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Provisional Peer-Reviewed Toxicity Values for

Methylene bromide
(CASRN 74-95-3)

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

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 METHYLENE BROMIDE (CASRN 74-95-3)

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

No RfD, RfC, or carcinogenicity assessment for methylene bromide (dibromomethane) is available on IRIS (U.S. EPA, 2008). No RfC for methylene bromide is available in the Health and Environmental Assessment Summary Tables (HEAST; U.S. EPA, 1997), although the HEAST does include RfDs based on the route-to-route extrapolation of inhalation toxicity data, originally derived in a Health and Environmental Effects Profile (HEEP; U.S. EPA, 1987). Subchronic and chronic RfDs of 1E-1 and 1E-2 mg/kg-day, respectively, were extrapolated from an inhalation NOAEL of 25 ppm for increased carboxyhemoglobin in rats exposed to methylene bromide vapor for 90 days (Keyes et al., 1982). The HEEP (U.S. EPA, 1987) did not produce a cancer assessment due to insufficient data. The HEEP is the only U.S. EPA document on methylene bromide in the Chemical Assessments and Related Activities (CARA) lists (U.S. EPA, 1991a, 1994a). The Drinking Water Standards and Health Advisories list (U.S. EPA, 2006) does not include methylene bromide. The Agency for Toxic Substances and Disease Registry (ATSDR, 2008) has not produced a Toxicological Profile for methylene bromide, and no World Health Organization Environmental Health Criteria document is available (WHO, 2008). The chronic toxicity and carcinogenicity of methylene bromide have not been assessed by the International Agency for Research on Cancer (IARC, 2008) or the National Toxicology Program (NTP, 2005, 2008). No occupational exposure limits are available for methylene bromide from the American Conference of Governmental Industrial Hygienists (ACGIH 2001, 2007), the National Institute of Occupational Safety and Health (NIOSH, 2008) or the Occupational Safety and Health Administration (OSHA, 2008). The California Environmental Protection Agency (Cal EPA, 2002, 2005a,b) has not derived a REL or cancer potency factor for methylene bromide.

Literature searches were conducted from 1960s through December 2007 for studies relevant to the derivation of provisional toxicity values for methylene bromide. Databases searched include MEDLINE, TOXLINE (Special), BIOSIS, TSCATS 1/TSCATS 2, CCRIS, DART/ETIC, GENETOX, HSDB, RTECS, and Current Contents. An updated literature search was conducted using PubMed through November 2008.

REVIEW OF PERTINENT DATA

Human Studies

No information was located regarding the toxicity of methylene bromide in humans.

Animal Studies

Oral Exposure

Groups of 10 male and 10 female young-adult Sprague-Dawley rats were exposed to methylene bromide (dibromomethane) in drinking water at concentrations of 1.0, 10.0, 100.0, or 1000.0 mg/L for 4 weeks (Komsta et al., 1988). Methylene bromide was initially solubilized with 0.5% by weight (w/v) Emulphor followed by dilution with tap water to appropriate concentrations. Control groups (10 per sex) were exposed to water alone or vehicle (0.5% Emulphor). Reported approximate chemical intakes were 0.1, 1.2, 11.9, and 124 mg/kg-day in males and 0.1, 0.9, 8.6, and 90 mg/kg-day in females (based on group water consumption and average body weight). Endpoints evaluated throughout the study consisted of clinical observations (daily), body weight (weekly), and food and water consumption (weekly). Endpoints evaluated at termination of exposure included hematology, serum chemistry, and hepatic microsomal enzyme activity (aniline hydroxylase, aminopyrine demethylase, and ethoxyresorufin deethylase). The hematology determinations consisted of hemoglobin, packed cell volume, erythrocyte count, total and differential leukocyte counts, and platelet counts. The serum chemistry determinations consisted of sodium, potassium, calcium, inorganic phosphate, total bilirubin, total protein, cholesterol, glucose, uric acid, alkaline phosphatase, aspartate aminotransferase, and lactate dehydrogenase. Gross necropsy and selected organ weight measurements (brain, heart, liver, spleen, and kidneys) were performed on all animals at termination of exposure. Comprehensive histological examinations (29 tissues, including male and female reproductive tissues) were conducted but limited to the control and high-dose groups.

