

**PUBLIC HEALTH GOALS FOR
CHEMICALS IN DRINKING WATER**

ETHYLENE DIBROMIDE

September 2003

**Governor of the State of California
Gray Davis**

**Secretary for Environmental Protection
California Environmental Protection Agency
Winston H. Hickox**

**Director
Office of Environmental Health Hazard Assessment
Joan E. Denton, Ph.D.**



**Public Health Goal for
Ethylene Dibromide
(1,2-Dibromoethane)
in Drinking Water**

Prepared by

**Office of Environmental Health Hazard Assessment
California Environmental Protection Agency**

**Pesticide and Environmental Toxicology Section
Anna M. Fan, Ph.D., Chief**

**Deputy Director for Scientific Affairs
George V. Alexeeff, Ph.D.**

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LIST OF CONTRIBUTORS

PHG PROJECT MANAGEMENT

REPORT PREPARATION

SUPPORT

Project Director

Anna Fan, Ph.D.

Author

Thomas Parker, M.Sc.

Administrative Support

Edna Hernandez
Coordinator

Public Workshop

Robert Howd, Ph.D.
Juliet Rafol

Primary Reviewer

Jolanta Bankowska, Ph.D.

Sharon Davis
Hermelinda Jimenez
Genevieve Vivar
Michelle St. Croix

Coordination of External Review

Yi Wang, Ph.D.
Moirra Sullivan, M.S.

Final Reviewers

Robert Howd, Ph.D.
Anna Fan, Ph.D.
George Alexeeff, Ph.D.

Library Support

Charleen Kubota, M.L.S.

Revisions/Responses

Robert Howd, Ph.D.

Web site Posting

Edna Hernandez
Laurie Monserrat

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PREFACE

**Drinking Water Public Health Goals
Pesticide and Environmental Toxicology Section
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency**

This Public Health Goal (PHG) technical support document provides information on health effects from contaminants in drinking water. PHGs are developed for chemical contaminants based on the best available toxicological data in the scientific literature. These documents and the analyses contained in them provide estimates of the levels of contaminants in drinking water that would pose no significant health risk to individuals consuming the water on a daily basis over a lifetime.

The California Safe Drinking Water Act of 1996 (amended Health and Safety Code, Section 116365), amended 1999, requires the Office of Environmental Health Hazard Assessment (OEHHA) to perform risk assessments and publish PHGs for contaminants in drinking water based exclusively on public health considerations. Section 116365 specifies that the PHG is to be based exclusively on public health considerations without regard to cost impacts. The Act requires that PHGs be set in accordance with the following criteria:

1. PHGs for acutely toxic substances shall be set at levels at which no known or anticipated adverse effects on health will occur, with an adequate margin of safety.
2. PHGs for carcinogens or other substances that can cause chronic disease shall be based upon currently available data and shall be set at levels that OEHHA has determined do not pose any significant risk to health.
3. To the extent the information is available, OEHHA shall consider possible synergistic effects resulting from exposure to two or more contaminants.
4. OEHHA shall consider the existence of groups in the population that are more susceptible to adverse effects of the contaminants than a normal healthy adult.
5. OEHHA shall consider the contaminant exposure and body burden levels that alter physiological function or structure in a manner that may significantly increase the risk of illness.
6. In cases of insufficient data to determine a level of no anticipated risk, OEHHA shall set the PHG at a level that is protective of public health with an adequate margin of safety.
7. In cases where scientific evidence demonstrates that a safe dose-response threshold for a contaminant exists, then the PHG should be set at that threshold.
8. The PHG may be set at zero if necessary to satisfy the requirements listed above.

9. OEHHA shall consider exposure to contaminants in media other than drinking water, including food and air and the resulting body burden.
10. PHGs published by OEHHA shall be reviewed every five years and revised as necessary based on the availability of new scientific data.

PHGs published by OEHHA are for use by the California Department of Health Services (DHS) in establishing primary drinking water standards (State Maximum Contaminant Levels, or MCLs). Whereas PHGs are to be based solely on scientific and public health considerations without regard to economic cost considerations, drinking water standards adopted by DHS are to consider economic factors and technical feasibility. Each standard adopted shall be set at a level that is as close as feasible to the corresponding PHG, placing emphasis on the protection of public health. PHGs established by OEHHA are not regulatory in nature and represent only non-mandatory goals. By federal law, MCLs established by DHS must be at least as stringent as the federal MCL if one exists.

PHG documents are used to provide technical assistance to DHS, and they are also informative reference materials for federal, state and local public health officials and the public. While the PHGs are calculated for single chemicals only, they may, if the information is available, address hazards associated with the interactions of contaminants in mixtures. Further, PHGs are derived for drinking water only and are not intended to be utilized as target levels for the contamination of other environmental media.

Additional information on PHGs can be obtained at the OEHHA Web site at www.oehha.ca.gov.

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PUBLIC HEALTH GOAL FOR ETHYLENE DIBROMIDE IN DRINKING WATER

SUMMARY

The Office of Environmental Health Hazard Assessment (OEHHA) establishes a public health goal (PHG) of 0.01 µg/L (0.01 ppb) for ethylene dibromide in drinking water. This PHG is based on the carcinogenic effects observed in an oral study performed by the National Cancer Institute in 1978. The authors reported cancer of the forestomach in rats and mice. Cancer potency was estimated by fitting the Doll-Armitage multistage model to the rat and mouse data. The geometric mean of the resulting cancer potency factors (the q_1^* for each species and sex) was used as the q_1^* for the calculation to derive the PHG. The resulting q_1^* value is $3.6 \text{ (mg/kg-day)}^{-1}$. The PHG was calculated assuming a *de minimis* theoretical excess individual cancer risk of one-in-a-million, or 1×10^{-6} from lifetime exposure to ethylene dibromide in drinking water.

In addition to the 0.01 ppb PHG value based on its carcinogenicity, a health-protective value of 50 µg/L (50 ppb) was calculated based on the noncancer effects of ethylene dibromide. The study selected for the determination of the noncancer value was that of Nitsche *et al.* (1981). In this inhalation study of ethylene dibromide in rats, the authors observed the absence of increases in relative liver and kidney weights in male F334 rats exposed to 3 ppm EDB for 6 hours per day, 5 days per week, for 13 weeks. For this non-cancer study, the ethylene dibromide NOAEL was 2.5 mg/kg-day. The noncancer health-protective value incorporates a 10-fold uncertainty factor to account for interspecies variation, a 10-fold uncertainty factor to account for human variability, and a third 10-fold uncertainty factor to account for the use of a subchronic study to calculate a chronic oral noncancer value.

The U.S. EPA has classified ethylene dibromide as a group B2, probable human carcinogen (U.S. EPA, 2000). The current U.S. EPA maximum contaminant level (MCL) for ethylene dibromide in drinking water is 0.05 ppb, based on analytical feasibility (U.S. EPA, 2002). The U.S. EPA determined a q_1^* value of $85 \text{ (mg/kg-day)}^{-1}$ based on the NCI (1978) study and calculated that the MCL corresponded to 1.25 times the 1×10^{-4} risk level (U.S. EPA, 1987a,b, 1991; ATSDR, 1991).

INTRODUCTION

The purpose of this document is to develop a PHG for ethylene dibromide in drinking water. Ethylene dibromide had been widely used in California as a fumigant and gasoline additive, but both uses have now been discontinued. Fumigant use was cancelled by the U.S. EPA in 1983 due to the possibility of residues in drinking water and stored grain (Alexeeff *et al.*, 1990). Ethylene dibromide use as a gasoline additive has decreased with the phase-out of leaded auto fuels (CARB, 1985). In this document, the available data on the toxicity of ethylene dibromide are evaluated, with a primary focus on the literature related to both oral and inhalation exposures that are appropriate for the

establishment of a PHG for drinking water. The studies that can be used to identify public health-protective levels are reviewed and summarized. Other studies that were retrieved in the literature search but were judged not to be relevant for development or support of a PHG value have not been discussed or cited.

CHEMICAL PROFILE

Chemical Identity

Ethylene dibromide is the common name for 1,2-dibromoethane, a colorless liquid with a sweet odor which has been used as a fumigant and gasoline additive. Ethylene dibromide is synthesized by reacting bromine with ethylene and has no natural sources (Alexeeff *et al.*, 1990; Reed *et al.*, 1987); see Table 1.

Table 1. Chemical Identity of Ethylene Dibromide

Attribute	Chemical Identity
Chemical name	1,2-dibromoethane
Synonyms	ethylene dibromide, EDB, glycol bromide, glycol dibromide, Bromofume, Dowfume, Nephis, Celmide, Soilbrom, Soilfume, Copfume, Pestmaster, Dibrome, dibromoethane, Unifume.
Chemical formula	C ₂ H ₄ Br ₂
CASRN	106-93-4

As shown in Figure 1, ethylene dibromide is a short-chain halogenated hydrocarbon.

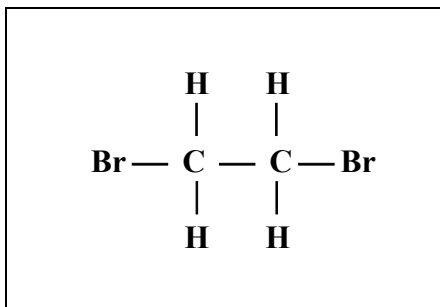


Figure 1. Chemical structure of ethylene dibromide

Physical and Chemical Properties

The properties of ethylene dibromide are summarized below in Table 2. It is a relatively volatile organic solvent that can solidify slightly below room temperature. The liquid is considerably more dense than water, and its vapors are much more dense than air.

Table 2. Physical and Chemical Properties of Ethylene Dibromide

Property	Value or Information	References
Molecular weight	187.9	NIOSH, 1994
Color	Clear	ATSDR, 1995
Physical state	Liquid (room temperature)	Merck Index, 1996
Odor	Sweet	ATSDR, 1995
Odor threshold	10-15 ppm	Reed <i>et al.</i> , 1987
Melting point	9.8 °C	CRC Handbook, 1988
Boiling point	131.3 °C	CRC Handbook, 1988
Flash point	N/A	NIOSH, 1994
Flammability limits	UEL & LEL, N/A	NIOSH, 1994
Autoignition temperature	Noncombustible	NIOSH, 1994
Solubility Water Organic solvents	0.43 g/100 ml water at 25 °C Soluble in alcohol, ether, benzene, and acetone	Alexeeff <i>et al.</i> , 1990 U.S. EPA, 1987a
Specific gravity, density	(liquid) 2.179 (25 °C) (gas, air =1) 6.5	Alexeeff <i>et al.</i> , 1990
Partition coefficient	Octanol/water, 86	Steinburg <i>et al.</i> , 1987
Log K _{ow}	1.76	Montgomery, 1993
Vapor pressure	12 Torr	NIOSH, 1994
Conversion factor	1 ppm = 7.69 mg/m ³	NIOSH, 1994

Production and Uses

Ethylene dibromide is not produced in California. The major uses of ethylene dibromide in California were as a fumigant pesticide and as a gasoline additive. In 1983, approximately 360 tons of ethylene dibromide were used in pesticidal applications including fumigation of soil, stored grains and fruits, grain mills and termite-infested areas. In September, 1983, the U.S. EPA cancelled most pesticidal uses of ethylene dibromide (CARB, 1985). There are currently no approved pesticidal uses in California.

Also in 1983, 2,100 tons of ethylene dibromide were used in leaded gasoline in California (CARB, 1985). Ethylene dibromide is a lead scavenger in tetra-alkyl leaded gasoline. Ethylene dibromide scavenges lead from tetraethyl lead antiknock agents and thereby prevents engine fouling (OEHHA, 1988). State law prescribed that lead be eliminated from motor gasoline and since 1992, all gasoline in California has been lead free (CARB, 1996). As leaded gasoline use in the state declined, ethylene dibromide use declined similarly for that application. We are not able to find any current use of ethylene dibromide in California.

