FINAL
9-16-2009

Provisional Peer-Reviewed Toxicity Values for

Bromodichloromethane
(CASRN 75-27-4)

Superfund Health Risk Technical Support Center
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Commonly Used Abbreviations

BMD	Benchmark Dose
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
LOAEL	lowest-observed-adverse-effect level
$LOAEL_{ADJ}$	LOAEL adjusted to continuous exposure duration
$LOAEL_{HEC}$	LOAEL adjusted for dosimetric differences across species to a human
NOAEL	no-observed-adverse-effect level
$NOAEL_{ADJ}$	NOAEL adjusted to continuous exposure duration
$NOAEL_{HEC}$	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
p-OSF	provisional oral slope factor
p-RfC	provisional inhalation reference concentration
p-RfD	provisional oral reference dose
RfC	inhalation reference concentration
RfD	oral reference dose
UF	uncertainty factor
UF_A	animal to human uncertainty factor
UF_C	composite uncertainty factor
UF_D	incomplete to complete database uncertainty factor
UF_H	interhuman uncertainty factor
UF_L	LOAEL to NOAEL uncertainty factor
UF_S	subchronic to chronic uncertainty factor

PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR BROMODICHLOROMETHANE (CASRN 75-27-4)

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (U.S. EPA) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1) U.S. EPA's Integrated Risk Information System (IRIS).
2) Provisional Peer-Reviewed Toxicity Values (PPRTVs) used in U.S. EPA's Superfund Program.
3) Other (peer-reviewed) toxicity values, including
   - Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
   - California Environmental Protection Agency (CalEPA) values, and
   - EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in U.S. EPA's IRIS. PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the U.S. EPA IRIS Program. All provisional toxicity values receive internal review by two U.S. EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multiprogram consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all U.S. EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because new information becomes available and scientific methods improve over time, PPRTVs are reviewed on a 5-year basis and updated into the active database. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV documents conclude that a PPRTV cannot be derived based on inadequate data.

Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and Resource Conservation and Recovery Act (RCRA) program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore,

users are strongly encouraged to read the entire PPRTV document and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the U.S. EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other U.S. EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the U.S. EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

IRIS (U.S. EPA, 1988) lists an RfD of 2×10^{-2} mg/kg-day for bromodichloromethane based on a LOAEL of 17.9 mg/kg-day for renal cytomegaly in male mice administered the chemical in corn oil by gavage for 102 weeks (National Toxicology Program [NTP], 1987) and a composite UF of 1,000 (10 for extrapolation from mice to humans, 10 for protection of sensitive individuals, and 10 for the use of a minimal LOAEL and database deficiencies). The Drinking Water Standards and Health Advisories list (U.S. EPA, 2006) includes an RfD of 3×10^{-3} mg/kg-day for bromodichloromethane without indicating a source. However, the source is likely to be a Drinking Water Criteria Document for Brominated Trihalomethanes (U.S. EPA, 2005) that derived an RfD of 3×10^{-3} mg/kg-day for bromodichloromethane based on a duration-adjusted $BMDL_{10}$ of 0.8 mg/kg-day for fatty degeneration in the liver of male rats in a 24-month dietary study (Aida et al., 1992). The Chemical Assessments and Related Activities (CARA) list (U.S. EPA, 1991, 1994a) includes a Health Effects Assessment (HEA) for Trihalogenated Methanes (U.S. EPA, 1987) that did not derive an RfD due to positive cancer results in the 2-year oral bioassay (gavage) in rats and mice (NTP, 1987). The Health Effects Assessment Summary Tables (HEAST; U.S. EPA, 1997) refers to IRIS for the RfD and lists the RfD also as the subchronic RfD. The Agency for Toxic Substances Disease Registry (ATSDR, 1989) derived a chronic oral minimal risk level (MRL; analogous to an RfD) of 0.018 mg/kg-day for renal effects in mice in the NTP (1987) study by the same method IRIS (U.S. EPA, 1988) used to derive the RfD. An intermediate oral MRL is not derived. A World Health Organization (WHO) Environmental Health Criteria Document for disinfectants and disinfectant by-products includes bromodichloromethane (WHO, 2000), but it does not derive any toxicity values.

An RfC for bromodichloromethane is not available on IRIS (U.S. EPA, 1988) or in the HEAST (U.S. EPA, 1997). ATSDR (1989) did not derive MRLs for inhalation exposure to bromodichloromethane. No occupational exposure limits for bromodichloromethane are available from the American Conference of Governmental Industrial Hygienists (ACGIH, 2001, 2007), the National Institute for Occupational Safety and Health (NIOSH, 2008) or the Occupational Safety and Health Administration (OSHA, 2008).

IRIS includes a cancer assessment for bromodichloromethane (verified 4/12/1992) in which the chemical was assigned to cancer weight-of-evidence Group B2 (probable human carcinogen), and an oral slope factor (OSF) of 6.2×10^{-2} $(mg/kg\text{-}day)^{-1}$ is presented based on an increased combined incidence of tubular cell adenoma and tubular cell adenocarcinoma in male B6C3F1 mice administered the test material by oral gavage for 2 years (NTP, 1987). The International Agency for Research on Cancer (IARC, 1991, 1999) classified bromodichloromethane as Group 2B (possibly carcinogenic to humans) with respect to carcinogenicity in humans based on inadequate evidence in humans and evidence of carcinogenicity in animals. Bromodichloromethane is listed in the NTP (2005) 11th Report on Carcinogens as "reasonably anticipated to be a human carcinogen." NTP (2006) recently published a new bioassay in male F/344 rats and female B6C3F1 mice in which the compound was administered in drinking water rather than by gavage.

The U.S. EPA (2005) Drinking Water Criteria Document for Trihalomethanes (DWCD) contains a comprehensive and recent review of the toxicological data for bromodichloromethane. The DWCD was used extensively in the development of this report.