The investigators used data from the water control group as baseline values for evaluation purposes because a comparison of data from the water and vehicle control groups showed that there were no significant differences between the groups (Komsta et al., 1988). The only effects attributable to methylene bromide exposure were reduced serum lactate dehydrogenase activity in females at ≥8.6 mg/kg-day and histological alterations in the liver and thyroid in males at 124 mg/kg-day. Serum lactate dehydrogenase activity in the 0.1, 0.9, 8.6, and 90 mg/kg-day females was 21.6, 17.2, 27.4, and 31.2% lower than water controls, respectively. Although statistically significant ($p \leq 0.05$) at ≥8.6 mg/kg-day, the investigators did not consider the decreases in serum lactate dehydrogenase to be biologically significant or related to any histological changes. Given the lack of a basis for toxicological concern of a decrease in serum lactate dehydrogenase (as opposed to an increase), the effect is discounted for determination of a LOAEL. In males, the histological changes in the liver were characterized as minimal-to-mild increases in perivenous cytoplasmic homogeneity and periportal cytoplasmic density; the incidence in the 124 mg/kg-day group was 8/10 compared to 3/10 in water controls and 5/10 in vehicle controls. The histological changes in the thyroid of the 124 mg/kg-day males were also minimal-to-mild in severity and included increased epithelial height (8/10 compared to 1/10 in water controls and 4/10 in vehicle controls) and reduced colloid density (2/10 compared to 0/10 in water controls and 0/10 in vehicle controls). Other histological findings included low incidences of minimal-to-mild renal tubular cytoplasmic inclusions in males at 124 mg/kg-day (4/10 compared to 2/10 in water controls and 1/10 in vehicle controls) and females at

90 mg/kg-day (3/10 compared to 0/10 in water controls and 0/10 in vehicle controls). Histology examinations were not performed in any tissues at lower doses in either sex. Although these effects were generally mild and not accompanied by overt functional changes, they are indicative of compound-related changes that could progress to more severe outcomes. Consequently, the highest doses tested in females (90 mg/kg-day) and males (124 mg/kg-day) are considered to be LOAELs. NOAELs of 8.6 mg/kg-day in females and 11.9 mg/kg-day in males are established in this study.

Inhalation Exposure

Keyes et al. (1982); 90-Day Inhalation Study in Rats and Dogs—An unpublished Dow Chemical Company study was conducted using groups of 115 male and 15 female Sprague-Dawley rats and three male Beagle dogs exposed to 0, 25, 75, or 150 ppm (0, 178, 533, or 1066 mg/m^3) of methylene bromide for 6 hours/day, 5 days/week, for a total of 62 or 63 exposures (rats) or 70 exposures (dogs) in approximately 90 days (Keyes et al., 1982). After 58 exposure days, five rats/sex/level were sacrificed for cytogenetic analysis of bone marrow. On the day after the last exposures, 10 rats/sex/level and all dogs were necropsied. The 100 remaining male rats were observed for up to 2 years following exposure. Endpoints that were evaluated during the exposure and observation parts of the study include clinical signs, body weight, hematology, and urinalysis indices (7 rats/sex/level, all dogs at termination of exposure, and 7 male rats/level after 1 year of observation) and clinical chemistry indices (10 rats/sex/level, all dogs at termination of exposure, and 10 male rats/level after 1 year of observation). The hematology determinations included red blood cell counts, hemoglobin concentration, packed cell volume, and total and differential white blood cell counts. The urinalysis determinations included pH, protein, ketones, bilirubin, urobilinogen, and occult blood. The clinical chemistry determinations included blood urea nitrogen, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase. Cytogenetic analyses were not performed because of inadequate slide preparation.

Additional endpoints included plasma bromine/bromide levels at various time points and blood carboxyhemoglobin (COHb) percentages. COHb was measured using an optical method. For each of these endpoints three rats/sex/level were evaluated preexposure and at 1, 3, 30, 41, 51, and 61 days of exposure. The authors did not specify whether the same or different animals were used for each measurement. All dogs were evaluated at similar time points. Complete gross necropsies and organ weight measurements (brain, heart, liver, kidneys, and testes) were performed on 10 rats/sex/level after 61or 62 exposures and all dogs after 70 exposures. Complete gross necropsies were also performed on 10 male rats/level at 1 and 2 years postexposure. Comprehensive histological examinations were performed in the 0- and 150-ppm groups after 90 days of exposure (five rats/sex/level and all dogs) and at 1 year postexposure (five male rats/level). No histological examinations were performed during the observation period on male rats that died spontaneously, were killed in moribund condition, or were sacrificed after 2 years. Histological examinations in the 25- and 75-ppm exposure groups were limited to the lungs, trachea, and bronchial lymph nodes in dogs after 90 days of exposure.

There were no exposure-related changes in most of the endpoints in either species. Dose-related increases in serum levels of free bromide ion and total bromine occurred at ≥25 ppm in the rats and dogs at all times of evaluation. There were no clinical signs of intoxication and no histopathological changes in both species. There was a slight increase in relative liver weight at the termination of the exposure period in female rats exposed to 75 ppm

(8.9% higher than controls, $p < 0.05$) and 150 ppm (6.1% higher than controls, not statistically significant). Male rats that were exposed to ≥75 ppm showed an equivocal decrease in body-weight gain during Study Days 121−361 in the postexposure observation period, but the decrease is not considered biologically significant.