ENVIRONMENTAL OCCURRENCE AND HUMAN EXPOSURE

Air

In 1983, 2,100 tons of ethylene dibromide were used in gasoline formulation in California. That same year, 362 tons of ethylene dibromide were used as pesticides for fumigation. The California Air Resources Board estimated that statewide air emissions for ethylene dibromide were 362 tons from pesticide application and about 15.5 tons from gasoline and motor vehicle-related sources (CARB, 1985).

Between January 1983 and May 1984 the California Air Resources Board collected 511 air samples from among four locations, El Monte, Los Angeles, Dominguez and Riverside. Thirty percent of the samples had concentrations above 5 ppt, the minimum reporting level. The average ethylene dibromide concentration was 7.4 ppt; however, all values below the reporting limit of 5.0 ppt were considered to be 2.5 ppt for each sample (CARB, 1985).

The breakdown of ethylene dibromide in the atmosphere is fairly slow, with a half-life of 50 days or longer. Airborne reactions of ethylene dibromide with hydroxyl radicals may be the most important atmospheric removal mechanism, although all of the main pathways of decomposition are not clearly established (Reed *et al.*, 1987).

The elimination of lead in motor fuels in California in 1982 and hence the elimination of ethylene dibromide as a gasoline additive, along with U.S. EPA's banning of the pesticidal use of ethylene dibromide in 1983, have reduced the potential for exposure to ethylene dibromide vapors in air.

Soil

Before its use as a pesticide was banned in 1983, ethylene dibromide was widely used as a soil fumigant for the control of nematodes and other pests, and was injected directly into the soil as a liquid (Reed *et al.*, 1987). The persistence of ethylene dibromide in soils varies greatly, with experimental soil half-lives varying from 1.5 to 18 weeks. With K_{oc} values ranging from 14 to 160, some ethylene dibromide can leach from soil. A moderate vapor pressure of 11.2 Torr suggests that some of the chemical will evaporate into the atmosphere from soil surfaces (HSDB, 2000). Ethylene dibromide is most likely, however, to remain sorbed by soil particles with the most likely losses due to soil

microbial degradation. Ethylene dibromide is degraded microbially under most redox conditions (Pignatello and Cohen, 1990; Reed *et al.*, 1987). Within soil, transport of ethylene dibromide to groundwater occurs both by vapor phase diffusion and by advective transfer via horizontal currents of infiltrating water. Ethylene dibromide has penetrated “many tens” of meters of soil to reach the water table (Pignatello and Cohen, 1990).

Water

Most pollution of groundwater was associated with the use of ethylene dibromide as a pesticide and to a lesser extent with leaks and spills of gasoline (Kloos, 1996). Ethylene dibromide can be fairly persistent as a ground water contaminant. Hydrolysis is likely the most significant abiotic reaction with half-life estimates varying from 2 to 15 years (Pignatello and Cohen, 1990). Ethylene dibromide is degraded by a variety of microbial cultures, with metabolism occurring under both oxic and anoxic conditions (Pignatello and Cohen, 1990). Between 1988 and 1993, 302 public wells were sampled in the Fresno and Clovis, California areas. From these wells, 13 active and 10 closed wells exceeded the U.S. EPA MCL of 0.05 ppb, with the concentrations in one well at 1.1 ppb. For most of the wells, agricultural application was the most likely source of the ethylene dibromide, although gasoline leaks were suspected in about five of them. In six wells for which data were adequate to track concentration versus time, the mean ethylene dibromide levels declined from 0.025 ppb to 0.015 ppb in 1993, and 60 percent over four years (Kloos, 1996).

Food

Shortly following the discovery of ethylene dibromide residues in commercial flour and cake mixes, the U.S. EPA in 1984 cancelled the use of ethylene dibromide as a fumigant in stored grain (Alexeeff *et al.*, 1990; Sharlin, 1986). The U.S. EPA estimated that before cancellation of ethylene dibromide use, 60 percent of wheat products consumed by humans in the United States was contaminated with ethylene dibromide residues (U.S. EPA, 1984). In one test, wheat was fumigated for 10 days, followed by either a 2-4 or 10-12 week aeration period, with higher concentrations corresponding with shorter aeration periods. Ethylene dibromide residues were 5-30 ppm in whole wheat, 18-23 ppm in shorts and bran, 0.002–0.04 ppm in white bread and 0.006–0.026 ppm in whole wheat bread (HSDB, 2000).

METABOLISM AND PHARMACOKINETICS

Absorption

The majority of an inhaled or oral dose of ethylene dibromide is rapidly absorbed when administered to laboratory animals. Dermally applied ethylene dibromide is also absorbed (OEHHA, 1988; WHO, 1996). Nachtomi and Alumot (1972) administered

110 mg/kg ethylene dibromide to rats and chicks via stomach tube and observed maximum liver and blood levels within 5 minutes for rats and within 30 minutes for chicks (Alexeeff *et al.*, 1990). In rats exposed to 75 ppm of ethylene dibromide, the rate of absorption via inhalation reached a plateau within 10-20 minutes and about 58 percent of the ethylene dibromide was absorbed, principally from the lower respiratory tract (Stott and McKenna, 1984). Plotnick *et al.* (1979) administered a single oral dose of 15 mg/kg of ¹⁴C labeled ethylene dibromide to male rats and observed radioactivity in the urine and feces in the subsequent 48 hours. The authors accounted for 1.7 percent of the administered radioactivity in the feces and 72.3 percent in the urine, suggesting that ethylene dibromide was almost completely absorbed from the gastrointestinal tract.

Distribution

Ethylene dibromide is readily and widely distributed in mammalian tissues (U.S. EPA, 1987a). Plotnick *et al.* (1979) examined the comparative ¹⁴C levels in selected tissues following oral administration of ethylene dibromide. The authors observed that ¹⁴C was distributed preferentially in the liver, kidney and spleen one day following administration.

Plotnick and Conner (1976) studied the ¹⁴C distribution in male guinea pigs following intraperitoneal administration of radiolabeled ethylene dibromide at 30 mg/kg. The authors observed that the liver, kidney and adrenal gland were the organs of highest radiolabel concentration (Plotnick and Conner, 1976).

Kowalski *et al.* (1985) investigated tissue distribution of ¹⁴C-ethylene dibromide and metabolites in rats and mice following intravenous and intraperitoneal injection. In mice, the highest levels of uptake occurred in the mucosa of the nasal, tracheobronchial and esophageal areas and the mucosa of the forestomach. Radiolabel remained 24 hours in the respiratory tract and persisted for four days in the forestomach (Alexeeff *et al.*, 1990).

Metabolism

Metabolism of ethylene dibromide occurs primarily in the liver and kidneys (Nachtomi, 1970). Both *in vitro* and *in vivo* metabolites of ethylene dibromide have been identified. The metabolism of ethylene dibromide occurs by one of two principal routes: an oxidative pathway catalyzed by cytochrome P450 and a conjugative pathway catalyzed by glutathione S-transferases (Ploemen *et al.*, 1997). Inorganic bromide is formed via oxidative catabolism by cytochrome P450 dependent mixed function oxidases or via the glutathione conjugation reaction (van Bladeren *et al.*, 1980; WHO, 1996). The oxidative pathway produces 2-bromoacetaldehyde, which is reactive and may be responsible for some of the toxic effects of ethylene dibromide (Hill *et al.*, 1978; OEHHA, 1988; WHO, 1996). 2-Bromoacetaldehyde may then be converted by dehydrogenase to 2-bromoacetic acid or, alternatively, react with GSH and GSH-S-transferase to yield S-carboxymethyl-GSH, which would undergo further metabolism to S-(β -hydroxyethyl) cysteine (Reed *et al.*, 1987; Alexeeff *et al.*, 1990; WHO, 1996). The other pathway is a direct reaction with glutathione involving GSH transferase enzymes. This produces the toxic intermediate S-

(2-bromoethyl)–GSH, which may be eventually detoxified via hydrolysis (WHO, 1996). Other metabolites isolated via the glutathione pathway include the reactive half mustards leading to 2-hydroxyethyl-L-cysteine (Van Bladeren *et al.*, 1980) and S-(β-hydroxyethyl) glutathione and its sulfoxide (Nachtomi, 1970), which are eliminated via the urinary tract (Alexeeff *et al.*, 1990). The conjugative metabolism pathway is associated with the mutagenicity and carcinogenicity of ethylene dibromide (Guengerich, 1994).

Excretion

Urinary excretion is the primary route of elimination for products of orally administered ethylene dibromide. Plotnick *et al.* (1979) observed that 24 hours following a single oral dose of ¹⁴C-ethylene dibromide, about 72 percent of the radiolabel was found in the urine and less than 2 percent in the feces. The final urinary elimination products were principally S-(β-hydroxyethyl) mercapturic acid and S-(β-hydroxyethyl) mercapturic acid sulfoxide or their conjugates (Nachtomi, 1970; Plotnick *et al.*, 1979).

TOXICOLOGY

Toxicological Effects in Animals

For non-cancer toxic effects, the formation of bromoacetaldehyde may be of more importance than the metabolic pathway involving direct conjugation with glutathione (Alexeeff *et al.*, 1990). Toxic effects of ethylene dibromide are primarily observed in the liver, kidneys, and testes. Inhaled ethylene dibromide produces nasal irritation and CNS depression. Ethylene dibromide also irritates the skin (U.S. EPA, 1987a; WHO, 1996). Reproductive and genotoxic effects also occur from exposure to ethylene dibromide.

Acute Toxicity

Rowe *et al.* (1952) performed single dose oral ethylene dibromide LD₅₀ studies on male and female rats, female mice, female rabbits, and mixed sex guinea pigs and chicks. The rabbit appears to be the most sensitive species, with a single oral dose, 14-day LD₅₀ value of 55 mg/kg. The lowest available acute LC₅₀ value for the inhalation route was 200 ppm, approximately 362 mg/kg, for a 9-hour exposure in the rat (Rowe and Spencer, 1952). The group of rats was mixed-sex and the strain was not mentioned by the authors. Our dose calculation assumed a breathing rate of 0.165 m³/d, a body weight of 0.152 kg, and an absorption rate of 58 percent (Stott and McKenna, 1984). McCollister *et al.* (1956) determined an oral LD₅₀ of 140 mg/kg in rats following the administration of ethylene dibromide at six dose levels (McCollister *et al.*, 1956; Alexeeff *et al.*, 1990). Thus, in the available data, the oral route has produced the lowest median lethal dose. All these authors gave little description of toxic effects other than lethality.

Broda and Nachtomi (1976) observed differences in liver morphology between rats and chicks treated with 110 mg/kg ethylene dibromide. At various time intervals up to 22 hours post exposure, groups of 5-8 animals were examined. In rats, the authors

observed centrosinuosidal dilations in liver tissue after 8 hours. At 17 hours post exposure, they observed degenerative changes in hepatocytes; and at 22 hours post exposure, chick livers exhibited substantial infiltration of eosinophilic granulocytes.

U.S. EPA (1987a) summarized the biochemical indices of animal liver damage from ethylene dibromide intoxication as including initially decreased and subsequently increased levels of free sulfhydryl groups, increased levels of triglycerides and total lipids, increased thymidine incorporation into DNA, increased liver and blood alkaline phosphatase, serum glutamic pyruvic transaminase (SGPT), and sorbitol dehydrogenase. The authors summarized the pathological indices of animal liver damage due to ethylene dibromide intoxication as including increased relative liver weight, sinusoidal dilation and centrilobular necrosis, with this evidence of liver damage evident 2-24 hours after ethylene dibromide administration (U.S. EPA, 1987a).

