FOREWORD INTRODUCTION

METHYL BROMIDE

CAS N°: 74-83-9

SIDS Initial Assessment Report for 13th SIAM

(Bern, Switzerland, November 6-9, 2001)

Chemical Name: Methyl bromide

CAS No.: 74-83-9

Sponsor Country: United States of America

National SIDS Contact Point (or Lead Organization which ever is applicable):

United States Environmental Protection Agency
Oscar Hernandez, Director
Risk Assessment Division (7403M)
1201 Constitution Ave, NW
Washington, DC 20460
(202) 564-7641
hernandez.oscar@epa.gov

Industry:

American Chemistry Council
Susan Lewis
1300 Wilson Blvd
Arlington, VA 22209
(703) 741-5635

History:

The work and review process undertaken for this chemical was done by industry and the United States Environmental Protection Agency. Documents were prepared and reviewed by industry prior to submission to sponsor country. Sponsor country conducted reviews of submitted data and offered comments to industry. Industry prepared and resubmitted documents for consideration at SIAM 13.

Testing: No Testing (X)
Testing ( )

Comments:

Deadline for Circulation: September 14, 2001
Updated: February 2002

Date of Circulation:

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	74-83-9
Chemical Name	Methyl bromide
Structural Formula	CH_3Br

RECOMMENDATIONS

The chemical is currently of low priority for further work in the SIDS program as it is subject to with-drawl under international activity (Montreal Protocol).

SUMMARY CONCLUSIONS OF THE SIAR

Human Health

Metabolism studies with radiolabeled methyl bromide show that it is rapidly metabolized and excreted. The primary route of excretion is exhalation as CO2 with lesser amount of radioactivity excreted in the urine and feces. Tissue distribution upon inhalation exposure showed that the liver contained the highest levels of radiolabel with appreciable amounts found in the lungs, nasal turbinates and kidneys. Upon oral or intraperitoneal injection, the liver was also the main organ for appreciable radiolabel with lesser amounts seen in the kidneys, testes, lung, heart, stomach and spleen.

Methyl Bromide (bromomethane) exhibits moderate acute toxicity by the oral and inhalation routes. The oral LD 50 in rats ranged from 104 to 214 mg/kg. Toxicity by the inhalation route is both time and concentration dependent. In mice, LC 50 values ranged from 1700 ppm (6,630 mg/m^3) for a 30 minute exposure to 405 ppm (1,575 mg/m^3) for a 4-hour exposure. Similarly in rats, the LC_{50} for a 30-minute exposure was reported as 2833 ppm (11,049 mg/m^3) while that for an 8-hour exposure was 302 ppm (1,178 mg/m^3). In repeated dose studies (4 weeks to 6 months duration) NOAELs of 5 – 33 ppm (20 - 129 mg/m^3) have been observed in inhalation studies using rats, mice, rabbits and dogs. Effects observed included decreased body weight, neurobehavioral changes and hematologic and clinical chemistry effects. Neurotoxic effects seen in experimental animals have included decreased locomotor activity, hyperactivity, depression, lethargy, ataxia, gait disturbances, tremor and convulsions. Methyl bromide was evaluated in 4 inhalation developmental toxicity studies (1 in rats, 3 in rabbits). No developmental effects were reported in these studies at exposure concentrations up to 70 ppm (273 mg/m^3) in both rats and rabbits. In one rabbit study, equivocal fetal effects were seen at a maternally toxic concentration of 80 ppm (312 mg/m^3). In a reproductive study by the inhalation route, no effects on reproductive performance were seen at exposure concentrations up to 90 ppm (351 mg/m^3). Neonate effects were limited to reduced body weights at day 28 post partum in F2 pups at 30 and 90 ppm (117 and 351 mg/m^3). The weight-of-evidence for all genetic toxicity testing indicates that methyl bromide is genotoxic, inducing gene mutations, chromosome mutations, DNA effects and other genotoxic effects both *in vitro* and *in vivo*. However, long-term and reproductive tests *in vivo* show no evidence of carcinogenic response. Methyl bromide is not considered to have produced heritable effects as no such effects were seen in reproductive studies in rats, or developmental studies in rats or rabbits at methyl bromide concentrations that did not induce maternal toxicity. This conclusion is further supported by negative results seen in the dominant lethal study in male rats.

In long-term inhalation bioassays, there were no statistically significant increases in tumors in rats exposed to concentrations up to 90 ppm (351 mg/m^3) for 29 months and mice exposed to concentrations up to 100 ppm (390 mg/m^3) for 2 years. The primary histological changes in both species were degeneration and hyperplasia of the nasal olfactory epithelium. Further, no evidence of oncogenicity was seen in a two-year dietary study in rats in which the animals were fed microencapsulated methyl bromide in order to maintain dietary concentrations.

Human exposure to methyl bromide may occur through inhalation of the gas or inadvertent contact with the liquid. The primary effects of methyl bromide in humans are on the nervous system, lung, nasal mucosa, kidney, eye, and skin. Effects on the central nervous system include blurred vision, mental confusion, numbness, tremors, and

speech defects. Topical exposure can cause skin irritation, burns, and eye injury. Exposure to high levels of methyl bromide causes pulmonary edema. Central nervous depression with respiratory paralysis and/or circulatory failure is the immediate cause of death generally preceded by convulsions and coma.

Environment

Although methyl bromide is very soluble in water (16.1 g/L at 25^0C), its high vapor pressure (1893 kPa) , log K_{ow} (1.94 at 25^0C) and log K_{oc} ranging from 2.1 to 2.2 in various soil types indicates a low tendency to absorb to soils causing it to rapidly evaporate from either water or soil. Methyl bromide has a half-life in air estimated between 0.3 and 1.6 years. The primary degradation is due to photolysis. In soils, projected half-lives are in the range of 0.2 to 0.5 days. In water, a half-life of 3 hours was calculated for a model river, this half-life relates to loss due to evaporation. As a result of evaporative transfer, abiotic and biotic processes are insignificant for methyl bromide due to the short residence time. Methyl bromide does not accumulate in aquatic species based on an estimated bioconcentration factor of 4.7 calculated from an octanol/water partition coefficient of 1.19. Rainbow trout and daphnid acute toxicity studies were conducted under static conditions with no headspace over the water column. A number of studies in several fish species indicate that methyl bromide causes acute lethality at concentrations of 0.7 to 20 mg/L. The most reliable 96-hour LC50 based on measured concentrations in the trout was 3.9 mg/l with NOECs of 1.9 and 2.9 mg/l for clinical signs and mortality, respectively. In daphnids, tests under similar conditions, the 48-hour LC50 for mortality and immobilization was 2.6 mg/l. Two studies have been reported in the literature for aquatic plants with a 48h-EC50 of 5 and 3.2 mg/l. A number of chronic studies in aquatic organisms are available, however, none were considered reliable to provide definitive results.

Exposure

In the United States, processing of the chemical is done in closed systems and is a chemical intermediate and a fumigant. In 1990, worldwide consumption of methyl bromide was reported to be 67,000 tonnes or approximately 74,000 US tons. In 1987, combined US production and import totaled 23,000 to 24,000 tonnes or approximately 26,000 US tons. Methyl bromide is used as a fumigant inside dwellings, office buildings, warehouses, silos, mills, vaults, ships and freight cars to control fungi, nematodes, insects and rats. Methyl bromide is also used outdoors as a fumigant, usually under gas-proof sheeting to control pests in soil and orchards. Soil fumigation consumes the bulk of methyl bromide production. In the US methyl bromide may only be applied and used by professional, certified applicators. Primary exposure would be via the inhalation route under occupational scenarios. Since methyl bromide is a gas at room temperature and dissipates rapidly from fumigation sites, non-occupational exposure to low residual amounts may occur to persons living in areas of methyl bromide fumigation. Most countries strictly regulate the application and handling of methyl bromide during fumigation operations to limit and protect the workers and public. Dermal exposure can result from direct contact to liquid methyl bromide through accidental splashing or contact with contaminated clothing.

