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DRINKING WATER CRITERIA DOCUMENT FOR BROMINATED TRIHALOMETHANES

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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 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 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 bromodichloromethane, 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-day)$^{-1}$	NTP (1987)
Concentration for excess cancer risk (10^{-6})	1.0 µg/L	NTP (1987)
Unit Risk	1×10^{-6} (µg/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-day)$^{-1}$	NTP (1985)
Concentration for Excess cancer risk (10^{-6})	0.8 µg/L	NTP (1985)
Unit Risk	1.2×10^{-6} (µg/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-day)$^{-1}$	NTP (1989a)
Concentration for Excess cancer risk (10^{-6})	8 µg/L	NTP (1989a)
Unit Risk	1.3×10^{-7} (µg/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