4-14-2005

Provisional Peer Reviewed Toxicity Values for

Bromoform

(CASRN 75-25-2)

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

Acronyms and Abbreviations

bw	body weight
cc	cubic centimeters
CD	Caesarean Delivered
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act of 1980
CNS	central nervous system
cu.m	cubic meter
DWEL	Drinking Water Equivalent Level
FEL	frank-effect level
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
g	grams
GI	gastrointestinal
HEC	human equivalent concentration
Hgb	hemoglobin
i.m.	intramuscular
i.p.	intraperitoneal
i.v.	intravenous
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
kg	kilogram
L	liter
LEL	lowest-effect level
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
m	meter
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mg/kg	milligrams per kilogram
mg/L	milligrams per liter
MRL	minimal risk level

MTD	maximum tolerated dose
MTL	median threshold limit
NAAQS	National Ambient Air Quality Standards
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**
PBPK	physiologically based pharmacokinetic
ppb	parts per billion
ppm	parts per million
PPRTV	**Provisional Peer Reviewed Toxicity Value**
RBC	red blood cell(s)
RCRA	Resource Conservation and Recovery Act
RDDR	Regional deposited dose ratio (for the indicated lung region)
REL	relative exposure level
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	Regional gas dose ratio (for the indicated lung region)
s.c.	subcutaneous
SCE	sister chromatid exchange
SDWA	Safe Drinking Water Act
sq.cm.	square centimeters
TSCA	Toxic Substances Control Act
UF	uncertainty factor
µg	microgram
µmol	micromoles
VOC	volatile organic compound

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PROVISIONAL PEER REVIEWED TOXICITY VALUES FOR BROMOFORM (CASRN 75-25-2)

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (EPA's) 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. EPA's Integrated Risk Information System (IRIS).

2. Provisional Peer-Reviewed Toxicity Values (PPRTV) used in 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 EPA's Integrated Risk Information System (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 EPA IRIS Program. All provisional toxicity values receive internal review by two EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multi-program consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because science and available information evolve, PPRTVs are initially derived with a three-year life-cycle. However, EPA Regions or the EPA Headquarters Superfund Program sometimes request that a frequently used PPRTV be reassessed. 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 manuscripts 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 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 manuscript and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other 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 EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

The HEAST (U.S. EPA, 1997) lists a subchronic oral reference dose (RfD) value of 2E-1 for bromoform. The subchronic RfD is based on a NOAEL of 25 mg/kg-day for hepatic vacuolization in male rats administered bromoform five days per week for 13 weeks (NTP, 1989a). The LOAEL in this study was 50 mg/kg-day. The NOAEL was adjusted to a continuous dose of 17.9 mg/kg-day and divided by a composite uncertainty factor (UF) of 100, which includes factors of 10 each to account for animal to human extrapolation and interhuman variability. The source document for derivation of the subchronic oral RfD is a Health and Environmental Effects Document (HEED) for Bromoform (U.S. EPA, 1989). The HEAST references IRIS (U.S. EPA, 2003) for the chronic RfD of 2E-2 mg/kg-day. The chronic RfD is also derived from the subchronic data for hepatic vacuolization in male rats obtained in the 13-week NTP (1989a) study. A composite UF of 1000, which includes an additional factor of 10 for

use of data from a subchronic study, was used in the calculation of the chronic value. The chronic RfD was verified by the RfD Work Group on August 13, 1987.

Several additional sources were reviewed for information on bromoform toxicity. The CARA list (U.S. EPA, 1991, 1994a) includes no documents for bromoform other than the 1989 HEED. A Health Effects Assessment (HEA) is available for trihalogenated methanes (U.S. EPA, 1987). The HEA lists subchronic and chronic RfDs of 0.056 mg/kg-day and 0.0056 mg/kg-day, respectively, for bromoform that were derived from subchronic oral exposure data published by Chu et al. (1982). The Drinking Water Standards and Health Advisories list (U.S. EPA, 2002) includes bromoform and lists the current chronic RfD value of 2E-2 mg/kg-day. ATSDR (1990) derived a chronic oral minimal risk level (MRL) for bromoform of 0.2 mg/kg-day based on a NOAEL of 20 mg/kg-day for hepatic pathology in a chronic study by Tobe et al. (1982). The World Health Organization (WHO, 2002) has not prepared an Environmental Health Criteria document on bromoform.