Subchronic Toxicity

Much of the subchronic data are from studies on the reproductive or genotoxic effects of ethylene dibromide. We present these studies on these effects later in this document.

Rowe and Spencer (1952) applied a 1.0 percent solution of ethylene dibromide in butylcarbitol acetate to a rabbit 10 times over 14 days. When applied to the ear the authors observed erythema and exfoliation of the skin. When treatment occurred on the abdomen, the authors observed that treatment resulted in erythema and edema that resulted in necrosis and sloughing of the skin. The lesions healed within seven days.

Adult hens maintained on feed containing 50-320 mg/kg ethylene dibromide laid smaller eggs; and after six weeks on the diet containing the highest dose, egg laying ceased irreversibly (Bondi *et al.*, 1955; IARC, 1977)

Reznik *et al.* (1980) exposed F344 rats and B6C3F₁ mice to ethylene dibromide for 13 weeks via inhalation. The rodents of each species were divided into three groups of 10 each, and were exposed to 3, 15, or 75 ppm (approximately 4.5, 22, and 110 mg/kg-day for the rat and 6.75, 34, and 120 mg/kg-day for the mouse) six hours per day, five days per week for 13 weeks. An additional group from each species was exposed only to room air. At the highest dose, the authors observed histomorphological alterations in the nasal cavity of both rats and mice, including cytomegaly, focal hyperplasia, squamous metaplasia, and loss of cilia. Only minor changes were observed at the lower dose levels (Reznik *et al.*, 1980). These effects are likely to be due to local irritant effects of ethylene dibromide, although some systemic dose influence cannot be discounted. The lowest LOAEL was that of the male rat of 21.45 mg/kg-day (at 0.18 kg body weight and 0.19 m³/d breathing rate). The highest NOAEL from the study was observed to be from the male mouse of 6.90 mg/kg-day (at 0.0316 kg and 0.053 m³/d breathing rate). The above LOAEL and NOAEL were not corrected for fractional uptake from inhalation because all of the toxic effects occurred in the respiratory tract, and as such, were apparently not systemic.

The NCI (1978) performed subchronic oral toxicity range-finding experiments in both the Osborne-Mendel rat and the B6C3F₁ mouse for ethylene dibromide, in order to establish maximum tolerated dosage levels for the subsequent carcinogen bioassays. Animals of

each species were distributed among six groups, each consisting of five males and five females. Technical grade ethylene dibromide in corn oil was introduced via gavage to five of the six groups of rats and mice at dosages of 40, 63, 100, 163, and 251 mg/kg-day. The sixth group received corn oil only. Intubation was performed five days per week for six weeks followed by a two-week observation period. For rats, mortality (one male and one female) and weight gain depression began at the 100 mg/kg-day level, and the authors selected 80 mg/kg-day as the initial MTD in rats. One mouse died at the 100 mg/kg-day level and two mice died at the 251 mg/kg-day level. Body weight trends were not clear in mice. The authors selected 120 mg/kg-day as the initial MTD for mice of both sexes (NCI, 1978).

The NTP (1982) sponsored subchronic inhalation studies in which F334 rats and B6C3F₁ mice were exposed to 0, 3, 15, or 75 ppm ethylene dibromide for 6 hours per day, 5 days per week for 13 weeks. For rats at 75 ppm, the authors reported swelling or vacuolization of the adrenal cortical cells of the zona fasciculata as well as slight decreases in follicular size in the thyroid. Male rats exhibited a dose-related weight gain depression to a 42 percent maximum at the conclusion of the study. There were no rat deaths. The authors selected 40 ppm as the initial MTD for the subsequent chronic study. For mice, 4 of 10 male mice died at 3 ppm and one of 10 female mice died at the 75 ppm exposure level. The authors observed evidence of eye irritation at 75 ppm. Also at the highest concentration, megalocytic cells were found lining the bronchioles in both sexes. The authors selected 40 ppm as the initial MTD for the subsequent chronic mouse inhalation study (NTP, 1982). The lowest LOAEL was that of the male rat of 36.1 mg/kg-d (at 0.18 kg body weight and 0.19 m³/d breathing rate). The highest NOAEL from the study was observed to be from the female rat of 13.5 mg/kg-d (at 0.201 kg and 0.21 m³/d breathing rate). The above LOAEL and NOAEL were corrected for 58 percent fractional uptake from inhalation (Stott and McKenna, 1984).

Nitsche *et al.* (1981) performed a 13-week inhalation study of ethylene dibromide in rats. Four groups of F344 rats, at 40 male and 20 female per group, were exposed to 0, 3, 10, or 40 ppm ethylene dibromide for 6 hours per day, 5 days per week, for 13 weeks. Male rats inhaling 40 ppm had significant body weight loss throughout the exposure period. Female rats exposed to 40 ppm for 13 weeks showed a significant decrease in specific gravity of the urine, and had elevated relative liver weights. Male rats exposed to 10 ppm and 40 ppm exhibited increases in relative liver and kidney weights and either showed single or multiple foci of hyperplasia of the nasal respiratory epithelium after 13 weeks. After 13 weeks at the high concentration the authors observed diffuse or focal non-keratinizing squamous hyperplasia and metaplasia of the respiratory epithelium. The LOAEL for this study, based on increased relative liver and kidney weights, is that of the male rat of 8.3 mg/kg-d (at 0.18 kg and 0.19 m³/d breathing rate). The NOAEL from the study, also from the male rat, was identified as 2.49 mg/kg-d. The above LOAEL and NOAEL were corrected for 58 percent fractional systemic uptake from inhalation exposure (Stott and McKenna, 1984).

Table 3. Selected Subchronic and Chronic LOAEL and NOAEL values*

LOAEL	NOAEL	Study	Comments
	2.5 mg/kg-d	Nitschke <i>et al.</i> (1981).	Male rats, absence of increased liver and kidney weights, reversible epithelial hyperplasia and squamous metaplasia of the nasal turbinates (subchronic)
21.4 mg/kg-d		Reznik <i>et al.</i> (1980)	Male rats, respiratory irritation (subchronic)
	6.9 mg/kg-d	Reznik <i>et al.</i> (1980)	Male mouse, absence of cytomegaly, focal hyperplasia, squamous hyperplasia, loss of cilia, and irritation localized in the nasal cavity (subchronic)
36.1 mg/kg-d		NTP (1982)	Male rat, nasal cavity alterations, e.g. epithelial hyperplasia, squamous metaplasia (subchronic)
	13.5 mg/kg-d	NTP (1982)	Absence of above indications (subchronic)
7.0 mg/kg-d		NTP (1982)	Female rat, retinal atrophy and adrenal cortex degeneration (chronic)

*Inhalation exposures, with estimated systemic doses.

Genetic Toxicity

Genotoxicity of ethylene dibromide has been clearly demonstrated. Ethylene dibromide binds to DNA following *in vivo* administration or *in vitro* incubation. A DNA adduct bioactivated by glutathione has been identified. DNA damage has been reported following single injections of ethylene dibromide in rats and mice, and also was observed when ethylene dibromide was added to cultured hepatocytes. Ethylene dibromide is mutagenic in a number of bacterial assays, in eucaryotes, plants, insect, and cultured mammalian cells (Alexeeff *et al.*, 1990). Ethylene dibromide can act as an initiator in the carcinogenic process.

Nachtomi and Sarma (1977) observed that oral administration of ¹⁴C-ethylene dibromide resulted in the incorporation of radiolabel into liver DNA, RNA, and protein (Nachtomi and Sarma, 1977). Similarly, Short *et al.* (1979) observed that the inhalation administration of ¹⁴C-ethylene dibromide resulted in the incorporation of radiolabel into DNA, RNA, and protein of liver, kidney, testes, and stomach (Short *et al.*, 1979).

The major DNA adduct (>95 percent of total) resulting from the bioactivation of ethylene dibromide by conjugation with glutathione is S-[2(N-guanyl) ethyl] GSH. This adduct is most likely responsible for the mutagenic activity of ethylene dibromide, which apparently reacts with DNA to produce base-pair mutations (Cmarik *et al.*, 1992; Guengerich, 1994).

In his review of the genotoxic effects of ethylene dibromide and ethylene dichloride, Rannug (1980) summarized the results of studies showing that in the absence of a metabolizing system, ethylene dibromide produces point mutations in *Salmonella*

typhimurium. The mutations are base-pair substitutions and strains G46, TA1530, TA1535 and TA100 are reverted by ethylene dibromide. Ethylene dibromide is a strong, direct mutagen in microorganisms (Rannug, 1980).

Novotna and Duverger-van Bogaert (1994) observed direct mutagenicity via the Ames mutagen bioassay in *S. typhimurium* strains TA1535 and TA100 in a dose related fashion. The authors also observed that the addition of liver S-9 yielded a higher mutagenic activity and that the activity was even higher with a kidney and liver S-9 fraction added. Interestingly, the addition of kidney S-9 alone did not modify the liver mutagenic activity of ethylene dibromide (Novotna and Duverger-van Bogaert, 1994).

Nachtomi and Farber (1978) confirmed that ethylene dibromide is an effective mitogen for the liver in rats. The authors intubated Wistar rats with 75 to 100 mg/kg of ethylene dibromide. The authors observed that ethylene dibromide induced about 15 percent of the hepatocytes to proliferate, with DNA synthesis beginning at about 24 hours post exposure and reaching a peak about 24 hours (Nachtomi and Farber, 1978).

From studies in *Drosophila*, Ballering *et al.* (1994) concluded that the glutathione mediated metabolic pathway, as opposed to the oxidative metabolic pathway, represents the major source for genotoxic effects (Ballering *et al.*, 1994). The authors observed sequence changes produced by ethylene dibromide using the vermilion locus of *Drosophila melanogaster*. When excision-repair-proficient males were exposed to ethylene dibromide and then were mated to excision-repair-deficient females, 14 mutation events were isolated from F₁ or F₂ progeny. The authors' data supported the conclusion that ethylene dibromide is genotoxic through modification at ring nitrogens in DNA, primarily at the N7 of guanine and to a lesser extent at the A1 of adenine. The importance for mutagenic action appears to be the formation of non-coding lesion or misrepair (Ballering *et al.* 1994).

Brimer *et al.* (1982) tested ethylene dibromide with the Chinese hamster ovary/hypoxanthine guanine phosphoribosyl transferase assay coupled with a rat liver metabolic activation system (S-9). Ethylene dibromide was mutagenic in the assay both with and without metabolic activation, with the mutagenic activity remaining unchanged. Metabolic activation did, however, increase the cytotoxicity of ethylene dibromide.

Developmental and Reproductive Toxicity

Ethylene dibromide is a reproductive toxicant. The compound crosses the placenta and binds to fetal tissue. Ethylene dibromide has not been found to be teratogenic. Ethylene dibromide affects spermatogenesis in bulls, rats, and rams. Fertility and egg size was affected in birds. Human studies have indicated some effects on spermatogenesis and fertility (Alexeeff *et al.*, 1990).

Short *et al.* (1979) studied reproductive effects on rats exposed to ethylene dibromide via inhalation. Male LD rats were exposed to normal daily concentrations of 19, 39, and 89 ppm of ethylene dibromide 7 hours/day, 5 days/week for 10 weeks. Mortality and morbidity occurred at the high concentration. Male rodents in this group had reduced testicular weights, reduced serum testosterone concentrations, and mating failure during a two-week mating period. The authors observed atrophy of the testes, epididymis,

prostate and seminal vesicles in these males. The reproductive performance of males exposed to the two lower concentrations was not impaired. Females inhaled average daily concentrations of 20, 30, or 80 ppm of ethylene dibromide for 7 hours/day, 7 days/week, for three weeks. Mortality and morbidity occurred in the high dose group. Females in this group did not cycle normally until several days following the termination of the exposure. The reproductive performance of females exposed to the lower concentrations was normal. Litters produced from pregnant females exposed in all dose groups were normal. The adverse reproductive effects on both sexes of rats were produced only at the high concentrations, which produced mortality and morbidity (Short *et al.*, 1979).