Recent monitoring studies in areas of high fumigation activity and coinciding with the time periods of fumigation showed ambient air concentrations of methyl bromide in the mean range of 0.099 to 7.68 ppb. Occupational exposures to methyl bromide in various types of soil fumigation show mean exposures ranging from 2 to 605 ppb.

Methyl bromide is highly regulated in various OECD countries based on its hazard data and use information. Under the Montreal Protocol, methyl bromide is considered to be an ozone depleting substance (ODS) and it has been agreed that a phase out of the consumption and production of this chemical is to occur by the year 2005 for industrialized countries. However, in order to satisfy the needs of developing countries, a decreasing level of production is authorized until 2010. Currently there are two exemptions to the 2005 phase out; they are: quarantine and pre-shipment exemption (= 18% of the uses); and critical and emergency exemptions. It should also be noted that under the Protocol, the amount of methyl bromide used as feedstock in the manufacture of other chemicals is not considered as production. It is anticipated that methyl bromide will be further investigated by individual OECD member countries participating in the Montreal Protocol. As a result, it does not appear that further work will be necessary in the SIDS Program regarding the collection of exposure or release data from use, as the need for this information is required to be investigated for "exemptions" from the phase out.

NATURE OF FURTHER WORK RECOMMENDED

No recommendation.

FULL SIDS SUMMARY

CAS NO: 74-83-9		SPECIES	PROTOCOL	RESULTS
PHYSICAL-CHEMICAL				
2.1	Melting Point	--	--	-93.6 °C
2.2	Boiling Point	--	--	3.56°C
2.3	Density	--	--	1.73 g/cm3 at 0°C
2.4	Vapour Pressure	--	--	1893 kPa (1420 mm Hg) at 20°C
2.5	Partition Coefficient (Log K_{ow})	--	--	1.94 $\pm$ 0.31 at 25°C
2.6 A.	Water Solubility	--	--	16.1 g/L at 25°C
B.	pH	--	--	No data
	pKa	--	--	No data
2.12	Oxidation: Reduction Potential	--	--	No data
ENVIRONMENTAL FATE AND PATHWAY				
3.1.1	Photodegradation	Atmospheric Soil	--	Half-lives of 0.29-1.1 years at 25°C. Half-lives of 0.4-1.6 at –8°C Half-life of 10 days at 25°C.
3.1.2	Stability in Water	--	--	Rapidly evaporates from water
3.2	Monitoring Data	--	--	Ambient air in field fumigation areas range from 0.099 to 7.68 ppb. Occupational exposures range from 2 to 605 ppb.
3.3	Transport and Distribution	--	--	Primarily distributes to the atmosphere.
3.5	Biodegradation	--	--	Negligible
ECOTOXICOLOGY				
4.1	Acute/Prolonged Toxicity to Fish	*Oncorhynchus mykiss*	96-hour lethality	LC50 = 3.9 mg/L
4.2	Acute Toxicity to Aquatic Invertebrates	*Daphnia magna*	48-hour lethality/	EC50 = 2.6 mg/L
4.3	Toxicity to Aquatic Plants e.g. Algae	*Chlorella pyrenoidosa* *Scenedesumus quadricauda*	48-hour growth inhibition 48-hr lethality	EC50 = 5 mg/L (exposure duration not specified) EC50 = 3.2 mg/L (exposure duration not specified)
4.5.1	Chronic Toxicity to Fish			No reliable data available.
4.5.2	Chronic Toxicity to Aquatic Invertebrates (*Daphnia*)			No reliable data available.
4.6.1	Toxicity to Soil Dwelling Organisms	--	--	No data
4.6.2	Toxicity to Terrestrial Plants	*Oryz sativa* *Zea mays*	Seed emergence and growth	NOEC= 4 mg/L LOEC = 10 mg/L (viability)
(4.6.3)	Toxicity to Other Non-Mammalian Terrestrial Species (Including Birds)	Bobwhite quail	Acute oral toxicity	LD50= 73 mg/kg

TOXICOLOGY				
5.1.1	Acute Oral Toxicity	Rat	Acute lethality	LD50 = 104 – 214 mg/kg
5.1.2	Acute Inhalation Toxicity	Rat	8-hour acute lethality	LC50 = 302 ppm
5.1.3	Acute Dermal Toxicity	--	--	No data
5.4	Repeated Dose Toxicity	Rat	13-week inhalation neurotoxicity	NOEC= 30 ppm (highest dose in study shown to have no effects)
5.5	Genetic Toxicity In Vitro			
A.	Bacterial Test (Gene mutation)	*Salmonella typhimurium* (TA 100, 98, 1535, 1537 and 1538)	Mutagenicity	With activation: Positive (TA 100 and 1535) Without activation: - Positive (TA 100 and 1535)
B.	Non-Bacterial In Vitro Test	Hamster ovary cells	Cell transformation assay.	Negative
		Rat hepatocytes	Unscheduled DNA Synthesis	Negative
		Human lymphocytes	Sister Chromatid Exchange (SCE)	Positive
		L5178Y -TK+/-	Mouse Lymphoma	Positive
5.6	Genetic Toxicity In Vivo	Micronucleus	Rat and mouse	Positive
		Dominant lethal	Rat	Negative
5.7	Carcinogenicity	Rat Mouse	Inhalation chronic toxicity and carcinogenicity	Negative in both species.
5.8	Toxicity to Reproduction	Rat	2-generation reproduction by inhalation exposure	NOEC= 3 ppm LOEC= 30 ppm
5.9	Developmental Toxicity/ Teratogenicity	Rat	Inhalation teratogenicity	NOEC ≥ 70 ppm (highest dose in study had no observed effects)
		Rabbit	Inhalation teratogenicity	NOEC= 40 ppm (highest dose in study shown to have no effects)
5.11	Experience with Human Exposure	Human	Inhalation and dermal	Neurologic effects and deaths have been reported upon exposure to high concentrations.

SIDS INITIAL ASSESSMENT REPORT

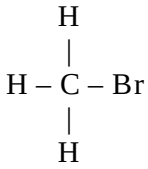
1. IDENTITY

IUPAC name: Bromomethane

CAS number: 74-83-9

Molecular Formula: CH_3Br

Structural Formula:



Synonyms: Bromomethane, monobromomethane, embafume, MeBr

Purity: > 99.5% [impurities: traces of chloromethane]
[odorant additives: chloropicrin (2%), amyl acetate (0.3%)

Table 1. Physical & Chemical properties:

Melting point:	-93.6 °C
Boiling point:	3.56 °C
Density:	1.73g/cm^3 (@ 0°C)
Vapor density:	3.974 kg/m^3 (@ 20°C)
Vapor pressure:	1893 kPa (1420 mmHg) (@ 20°C)
Partition coefficient (Log Pow): Water solubility:	1.19 (@ 25°C) estimated 1.94 (@25°C) measured 16.1 g/l (@ 25°C)
Henry's law constant:	6.24 x 10^{-3} atm-m^3/mol
Flash point:	194 °C
Autoignition temperature:	537 °C (999 °F)
Flammability limits:	10 – 16% by volume

2. GENERAL INFORMATION ON EXPOSURE

The major source of methyl bromide is stated to be from the oceans (Howard, 1989; IPCS, 1995). Anthropogenic sources of this chemical include fumigation, degreasing and extraction operations, as well as automobile and turbine exhaust. In 1990, worldwide consumption of methyl bromide was reported to be 67,000 tonnes or approximately 74,000 U.S. tons (IPCS, 1995). In 1987, combined U.S. production and import totaled 23,000 to 24,000 tonnes or ~26,000 U.S. tons (HSDB, 2000). U.S. production volumes after 1990 are not available due to data being claimed confidential business information.

