the highest power on dose in the above equation, n) was set equal to one less than the number of dose groups in the experiment being analyzed.
Weibull model[1]	$P(d) = \gamma + (1 - \gamma)(1 - \exp\{-\beta d^{\alpha}\})$	The background parameter γ is restricted to fall between 0 (inclusive) and 1, and β is greater than or equal to 0. For these analyses, the parameter α is constrained to be greater than or equal to 1.[1]

[1]The linear model is a special case of the Weibull model obtained by fixing the parameter α equal to 1. The quadratic model is a special case of the Weibull model obtained by fixing the parameter α equal to 2.

When fitting all of the above-mentioned quantal models, maximum likelihood methods were used to estimate the parameters of the models. That method maximizes the log-transformed likelihood of obtaining the observed data, which is (except for an additive constant) given by

$$L = \sum [n_i \cdot \ln\{P(d_i)\} + (N_i - n_i) \cdot \ln\{1 - P(d_i)\}]$$

where the sum runs over i from 1 to k (the number of dose groups), and for group i, d_i is the dose (exposure level), N_i is the number of individuals tested, and n_i is the number of individuals responding (U.S. EPA, 2000a).

Continuous Models

The continuous endpoints of interest with respect to brominated trihalomethanes toxicity were quantitatively summarized by group means and measures of variability (standard errors or standard deviations). The models used to represent the dose-response behavior of those continuous endpoints are those implemented in EPA's Benchmark Dose Software (U.S. EPA,

2000a). These models were the power model, the Hill model, and the polynomial model. These mathematical models fit to the data are defined here. In all cases, $\mu(d)$ indicates the mean of the response variable following exposure to "dose" d.

The power model is represented by the equation

$$\mu(d) = \gamma + \beta d^{\alpha}$$

where the parameter α is restricted to be nonnegative. [The linear model is obtained when α is fixed at a value of 1. The linear model was not separately fit to the data; if the result of fitting the power model does not result in the linear form, $\alpha = 1$, then the linear model does not fit as well as the more general power model, by definition.]

The Hill model is given by the following equation:

$$\mu(d) = \gamma + (vd^n) / (d^n + k^n))$$

where the parameters n and k are restricted to be positive. Because the Hill model has four parameters to be estimated (γ, v, n, and k), the power n was fixed equal to 1 when the model was fit to data sets with only three dose groups, so that the number of estimated parameters did not exceed the number of data points.

The polynomial model is defined as

$$\mu(d) = \beta_0 + \beta_1 d + ... + \beta_n d^n$$

where the degree of the polynomial, n, was set equal to one less than the number of dose groups in the experiment being analyzed. Note that U.S. EPA (2000a) recommends the use of the most parsimonious model that provides an adequate fit to the data. It may appear that use of a polynomial model with degree equal to one less than the number of dose groups would not yield the most parsimonious model. However, allowing the model to have that degree is not the same as forcing the model to have that degree; in the model fitting, if fewer parameters (e.g., a lower degree polynomial) is adequate and consistent with the data, then the fitting will reflect that fact and a more parsimonious model will be the result. For these analyses, the values of the β parameters allowed to be estimated were constrained to be either all nonnegative or all nonpositive (as dictated by the data set being modeled, i.e., nonnegative if the mean response increased with increasing dose or nonpositive if the mean response decreased with increasing dose).

In the case of continuous endpoints, one must assume something about the distribution of individual observations around the dose-specific mean values defined by the above models. The assumptions imposed by BMDS were used in this analysis: individual observations were assumed to vary normally around the means with variances given by the following equation:

$$\sigma_i^2 = \sigma^2 \cdot [\mu(d_i)]^{\eta}$$

where both σ^2 and ρ were parameters estimated by the model.

Given those assumptions about variation around the means, maximum likelihood methods were applied to estimate all of the parameters, where the log-likelihood to be maximized is (except for an additive constant) given by

$$L = \sum [(N_i/2)\cdot\ln(\sigma_i^2) + (N_i - 1)s_i^2/2\sigma_i^2 + N_i\{m_i - \mu(d_i)\}^2/2\sigma_i^2]$$

where N_i is the number of individuals in group i exposed to dose d_i, and m_i and s_i are the observed mean and standard deviation for that group. The summation runs over i from 1 to k (the number of dose groups).

Goodness of Fit Analyses

For the quantal models, goodness of fit was determined by the modeling software using the chi-square test. This test is based on sums of squared differences between observed and predicted numbers of responders. The degrees of freedom for the chi-square test statistic are equal to the number of dose groups minus the number of parameters fit by the method of maximum likelihood (ignoring those parameters that are estimated to be equal to one of the bounds defining their constraints -- see the discussion above about constraints imposed on the model parameters). When the number of parameters estimated equals the number of dose groups, there are no degrees of freedom for a statistical evaluation of fit.

For the continuous models, goodness of fit was determined based on a likelihood ratio statistic. In particular, the maximized log-likelihood associated with the fitted model was compared to the log-likelihood maximized with each dose group considered to have a mean and variance completely independent of the means and variances of the other dose groups. It is always the case that the latter log-likelihood will be at least as great as the model-associated log-likelihood, but if the model does a "reasonable" job of fitting the data, the difference between the two log-likelihoods will not be too great. A formal statistical test reflecting this idea uses the fact that twice the difference in the log-likelihoods is distributed as a chi-square random variable. The degrees of freedom associated with that chi-squared test statistic are equal to the difference between the number of parameters fit by the model (including the parameters σ^2 and ρ defining how variances change as a function of mean response level) and twice the number of dose groups (which is equal to the number of parameters estimated by the "model" assuming independence of dose group means and variances).

Visual fit, particularly in the low-dose region, was assessed for models that had acceptable global goodness-of-fit. Acceptable global goodness of fit was either a p-value greater than or equal to 0.1, or a perfect fit when there were no degrees of freedom for a statistical test of fit. Choice of 0.1 is consistent with current U.S. EPA guidance for BMD modelling (U.S. EPA, 2000b). Local fit was evaluated visually on the graphic output, by comparing the observed and estimated results at each data point.

Goodness-of-fit statistics are not designed to compare different models, particularly if the different models have different numbers of parameters. Within a family of models, adding

parameters generally improves the fit. BMDS reports the Akaike Information Criterion (AIC) to aid in comparing the fit of different models. The AIC is defined as –2L+2p, where L is the log-likelihood at the maximum likelihood estimates for the parameters, and p is the number of model parameters estimated. When comparing the fit of two or more models to a single data set, the model with the lesser AIC was considered to provide a superior fit.

Definition of the BMR and Corresponding BMD and BMDL

For all of the quantal endpoints analyzed here, the BMDs and BMDLs were defined based on BMRs of 5% and 10% extra risk. BMDLs were defined as the 95% lower bound on the corresponding BMD estimates. Confidence bounds were calculated by BMDS using a likelihood profile method.

Although the 10% response level was selected as the "point of departure" for all the quantal endpoints analyzed here, we have chosen to follow standard practice and include results for both the 5% and 10% level of response. In some cases (see discussions below), a comparison of the 5% and 10% results gives clues about problems with some of the models. In general, we have included both for completeness, as there is no current consensus concerning the most appropriate point of departure except in some particular cases (e.g., use of 5% risk for developmental toxicity tests where the nesting of effects has been modeled using models specifically designed for such experimental designs).

For the continuous models, BMDs were implicitly defined as follows:

$$|\mu(\text{BMD}) - \mu(0)| = \delta \cdot \sigma_1$$

where σ_1 is the model-estimated standard deviation in the control group. In other words, the BMR was defined as a change in mean corresponding to some multiplicative factor of the control group standard deviation.

The value of δ used in this analysis was 1.1. This value was chosen based on the work of Crump (1995), who showed that this choice corresponded to an additional risk of 10% when the background response rate was assumed to be 1%, with normal variation around the mean (and constant standard deviation). Although the current analyses allowed for nonconstant standard deviations and estimated extra risk, while the Crump (1995) comparison was based on constant standard deviations and additional risk, the values of 1.1 was used for two reasons. First, the difference between additional and extra risk is small when the background rate is 1% or less, so that the change from additional to extra risk will have minimal impact on the correspondences proven by Crump (1995). Second, there can be no such generic, a priori correspondences when standard deviations are allowed to vary in a manner determined only after the model fitting is accomplished. Thus, to avoid data set- and model-specific choices for δ, the correspondences proven by Crump (1995) can be used as the best available, consistent definition of the benchmark response. The definition of the BMR as a change in mean of 1.1 times the control standard deviation is very close to the default value of 1 standard deviation recommended by recent draft EPA guidelines (U.S. EPA, 2000b). In the following, BMDs and BMDLs

corresponding to $\delta = 1.1$ are denoted BMD_{10} and $BMDL_{10}$, because of the just-noted association of that value of δ with 10% risk.

As for the quantal models, for all of the continuous models BMDLs were defined as the 95% lower bound on the corresponding BMD. Confidence intervals were calculated using a profile likelihood method.

Choice of BMDL

The following guidance was followed with regard to the choice of the BMDL to use as a point of departure for calculation of a health advisory. This guidance is consistent with recommendations in U.S. EPA (2000b). For each endpoint, the following procedure is recommended:

1. Models with an unacceptable fit (including consideration of local fit in the low-dose region) are excluded. Visual fit, particularly in the low-dose region, was assessed for models that had acceptable global goodness-of-fit.

2. If the BMDL values for the remaining models for a given endpoint are within a factor of 3, no model dependence is assumed, and the models are considered indistinguishable in the context of the precision of the methods. The models are then ranked according to the AIC, and the model with the lowest AIC is chosen as the basis for the BMDL.

3. If the BMDL values are not within a factor of 3, some model dependence is assumed, and the lowest BMDL is selected as a reasonable conservative estimate, unless it is an outlier compared to the results from all of the other models. Note that when outliers are removed, the remaining BMDLs may then be within a factor of 3, and so the criteria given in item 2. would be applied.

4. The BMDL values from all modeled endpoints are compared, along with any NOAELs or LOAELs from data sets that were not amenable to modeling, and the lowest NOAEL or BMDL is chosen.

5. Models with an unacceptable fit (including consideration of local fit in the low-dose region) are excluded.

D. MODELING RESULTS

1. Bromodichloromethane

The majority of endpoints modeled consisted of dichotomous data. BMDS modeling results for bromodichloromethane dichotomous endpoints are summarized in Table A-5 below. Four sets of continuous data were modeled. These results are summarized in Section f below. Detailed output for each model run is compiled in Appendix B, provided in electronic format on compact disk.

a. Developmental and Reproductive studies

Three data sets for developmental or reproductive toxicity were modeled. When the data for full litter resorption (FLR) in rats reported by Bielmeier et al. (2000) were analyzed, the BMDL results for the log-logistic model were low relative to the corresponding BMD estimates (compared to the estimates obtained from the other models); the results from the log-logistic model might be that is considered qualitative outliers. The remaining values are still not within factor of 3, indicating some model dependence of the results. In any case, the multistage model was chosen as it gave the smallest value for the AIC.

Modeling of FLR data from Narotsky et al. (1997) also gave the same type of questionable results for the log-logistic model (very low BMDLs relative to the BMD). Here, as in the case of the Bielmeier et al. (2000) modeling, the initial fit of the log-logistic model does not appear to be suspect; the goodness of fit evaluations and visual examinations of the model predictions are consistent with the data and with the other models. It appears that there is some error or problem with the log-logistic model in the BMDS software that affects the calculation of BMDLs for some data sets. When the log-logistic model was eliminated from consideration, the remaining BMDLs are within a factor of 3. The log-probit model was selected because it has the lowest value for the AIC.

Data from the study by Ruddick et al. (1983) consisted of the count of the numbers of litters that had one or more fetuses with sternebral variations. Although this expression of the response rates does not correspond directly to the probability of a response in the offspring of treated dams, it is consistent with the full litter resorption results from Bielmeier et al. (2001) and Narotsky et al. (1997) in the sense that it relates to effects recorded at the level of the dam. The log-logistic and log-probit models could not determine BMDLs for this data set. However, the other models did provide estimates of the BMDLs, all of which were within a factor of three of one another. The multistage, having the lowest AIC of all the models was selected as the basis for the BMDL estimate for this data set.

b. One-day Health Advisory

Four data sets were modeled in support of the One-day HA for bromodichloromethane. For the Lilly et al. (1994) data on vacuolar degeneration in male rats, the multistage model gave questionable results (very high AIC and a goodness of fit p value that appeared unrealistically high when the model fit was examined visually) and was eliminated from consideration. The

BMDS software gave warnings on bound calculation for the probit model. All of the remaining BMDLs are within factor of 3, so the log-probit model was selected because it has the lowest value for the AIC.

When the data from the same study for renal tubular degeneration were modeled, the multistage and log-logistic gave questionable results and were eliminated from consideration. The remaining BMDLs are not within a factor of 3, indicating some model dependence of the results. The lowest BMDL was thus selected as a reasonable conservative estimate. The gamma or Weibull models predict the same BMDL. The gamma model was selected on the basis of having the lowest AIC.