Hsu *et al.* (1985) studied the effects of paternal exposure to ethylene dibromide on regional brain enzyme activity in development of brains of F₁ progeny. The authors observed that adult male rats mated at 7-14 days following subacute *i.p.* dosing of 1 mg/kg-day over five successive days resulted in significant changes in the enzyme choline acetyltransferase in various brain regions of F₁ progeny at 21 days after birth, but not at 7, 14, or 90 days following birth. In the 21-days-old progeny, the specific choline acetyltransferase was increased in the cerebellum by 25 percent, corpus striatum by 29 percent, hippocampus by 45 percent and the hypothalamus by 28 percent. The authors also observed that specific acetylcholinesterase activity was altered in various brain regions of the F₁ progeny at 7, 14, and 21 days after birth, but not at 90 days following birth. For example, at seven days following birth, acetylcholinesterase activity increased in corpus striatum by 37 percent and in the hippocampus by 29 percent, but was not affected in the cerebellum, frontal cortex, or hypothalamus. At 14 days of age, acetylcholinesterase activity was decreased in the cerebellum by 14 percent, corpus striatum by 16 percent and hippocampus by 18 percent (Hsu *et al.*, 1985). This low effect level was not used for risk assessment because of uncertainty about the relevance of both the exposure route and the nature of the endpoints.

Edwards *et al.* (1970) investigated the antifertility effects of ethylene dibromide in rats. Ten mg/kg ethylene dibromide in arachis oil was administered *i.p.* to six male Wistar rats over five successive days for a total dosage of 50 mg/kg. Ethylene dibromide showed a selective disposition to damage spermatogenic cells. The effect was temporary, with the greatest anti-fertility effects observed in weeks three and four following exposure.

A number of studies examined the antifertility effects of ethylene dibromide on bull spermatozoa. Amir and Volcani (1965) fed bull calves approximately 2 mg/kg-day ethylene dibromide for 14-16 months. The authors observed abnormal spermatozoa shape and poor motility. Recovery was observed three months allowing termination of treatment. Follow-up studies showed that 4 mg/kg ethylene dibromide given orally every other day for 20 days induced changes in the sperm of bulls (Amir and Volcani, 1965; Amir *et al.*, 1977; Amir, 1973; Courtens *et al.*, 1980; Reed *et al.*, 1987).

Eljack and Hrudka (1979) observed a dose-dependent decline in sperm mortality and an increase in abnormal sperm following subcutaneous administration of 0, 7.8, 9.6, and 13.5 mg/kg-d ethylene dibromide to groups of 4-6 Colombian rams over 12 consecutive days. The effects occurred following a 4-week latency period and returned to normal 15 weeks following treatment.

Biochemical and mutational effects of ethylene dibromide were measured in rat sperm (Zenz, 1994). Ethylene dibromide significantly depressed glutathione concentrations in the caput and caudal epididymis, but not the testes. Ethylene dibromide enhanced the production of ethylmethane sulfonate-induced dominant lethal mutations in matings 2 and 3 weeks post exposure. This latter effect corresponded with depression of glutathione in reproductive tissue and with increased binding of ethylmethane sulfonate to sperm heads.

Immunotoxicity

Little information could be located regarding immunotoxicity of EDB in animals.

In a chronic inhalation study in which Sprague-Dawley rats were exposed to 20 ppm ethylene dibromide (7 hr/day, 5 days/week for 18 months), significant spleen atrophy was observed in male rats compared with controls (Wong *et al.*, 1982).

Neurotoxicity

As described earlier, Hsu *et al.* (1985) studied the effects of paternal exposure to ethylene dibromide on regional brain enzyme activity in developing brains of F₁ progeny. The authors described significant changes in acetylcholinesterase and choline acetyltransferase at various early development stages in the F₁ progeny.

Albrecht (1987) evaluated central nervous system toxicity of ethylene dibromide and several other chemicals that may produce environmental residues in Swiss Webster mice over a four-week administration period. The differential effects of the tested chemicals on CNS excitability caused by a known CNS stimulant, pentylenetetrazol, was studied. Ethylene dibromide antagonized the stimulatory effect of pentylenetetrazol, but not as potently as other compounds such as chlordecone and heptachlor epoxide. Ethylene dibromide was roughly equivalent in potency to pentachlorophenol.

Chronic Toxicity

NCI (1978) performed a carcinogenicity bioassay for ethylene dibromide via gavage in rats and mice, at two doses, to groups of 50 animals of each sex. The time weighted average high and low doses of ethylene dibromide were 41 and 38 mg/kg-day for male rats, 39 and 37 mg/kg-day for female rats, and 107 and 62 mg/kg-day for mice of both sexes. Twenty animals per species were vehicle controls and twenty more were untreated controls. In addition to carcinogenicity information, the bioassay also yielded chronic, non-cancer toxicity information, which we summarize here. Male and female rats were terminated early, at 49 and 61 weeks, respectively, due to the high mortality. Male mice and high dose female mice were terminated by week 78 due to excessive mortality. Low dose female mice were treated for 53 weeks followed by observation for 37 weeks.

For both sexes and doses in rats NCI (1978) observed a compound-related body weight depression, increased mortality, degeneration of liver and adrenal gland, forestomach hyperkeratosis, and forestomach acanthosis. Reddened ears and a hunched posture were

noted in all treated groups by week five, and firm distended abdomens and abdominal urine stains by week 38. In both sexes of mice, dose-related mean body weight depressions were observed from week ten onward, and the treated animals had soft feces, alopecia, and body sores. A thin, hunched appearance was noted in the high dose mice as the study progressed. Increased mortality was observed at both doses in both sexes.

Wong *et al.* (1982) chronically treated rats with ethylene dibromide by inhalation to determine the toxicity and carcinogenic potential at the OSHA standard of 20 ppm in air. Groups of 48 male and female Sprague-Dawley rats were exposed to 20 ppm ethylene dibromide with and without 0.5 percent disulfiram (tetraethylthiuram disulfide) in the diet. The disulfiram portion of the study was to ascertain the potential toxicity-promoting effect of this alcoholism treatment drug on chronic ethylene dibromide exposure. Inhalation exposure was 7 hr/day, 5 days/week for 18 months. Ethylene dibromide treated rats showed significant mortality, with 90 percent of males and 77 percent of females dying by the 19th month. The authors reported that the spleens of ethylene dibromide treated male rats exhibited atrophy and hemosiderosis (Wong *et al.* 1982). The neoplasia results from this study are discussed later in this document.

NTP (1982) performed a carcinogenesis bioassay for ethylene dibromide via inhalation at two doses with 50 rats and mice of each sex/group. The treatment concentrations were 10 or 40 ppm for six hours/day 5 days/week for up to 103 weeks (low dose) or 91 weeks (high dose). The authors reported that among the compound-related non-neoplastic lesions observed in the rat were hepatic necrosis and toxic nephropathy (both sexes), testicular degeneration and atrophy, and retinal degeneration in female rats. In treated mice, the most prominent lesion reported was epithelial hyperplasia of the respiratory system (NTP, 1982). For this chronic study, the lowest LOAEL (7 mg/kg-d at 0.124 kg body weight and 0.14 m³/d breathing rate) was that of the female rat for retinal atrophy and adrenal cortex degeneration at the low dose. The LOAEL was corrected for 58 percent fractional systemic uptake from inhalation exposure (Stott and McKenna, 1984).

Carcinogenicity

Carcinogenic activity of ethylene dibromide has been demonstrated in laboratory experiments on mice and rats via the dermal, oral, inhalation, and intraperitoneal routes of administration. Tumors occurred at both local and distant sites from the original location of chemical contact in both test species (NCI, 1978; CDHS, 1985, 1988; Alexeeff *et al.*, 1990).

Oral

NCI (1978) performed a bioassay for possible carcinogenicity of orally administered ethylene dibromide in Osborne-Mendel rats and B6C3F₁ mice. Rats received time-weighted average doses of 38 and 41 mg/kg-day for males, and 37 and 39 mg/kg-day for females. Both sexes of mice received 62 and 107 mg/kg-day day of ethylene dibromide. During the course of the study, the dosing regimen changed for both rats and mice, which is why dosages are given as time-weighted averages.

Initially groups of male and female rats in the NCI (1978) study were dosed at levels of 40 and 80 mg/kg-day per group of 50 rodents each. In week 17, intubation of the high

dose group was discontinued due to too many deaths at this dosage level, as 18 high dose males and 20 high dose females were dead by about week 15. The remaining high dose rodents were given no additional ethylene dibromide treatments for 13 weeks, and thereafter were intubated at the low dosage level. All surviving treated male rats were terminated in week 49 and females at week 61, because there were too few left surviving to justify continuation. The significant incidence of neoplasia, i.e., squamous cell carcinoma of the stomach, hepatocellular carcinoma, hemangiosarcoma, and follicular-cell adenoma or carcinoma of the thyroid, is provided in Table 4. The authors observed a dramatically increased incidence of squamous cell carcinomas originating in the forestomach of male and female rats. This neoplasm occurred in 90 percent of the low dose males and 80 percent of the low dose females, 66 percent of the high dose males and 58 percent of the high dose females. None were observed in control animals. The authors reported that metastatic neoplasms, both by peritoneal seeding and through blood vessels, were widespread and usually multiple, involving nearly every organ of the abdominal cavity, and the lung in some animals (NCI, 1978). The higher tumor incidence in the lower dose group appeared due to the excessive mortality in the high dose group (Alexeeff *et al.*, 1990); see Table 4.

Table 4. Incidence of Tumors in Rats as Reported in the NCI (1978) Study

Dosage group	Sex	Tumor Incidence	Percent tumor-bearing animals
Stomach squamous-cell carcinoma			
Vehicle control	Male	0/20	0
	Female	0/20	0
Low dose	Male	45/50	90
	Female	40/50	80
High dose	Male	33/50	66
	Female	29/50	58
Hepatocellular carcinoma or neoplastic nodule			
Vehicle control	Male	0/20	0
	Female	0/20	0
Low dose	Male	3/50	6
	Female	1/41	2
High dose	Male	2/50	4
	Female	8/48	13
Hemangiosarcomas of the circulatory system			
Vehicle control	Male	0/20	0
	Female	0/20	0
Low dose	Male	11/50	22
	Female	1/49	2
High dose	Male	4/50	8
	Female	3/48	6

Table adapted from Alexeeff *et al.*, 1990

In the same study, groups of 50 mice of both sexes were initially dosed at 60 and 120 mg/kg-day. In week 11, low and high dose groups were increased to 100 and 200 mg/kg-day, respectively. In week 13, doses for all mice were decreased to the original levels. The study was further modified in week 40, when the dosage administered to the high dose groups was decreased to 60 mg/kg-day, the same as the low dose group. All ethylene dibromide administration ceased after 54 weeks because of high mortality, and surviving mice were killed by week 78 except low dose and untreated control females, which were permitted to live an additional 13 weeks. As with rats, the authors observed a dramatic increase in incidence of squamous cell carcinoma of the forestomach in treated male and female mice, with none observed in control rodents. The malignant gastric neoplasm was observed in 59 percent of high dose males, 56 percent of high dose females, 90 percent of low dose males and 94 percent of low females (NCI, 1978). Excessive mortality and variations in the dosing schedule may have confounded the results, especially the response versus the two dose levels (see Table 5). The combined rat and mouse forestomach carcinoma data were used to model the risk of ethylene dibromide exposure in drinking water.