Regarding the major anthropogenic source, methyl bromide is used as a fumigant inside dwellings, office buildings, and warehouses/silos/mills/vaults/ships/freight cars to control fungi, nematodes, insects and rats (Howard, 1989; IPCS, 1995). Methyl bromide also is used outdoors as a fumigant, usually under gas-proof plastic sheeting, to control pests in soil and orchards. Methyl bromide is also a chemical intermediate.
Soil fumigation consumes the bulk of methyl bromide production. Significant contact with this chemical could occur as a consequence of occupational exposure during fumigation operations if it is not handled safely. Since methyl bromide is a gas at room temperature and dissipates rapidly from fumigation sites, minor exposure to the general public may result from inhalation of residual amounts left after fumigation operations are completed. Most countries strictly regulate the application and handling of methyl bromide during fumigation operations to protect the worker and public (IPCS, 1995).

Recent monitoring studies (CDPR, 2000; MBIP, 2000) in areas of high fumigation activity and coinciding with the time periods of fumigation showed ambient air concentration of methyl bromide in the mean range of 0.099 to 7.68 ppb. Occupational exposures to methyl bromide in various types of soil fumigation show mean exposures ranging from 2 to 605 ppb. Table 2 shows the TRI on-site and off-site reported releases from manufacturing. Soil fumigation releases are not included.

In the United States, the applicable regulations for methyl bromide may be found in Annex 1. Methyl bromide is highly regulated in the United States based on its hazard data and use information. Currently the United States Environmental Protection Agency has authority to regulate methyl bromide under the Clean Air Act. Under the Montreal Protocol, methyl bromide is considered to be an ozone depleting substance (ODS) and it has been agreed that a phase out of the consumption and production of this chemical is to occur by the year 2005 for industrialized countries. However, in order to satisfy the needs of developing countries, a decreasing level of production is authorized until 2010. Currently there are two exemptions to the 2005 phase out; they are: quarantine and pre-shipment exemption (= 18% of the uses); and critical and emergency exemptions. It should also be noted that under the Protocol, the amount of methyl bromide used as feedstock in the manufacture of other chemicals is not considered as production. Reductions in the European Union are as follows: from 1/1/2001 to 12/31/2003: 60% reduction from 1991 production volume; from 1/1/2004 to 12/31/2004; 75% reduction from 1991 production volume; and from 1/1/2005 – onwards: production is prohibited except where indicated above.

Table 2 - TRI On-site and Off-site Reported Releases (in pounds), of BROMOMETHANE, U.S., 1999, All Industries

Chemical Bromomethane	
	Pounds
Total Air Emissions	**1,428,908**
Surface Water Discharges	**29**
Underground Injections	**0**
Releases to Land	**4**
Total On-site releases	**1,428,941**
Total Off-site releases	**1,603**
Total On-and Off- site Releases	**1,430,544**

(not including fumigation releases)

2.1. Environmental Fate and Photodegradation

Although methyl bromide is very soluble in water, its high vapor pressure (i.e., it is a gas at room temperature) and low tendency to absorb to soils causes it to rapidly evaporate from either soil or water into the air (Howard, 1989). In the lower atmosphere, methyl bromide has a long half-life that has been estimated to range between 0.4 to 1.6 years at -8°C and 0.29-1.1 years at 25°C due to its low rate of photolysis in the troposphere. As a result, this chemical eventually will diffuse upward to the stratosphere where it is expected to degrade by photolysis from higher energy and shorter wavelength solar radiation (Howard, 1989). In the stratosphere, the estimated "lifetime" of the compound was reported as 30–40 years. Photolysis in the stratosphere may produce active bromine species that may be related to ozone depletion (IPCS, 1995). Methyl bromide is considered to be a Class 1 ozone depleting substance (ODS) under the Montreal Protocol.

In soils, methyl bromide evaporates rapidly into the atmosphere. Measured Log Koc's range from 2.1 to 2.2 for various soil types, indicating that methyl bromide will not absorb strongly to soils (Howard, 1989). One model projected half-lives of 0.2 and 0.5 days for methyl bromide in soils at depths of 1 and 10 cm, respectively. Fumigation of soils may result in methyl bromide penetration to depths of 0.8 meters (IPCS, 1995). Dry soils have a greater tendency than moist soils to adsorb methyl bromide. In soil, methyl bromide may hydrolyze abiotically (i.e., chemically) to methanol and bromide ion with an experimentally derived half-life of 20 to 26 days. In a real-life setting, this rate would depend to a high degree on soil type and moisture content; soils having higher organic content would more likely produce bromide ion (Howard, 1989; IPCS, 1995). While some methylotrophic bacteria have been shown to oxidize methyl bromide to formaldehyde, biodegradation of methyl bromide is not believed to be a major process in soils, perhaps largely due to its short residence time in soils (Howard, 1989). Data were also reported for direct photolysis on soil. Methyl bromide was reported to photodegrade with a half-life of 10 days (25°C) on an unidentified soil. The reliability of the data could not be determined due to insufficient data on the test method.

In water, methyl bromide will evaporate rapidly into the atmosphere, due largely to its high vapor pressure. Also, this chemical may partition to groundwater where, depending on the amount of interaction between groundwater and air, methyl bromide would, again, partition rapidly into the atmosphere (ibid.). In one projection, a half-life of 3 hours was calculated from a model river. While abiotic and biotic processes occur in water, their rates are insignificant compared with evaporative transfer, unless evaporation is inhibited. Abiotic hydrolysis of methyl bromide is expected to occur with calculated half-lives of 20 to 27 days. Reaction products from abiotic hydrolysis are methanol and bromide ion. In "surface water," methyl bromide was reported to

degrade with half-lives (25°C) of 256–361 hours; no information on the light source was provided. It should be noted that the half-lives are longer than the hydrolysis half-lives; the water source was not identified and the water samples were reported to be of pH 5, 7 and 9. Also, the reliability of the data could not be determined due to insufficient data on the test method. Biodegradation in water is expected to be insignificant (Howard, 1989). Methyl bromide is not expected to bioaccumulate in aquatic species. This conclusion is based on an estimated bioconcentration factor of 4.7, estimated from a calculated log Kow of 1.19 (ibid). The measured log Kow is 1.94+/-0.31.

In bulk foods that have been fumigated with methyl bromide, such as wheat, cereals, spices, nuts, dried and fresh fruits, and tobacco, levels decrease rapidly with aeration and are not detectable after several weeks. Nuts, seeds, and fatty foods (e.g., cheese) tend to retain methyl bromide longer (IPCS, 1995). Levels were undetectable after a few days in asparagus, avocados, peppers, or tomatoes after a 2-hr fumigation with 320 mg MeBr/m^3. In wheat, flour, raisins, corn, sorghum, cottonseed meal, rice, and peanut meal, methyl bromide levels were reduced to less than 1 mg/kg within days (Howard, 1989).

2.2. Human Exposure

The major route of exposure for methyl bromide is via occupational exposure by the inhalation route. Based on current work practices and physical chemistry, workers are not likely to incur dermal exposure. Fumigation of soils and agricultural products is highly regulated in sponsor countries (IPCS, 1995). Thus, worker exposures should be minimal when appropriate handling procedures are followed.