Exposure to methylene bromide also caused increased blood COHb levels in both species as shown in Table 1. Increases in mean COHb percentages that were statistically significant ($p < 0.05$ compared to control group) occurred at ≥75 ppm methylene bromide; there was only one statistically significant increase at 25 ppm (after 61 exposures in females). However, due to the small group sizes ($n = 2$ or 3 rats/sex/level at each time point), the statistical tests performed by the researchers had little power to detect an effect. Dogs were less sensitive than the rats, as indicated by a small increase in percent COHb saturation relative to controls detectable only at 150 ppm (mean 6.0% increase at the only sampling time where the difference from the control mean was statistically significant). Regression analysis was performed by the researchers to quantitatively assess the effect of increasing exposure concentration on percent COHb saturation. The analysis used pooled percentage COHb saturation values after 30 to 61 exposures; data for each concentration were pooled to improve the precision of analysis and because COHb percentages did not increase appreciably after more than 30 exposures. The slopes of the regression lines predicted that the average increase in percent COHb saturation (relative to controls) in the animals repeatedly exposed to 25, 75, and 150 ppm would be 0.4, 1.2, and 2.4%, respectively, in dogs, 2.6, 8.3, and 16.5% in male rats, and 2.3, 6.8, and 13.5% in female rats. Baseline COHb in untreated control animals was 2.8%. Table 1 shows selected data from Table 28 of Keyes et al. (1982) that correspond to the data used by the researchers for their regression analysis. Statistical analysis performed by Syracuse Research Corporation for this review of the data pooled across the selected time points showed highly statistically significant changes in COHb from control in all treated groups (two-tailed t-test, $p < 0.01$). The lowest exposure level in this study of 25 ppm (178 mg/m^3) is identified as a LOAEL for increased COHb in rats; a NOAEL is not identified.

Table 1. Carboxyhemoglobin Concentrations (%) in Rats Exposed to Methylene Bromide[a]

	Exposure Concentration (ppm)							
	0	**0**	**25**	**25**	**75**	**75**	**150**	**150**
Exposures	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
30	---	2.7	6.4	5	9	8	20.6	20
	1.3	0.6	6.9	6	7.9	8	21.7	23.2
	2.5	1.1	7.9	6	16.7	8.2	20.4	23.1
41	4.5	3.9	3	2.4	6.3	4.5	15.9	12.6
	0.9	4.2	5.1	3.9	4.2	2.4	14.7	14.1
	1.4	---	5.4	5.1	6	6.3	14.7	17.8
61	4.1	3.1	9.2	7.8	10.8	9.2	18	14.3
	2.4	2	6.1	10.2	14.6	10.2	23.8	15.6
	5.1	2.7	7.5	10.9	9.5	8.5	23.8	14.3
n	8	8	9	9	9	9	9	9
Mean[b]	2.8	2.5	6.4[c]	6.4[c]	9.4[c]	7.3[c]	19.3[c]	17.2[c]
SD[b]	1.6	1.3	1.8	2.8	4.1	2.5	3.6	4.0

[a]Keyes et al., 1982, Table 28.
[b]Means and standard deviations (SDs) were calculated from the Keyes et al. (1982) data for this review; the selected data set is identical to that used by Keyes et al. (1982) to generate Figure 3 of that report and the accompanying regression formulas for methylene bromide-induced COHb formation.
[c]Statistically significant difference from control ($p < 0.01$) by the two-tailed t-test performed for this review.

Toxicokinetics and Toxicodynamics

The available data indicate that COHb induction is the most sensitive effect of subchronic inhalation exposure to methylene bromide. This effect is a consequence of the cytochrome P-450-mediated metabolism of methylene bromide to carbon monoxide and inorganic bromide (Fozo and Penney, 1993; Kubic and Anders, 1975, 1978; Kubic et al., 1974; Stevens et al., 1980) and is consistent with the metabolism of methylene chloride and other dihalomethanes, which characteristically produce carbon monoxide and increases in COHb that can lead to tissue hypoxia (ATSDR, 2000; U.S. EPA, 2008). The formation of COHb is a reversible process, but the elimination half-time is relatively long (2−6.5 hours, depending on initial COHb levels) because carbon monoxide is tightly bound to hemoglobin (McKee and Bachman, 2000). The long elimination half-time may lead to accumulation of COHb, indicating that even relatively low concentrations of carbon monoxide could produce high blood levels of COHb.

The cytochrome P-450 pathway is the major metabolic route of methylene chloride at low exposure levels similar to the levels of methylene bromide that caused the increased COHb levels (Keyes et al., 1982). Studies with methylene chloride have established that the mixed-function oxidases (MFO) pathway is a high-affinity, limited-capacity pathway that saturates at air concentrations of about 200−500 ppm (ATSDR, 2000). Thus, at low-exposure concentrations, most of the methylene chloride is likely to be metabolized by the MFO pathway.