Table 5. Incidence of Tumors in Mice as Reported in the NCI (1978) Study

Dosage Group	Sex	Time-adjusted ^a incidence	Percent
Squamous-cell carcinoma of the forestomach			
Vehicle control	Male	0/19	0
	Female	0/19	0
Low dose	Male	29/31	94
	Female	9/10	90
High dose	Male	24/34	71
	Female	11/14	79
Alveolar/bronchiolar tumors			
Vehicle control	Male	0/19	0
	Female	0/19	0
Low dose	Male	1/30	3
	Female	2/10	20
High dose	Male	8/34	24
	Female	4/14	29

^aNumber only considers animals after observation of first tumor.

Table adapted from Alexeeff *et al.*, 1990

Inhalation

NTP (1982) performed a carcinogenesis bioassay of ethylene dibromide by exposing groups of 50 F334 rats and B6C3F₁ mice of each sex by inhalation to concentrations of 10 or 40 ppm of ethylene dibromide for 78-103 weeks. Survival of the high dose rats of either sex and of the low and high dose mice was significantly shorter than that of control rodents. Converted to a mass/mass basis, the doses for rats are 4.6, 18.4, 4.9, and 19.6 mg/kg-day for low dose male, high dose male, low dose female and high dose female, respectively. For mice, the estimated doses are 8.5, 34.1, 9.0 and 35.9 for low dose male, high dose male, low dose female, and high dose female, respectively (Reed *et al.*, 1987).

In the above study, the authors observed significant increases in carcinomas and adenocarcinomas of high-dose rats of both sexes. Adenocarcinomas and adenomas of the nasal cavity also increased significantly in low dose rats of both sexes. The combined incidence of alveolar/bronchiolar adenomas and carcinomas was statistically significant for high dose female rats. Hemangiosarcomas of the circulatory system (mainly spleen) and mesotheliomas of the tunica vaginalis occurred in high dose males. Fibroadenomas of the mammary glands were significantly increased in females (Table 6) (NTP, 1982).

Table 6. Tumors in Rats from NTP Inhalation Bioassay (1982)

Site	Male			Female		
	Control	10 ppm	40 ppm	Control	10 ppm	40 ppm
Nasal cavity	0/50	39/50	41/50	0/50	34/50	43/50
Lung tumors	1/50	2/50	1/50	0/50	0/50	5/50
Testis	35/50	45/50	10/50	-- ^a	--	--
Tunica vaginalis	1/50	8/50	25/50	--	--	--
Mammary gland fibroadenoma	--	--	--	4/50	29/50	24/50
Spleen hemangiosarcoma	0/50	1/50	15/50	0/50	0/49	5/48

^a Information not available.

Table adapted from Alexeeff *et al.*, 1990

For the mouse portion of the above study, the authors observed alveolar and bronchiolar adenoma and carcinoma in high dose males and females. Additionally, for female mice, hemangiosarcomas were observed at both the low and high dose. Female mice also showed a significantly greater incidence of subcutaneous fibrosarcoma and nasal cavity carcinomas at the high dose as well as increased mammary gland adenocarcinomas at the low dose; see Table 7.

Table 7. Tumors in Mice from NTP Inhalation Bioassay (1982)

Site	Male			Female		
	Control	10 ppm	40 ppm	Control	10 ppm	40 ppm
Nasal cavity	0/45	0/50	0/50	0/50	0/50	11/50
Lung tumors	0/50	3/48	25/46	4/49	11/49	42/50
Hemangiomas (numerous locations)	0/50	0/50	2/50	0/50	1/50	23/50
Hemangiosarcomas (numerous locations)	0/50	0/50	2/50	0/50	11/50	23/50
Subcutaneous tissue	0/50	0/50	2/50	0/50	5/50	15/50

Table adapted from Alexeeff *et al.*, 1990

Wong *et al.* (1982) performed a chronic inhalation study to examine the carcinogenicity and toxicity of ethylene dibromide in Sprague-Dawley rats. Coincidentally, the study also examined the effect of orally administered disulfiram both in combination with ethylene dibromide administration, and the effect of each chemical administered individually. This study was described earlier in this document.

Four groups of about 48 rats each received no treatment, 0.05 percent dietary disulfiram, 20 ppm ethylene dibromide, or both chemicals combined for 18 months. Rats in the two groups receiving ethylene dibromide had high mortality and an increased tumor incidence in mammary glands (females), spleen (both sexes), adrenal (both sexes), and subcutaneous tissue (males). As a note of interest, disulfiram administered in combination with ethylene dibromide also produced a significant excess of tumors in liver, mesentery, kidney, testes, thyroid, and lungs of one or both sexes (Wong *et al.*, 1982).

Toxicological Effects in Humans

Acute Toxicity

Letz *et al.* (1984) described two cases of human fatalities following acute exposure to ethylene dibromide. Both exposures were dermal and inhalation, and occurred in the same industrial setting. The first worker collapsed while working inside a large storage tank known to contain residues of ethylene dibromide. The second worker collapsed inside the same tank while trying to rescue the first worker. The first worker was inside the tank for 45 minutes before rescue. He died 12 hours later with metabolic acidosis, CNS depression, and clinical evidence of liver damage. The second worker died 64 hours later with intractable metabolic acidosis and hepatic and renal failure. The mean ethylene dibromide concentration in the tank was subsequently measured, over

5 samples, as 215 µg/L, which was considered not likely to be a sufficient concentration to cause unconsciousness. The liquid pooled at the bottom of the tank ranged between 0.1 and 0.3 percent ethylene dibromide, and intoxication most likely occurred dermally, as the victims lay unconscious (Letz *et al.*, 1984).

Letz *et al.* (1984) also described two cases, previously reported in the literature, of acute ethylene dibromide fatalities. In the early 1900s in Germany, a woman was mistakenly given ethylene dibromide as an anesthetic, instead of ethyl bromide. The patient died 44 hours later from uterine hemorrhage and parenchymatous degeneration of the heart, liver and kidneys. Also described was the suicide of a 43 year-old woman who intentionally ate ethylene dibromide capsules designed for soil implantation, at a dose of 140 mg/kg. The woman died 54 hours following ingestion. Autopsy revealed extensive hepatic and renal damage. The liver showed massive centrilobular necrosis without inflammation and the proximal renal tubules were congested and necrotic (Olmstead, 1960; Letz *et al.*, 1984).

Saraswat *et al.* (1986) reviewed six cases of human intoxication by ethylene dibromide ingestion. All cases were suicide attempts and 2 attempts were successful. The four non-fatal cases complained of nausea, burning throat and were confused or drowsy when admitted to the hospital. Stomach washes had a chloroform-like odor. Three of the four showed blisters on the lips and oral mucosa. Post-mortem examination of the fatal cases revealed oral cavity and throat ulceration; stomach mucosa erosion; liver, kidney, spleen, brain and lung congestion; centrolobular necrosis of the liver; cloudy swelling of the distal tubules; and in one of the two cases, meningeal congestion with interstitial cortical edema of the brain.

Subchronic Toxicity

Studies of subchronic toxicity of ethylene dibromide to humans could not be located.

Genetic Toxicity

Steenland *et al.* (1985) examined the peripheral blood lymphocytes of 14 workers, both before and after pesticide application exposure to daily average ethylene dibromide concentrations of 60 ppb for an average of 14 days. The authors observed no statistically significant difference between the frequencies of either sister chromatid exchange or chromosomal aberrations before and after spraying.

Perocco and Prodi (1981) observed DNA damage by ethylene dibromide in human lymphocytes cultured *in vitro*. The authors measured the rate of reparative synthesis of human lymphocytes cultured *in vitro* in the presence of 10 mM hydroxyurea, S-9, and 2.5, 5, and 10 µL/mL ethylene dibromide as tritiated thymidine uptake. The authors reported that ethylene dibromide (in addition to other compounds similarly tested) elicits DNA reparative synthesis in human lymphocytes as a result of the chemicals' DNA damaging and mutagenic power.

Crespi *et al.* (1985) tested ethylene dibromide (along with ethylene dichloride) for the ability to induce gene mutations in two human lymphoblastoid cell lines (TK6 and

AHH-1). The authors hypothesized that even though many active mutagen metabolites may be inactivated via glutathione S-transferase, which catalyzes nucleophilic attack by reduced glutathione on certain electrophilic compounds, this enzyme can enhance the mutagenic effect of vicinal dihalogen compounds such as ethylene dibromide. The authors concluded that ethylene dibromide is a potent mutagen for both cultured human cell lines.

DeLeve (1997) studied whether variations in endogenous glutathione in human cells could modify the genotoxicity of ethylene dibromide. The author observed significantly higher genotoxicity, as measured by sister chromatid assays in normal as opposed to glutathione synthetase-deficient human skin fibroblasts. The author concluded that low endogenous levels of glutathione are protective against ethylene dibromide-induced genotoxicity.

Developmental and Reproductive Toxicity

Wong *et al.* (1979) attempted to examine retrospectively any antifertility influence of ethylene dibromide on the reproductive performance of male humans occupationally exposed to this chemical. The authors evaluated reproductive fitness based on reproductive histories of the men's wives, subsequent to their occupational exposure to ethylene dibromide. The number of live, apparently legitimate, natural children born to the wives was compared to the expected value published by the National Center for Health Statistics. Corrections to the data were made to adjust for the confounding effects of maternal age, parity, race, and calendar year. Of the four ethylene dibromide manufacturing plants studied, the authors observed that workers at one plant showed a significant decrease in fertility. Wong *et al.* (1985) performed an epidemiological surveillance program to evaluate the reproductive hazards of ethylene dibromide and DBCP. Using the data from the above study, the authors pooled the results from the four chemical plants. The observed standardized birth ratio was 0.82, with a 95 percent confidence interval of 0.61-1.03. Since the p-value was .06 for a one-sided test, the observed effect of reduced fertility did not meet statistical significance. The authors referred to the reduced fertility observation as of "borderline statistical significance."

Ratcliffe *et al.* (1987) examined 46 male human papaya workers to determine whether long-term ethylene dibromide exposure affected semen condition. The authors observed significant sperm count decrease, decreases in sperm viability and motility, and increases in proportion of morphological abnormalities when compared to a control group of unexposed workers. The authors concluded that ethylene dibromide exposure at levels near the NIOSH recommended limit of 45 ppb (TWA over 8 hours) may increase the risk of reproductive impairment.

Kulkarni *et al.* (1992) examined the metabolism of ethylene dibromide in human fetal liver tissue to determine if the relative abundance of glutathione S-transferase in developing hepatic tissue would modify the toxicity to the fetus. Ethylene dibromide metabolism by glutathione S-transferase increased directly with enzyme concentration, with incubation time, and with ethylene dibromide concentration. The significant bioactivation via the glutathione S-transferase pathway, and the resulting generation of toxic metabolites occur in the fetal liver. This was observed especially in light of the fact

that detoxification of ethylene dibromide via the cytochrome P450-dependant oxidative pathway is relatively limited in fetal livers. Thus, the human fetus may be at greater risk from ethylene dibromide toxicity than the adult (Kulkarni *et al.*, 1992).

Immunotoxicity

Studies relating to immunotoxicity of ethylene dibromide in humans could not be located.

Neurotoxicity

Data on human neurological effects from exposure to ethylene dibromide are sparse. As mentioned earlier, Saraswat *et al.* (1986) described a suicidal dose of ethylene dibromide leading to non-specific brain lesions such as meningeal congestion and interstitial cortical edema. Letz (1984) described fatal occupational exposure in two humans, both of whom were exposed in the same storage tank. The first worker was initially described as intermittently comatose or incoherent and combative, but lethargic. Among the other postmortem indications observed was severe acute passive congestion of the brain. Less information was obtained about the second worker, save for deliriousness, combativeness, and confusion.