Literature searches were conducted from 1960s through May 2009 for studies relevant to the derivation of provisional toxicity values for bromodichloromethane. Databases searched include: MEDLINE, TOXLINE (Special), BIOSIS, TSCATS 1/TSCATS 2, CCRIS, DART/ETIC, GENETOX, HSDB, RTECS, and Current Contents (September 2008–May 2009).

REVIEW OF PERTINENT DATA

Human Studies

No studies examining the toxicological effects of human exposure to bromodichloromethane alone were identified in the literature search. A number of epidemiological studies have examined potential associations between exposure to trihalomethanes (including bromodichloromethane) and reproductive or developmental effects, or between exposure to chlorinated drinking water and cancer. All of the studies identified in the literature search were reviewed by U.S. EPA recently in the DWCD (U.S. EPA, 2005). An overview of the human data, adapted from the executive summary of the DWCD, is presented here.

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 only possible increased cancer incidence in bladder was suggested (Villanueva et al., 2003, 2004). 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 (Waller et al., 1998), however contrary data have recently been reported (MacLehose et al., 2008). 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 ≥ 20 μg/L of bromodichloromethane prior to conception through the first month of pregnancy (Kramer et al., 1992; King et al., 2000; Dodds and King, 2001). An association has been reported for total brominated trihalomethanes and reduced menstrual cycle and follicular phase length in women of child-bearing age (Windham et al., 2003). A study of semen quality in healthy men found an association between increased exposure to bromodichloromethane in residential tap water and decreased sperm linearity (Fenster et al., 2003).

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.

Because of potential confounding by coexposure to other compounds, none of the human studies reviewed was used by U.S. EPA (2005) for dose-response assessment; thus, these studies were not summarized for this review.

Animal Studies

Oral Exposure

Subchronic Studies—All studies of subchronic oral exposure to bromodichloromethane that were identified in the literature search were reviewed by U.S. EPA recently in the DWCD (U.S. EPA, 2005). The study summaries included in this PPRTV are adapted from that document.

Chu et al. (1982) 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 study 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 was clearly dose-related. Specifically, the incidence of hepatic lesions was increased in males at concentrations equal to or greater than 6.5 mg/kg-day, with similar statistically significant increases in the severity of these lesions in these dose groups compared to the control. The study authors 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 6.5 mg/kg-day. 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 increased compared to that of the control group (0/10) with the severity significantly increased in the 6.9 and 219 mg/kg-day treatment groups, but not in the 57 mg/kg-day 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 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 B6C3F1 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.

Chronic Studies—A large number of chronic oral exposure studies to bromodichloromethane have been published and reviewed by U.S. EPA (2005). Because the IRIS record for bromodichloromethane includes both a chronic RfD and cancer assessment with an OSF, the chronic oral studies of this compound are not summarized in this PPRTV. There are two newer studies (published since the U.S. EPA, 2005 Drinking Water Criteria Document) of chronic duration that have been identified in the literature search: a 2-year cancer bioassay using drinking water exposure (NTP, 2006) and a 6-month neurotoxicity study (Moser et al., 2007).

The neurotoxicity study is discussed below under Other Studies, while the cancer bioassay is noted briefly in the context of the cancer weight-of-evidence assessment for bromodichloromethane.

Reproductive/Developmental Studies—All of the reproductive and developmental toxicity studies of bromodichloromethane that were identified in the literature search were reviewed by U.S. EPA recently in the DWCD (U.S. EPA, 2005). The study summaries included in this review are adapted from that document.

Developmental Toxicity

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 bromodichloromethane, not all females became pregnant and/or delivered litters (Ruddick et al., 1983). 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.

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 (Narotsky et al., 1997). 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 (Narotsky et al., 1997). 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 study 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 (Narotsky et al., 1997). 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 potential toxicity of bromodichloromethane (98.2% pure) 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 1,300-ppm bromodichloromethane using the study design described in Table 1. Feed and water consumption, body weight, hematology, clinical chemistry, cell proliferation, and pathology were evaluated in addition to developmental and reproductive endpoints. 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 postimplantation loss. 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).

Table 1. NTP (1998) Study Design

Gender	Group	Description	# Animals per Dose Group			
			0 ppm[a]	100 ppm	700 ppm	1,300 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

[a]Control animals received deionized water

Based on measured water consumption, the study authors estimated that the nominal concentrations of 0, 100, 700, and 1,300 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 (NTP, 1998). The calculated doses for Group B females were 0, 13, 54, and 90 mg/kg-day. However, analysis of the exposure solutions indicated that the concentrations of bromodichloromethane were much lower than the nominal concentrations (69, 608, and 1141 ppm for the 100-, 700-, and 1,300-ppm groups, respectively). Based on water consumption and analytical measurements of bromodichloromethane in the provided drinking water, the calculated average daily doses were 0, 6, 36, and 60 mg/kg-day for all male rats; 0, 10, 63, and 102 mg/kg-day for all female rats in Groups A and C; and 0, 9, 47, and 79 mg/kg-day for Group B females.

All animals survived the treatment period, with the exception of one Group A male in the 36 mg/kg-day dose group (NTP, 1998). Body weight and food and water consumption were decreased at many time points for animals dosed with 700- and 1,300-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. Alterations in hematological endpoints or clinical chemistry were not observed following bromodichloromethane exposure, with the exception of a 14% drop in creatinine in the 6 mg/kg-day Group A males and a 43% increase in 5'-nucleotidase in the 60 mg/kg-day 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 phosphatase 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 treated with 36 and 60 mg/kg-day bromodichloromethane and in Group B males treated with 60 mg/kg-day bromodichloromethane. Hepatic necrosis was dose-dependent, with incidences of 0/10, 0/10, 4/9, and 10/10 observed at 0, 6, 36, and 60 mg/kg-day, 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 60 mg/kg-day 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 102 mg/kg-day dose group.

Because exposure to concentrations of 36 and 60 mg/kg-day produced changes in liver histopathology in male rats and resulted in decreases in body weight and food and water consumption in both sexes, 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 males exposed at the 6 and 36 mg/kg-day concentrations, these data identify NOAEL and LOAEL values of 6 and 36 mg/kg-day, respectively, for occurrence of hepatic cell necrosis.