As exposure concentrations increase and the MFO pathway saturates, metabolism proceeds by a second pathway that is glutathione (GSH)-mediated (ATSDR, 2000). The GSH-dependent pathway produces formaldehyde and carbon dioxide but no carbon monoxide. In vitro and in vivo comparisons of methylene chloride metabolism in rats, mice, hamsters, and humans indicate that metabolic rates for the MFO pathway are similar among the four species and saturated at concentrations of 500 ppm and above (Green, 1997; Reitz et al., 1989).

Physiological baseline COHb saturations in the general population are estimated to average 0.5% (range 0.3−0.7%) among nonsmokers and 4% in smokers, with a usual range of 3−8% for one- to two-packs-per-day smokers (McKee and Bachman, 2000; U.S. EPA, 2000a). Carbon monoxide studies indicate that adverse CNS and cardiovascular effects are likely to be associated with blood COHb levels of approximately 5% and above in healthy humans (McKee and Bachman, 2000). CNS effects (e.g., reductions in eye-hand coordination, attention, and vigilance) were observed at peak COHb levels of ≥5% in some historical reports, although more recent investigations indicate that significant behavioral impairments in healthy individuals are not expected until COHb levels exceed 20% (McKee and Bachman, 2000; U.S. EPA, 2000a). COHb concentrations of 5−20% were associated with significant decreases in VO_{2_max} (the rate of maximal oxygen consumption during strenuous exercise) following exposure to carbon monoxide in healthy young adult nonsmokers (U.S. EPA, 2000a). Maximal exercise duration and performance in healthy individuals were reduced at COHb concentrations of ≥2.3% and ≥4.3%, respectively, but the performance decrements at these COHb levels were small and likely to affect only competing athletes rather than people in everyday activities (McKee and Bachman, 2000; U.S. EPA, 2000a). No effects were observed during submaximal exercise in healthy individuals at COHb levels as high as 15−20% (U.S. EPA, 2000a). Blood COHb concentrations as low as 2.4−3.0% have been associated with adverse effects in people with coronary artery disease; at these COHb levels, individuals with reproducible exercise-induced angina are likely to experience a reduced capacity to exercise because of decreased time to onset of angina (U.S. EPA, 2000a; McKee and Bachman, 2000).

Following a review of the toxicity database for carbon monoxide, U.S. EPA (2000a) concluded that myocardial ischemia[1] during exercise in nonsmokers with coronary artery disease (i.e., the sensitive human population) is the endpoint of most concern and established it as the basis for the derivation of the National Ambient Air Quality Standard (NAAQS) for carbon monoxide. In addition, U.S. EPA (2000a) advised caution in comparing results across the studies as they used different methods for determining COHb with widely varying sensitivities. The lowest levels of COHb (≥2.4%) associated with myocardial ischemia in the sensitive population were determined by gas chromatography and reported by Allred et al. (1989a,b; 1991). When COHb was measured by carbon monoxide oximetry (CO-OX: spectrophotometric or optical method with a precision of ± 1% COHb), myocardial ischemia in the sensitive population was associated with COHb ≥2.9% (Anderson et al., 1973; Sheps et al.,1987; Adams et al., 1988; Kleinman et al., 1989, 1998; Allred et al., 1989a,b, 1991). Based on an evaluation of these studies, U.S. EPA (2000a) concluded that when optical methods are used to determine COHb, (1) a COHb range of 2.9−5.9% (rounded to 3−6%) is associated with myocardial ischemia and (2) this range is associated with incremental increases of 1.5−4.4% COHb from preexposure baseline values. The current 1-hour (35 ppm) and 8-hour (9 ppm)

[1]Measured as a decrease in the time to onset of angina pain and by decreased time to onset of significant EKG S-T segment depression during exercise.

NAAQS for carbon monoxide are intended to keep COHb levels below 2.1% to protect the most cardiovascular-sensitive members of the general population. These values were determined from models that quantify the relationship between carbon monoxide exposure and COHb concentrations.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC ORAL p-RfD VALUES FOR METHYLENE BROMIDE

Subchronic p-RfD

The only available study is the 28-day drinking water study in male and female rats (Komsta et al., 1988). Effects included reduced serum lactate dehydrogenase in females at 8.6 and 90 mg/kg-day (highest tested dose in females) and histological changes in the liver (minimal-to-mild increases in perivenous cytoplasmic homogeneity and periportal cytoplasmic density) and thyroid (minimal-to-mild increase in epithelial height) in males at 124 mg/kg-day (highest tested dose in males) and in the kidney (minimal-to-mild increases in tubular cytoplasmic inclusions) in females at 90 mg/kg-day. LOAELs of 90 and 124 mg/kg-day are established for females and males, respectively, based on kidney effects in male and female rats and liver and thyroid effects in males. The corresponding NOAELs are 8.6 mg/kg-day (females) and 11.9 mg/kg-day (males).