Neither the HEAST, the HEED, nor the HEA report a subchronic inhalation reference concentration (RfC) for bromoform. The HEAST references IRIS for the chronic RfC. IRIS indicates that the inhalation database for bromoform is inadequate to calculate an inhalation reference concentration and lists the current RfC status as *not verifiable* (U.S. EPA, 2003). The RfD/RfC Workgroup assigned this status on February 11, 1993. ACGIH (2001) lists a TLV-TWA of 0.5 ppm (5.2 mg/m^3) with skin and A3 cancer notations for bromoform. This occupational exposure limit is intended to minimize the potential for lacrimation, salivation, and irritation of the respiratory tract, and possible liver damage as reported in rodent studies. The A3 notation identifies bromoform as a confirmed animal carcinogen with unknown relevance to humans. NIOSH (2002) and OSHA (2002) have adopted 0.5 ppm (5 mg/m^3) with a skin notation for the bromoform REL-TWA and PEL-TWA, respectively.

The HEAST references IRIS (U.S. EPA, 2003) for the oral cancer slope factor, drinking water unit risk, and inhalation unit risk values for bromoform. These values were verified by the CRAVE Work Group on August 2, 1989. The oral cancer slope factor of 7.9E-3 $(mg/kg\text{-}day)^{-1}$ and oral unit risk value of 2.3E-7 $(\mu g/L)^{-1}$ are derived from dose-response data for neoplastic lesions in the large intestine of female F344/N rats (NTP, 1989a). Appropriate inhalation exposure data were not available for estimation of carcinogenic potency. Therefore, the inhalation unit risk of 1.1E-6 $(\mu g/m^3)^{-1}$ was derived from the oral exposure tumor data by route-to-route extrapolation. The HEAST lists an inhalation slope factor of 3.9E-3 $(mg/kg\text{-}day)^{-1}$ for bromoform. The calculation of this factor is not clearly described in the documents referenced in the HEAST. Handwritten comments on a draft of the IRIS file dated May 19, 1989 suggest that the inhalation slope factor was obtained by adjusting the oral slope factor for a default bromoform absorption of 50% via the inhalation route. U.S. EPA (1989, 2003) evaluated the weight of evidence for human carcinogenicity and assigned bromoform to carcinogenicity group B2 (probable human carcinogen). NTP (2002) does not list bromoform among the chemicals it

considers to be known human carcinogens or reasonably anticipated to be human carcinogens. IARC (1991, 1999) has assigned bromoform to weight of evidence Group 3, not classifiable as to its carcinogenicity to humans.

Literature searches to identify studies relevant to the derivation of provisional toxicity values for bromoform were conducted for the period 1988 through September 19, 2002. Databases searched included: TOXLINE, MEDLINE, TSCATS, RTECS, CCRIS, DART, EMIC/EMICBACK, HSDB, GENETOX and CANCERLIT. Additional literature searches from 2002 through May 2004 were conducted by NCEA-Cincinnati using TOXLINE, MEDLINE, Chemical and Biological Abstracts databases.

REVIEW OF PERTINENT DATA

Human Studies

No adequate data on the subchronic or chronic effects of bromoform in humans were located. Some data on the acute or short-term effects of oral exposure are available because bromoform was used in the early 1900s to sedate children suffering from whooping cough (U.S. EPA, 1989, 1994b). A typical dose for mild sedation was one drop (about 180 mg) given three to six times daily, or approximately 54 mg/kg-day (U.S. EPA, 1994b). Several deaths or near-deaths related to this practice have been documented. The most prominent clinical sign of poisoning in fatal cases was profound depression of the central nervous system. Death usually resulted from respiratory failure. The fatal doses were not quantified, but have been estimated to be 20 to 40 drops of bromoform or approximately 150 to 300 mg/kg-day (U.S. EPA, 1989, 1994b). Low-level inhalation exposure is reported to result in irritation, lacrimation, and reddening of the face (Sax and Lewis, 1989). Dykan (1964) reported that workers in bromoform production exhibited changes in the central nervous system and liver. No supporting details were provided on the duration or level of exposure, the number of workers evaluated, or potential confounding factors.

A number of epidemiological studies have examined the potential association of reproductive or developmental outcomes with consumption of tap water containing trihalomethanes, including bromoform (reviewed in Nieuwenhuijsen et al., 2000 and Bove et al., 2002). Studies that have examined associations of reproductive or developmental outcomes with trihalomethanes have in most cases used total trihalomethane concentration (a composite measure which includes the brominated trihalomethanes and chloroform) as the exposure metric. One study is available which examined the potential association specifically between bromoform concentration in disinfected tap water and reproductive outcome. Waller et al. (1998) conducted a prospective study in pregnant women to examine the association between exposure to trihalomethanes in drinking water and spontaneous abortion (pregnancy loss at 20 or less

completed weeks of gestation). No association with incidence of spontaneous abortion was observed for bromoform when data were adjusted for gestation or maternal age at interview, smoking, history of pregnancy loss, and maternal race or after adjustment for the presence of other trihalomethanes in tap water.