It is perhaps informative to compare the considerations applied here, in the case of the renal endpoint, to those applied above to the Narotsky et al. (1997) modeling results. In the case of the Narotsky et al. (1997) results, a single model (log-logistic) gave a $BMDL_{10}$ that was about 8-fold lower than the corresponding BMD_{10}, whereas the other models gave BMDLs that were within a factor of about 2 of the corresponding BMDs. The discrepancy was even greater at a BMR of 5%, suggesting that a problem may be associated with that one model. In the case of the Lilly et al. (1994) renal effect, after eliminating the obviously problematic model results (log-logistic and multistage), the differences between BMDs and corresponding BMDLs are present and consistent for all the models at both 5% and 10% response. It is true that some models have a greater difference between the BMD and the BMDL than do other models and that this model dependence is due, at least in part, to the fact that the BMD estimates fall below the lowest nonzero experimental dose. But the choice of the most conservative BMDL is intended to cover that model dependence: if there is little information in the region of interest, so that otherwise reasonable (good-fitting) models disagree as to the BMDL because of differences in possible curve shapes, the most conservative choice is a good one since we can not rule out the possibility that the true curve shape is described by the most conservative model. This use of the BMD methodology and treatment of model-dependence is much superior to the choice of some other (higher) BMR to use in cases where response rate at the lowest nonzero experimental dose is greater than 10%. Such an alternative would entail additional arbitrary decisions about what the higher BMR should be and how to scale the results corresponding to that BMR so as to be consistent with results from studies in which $BMDL_{10}$s were estimated.

Renal and hepatic histopathology data were modeled from the study of Thornton-Manning et al. (1994). The data for hepatic centrilobular vacuolar degeneration were not satisfactorily fit (all goodness of fit p values were less than 0.1) by any model. These data displayed some peculiarities: 0% response at the lowest nonzero dose, 100% response at the next dose, and then a drop to 75% response at the highest dose. Because of the poor fit, the BMDLs for this endpoint can not be used. For renal data, the multistage model gave questionable results (very high AIC and a goodness of fit p value that appeared unrealistically high when the model fit was examined visually) and was eliminated from consideration. The remaining BMDLs were within a factor of 3, and the log-logistic model was selected because it has the smallest AIC.

c. Ten-day Health Advisory

Five data sets were modeled using the BMDS software in support of the Ten-day HA for bromodichloromethane. For the Condie et al. (1983) data on liver histopathology, the logistic and probit models were eliminated on the basis of poor fit. The rest of the BMDLs were within a factor of 3, so the log-logistic model was selected on the basis of the smallest AIC. When renal histopathology data from the same study were modeled, the logistic and probit models were eliminated for lack of fit. The remaining BMDLs were within a factor of 3, so the models with the lowest AIC (Weibull and log-logistic) were examined. The Weibull model results were selected on the basis of the smallest BMDL. For the Aida et al. (1992a) data set for liver cell vacuolation, questionable results were obtained with the log-logistic model (very low BMDLs relative to the BMD). The remaining BMDLs are within a factor of 3 and the multistage model was selected because it had the smallest AIC value. When the Melnick et al. (1998) data were analyzed, the multistage model was eliminated because it gave a goodness of fit p-value that was unrealistically high when the curve fit was evaluated by visual inspection and because the AIC was very large. The BMDL values of the remaining models were within a factor of three. The result from the Weibull model was selected on the basis of having the lowest AIC value. When results for the NTP (1998) study were examined, all models gave acceptable fit. Because all BMDLs were within a factor of three of one another, the log-logistic model was selected on the basis of the smallest AIC value.

d. Longer-term Health Advisory

A single data set (NTP, 1987) was analyzed using the BMDS software in support of the Longer-term HA. Results for the logistic and probit models were rejected for lack of fit (goodness of fit p values less than 0.1). The remaining p values were within a factor of 3, so the log-probit model was selected on the basis of the smallest AIC.

e. RfD

Several data sets that had previously given low $BMDL_{10}$ estimates when modeled using the Crump benchmark dose software (THC and THWC programs; K.S. Crump, Inc.) were reanalyzed using the BMDS software and current guidance for evaluation of results. An advantage of the BMDS package is that it includes several additional model options for data analysis. Results for the models (Weibull, multistage) common to both programs were in close agreement. However, one or more of the additional models available in the BMDS sometimes fit the data better when analyzed by the criteria set forth in Section C (above). In these cases the BMD and BMDL values changed by a small amount. Where appropriate, these revised values were used to calculate health advisories and RfDs.

Data for kidney cytomegaly from the chronic NTP (1987) study in male mice were remodeled using the BMDS program. The results from the logistic and probit models were rejected for lack of fit ($p < 0.10$). Estimates of the $BMDL_{10}$ calculated by the Weibull and multistage models were identical to estimates derived using the Crump benchmark dose software (0.96 mg/kg-day). The results for the $BMDL_{10}$ varied by more than a factor of 3, indicating a degree of model dependence. The log-logistic model gave a very low value for the $BMDL_{10}$ and

was eliminated from further consideration. Of the remaining models, the log-probit model gave the lowest value for the AIC and the corresponding $BMDL_{10}$ was thus selected as a candidate for derivation of the RfD.

When data for fatty degeneration in the liver of female rats (Aida et al., 1992b) were remodeled using the BMDS program, all models fit the data adequately. The resulting $BMDL_{10}$ values were within a factor of three, indicating model independence. The $BMDL_{10}$ calculated using the probit model was selected as a candidate for derivation of the RfD because it had the lowest AIC value.

6. Modeling of Continuous Endpoints

Four continuous data sets for maternal body weight gain were modeled in support of Health Advisory and RfD derivation: Narotsky et al. (1997); CCC (2000b) and CCC (2000d). Data fitting problems were encountered when attempting to model these data sets using BMDS Version 1.2. Therefore, these data sets were modeled using BMDS Version 1.3. This version was not available when the analysis of dichotomous data sets for other endpoints was performed.

For the Narotsky et al. (1997) study, body weight gain data for gestation days 6 to 8 were modeled. To facilitate modeling, a constant value of 20 was added to each mean so that all modeled data were positive. This procedure is considered an acceptable approach for transforming data prior to modeling continuous data with the BMDS software (W. Setzer, U.S. EPA, personal communication). The BMDS tests for variance rejected the hypothesis that there is a constant variance for this data set. The modeled variance available in BMDS did a good job of describing the variation in the variances (p-value of 0.76), when a constant value of 20 was added to the means.

When the models were fit to the transformed data, modeling the nonconstant variance in terms of the means plus a constant value of 20, the fits to the means (plus 20) were all good. Note that the number of parameters for the power and polynomial models are misspecified in BMDS (because the power hits the bound of 1 and the polynomial is linear), and so the AIC and p-value for fit are incorrect. The correct values can be found in the output for the linear model. The results for the Narotsky et al. (1997) body weight gain data are summarized below:

Model	**Log-likelihood**	**AIC**	**BMD**	**BMDL**
Power (linear)	-84.84	177.68	12.0	9.0
Polynomial	-84.84	177.68	12.0	9.0
Hill	-82.83	177.66	18.3	10.2

Even though the Hill model has two more parameters than the power (linear) model, the decrease in the log-likelihood gained by those extra parameters is enough to give the slight edge to the Hill model in terms of AIC. Thus, the model of choice is the Hill model, since the BMDs were within a factor of 3 of one another).

To validate the choice of the constant used in the modeling of this data set, we investigated the effect of adding different constants to the means, with respect to the estimates from the Hill model. Different constants added to the mean change the parameter estimates obtained in the maximization of the likelihood. In fact, the choice of the constant can be viewed as the determination of another parameter that gives the best fit of the model to the data – in this case allowing the model for the variance to be improved. Note that benchmark responses (BMRs) defined in terms of a change in the mean equal to some multiple of the control standard deviation will be appropriate even with the transformed data, because adding a constant to a set of observations does not alter the standard deviation of the transformed data. Thus, the choice of the BMR defined as 1.1 standard deviations is consistent for any choice of added constant. (Any differences in BMDs and BMDLs noted with different added constants is due to differences in the fitted model parameters, not the definition of the BMR).

In addition to the added constant of 20 that was used for the comparisons above, we also examined adding constants of 10, 15, 25, or 30. The results of adding the different constants on the outcome of the Hill model are summarized below:

Constant Added	**Log-likelihood**	**BMD**	**BMDL**
10	-82.83	18.7	11.1
15	-82.73	18.4	10.6
20	-82.71	18.3	10.2
25	-83.37	19.2	10.4
30	-84.22	19.9	8.5

Because the log-likelihood measures the goodness of fit, it can be seen that the constant of 20 is the best choice from among those that we tried. Since the changes in the BMDs and BMDLs are minor in the region of 20, we did not attempt to fine-tune the choice of the constant any further. For the Narotsky et al. (1997) data set, the Hill model applied to the data (with a constant of 20 added to the means) was selected as the best basis for BMD estimation. We confirmed that the polynomial and power models (which both still defaulted to a linear form) did not yield a log-likelihood as large as that from the Hill model, for the choice of 20 as the added constant. The Hill model yielded a BMD of 18, with a BMDL of 10.

For the CCC (2000b) study, data for body weight gain in rabbits on gestation days 6 to 29 (corrected for gravid uterine weight) were modeled. For this data set, the hypothesis of constant variance could not be rejected at the 0.05 level ($p = 0.20$). Thus, for all of the modeling considered, we have assumed constant variance.

The best-fitting polynomial model was linear. Unfortunately, the linear model did not describe the data well (p-value for goodness of fit less than 0.001). In contrast, both the power model and the Hill model gave adequate fits to the data (p-values of 0.28 and 0.52, respectively). The following table summarizes the outputs for the various models:

Model	Log-likelihood	AIC	BMD	BMDL
Polynomial (linear)	141.10	-276.2	35.4	29.3
Power	146.47	-284.94	50.3	Failed
Hill	147.04	-284.08	53.7	Failed

Even though the Hill model provided a slightly larger log-likelihood, the gain was not sufficient to decrease the AIC below that associated with the power model (the Hill model uses 1 extra parameter, and thus the comparison of the AICs says that the improvement in the fit -- the log-likelihood -- is not enough to make up for the fact that there is that one extra parameter). [Note: the AICs in the BMDS output files are incorrectly calculated because the log-likelihoods are positive numbers rather than negative numbers.] The power model would be the model of choice, given that the BMDs for the two models that fit the data are within a factor of 3. However, BMDS did not complete the calculation of the BMDL for either the power or the Hill model. Nevertheless, it appears that the magnitude of the BMD (around 50 mg/kg) would not make this endpoint the critical one with respect to finding a health-protective starting point for RfD determination (i.e., compare 50 mg/kg to the BMDs from other endpoints).

Two data sets for body weight gain were modeled for the CCC (2000d) study in rats. The decrease in body weight gain was most severe on gestation days 6 to 7. Here, as in the case of the Narotsky et al. (1997) data set, a constant needed to be added to make the model of the variance acceptable. However, this data set was problematic. No model available in BMDS fit the data, regardless of how they were transformed by adding a constant. The power and polynomial models defaulted to the linear form which clearly did not describe the change in the means as a function of dose. The Hill model had the required curvature to describe the pattern of the means as a function of dose, but the problem for that model (as well as for the others) was that no good model of the variance can be determined just by adding a constant to observations. The best constant found was around 10, which reduced the log-likelihood for the fitted model to -246.17. This is compared to the log-likelihood for the independent means, independent variances model of -240.49. The comparison of these two models would not accept the fitted model (p-value slightly less than 0.025). As can be seen on the figure in the electronic Appendix B for this particular choice, the means are well fit by the model, but the variances are not well-modeled, especially in the control group, for which the estimated standard deviation is greater than the observed standard deviation. The BMD for that run is 17.7 and the BMDL is 14.5. These are the smallest from among the runs that we made (using added constants of between 0 and 100, and also the constant variance model), for which the BMDs ranged between 18.3 and 19, and the BMDLs ranged between 15.3 and 15.7. It is clear that the BMD and BMDL estimates are not especially sensitive to the choice of the added constant. Considering that the models overpredict the observed control standard deviation, these BMD estimates may be viewed as slight overestimates, if anything. But because the fits are not particularly good, some caution might be warranted if one is considering using these results as the basis for regulation.

To obtain a more reliable estimate of the BMD for decreased body weight observed in CCC (2000d), body weight gain data were also modeled for gestation days 6 to 9. These data also required transformation with a constant. This was accomplished by starting the search for the constant with a minimum of 30 and a maximum of 500. In this case, it was determined that a value of about 250, added to the means, produced an acceptable model of the change in variance as a function of the mean (p-value of about 0.21), and yielded the largest maximized likelihood for the power model. This result was first compared to other choices of constants around 250 (e.g., 240 and 260), then to values that were progressively further away (e.g., values of 200 and 300, 90 and 400, etc.). We did not fine-tune the estimate of the constant, since the BMD estimates were stable when the added constant was 240, 250, or 260.