While inhalation of methyl bromide by the general public near fumigation operations is possible, this group may be more likely to ingest this chemical from food substances previously fumigated. Residual levels of methyl bromide are highly regulated in fumigated foods. In addition, ingestion may also occur from groundwater contaminated by methyl bromide during soil fumigation operations.

3. EFFECTS ON HUMAN HEALTH

3.1 Toxicokinetics and Metabolism

Methyl bromide was rapidly absorbed when rats, beagles, and humans were exposed by inhalation. Rats are believed to be more efficient than humans at absorbing methyl bromide by inhalation exposure (IPCS, 1995), absorbing 50% of the dosage up to approximately 180 ppm (ATSDR, 1992). Also in this species, absorption was found to be directly proportional to air concentrations up to about 300 ppm. Absorption also was rapid and extensive (97%) in rats after oral administration (ibid.). After oral or intraperitoneal (ip) administration, methyl bromide also was rapidly absorbed and distributed to various tissues (Hayes, 1991).

Hayes (1991) further reports that, once absorbed after oral or ip administration, methyl bromide is distributed in rats to fat, lung, liver, adrenals, and kidney, with less found in the brain. Thereupon, methyl bromide is rapidly and extensively metabolized to methanol (ultimately to CO_2) and bromide. After oral or inhalation exposure, 85% of the dose was eliminated in rats within 65 to 72 hours. Most of the ^{14}C-radiolabeled dose (30-50%) was recovered as expired CO_2, 4-20% as expired parent compound, 16-40% was recovered in the urine, and a small percentage was found in the feces. Extensive enterohepatic circulation was indicated since 46% of the radioactivity was found in bile within the first day after dosing. Methyl bromide depletes glutathione and other sulfhydral proteins suggesting the formation of metabolite conjugates that have not yet been characterized. Tissue half-lives of radiolabeled methyl bromide range from 0.5 to 8 hours (ibid.).

3.2 Acute Toxicity

Data in Experimental Animals

Oral: Because methyl bromide is a gas above 4°C, determination of an oral lethal dose is problematic in experimental animals. Moreover, toxicity by this route of exposure is not likely in humans. Nonetheless, methyl bromide was determined to have an acute oral LD50 in rats of 214 mg/kg in a study by Danse et al. (1984) and approximately 100 mg/kg in a study by Kipplinger et al. (1994) indicating moderate toxicity by this route of exposure. An earlier study revealed an acute oral LD50 of 60-65 mg/kg in rabbits (Dudley et al., 1940). Other acute oral toxicity studies in these and other species reveal similar lethal doses. In beagle dogs, toxic signs from oral exposure included vomiting of reddish material (Naas, 1990).

Inhalation: Inhalation rat LC50's range from 781 ppm (4 hr) to 302 ppm (8 hr) (Kato, 1986; Honma, 1985). In mice, the 4-hour LC50 was determined to be 405 ppm by Yamano (1991). Methyl bromide exhibits a steep dose-response curve for mortality in many experimental animals (Hayes, 1991; IPCS, 1995). Signs and symptoms of methyl bromide exposure in mice exposed for 4 hours, at concentrations of 338 ppm and greater, included: decreased locomotor activity, ataxia, nasal discharge, lacrimation, diarrhea, irregular breathing, and bradypnea (Japanese Ministry of Labor, 1992). In mice exposed to 500 ppm and higher, autopsy revealed necrosis in the liver, kidneys, and olfactory epithelium, as well as keryorrhexis (disruption of the nuclei) in cells of the thymus and lymph nodes (ibid.). Also at lethal concentrations, findings were similar in rats but, in addition, included myocardial hemorrhage and adrenal gland hemorrhage and necrosis and excluded changes in lymph nodes (ibid.).

Dermal: Acute dermal toxicity studies have not been reported in experimental animals.

Data in Humans

Considerable anecdotal and clinical case-study information exists for acute exposure to methyl bromide in humans from an occupational exposure perspective since this chemical has long been used as a fumigant and, formerly, as a fire extinguisher. While such information is useful to characterize the type of injury resulting from overexposure, it is limited in defining the exposure level or duration of exposure causing damage since exposure levels/durations very often are difficult to define retrospectively from an over-exposure incident.

A number of fatalities have occurred in humans from inhalation of methyl bromide (Gosselin, 1984). Overwhelming exposure may cause death from narcosis and respiratory failure (Torkelson, 1994). In addition, inhalation exposure to high levels of methyl bromide may cause direct damage to lungs resulting in chemical pneumonia and edema that also may be fatal (Gosselin, 1994). Secondarily, inhalation exposure may damage the central and peripheral nervous system. In cases where damage to the lung is not fatal, death may result from convulsions or coma (ibid.). Where death has not resulted, CNS and PNS damage may persist, the former as organic brain syndrome and the latter as a peripheral neuropathy in cases of heavy exposure. CNS and PNS damage results more commonly from episodic or high level chronic rather than acute exposure, according to Ellenhorn (1988). More rarely, renal and hepatic sequelae may ensue from acute overexposure to methyl bromide (Ellenhorn, 1988; Gosselin, 1984; Torkelson, 1994).

Humans exposed to high concentrations of methyl bromide may exhibit coughing, chest tightness or pain, dyspnea, and cyanosis reflecting lung damage (IARC, 1986). Other symptoms, many of which are neurological in origin, may include gastric and abdominal pain, nausea, vomiting, headache, speech impairment, visual difficulties, tremors, seizures, unconsciousness, and convulsions (Gosselin, 1984; IARC, 1986; Torkelson, 1994). Still other symptoms may include dizziness, fainting, apathy, weakness, tiredness, giddiness, delirium, stupor, memory loss, mental confusion, psychosis, limb numbness, muscle twitching, loss of coordination including ataxia, and paralysis (IRIS, 2000).

3.3 Irritation

Eye Irritation: Grant (1993) reports the results of experimental exposure of methyl bromide gas directly to rabbit eyes. This exposure resulted in severe irritation that began to clear within five days. In older inhalation studies, lacrimation was observed in rats exposed to concentrations of 2570 ppm and undefined irritation was observed in mice at concentrations of 823 ppm (Irish, 1940; Balander and Polyak, 1962). In humans, splashes or direct exposure to vapors has not been reported to cause severe eye irritation. Severe acute inhalation exposure in humans has resulted in neurological sequelae that include visual impairment that is delayed in onset and usually reversible (Grant, 1993).

Skin Irritation: No information was found evaluating the dermal irritation of methyl bromide in experimental animals. In humans, Torkelson (1994) reports that workers have incurred skin irritation (including severe, second degree burns with large blisters that do not completely penetrate the dermis) when methyl bromide was confined under shoes, gloves, or other such items of clothing. Methyl bromide has a vesicant effect on skin as well as a freezing effect common to liquefied gases suddenly contacting and rapidly evaporating from tissue. In addition, methyl bromide is considered an alkylating agent capable of reacting with cellular proteins. All three effects, but especially its intense vesicant effect, would contribute to the irritating effect of methyl bromide on skin.

3.4 Sensitization

No information was located indicating that methyl bromide may cause either skin or respiratory sensitization.