Chronic Toxicity

Some epidemiological studies exist describing the health effects of chronic exposure to ethylene dibromide in the workplace. The previously described studies by Wong *et al.* (1979, 1985) and Ratcliffe *et al.* (1987) reported the antifertility influence of chronic exposure to ethylene dibromide on human male reproductive fitness.

Ott *et al.* (1980) examined the mortality data of 161 employees exposed to ethylene dibromide in two production units for up to 35 years. Although deaths due to cancer were the central focus of the study, the authors also considered diseases of the cardiovascular system, pulmonary disease and influenza, including related pneumonia. Of these non-cancer deaths, none of the observed versus expected values appeared to be significantly different, although the low number of human deaths evaluated for both cancer and non-cancer related causes (36) yielded a relatively low statistical power for the study (Ott *et al.*, 1980).

Sweeney and colleagues (1986) investigated mortality from cancer and other causes of death among workers employed at an east Texas chemical plant. The authors examined the cause-specific mortality of 2,510 males working with ethylene dibromide or other specified chemicals and compared the values to the United States population. There were no significant increases in mortalities from malignancies or non-cancer causes. For the non-cancer-related causes of death, cardiovascular diseases and nonmalignant respiratory diseases, the observed number of deaths was slightly lower than the expected number (Sweeney *et al.*, 1986).

Carcinogenicity

Ott *et al.* (1980), in a retrospective study mentioned above, examined the mortality of men occupationally exposed to ethylene dibromide in two chemical plants which, respectively, operated from 1924-1969 and from the mid 1920s-1976. In the first facility, two deaths occurred from malignant neoplasia against 3.6 expected (based upon white male mortality). In the second plant, five deaths from malignant neoplasia were observed versus 2.2 expected. A possible confounder to the exposure data for the second plant was exposure to “various bromide products” including vinyl bromide, trimethylene chlorobromide, ethyl bromoacetate, and several others. The authors concluded that they observed far fewer malignant neoplasms than might have been expected from a direct extrapolation of animal carcinogen bioassays. The study neither established nor ruled-out ethylene dibromide as a human carcinogen.

Apfeldorf and Infante (1981) reviewed epidemiological study results for six compounds, including ethylene dibromide. Reviewing the Ott *et al.* (1980) study, the authors concluded that it lacked sufficient statistical power due to small sample size. No other epidemiological studies for ethylene dibromide were available for study at the time (Apfeldorf and Infante, 1981).

Sweeney *et al.* (1986), in a study mentioned previously, examined cause-specific mortality from a work force of 2,510 males who worked at an east Texas chemical plant for at least part of the period from 1952-1957. The authors reported no significant increase in mortality from malignancies. The authors cited the low number of total deaths, low power for detecting excess risk for rare causes of death, and incomplete exposure data as potential deficiencies of the study.

Recently, Hansen (2000) described an elevated risk of male breast cancer from occupational exposure to gasoline and vehicular combustion products, which might be relevant to ethylene dibromide due to its use in leaded gasoline. The author performed a nationwide register-based case control study on male breast cancer mortality in Denmark. The author examined histories from 230 cases and 12,880 control subjects to develop odds ratios for breast cancer among Danish men with greater than three months potential exposure to gasoline and combustion products. A significantly increased risk ($P < 0.01$) odds ratio of 2.2 was found for men who had been exposed for greater than three months. Men younger than 40 years had a significantly greater odds ratio of risk of 3.7, which increased to 5.4 when at least ten years lag time was taken into account. The author concluded that a significantly increased risk for breast cancer was found among male workers who had potentially been exposed to automotive gasoline and its combustion products (Hansen, 2000).

Since gasoline contained other carcinogenic compounds such as benzene, 1,3-butadiene, 1,2-dichloroethane, and since exposure included carcinogenic combustion products of gasoline such as PAHs, the study lacks the ability to differentiate the individual risks. Accordingly, the study cannot establish an epidemiological link between ethylene dibromide exposure and male breast cancer.

DOSE-RESPONSE ASSESSMENT

Noncancer Effects

We derive an animal non-cancer NOAEL from Nitsche *et al.* (1981). As described earlier, the authors performed a 13-week inhalation study of ethylene dibromide in rats. Four groups of F344 rats, at 40 males and 20 females per group, were exposed to 0, 3, 10, or 40 ppm ethylene dibromide for 6 hours/day, 5 days/week, for 13 weeks. Male rats inhaling 40 ppm had significant body weight loss throughout the exposure period. Female rats exposed to 40 ppm for 13 weeks showed a significant decrease in specific gravity of the urine, and had elevated relative liver weights. Male rats exposed to 10 ppm and 40 ppm exhibited increases in relative liver and kidney weights and either showed single or multiple foci of hyperplasia of the nasal respiratory epithelium after 13 weeks. After 13 weeks at the high concentration, the authors observed diffuse or focal non-keratinizing squamous hyperplasia and metaplasia of the respiratory epithelium. The LOAEL for this study, based on increased relative liver and kidney weights, is that of the male rat of 8.3 mg/kg-d (at 0.18 kg and 0.19 m³/d breathing rate), with a corresponding NOAEL of 2.49 mg/kg-d. The LOAEL and NOAEL were corrected for 58 percent fractional systemic uptake from inhalation exposure (Stott and McKenna, 1984).

The study of NTP (1982) is considered to provide important supportive data. As reported earlier in this document, the authors performed a carcinogenesis bioassay for ethylene dibromide in which they also observed non-cancer chronic toxicity. EDB was administered via inhalation at two doses with 50 rodents of each sex in both rats and mice. The treatment concentrations were 10 or 40 ppm for six hours/day 5 days/week for up to 103 weeks (low dose) or 91 weeks (high dose). The authors reported that among the compound-related non-neoplastic lesions observed in the rat were hepatic necrosis and toxic nephropathy (both sexes), testicular degeneration and atrophy, and retinal and adrenal cortex degeneration in female rats. In treated mice, the most prominent lesion reported was epithelial hyperplasia of the respiratory system (NTP, 1982). For this chronic study, the lowest LOAEL (7 mg/kg-d at 0.124 kg body weight and 0.14 m³/d breathing rate, corrected for 58 percent fractional systemic uptake from inhalation exposure) was that of the female rat for retinal atrophy and adrenal cortex degeneration at the low dose.

Carcinogenic Effects

Estimation of Cancer Potency

The cancer potency factor (CPF) is a slope derived from a mathematical function used to extrapolate the cancer incidence from a bioassay in animals using high doses to an expected value at the low doses that are likely to be found in chronic human exposure. Mathematical models, such as the linearized multi-stage (LMS) model, can be used in quantitative carcinogenic risk assessments in which the risk is assumed to be proportional

to the exposure to the chemical agent at very low doses. Cancer potency is estimated using the q_1^* , which is the upper 95 percent confidence limit on the cancer potency slope calculated by the LMS model.

Basis for Cancer Potency

Oral potency.

Two sets of animal cancer bioassays (NCI, 1978; NTP, 1982) were considered by Reed *et al.* (1987) and OEHHA (1988) for quantitative risk assessment. Reed *et al.* (1987) fit the Doll-Armitage multistage (Crump and Howe, 1984) analysis to correct for the erratic dosing pattern used in the NCI experiment. For this bioassay, the modeled theoretical human potencies ranged from 0.7 to 97 (mg/kg-day)⁻¹ depending upon the data set used and the assumptions used regarding tumor lethality and animal-to-human dose equivalence (please see Table 8). In estimating risks from consumption of ethylene dibromide contaminated drinking water, OEHHA chose to use the geometric mean of potencies (q_1^*) derived from squamous cell carcinomas of the stomach in each species and strain tested by gavage for those tumors assumed to cause death (OEHHA, 1988), one of the options in the Doll-Armitage model calculations. These data correspond to the values in bold in the q_1^* human column in Table 8. Potency calculations based on the assumption that deaths are incidental to the tumors (the “incidental” column in Table 8) were more variable, with poorer fits to the cancer models. For the purposes of Proposition 65, OEHHA (then part of the California Department of Health Services) recommended that this geometric mean oral potency value of 3.6 (mg/kg-day)⁻¹ be used as the basis of risk assessment due to ingestion (OEHHA, 1988).

Inhalation potency.

OEHHA additionally examined the NTP (1982) inhalation study (described earlier) and fitted the Weibull-multistage model to describe tumor incidence at two sites: tumors at the site of first contact (nasal malignancies in male rats), and tumors at a remote site (hemangiosarcomas in female mice). For comparison to the oral value selected above, the geometric mean of q_1^* values determined from potencies in each species and strain tested by inhalation for those tumors assumed to cause death is 0.34 (mg/kg-day)⁻¹, or about one-tenth that calculated for the oral route (OEHHA, 1988).

Table 8. EDB Cancer Potency Estimates, Upper 95 Percent Confidence Limits, in (mg/kg-day)⁻¹

Sex/Species	Tumors	Crump Multistage		Time-to-tumor ^a			
		q ₁ *A	q ₁ *H	Lethal		Incidental	
		q ₁ *A	q ₁ *H	q ₁ *A	q ₁ *H	q ₁ *A	q ₁ *H
NCI (1978) Gavage Study							
Male rat	Stomach squamous cell carcinoma	1.09 ^b	5.66 ^b	2.48	12.87^C	.033	1.71
Female rat	Stomach squamous cell carcinoma	0.04 ^d	2.41 ^d	1.74	10.49^C	16.10	97.08
Male mouse	Stomach squamous cell carcinoma	.060 ^{b,e}	7.67 ^{b,e}	0.15	1.93^C	0.69	8.87
Female mouse	Stomach squamous cell carcinoma	.028 ^{b,e}	3.75 ^{b,e}	0.05	0.68^C	0.80	10.86
NTP (1982) Inhalation Study							
Male rat	Nasal combined	0.44 ^b	2.63 ^b	0.07	0.39	0.62	3.75
Male rat	Nasal adenocarcinoma	0.16 ^b	0.95 ^b	0.03	0.20	0.16	0.98
Female rat	Nasal combined	0.31 ^b	1.98 ^b	---	---	0.45	2.91
Female rat	Nasal adenocarcinoma	0.07	0.47	0.07	0.42	0.16	1.00
Male mouse	Lung combined	0.02	0.21	0.04	0.50	0.14	1.73
Female mouse	Lung combined	0.03	0.44	0.02	0.27	0.03	0.38

^aFor the NCI bioassay data, the Armitage-Doll Model was fit to account for variable dose rate (Reed *et al.*, 1987), based on tumors assumed to cause death (Lethal columns) and those for which deaths were assumed not directly related to the tumors (Incidental columns). "A" Denotes Animal, "H" Denotes Human.

^bHigh dose group removed due to lack of fit.

^cA geometric mean of 3.6 (mg/kg-d)⁻¹ was derived from these values and used as the basis of the risk assessment.

^dHigh dose group removed in fitting Crump Multistage due to the similarity of the lifetime average of the high and low dose levels.

^eThe incidence data used included only animals surviving up to or longer than the time of first observed tumor.

Table modified from CDHS, 1988, and Reed *et al.*, 1987.

CALCULATION OF THE PHG

Calculations of concentrations of chemical contaminants in drinking water associated with negligible risks for carcinogens or noncancer toxicants must take into account the toxicity of the chemical itself, as well as the potential exposure of individuals using the water. Tap water is used directly as drinking water, and for preparing foods and beverages. It is also used for bathing or showering, and in washing, flushing toilets, and other household uses resulting in potential dermal and inhalation exposures.