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 10 mg/kg-day concentration in Group C females, and a slight decrease in the number of corpora lutea at the 63 mg/kg-day concentration in Group A females (NTP, 1998). 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 60 and 102 mg/kg-day for male and female rats, respectively.

Bielmeier et al. (2001, 2004) 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 (Bielmeier et al., 2004). The strain comparison and critical period studies (Bielmeier et al., 2001) are summarized in Table 2 and discussed below.

In the strain comparison experiment, female SD rats (13 to 14/dose group) were dosed with 0, 75, or 100 mg/kg-day by aqueous gavage in 10% Emulphor® on GD 6 to 10 (Bielmeier et al., 2001). 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 PND 1 and 6. Surviving litters appeared normal and no effect on postnatal survival, litter size, or pup weight was observed.

Table 2. Summary of experiments conducted by Bielmeier et al. (2001)[a]

Study/Strain	Dose (mg/kg-day)	Treatment Period	Number of animals			
			Treated	Pregnant	Resorbed	%FLR
Strain comparison						
F344	0	GD 6–10	12	11	0	0
F344	75	GD 6–10	14	13	8	62[b]
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[c]
F344	75	GD 6–10	12	12	9	75[b]
F344	75	GD 11–15	13	13	0	0

[a]Source: Table 1 in Bielmeier et al. (2001)
[b]$p < 0.01$ for significant differences from controls (Fisher's Exact Test)
[c]$p < 0.05$

Abbreviations: GD, gestation day; FLR, full litter resorption; SD, Sprague-Dawley.

Bielmeier et al. (2001) conducted a second experiment to identify the critical period for bromodichloromethane-induced FLR in F344 rats. Two different 5-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 postnatal 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 (CCC) sponsored a developmental toxicity study of bromodichloromethane in rats (CCC, 2000a). 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, 1998) 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 (CCC, 2000a; Christian et al., 2001a). 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 18.4, 45.0, and 82.0 mg/kg-day 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 45.0 and 82.0 mg/kg-day exposure groups. In contrast, the effect was transient in the 2.2 and 18.4 mg/kg-day exposure groups. Average body weights were significantly reduced in the 45.0 and 82.0 mg/kg-day 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 (CCC, 2000a; Christian et al., 2001a). 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 18.4, 45.0, and 82.0 mg/kg-day groups. In the 18.4 mg/kg-day 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 45.0 and 82.0 mg/kg-day 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 82.0 mg/kg-day (CCC, 2000a; Christian et al., 2001a). 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 mg/kg-day, 1/182; 2.2 mg/kg-day, 0/199; 18.4 mg/kg-day, 0/200; 45.0 mg/kg-day, 0/188; 82.0 mg/kg-day, 4/194; litter incidence: 0 mg/kg-day, 1/23; 2.2 mg/kg-day, 0/25; 18.4 mg/kg-day, 0/25; 45.0 mg/kg-day, 0/25; 82.0 mg/kg-day, 2/25) of wavy ribs in the 82.0 mg/kg-day 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 18.4 and 45.0 mg/kg-day, respectively, based on statistically significant, persistent reductions in maternal body weight and body weight gains. The concentration-based developmental NOAEL and LOAEL were 45.0 and 82.0 mg/kg-day, respectively, based on a significantly decreased number of ossification sites per fetus for the forelimb phalanges and the hindlimb metatarsals and phalanges.

The Chlorine Chemistry Council sponsored a range-finding developmental toxicity study in New Zealand White rabbits (CCC, 2000b). 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, 1998) 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 1,350 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. 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 (CCC, 2000b; Christian et al., 2001b). 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 4.9 and 13.9 mg/kg-day 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 32.3 and 76.3 mg/kg-day 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) (CCC, 2000b; Christian et al., 2001b). All placentas were normal in appearance. No gross external fetal alterations were observed in the control or treatment groups.

In the satellite study, analytical analyses detected trace amounts of bromodichloromethane in placental samples from two litters in the 76.3 mg/kg-day group and in one fetus from the 76.3 mg/kg-day group (CCC, 2000b; Christian et al., 2001b). Bromodichloromethane was not detected in amniotic fluid or maternal plasma. One litter in the 32.3 mg/kg-day satellite exposure group consisted of only early resorptions. The concentration-based LOAEL for maternal toxicity in this study is 4.9 mg/kg-day, the lowest concentration tested, based on reduced body weight gain. The concentration-based NOAEL for developmental effects was 76.3 mg/kg-day (the highest dose tested).

The Chlorine Chemistry Council (CCC, 2000c) 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 55.3 mg/kg-day dose group was sacrificed moribund with hindlimb paralysis caused by a back injury. Another rabbit in the 55.3 mg/kg-day 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 35.6 and 55.3 mg/kg-day 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 13.4 and 35.6 mg/kg-day 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 and 35.6 mg/kg-day, respectively, based on decreased body weight gain. The developmental NOAEL was 55.3 mg/kg-day based on absence of statistically significant, dose-related effects at any tested concentration.

Reproductive Toxicity

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 study authors estimated the doses to be 0, 22, and 39 mg/kg-day. At 52 weeks, the study 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), 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.

The Chlorine Chemistry Council sponsored a range finding reproductive toxicity study of bromodichloromethane in rats (CCC, 2000d), which was conducted according to standard U.S. EPA test guidelines (U.S. EPA, 1998) and GLP standards. This study is summarized in Christian et al. (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. Bromodichloromethane was administered to parental rats (P generation) in drinking water at concentrations of 0, 50, 150, 450, or 1,350 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 (CCC, 2000d; Christian et al., 2001b). Lactation was extended for one week (LD 22-29) beyond the normal 3-week period because F1 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 F1 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 (CCC, 2000d; Christian et al., 2001b). In addition, testes and epididymides were excised from males and paired organ weights were measured. F1 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 F1 generation.