Animal Studies

NTP (1989a) administered bromoform to male and female F344/N rats by corn oil gavage for 13 weeks (5 days/week). The study was conducted using a standard NTP protocol and was GLP compliant. The test animals (10/sex/dose) received doses of 0, 12, 25, 50, 100, or 200 mg/kg-day. The administered doses in both male and females correspond to duration-adjusted doses of 0, 8.6, 17.9, 35.7, 71.4, and 143 mg/kg-day, respectively. Complete histopathology was performed on rats in the vehicle control and high-dose groups. Liver histopathology was performed on rats in all treatment groups. No rats died before the end of the study and body weights were not significantly affected in either sex. All high-dose animals and male rats dosed with 100 mg/kg-day were lethargic. A dose-related increase in the incidence of hepatocellular vacuolization was observed in male rats (incidence: vehicle control, 3/10; 12 mg/kg-day, 6/10; 25 mg/kg-day, 5/10; 50 mg/kg-day, 8/10; 100 mg/kg-day, 8/10; 200 mg/kg-day, 10/10). The response in males reached statistical significance at 50 mg/kg-day when data were analyzed using Fisher's Exact Test (U.S. EPA, 2003). This lesion was characterized by the presence of well-demarcated vacuoles in the cytoplasm of hepatocytes. The number of these vacuoles was reported to be greater in the 200 mg/kg-day group than in other dose groups. Hepatocellular vacuolization was not observed in female rats. No data were presented for any other histopathologic lesion in males or females. NOAEL and LOAEL values of 25 mg/kg-day and 50 mg/kg-day (duration adjusted values of 17.9 and 35.7 mg/kg-day) were identified in this study based on hepatocellular vacuolization in male rats.

In a parallel study, NTP (1989a) administered bromoform to male and female $B6C3F_1$ mice by corn oil gavage for 13 weeks (5 days/week). The study was conducted using a standard NTP protocol and was GLP compliant. Test animals (10/sex/dose) received doses of 0, 25, 50, 100, 200, or 400 mg/kg-day. The administered doses correspond to duration-adjusted doses of 0, 17.9, 35.7, 71.4, 143, and 286 mg/kg-day, respectively. Complete histopathology was conducted on the vehicle control and high-dose groups. In addition, liver histopathology was conducted on male mice receiving doses of 100 mg/kg-day and higher. One female mouse died at 100 mg/kg-day; no other deaths occurred at any dose level. Final mean body weights in dosed animals did not differ statistically from the vehicle control, although the final body weight in high-dose males was reduced by 8%. A dose-related increase in the incidence of minimal to moderate hepatocellular vacuolization was seen in male mice. This lesion was not observed in female mice. The histologic changes associated with this lesion in males involved only a few cells or were diffuse. The incidence of this lesion was 5/10 at 200 mg/kg and 8/10 at 400 mg/kg. The incidences of hepatocellular vacuolization in the vehicle control and 100 mg/kg-day groups were

not explicitly reported and are assumed to be 0/10. NOAEL and LOAEL values of 100 mg/kg-day and 200 mg/kg-day (duration adjusted values of 71.4 and 143 mg/kg-day), respectively, were identified in this study based on hepatocellular vacuolization in male mice.

Chu et al. (1982) administered bromoform to male and female weanling Sprague-Dawley rats (20 rats/sex/group) in drinking water for 90 days. The tested concentrations were 0, 5, 50, 500, or 2500 ppm. No information was provided on measures taken to monitor or validate the concentrations of bromoform in drinking water. Half of the animals in each group (10/sex/dose) were sacrificed at the end of the exposure period and the remaining animals were given tap water for another 90 days to assess recovery. Based on estimation of bromoform intake per rat by the study authors and data provided for body weight gain, the administered concentrations resulted in doses of approximately 0, 0.65, 6.1, 57, and 218 mg/kg-day for males and 0, 0.64, 6.9, 55, and 283 mg/kg-day for females. A trend toward decreased body weight gain was observed, but this response did not reach statistical significance in any exposure group. Lymphocyte counts were significantly decreased in high-dose males and females when evaluated 90 days after cessation of treatment. Lactate dehydrogenase activity was significantly decreased in high-dose males and females at cessation of treatment and the effect was still evident after the 90-day recovery period. Mild histologic changes occurred in the liver and thyroid of male and female animals. The observed hepatic lesions included increased cytoplasmic volume and vacuolization due to fatty infiltration. The incidence and severity of hepatic lesions tended to increase with dose (Table 1). The incidences of combined hepatic lesions in the 500 ppm and 2500 ppm exposure groups were significantly greater ($p \leq 0.05$) than the vehicle control incidence for both male and female rats when independently analyzed using Fisher's Exact Test by SRC. The severity of combined hepatic lesions was significantly greater in 2500 ppm males and in 500 and 2500 ppm females. Lesions of the thyroid included decreased follicular size and colloid density and occasional focal collapse of follicles. Neither the incidence nor severity of thyroid lesions in treated animals differed significantly from the controls. Histologic changes in most dose groups were mild and similar to controls when evaluated after the 90-day recovery period, although males in the high-dose group continued to exhibit an increased incidence of hepatic lesions with greater severity relative to the vehicle control. The NOAEL and LOAEL values in this study are approximately 7 mg/kg-day and 55 mg/kg-day, respectively, based on increased incidence and severity of hepatic lesions (combined) in male and female rats.