The 8.6 mg/kg-day NOAEL for female rats (Komsta et al., 1988) was used to derive the subchronic p-RfD. The NOAEL of 8.6 mg/kg-day was divided by a composite uncertainty factor (UF) of 1000 to derive a **subchronic p-RfD of 9×10^{-3} mg/kg-day**, as follows:

$$\begin{aligned} \textbf{Subchronic p-RfD} &= \text{NOAEL} \div \text{UF} \\ &= 8.6 \text{ mg/kg-day} \div 1000 \\ &= \mathbf{0.009 \text{ or } 9 \times 10^{-3} \text{ mg/kg-day}} \end{aligned}$$

The composite UF of 1000 is composed of the following:

- A full UF of 10 for intra-species differences is used to account for potentially susceptible individuals in the absence of information on the variability of response in humans.
- A full UF of 10 is applied for inter-species extrapolation to account for potential pharmacokinetic and pharmacodynamic differences between rats and humans.
- A full UF of 10 is employed to account for deficiencies in the database. The toxicological database for oral exposure to methylene bromide is limited to a single, but adequate, 4-week study in one species. The database lacks true subchronic, reproductive, and developmental toxicity studies.
- Although the study duration of 28 days is much shorter than a standard subchronic study (90 days), an adjustment for exposure duration is not considered necessary as the 10-fold difference between the LOAEL and NOAEL provides a sufficient margin for both exposure-duration uncertainty and the minimal toxicological effects at the LOAEL.

Confidence in the key study is low-to-medium. This study tested a range of doses and a variety of endpoints, but the histopathology examinations were limited to the high-dose group of each sex and the study duration was only 28 days. Confidence in the database is low because it lacks reproductive and developmental toxicity data. Low confidence in the subchronic p-RfD results.

Chronic p-RfD

No chronic oral toxicity studies of methylene bromide were located. The only adequate repeated-dose oral study is the 28-day drinking water study in rats (Komsta et al., 1988) used to derive the subchronic p-RfD. The short duration of this study precludes using it for derivation of a chronic p-RfD.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION p-RfC VALUES FOR METHYLENE BROMIDE

It is inappropriate to derive provisional toxicity values for methylene bromide. However, information is available for this chemical which, although insufficient to support derivation of a provisional toxicity value, under current guidelines, may be of limited use to risk assessors. In such cases, the Superfund Health Risk Technical Support Center summarizes available information in an Appendix and develops a "screening value." Appendices receive the same level of internal and external scientific peer review as the PPRTV documents to ensure their appropriateness within the limitations detailed in the document. Users of screening toxicity values in an appendix to a PPRTV assessment should understand that there is considerably more uncertainty associated with the derivation of an appendix screening toxicity value than for a value presented in the body of the assessment. Questions or concerns about the appropriate use of screening values should be directed to the Superfund Health Risk Technical Support Center.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR METHYLENE BROMIDE

Weight-of-Evidence Descriptor

The only study providing information on the carcinogenicity of methylene bromide is the previously summarized Keyes et al. (1982) inhalation study in rats. Male rats (100 per group) were exposed to 0, 25, 75, or 150 ppm of methylene bromide by inhalation 6 hours/day, 5 days/week, for 90 days and observed for up to 2 years (Keyes et al., 1982). Complete gross necropsy was performed on 10 rats from each group at 1 year, all rats dying spontaneously during the study, and all surviving rats at 2 years. Histopathological examination was limited to 5 rats in the control and 150-ppm groups sacrificed at 1 year. Therefore, assessment of tumorigenic potential is based mainly on evaluation of gross pathology data. No exposure-related effect on tumor incidence was observed. This study is not an adequate evaluation of the carcinogenic potential of methylene bromide due to the short (90-day) exposure duration and insufficient histopathological follow-up.

Mutagenicity studies showed that methylene bromide was mutagenic in *S. typhimurium* strains TA100, TA1950, and BA13 (but not TA1535) when tested without metabolic activation and mutagenicity in *S. typhimurium* TA100 and TA1535 was enhanced by the addition of S9 or GST metabolic activation preparations or by the expression of GSTs (Buijs et al., 1984; Kundu et al., 2004; Osterman-Golkar et al., 1983; Roldan-Arjona and Pueyo, 1993; Simmon et al., 1977; Thier et al., 1993, 1996; Van Bladeren et al., 1980, 1981; Wheeler et al., 2001). Methylene bromide was mutagenic in *E. coli* strains K39 and WU361189 (but not Sd-4) without metabolic activation and mutagenicity in *E. coli* K12 and TRG8, as well as in Chinese hamster fibroblasts, was enhanced by the expression of DNA alkyltransferases (Abril et al., 1995, 1997, 1999; Liu et al., 2004; Osterman-Golkar et al., 1983). Aneuploidy was not induced in *A. nidulans* diploid strain P1 (Parry et al., 1996) and inhalation exposure did not cause sex-linked recessive lethal mutations in *D. melanogaster* (Kramers et al., 1991).