The power model was selected for the above search for the constant because it provided a much better fit to the transformed data than did the Hill model or the polynomial model for preliminary choices for the added constant (30 and 55). Because the final choice of the constant was so different from the preliminary choices, we compared the fits of the models to the transformed data using 250 as the added constant and the results are summarized here:

Model	**Log-likelihood**	**AIC**	**BMD**	**BMDL**
Power	-304.942	619.885	22.9	18.4
Polynomial	-336.201	682.403	34.0	Failed
Hill	-413.929	839.858	Failed	Failed

The Hill model did not fit the data at all (perhaps due to a vanishingly small estimate of the parameter k, which may be due to the fact that the model did not correctly maximize the likelihood). The polynomial model did not fit the data well and is much less satisfactory than the power model (compare the AICs in the table above). Even for the power model, the p-value for goodness of fit was 0.036, which is less than the standard critical p-value of 0.05. However, for this data set which is nonmonotonic, the power model does a satisfactory job. Consequently, the reasonable choice for the BMD is 23 and for the BMDL is 18, for this data set.

Table A-5 Benchmark Dose Modeling Results for Bromodichloromethane (Dichotomous Endpoints)

Model	G-O-F p value	AIC	No. paramet.	05		10	
				BMD	BMDL	BMD	BMDL
DEVELOPMENTAL OR REPRODUCTIVE STUDIES							
Bielmeier et al. (2001) Rat Female Full Litter Resorption							
Gamma	1.0	24.9923	3 (2)	36	2.1	42	4.3
Logistic	0.77	25.0528	2 (2)	31	8.6	40	16
Log-logistic	1.0	24.9223	3 (2)	40	0.8	46	1.6
Log-probit	1.0	24.9223	3 (2)	41	5.3	45	7.7
Multistage	0.91	23.1120	1	16	2.1	23	4.2*
Probit	0.83	24.9874	2 (2)	28	7.9	37	15
Weibull	1.0	24.9223	3 (2)	26	2.1	34	4.3
Narotsky et al. (1997) Rat Female Full Litter Resorption							
Gamma	0.68	30.3049	3 (2)	36	11	49	22
Logistic	0.47	31.0964	2 (2)	41	25	54	39
Log-logistic	0.68	30.3427	3 (2)	36	0.13 (?)	48	5.9 (?)
Log-probit	0.72	30.1687	3 (2)	36	21	48	30*
Multistage	0.68	30.4257	2	33	10	47	21
Probit	0.53	30.8108	2 (2)	40	23	52	37
Weibull	0.67	30.3983	3 (2)	35	10	49	22
Ruddick et al. (1983) Rat Female Sternebral Aberrations							
Gamma	0.44	64.2926	3 (3)	16	7.1	30	15
Logistic	0.66	62.5139	2 (2)	22	14	43	28
Log-logistic	0.47	64.2131	3 (3)	19	Failed	33	Failed
Log-probit	0.49	64.1729	3 (3)	23	Failed	37	Failed
Multistage	0.74	62.2980	2	13	7.1	27	15*
Probit	0.67	62.5023	2 (2)	22	14	42	27
Weibull	0.44	64.2965	3 (3)	14	7.1	29	15

Table A-5 (cont.)

Model	G-O-F p value	AIC	No. paramet.	05		10	
				BMD	BMDL	BMD	BMDL
CANDIDATE STUDIES FOR 1-DAY HA							
Lilly et al. (1994) Rat Male Hepatic midzonal vacuolar degeneration (Aqueous vehicle)							
Gamma	0.63	3.8029	3 (1)	187	135	206	156
Logistic	1.0	4.0000	2 (2)	290	145	292	174
Log-logistic	0.99	2.0468	3 (1)	240	164	250	182
Log-probit	1.0	2.0000	3 (1)	258	168	263	182*
Multistage	1.0 (?)	298.31	2	6.4	0.1	9.2	0.28
Probit	1.0	4.0000	2 (2)	281	184 (W)	285	187 (W)
Weibull	1.0	2.0006	3 (1)	294	144	307	170
Lilly et al. (1994) Rat Male Renal Tubule degeneration (Aqueous vehicle)							
Gamma	1.0	9.6389	3 (1)	119	4.3	131	8.9*
Logistic	1.0	11.6382	2 (2)	163	19	171	35
Log-logistic	1.0	9.6382	3 (1)	163	0.00078 (?)	170	0.00625 (?)
Log-probit	1.0	11.6382	3 (2)	152	11	160	16
Multistage	1.0 (?)	102.1000	2	6.4	0.13	9.2	0.12
Probit	1.0	11.6302	2 (2)	132	17	144	33
Weibull	1.0	11.6302	3 (2)	92	4.4	110	8.9
Thornton-Manning et al. (1994) Rat Female Hepatic centrilobular vacuolar degeneration (Poor fit: no model selected							
Gamma	0.01	19.0705	3 (2)	40	5.5	53	11
Logistic	<0.01	21.4063	2 (2)	34	16	54	29
Log-logistic	<0.01	17.3420	3 (2)	55	13	67	22
Log-probit	0.02	17.9809	3 (2)	53	16	64	22
Multistage	0.01	20.1038	2	17	5.0	31	10
Probit	<0.01	219.846	2 (2)	31	15	52	29
Weibull	0.02	19.6598	3 (2)	23	5.1	37	10
Thornton-Manning et al. (1994) Rat Female Renal tubular degeneration							
Gamma	1.0	10.3742	3 (1)	99	45	109	60

Table A-5 (cont.)

Model	G-O-F p value	AIC	No. paramet.	05		10	
				BMD	BMDL	BMD	BMDL
Logistic	1	12.3178	2 (2)	138	42	141	63
Log-logistic	1	10.3178	3 (1)	127	51	133	65*
Log-probit	1	12.3178	3 (2)	123	54	128	66
Multistage	1 (?)	445.4650	2	3.4	0.06	4.4	0.08
Probit	1	12.3178	2 (2)	128	38	133	58
Weibull	1	12.3179	3 (2)	127	39	133	56
CANDIDATE STUDIES FOR THE 10-DAY HA							
Condie et al. (1983) Mouse Male Hepatic centrilobular pallor							
Gamma	0.19	30.0268	3 (2)	11	2.2	17	4.5
Logistic	<0.01	33.9633	2 (2)	14	7.9	23	14
Log-logistic	0.30	28.7294	3 (2)	19	4.4	24	7.5*
Log-probit	0.26	29.2521	3 (2)	18	6.0	23	8.6
Multistage	0.16	30.6962	2	4.2	2.1	8.5	4.3
Probit	<0.01	34.7900	2 (2)	13	7.6	22	14
Weibull	0.18	30.3429	3 (2)	7.2	2.1	12	4.4
Condie et al. (1983) Mouse Male Renal epithelial hyperplasia							
Gamma	0.88	35.6018	3 (2)	75	46	83	56
Logistic	0.10	40.0819	2 (2)	25	15	40	27
Log-logistic	0.98	35.3785	3 (2)	112	48	117	58
Log-probit	0.84	37.3705	3 (3)	109	51	113	59
Multistage	0.38	37.5968	2	46	17	58	31
Probit	0.08	40.7541	2 (2)	21	13	35	24
Weibull	0.98	35.3785	3 (2)	120	41	125	53*
Aida et al. (1992a) Rat Female Liver cell vacuolization							
Gamma	0.56	23.4101	3 (2)	18	8.5	36	17
Logistic	0.23	25.2544	2 (2)	50	27	83	49
Log-logistic	0.60	23.2879	3 (2)	19	0.0015 (?)	35	0.1 (?)
Log-probit	0.65	21.5114	3 (2)	34	20	49	28

Table A-5 (cont.)

Model	G-O-F p value	AIC	No. paramet.	05		10	
				BMD	BMDL	BMD	BMDL
Multistage	0.78	21.4146	1	16	8.5	34	17*
Probit	0.25	25.0481	2 (2)	46	25	76	46
Weibull	0.57	23.4143	3 (2)	17	8.5	34	17
Melnick et al. (1998) Mouse Female Hepatocyte hydropic degeneration							
Gamma	0.99	26.2625	3 (2)	28	4.2	35	8.5
Logistic	0.82	26.8455	2 (2)	24	11	36	20
Log-logistic	0.91	26.5313	3 (2)	31	11	38	16
Log-probit	0.96	26.3532	3 (2)	32	12	38	17
Multistage	1.0?	445.4650	2	3.4	0.076	4.4	0.082
Probit	0.89	26.5905	3(2)	24	11	34	19
Weibull	1.0	26.2278	3(2)	23	4.2	31	8.4*
NTP (1998) Rat Male Single cell hepatic necrosis							
Gamma	1.0	14.4941	3 (1)	26	12	28	15.125
Logistic	1.0	16.3653	2 (2)	34	12	35	16.9848
Log-logistic	1.0	14.3662	3 (1)	33	15	34	18.4508*
Log-probit	1.0	16.3653	3 (2)	33	15	334	17.4679
Multistage	0.93	15.1127	1	16	4.8	20	9.4
Probit	1.000	16.3653	2 (2)	31	10	32	15
Weibull	1.000	16.3653	3 (2)	30	9.8	32	14
CANDIDATE STUDIES FOR THE LONGER-TERM HA							
NTP (1987) Mouse Female Hepatic Vacuolated Cytoplasm							
Gamma	0.27	32.2103	3 (2)	57	28	73	42
Logistic	0.04	37.1630	2 (2)	54	33	80	54
Log-logistic	0.38	31.1109	3 (2)	60	33	74	46
Log-probit	0.41	30.9082	3 (2)	62	35	75	47*
Multistage	0.32	31.6422	1	44	20	63	37
Probit	0.05	36.4925	2 (2)	55	32	80	54
Weibull	0.20	33.6311	3 (2)	46	21	65	35

Table A-5 (cont.)

Model	G-O-F p value	AIC	No. paramet.	05		10	
				BMD	BMDL	BMD	BMDL
CANDIDATE STUDIES FOR THE RfD							
Aida et al. (1992b) Rat Female Hepatic Fatty Degeneration							
Gamma	1.0	46.8266	--	5.1	0.71	5.8	1.4
Logistic	0.99	44.8652	--	2.0	1.3	3.4	2.3
Log-logistic	1.0	42.8266	--	6.7	2.0	7.1	2.9
Log-probit	1.0	46.8266	--	5.9	2.0	6.4	2.7
Multistage	1.0	48.8266	--	2.9	0.56	4.0	1.1
Probit	1.0	44.8296	--	1.8	1.2	3.1	2.1*
Weibull	1.0	46.8266	--	3.3	0.65	4.4	1.3
Aida et al. (1992b) Rat Male Hepatic Fatty Degeneration							
Gamma	1.0	29.3001	3 (2)	1.2	0.39	2.1	0.80
Logistic	0.15	34.2633	2 (2)	2.6	1.5	4.4	2.7
Log-logistic	0.98	29.3760	3 (2)	2.1	0.51	3.0	0.94
Log-probit	1.0	29.3074	3 (2)	2.2	0.99	3.0	1.4
Multistage	1.0	29.3001	2	0.73	0.43	1.5	0.88
Probit	0.17	33.8989	2 (2)	2.6	1.6	4.4	2.9
Weibull	1.0	29.3001	3 (2)	1.1	0.39	1.9	0.80*
Aida et al. (1992b) Rat Male Hepatic Granulomas							
Gamma	0.99	34.9241	3 (1)	1.0	0.67	2.1	1.4
Logistic	0.13	42.0098	2 (2)	4.2	2.6	7.0	4.6
Log-logistic	0.66	38.0328	3 (2)	1.9	0.37	3.0	0.82
Log-probit	0.87	35.6192	3 (1)	2.4	1.6	3.5	2.3
Multistage	0.95	36.8960	2	1.1	0.67	2.2	1.4
Probit	0.15	41.5172	2 (2)	3.8	2.4	6.4	4.4
Weibull	0.99	34.9241	3 (1)	1.0	0.67	2.1	1.4*
NTP (1987) Mouse Male Hepatic Focal Necrosis							
Gamma	1.0	15.6352	3 (1)	44	27	49	34
Logistic	1.0	17.4602	2 (2)	65	30	66	38

Table A-5 (cont.)