3.5 Repeat Dose Toxicity

3.5.1 Subacute and Subchronic Effects

Data in Experimental Animals

In a series of early inhalation studies with a variety of species (135 rats, 98 guinea pigs, 104 rabbits, and 13 monkeys total, spread over six exposure levels), Irish (1940) established that at concentrations of 0, 17, 33, 65, 100, and 200 ppm administered 6 hr/day, 5 d/wk for up to 6 months, neurotoxicity occurred in rabbits and monkeys. Neurotoxicity appeared at 100 ppm and less (where lung injury was not lethal), characterized by paralysis of the extremities and, in one monkey exposed to 100 ppm for 6 months (6 hr/day, 5 d/wk), by paralysis and convulsions. At 33 and 65 ppm, rabbits exhibited limb paralysis (and some lung damage) while monkeys showed paralysis only at 65 ppm; rats showed no effects at 65 ppm or less and guinea pigs showed nothing at 100 ppm and less. No effects occurred at 17 ppm in any species. The Irish studies are a secondary reference with a Klimisch score of 4 but they are widely referenced and the results are considered relevant and consistent. A subsequent study by Anger et al. (1981) confirmed a neurotoxic response in rabbits, but not rats, inhaling 65 ppm methyl bromide for 4 weeks. In contrast to the Irish study, which found limb paralysis in rabbits at 33 ppm, Russo et al. (1984) found no neurotoxicity (measured by the latency rates of the ulnar and sciatic nerves and amplitude of the eyeblink reflex) in rabbits exposed to methyl bromide concentrations of 27 ppm for 7.5 hr/day, 4 day/wk for 8 months. Other investigators have found neurological effects in rats and mice inhaling methyl bromide (Ikeda, 1980; Honma, 1982; Kato et al., 1986; Eustis, 1988; WHO, 1991; NTP, 1992; Norris et al. 1993).

Kato et al. (1986) also found focal necrosis and fibrosis of coronary ventricles and papillary muscle in rats (10 to 12 animals per concentration level) exposed by inhalation to 0, 200, 300, or 400 ppm methyl bromide 4 hr/day, 5 day/week for 6 weeks (and 150 ppm for 11 weeks). This effect occurred at all exposure levels. At 300 ppm and higher, ataxia and paralysis was observed; at 400 ppm, necrosis was found at autopsy of the bilateral regions of the dorso-external cortex of the brain. In 1 of 10 rats at 200 ppm and 6 of 10 at 300 ppm, and 6 of 8 at 400 ppm, adverse effects on the testes were seen. These effects were unilateral and included: atrophy of seminal epithelium, incomplete spermatogenesis, giant cells in seminal tubules. Necrotic spermatocytes occurred in seminal fluid without spermatozoa in tubules of epididymis adjacent to atrophied testes. Morrissey et al. (1988) reported testicular damage in rats and mice exposed by inhalation to methyl bromide concentrations of 10 to 120 ppm, but did not specify what level(s) caused testicular effects. The effects were an increased relative weight of the epididymis and testes, a decreased sperm density and an increased percentage of abnormal sperm in mice; in rats, a decreased weight of the cauda epididymis, increased relative weight of the testis and decreased sperm motility was reported. Hurtt and Working (1988) found transient depression of plasma testosterone and testicular glutathione levels without effects on spermatogenesis, testicular weight, and several other reproductive parameters in rats (10/sacrifice group) exposed to 200 ppm methyl bromide 6 hr/day, 5 day/week for 5 days and followed for up to 68 days post-exposure.

Specifics of repeat-dose animal studies are shown in Annex.2, Table 1 (some subchronic toxicity studies are reported in Annex 2, Table 4: Reproductive/Developmental Toxicity or Annex 2, Table 5: Neurotoxicity).

Data in Humans

See "Chronic effects (non-neoplastic)"

3.5.2 Chronic effects (non-neoplastic)

Data in Experimental Animals

In a chronic inhalation study, Wistar rats (50-60/sex/group) were exposed to atmospheres containing methyl bromide concentrations of 0, 3, 30, or 90 ppm 6 hr/day, 5 days/week for 29 months (Reuzel et al., 1991). Monitored toxicological parameters included: behavioral signs, body weights, hematology, clinical chemistries, urinalyses, mortality, complete necropsy including organ weights, and histopathology. The major findings were: 1) degeneration and slight to moderate hyperplasia in the nasal olfactory epithelium (dose-response at all dose levels), 2) damage to heart tissue (significant at the 90 ppm level), 3) esophageal hyperkeratosis at 90 ppm in males only at 29 months, and 4) forestomach lesions that were not statistically significant. No treatment-related gross or microscopic changes were observed in the brains or lungs of exposed animals.

In the Reuzel study, irritation of the nasal olfactory epithelium was characterized by degeneration and hyperplasia. This lesion increased in severity in a concentration-dependent manner, ranking from very slight to moderate. Irritation also increased with time, even in controls, suggesting an age-related effect. A statistically significant increase was found between controls and the low-exposure group (3 ppm) at the end of the 29-month exposure period but not before. The frequency of this lesion also increased with age in the controls from 12 through 24 and 29 months. All but one of the lesions in the 3-ppm group was described as slight or very slight. One moderate lesion of the nasal mucosa was also observed in a control animal at the 24-month sacrifice interval. The NOEL for this lesion was > 90 ppm after 12 months of exposure, 3 ppm after 24 months, and < 3 ppm after 29 months (Reuzel, 1991).

In a combined carcinogenesis/chronic toxicity bioassay, the Japanese Ministry of Labor exposed rats and mice by inhalation to methyl bromide (JML, 1992). Rats (50/sex/dose) were exposed to methyl bromide concentrations of 0, 4, 20, or 100 ppm, 6 hr/day, 5 days/week for 2 years. Survival was not affected, however, high-dose rats gained weight more slowly than controls. High exposure animals also exhibited various alterations in hematological, clinical chemical, and urinalyses parameters. Rats at all exposure concentrations showed inflammation of the nasal olfactory epithelium. Necrosis and metaplasia of the olfactory epithelium was detected at 100 ppm. However, a high control incidence of metaplasia is noted in aged rats.

Mice (50/sex/dose) were exposed to methyl bromide concentrations of 0, 4, 16, or 64 ppm 6 hr/day, 5 days/week for 2 years. Survival was not affected. At the highest exposure concentrations, both sexes showed reduced weight gain and slight atrophy of the granular layer of the cerebellum (JML, 1992).

The U.S. National Toxicology Program conducted a combined carcinogenesis/chronic toxicity study with mice (NTP, 1992). Mice (86/sex/group) were exposed to methyl bromide concentrations of 0, 10, 33, or 100 ppm 6 hr/day, 5 days/week for 2 years. Clinical signs, body weight, survival, hematology, clinical chemistries, urinalyses, organ weights and histopathology were evaluated. In addition, neurobehavioral and neuropathological assessments were included in some animals at

various time points throughout the treatment period. Survival was poor in the high exposure group at 20 weeks (mortality was 47% in males and 10% in females); therefore exposure was discontinued in this group and animals were observed thereafter. Body weights were decreased in high exposure animals, which also exhibited tremors, abnormal posture, and limb paralysis and performance decrements in neurobehavioral tests. Target organs included brain, sternum (dysplasia), heart, and nasal epithelium; these organs were affected in the high exposure group only. In addition, the severity of effects in target organs was more pronounced in male mice. Brain lesions were characterized by cerebral degeneration; heart lesions by myocardial degeneration and chronic cardiomyopathy; and nasal epithelial changes by necrosis and metaplasia.

Details of these tests are shown in Annex 2, Table 3.

Data in Humans

Verberk et al. (1979) performed EEG's and general neurological examinations on 33 methyl bromide workers (greenhouse fumigators) and gathered symptom incidence by means of a questionnaire. Except for EEG's where exposed workers showed a slight diffuse increase in beta and theta activity, no differences were found when compared to control workers. Workers with altered EEG's, when compared to unaffected workers, had statistically increased levels of blood bromide (10.9 mg Br/l versus 8.2 mg Br/l).