The consumption of bromodichloromethane was calculated from measured water intake and measured concentrations of the test article (CCC, 2000d; Christian et al., 2001b). Mean consumed dosages of bromodichloromethane for P generation male rats during the entire exposure period, P generation female rats during different physiologic stages, and

F1 postweaning rats are summarized in Table 3. 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 F1 female rats during the 1-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.

Table 3. Mean Consumed Doses (mg/kg-day) of Bromodichloromethane in the Range Finding Study Conducted by CCC (2000d) and Summarized in Christian et al. (2001b)[a]

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	Premating 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
F1	M	Postweaning days 1–8	0.0	13.6 ± 3.5	41.4 ± 7.1	106.9 ± 20.8	297.8 ± 113.8
F1	F	Postweaning days 1–8	0.0	13.9 ± 2.6	40.1 ± 6.8	117.9 ± 42.7	333.6 ± 110.6

[a]Source: CCC, 2000d; Christian et al., 2001b.

In the P generation, all male rats and all females except one survived to scheduled sacrifice (CCC, 2000d; Christian et al., 2001b). 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 postcohabitation 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 1,350-ppm exposure groups, and persisted in the 450- and 1,350-ppm females during gestation and lactation. Treatment-related clinical signs of toxicity were observed in both sexes in the 1,350-ppm exposure groups and were considered to be generally associated with reduced water consumption. Males exhibited dehydration, emaciation, chromorhinorrhea, and chromodacryorrhea during the premating, cohabitation, and postcohabitation 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 1,350-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 1,350-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 1,350 ppm, respectively, relative to control values. In female rats, reductions in body weight gain and body weight occurred in 150-, 450-, and 1,350-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 1,350-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.

No gross lesions attributable to bromodichloromethane were observed in the P generation male or female rats at necropsy (CCC, 2000d; Christian et al., 2001b). The absolute paired epididymal weights were slightly reduced (93.2% and 92.5%, respectively) in the 450- and 1,350-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 F1 pup body weights in the 150-, 450-, and 1,350-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 1,350-ppm exposure groups, respectively. Reduced body weight gain continued to occur in the F1 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 F1 pup litters in the 50-ppm group during lactation or the 1-week postweaning period.

Statistical analysis was not conducted in this range finding study (CCC, 2000d; Christian et al., 2001b). 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 (premating, mating, gestation, and lactation), it is not possible to convert the administered drinking water concentrations into biologically meaningful average daily doses.

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, 1998) 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 premating 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. F1 generation rats were exposed to bromodichloromethane in utero and by consumption of the dam's drinking water during the lactation period. At weaning, F1 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. F1 generation females delivered litters and the F2 litters were sacrificed on LD 22.

During the course of the experiment, parental and F1 generation rats were evaluated for viability, clinical signs, water and feed consumption, and body weight (CCC, 2002; Christian et al., 2002). Parental and F1 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. F1 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. F1 weanlings not selected for continued evaluation (3 pups/sex/litter, when available) and all F2 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 F1 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 F1 generation males and females (CCC, 2002; Christian et al., 2002). 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 F1 generation males and females at all concentrations of bromodichloromethane. Water intake by parental and F1 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; CCC, 2002) were not significantly affected by bromodichloromethane treatment. However, F1 and F2 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 F1 generation had statistically significant reductions in pup body weight at weaning on lactation day 22. Reductions in F2 pup body weight did not reach statistical significance. Small (6%), but statistically significant, delays in F1 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 F1 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 F2 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 F1 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 F0 females and F1 males and females.

Inhalation Exposure

The literature search identified only one publication, Torti et al. (2001), containing pertinent inhalation toxicity data for bromodichloromethane. This publication reported the results of 1-week and 3-week studies of wild-type and genetically modified mice, as well as 13-week interim sacrifice results from a chronic inhalation bioassay of genetically modified mice. The studies reported by Torti et al. (2001) have all been reviewed by U.S. EPA recently in the DWCD (U.S. EPA, 2005). The study summaries included in this review are excerpted from that document.

Torti et al. (2001) conducted a 1-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 (0, 6.7, 67, 201, 670, and 1,005 mg/m^3) for 6 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 (0, 2.0, 6.7, 67, or 201 mg/m^3) 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 (1,005 mg/m^3), the average measured concentrations were 102 to 114% of the target concentrations. The average high dose concentration was 78.8% of the target concentration (670 mg/m^3) 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 201 mg/m^{3}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 670 and 1,005 mg/m^3 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 67 mg/m^3 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 degeneration in the liver as compared to C57BL/6 mice (Torti et al., 2001). 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 (67 mg/m^3) 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 6.7 and 67 mg/m^3, 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, 0.3, 1, 3, 10, or 30 ppm (0, 2.0, 6.7, 20, 67, or 201 mg/m^3) 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 201 mg/m^{3}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 201 mg/m^3. 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 201 mg/m^3. Histopathologic evaluation revealed near-normal kidney architecture. Minimal to moderate degenerative tubular change and regenerative tubules were observed in the 67 and 201 mg/m^3 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 201 mg/m^3 and in heterozygous FVB/N mice exposed at 67 or 201 mg/m^3. 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 201 mg/m^3 groups showing small elevations. These elevations were statistically significant in all 300-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 20 and 67 mg/m^3, 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.

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 (0, 3.4, 20, 67, or 101 mg/m^3), 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.

Storer et al. (2001) reported negative findings in the inhalation cancer bioassay using p53 heterozygous C57BL/6 and FVB/N male mice referred to by Torti et al. (2001). No further details were provided, and a full report on the findings was not identified in the literature search.

Other Studies

Toxicokinetics

U.S. EPA (2005) recently reviewed the available data on the toxicokinetics of bromodichloromethane and other trihalomethanes. The information provided herein is adapted from the executive summary of the DWCD (U.S. EPA, 2005). Further details on the studies noted in the summary are available in the DWCD. Pertinent studies published after the DWCD are also discussed.

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. 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 posttreatment. 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 principal route of excretion [of trihalomethanes] 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.