Model	G-O-F p value	AIC	No. paramet.	05		10	
				BMD	BMDL	BMD	BMDL
Log-logistic	1.0	15.4604	3 (1)	59	28	61	34
Log-probit	1.0	17.4602	3 (2)	57	28	60	34
Multistage	1.0	16.0769	1	40	23	47	32
Probit	1.0	17.4602	2 (2)	60	28	62	36
Weibull	1.0	15.4603	3 (1)	60	27	63	35*
NTP (1987) Mouse Male Kidney Cytomegaly							
Gamma	0.76	72.3705		0.58	0.47	1.2	0.96
Logistic	<0.01	88.5494		3.3	2.1	5.3	3.8
Log-logistic	1.0	73.8361		1.5	3.6E-9	2.2	1.1E-7
Log-probit	0.99	71.8572		1.4	1.1	2.0	1.5*
Multistage	0.45	74.3705		0.58	0.47	1.2	0.96
Probit	<0.01	92.6760		2.8	2.0	4.8	3.6
Weibull	0.76	72.3705		0.58	0.47	1.2	0.96

* Selected model result for endpoint.
AIC Akaike Information Criterion
BMD Benchmark Dose
BMDL 95% lower confidence level on BMD
G-O-F Goodness-of-Fit
HA Health Advisory
? Results questionable on the basis of visual inspection or probable calculation error
W BMDS gave a warning message: “BMDL computation is at best imprecise for these data”

2. Dibromochloromethane

BMDS modeling results for dibromochloromethane are summarized in Table A-6 below. Detailed output for each model run is compiled in Appendix B, provided in electronic format on compact disk.

a. Developmental and Reproductive Studies

No data were modeled. The generation F2b day 14 postnatal body weight data of Borzelleca and Carchman (1982) were considered for modeling. However, the study authors did not report the number of litters examined for this continuous endpoint. Since this information is required as input, the data could not be modeled.

b. One-day Health Advisory

No data were modeled for the One-day HA.

c. Ten-day Health Advisory

Seven data sets were modeled in support of the Ten-day HA. When data were analyzed for the liver cell vacuolation in female rats (Aida et al. 1992a), the multistage model gave questionable results and was eliminated from consideration. The remaining BMDL values were within a factor of 3, so the estimate from the Weibull and gamma models was selected on the basis of the smallest AIC. For the same endpoint in male rats (Aida et al. 1992a), model fit was adequate in all cases and all BMDL values were within a factor of 3. The multistage model was selected on the basis of the smallest AIC.

Liver and kidney histopathology data from the study by Condie et al. (1983) were analyzed. In the analysis of kidney data, the BMDLs calculated by the logistic and probit models were eliminated because the models fit the data poorly. Among the remaining models, the log-logistic BMDLs are smallest by more than a factor of 3. Thus, this result was examined as a possible outlier. Although this model does at times have difficulty calculating reasonable lower bounds, the BMDs in this case are also smaller than the BMDs from the other models (although not by a factor of 3 for all them – the BMDLs calculated by the logistic model are more than 3-fold below any other BMDL). In addition, the AIC value is much lower for log-logistic than for the other models. Thus, the BMDL calculated by the log-logistic model was selected.

With respect to the Condie et al. (1983) liver histopathology data, the log-logistic model gives the smallest BMDL by more than a factor of 3. However, the BMDs are within a factor of 3 of the BMDs generated by other models and the AIC for the log-logistic model is not the lowest. The log-logistic results were therefore considered as outliers. Among the remaining options, the Weibull and gamma models have the lowest AIC, and the BMDL calculated by these models was selected.

Data for stomach nodules in male and female rats (NTP, 1985) were also analyzed. In females, all calculated BMDLs are within a factor of 3, so the result from the multistage model was selected on the basis of having the smallest AIC. In males, the log-logistic model has the smallest AIC and the lowest BMDL.

In the analysis of the Melnick et al. (1998) results for hepatic hydropic degeneration in female mice, the multistage model failed to fit the data. All remaining results were similar and the BMDL calculated by the log-logistic model was selected on the basis of the lowest AIC.

d. Longer-term Health Advisory

Two data sets for hepatic lesions reported in Chu et al. (1982b) were modeled in support of the Longer-term HA. When liver histopathology data for male rats was analyzed, the log-logistic model calculated a BMDL that was more than 3-fold lower than those from some other models. However, both the AIC and the BMD calculated by the log-logistic model were the lowest among all models. The BMDL calculated by this model was thus selected. When data for liver lesions in female rats were analyzed, no model adequately fit the data (all p values were less than 0.1). Thus, no BMDL was selected from this data set.

Data for fatty metamorphosis in the liver of male rats that had previously been modeled using the Crump software was reanalyzed using the BMDS program. All models adequately fit the data for this endpoint. All resulting BMDL values were within a factor of three, with the exception of the estimate calculated using the log-probit model The value calculated using the probit model was selected on the basis of the lowest AIC.

e. RfD

Two data sets from the NTP (1985) chronic oral exposure study were modeled using the BMDS software in support of the RfD for dibromochloromethane. These data sets were selected after inspection of the results for BMD modeling of key dibromochloromethane endpoints using the Crump software (K. S. Crump, Inc.). For fatty metamorphosis in the liver of male rats, all models fit the data acceptably, although the p value for the probit model was marginal ($p = 0.16$). All BMDL values were within a factor of three, with the exception of the log-logistic model. Results from the log-logistic model were selected on the basis of the lowest AIC value. For ground glass cytoplasm in the liver of male rats, all models fit the data acceptably and the BMDL values were within a factor of three. The results for the probit model were selected on the basis of the lowest AIC.

Table A-6 Benchmark Dose Modeling Results for Dibromochloromethane

Model	G-O-F p value	AIC	No. paramet.	05		10	
				BMD	BMDL	BMD	BMDL
DEVELOPMENTAL AND REPRODUCTIVE STUDIES (NONE)							
CANDIDATE STUDIES FOR 1-DAY HA (NONE)							
CANDIDATE STUDIES FOR 10-DAY HA							
Aida et al. (1992a) Rat Female Liver cell vacuolization							
Gamma	1.0	15.4833	3 (2)	24	3.2	30	6.7
Logistic	0.91	15.7862	2 (2)	24	10	34	17
Log-logistic	0.99	15.5265	3 (2)	24	7.5	30	12
Log-probit	1.0	15.4078	3 (2)	25	8.4	30	12
Multistage	1 (?)	347	2	3.4	0.063	4.4	0.064
Probit	0.95	15.6586	2 (2)	22	8.9	32	16
Weibull	1.0	15.4833	3 (2)	21	3.2	29	6.7*
Aida et al. (1992a) Rat Males Liver cell vacuolization							
Gamma	0.76	20.0153	3 (2)	12	2.5	18	5.1
Logistic	0.77	20.0459	2 (2)	17	8.0	26	14
Log-logistic	0.56	20.8287	3 (2)	14	2.7	20	5.3
Log-probit	0.57	20.7294	3 (2)	14	6.0	19	8.6
Multistage	0.98	19.3422	2	7.0	2.7	14	5.5*
Probit	0.80	19.9157	2 (2)	15	7.4	24	13
Weibull	0.83	19.7280	3 (2)	12	2.6	18	5.3
Condie et al (1983) Mouse Male Renal mesangial hypertrophy							
Gamma	0.12	36.8675	3 (1)	3.8	2.6	7.8	5.3
Logistic	<0.01	47.0265	2 (2)	12	7.7	22	15
Log-logistic	0.49	33.8237	3 (1)	16	0.7	3.5	1.6*
Log-probit	0.11	36.7799	3 (1)	8.9	5.6	13	8.1
Multistage	0.12	36.8675	2 (1)	3.8	2.6	7.8	5.3
Probit	<0.01	46.9299	2 (2)	12	8.0	22	16
Weibull	0.12	36.8675	3 (1)	3.8	2.6	7.8	5.3
Condie et al. (1983) Mouse Male hepatic cytoplasmic vacuolization							

Table A-6 (cont.)

Model	G-O-F p value	AIC	No. paramet.	05		10	
				BMD	BMDL	BMD	BMDL
Gamma	0.32	43.8699	3 (2)	5.4	3.4	11	6.9
Logistic	0.15	45.1424	2 (2)	15	9.4	27	18
Log-logistic	0.33	43.9124	3 (2)	3.3	1.5	7.0	3.3
Log-probit	0.23	44.4103	3 (2)	14	8.5	20	12
Multistage	0.13	45.8578	3	5.7	3.4	12	6.9
Probit	0.16	44.9950	2 (2)	14	9.2	26	18
Weibull	0.32	43.8699	3 (2)	5.4	3.4	11	6.9*
NTP (1985) Mouse Female Stomach nodules							
Gamma	0.98	16.2743	3 (2)	167	38	225	78
Logistic	0.86	17.1462	2 (2)	209	104	284	170
Log-logistic	0.98	16.2955	3 (2)	163	34	222	73
Log-probit	0.99	16.1399	3 (2)	166	74	218	106
Multistage	0.99	14.3879	1	152	37	218	77*
Probit	0.90	16.8628	2 (2)	197	95	267	158
Weibull	0.97	16.3761	3 (2)	162	37	227	77
NTP (1985) Mouse Male Stomach nodules							
Gamma	0.91	18.5058	3 (1)	75	33	153	67
Logistic	0.64	21.8294	2 (2)	191	97	306	168
Log-logistic	0.93	18.5021	3 (1)	68	26	143	54*
Log-probit	0.68	19.4319	3 (1)	122	69	176	99
Multistage	0.91	18.5858	1	75	33	153	67
Probit	0.65	21.7110	2 (2)	174	88	284	154
Weibull	0.91	18.5858	3 (1)	75	33	153	67
Melnick et al (1998) Mouse Female hepatic hydropic degeneration							
Gamma	0.99	12.6487	3 (1)	76	55	84	64
Logistic	1.0	14.0080	2 (2)	123	56	126	70
Log-logistic	1.0	12.0080	3 (1)	108	59	112	68*
Log-probit	1.0	14.0080	3 (2)	107	60	111	68

Table A-6 (cont.)

Model	G-O-F p value	AIC	No. paramet.	05		10	
				BMD	BMDL	BMD	BMDL
Multistage	program	failed!	--	--	--	--	--
Probit	1.0	14.0080	2 (2)	112	56	116	68
Weibull	1.0	14.0080	3 (2)	112	56	117	68
CANDIDATE STUDIES FOR LONGER-TERM HA							
Chu et al. (1982b) Rat Male Hepatic lesions							
Gamma	0.84	65.3956	3 (2)	14	6.2	29	13
Logistic	0.79	65.6217	2 (2)	22	12	43	23
Log-logistic	0.88	65.1734	3 (2)	8.6	2.5	18	5.3*
Log-probit	0.72	65.8723	3 (2)	37	15	54	22
Multistage	0.84	65.3956	2	14	6.2	29	13
Probit	0.79	65.6176	2 (2)	22	12	43	24
Weibull	0.84	65.3956	3 (2)	14	6.2	29	13
Chu et al. (1982b) Rat Female Hepatic lesions							
Gamma	0.04	67.0864	3 (3)	Flat Curve	Estimated	No BMDs	–
Logistic	0.09	64.3869	2 (2)	66	26	127	49
Log-logistic	0.09	64.3474	3 (2)	48	9.8	101	21
Log-probit	0.04	67.0864	3 (3)	4800	38	6900	54
Multistage	0.09	64.3615	2	54	15	110	30
Probit	0.09	64.3843	2 (2)	64	25	125	48
Weibull	0.09	64.3615	3 (2)	54	15	110	30
NTP 1985 Rat Male Fatty Metamorphosis (Subchronic)							
Gamma	0.90	42.3900	3 (3)	2.6	0.44	3.9	0.91
Logistic	0.97	40.3442	2 (2)	1.2	0.76	2.4	1.5
Log-logistic	0.81	42.9172	3 (3)	4.1	0.20	5.5	0.42
Log-probit	0.85	42.6546	3 (3)	4.3	1.1	5.4	1.6
Multistage	0.92	43.8670	4	1.0	0.49	2.1	1.0
Probit	0.98	40.1651	2 (2)	1.3	0.84	2.5	1.7*
Weibull	0.92	42.2885	3 (3)	2.4	0.45	3.9	0.92

Table A-6 (cont.)

Model	G-O-F p value	AIC	No. paramet.	05		10	
				BMD	BMDL	BMD	BMDL
CANDIDATE STUDIES FOR THE RfD							
NTP (1985) Rat Male Hepatic Fatty Metamorphosis - Chronic							
Gamma	0.53	105.8690	3 (2)	0.81	0.57	1.7	1.2
Logistic	0.34	106.2850	2 (2)	1.2	0.86	2.4	1.7
Log-logistic	1.0	107.4950	3 (3)	1.7	0.071	2.7	0.15
Log-probit	0.86	105.5270	3 (2)	1.9	1.1	2.7	1.6*
Multistage	0.53	105.8690	2	0.81	0.57	1.7	1.2
Probit	0.16	107.2230	2 (2)	1.4	1.1	2.8	2.2
Weibull	0.53	105.8690	3 (2)	0.81	0.57	1.7	1.2
NTP (1985) Rat Male Ground Glass Cytoplasm - Chronic							
Gamma	1.0	181.2470	3 (3)	6.1	2.4	10	5.0
Logistic	0.58	179.5560	2 (2)	6.3	5.1	12	9.7
Log-logistic	1.0	181.2470	3 (3)	7.6	1.6	12	3.5
Log-probit	1.0	181.2470	3 (3)	9.1	6.3	13	9.1
Multistage	1.0	181.2470	3	4.3	2.4	8.6	5.0
Probit	0.62	179.4870	2 (2)	6.0	4.9	11	9.4*
Weibull	1.0	181.2470	3 (3)	5.5	2.4	9.8	5.0

*Selected model result for endpoint.