Table 1. Incidence and Severity of Hepatic Lesions in the Subchronic Oral Exposure Study Conducted by Chu et al., 1982

Concentration (ppm)	Estimated Dose (mg/kg-day) Males/Females	Males		Females	
		Incidence	Severity [a]	Incidence	Severity
0 (vehicle control)	0	2/9 (22%)	1.2 ± 0.44	0/10 (0%)	1.0 ± 0.0
5	0.65/0.64	5/10 (50%)	1.6 ± 0.70	3/10 (30%)	1.3 ± 0.48
50	6.1/6.9	4/10 (40%)	1.4 ± 0.52	0/10 (0%)	1.0 ± 0.0
500	57/55	7/10[b] (70%)	1.8 ± 0.63	4/10[b] (40%)	1.5 ± 0.71[c]
2500	218/283	9/9[b] (100%)	2.7 ± 0.71[c]	6/10[b] (60%)	1.7 ± 0.67[c]

[a] Severity grading ranges from 1 (normal) to 10 (malignant tumors or complete necrosis)
[b] Significantly different from vehicle control ($p \leq 0.05$) by Fisher's Exact Test (analysis performed by SRC)
[c] Significantly different from vehicle control ($p \leq 0.05$) (analysis performed by study authors)

Tobe et al. (1982) evaluated the chronic effects of bromoform administered in the diet to male and female Slc:Wistar SPF rats (40/sex/group) for 24 months. Bromoform was administered in microencapsulated form at dietary levels of 0.0%, 0.04%, 0.16%, or 0.65%. Control groups (70 rats/sex) received feed amended with microcapsules that did not contain bromoform. Data were reported from interim sacrifice of 9 animals/sex in the control group and 5 animals/sex/dose in the exposure groups at 18 months. All surviving animals were sacrificed at 24 months. Necropsy, hematology, and serum biochemistry were conducted at the interim and final sacrifices. Based on the published data for body weight and food consumption, the administered levels of bromoform correspond to doses of approximately 0, 22, 90, and 364 mg/kg-day for males and 0, 38, 152, and 619 mg/kg-day for females. Marked suppression (>65%) of body weight gain was seen in males and females at the high dose (364 and 619 mg/kg-day) and mild suppression of body weight gain (roughly 15-20%) was seen in males and females at the mid dose (90 and 152 mg/kg-day). No dose-related hematological changes were observed at either 18 or 24 months. At the 18 month interim time point, dose-related increases in absolute and relative liver weights occurred in females at $\geq$ 152 mg/kg-day. Dose-related changes in serum parameters in males included decreased glucose concentration, non-esterified fatty acid (NEFA) concentration, and cholinesterase (CHE) activity and increased gamma-glutamyl

transpeptidase (GGTP), leucine aminopeptidase (LAP), and glucose-6-phosphate dehydrogenase (G6PDH) activities. Dose-related changes in females included decreased serum albumin/globulin ratio, glucose concentration, and CHE activity and increased total protein concentration, GGTP activity, and G6PDH activity. Prominent necropsy findings at 18 months included dose-related yellowing of the liver males and females; roughening of the liver surface in males; and hypertrophy and transparency of the lobules of the liver in females. At study termination, increased absolute and relative liver weights were observed in mid- and high-dose (at $\geq$ 152 mg/kg-day) females. Dose-related alterations in serum biochemistry in males included decreased levels of NEFA and CHE activity. Decreased glucose and NEFA concentrations and increased GGTP activity, LAP activity, glycylproline-dipeptidyl aminopeptidase activity, and potassium concentration showed dose-related trends in females. The most prominent findings at necropsy were yellowing of the surface of the liver in all high-dose animals and mid-dose females; roughening of the liver surface in all high-dose animals; small white spots on the liver in some high-dose (364 mg/kg-day) males and mid- and high-dose (at $\geq$ 152 mg/kg-day) females; and transparent lobules in mid- and high-dose females. No histopathology data for bromoform have been published from this study. The body weight and serum biochemistry data obtained for male animals in this study suggest NOAEL and LOAEL values of 22 mg/kg-day and 90 mg/kg-day, respectively.