In a study published after the DWCD, Leavens et al. (2007) compared the pharmacokinetics of ^{13}C-bromodichloromethane (99% pure) in ten humans (nine males, one female) exposed via ingestion (in sterile, distilled water) and dermal contact (1-hour forearm submersion). A bromodichloromethane concentration of 36 µg/L was used for both exposures. Blood was collected before—and up to—24 hours after exposure for gas chromatographic analysis of ^{13}C-bromodichloromethane. The study authors estimated doses of bromodichloromethane as ranging from 81 to 257 ng/kg body weight via oral exposure and from 87.4 to 321 ng/kg body weight via dermal exposure. Blood levels of ^{13}C-bromodichloromethane were higher after dermal exposure than after oral exposure. The average C_{max} after oral exposure, occurring by 11 minutes after exposure, was 2.6 ng/L. In contrast, the concentration of ^{13}C-bromodichloromethane at the end of the dermal exposure period of 1 hour averaged 94.9 ng/L. Blood levels of bromodichloromethane declined rapidly after oral exposure, with levels approaching the detection limit within 3–4 hours after exposure; the study authors estimated the half-life in blood to be 47 minutes. Bromodichloromethane was measurable in blood up to 24 hours after dermal exposure, and a biphasic decline in blood levels was observed; half-lives of 32.6 and 309 minutes were calculated for the two elimination phases. The study authors concluded that the differences in blood levels after oral and dermal exposure to

bromodichloromethane were largely attributable to a significant first-pass liver metabolism of the compound after oral exposure.

PBPK Models of Bromodichloromethane

Lilly et al. (1998) developed a physiologically based pharmacokinetic (PBPK) model for orally administered bromodichloromethane. The study authors collected toxicokinetic data from male F344 rats exposed via gavage to 0, 50, or 100 mg bromodichloromethane/kg in either corn oil or 10% Emulphor® to characterize the PBPK parameters. In a recent study, Tan et al. (2007) adapted a human PBPK model for chloroform for use in modeling the pharmacokinetics of bromodichloromethane and other trihalomethanes. The study authors derived chemical-specific partition coefficients from available data in humans and rats. They used the model with a probabilistic exposure scenario to estimate trihalomethane concentrations in blood. A PBPK model for bromodichloromethane in the mouse was not located in the available literature.

Immunotoxicity

U.S. EPA (2005) reviewed a 26-week immunotoxicity study of bromodichloromethane in drinking water (French et al., 1999); the study summary below is adapted from the DWCD.

French et al. (1999) investigated the immunotoxicity of bromodichloromethane in male Fisher 344 rats. 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 study 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.

Other studies of shorter exposure duration reported that bromodichloromethane decreased antibody-forming cells in the serum of female mice exposed to gavage doses of ≥125 mg/kg-day for 14 days (Munson et al., 1982), but they did not affect antibody response to injected sheep red blood cells, or T and B lymphocyte proliferation, in female mice exposed to doses up to 62 mg/kg-day in drinking water (for 14–28 days) or to doses up to 250 mg/kg-day via gavage (for 16 days; French et al., 1999). In female rats exposed by gavage to bromodichloromethane, doses of 300 mg/kg-day (the highest dose tested) for 5 days and lymphocyte proliferation in spleen cells (in response to the mitogens concanavalin A and phyto-hemagglutin-p) was depressed (French et al., 1999).

Neurotoxicity

Balster and Borzelleca (1982) observed no changes in neurobehavioral measures (motor coordination or exploratory behavior) in mice exposed to bromodichloromethane via gavage at doses up to 10 mg/kg-day for 90 days. In additional experiments, no effect was observed on passive-avoidance learning after 30 days of exposure to 100 mg/kg-day, but operant behavior (use of a lever to access a food reward) was adversely affected by 60 days of exposure to doses of 100 or 400 mg/kg-day.

Moser et al. (2007) evaluated the potential neurotoxicity of bromodichloromethane administered in drinking water to male and female F-344 rats for 6 months. The study authors estimated doses of 0, 9, 27, and 72 mg/kg-day of bromodichloromethane. A functional observation battery and motor activity were assessed at Weeks 4, 9, 17, and 26. After exposure was terminated, the animals were sacrificed for histopathologic examination of central and peripheral nervous system tissues. The data showed that there were no toxicologically significant alterations in neurobehavioral parameters or neuropathology.

DERIVATION OF PROVISIONAL SUBCHRONIC ORAL RfD FOR BROMODICHLOROMETHANE

Subchronic p-RfD

Two subchronic toxicity studies (both reported by NTP, 1987) and eight developmental or reproductive toxicity studies (Ruddick et al., 1983; Narotsky et al., 1997; Bielmeier et al., 2001; CCC, 2000a,b,c,d and CCC, 2002, also published as Christian et al. 2001a,b, and Christian et al., 2002) are available for use in deriving a subchronic p-RfD for bromodichloromethane. The DWCD (U.S. EPA, 2005) considered these same studies in deriving the longer-term health advisory for bromodichloromethane. After careful consideration of these studies and the benchmark dose (BMD) modeling of a number of endpoints from several different studies as reported in the DWCD (U.S. EPA, 2005), dose-dependent pregnancy loss [i.e., full litter resorption (FLR)] in gavage-treated female F344 rats has been identified as the most sensitive endpoint from a strain comparison, a critical period, and two hormone profile experiments on the developmental toxicity of bromodichloromethane (Bielmeier et al., 2001). Support for the choice of FLR as a critical effect is that this endpoint has also been observed in other rat studies of bromodichloromethane (Narotsky et al., 1997). Additionally, epidemiologic studies show an association between an increased risk of spontaneous abortion with consumption of bromodichloromethane in drinking water (Waller et al., 1998). In the Bielmeier et al. (2001) study, the strain comparison experiments show a significantly increased incidence of FLR occurred in F344 rats treated with bromodichloromethane, whereas Sprague-Dawley rats maintained their litters. In the critical period experiments, F344 rats treated on GD 6−10 at 75 mg/kg-day had a significantly increased incidence of FLR, but rats treated on GD 11−15 at 75 or 100 mg/kg-day were unaffected. Because significant increases in incidences of FLR were consistent between F344 rats treated on GD 6−10, GD 6−15, GD 8, and GD 9, all available dichotomous models in the U.S. EPA BMD Software (BMDS) version 2.1 beta were applied to the FLR incidence data from a hormone profile experiment that treated F344 rats with three doses of bromodichloromethane on GD 9 (Bielmeier et al., 2001). For dose-dependent incidences of FLR observed in treated female rats, a benchmark response (BMR) of 5% extra risk was used in modeling this endpoint because of its severity and occurrence during fetal development. A $BMDL_{05}$ of 0.76 mg/kg-day was calculated for bromodichloromethane-induced FLR and identified as the point of departure (POD) for the subchronic p-RfD derivation. Details of BMD modeling are presented in Appendix A.