AIC Akaike Information Criterion
BMD Benchmark Dose
BMDL 95% lower confidence level on BMD
G-O-F Goodness-of-Fit
HA Health Advisory
? Results questionable on the basis of visual inspection or probable calculation error
W BMDS gave a warning message: “BMDL computation is at best imprecise for these data”

3. Bromoform

BMDS modeling results for dibromochloromethane are summarized in Table A-7 below. Detailed output for each model run is compiled in Appendix B, provided in electronic format on compact disk.

a. Developmental and Reproductive Studies

Data from the study by Ruddick et al. (1983) consisted of the count of the numbers of litters that had one or more fetuses with sternebral variations. This expression of the response rates does not correspond directly to the probability of a response in the offspring of treated dams. All of the model fit the data and all of the BMDL results for 10% extra risk are within a factor of three of one another, so the results from the log-probit model were selected, because that model had the lowest AIC.

b. One-day Health Advisory

BMD calculations were not conducted in support of the One-day HA due to a lack of appropriate data.

c. Ten-day Health Advisory

Six data sets were modeled in support of the Ten-day HA. Modeling results for each data set were evaluated using the criteria given in Section C. For the Aida et al. (1992a) data on liver cell vacuolation in female rats, all models fit well and (with one exception) give the same AIC. The log-logistic results appear to be qualitative outliers because they are more than 3 times less than the next closest BMDLs, even though the BMD is the second largest. The BMDL calculated by this model was thus rejected in favor of the next lowest BMDL (Weibull model). When the same endpoint was modeled in male rats (Aida et al. 1992), the probit model either failed or gave a warning message for the lower bound calculations and results were thus eliminated. The remaining models calculated very similar BMDLs. The Weibull model was selected because it gave the lowest AIC among the remaining models.

For histopathological effects in the kidney of male mice (Condie et al., 1983), two models (logistic and probit) had somewhat higher BMDLs and the largest values for AIC. If these results are eliminated as qualitative outliers, the remaining BMDLs are within a factor of 3. The Log-probit model was selected from among the remaining models because it gave the lowest AIC. Modeling of data on liver histopathology from the same study gave a similar pattern of results. Results from the probit and logistic models were eliminates as qualitative outliers (high AICs and BMDLs that were higher by more than a factor of 3 from the lowest BMDL). The remaining BMDLs are within a factor of 3 and so the Log-probit model was selected because it had the lowest AIC.

Melnick et al. (1998) reported data for hydropic degeneration in the liver of female mice. The multistage model gave questionable results (very high AIC and a goodness of fit p value that appeared unrealistically high when the model fit was examined visually) for this data set that

appeared to reflect a calculation error in the BMDS software. The BMDLs estimated by the remaining models are very close. The Log-probit model was selected because it has the lowest AIC. When data for stomach nodules in male mice were modeled (NTP, 1989a), all models gave an acceptable fit and all BMDLS were within a factor of three. The results from the multistage model were selected because it had the lowest AIC.

d. Longer-term Health Advisory

Three data sets were modeled in support of the longer-term Health Advisory for Bromoform. No models adequately fit (i.e. all p values for goodness of fit were less than 0.1) the Chu et al. (1982b) data for hepatic lesions in female rats (nonmonotonic dose response). Thus, none of the calculated BMDLs were candidates for deriving the Longer-term Health Advisory. For the same endpoint in male rats (Chu et al. 1982b), the multistage model gave a bad fit and was eliminated from consideration. The log-logistic BMDLs are the lowest of the remaining values, but there is a spread of greater than 3. The result for the log-logistic model was eliminated as a qualitative outlier, since this model gave the largest BMDs but the BMDLs were among the lowest observed (i.e. gave a wide confidence interval). The remaining BMDL values were similar and the probit model was selected because it gave the lowest AIC value. Modeling of the NTP (1989a) data for hepatic vacuolation in female mice gave similar results across all models. The Log-probit model was selected because it gave the lowest AIC .

e. RfD

Two data sets from the oral exposure study conducted in rats by NTP (1989a) were modeled using the BMDS program for consideration in derivation of the RfD. For hepatic vacuolization in male rats exposed to bromoform for 13 weeks, no fit was obtained for the multistage model. The BMDL values calculated using the remaining models were with a factor of 3, with the exception of the log-logistic model. The results from the Weibull model were selected on the basis of the lowest AIC value. With respect to data for fatty changes in the liver of male rats chronically exposed to bromoform (NTP, 1989a), all models gave acceptable fits. BMDLs calculated by all models except the log-logistic were within a factor of 3. The log-logistic model was eliminated as a qualitative outlier, since it gave the highest BMDs but very low BMDLs (i.e. it resulted in a very wide confidence interval). Of the remaining models, the lowest AIC was observed for the multistage and it was therefore selected.

Table A-7 Benchmark Dose Modeling Results for Bromoform

Model	G-O-F p value	AIC	No. paramet.	05		10	
				BMD	BMDL	BMD	BMDL
REPRODUCTIVE STUDIES							
Ruddick et al. (1983) Rat Females Sternebral Aberrations							
Gamma	0.85	67.302	3 (3)	16	9.2	32	19
Logistic	0.70	66.0887	2 (2)	33	23	59	42
Log-logistic	0.90	67.3517	3 (3)	19	6.4	35	14
Log-probit	0.87	65.6053	3 (2)	35	23	50	33*
Multistage	0.85	67.339	3	15	9.1	31	19
Probit	0.74	65.9551	2 (2)	30	21	55	40
Weibull	0.85	67.3711	3 (3)	16	9.2	32	19
CANDIDATE STUDIES FOR 1-DAY HA (NONE)							
CANDIDATE STUDIES FOR 10-DAY HA							
Aida et al. (1992a) Rat Females Liver cell vacuolization							
Gamma	1.0	12.3758	3(2)	23	1.1	28	2.3
Logistic	1.0	12.3758	2 (2)	45	5.2	47	9.6
Log-logistic	1.0	12.3758	3 (2)	42	0.29	45	0.61
Log-probit	1.0	12.3758	3 (2)	32	2.6	35	3.7
Multistage	1.0	14.3758	3	9.1	1.9	14	2.4
Probit	1.0	12.3758	2 (2)	36	4.8	40	9.0
Weibull	1.0	12.3758	3 (2)	11	1.1	16	2.3*
Aida et al. (1992a) Rat Males Liver cell vacuolization							
Gamma	0.99	2.2426	3 (1)	73	44	81	53
Logistic	1.0	4.0000	2 (2)	116	56	118	57
Log-logistic	1.0	2.0014	3 (1)	91	48	95	56
Log-probit	1.0	4.0000	3 (2)	95	49	93	56
Multistage	0.76	4.2093	1	46	14	59	28
Probit	1.0	4.0000	2 (2)	118	failed	121	58 (W)
Weibull	1.0	2.0000	3 (1)	134	40	140	51*
Condie et al. (1983) Mouse Male Renal mesangial nephrosis							

Table A-7 (cont.)

Model	G-O-F p value	AIC	No. paramet.	05		10	
				BMD	BMDL	BMD	BMDL
Gamma	0.46	32.2294	3 (2)	45	9.1	64	19
Logistic	0.13	38.0766	2 (2)	54	30	85	54
Log-logistic	0.51	31.9498	3 (2)	49	6.6	68	14
Log-probit	0.53	31.8168	3 (2)	57	23	73	34*
Multistage	0.40	32.6120	2	29	8.7	51	18
Probit	0.16	34.4823	2 (2)	52	29	82	52
Weibull	0.44	32.4190	3 (2)	35	8.9	56	18
Condie et al. (1983) Mouse Male Centrilobular pallor							
Gamma	0.38	30.0854	3 (2)	44	7.4	61	15
Logistic	0.10	33.0715	2 (2)	46	25	73	45
Log-logistic	0.46	29.5813	3 (2)	51	8.8	67	17
Log-probit	0.46	29.5362	3 (2)	56	20	70	28*
Multistage	0.31	30.6200	2	29	7.0	49	14
Probit	0.11	32.6482	2 (2)	45	25	71	44
Weibull	0.35	30.4002	3 (2)	32	7.2	50	15
Melnick et al. (1998) Mouse Female Liver hydropic degeneration							
Gamma	0.99	8.3184	3 (1)	177	111	196	135
Logistic	1.0	10.2790	2 (2)	190	85	199	123
Log-logistic	1.0	8.2790	3 (1)	191	123	199	146*
Log-probit	1.0	10.2790	3 (2)	189	127	198	146
Multistage	1.0 (?)	396.414	2	64	0.099	9.2	0.16
Probit	1.0	10.2790	2 (2)	182	78	197	115
Weibull	1.0	10.2790	3 (2)	172	88	196	118
NTP (1989a) Mouse Male Stomach nodules							
Gamma	0.43	20.0036	3 (2)	165	46	208	82
Logistic	0.22	22.0653	2 (2)	158	77	223	131
Log-logistic	0.48	19.6930	3 (2)	165	55	206	89
Log-probit	0.50	19.4922	3 (2)	176	66	211	95

Table A-7 (cont.)

Model	G-O-F p value	AIC	No. paramet.	05		10	
				BMD	BMDL	BMD	BMDL
Multistage	055	18.7358	1	116	32	167	66*
Probit	0.25	21.5725	2 (2)	162	74	222	127
Weibull	0.38	20.6462	3 (2)	137	35	188	68
CANDIDATE STUDIES FOR THE LONGER-TERM HA							
Chu et al. (1982b) Rat Female Hepatic lesions							
Gamma	flat	curve	fit	no BMD	–	--	--
Logistic	0.06	53.0088	2 (2)	38	23	69	44
Log-logistic	0.07	51.4919	3 (2)	10	4.1	21	8.6
Log-probit	0.05	52.6957	3 (2)	36	18	51	26
Multistage	0.07	51.9025	2	16	8.2	32	17
Probit	0.06	52.9040	2 (2)	35	22	65	42
Weibull	0.07	51.9025	3 (2)	16	8.2	32	17
Chu et al.(1982b) Rat Male Liver lesions							
Gamma	0.45	56.7134	3 (3)	31	1.6	36	3.3
Logistic	0.66	54.7588	2 (2)	5.1	2.8	10	5.6
Log-logistic	0.45	56.7134	3 (3)	45	0.8	48	1.6
Log-probit	0.45	56.7134	3 (3)	37	4.3	41	6.1
Multistage	<0.01	202.7	2	2.5	1.1	3	1.9
Probit	0.67	54.6647	2 (2)	5.3	3.0	10	5.9*
Weibull	0.45	56.7024	3 (3)	13	1.6	19	3.3
NTP (1989a) Mouse Female Hepatic vacuolization							
Gamma	0.73	30.3981	3 (2)	69	35	82	51
Logistic	0.27	33.9813	2 (2)	68	41	96	66
Log-logistic	0.80	30.0241	3 (2)	70	38	87	54
Log-probit	0.85	29.6545	3 (2)	74	41	88	55*
Multistage	0.62	31.6064	2	53	24	76	45
Probit	0.33	33.0847	2 (2)	68	40	95	64

Table A-7 (cont.)

Model	G-O-F p value	AIC	No. paramet.	05		10	
				BMD	BMDL	BMD	BMDL
Weibull	0.61	31.3715	3 (2)	56	28	81	46
CANDIDATE STUDIES FOR THE RfD							
NTP (1989a) Rat Male Hepatic vacuolation - Subchronic							
Gamma	0.70	65.8698	3 (2)	2.2	1.3	4.4	2.6
Logistic	0.65	66.1399	2 (2)	3.9	2.4	7.3	4.7
Log-logistic	0.49	68.4402	3 (3)	2.5	0.45	4.4	0.94
Log-probit	0.61	66.4811	3 (2)	5.5	2.9	8.0	4.2
Multistage	-	661.889	6	2.1	0.0064	2.4	0.0084
Probit	0.65	66.0888	2 (2)	3.9	2.6	7.8	5.3
Weibull	0.70	65.8698	3 (2)	2.2	1.3	4.4	2.6*
NTP (1989a) Rat Male Fatty Changes in Liver - Chronic							
Gamma	1.0	84.7983	3 (3)	29	0.66	32	1.4
Logistic	0.89	82.8323	2 (2)	1.9	1.2	3.8	2.4
Log-logistic	1.0	82.7983	3 (2)	51	0.016	53	0.033
Log-probit	1.0	84.7983	3 (3)	38	0.97	41	1.4
Multistage	0.99	82.7983	2	8.9	0.66	13	1.4*
Probit	0.98	82.7996	2 (2)	2.2	1.6	4.5	3.2
Weibull	1.0	84.7983	3 (3)	12	0.66	16	1.4

*Selected model results for endpoint.