NTP (1989a) administered bromoform to male and female F344/N rats (50/sex/group) by gavage in corn oil at doses of 0, 100, or 200 mg/kg-day for 103 weeks (5 days/week). The study was conducted using a standard NTP protocol and was GLP compliant. The administered doses correspond to duration-adjusted doses of 0, 71.4, and 143 mg/kg-day, respectively. Necropsy and histopathological examination were performed on all animals at study termination. Final mean body weight was decreased by 25% in high-dose females and by 21% in high-dose males when compared to the corresponding controls. Survival of high-dose males was significantly lower than that of the vehicle controls after week 91 of the study. Survival of dosed female rats was similar to the vehicle control group. Treatment-related clinical signs included lethargy in males and females and increased aggressiveness in males. Increased incidences of hepatic fatty change and chronic inflammation were observed in males and females at both doses (Table 2). The incidence of mixed cell foci were increased in females at both doses. The incidence of hepatic necrosis was increased in high-dose males, while a dose-related decrease in the incidence of hepatic necrosis was observed in females. Nonneoplastic changes in other tissues were not reported. This study identified a LOAEL of 100 mg/kg-day (duration-adjusted value of 71.4 mg/kg-day), the lowest dose tested, for hepatic fatty change and chronic inflammation.

NTP (1989a) administered bromoform doses of 0, 50, or 100 mg/kg-day to male $B6C3F_1$ mice (50/dose) by corn oil gavage for 103 weeks (5 days/week). Female $B6C3F_1$ mice (50/dose) received corn oil gavage doses of 0, 100, or 200 mg/kg-day for 103 weeks (5 days/week). The study was conducted using a standard NTP protocol and was GLP compliant. The administered

Table 2. Incidence of Hepatic Lesions in Rats in the Two-Year Corn Oil Gavage Study of Bromoform (NTP, 1989a)

Lesion	Males			Females		
	Vehicle Control	100 mg/kg-day	200 mg/kg-day	Vehicle Control	100 mg/kg-day	200 mg/kg-day
Fatty Change	23/50 (46%)	49/50 (98%)	50/50 (100%)	19/50 (38%)	39/49 (80%)	46/50 (92%)
Active Chronic Inflamation	0/50 (100%)	29/50 (58%)	23/50 (46%)	9/50 (18%)	8/49 (16%)	27/50 (54%)
Necrosis	7/50 (14%)	3/50 (6%)	20/50 (40%)	11/50 (22%)	3/49 (6%)	2/50 (4%)
Mixed Cell Focus	10/50 (20%)	11/50 (22%)	8/50 (16%)	8/50 (16%)	25/49 (51%)	28/50 (56%)

doses correspond to duration-adjusted doses of 0, 71.4, and 143 mg/kg-day, respectively. At study termination, all animals were necropsied and a complete histological examination of tissues was performed on vehicle control and high-dose mice. Selected tissues were examined in low-dose mice. Decreased survival was observed in females of both dosed groups when compared to the vehicle control group. Survival in dosed males was comparable to the vehicle control group. The decline in survival of females was attributed in part to a utero-ovarian infection in the test animals. No treatment-related clinical signs were noted in either sex. Final mean body weights for low- and high-dose females were 10% and 16% lower than the control values, respectively; final mean body weight was not affected in males. Increased incidences of minimal to mild fatty change were noted in the livers of dosed females relative to controls (vehicle control, 1/49; low-dose, 9/50; high-dose, 24/50). The incidence of focal or multifocal follicular cell hyperplasia was increased in the thyroid of high-dose females (vehicle control, 5/49; low-dose, 4/49; high-dose, 19/47). Hyperplasia of the glandular stomach occurred at slightly increased incidences in dosed mice. No other statistically or biologically significant nonneoplastic changes were reported in mice of either sex in response to treatment. The LOAEL for this study is 100 mg/kg-day (duration-adjusted value of 71.4 mg/kg-day), the lowest dose tested, based on decreased body weight and histologic changes in female rats.