For derivation of the **subchronic p-RfD**, the $BMDL_{05}$ of 0.76 mg/kg-day was divided by a UF of 100 to yield a subchronic p-RfD for bromodichloromethane, as follows:

Subchronic p-RfD = $BMDL_{05} \div UF$
= 0.76 mg/kg-day ÷ 100
= **0.008 or 8×10^{-3} mg/kg-day**

The UF of 100 is composed of the following:

- A full 10-fold UF_H for intraspecies differences is applied to account for potentially susceptible individuals in the absence of information on the variability of response in humans.
- A full 10-fold UF_A for interspecies extrapolation is applied to account for potential pharmacokinetic and pharmacodynamic differences between rats and humans.
- A UF_D of 1 for database deficiencies is applied. The database for oral exposure to bromodichloromethane includes subchronic and chronic toxicity studies in two species, developmental toxicity studies in two species, a multigeneration reproductive toxicity study in rats, and a 6-month neurotoxicity study in rats.

Confidence in the key study (Bielmeier et al., 2001) is high; the study uses 8–11 animals per dose, examines several developmental toxicity endpoints, is well designed and well documented, identifies both a NOAEL and LOAEL, and the data were amenable to BMD modeling. Confidence in the database is also high; it includes subchronic and chronic toxicity studies in two species, developmental toxicity studies in two species, a multigeneration reproductive toxicity study in rats, and a 6-month neurotoxicity study in rats. High confidence in the subchronic p-RfD for bromodichloromethane follows.

Chronic p-RfD

A chronic oral RfD of 2×10^{-2} mg/kg-day for bromodichloromethane is available on IRIS (U.S. EPA, 1988). This chronic oral RfD is based on a LOAEL of 17.9 mg/kg-day for renal cytomegaly in male mice administered the chemical in corn oil by gavage for 102 weeks (National Toxicology Program [NTP], 1987) and a composite UF of 1000 (10 for extrapolation from mice to humans, 10 for protection of sensitive individuals, and 10 for the use of a minimal LOAEL and database deficiencies). Although the subchronic p-RfD for bromodichloromethane of 8×10^{-3} mg/kg-day in this PPRTV document is lower than the IRIS chronic oral RfD of 2×10^{-2} mg/kg-day (U.S. EPA, 1988), the p-RfD is based on a study (Bielmeier et al., 2001) that did not exist when the IRIS assessment was initially performed. Additionally, a 2-generation reproductive study has also been published, (CCC, 2002; Christian et al., 2002), that changed the database UF from 10 (which was used in the IRIS assessment) to one (1) for the purposes of this PPRTV document.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION RfC VALUES FOR BROMODICHLOROMETHANE

The only study available for use in deriving an inhalation p-RfC for bromodichloromethane is a 3-week inhalation study in mice (Torti et al., 2001). This study used both wild–type (p53 homozygous) and genetically modified (p53 heterozygous) mice of two different strains (C57BL/6 and FVB/N). This 3-week inhalation study, although of shorter

duration than is typically used for derivation of subchronic toxicity studies, is supported by a 1-week study that identifies the same target organs and effect levels (also reported by Torti et al., 2001).

Subchronic p-RfC

For the purpose of deriving the subchronic p-RfC, the toxicological findings in wild-type C57BL/6 and FVB/N mice were considered the most relevant because findings in the genetically modified mice are of uncertain relevance to humans. In wild-type mice exposed for 3 weeks (Torti et al., 2001), the NOAEL and LOAEL values were 20 and 67 mg/m^3 based on histopathologic evidence of kidney degeneration identified as the most sensitive effect (note that the liver findings noted at the LOAEL in the study summary were limited to the genetically modified FVB/N mice). Torti et al. (2001) reported mean severity scores for kidney degeneration in the 3-week study but did not report incidences of this effect; thus, the data are not amenable to BMD modeling. The NOAEL from this study, 20 mg/m^3, is selected as the POD for subchronic p-RfC derivation. The NOAEL is adjusted for continuous exposure as follows:

$$\begin{aligned} NOAEL_{ADJ} &= NOAEL \times 6/24 \text{ hours} \\ &= 20\ mg/m^3 \times 6/24 \\ &= 5\ mg/m^3 \end{aligned}$$

The human equivalent concentration ($NOAEL_{HEC}$) is then calculated using the dosimetric adjustment outlined in U.S. EPA (1994b). As kidney histopathology is an extrarespiratory effect, bromodichloromethane was treated as a Category 3 gas and the ratio of blood:gas partition coefficients was used to make the dosimetric adjustment. A blood:gas partition coefficient for bromodichloromethane in humans is identified (26.3; Abraham et al., 2005), but a corresponding value for mice has not been located; thus, the default ratio of 1.0 is used. The resulting $NOAEL_{HEC}$ is 5 mg/m^3 (5 $mg/m^3 \times 1.0$). The $NOAEL_{HEC}$ is divided by a UF of 300 to give a **subchronic p-RfC** for bromodichloromethane as shown below:

$$\begin{aligned} \textbf{Subchronic p-RfC} &= NOAEL_{HEC} \div UF \\ &= 5\ mg/m^3 \div 300 \\ &= \mathbf{0.02\ mg/m^3 \text{ or } 2 \times 10^{-2}\ mg/m^3} \end{aligned}$$

The composite UF of 300 consisted of the following:

- A UF_A of 3 ($10^{0.5}$) is used for extrapolation from rats to humans using dosimetric adjustments. The interspecies UF includes a factor of 1 (one) for species differences in pharmacokinetic considerations (as a dosimetric adjustment was used) and 3 for pharmacodynamic considerations.
- A full 10-fold UF_H is used for protection of sensitive individuals in the absence of information on the variability in response to bromodichloromethane in the human population.
- A database UF_D of 10 is used; the toxicological database for inhaled bromodichloromethane contains only 1- and 3-week studies in one species, and a chronic bioassay in genetically modified mice that has not been fully published anywhere. While the database for oral exposure to bromodichloromethane is extensive, evidence for a significant first-pass metabolism of orally administered

bromodichloromethane limits the value of the oral database for interpreting inhalation toxicity.