AIC Akaike Information Criterion
BMD Benchmark Dose
BMDL 95% lower confidence level on BMD
G-O-F Goodness-of-Fit
HA Health Advisory
? Results questionable on the basis of visual inspection or probable calculation error
W BMDS gave a warning message: “BMDL computation is at best imprecise for these data”

APPENDIX B (Electronic Format)

Appendix B contains BMD Modeling Output in Electronic Format (compact disk)

APPENDIX C

Determination of the Relative Source Contribution for Dibromochloromethane (DBCM)

The relative source contribution (RSC) is the percentage of total daily exposure that is attributable to tap water when all potential sources are considered (e.g., air, food, soil, and water). Ideally, the RSC is determined quantitatively using nationwide, central tendency and/or high-end estimates of exposure from each relevant medium. In the absence of such data, a default RSC ranging from 20% to 80% may be used. The RSC used in the current and previous drinking water regulations for DBCM is 80%. This value was determined by use of a screening level approach to estimate and compare exposure from various sources. Information considered for DBCM during this process is summarized below.

The initial step in RSC determination is problem formulation, including identification of population(s) of concern, critical health effects, and relevant exposure sources and pathways. The occurrence of DBCM in tap water is reasonably well documented. Occurrence is widespread as a result of disinfection of drinking water, resulting in broad exposure of the U.S. general population. For chronic exposure to DBCM, the most sensitive responses in animal studies are histopathological changes in the liver. There is no evidence that children or the fetus are more sensitive to these effects than are adults. Although polymorphisms in metabolizing enzymes might predispose some groups to greater sensitivity to this compound, no sensitive subpopulations have yet been clearly identified. Therefore, the population of concern for exposure to DBCM is considered to be the U.S. general population.

Production and use of DBCM occur mainly on a limited scale in the United States. In the past, brominated trihalomethanes have been used in pharmaceutical manufacturing and chemical synthesis, as ingredients in fire-resistant chemicals and gauge fluids, and as solvents for waxes, greases, resins, and oils (U.S. EPA, 1975). However, use patterns have changed over time. DBCM is now reportedly used in laboratory quantities only (ATSDR, 1990). Thus, releases to the environment are not anticipated to be significant on a nationwide basis when compared to occurrence in disinfected tap water.

DBCM has been detected in air and food in a few studies, in addition to its presence in tap water. No data were available in the materials reviewed for levels of DBCM in soil. DBCM is expected to volatilize readily from wet or dry soil surfaces based on its Henry's Law constant and vapor pressure (U.S. EPA, 1987). For this reason, exposure via ingestion of soil is not expected to be a significant route of exposure. Therefore, water, food, and air are considered to be the relevant pathways for this analysis.

Evaluation of Occurrence Data

The next step in RSC determination is to judge whether or not adequate data exist to characterize exposure from relevant exposure pathways. Factors to be considered in the evaluation of data adequacy include sample size; whether the data represent a random sample and are representative of the target population; acceptable analytical detection limits; statistical distribution of the data, and estimator precision. In addition, it is important to know whether the data are representative of current conditions. The available occurrence data for DBCM in water, air, food, and soil are summarized in Chapter IV of this document. Relevant information from that chapter is also presented below.

Occurrence Data for Water

Adequate data are available to estimate central tendency and high-end values for exposure to DBCM from treated surface and ground water. Numerous studies (summarized in Chapter IV of this document) have examined the levels of DBCM in disinfected water. Of these studies, the Information Collection Rule (ICR) data (U.S. EPA, 2001) most closely met the requirements for sample size, geographic representation, reporting of analytical limits, and relevance to current conditions. This survey examined the occurrence of brominated trihalomethanes in public water supplies (PWSs) serving at least 100,000 persons as required by the Information Collection Rule promulgated by U.S. EPA in May of 1996 for disinfectants and disinfection byproducts (D/DBPs). The rule covered both surface and ground water systems. Monitoring data were collected from about 300 water systems operating 501 plants over the18-month period between July 1997 and December 1998. At each plant, samples were collected monthly and analyzed for a variety of D/DBPs on a monthly or quarterly basis. DBCM was among the analytes evaluated quarterly (U.S. EPA, 2001). Five samples were taken each quarter at each plant – one of the finished water and four of the water in the distribution system. Of the four samples from the distribution system, one represented a sample with the same residence time as a finished water sample held for a specific period of time, two represented approximate average water residence times in the system, and one sample was taken where water residence time in the system is the longest. For each plant and reporting period, EPA compiled several summary statistics. The Distribution System (DS) Average value is the average of the four distribution system samples. The DS High Value is the highest concentration of the four distribution system samples collected by a plant in a given quarter. The DS High Value might be from any of the four samples and could vary from quarter to quarter depending on which sample yielded the highest concentrations in each quarter (U.S. EPA, 2001a). Table C-1 summarizes the results of all six of the quarterly reporting periods. The DS average and 90^{th} percentile values for DBCM in surface water were 4.72 μg/L and 5.57 μg/L, respectively. The DS average and 90^{th} percentile values for dibromochloromethane in groundwater water were 3.09 μg/L and 8.94 μg/L, respectively.

U.S. EPA set a minimum reporting level (MRL) for DBCM of 1.0 μg/L for the ICR. The MRL is a level below which systems were not required to report their monitoring results, even if there were detectable levels. Values below the MRL were assigned a value of zero for the purpose of calculating averages; this assignment affects the calculation of mean values for finished water and DS high results and calculation of all DS average values.

Data for Occurrence in Air

Occurrence data for DBCM in ambient outdoor air were available from three reports (Brodzinsky and Singh, 1983; Shikiya et al., 1984; Atlas and Shauffler, 1991). Brodzinsky and Singh (1983) reviewed and summarized existing data for DBCM concentrations in ambient outdoor air for several urban/suburban or source dominated locations across the United States (Table C-2). No concentration data were available for rural or remote areas. The authors reported mean, median, first and third quartile values, and minimum and maximum values by city. In addition, they reported the same measures when the data were grouped by type of location (i.e.,

Table C-1 DBCM Concentrations Measured in U.S. Public Drinking Water Systems Serving 100,000 or More Persons

Source	Data Type[a]	Number of Samples	Median[b]	Mean[b]	90th Percentile	Range
DBCM (µg/L)						
Surface Water	Finished	1853	1.9	4.03	12.0	<1.0 - 55.1
	DS Average	1655	2.40	4.72	13.2	0 - 67.3
	DS High	1655	2.9	5.57	15.0	< 1.0 - 67.3
Ground Water	Finished	604	< 1.0	1.38	4.10	<1.0 - 33
	DS Average	602	1.35	3.09	8.94	0 - 37.5
	DS High	602	2.1	4.60	12.9	<1.0 - 85

Source: Disinfectants and Disinfection Byproducts (D/DBPs) ICR Data, U.S. EPA (2001).

[a] Finished = sample location after treatment, before entering the distribution system (DS); DS Average = average of four sample locations in the DS; DS High = the highest concentration of the four distribution system samples collected by a plant in a given quarter. For purposes of calculations, all values below the minimum reporting level (MRL) of 1.0 µg/L for all three compounds were assigned a value of zero.

[b] Median and mean of all samples including those below the MRL.

urban/suburban or source dominated), and when all data were combined. Dibromochloromethane was detected in the air samples from Magnolia, AR, El Dorado, TX, Chapel Hill, NC, Beaumont TX, and Lake Charles, LA at mean concentrations of 0 ppt, 0.48 ppt, 14 ppt, 14 ppt, and 19 ppt, respectively. Data from these sites were combined for additional statistical analyses. The study authors indicated that a value of 0.0 was entered for samples below the detection limit. The detection limits from individual studies were not reported. Mean (± standard deviation) outdoor air concentrations in urban/suburban and source dominated locations, respectively, were 15 ± 4 ppt and 0.28 ± 0.67 ppt for DBCM. Brodzinsky and Singh (1983) also calculated overall (grand) means based on data from all sites. The grand mean value for DBCM was 3.8 ppt (n = 89, with 63 nondetects). When expressed on a ng/m^3 basis, the corresponding mean value was 0.032 µg/m^3. Assuming an inhalation rate of 20 m^3/day, this concentration results in a daily intake of 0.6 µg/day. Assuming a rate of 13.2 m^3/day, this concentration results in a daily intake of 0.43 µg/day.

Shikiya et al. (1984) analyzed ambient air samples collected at four urban/industrial locations in the California South Coast Air Basin from November 1982 to December 1983 for the presence of DBCM. The sampling locations were El Monte, downtown Los Angeles, Dominguez, and Riverside. The air samples were analyzed using gas chromatography with detection by electron capture. The quantitation limit, defined as a level 10 times greater than the noise level, was 10 ppt by volume. The detection limit was defined as three times the noise level. Most data in this report were presented graphically. A few additional details were

presented in a short summary statement for each chemical. Summary data for each compound included monthly means and composite means. The monthly means were calculated as the average of all data at a site that were above the quantitation limit for a single month; samples with concentrations below the limit of detection were not included in the calculations. The composite means were calculated as the average value of all data for each compound above the quantitation limit at each site. Only seventeen percent of the samples had DBCM levels above the quantitation limit of 10 ppt (0.085 $\mu g/m^3$). The highest reported concentration, monthly mean, and mean composite for DBCM were 290 ppt (2.5 $\mu g/m^3$), 280 ppt (2.4 $\mu g/m^3$), and 50 ppt (0.43 $\mu g/m^3$), respectively; all were recorded in downtown Los Angeles in June. Only two monthly means were above 160 ppt; the remainder of the monthly means were below 60 ppt.

Table C-2 Selected Concentration Data for Individual Brominated Trihalomethanes (ppt) in Outdoor Air as Summarized in Brodzinsky and Singh (1983)[a,b]

City	n	Non-detects	Mean (Std dev.)	Median	3rd Quartile	Maximum	Reference
DBCM							
Individual Sites							
Beaumont, TX	11	0	14 (0.0)	14	14	14	Wallace (1981)
Chapel Hill, NC	6	0	14 (0.0)	14	14	14	Wallace (1981)
El Dorado, AR	40	35	0.48 (0.82)	0.0	0.82	2.5	Pellizzari et al. (1978)
Lake Charles, LA	4	0	19 (9.6)	21	27	27	Pellizzari (1979)
Magnolia, AR	28	28	0.0 (0.0)	0.0	0.0	0.0	Pellizzari et al. (1978)
Totals							
Urban/Suburban	21	0	15 (4.2)	14	14	27	-
Source Areas	68	63	0.28 (0.67)	0.0	0.0	2.5	-
Grand Totals	89	63	3.8 (6.7)	0.0	2.5	27	-

[a] Includes only data considered to be of adequate, good, or excellent quality by the study authors.
[b] Concentrations are reported as parts per trillion by volume

Atlas and Schauffler (1991) collected replicate air samples at various locations on the Island of Hawaii during a month-long field experiment to test an analytical method for determining halocarbons in ambient air. DBCM was found at a mean level of 0.27 ppt. This information was obtained from a secondary source which did not report the detection limit.

Wallace et al. (1982) conducted a pilot study designed to field test personal air-quality monitoring methods. Personal air samples were collected from students at two universities: Lamar University, Texas, located near a petrochemical manufacturing area, and the University of North Carolina (UNC), located in a nonindustrialized area. The samples were analyzed for a number of volatile organic compounds, including brominated trihalomethanes. DBCM was not detected at either location. Based on an analytical limit of 0.12 $\mu g/m^3$ or 0.018 ppb, these data suggest that exposure via personal air is less than 2.4 μg/day.

There are several limitations associated with the available data on occurrence of DBCM in outdoor air. The available studies were collectively limited to five states (Arkansas, California, Hawaii, North Carolina, and Texas). With the possible exception of the Hawaiian study (Atlas and Schauffler 1991), all data were collected from urban/suburban or source dominated locations. Thus, the data from these studies are not considered to be geographically representative of the United States. In addition, sample size was not explicitly reported in the Shikiya et al. (1984) study and the reported means were based only on data above the detection limit (only 17% of total samples). An independent statistical evaluation could not be performed because raw data were not presented. The data presented by Brodzinsky and Singh (1983) were obtained from multiple sources and combined results for sampling periods ranging from instantaneous grab samples to 24 hour averages (Wallace, 1997). The data from the Shikiya et al. (1984) and Brodzinsky and Singh (1983) reports are approximately 20 years old and may not accurately reflect current conditions.

Relatively few studies have reported the concentrations of trihalomethanes in indoor air of homes. Kostiainen (1995) identified over 200 volatile organic compounds in indoor air of 26 houses identified by residents as causing symptoms such as headache, nausea, irritation of the eyes, drowsiness, and fatigue. DBCM was not reported among the detected compounds.

Weisel et al. (1999) measured brominated trihalomethane concentrations in indoor air in 49 New Jersey residences selected to represent low and high levels of drinking water contamination with trihalomethanes. Descriptive statistics for DBCM concentration in water were provided for the combined high and low concentration groups, but not for the individual groups. One valid 15-minute air sample was collected at each of 48 residences. The indoor air concentrations of DBCM averaged 0.44 ± 0.95 $\mu g/m^3$ (0.052 ± 0.11 ppb) and 0.53 ± 0.84 $\mu g/m^3$ (0.062 ± 0.09 ppb) in the low and high water concentration residences with detection frequencies of 5/25 and 7/23, respectively. The detection limit was 0.14 $\mu g/m^3$ (C. Weisel, personal communication). It was not clear whether the averages were based on all measured samples or only those samples that were above the detection limit. For this reason, the data were not used for calculation of exposure to DBCM from indoor air.