Noncancer Effects

The principal study selected for the derivation of the noncancer value for ethylene dibromide was the subchronic inhalation study in rats of Nitschke *et al.* (1981). In this study, 2.49 mg/kg-day was the NOAEL for the endpoints of increased relative liver and kidney weights as well as reversible epithelial hyperplasia and squamous metaplasia of the nasal turbinates in male rats. The study was slightly over 90 days in length. We selected this subchronic study over the chronic rat study of NTP (1982) as the former offers a NOAEL, thus is more scientifically relevant than the LOAEL of NTP (1982).

The calculation of the public health-protective concentration (C, in mg/L) for ethylene dibromide follows a general formula for noncancer endpoints:

$$C = \frac{\text{NOAEL} \times \text{RSC} \times \text{BW}}{\text{UF} \times \text{L/day}} = \text{mg/L}$$

where,

NOAEL = no-observed-adverse-effect-level, 2.5 mg/kg-day for absence of increased liver and kidney weights in a subchronic rat inhalation study;

RSC = relative source contribution. The default is 0.2; however, since EDB has been removed from commerce for nearly 20 years, exposure via other than drinking water sources is highly unlikely. Accordingly, OEHHA employs a more realistic RSC value of 0.6.

BW = body weight for an adult male (70 kg);

UF = uncertainty factor (typical defaults are 10 for inter-species variation, 10 for human variability, and 10 for using a subchronic study to derive a chronic value);

L/day = volume of drinking water consumed by an adult (the default is 2 L/day).

Therefore,

$$C = \frac{2.5 \text{ mg/kg-day} \times 0.6 \times 70 \text{ kg}}{1000 \times 2 \text{ L/day}}$$

$$= 0.00525 \text{ mg/L} = 5.25 \text{ } \mu\text{g/L} = 50 \text{ ppb (rounded)}$$

The health protective concentration for ethylene dibromide in water, based on noncancer effects, is therefore 50 $\mu\text{g/L}$ (50 ppb).

Carcinogenic Effects

Cancer potency for ethylene dibromide is estimated from the q_1^* (or cancer potency factor, CPF) for the oral route of $3.6 \text{ (mg/kg-day)}^{-1}$, calculated in this case by the Doll-Armitage multi-stage model assuming that the tumors were the cause of the early deaths in the NCI (1978) study. The indicated potency value is the geometric mean of human-equivalent q_1^* values calculated from on the mouse and rat stomach squamous cell carcinoma data, as shown earlier in Table 8. This value was adopted by the (then) California Department of Health Services (OEHHA, 1988) following a comprehensive evaluation for Proposition 65 Risk Specific Intake Levels.

For cancer endpoints, the following equation is commonly used to calculate cancer risk levels when a non-threshold mechanism is assumed:

$$C = \frac{R \times BW}{q_1^* \times L/\text{day}} = \text{mg/L}$$

where,

- R = the *de minimis* level for lifetime excess individual cancer risk (a default of 10^{-6});
- BW = adult body weight (default 70 kg);
- q_1^* = the upper 95 percent confidence limit of the cancer potency slope;
- L/day = the adult daily volume of drinking water consumed (the default is 2 L/d).

The default values are used in this case, with the cancer potency indicated above of $3.6 \text{ (mg/kg-day)}^{-1}$; therefore:

$$C = \frac{1 \times 10^{-6} \times 70}{3.6 \text{ (mg/kg-day)}^{-1} \times 2 \text{ L/day}} = 0.0000097 \text{ mg/L}$$

$$C = 0.01 \text{ } \mu\text{g/L (rounded)} = 0.01 \text{ ppb} = 10 \text{ ppt}$$

The health protective concentration of ethylene dibromide is based on a rodent bioassay conducted by NCI (1978), which was previously used for the Proposition 65 Risk Specific Intake Level calculation (OEHHA, 1988). We again conclude that this is the most relevant calculation method for the available data. To derive a PHG for ethylene

dibromide in drinking water, the calculated value of 0.0097 ppb is rounded to one significant figure, or 0.01 ppb.

RISK CHARACTERIZATION

OEHHA recommends that the PHG value be based on cancer endpoints (0.01 ppb) as opposed to the noncancer endpoint (50 ppb), because the former is: a) more health-protective, and b) is based on more severe and life-threatening impacts.

There are some areas of uncertainty regarding the PHG for ethylene dibromide. Animal survival was poor in the NCI (1978) gavage study. In addition, because dosing schedule varied, the typical linearized multistage model was not sufficient to accurately reflect the relationship between lifetime exposure and tumor incidence. To address this uncertainty, a Doll-Armitage model was included as a time-dependent analysis, sometimes called a time-to-tumor analysis. For this model, we assume that under the *lethal* analysis, the tumor in question is the cause of death; and under the *incidental* analysis, that the animals died of a cause unrelated to the particular tumor of interest (OEHHA, 1988). Survival was also poor in the NTP (1982) inhalation bioassay, so use of this data set would not likely improve reliability of the cancer potency assessment.

Some controversy may exist with the use of tumors at the site of contact, such as the forestomach, for risk assessment. This is particularly relevant when the administration is by gavage, since this results in a relatively high concentration being delivered to the rodent forestomach, compared to administration of a chemical in the food or drinking water. However, the low solubility and high volatility of ethylene dibromide make it problematic to deliver high doses in drinking water; such chemicals have commonly been administered by gavage in NCI/NTP studies. Ethylene dibromide is a severe skin irritant (NCI, 1978; U.S. EPA, 1987), so acute toxic effects on the stomach lining would be expected.

However, there is much evidence indicating that ethylene dibromide is a systemic carcinogen; and as such, tumors are generally modeled via linear extrapolation. As mentioned previously, the genotoxicity of ethylene dibromide has been clearly demonstrated in bacterial assays, eucaryotes, plants, insects, and cultured mammalian cells. Ethylene dibromide administration to animals has produced a variety of tumors including lung tumors in mice and rats, nasal and respiratory tumors in mice and rats, tumors of the spleen, adrenals, testes, and mammary glands in rats, and hemangiosarcomas at numerous locations in the mouse. Ethylene dibromide is a multi-organ carcinogen via both the inhalation and oral routes of administration.

An additional element of uncertainty arises in the NCI (1978) study from the use of technical grade compound, possibly containing higher levels of bioactive impurities than more purified forms of the chemicals. Purity was measured using gas-liquid chromatographic analyses on three separate occasions with the results of 96.3, 99.1 and 99.6 percent purity reported (NCI, 1978).

Epidemiologic studies have not been adequate to establish or rule out carcinogenicity of ethylene dibromide in humans. Thus, the PHG value developed here relies on animal data with no human epidemiological corroboration. However, we believe that this value

is adequate to protect humans, including sensitive subpopulations such as infants and the elderly, from adverse effects resulting from exposure to ethylene dibromide in drinking water.

OTHER REGULATORY STANDARDS

The current U.S. EPA maximum contaminant level for ethylene dibromide is 0.05 µg/L (ppb) based on carcinogenic hazard, limited by analytical feasibility (U.S. EPA, 2002). The U.S. EPA has listed ethylene dibromide as a group B2 probable human carcinogen (U.S. EPA, 2000). The International Agency for Research on Cancer has listed ethylene dibromide as a group 2A (probable human) carcinogen (IARC, 1999). In 1994, the California Department of Health Services established a maximum contaminant level of 0.05 µg/L. In California, ethylene dibromide is listed under the Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65) as a chemical known to the state to cause cancer. The following table includes selected national and state regulations and guidelines for comparison to the PHG.

Table 9. Selected Guidelines and Regulations for Ethylene Dibromide

Agency	Standard or Criterion	Level	Comment
California Department of Health Services	MCL (maximum contaminant level)	0.05 µg/L (ppb) in water	1994
National Institute of Occupational Safety and Health	REL (recommended exposure level)	TWA (time-weighted average) 0.045 ppm in air	lowest feasible concentration for occupational exposure to carcinogens
Occupational Safety and Health Administration	PEL (permissible exposure limit)	TWA 20 ppm in air	occupational, inhalation level
U.S. EPA	MCL (maximum contaminant level)	0.05 µg/L (ppb) in water	national drinking water standard
U.S. EPA	MCLG (maximum contaminant level goal)	0 mg/L in water	set at zero for carcinogens
U.S. EPA	10-day Health Advisory	8 µg/L (ppb) in water	10 kg child
OEHHA	Proposition 65, no significant risk level	0.2 µg/day	Prop. 65 levels are set at a 1x10 ⁻⁵ risk level

Table adapted from: ATSDR, 1995; NIOSH, 1994; IARC, 1999; U.S. EPA, 2000.

REFERENCES

- Albrecht WN (1987). Central nervous system toxicity of some common environmental residues in the mouse. *J Toxicol Environ Health* 21(4):405-21.
- Alexeeff G, Kilgore W, Li M (1990). Ethylene dibromide: toxicology and risk assessment. *Rev Environ Contam Toxicol* 112:49-122.
- Amir D (1973). The sites of the spermicidal action of ethylene dibromide in bulls. *J Reprod Fert* 35:519-25.
- Amir D, Esnault C, Nicolle J, Courot M (1977). DNA and protein changes in the spermatozoa of bulls treated orally with ethylene dibromide. *J Repro Fert* 51: 453-56.
- Amir D, Volcani R (1965). Effect of dietary ethylene dibromide on bull semen. *Nature* 206:99-100.
- Apfeldorf R, Infante PF (1981). Review of epidemiologic study results of vinyl chloride-related compounds. *Environ Health Perspect* 41:221-6.
- ATSDR (1991). Toxicological profile for 1,2-dibromoethane. Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services. TP-91/13.
- ATSDR (1995). ToxFAQs, fact sheet for frequently asked questions about 1,2-dibromoethane. Agency for Toxic Substances and Disease Registry. Atlanta, Georgia. Available online at www.atsdr.cdc.gov/tfacts37.html.
- Ballering LA, Nivard MJ, Vogel EW (1994). Mutation spectra of 1,2-dibromoethane, 1,2-dichloroethane and 1-bromo-2-chloroethane in excision repair proficient and repair deficient strains of *Drosophila melanogaster*. *Carcinogenesis* 15(5):869-75.
- Bondi A, Olomucki E, Calderon M (1955). Problems connected with ethylene dibromide fumigation of cereals. II Feeding experiments with laying hens. *J Sci Fd Agric* 6:600-2. (As cited in IARC, 1977.)
- Brimer PA, Tan EL, Hsie AW (1982). Effect of metabolic activation on the cytotoxicity and mutagenicity of 1,2-dibromoethane in the CHO/HGPRT system. *Mutat Res* 95:377-88.
- Broda C, Nachtomi EAE (1976). Differences in liver morphology between rats and chicks treated with ethylene dibromide. *Gen Pharmacol* 7:345-8. (As cited in Alexeeff *et al.*, 1990.)
- CARB (1985). Report on ethylene dibromide to the scientific review panel. Part A - Review of ethylene dibromide uses, emissions, and public exposure. California Air Resources Board, Sacramento, California.
- CARB (1996). Motor Fuels. California Air Resources Board, Cal/EPA, Compliance Assistance Program Report.
- CDHS (1985). Health effects of ethylene dibromide. Report on Ethylene Dibromide to the Scientific Review Panel. Part B. California Department of Health Services.