Confidence in the key study (Torti et al., 2001) is low. Although, the study is well designed, thoroughly documented, and carefully conducted, and both a NOAEL and LOAEL are identified, the brevity of the exposure duration, the use of only one animal species (mouse), the use of only one sex in the control groups, and the unknown distribution of sexes in the exposure groups limit confidence in the findings. Confidence in the database for inhaled bromodichloromethane is low, as noted above. The database lacks subchronic- and chronic-duration inhalation toxicity data as well as reproductive and developmental toxicity studies. Available studies have not identified neurotoxicity after acute exposure to high concentrations, indicating that the lack of a neurotoxicity study may not be a concern. Low confidence in the subchronic p-RfC follows.

Chronic p-RfC

The brevity of the exposure duration (3 weeks) in the only available inhalation toxicity study of bromodichloromethane (Torti et al., 2001) precludes its use for derivation of a chronic p-RfC. Route-to-route extrapolation from the IRIS chronic RfD for bromodichloromethane (U.S. EPA, 2008) was considered, but it was concluded to be unfeasible given the available toxicokinetic information. There are few toxicokinetic data for inhalation exposure to bromodichloromethane; no studies of absorption, distribution, metabolism or excretion after inhalation exposure were identified. The chronic RfD on IRIS is based on kidney effects in a chronic mouse study (NTP, 1987). While a rat PBPK model has been developed for oral exposure to bromodichloromethane (Lilly et al., 1998), and a human PBPK model is available for all exposure routes (Tan et al., 2007), no model is yet available for the mouse. In addition, Leavens et al. (2007) compared human blood levels of bromodichloromethane after oral and dermal exposure and concluded that there is a considerable first-pass effect via oral exposure, as well as a significant difference in absorption and distribution between the oral and dermal routes. The evidence for first-pass metabolism of bromodichloromethane after oral exposure provides a strong argument against route-to-route extrapolation in the absence of a PBPK model for the relevant species.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR BROMODICHLOROMETHANE

Weight-of-Evidence Descriptor

IRIS includes a cancer assessment for bromodichloromethane (verified 4/12/1992) in which the chemical was assigned to the cancer weight-of-evidence Group B2 (probable human carcinogen under the U.S. EPA [1986] *Guidelines for Cancer Risk Assessment*), and an OSF of 6.2×10^{-2} (mg/kg-day)$^{-1}$ was derived based on an increased combined incidence of tubular cell adenoma and tubular cell adenocarcinoma in male B6C3F1 mice exposed to bromodichloromethane by oral gavage for 2 years (NTP, 1987). Three cancer bioassays of bromodichloromethane have been published since the IRIS cancer assessment for this compound. Of these, two (Aida et al., 1992 and George et al., 2002) were reviewed by U.S. EPA (2005); brief summaries of these were adapted from the DWCD.

Aida et al. (1992) administered microencapsulated bromodichloromethane mixed with powdered feed to Wistar rats for up to 24 months. The mean doses were estimated to be 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 (40 males and 40 females for each treatment group and 70 males and 70 females for the control group). 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. The study authors concluded that there was no clear evidence that microencapsulated bromodichloromethane administered in the diet was carcinogenic in Wistar rats.

George et al. (2002) conducted a chronic cancer bioassay of bromodichloromethane administered in drinking water to mice and rats. The study authors observed a significantly increased prevalence of neoplastic lesions in the liver of male rats at 3.9 and 20.6 mg/kg-day bromodichloromethane, but not at 36.3 mg/kg-day. In mice, hepatocellular adenomas and carcinomas were seen in all treatment groups, but neither prevalence nor multiplicity was increased by exposure to bromodichloromethane compared to controls.

U.S. EPA (2005) assessed the weight of evidence for bromodichloromethane carcinogenicity under the Draft Revised 1999 Cancer Guidelines (U.S. EPA, 1999), including the studies published by Aida et al. (1992) and George et al. (2002), and concluded that bromodichloromethane is "*Likely to be Carcinogenic to Humans"* by the oral route. In 2006, NTP published the findings of a new 2-year cancer bioassay for bromodichloromethane administered in drinking water to male F344/N rats and female B6C3F1 mice (50/species/dose). In the NTP study, bromodichloromethane was administered at concentrations of 0, 175, 350, and 700 mg/L for 105 weeks. NTP (2006) estimated average daily doses of 6, 12, and 25 mg/kg-day but acknowledged that these values are likely overestimates based on the difference between nominal concentrations and actual concentrations measured in the animals' water supplies. Bromodichloromethane exposure via drinking water did not result in a statistically significant increase in the incidence of any neoplasm in male rats or female mice (NTP, 2006). Possible explanations between the differential tumor responses observed between the NTP (1987) and NTP (2006) carcinogenicity bioassays include the influence of the vehicle (i.e., corn oil versus drinking water), the stability of bromodichloromethane in drinking water, and different absorption rates that may lead to variability in organ dosimetry after exposure by gavage versus drinking water.