The available inhalation exposure data for animals are reviewed in IRIS (U.S. EPA, 2003) and the HEED for Bromoform (U.S. EPA, 1989). von Oettingen (1955) reported that exposure of rats to bromoform vapors may result in irritation of the respiratory tract and lacrimation. As reported in IRIS, bromoform had a narcotic effect on rabbits subjected to a

single inhalation exposure at concentrations ranging from 1064 to 1741 ppm (Dykan, 1964). Rats exposed to a bromoform vapor concentration of 240 ppm for 10 days developed central nervous system effects and dystrophic and vascular alterations of the liver and kidney (Dykan, 1964). Exposure of rats to a vapor concentration of 24 ppm (4 hours/day) for two months induced hepatic disorders characterized by decreased blood clotting and impaired glycogenesis in the liver and altered renal filtration capacity (Dykan, 1962). No adverse effects were evident after two months of exposure to a vapor concentration of 4.8 ppm (Dykan, 1964). U.S. EPA (1989, 2003) noted that these data were obtained from study abstracts that did not provide sufficient information for critical evaluation of the studies.

The developmental toxicity of bromoform has been assessed in rats. Ruddick et al. (1983) administered 0, 50, 100, or 200 mg/kg-day to pregnant Sprague-Dawley rats (15/dose) by corn oil gavage on gestation days 6 through 15. Maternal weight gain, organ weights, hematology, and clinical chemistry parameters were unaffected by treatment. Increased incidences of a 14^{th} rib, wavy ribs, interparietal deviations, and sternebral variations were reported in treated animals. The study authors did not report statistical analysis of these data. Analysis of the published data using Fisher's Exact Test indicates that the litter incidence of sternebral variations (control, 1/15; 50 mg/kg-day, 3/15; 100 mg/kg-day, 5/14; 200 mg/kg-day, 8/15) was significantly different ($p = 0.007$) from the control at 200 mg/kg-day. The responses for other fetotoxic endpoints did not reach statistical significance ($p \leq 0.05$) by this test. Based on this analysis, the NOAEL for developmental toxicity in this study is 100 mg/kg-day and the LOAEL is 200 mg/kg-day for increased incidence of sternebral variations in the offspring of pregnant females treated with bromoform. The NOAEL for maternal toxicity is 200 mg/kg-day, the highest dose tested.

NTP (1989b) evaluated the reproductive toxicity of bromoform in CD-1 mice using a two generation continuous breeding protocol. Male-female pairs (20 pairs/dose) were administered daily doses of 50, 100, or 200 mg/kg-day by corn oil gavage during a seven-day precohabitation and a 98-day cohabitation period. Vehicle control animals (40 pairs/dose) received corn oil only. The F_1 generations from the vehicle control and 200 mg/kg-day groups were also treated and evaluated. In the F_0 generation, the body weights at delivery of dams in the 200 mg/kg-day group were consistently less than the value for the control group. The reduction in body weight was statistically significant after the delivery of four out of the five total litters. No evidence of systemic toxicity was reported for F_0 males. There was no apparent effect of treatment on fertility, litters per pair, live pups per litter, proportion of live births, sex of live pups, or pup body weights. In the F_1 generation, male and female mice receiving 200 mg/kg-day had increased relative liver weights and decreased relative kidney weights. Terminal body weights of dosed F_1 males were significantly lower than the corresponding control values. Minimal to mild hepatocellular degeneration was observed in 19/20 dosed males. Postnatal survival was significantly decreased in the 200 mg/kg-day group. No other effects on fertility or reproduction

were observed. The NOAEL and LOAEL values in this study are 100 mg/kg-day and 200 mg/kg-day, respectively, for reproductive toxicity.

Other Studies

The available toxicokinetic data for bromoform are limited. Absorption of bromoform occurs from the respiratory tract, gastrointestinal tract, and skin (von Oettingen, 1955). Mink et al. (1986) estimated gastrointestinal absorption of radiolabeled bromoform to be 60 to 90% in mice and rats given a single oral dose, as determined from recovery of radioactivity from exhaled air, urine, and tissues. No quantitative data are available for absorption via the inhalation or dermal routes. Once absorbed, bromoform and its metabolites are rapidly distributed. In rats, bromoform distributed to adipose tissue, kidney, brain, and liver after a single gavage dose of radiolabeled compound (Parra et al., 1986). Radioactivity has also been detected in the urinary bladder, lungs, skeletal muscle, pancreas, stomach, and thymus of rodents after administration of a single gavage dose of radiolabeled bromoform (Mink et al., 1986). Bromoform is metabolized to carbon dioxide, carbon monoxide, and hydrogen bromide via a cytochrome P-450-dependent pathway (U.S. EPA, 1989). The primary site for metabolism is the liver. Metabolism to toxic species appears to be mediated by both oxidative and reductive pathways, as inferred from studies of bromoform and structurally-related trihalomethanes. Preliminary evidence obtained using transfected strains of *Salmonella typhimurium* suggests that production of toxic metabolites may also be mediated by a glutathione S-transferase- dependent pathway (DeMarini et al., 1997). Excretion of bromoform and its metabolites occurs primarily via the lungs, with a small fraction excreted in the urine (Mink et al., 1986).