Anger et al. (1986) conducted a cross-section occupational study of soil and structure fumigation workers chronically exposed to methyl bromide. Four groups were evaluated including: 1) structural fumigators using methyl bromide >80% of the time and soil fumigators exclusively using methyl bromide/chloropicrin combination (total n = 32), 2) structural fumigators using sulfuryl fluoride >80% of the time (n = 24), 3) fumigators using both methyl bromide and sulfuryl fluoride (n = 18), and 4) a control group consisting of workers in the fumigation industry but not directly applying fumigants (n = 29). Exposed workers had been fumigators for more than one year and had been engaged in fumigation operations within 50 days of neurological evaluations. The methyl bromide groups performed less well than controls in tests of cognitive function, reflexes, and sensory and visual performance. The results were confounded by co-exposures to other fumigants or other chemicals, poor age-matching with controls, lack of knowledge of (lack of control for) exposure levels, industrial hygiene practices, and other factors.

Kishi et al. (1991) conducted a questionnaire survey of workers (75 males employed from 1 to 25 years) in a methyl bromide manufacturing plant measuring the incidence of acute and general symptoms compared to a group of non-exposed control (railway) workers. Methyl bromide levels were monitored every six months and averaged less than 4 mg/m^3 (< 1 ppm) with excursions during accidents up to 20 mg/m^3 (5-6 ppm). While the incidence of symptoms were higher in exposed compared to non-exposed workers, mean bromide ion concentration in the urine of workers did not correlate with symptom incidence.

Calvert et al. (1998) conducted a study with 123 structural fumigation workers predominantly exposed either to methyl bromide or sulfuryl fluoride for an average of 1.20 and 2.85 years, respectively. A reference cohort of 120 workers had no pesticide exposure. All participants were evaluated for 1) nerve conduction velocity and amplitude, 2) vibrotactile threshold, 3) neurobehavioral parameters (hand-eye coordination, reaction time, continuous performance, symbol digit, pattern memory, serial digit learning, and mood scales), 4) visual acuity, 5) olfactory function, and 6) renal function. All fumigation workers performed less well in dexterity tests and had a higher incidence of carpel tunnel syndrome than non-exposed subjects. Although sulfuryl fluoride workers showed CNS effects including pattern memory deficits and olfactory thresholds, methyl bromide workers did not. Other parameters evaluated in this study were not affected by exposure to

fumigants. The authors ascribed decrements in dexterity and the increased incidence of carpel tunnel syndrome to ergonomic stresses rather than to exposure to fumigants.

3.6 Genetic Toxicity

Many bacterial *in vitro* tests for genotoxicity have been conducted with methyl bromide. Several Ames assays have shown that methyl bromide induces reverse mutations in Salmonella typhimurium with or without metabolic activation. Mutagenic responses also have been found using Escherichia coli as the test organism. In vitro tests using mammalian cells in culture, such as the mouse lymphoma assay, and the Sister Chromatid Exchange assay, also have shown positive genotoxic responses. A cell transformation assay, using Syrian hamster embryo cells, showed no enhanced transformation to the malignant state when incubated with methyl bromide and methyl bromide did not induce Unscheduled DNA Synthesis in rat primary hepatocytes or human fibroblasts.

In vivo tests, such as the Mouse Micronucleus Assay, the Sister Chromatid Exchange Assay and the Mouse DNA Alkylation Assay have yielded positive responses indicating that methyl bromide is clastogenic and otherwise able to disrupt DNA. Methyl bromide however was negative in the sexlinked recessive assay in drosophila melanogaster and the dominant lethal assay in rats.

The weight of evidence for all genetic toxicity studies shows that the overall genotoxicity potential for methyl bromide is equivocal. Positive and negative studies were both seen *in vivo* and *in vitro*. As these studies may be predictive of either carcinogenic or heritable effects, it is important to evaluate the appropriate *in vivo* studies. Long-term studies in mice and rats showed no evidence of a carcinogenic response. Methyl bromide is not considered to have produced heritable effects as no such effects were seen in reproductive studies in rats, or developmental studies in rats or rabbits at methyl bromide concentrations that did not induce maternal toxicity. This conclusion is further supported by negative results seen in the dominant lethal study in male rats.

Individual genotoxicity tests and results are presented in Annex 2 Table 2.

3.7 Carcinogenicity

Data in Experimental Animals

In the three inhalation carcinogenicity/chronic toxicity studies described in the previous section (3.5.2 Chronic effects (non-neoplastic)), no increased incidence of malignancies were found (Reuzel et al., 1991; JML, 1992; NTP, 1992).

Oral administration of methyl bromide by gavage has resulted in forestomach tumors in rats, presumably due to irritation of the lining of the forestomach. Danse et al., (1984) administered 0, 0.4, 2, 10, or 50 mg/kg/day methyl bromide 5 d/wk by gavage in arachis oil for 90 days to Wistar rats (10/sex/dose). Squamous cell carcinomas of the forestomach were reportedly found in 13 of 20 rats receiving the highest dose. However, a panel of NTP pathologists re-evaluated the slides from this study and concluded that the lesions were non-neoplastic. Marked inflammation and hyperplasia occurred at all dose levels except those in the 0.4 mg/kg group. Boorman et al. (1986) administered 50 mg/kg methyl bromide in arachis oil by gavage to groups of fifteen male Wistar rats, 5 time/wk for periods of 13, 17, 21, or 25 weeks whereupon, subjects were sacrificed. In order to investigate the reversibility of irritation and hyperplasia and their effects on subsequent tumor development, other groups were treated for 13, 17, or 21 weeks, then observed for 25 weeks. In the

group treated for 25 weeks then immediately sacrificed, a carcinoma of the forestomach was observed in one of 10 rats. No other malignancies were found in the other groups. Hyperplasia occurred in all groups but regressed after 25 weeks in the groups observed for 25 weeks post-treatment. A third gavage study by Hubbs and Harrington (1986) showed similar regression of pre-neoplastic lesions. In this study, rats were gavaged with 25 or 50 mg/kg methyl bromide in peanut oil for 30, 60, 90, or 120 days, then observed for 30 or 60 days (those treated for 90 days). Ulceration was reported in treated rats that reversed after treatment was discontinued. No neoplasms were reported in this study.

In dietary studies, methyl bromide has not caused neoplasia. Methyl bromide was evaluated for chronic toxicity and oncogenicity in a 24-month dietary toxicity study in Sprague-Dawley rats (N.S./sex/group) (Mertens, 1997). Because of the volatile nature of methyl bromide and the feeding characteristics of rats, it was not considered feasible to conduct the study using feed fumigated with methyl bromide. For purposes of this study, methyl bromide was microencapsulated and mixed into the rodent diet. Methyl bromide dietary concentrations were 0.5, 2.5, 50, or 250 ppm (0.02, 0.11, 2.20, or 11.1 mg/kg/day for males and 0.03, 0.15, 2.92, or 15.1 mg/kg/day for females, respectively). Two control groups on a comparable regimen received 1) basal diet or 2) placebo (microcapsules without methyl bromide). No methyl bromide related effects were seen on survival, clinical condition, hematology, serum chemistry, urinalysis, organ weights, ophthalmologic assessments, or macroscopic and microscopic pathology evaluations. Food consumption, mean body weights and mean body weight gains were reduced in the 250 ppm males and females during the rapid growth phase for the animals during the first 12 to 18 months of the study. During the first 18 months of the study, mean body weight gain for high-dose males was 9% to 21% lower than the male control groups while mean body weight gain for females was 7% to 22% lower than the female control groups. Typical of chronic toxicity studies, food consumption and body weight gains during the second year of the study were comparable to controls as the mature animals reached adult body weight plateau. No evidence of oncogenicity was seen in this study.