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APPENDIX A. BENCHMARK DOSE MODELING FOR THE SUBCHRONIC p-RfD

The benchmark dose (BMD) modeling for incidence of FLR in bromodichloromethane gavage-treated female F344 rats (Bielmeier et al., 2001) was conducted with the U.S. EPA's BMD software (BMDS version 2.1 beta). The original data were modeled with all the dichotomous models (i.e., Gamma, Multistage, Logistic, Log-Logistic, Probit, Log-Probit, Weibull, and Quantal Linear models) available within BMDS 2.1 beta with a benchmark response (BMR) of 5% extra risk because of the severity of FLR and its occurrence during fetal development (see Table A-1). An adequate fit was determined based on the goodness-of-fit *p*-value ($p > 0.1$), scaled residual at the range of benchmark response (BMR), and visual inspection of the model fit. Among all the models that provided adequate fit to the data, the lowest $BMDL_{05}$ was selected if the $BMDL_{05}$s estimated from the different models varied >3-fold; otherwise, the $BMDL_{05}$ from the model with the lowest Akaike's Information Criterion (AIC) was considered to be appropriate for the data set.

As assessed by the goodness-of-fit *p*-values, all dichotomous models available in the BMDS adequately fit the FLR data (see Table A-2). Because the $BMDL_{05}$s estimated from the different models varied >3-fold, the lowest $BMDL_{05}$ calculated by the Log-Logistic model was selected as the POD. The estimated BMD_{05} and $BMDL_{05}$ from this model for FLR are 40.53 and 0.76 mg/kg-day, respectively (see Table A-2 and Figure A-1).

Table A-1. Incidence of Full Litter Resorption (FLR) in Female F344 Rats Given Bromodichloromethane by Gavage on Gestational Day 9[a]

Dose (mg/kg-day)	0	75	100
FLR incidence	0/8	7/11	9/10

[a]Bielmeier et al., 2001

Table A-2. Dose-Response Modeling of Incidence of FLR in Female F344 Rats Given Bromodichloromethane by Gavage on Gestational Day 9[a]

Model	Goodness of fit *p*-value	AIC	BMD_{05}	$BMDL_{05}$
Gamma[b]	1.0000	24.922	36.32	2.10
Multistage[c]	0.9122	23.112	15.95	2.06
Logistic	0.7706	25.053	30.74	8.64
Log-Logistic[d]	**1.0000**	**24.922**	**40.53**	**0.76**
Probit	0.8337	24.987	28.22	7.92
Log-Probit[d]	1.0000	24.922	40.55	5.34
Weibull[b]	1.0000	24.922	26.43	2.10
Quantal-Linear	0.6537	23.819	3.00	1.94

[a]Bielmeier et al., 2001
[b]Restrict power ≥ 1
[c]Restrict betas ≥ 0; degree of polynomial = 2 (lowest degree of polynomial with an adequate fit reported).
[d]Slope restricted to >1

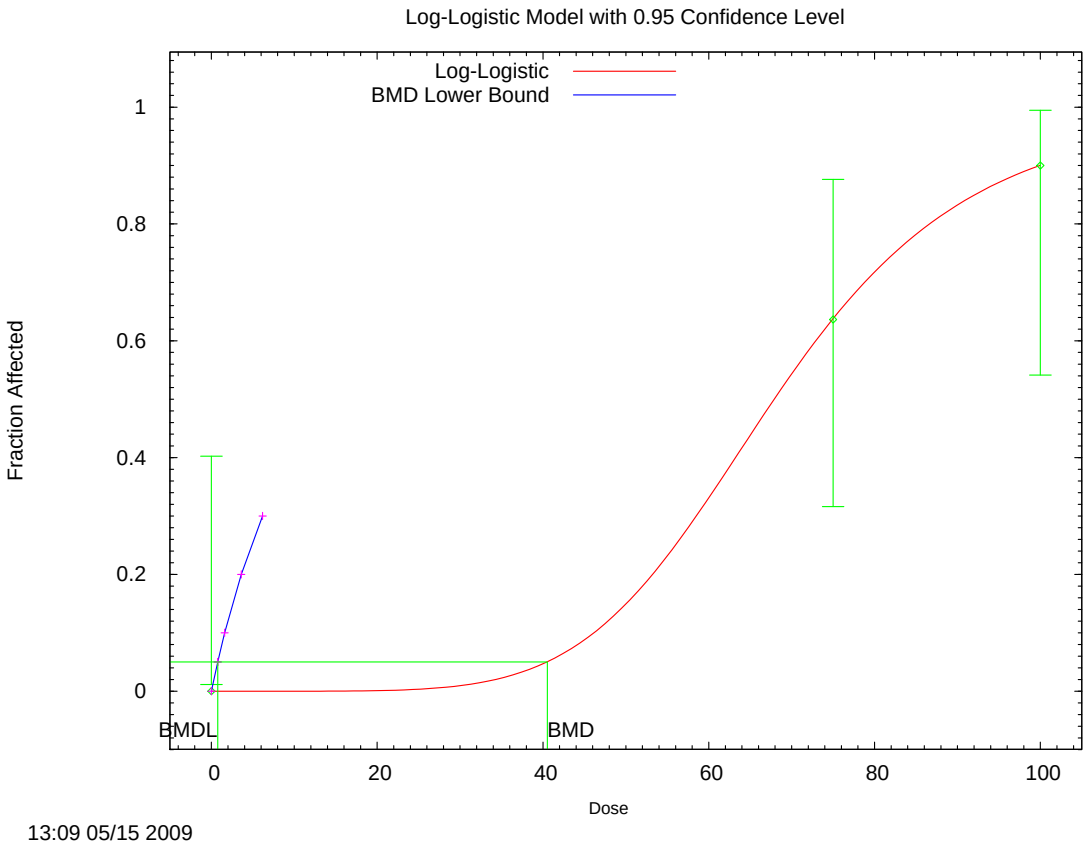

Figure A-1. Dose-Response Modeling of FLR in Female F344 Rats Given Bromodichloromethane by Gavage on Gestational Day 9 (Bielmeier et al., 2001).

The BMDs and BMDLs are associated with a BMR of 5% extra risk and are in units of mg/kg-day.