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC ORAL RfD VALUES FOR BROMOFORM

A **chronic RfD of 2E-2 mg/kg-day** is listed for bromoform on IRIS (U.S. EPA, 2003), based a NOAEL of 25 mg/kg-day for hepatic vacuolization in male rats administered bromoform five days per week for 13 weeks (NTP, 1989a). The presence of a chronic RfD on IRIS precludes derivation of a provisional chronic RfD for this chemical.

In considering derivation of a provisional subchronic RfD, no new human or animal data on the subchronic toxicity of bromoform were identified in the literature search. None of the existing human data are adequate for derivation of a subchronic RfD value for bromoform. The existing subchronic animal data include well-conducted subchronic gavage studies in rats and mice (NTP, 1989a) and data from a drinking water exposure study conducted in rats (Chu et al., 1982). Additional data are available from a reproductive toxicity study in mice (NTP, 1989b) and a developmental toxicity study in rats (Ruddick et al., 1983). The NTP (1989a) data for rats

identify histologic changes in the liver as the most sensitive response to bromoform exposure among the endpoints evaluated in these studies.

As previously noted, the subchronic RfD currently listed in the HEAST was derived by U.S. EPA (1989) using a NOAEL of 25 mg/kg-day for hepatocellular vacuolization in male F344/N rats administered bromoform by corn oil gavage five days per week for 13 weeks (NTP, 1989a). The LOAEL for this lesion was 50 mg/kg-day. A composite UF of 100 was applied to the duration adjusted dose of 17.9 mg/kg-day to give the subchronic RfD of 0.2 mg/kg-day. A modifying factor (MF) was not used in the calculation.

A benchmark dose (BMD) modeling approach (U.S. EPA, 2000) was used as an alternative to the conventional NOAEL/LOAEL approach for derivation of the provisional subchronic RfD. The NTP (1989a) data for hepatocellular vacuolization in male rats exposed to bromoform for 13 weeks were selected for this purpose (3/10, 6/10, 5/10, 8/10, 8/10, and 10/10 in the time-adjusted dosage as 0, 12, 25, 50, 100, and 200 mg/kg-day dose groups, respectively). These data were obtained in a well-documented study conducted under Good Laboratory Practice guidelines and describe a statistically and biologically significant dose-related trend in the selected endpoint. In addition, this study utilized a larger number of doses than other candidate studies, including three doses below 100 mg/kg-day that provide information on the shape of the dose-response curve in the region of greatest interest. Available models in the U.S. EPA Benchmark Dose Software (BMDS) program (Version 1.3.1) were fit to the incidence data for hepatocellular vacuolization to estimate the duration-adjusted dose producing a 10 percent extra risk of developing the lesion (i.e., the BMD). BMDS modeling results were evaluated according to the criteria outlined in U.S. EPA (2000). The best fit to the data was obtained with the Quantal-Linear model (Table 3). This model gave values of 4.4 mg/kg-day for the BMD and 2.6 mg/kg-day for the $BMDL_{10}$, the lower 95 percent confidence interval on the BMD. Figure 1 plots predicted (from the fitted Quantal-Linear model) and observed lesion incidence as a function of the duration-adjusted dose, as well as the BMD and the $BMDL_{10}$.

Table 3. Summary of BMD Modeling Results Obtained for Incidence of Hepatocellular Vacuolization in Male Rats Using the U.S. EPA Benchmark Dose Software

Model[a]	Chi Square	p	AIC[b]	BMD[c]	$BMDL_{10}$[d]
Gamma	1.92	0.589	67.7261	1.9	0.00031
Logistic	2.48	0.648	66.1399	7.3	4.7
Log Logistic	2.40	0.494	68.4402	4.4	0.039
Probit	2.45	0.654	66.0888	7.8	5.3
Quantal Linear	2.18	0.703	65.8698	4.4	2.6
Quantal Quadratic	3.45	0.483	67.1516	19.9	13.6
Weibull	2.02	0.568	67.8044	2.8	0.013

[a] Available models in the U. S. EPA Benchmark Dose Software Program (version 1.3.1)
[b] AIC, Aikake Information Criterion
[c] BMD, Benchmark Dose. The BMD is a maximum likelihood estimate of the dose producing a 10 percent extra risk of hepatic vacuolization in male F344/N rats.
[d] $BMDL_{10}$ = lower 95 percent confidence interval on the BMD.