- CDHS (1988). Proposed Maximum Contaminant Level for Ethylene Dibromide. Appendix A. Hazard Evaluation Section (now Office of Environmental Health Hazard Assessment), California Department of Health Services.
- Cmarik JL, Humphreys WG, Bruner KL, Lloyd RS, Tibbetts C, Guengerich FP (1992). Mutation spectrum and sequence alkylation selectivity resulting from modification of bacteriophage M13mp18 DNA with S-(2-chloroethyl)glutathione. Evidence for a role of S-(2-N7-guanyl)ethyl)glutathione as a mutagenic lesion formed from ethylene dibromide. *J Biol Chem* 267(10):6672-9.
- Courtens J, Amir D, Durrand J (1980). Abnormal spermiogenesis in bulls treated with ethylene dibromide: an ultrastructural and ultracytochemical study. *J Ultrastr Res* 71:103-15.
- CRC Handbook (1988). Handbook of Chemistry and Physics, 69th Ed. RC Weast, Ed., CRC Press, Inc., Boca Raton, Florida.
- Crespi CL, Seixas GM, Turner TR, Ryan CG, Penman BW (1985). Mutagenicity of 1,2-dichloroethane and 1,2-dibromoethane in two human lymphoblastoid cell lines. *Mutat Res* 142(3):133-40.
- Crump K, Howe R (1984). The multistage model with time-dependent dose pattern; Applications to carcinogenic risk assessment. *Risk Analysis* 4(3):163-76. (As cited in OEHHA, 1988.)
- DeLeve LD (1997). Effect of decreased glutathione levels in hereditary glutathione synthetase deficiency on dibromoethane-induced genotoxicity in human fibroblasts. *Mutat Res* 389(2-3):291-7.
- Edwards K, Jackson H, Jones A (1970). Studies with alkylating esters. II. A chemical interpretation through metabolic studies of the antifertility effects of ethylene dimethane sulphonates and ethylene dibromide. *Biochem Pharmacol* 19(5):1783-9.
- Eljack A, Hrudka F (1979). Pattern and dynamic of teratospermia induced in rams by parenteral treatment with ethylene dibromide. *J Ultrastr Res* 67:124-34.
- Guengerich FP (1994). Metabolism and genotoxicity of dihaloalkanes. *Adv Pharmacol* 27:211-36.
- Hansen J (2000). Elevated risk for male breast cancer after occupational exposure to gasoline and vehicular combustion products. *Am J Ind Med* 37(4):349-52.
- Hill D, Shih T, Johnston T, Struck R (1978). Macromolecular binding and metabolism of the carcinogen 1,2-dibromoethane. *Cancer Res* 38(8):2438-42.
- HSDB (2000). Hazardous Substances Data Bank. ToxNet, National Library of Medicine. URL: <http://toxnet.nlm.nih.gov>.
- Hsu LL, Adams PM, Fanini D, Legator MS (1985). Ethylene dibromide: effects of paternal exposure on the neurotransmitter enzymes in the developing brain of F1 progeny. *Mutat Res* 147(4):197-203.
- IARC (1977). IARC Monographs on the evaluation of the carcinogenic risk of chemicals to man: Some fumigants. World Health Organization, Lyon, France. Vol. 15:195-209.

IARC (1999). IARC monographs on the evaluation of carcinogenic risks to humans. Re-evaluation of some organic chemicals. World Health Organization, Lyon, France. Vol. 71:641-99.

Kloos H (1996). 1,2 Dibromo-3-chloropropane (DBCP) and ethylene dibromide (EDB) in well water in the Fresno/Clovis metropolitan area, California. Arch Environ Health 51(4):291-9.

Kulkarni AP, Edwards J, Richards IS (1992). Metabolism of 1,2-dibromoethane in the human fetal liver. Gen Pharmacol 23(1):1-5.

Letz GA, Pond SM, Osterloh JD, Wade RL, Becker CE (1984). Two fatalities after acute occupational exposure to ethylene dibromide. JAMA 252(17):2428-31.

McCullister D, Hollingsworth R, Oyen F (1956). Comparative inhalation toxicity of fumigant mixtures. Ind Hlth 13:1-7. (As cited in Alexeeff *et al.*, 1990.)

Merck Index (1996). The Merck Index, 12th Ed., S Budavari, Ed., Merck and Co., Inc., Whitehouse Station, New Jersey.

Montgomery JH (1993). Agrochemicals Desk Reference: Environmental Data. Lewis Publishers, Boca Raton, Florida, pp. 210-1.

Nachtomi E, Alumot E (1972). Comparison of ethylene dibromide and carbon tetrachloride toxicity in rats and chicks: blood and liver levels; lipid peroxidation. Exp Mol Pathol 16(1):71-8.

Nachtomi E, Sarma D (1977). Repair of rat liver DNA *in vivo* damaged by ethylene dibromide. Biochem Pharmacol 26:1941-5.

Nachtomi E (1970). The metabolism of ethylene dibromide in the rat: the enzymic reaction with glutathione *in vitro* and *in vivo*. Biochem Pharmacol 19(11):2853-60.

NCI (1978). Bioassay of 1,2 dibromoethane. National Cancer Institute. Technical Report No. 86. U.S. Department of Health, Education, and Welfare, Bethesda, Maryland. NCI-CG-TR-86.

NIOSH (1994). Pocket guide to chemical hazards. U.S. Department of Health and Human Services, pp. 36-7.

Nitschke KD, Kociba RJ, Keyes DG, McKenna MJ (1981). A thirteen week repeated inhalation study of ethylene dibromide in rats. Fundam Appl Toxicol 1(6):437-42.

Novotna B, Duverger-van Bogaert M (1994). Role of kidney S9 in the mutagenic properties of 1,2-dibromoethane. Toxicol Lett 74(3):255-63.

NTP (1982). Carcinogenesis bioassay of 1,2-dibromoethane in F344 rats and B6C3F1 mice (inhalation study). Technical report No. 210. National Toxicology Program, U.S. Dept of Health and Human Services.

OEHHA (1988). Proposition 65 risk-specific intake level for ethylene dibromide. Reproductive and Cancer Hazard Assessment; Office of Environmental Health Hazard Assessment; Cal/EPA. Sacramento, California.

Olmstead E (1960). Pathological changes in ethylene dibromide poisoning. Arch Ind

Health 21:45-9.

Ott MG, Scharnweber HC, Langner RR (1980). Mortality experience of 161 employees exposed to ethylene dibromide in two production units. *Br J Ind Med* 37(2):163-8.

Perocco P, Prodi G (1981). DNA damage by haloalkanes in human lymphocytes cultured *in vitro*. *Cancer Lett* 13:213-8.

Pignatello JJ, Cohen SZ (1990). Environmental chemistry of ethylene dibromide in soil and ground water. *Rev Environ Contam Toxicol* 112:1-47.

Ploemen JP, Wormhoudt LW, Haenen GR, Oudshoorn MJ, Commandeur JN, Vermeulen NP, *et al.* (1997). The use of human *in vitro* metabolic parameters to explore the risk assessment of hazardous compounds: the case of ethylene dibromide. *Toxicol Appl Pharmacol* 143(1):56-69.

Plotnick H, Conner W (1976). Tissue distribution of ¹⁴C-labeled ethylene dibromide in the guinea pig. *Res Commun Chem Pathol Pharmacol* 13(2):251-8.

Plotnick H, Weigel W, Richards D, Cheever K (1979). The effect of dietary disulfiram upon the tissue distribution and excretion of ¹⁴C-1,2-dibromoethane in the rat. *Res Commun Chem Pathol Pharmacol* 26(3):535-45.

Rannug U (1980). Genotoxic effects of 1,2-dibromoethane and 1,2-dichloroethane. *Mutat Res* 76(3):269-95.

Ratcliffe J, Schrader S, Steenland K, Clapp D, Turner T, Hornung R (1987). Semen quality in papaya workers with long term exposure to ethylene dibromide. *Br J Ind Med* 44:317-26.

Reed N-M, Narloch B, Olsen H, Marty M, Tabalanta N, Reed W, *et al.* (1987). Health risk assessment for 1,2-dibromoethane (EDB) in California drinking water. Department of Environmental Toxicology, University of California, Davis, California.

Reznik G, Stinson SF, Ward JM (1980). Respiratory pathology in rats and mice after inhalation of 1,2-dibromo-3-chloropropane or 1,2-dibromoethane for 13 weeks. *Arch Toxicol* 46(3-4):233-40.

Rowe B, Spencer H (1952). Toxicity of ethylene dibromide. *Ind Hyg Occup Med* 6:158-73.

Saraswat PK, Kandara M, Dhruva AK, Malhotra VK, Jhanwar RS (1986). Poisoning by ethylene dibromide -- six cases: a clinicopathological and toxicological study. *Indian J Med Sci* 40(5):121-3.

Sharlin HI (1986). EDB: a case study in communicating risk. *Risk Anal* 6(1):61-8.

Short RD, Winston JM, Hong CB, Minor JL, Lee CC, Seifter J (1979). Effects of ethylene dibromide on reproduction in male and female rats. *Toxicol Appl Pharmacol* 49(1):97-105.

Steenland K, Carrano A, Clapp D, Ratcliffe J, Ashworth L, Meinhardt T (1985). Cytogenetic studies in humans after short-term exposure to ethylene dibromide. *J Occup Med* 27(10):729-32.

- Steinburg SM, Pignatello JJ, Sawhney BL (1987). Persistence of 1,2-dibromoethane in soils: entrapment in intraparticle micropores. *Environ Sci Technol* 21:1201-8. (As cited in Alexeeff *et al.*, 1990)
- Stott WT, McKenna MJ (1984). The comparative absorption and excretion of chemical vapors by the upper, lower, and intact respiratory tract of rats. *Fund Appl Toxicol* 4(4):594-602.
- Sweeney MH, Beaumont JJ, Waxweiler RJ, Halperin WE (1986). An investigation of mortality from cancer and other causes of death among workers employed at an east Texas chemical plant. *Arch Environ Health* 41(1):23-8.
- U.S. EPA (1984). Ethylene dibromide: Scientific report and decision document for grain and grain milling fumigation uses. Office of Pesticide Programs, U.S. Environmental Protection Agency, pp. 1-118. (As cited in Alexeeff *et al.*, 1990.)
- U.S. EPA (1987a). Drinking water criteria document for ethylene dibromide (EDB). Environmental Criteria and Assessment Office, U.S. Environmental Protection Agency, Cincinnati, OH. PB-89-192181.
- U.S. EPA (1987b). Health effects assessment for ethylene dibromide. U.S. Environmental Protection Agency. EPA/600/8-88/037.
- U.S. EPA (1991). Establishment of MCLs. *Federal Register* Vol. 56, No. 20, pp. 3523-97.
- U.S. EPA (2000). Ethylene dibromide, hazard summary. Office of Air Quality Planning and Standards. U.S. Environmental Protection Agency. Available online at: www.epa.gov/ttnuatw1/hlthef/ethyl-di.html.
- U.S. EPA (2002). National Primary Drinking Water Regulations, Announcement of the Results of EPA's Review of Existing Drinking Water Standards and Request for Public Comment. *Federal Register* April 17, 2002, 67(74):19029-19090.
- van Bladeren PJ, Breimer DD, Rotteveel-Smijds GM, de Jong RA, Buijs W, van der Gen A, *et al.* (1980). The role of glutathione conjugation in the mutagenicity of 1,2-dibromoethane. *Biochem Pharmacol* 29(21):2975-82.
- WHO (1996). Environmental Health Criteria # 177, 1,2-Dibromoethane. World Health Organization. Geneva.
- Wong LC, Winston JM, Hong CB, Plotnick H (1982). Carcinogenicity and toxicity of 1,2-dibromoethane in the rat. *Toxicol Appl Pharmacol* 63(2):155-65.
- Wong O, Morgan RW, Whorton MD (1985). An epidemiologic surveillance program for evaluating occupational reproductive hazards. *Am J Ind Med* 7(4):295-306.
- Wong O, Utidjian HM, Karten VS (1979). Retrospective evaluation of reproductive performance of workers exposed to ethylene dibromide (EDB). *J Occup Med* 21(2):98-102.
- Zenz C (1994). Reproductive toxicology and occupational exposure. In: *Occupational Medicine*. Zenz C, ed. Mosby, St. Louis, pp. 850-1.