Other investigators have considered diets fumigated with methyl bromide (rather than microencapsulation) to be an acceptable experimental approach. In a study by Mitsumori et al. (1990), the diet of Fischer 344 rats was fumigated with methyl bromide to achieve total bromide concentrations of 0, 80, 200, or 500 ppm. At the high dose, this corresponded to methyl bromide concentrations of less than 20 mg/kg food. Rats (60/sex/group) were fed this diet for 2 years. Controls received either unfumigated diet or diet containing 500 ppm bromide as KBr. Clinical signs, body weight, and food/water consumption were monitored and clinical chemistry, hematology, urinalysis, and ophthalmic examinations were conducted at the 2-year sacrifice. An interim 12-month sacrifice group also was evaluated. Rats fed diets fumigated with methyl bromide showed only slightly depressed body weight in males only in the 500 ppm group from 60 weeks onward. Females were unaffected. No increased in tumor incidence was found.

In a 12-month dietary safety study (Newton, 1995; Wilson et al., 1998), beagle dogs were exposed to methyl bromide fumigated feed at dietary concentrations of 0, 0.5, 1.5, or 5 ppm (0, 0.06, 0.13, or 0.28 mg/kg/day for males and 0, 0.07, 0.12, or 0.27 mg/kg/day for females, respectively). Prestudy trials were conducted to determine the methyl bromide fumigation concentrations and post fumigation intervals required to achieve the desired concentrations over a 1-hour feeding period. Dogs did not receive methyl bromide treated diets on weekends. No toxicologically significant effects from methyl bromide exposure were seen on clinical observations, body weight, body weight gain, food consumption, clinical pathology, urinalysis, ophthalmology, absolute or relative organ weights, or macroscopic or microscopic pathology. Based on the results of this study, the NOEL for methyl bromide when administered via fumigated feed to beagle dogs was greater than 5 ppm (> 0.28 mg/kg/day for males and > 0.27 mg/kg/day for females).
Details of carcinogenicity studies in animals are shown in Annex 2. Table 3.

Data in Humans

The International Agency for Research on Cancer (IARC, 1986) has reviewed the epidemiological data up to 1986 and concluded from two epidemiological studies evaluating a variety of brominated compounds that no conclusions could be reached regarding methyl bromide per se. The International Program on Chemical Safety (IPCS, 1995) has extended this review up to 1995 and not found an association between methyl bromide exposure and excess rates of cancer. Currently, IARC (1999) ranks methyl bromide as Class 3 (not classifiable as to carcinogenicity in humans) and EPA ranks this chemical as Class D (not classifiable as to human carcinogenicity: inadequate human and animal evidence of carcinogenicity or no data are available).

3.8 Reproduction/Developmental Toxicity

Reproduction

In a 2-generation reproduction study, parental rats (F0; 25/sex/exposure level) and a following generation of offspring (F1) were exposed by inhalation to methyl bromide concentrations of 0, 3, 30, or 90 ppm 6 hr/day, 5 days/week over the premating, gestation, and lactation periods to evaluate toxicity and reproduction in parental generations (American Biogenics Corporation, 1986). Exposure for the F1 generation differed from the F0 generation in that F1 rats received the additional exposure to methyl bromide as pups during lactation. The progeny of the F1 generation (F2) were exposed to methyl bromide only during their gestation period as embryos/fetuses and their lactation period as pups. Toxicity, reproductive performance, and reproductive organ status were evaluated in parental generations, including reproductive organ histopathology, fertility rates, number of live pups/litter, etc. Various developmental parameters were evaluated in the two offspring generations (F1 and F2) including gross appearance, viability, growth rate, etc. In the F0 generation at 90 ppm, methyl bromide exposure caused a statistically significant decrease in body weight gain and some organ weights (not associated with histopathological changes) with no effects on reproductive organ appearance or weights, reproductive organ histopathology, or reproductive performance. The F1 generation appeared normal except for decreases in some organ weights (no associated histopathology). Neither the F0 nor the F1 generation showed decrements in reproductive performance. Neonates comprising the F2 generation showed reduced growth rates at 30 and 90 ppm at 28 days of life but no overt developmental anomalies.

Effects in males: Of eight studies that evaluated testicular effects, most showed an adverse effect on testicular tissue and sperm function and morphology in rats or mice exposed to methyl bromide levels exceeding 100 ppm. Morrissey et al. (1988) evaluated reproductive parameters from the NTP study where rats and mice were exposed to methyl bromide concentrations up to 120 ppm. While these investigators also found altered male reproductive parameters, exposure levels were not reported. Thus, it cannot be firmly concluded that concentrations below 100 ppm will not cause testicular effects.

Effects in females: No adverse effects have been reported on reproductive tissues or reproductive performance in females exposed to methyl bromide.

Developmental Effects

The potential for developmental toxicity was evaluated in five inhalation studies (two with rats and three with rabbits) (Sikov et al. 1981; Breslin et al. 1990a & b; Peters et al., 1982). No developmental anomalies were reported in any of these studies except for Breslin et al. (1990) where

anomalies were observed in rabbit pups at 80 ppm that may have been due to the significant maternal toxicity at this exposure level.
Details of reproduction/developmental studies in animals are shown in Annex 2, Table 4.

3.9 Neurotoxicity

In earlier subchronic toxicity studies (e.g., Irish, 1940, Anger, 1981), signs of neurotoxicity were observed (see Section 3.5.1). Several later studies have focused on this toxicity endpoint. In an acute neurotoxicity study (Driscoll and Hurley, 1993), male and female CD (Sprague-Dawley) rats were exposed via inhalation for six hours to methyl bromide at concentrations of 0, 30, or 350 ppm. Animals were assessed for clinical signs and changes in body weights. Neurobehavioral evaluations (functional observation battery and motor activity) were performed within three hours of exposure and at seven and 14 days post-exposure. After 15 days, gross pathological evaluation was performed and brains were weighed. Microscopic evaluations were performed on central and peripheral nervous tissue. All animals survived to study termination. No methyl bromide induced effects were noted for body or brain weights. Neurobehavioral effects were observed only in the 350 ppm exposed group at the 3-hour post exposure assessment only. Effects noted in male and female rats consisted of decreased arousal, increased incidences of drooping or half-shut eyelids, piloerection, decreased rearing, depressed body temperature, and markedly decreased motor activity. The 350 ppm males had a decreased tail pinch response while females from this group showed increased urination and abnormal air righting response. No treatment related histological findings were seen in nervous system or nasal tissues.