Methylene chloride, a structural analog of methylene bromide, is carcinogenic in long-term rodent inhalation and drinking water bioassays and there are metabolic similarities between methylene chloride and methylene bromide (ATSDR, 2000; Fozo and Penney, 1993; IARC, 1999; Kubic and Anders, 1975, 1978; Kubic et al., 1974; U.S. EPA, 1987). The metabolism of both dihalomethanes occurs through the same pathways, producing potentially reactive intermediates that can bind to DNA (summarized in ATSDR, 2000 and IARC, 1999).

In accordance with current EPA cancer guidelines (U.S. EPA, 2005), there is "*Inadequate Information to Assess the Carcinogenic Potential*" of methylene bromide.

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APPENDIX A. DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION p-RfC VALUES FOR METHYLENE BROMIDE

For reasons noted in the main PPRTV document, it is inappropriate to derive provisional toxicity values for methylene bromide. However, information is available for this chemical which, although insufficient to support derivation of a provisional toxicity value, under current guidelines, may be of limited use to risk assessors. In such cases, the Superfund Health Risk Technical Support Center summarizes available information in an Appendix and develops a "screening value." Appendices receive the same level of internal and external scientific peer review as the PPRTV documents to ensure their appropriateness within the limitations detailed in the document. Users of screening toxicity values in an appendix to a PPRTV assessment should understand that there is considerably more uncertainty associated with the derivation of an appendix screening toxicity value than for a value presented in the body of the assessment. Questions or concerns about the appropriate use of screening values should be directed to the Superfund Health Risk Technical Support Center.

Screening Subchronic p-RfC

The unpublished Keyes et al. (1982) study is the only adequate evaluation of the inhalation toxicity of methylene bromide in animals. The only effect observed was an increase in blood COHb levels in both rats and dogs. The rats were more sensitive than the dogs and had mean COHb percentages that were significantly higher than control values at all exposure levels; COHb percentages were significantly increased in the methylene bromide-exposed rats at ≥25 ppm, where mean values ranged from 3−7% compared to 2−4% in unexposed controls (Keyes et al., 1982). Regression analysis by the researchers predicted that the average increase in percent COHb saturation (above controls) at 25 ppm was 2.6 in male rats and 2.3 in female rats. Assessment of the toxicological significance of the 2−3% absolute increase in average COHb levels in rats exposed to 25 ppm methylene bromide is complicated by the fact that most of the relevant data on effects associated with increased COHb levels are in humans exposed to methylene chloride or carbon monoxide (ATSDR, 2000; McKee and Bachman, 2000; U.S. EPA, 1987, 1991b). Methylene chloride and carbon monoxide do not necessarily produce the same effects at the same COHb saturation percentages (ATSDR, 2000; Fozo and Penney, 1993).

Examination of other study endpoints in Keyes et al. (1982) showed no overtly adverse effects or any other changes that could be related to increased COHb levels, but endpoints known to be particularly sensitive to carboxyhemoglobinemia were not investigated. In particular, based on human studies with methylene chloride and carbon monoxide, as summarized above, the most sensitive health effects likely to be associated with increased COHb from exposure to methylene bromide are subtle CNS and cardiovascular effects.

Based on the association of COHb and adverse effects in humans, the data on COHb levels in rats (Keyes et al., 1982) are used as the basis for a provisional RfC for methylene bromide. The data in Table 1 are amenable to Benchmark Concentration (BMC) analysis. Pooling the data by sex across timed measurements for 30 days or longer, as was done in the original study for regression analysis, appears reasonable on the basis of similarity of mean COHb values. However, the study authors were not explicit about how the animals were selected for each of the timed measurements, such that some of the measurements may have been from the same animals. If that were the case, treating the measurements as independent

observations would not be valid. This is particularly an issue for females, in which the population from which to select was small (15 for the first two measurements, 10 for the last). The same would hold for males if the selection was made from the animals set aside for the 90-day sacrifice but would not be considered an issue if animals were randomly selected from the total population of 115 animals. Assuming the latter, BMC analysis was conducted on the pooled male data as shown in Table 1. BMC analysis of female COHb levels was conducted on the pooled data and on data from the last timed measurement (61 days). All BMC analyses were performed using all the continuous models in BMDS (Ver. 2.12; U.S. EPA, 2008).