It is possible that DBCM has been surveyed in studies of volatile organic compounds in air and not reported because it was below detection limits. This has been suggested by Dr. Joachim Pleil of the U.S. EPA Office of Research and Development, who is highly experienced in air monitoring of volatiles including trihalomethanes. According to Dr. Pleil, the analytical methods used in analysis of volatile organic compounds are sufficiently sensitive to detect DBCM even if is present only in minute quantities. For example, a survey of volatile organic compounds in indoor air by Pleil et al. (1985) using EPA Method TO-14 (detection limit

approximately 100 ppt by volume, or 0.85 μg/m^3, for trihalomethanes) would certainly have detected DBCM had it been present (J. Pleil, personal communication).

To accurately estimate total daily inhalation exposures from indoor and outdoor air, the following data needs to be evaluated: location and season, the time spent indoors compared with outdoors, potential exposures of individuals while showering or bathing, potential exposure from volatilization of DBCM during other household activities (e.g., use of dishwashers, toilet flushing), exposures of individuals who spend large amounts of time at indoor pools or in hot tubs, and potential for occupational exposures (e.g., for laundromat or sewage treatment plant workers). The existing measurement data are not adequate for such a refined analysis, but may be used to roughly estimate intake from outdoor air.

Data for Occurrence in Food

Information on the levels of DBCM in foods and beverages is limited. Chlorine is used in food production for applications such as the disinfection of chicken in poultry plants and the superchlorination of water at soda and beer bottling plants (Borum, 1991). Therefore, the possibility exists for contamination of food from chlorination by-products in foods with resulting dietary exposure.

Two studies have reported analyses of commercial beverages for DBCM. In Italy, Cocchioni et al. (1996) analyzed 61 samples of different commercially prepared beverages and 94 samples of mineral waters for volatile organo-halogenated compounds. Maximum DBCM concentrations of 13.9 μg/L (ppb) were found in prepared beverages, with a frequency of detection of 43% (26/61), with a detection limit of less than 1 μg/L (ppb). McNeal et al. (1995) examined 27 different prepared beverages and mineral waters in the United States for DBCM at a detection limit of 0.1 ng/g (ppb). DBCM was detected at 1 ng/g (ppb) in only one of seven types of mineral and sparkling waters examined. DBCM was not detected in any of 5 flavored noncarbonated beverages examined. DBCM was detected in only 4 of the 13 carbonated soft drinks examined at levels of 0.5 to 2 ng/g (ppb). DBCM was not detected in either of the two types of beer examined.

Two studies have tested for DBCM in individual food items. McNeal et al. (1995) tested several types of food products and water from canned vegetables in the United States for DBCM. DBCM was not detected in any of the samples. The foods examined included two types of canned tomato sauce, canned pizza sauce, canned vegetable juice, vegetable waters from two types of canned green beans and one type of sweet corn, duck sauces, beef extract, and Lite syrup product. Imaeda et al. (1994) examined bean curd commercially available in Japan for trihalomethanes. DBCM was not found in any of ten samples analyzed at a detection limit of 0.1 ppb.

Kroneld and Reunanen (1990) analyzed pasteurized and unpasteurized cow's milk for DBCM content in a study conducted in Turku, Finland. DBCM was detected in only one sample of pasteurized milk at 5 μg/L (ppb). The detection limit was not specified and information sample size was unavailable in the secondary source that reported this study (U.S. EPA, 1994).

DBCM was not detected in unpasteurized milk. The presence of the DBCM in pasteurized milk may have resulted from the use of chlorinated water during processing.

Estimates for dietary intake of DBCM by residents of the United States were not identified in the materials reviewed for this document. Information on the levels in U.S. foods is too limited to independently calculate a reliable estimate. However, the available data suggest that the concentration of DBCM in foods is low.

Data for dietary intake of DBCM are available from a study conducted in Japan. Toyoda et al. (1990) analyzed the dietary intake of DBCM by 30 housewives in Nagoya and Yokohama. Duplicate portions of daily meals were collected for three consecutive days, sampled for DBCM and analyzed at a detection limit of 0.2 ppb. The amount and types of food consumed were not reported. This omission prevents a comparison of the studied diet to that consumed by the U.S. population. The concentration of DBCM in the Japanese diet ranged from undetectable to 0.6 ppb (average, 0.1 ± 0.2 ppb), and the mean dietary intake was estimated to be 0.3 ± 0.3 μg/day. These data are considered adequate only for a rough estimate of the dietary intake of DBCM.

Evaluation

The occurrence data base for DBCM in tap water consists of nationally aggregated data and is considered adequate for determination of the RSC. In comparison, fewer occurrence data are available for DBCM in food and outdoor air. The available air and food occurrence data, although limited, permit rough estimates of intake.

Determination of the RSC

The RSC is calculated as follows:

$$RSC = \frac{DI_{water}}{DI_{total}} \qquad (1)$$

where:

$$DI_{water} = DI_{water,\ ingestion} + DI_{water,\ inhalation} + DI_{water,\ dermal} \qquad (2)$$

$$DI_{total} = DI_{water,\ total} + DI_{outdoor\ air} + DI_{food} \qquad (3)$$

The estimation of individual terms in these equations is described below.

Exposure Associated with Tap Water Uses

Exposure to DBCM as a chlorination by-product in residential water can occur via three primary exposure routes: 1) by ingestion; 2) by inhalation of DBCM volatilized during use of tap water for bathing, showering, and other household activities; and 3) by dermal exposure during showering and bathing. The existence of these routes for DBCM is supported by recent study data. Kerger et al. (2000) demonstrated that levels of DBCM in indoor air are related to the use of tap water for showering and bathing. Increases in the level of DBCM in the breath or blood after showering or bathing have been documented in human subjects (Weisel et al., 1999; Backer et al., 2000; Lynberg et al., 2001). Quantitative estimates of average daily exposure from volatilized DBCM or dermal contact have been calculated and described in the following pages for comparison with other routes of intake. These derived values have solid scientific support and are sufficient for a reliable estimate. It is important to note that these estimates are for exposure of the general population via tap water. Individuals who participate in activities such as swimming or hot tub use may experience increased dermal and or inhalation uptake or brominated trihalomethanes as a result of increased contact time with disinfected water. It is important to note that water in hot tubs and swimming pools is routinely subjected to additional disinfection and may not be representative of tap water using for drinking, cooking, and other household activities.

a. Ingestion of DBCM in Drinking Water

Ingestion of DBCM is calculated by multiplying an appropriate intake rate for tap water by the concentration of the compound found in tap water. Mean water intake rates of 1.2 and 0.6 L/day (NRC, 1999) were used for total mean ingestion from all uses and for direct ingestion (i.e., direct ingestion from tap, does not include use of tap water for making coffee and tea, soup, etc.), respectively. An adjustment for intake of commercial beverages (e.g., soft drinks or mineral waters) was not applied, because the intake of these beverages would be subtracted from the daily tap water intake and the available data (e.g., McNeal, 1995) suggest that the level of DBCM in such beverages is usually less than or similar to the level found in tap water. Assuming a tap water concentration of 4.72 μg/L (the distribution system average for DBCM in treated surface water), the total and direct intakes of DBCM via ingestion of tap water are 5.7 μg and 2.8 μg, respectively.

b. Inhalation of Waterborne DBCM

A three-compartment model approach was used to investigate the exposure from water-related dibromochloromethane in indoor air. The three-compartment model employed was that of McKone (1987). This model predicts the concentration of a volatile chemical in water (in this case, DBCM) in each of three compartments of a house: the shower, the bathroom, and the remainder of the house. The three-compartment model recognizes that most household water uses are episodic rather than continuous, and room barriers (walls, doors) may restrict the rapid mixing of DBCM released into air in one location with whole-house air, leading to occasional high levels of DBCM in some rooms (especially those with high water usage, such as the shower or laundry). Because concentrations are not constant, results are calculated as a function of time throughout the day. Based on the time- and compartment-specific concentration values, human

exposure levels in each compartment can then be calculated based on an assumed pattern of human occupancy and behavior within the house. McKone (1987) estimated the source term for the release of VOCs from water to air in each of the three compartments by extrapolation from measurements of radon release using the VOC-specific Henry's law constant and the liquid- and gas-phase diffusion coefficients. Basic equations and inputs to the model are provided below:

Transient three-compartment model based on transfer efficiency developed by McKone (1987)

$$V_i \frac{dy_i(t)}{dt} = S_i(t) + \sum Q_{ji} \cdot y_j(t) - \sum Q_{ij} \cdot y_i(t)$$

$$S_i(t) = \frac{WU_i\left(\tau_\iota^0, \tau_\iota^*\right) \cdot TE_i^{VOC} \cdot F\left(t, \tau_\iota^0, \tau_\iota^*\right) \cdot}{\tau_\iota^* - \tau_\iota^0}$$

$$TE_i^{VOC} = TE_i^{Rn} \cdot \frac{K_{OL} A_{VOC}}{K_{OL} A_{Rn}} = TE_i^{Rn} \cdot \frac{\left(\frac{2.5}{D_L^{2/3}} + \frac{1}{H \cdot D_G^{2/3}}\right)_{Rn}}{\left(\frac{2.5}{D_L^{2/3}} + \frac{1}{H \cdot D_G^{2/3}}\right)_{VOC}}$$

$$E_{inh} = \frac{1}{ED} \int y_i(t) \cdot OF_i(t) dt$$

$$Dose = E_{inh} \cdot BR$$

Where:

A	interfacial area existing between water and air (cm^2)
BR	breathing rate (L/min)
C	aqueous-phase concentration (mg/L)
D_L	liquid-phase diffusion coefficient (cm^2/sec)
D_G	gas-phase diffusion coefficient (cm^2/sec)
ED	exposure duration (min)
E_{inh}	inhalation exposure (mg/L, except for radon, which is in pCi/L)
$F(t, \tau_i^0, \tau_i^*)$	function = 1 when time t is between τ_i^0 and τ_i^*, and 0 otherwise

H	Henry's law constant ($mg/L_{air}/mg/L_{water}$)
K_{OL}	overall mass-transfer coefficient (cm/min)
$K_{OL}A$	overall interfacial mass-transfer coefficient (L/min)
$OF_i(t)$	occupancy factor for compartment i at time t (1 if present, 0 if absent)
Q_{ij}	ventilation rate from compartment i to compartment j (L/min)
S_i	emission rate from source in compartment i (mg/min)
TE_i^j	transfer efficiency of chemical j during water use in compartment i (1)
τ_i^0	time when water device in compartment i starts (min)
τ_i^*	time when water device in compartment i ends (min)
V_i	volume of compartment i (L)
$WU_i(\tau_i^0, \tau_i^*)$	volume of water used in compartment i between time τ_i^0 and τ_i^* (L)
y_i	gas-phase concentration in compartment i (mg/L)
Rn	radon
voc	volatile organic compound

The following properties of dibromochloromethane at 20°C were used as inputs to the model:

H ($mg/L_{air}/mg/L_{water}$) = 0.036
D_L (cm^2/s) = 9.63×10^{-6}
D_G (cm^2/s) = 8.24×10^{-2}

The following human activity and water use patterns were assumed. Four people living in the house each take an 8-minute shower every morning, and spend 12 minutes in the bathroom immediately thereafter. Each person spends an additional 20 minutes in the bathroom some time during the remainder of the day. The second person taking a shower is selected for the purpose of comparing exposure estimates. The person being modeled is assumed to spend 75% their time indoors by assigning an average daily occupancy factor of 0.75.

The following specific parameters were used to implement the calculations of the three-compartment model developed by McKone (1987):

Variable	Description	Value
PNUM	Number of people in the house	4
Person	Designated person for exposure calculation	2nd showerer
V_s	Volume of shower	2000 L
V_b	Volume of bathroom	10000 L
V_a	Volume of main house	400000 L
I_s	Volume of water used in shower	350 L
I_b	Volume of water used in bathroom	350 L
I_a	Volume of water used in main house	450 L
R_s	Shower air residence time	3 min
R_b	Bathroom air residence time	12 min
R_a	Main house air residence time	78 min

Variable	Description	Value
Q_{sb}	Ventilation rate from shower to bathroom	40000 L/hr
Q_{bs}	Ventilation rate from bathroom to shower	40000 L/hr
Q_{ab}	Ventilation rate from main house to bathroom	50000 L/hr
Q_{ba}	Ventilation rate from bathroom to main house	45000 L/hr
Q_{bo}	Ventilation rate from bathroom to outside	5000 L/hr
Q_{ao}	Ventilation rate from main house to outside	258000 L/hr
SFR	Shower flow rate	11 L/min
TE_s^{Rn}	TE from shower to air for radon	0.7
TE_b^{Rn}	TE from bathroom to air for radon	0.3
TE_a^{Rn}	TE from household water to air for radon	0.66
t_s	Time in shower	8 min
t_b	Time in bathroom after shower	12 min
t_b'	Time in bathroom during rest of day	20 min
t_s^0	Time when first shower water use starts	7:00 a.m.
t_s^*	Time when first shower water use ends	7:08 a.m.
t_b^0	Time when toilet water use starts	12:00 a.m.
t_b^*	Time when toilet water use ends	12:00 a.m.
t_a^0	Time when other household water use starts	7:00 a.m.
t_a^*	Time when other household water use ends	11:00 p.m.
OF	Daily average occupancy factor	0.75
BR	Breathing rate	9.2 L/min

Source for BR is U.S. EPA (1995) and for all other values is U.S. EPA (1993).