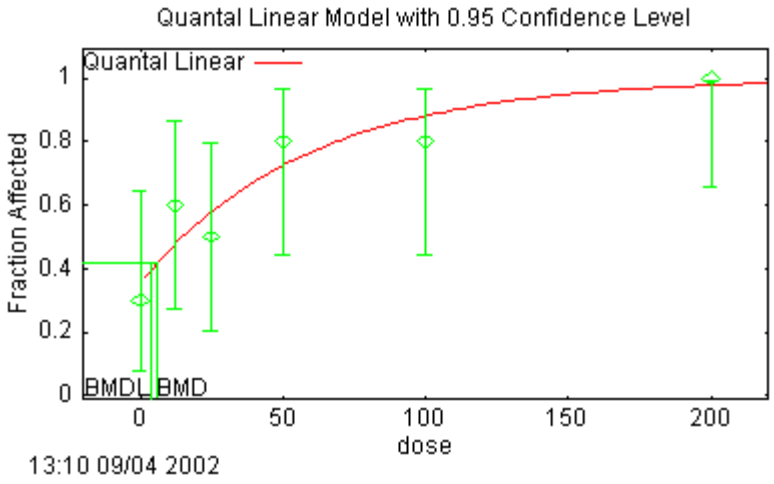

Figure 1. BMD Modeling Results for Hepatocellular Vacuolization in Male Rats Administered Bromoform by Corn Oil Gavage for 13 weeks (NTP, 1989a)

A provisional **subchronic RfD of 0.03 mg/kg-day** for bromoform is calculated using the duration adjusted $BMDL_{10}$ of 2.6 mg/kg-day for hepatocellular vacuolization in male rats exposed to bromoform for 13 weeks (NTP, 1989a):

$$\text{subchronic p-RfD} = \frac{BMDL_{10}}{UF \times MF}$$

$$= \frac{2.6 \text{ mg/kg-day}}{100 \times 1}$$

$$= 0.03 \text{ mg/kg-day (3E-2 mg/kg-day)}$$

A composite UF of 100 incorporating factors of 10 each for extrapolation from animals to humans and for human variability; and a modifying factor (MF) of 1 are used in the calculation. Use of the $BMDL_{10}$ in this calculation accounts for inherent uncertainty in the study results and ensures (with 95% confidence) that the desired benchmark response of 10% extra risk is not exceeded. A database uncertainty factor was not applied because supporting systemic studies were available and developmental (in only one species, however) and reproductive endpoints had been studied and found not to be sensitive targets for this chemical.

Confidence in the principal study for the subchronic RfD (NTP, 1989a) is medium-to-high. The study used both sexes of two species, included comprehensive histopathological examination of livers in all animals, identified liver lesions as the critical effect in both species with associated NOAELs and LOAELs, and was reported in adequate detail, including the lesion incidence data. However, the study did not investigate clinical chemistries or perform urinalysis. Confidence in the database is medium-to-high. Several studies support the choice of hepatic lesions as the critical effect, and reproductive and developmental endpoints have been studied and found not to be sensitive targets. Medium-to-high confidence in the subchronic p-RfD follows.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION RfC VALUES FOR BROMOFORM

U.S. EPA (2003) concluded in a previous review of inhalation data for bromoform that insufficient data were available for derivation of a chronic p-RfC. The available inhalation data are from abstracts of studies conducted by Dykan (1962, 1964). In summarizing these studies, U.S. EPA (2003) noted that no details were available for exposure generation or characterization, specific effect measures, or results. In addition, there were insufficient toxicokinetic data to perform a route-to-route extrapolation from existing oral data, and the potential for local respiratory (portal-of-entry) effects was not adequately characterized. No new subchronic or chronic inhalation studies, reproductive or developmental studies that employed an inhalation route of exposure, or relevant toxicokinetic studies were identified in the literature search. The database for bromoform, therefore, remains inadequate for derivation of subchronic or chronic p-RfC values.

DERIVATION OF A PROVISIONAL CARCINOGENICITY ASSESSMENT FOR BROMOFORM

A cancer assessment, including derivation of an oral slope factor and inhalation unit risk, is available for bromoform on IRIS (U.S. EPA, 2003), precluding derivation of a provisional carcinogenicity assessment for this chemical.

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