In the absence of information on a clear COHb increase that would be considered adverse in rats, the default BMR, recommended for continuous data, of one estimated standard deviation (SD) from the control mean was selected (U.S. EPA, 2000b); resulting in a 1 SD (1.6%) change from the control mean (2.8%). This BMR is at or below the range of percent increase in COHb (from baseline) associated with cardiovascular effects in sensitive human subpopulations discussed previously. The linear and power models with nonhomogeneous (modeled) variance provide the best fits (identical) and lowest BMCL for the male data set. None of the BMDS models adequately fit the female data. None of the BMCLs for the female data were lower than the lowest male BMCL. Otherwise, the data did not suggest that the females were more sensitive than the males. Details of the modeling are presented in Appendix B.

The BMC results for the rats are converted to human equivalent concentrations (HEC) by standard U.S. EPA methods (U.S. EPA, 1994b). The rat BMCL in mg/m^3 is duration-adjusted for intermittent exposure. Exposure in the Keyes et al. (1982) study was 6 hours per day, 5 days per week. The resulting exposure adjustment factor is 0.1786. Because methylene bromide exhibits its toxic effects outside of the respiratory tract, it is treated as a Category 3 gas for purposes of calculating the p-RfC. The HEC for extrarespiratory effects produced by a Category 3 gas is calculated by multiplying the duration-adjusted BMCL by the ratio of blood:gas partition coefficients ($H_{b/g}$) in animals and humans. $H_{b/g}$ values were not available for methylene bromide in rats or humans. Using the default value of 1 for the ratio of partition coefficients (U.S. EPA, 1994b), the $BMCL_{HEC}$ is equivalent to the $BMDL_{ADJ}$. The $BMDL_{HEC}$ derivation is shown in Table A-1.

Table A-1. BMC Modeling Results[a] for Carboxyhemoglobin (%) in Male Rats Exposed to Methylene Bromide[b]

BMR	BMC (ppm)	BMCL (ppm)	BMCL (mg/m^3)[c]	$BMCL_{HEC}$ (mg/m^3)[d]
1 SD	14.67	10.15	72.17	12.9

[a]Linear model, modeled variance, AIC = 111.32; means $p = 0.3598$; variance $p = 0.1374$; details are shown in Appendix B.
[b]Keyes et al., 1982.
[c]1 ppm = 7.1 mg/m^3; unadjusted for exposure protocol.
[d]$BMCL_{HEC}$ = BMCL × 6/24 hr × 5/7 days/week × $[(H_{b/g})_{RAT} / (H_{b/g})_{HUMAN}]$, where $[(H_{b/g})_{RAT} / (H_{b/g})_{HUMAN}] = 1$.

The POD ($BMCL_{HEC}$) of 12.9 mg/kg-day, based on increased COHb in rats (Keyes et al., 1982), is used to derive the subchronic p-RfC as follows:

Screening Subchronic p-RfC = POD ÷ UF
= 12.9 mg/m^3 ÷ 300
= **0.04 or 4 × 10^{-2} mg/m^3**

The UF of 300 is composed of the following factors:

- A partial UF of 3 ($10^{0.5}$) is applied for interspecies extrapolation to account for potential pharmacodynamic differences between rats and humans. Converting the rat data to human equivalent concentrations by the dosimetric equations accounts for pharmacokinetic differences between rats and humans; thus, a full UF of 10 for interspecies extrapolation is not used.
- A UF of 10 is applied for protection of sensitive human subpopulations.
- A UF of 10 is applied to account for deficiencies in the database. Developmental and reproductive toxicity studies are missing.

Screening Chronic p-RfC

No chronic inhalation toxicity study of methylene bromide was located. The critical effect (increased COHb; Keyes et al, 1982) and the POD (12.9 mg/m^3) is appropriate for the screening chronic p-RfC derived as follows:

Screening Chronic p-RfC = POD ÷ UF
= 12.9 mg/m^3 ÷ 3000
= **0.004 or 4 × 10^{-3} mg/m^3**

The UF of 3000 is composed of the following factors:

- A partial UF of 3 ($10^{0.5}$) is applied for interspecies extrapolation to account for potential pharmacodynamic differences between rats and humans. Converting the rat data to human equivalent concentrations by the dosimetric equations accounts for pharmacokinetic differences between rats and humans; thus, a full UF or 10 for interspecies extrapolation is not used.
- An UF of 10 is applied for protection of sensitive human subpopulations.
- An UF of 10 is applied to account for deficiencies in the database. Developmental and reproductive toxicity studies are missing.
- An UF of 10 is applied for extrapolation from subchronic to chronic duration.

APPENDIX B. DETAILS OF BENCHMARK DOSE MODELING FOR SUBCHRONIC INHALATION p-RfC

Results of Model Fitting for Blood COHb in Male and Female Rats (Keyes et al., 1982)

BMD modeling was conducted for increased blood carboxyhemoglobin (COHb) levels in male and female rats observed by Keyes et al. (1982) using BMDS version 1.4.1 with default parameter restrictions. The model-fitting procedure was run initially with a BMR of 1 standard deviation change from the control mean as recommended by U.S. EPA (2000b).