Based on these assumptions and parameter values, the model predicts an inhaled dose for DBCM of 540 μg/year per μg/L_{water}. Dividing this number by 365 days/year and multiplying the result by 4.72 μg/L (the distribution system average for DBCM in treated surface water) gives an estimate of 7 μg/day for inhalation intake of DBCM volatilized from tap water.

c. Dermal Absorption of DBCM via Bathing or Showering

Estimates of dermal uptake of DBCM were obtained using a membrane model approach as described in Cleek and Bunge (1993) and Bunge and McDougal (1998). The approach assumes that the skin is composed of stratum corneum and viable epidermis. If the concentration of the vehicle remains constant and the systemic concentration remains small, the dermal absorption of chemicals can be divided into two periods: non-steady state and steady state. In the non-steady state period, the chemical is absorbed in the lipophilic stratum corneum. The viable dermis acts like a sink for the chemical once the steady-state is achieved. Equations are available for calculation of uptake under both steady state and non-steady state conditions. Because the duration of exposure to DBCM during showering and bathing (10 minutes; U.S. EPA, 1992c) is substantially less than the time to steady state for DBCM absorption through skin (3.9 hours; U.S. EPA; 1992c), the non-steady state approach is the default procedure for estimating uptake:

$$DA_{event} = 2AC_w\sqrt{\frac{K_p K_{sc/w} L_{sc} t_{event}}{\pi}}$$

(4)

where:

DA_{event} = the amount of DBCM (μg) absorbed in a 10-minute shower or bath
A = the surface area exposed during the bath or shower event = 20,000 cm^2 (U.S. EPA, 1992)
C_w = concentration of DBCM in tap water = 4.72 x 10^{-3} μg/cm^3 (U.S. EPA, 2001)
K_p = permeability coefficient = 3.9 x 10^{-3} cm/hr (U.S. EPA, 1992c)
$K_{sc/w}$ = stratum corneum-water partition coefficient = 38 (see equation 5)
L_{sc} = diffusion length of the stratum corneum = 1.5 x 10^{-3} cm (Bunge, personal communication)
t_{event} = duration of exposure event = 10 minutes = 0.1667 hours (U.S. EPA, 1992c)

The stratum corneum-water partition coefficient, *Ksc/w*, was estimated as recommended by Bunge and McDougal (1999):

$$K_{sc/w} = K_{ow}^{0.71} = 38 \quad (5)$$

where

K_{ow} = octanol water partition coefficient = 170 for DBCM (U.S. EPA, 1992c)

When calculated using equation (4) and the input values above, the dermal uptake of DBCM during a 10-minute showering or bathing event is approximately 0.65 μg.

Perhaps the most difficult part of estimating dermal exposure is the determination of the permeability coefficient, K_p, in the equation above. Estimates have been obtained from skin penetration experiments. Most skin penetration experiments fall into one of two categories: *in vivo* experiments performed on living humans or animals, and *in vitro* experiments made in diffusion cells with excised skin from humans and animals. Determination of permeability coefficients *in vitro* and *in vivo* generally requires that the exposure concentrations and surface area are known and consistent. There is scientific debate over whether *in vitro* or *in vivo* measurements are the most appropriate way to measure absorption of chemicals. *In vitro* methods can provide quick and direct measures of flux and permeability coefficients. It is also advantageous that human skin can be used *in vitro* when chemicals would be too toxic in *in vivo* studies. Although the actual *in vitro* experiments are simpler, their use includes many important variables (e.g. animal species, thickness of skin, use of fresh or frozen skin, and receptor solution) and uncertainties which influence the representativeness of the data. *In vivo* studies are often more elaborate and require more data analysis.

The U.S. EPA (1992) *Dermal Exposure Assessment* document used *in vitro* data to estimate the permeability coefficient (K_p) for multiple chemicals. An equation developed by Potts and Guy (1993) was used to estimate the K_p of over 200 chemicals, including DBCM, as a

function of the octanol:water partition coefficient (K_{ow}) and molecular weight (MW). The equation was derived from an experimental data base compiled by Gordon Flynn (1990), which includes data for *in vitro* dermal absorption of about 90 chemicals from water:

$$\log K_p = -2.72 + 0.71(\log K_{ow}) - 0.006MW \quad (6)$$

where,

$\log K_{ow}$ = logarithm of the octanol water partition coefficient = 2.23 for DBCM (U.S. EPA, 1992)
MW = molecular weight = 208.28 for DBCM

Despite the adequate correlations for representing experimental permeability data for a broad rage of chemicals, experimental data may deviate from predictions made using the Potts and Guy equation by one to two orders of magnitude. This variability was clearly demonstrated by Vecchia (1997). Although uncertainty in experimental temperature and other data are partly responsible, other known/unknown factors may also contribute to this discrepancy. For example, the correlation assumes that MW is a good predictor for molecular size. This assumption may not be appropriate for groups of compounds with chemical diversities affecting molecular size. Halogenated hydrocarbons will occupy the same molar volume as a hydrocarbon molecule with a much lower MW. As a result, equations based on MW that are developed from databases consisting primarily of hydrocarbons will tend to systematically underestimate permeability coefficients for chemically dense compounds such as DBCM, by an order of magnitude or perhaps even more for compounds with specific gravity values larger than about 2.5 and MW greater than 200.

The K_p was calculated using the procedure of Vecchia and Bunge (2002) to address potential underestimation of the K_p for DBCM by the prediction method used in U.S. EPA (1992). This calculation used a modification (equation 7) of the Potts and Guy equation (equation 6) which incorporates an adjustment factor for density of halogenated compounds when compared to non-halogenated hydrocarbons:

$$\log K_p = -2.72 + 0.71(\log K_{ow}) - 0.006\frac{MW\rho_{hc}}{\rho_{halo}} \quad (7)$$

where:

log K_{ow} = logarithm of the octanol water partition coefficient = 2.23 for DBCM (U.S. EPA, 1992c)
MW = molecular weight = 208.28 for DBCM
ρ_{hc} = estimated liquid density of the compounds in the Flynn equation upon which equation (6) was developed = 0.9 (Vecchia and Bunge, 2002)
ρ_{halo} = density of a halogenated hydrocarbon = 2.38 for DBCM (U.S. EPA, 1994)

The resulting value for K_p is 0.03 cm/hr. Substitution of this adjusted value in equation (4) results in an uptake estimate of 2.0 μg for a 10-minute showering or bathing event This value is

about 3-fold higher than the estimate obtained for dermal absorption using the unadjusted K_p value reported in U.S. EPA (1992). The 2 μg value was selected for determination of the RSC for DCBM.

Exposure Associated Via Food and Outdoor Air

Dietary intake data for DBCM from a Japanese study were used in the absence of intake data for U.S. residents. These data were used with the understanding that the composition (and thus levels of DBCM) of U.S. and Japanese diets may differ. The grand mean for outdoor air concentration calculated by Brodzinsky and Singh (1983) was used to estimate intake from outdoor air. Although these data for food intake and air concentrations have limitations, they were considered sufficient for a screening level estimate of the RSC.

Calculation of the RSC

An example calculation of the RSC is shown below. Results of calculations using different exposure assumptions are summarized in Table C-3.

$$DI_{water} = DI_{water,\ ingestion} + DI_{water,\ inhalation} + DI_{water,\ dermal} \quad (2)$$

$$= 5.7\ \mu g/day + 7.0\ ug/day + 2.0\ \mu g/day = 14.7\ \mu g/day$$

$$DI_{total} = DI_{air} + DI_{food} + DI_{water} \quad (3)$$

$$= 0.4\ \mu g/day + 0.3\ \mu g/day + 14.7\ \mu g/day$$

$$= 15.4\ \mu g/day$$

$$RSC = \frac{DI_{water}}{DI_{total}} = \frac{14.7\ \mu g}{15.4 \mu g} = 0.96 \times 100 = 95\%$$

where:

DI_{water} = Intake of DBCM from tap water
DI_{total} = Intake of DBCM from all relevant sources (i.e., water, air, and food for DBCM)
DI_{air} = Intake of DBCM from outdoor air, assuming 0.032 μg/m^3 and intake of 13.2 m^3/day
DI_{food} = Intake of DBCM from food = 0.3 μg/day after Toyoda (1990)
$DI_{water,\ ingestion}$ = Intake of DBCM from tap water by ingestion (assumes intake of 1.2 L/day)
$DI_{water,\ inhalation}$ = Intake of DBCM from tap water by inhalation, as determined using 3-compartment model; assumes intake of 13.2 m^3/day (U.S. EPA, 1995)
$DI_{water,\ dermal}$ = Dermal absorption of DBCM during bathing or showering using adjusted value of K_p; assumes 1 shower or bath/day

The RSC calculated using the ICR distribution system mean for surface water and U.S. EPA default values for inhalation and drinking water ingestion was 0.95 or 95%. Substitution of

groundwater concentration data and/or the direct value for drinking water intake also resulted in RSC values greater than 90%. These calculations suggest that an RSC as high as the default ceiling of 80% is justified for DBCM.

The uncertainty in use of 80% for the RSC is related to the quality of data for intake from food and outdoor air. The primary concern is that the available data might result in a significant underestimate of the actual exposure via these media, resulting in an RSC value that was not appropriately protective of health. A series of calculations was performed to test the effect of underestimating exposure from outdoor air and/or food on the RSC. An arbitrary 10-fold increase in the dietary intake of DBCM while holding intake from other sources constant resulted in RSC values of 78% or 81%, depending upon the intake values selected. Increasing the intake of DBCM from outdoor air by 10-fold resulted in RSC values of 72% or 76%. Increasing both food and air intake of DBCM by 10-fold gave RSC values of 62% or 67%. These calculations assumed a tap water concentration of 4.72 μg/L, which is the Information Collection Rule distribution system mean for surface water (U.S. EPA, 2001). These calculations suggest that an RSC of 80% would be protective of human health even if concentrations of DBCM in food or outdoor air were underestimated by a factor of almost 10-fold.

Conclusion

An RSC of 80% is recommended based on the analysis of available data on occurrence of DBCM in tap water and other media. The major uncertainties in this analysis are related to limited measurement data for DBCM in outdoor air and in food.

Table C-3 Results of RSC Calculations for DBCM

Water Source	C_w (µg/L)[a]	Condition	IR_{water} (L/day)[b]	IR_{air} (m^3/day)[c]	DI_{food} (µg/day)[d]	DI_{air} (µg/day)[e]	DI_{water} (µg/day)[f]	DI_{total} (µg/day)[g]	RSC (%)
Surface	4.72	Surface Water	0.6	13.2	0.3	0.42	11.8	12.6	94
			1.2	13.2	0.3	0.42	14.7	15.4	95
Ground	3.09	Ground Water	0.6	13.2	0.3	0.42	10.9	11.6	94
			1.2	13.2	0.3	0.42	12.7	13.4	95
Surface	4.72	If intake of DBCM from food increased by 10-fold	0.6	13.2	3.0	0.42	11.8	15.3	78
			1.2	13.2	3.0	0.42	14.7	18.1	81
Surface	4.72	If intake of DBCM from air increased by 10-fold	0.6	13.2	0.3	4.2	11.8	16.4	72
			1.2	13.2	0.3	4.2	14.7	19.2	76
Surface	4.72	If intake of DBCM from air and food increased by 10-fold	0.6	13.2	3.0	4.2	11.8	19.1	62
			1.2	13.2	3.0	4.2	14.7	21.9	67

[a] Concentration of DBCM in water: ICR distribution system mean (U.S. EPA, 2001).

[b] Daily intake rate for water. Values are for direct (0.6 L/day; NRC. 1999) or total mean (1.2 L/day; NRC, 1999) ingestion rates.

[c] Daily inhalation rate. Value used is consistent with the input value of 9.2 L/min for the three-compartment model used to estimate intake of DBCM from indoor air.

[d] Daily intake of DBCM in food, based on data from Toyoda et al. (1990).

[e] Daily intake of DBCM in air, based on grand mean for DBCM concentration in outdoor air calculated by Brodzinsky and Singh (1983)

[f] Daily intake of DBCM in water from ingestion, inhalation of volatilized compound, and dermal absorption. See text for details of calculation.

[g] Total daily intake of DBCM from water, outdoor air, and food.

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