The data for male rats were successfully fit to a linear model with modeled variance, as shown in Table B-1. The best fitting model is illustrated in Figure B-1. The female data were not amenable to BMD modeling, even with the highest concentration dropped. For females, both with or without the high dose, the assumption of constant variance was not met. Although the variance model built into the BMDS fit the data adequately, none of the available models fit the means adequately with the variance model applied.

Table B-1. Model Predictions for Increased Carboxyhemoglobin in Rats

Model	Variance *p*-Value[a]	Means *p*-Value[a]	AIC	BMC (ppm)	BMCL (ppm)
Males, All dose groups, BMR = 1 Standard Deviation					
Linear (modeled variance)[b]	0.137	0.360	111.32	14.676	10.155
Polynomial (2nd degree)	0.137	0.157	113.28	15.678	10.183
Polynomial (3rd degree)	0.137	0.173	113.13	16.433	10.279
Power[c]	0.137	0.360	111.32	14.676	10.155
Hill[d]	0.137	0.336	111.46	14.405	11.406
Females, All dose groups, BMR = 1 Standard Deviation					
Linear (modeled variance)[b,e]	0.745	0.0041	114.50	19.130	12.607
Females, 61-day, BMR = 1 Standard Deviation					
Linear (constant variance)[b,e]	0.230	0.00032	30.92	58.635	35.097

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]The Constant Variance model did not fit.
[c]Power restricted to ≥ 1.
[d]Intercept specified at control level to obtain sufficient degrees of freedom.
[e]Poorer fit obtained with other models (polynomial, power, Hill).

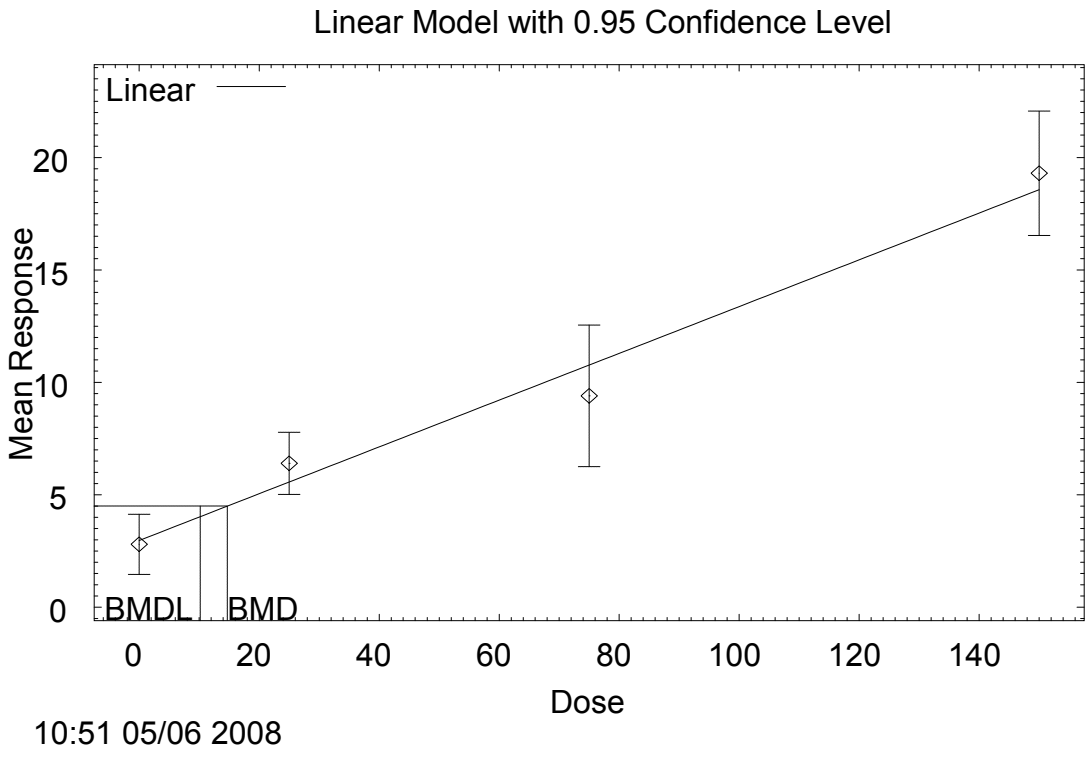

Figure B-1. Fit of Linear Model with Nonhomogeneous (Modeled) Variance to Data on Increased Carboxyhemoglobin in Male Rats from Keyes et al., 1982

BMDs and BMDLs indicated are associated with a change of 1 SD from the control and are in units of ppm.