In a subchronic inhalation neurotoxicity study (Norris et al., 1993), CD (Sprague-Dawley) rats (N.S./sex/dose) were exposed to methyl bromide concentrations of 0, 30, 70, or 140 ppm six hours/day, five days/week for 13 weeks. At the 140 ppm concentration, two male rats died during the first month. Clinical signs observed for these two rats included convulsions, tremors, hyperactivity, rapid respiration, and salivation. Mean body weights were significantly lower than the controls. Neurological evaluations for males revealed increased hind limb splay (weeks 4, 8, 13), abnormal air righting reflex (week 13), and decreased forelimb grip strength (week 13). Female rats demonstrated lower arousal scores (weeks 8 and 13), decreased rearing (weeks 4, 8, and 13), and significantly decreased motor activity (week 13). Mean absolute brain weights were significantly lower for both sexes; no differences were noted for the relative brain weights indicating that lower absolute brain weights were a reflection of the generally lower body weights for the treated animals. Gross lesions were limited to moderate to severe brain hemorrhage in the two 140 ppm male animals that died. Microscopic lesions in the brain were found in these two males and in one 140 ppm male that survived the 13-week exposure. Microscopic lesions in the brain were seen in these three males and consisted of neuronal necrosis in the hippocampus, necrosis and malacia in the cerebral cortex and basal ganglia, and malacia and/or necrosis in the thalamus and midbrain. One additional 140 ppm male had slight neuronal edema in the hippocampus. Other lesions in the 140 ppm group consisted of minimal regenerative dysplasia of the olfactory epithelium of the nasal cavity in three males and three females and minimal peripheral nerve degeneration in two males and two females. In the 70 ppm group, lower mean body weights and weight gain were reported for females from week 9 onward. Neurological findings were limited to slightly decreased forelimb grip strength (week 13) in males and decreased motor activity (week 13) in females. Although the mean absolute brain weights for females were statistically significantly decreased (5% lower than the control group), no difference was seen for the relative brain weight and no microscopic pathology was observed. At 30 ppm, the mean absolute brain weight for females was statistically significantly lower than control (5% lower than the control group), however, no difference was seen in relative brain weight and no microscopic pathology in the brain was found.

In a series of inhalation studies (Newton, 1994a), beagle dogs received one to four days exposure to methyl bromide. The purpose of this study was to determine tolerable inhalation exposure levels to be used in a four-week inhalation study. In the initial phase of the study, three males and three females were exposed for six to seven hours to methyl bromide concentrations of 233 (1 male), 314 (1 male, 1 female), 345/350 (1 male, 1 female), or 394 ppm (1 female). Signs of toxicity were observed at all concentrations, therefore, the one-day NOAEL was < 233 ppm. In the second phase of the study, dogs were exposed to 55 ppm (1 male, 1 female), 156 ppm (1 male, 1 female), 268 ppm (1 male, 2 females), or 283 ppm (2 males, 1 female) for seven hours/day for up to four days. The 268 ppm and 283 ppm dogs were exposed to methyl bromide for two days and developed clinical signs of toxicity. Therefore, the two-day NOAEL for beagle dogs exposed to methyl bromide for this duration was < 268 ppm. The 55 ppm and 156 ppm dogs were exposed to methyl bromide seven hours/day for four consecutive days. No effects were seen in either the 55 ppm or 156 ppm dogs during days 1 and 2 of exposure. However, the 156 ppm animals showed decreased activity during exposure on days 3 and 4 and irregular gait during the post-exposure period on day 4.

In the subsequent study, Newton et al. (1994b) exposed beagle dogs (4/sex/group) 7 hours/day, 5 days/week for 4 weeks to methyl bromide concentrations of 0, 5, 10, 25, 50, or 100 ppm. No clinical evidence of neurotoxicity was seen in any group throughout the four weeks of exposure. After four weeks, four of the controls (two females and two males) and all dogs in the 5 ppm group continued the test for an additional two weeks and the exposure concentration for the 10 ppm group was increased to 150 ppm. Dogs exposed to 150 ppm methyl bromide showed severe body weight loss over the first few days of exposure. After five or six exposures, evaluation of the 150 ppm animals by a veterinary neurologist revealed ataxia, a base-wide stance, intention tremor, nystagmus, marked depression, and inability (unwillingness) to stand and perform postural responses. Due to the severity of these effects, the dogs were sacrificed. Neurological evaluation revealed no treatment related effects for dogs exposed to the lower methyl bromide concentrations. Significant neurological microscopic findings were found in the 150 ppm group, which consisted of vacuoles in the granular layer of the cerebellum.

Neurotoxicity studies are summarized in Annex 2, Table 5.

3.10 Specific Target Organ Effects

Methyl bromide is an unusual respiratory toxicant in the rat in that it is specifically toxic to the olfactory epithelium while other nasal epithelia are unaffected (Hurtt et. al., 1988). Within the olfactory epithelium, methyl bromide only affects specific cell types. Studies using histochemical techniques showed clearly that methyl bromide specifically induced degeneration of the sensory and sustentacular cells while sparing the basal cells from which the former cells are regenerated. Hurtt et al. (1988) evaluated the time course for the regeneration of the olfactory epithelium following short term exposure to methyl bromide. Male rats were exposed to 200 ppm methyl bromide for six hours/day, for one to five days. Air-exposed animals served as controls. In a companion study, animals were exposed to 0, 90, or 200 ppm for six hours/day, and olfactory function was assessed by the ability of food deprived animals to locate buried food pellets. Destruction of the olfactory epithelium was evident after a single six-hour exposure to 90 or 200 ppm. As discussed previously, severe effects were seen in the sustentacular and mature sensory cells while basal cells remained intact. Regeneration of the olfactory epithelium was seen as early as day three of exposure despite continued exposure at these high methyl bromide concentrations. The recovery of the olfactory epithelium was essentially complete 10 weeks post-exposure. The rapid recovery would be expected since the nonaffected basal cells regenerate sensory and sustentacular cells. Olfactory function, as measured by food finding activity, was impaired in

animals exposed to 200 ppm only. Recovery of this function was evident four to six days post-exposure, much earlier than the time course for histological recovery.
In another study (Hastings, 1994), morphologic and biochemical (carnosine content of the olfactory bulb, a biomarker for integrity of the olfactory epithelium) evaluations were conducted to further explore methyl bromide exposure effects and recovery. Prior to treatment, rats were food-deprived and trained to find buried food pellets as a measure of olfactory function. The rats were exposed to a methyl bromide concentration of 200 ppm on a four hour/day, four day/week, two-week regime. A single exposure resulted in extensive damage to the olfactory epithelium, reduced carnosine content, and impaired olfactory function. Even though exposure continued, olfactory function began to improve after the first exposure. This recovery proceeded even though persistent thinning and disorganization of the olfactory epithelium and decreased carnosine levels in the olfactory bulb were present. Regeneration of the olfactory epithelium was complete approximately 30-40 days after the last exposure.

3.11 Initial Assessment for Human Health

Methyl bromide exists as a gas at ambient temperatures; therefore, inhalation is the predominant form of exposure. Since the bulk of methyl bromide production is used in fumigation operations of soils and commodity products such as cereals and grains, fumigation workers are the principal exposed population. The public who live near fumigation operations or who consume fumigated food commodities may be exposed to low levels of this chemical. Methyl bromide is rapidly absorbed via most routes of exposure, including inhalation, rapidly distributed to all tissues, and rapidly metabolized and excreted.

Acute overexposure to methyl bromide may damage lungs and the central and peripheral nervous systems. In severe cases, injury to these target organs may be irreversible or only slowly reversible. Over the past century, numerous fatalities have resulted from mishandling of methyl bromide, usually in a work-related environment. Less frequently, overexposure to methyl bromide may injure the liver and kidneys, which is usually reversible. If the liquid from pressurized containers is splashed onto skin or eyes, burns may result. Such burns may be severe due to the vesicant, freezing, and direct chemical reactivity of methyl bromide. Neither odor nor sensory irritation is a good warning indicator for exposure to methyl bromide.

Effects from long-term exposure to methyl bromide may be more related to episodic acute overexposure incidents since symptoms from repeated exposure in workers are similar to those from acute overexposure. Repeated exposure of animals to methyl bromide has been shown to injure the lungs, CNS and PNS, heart, liver and kidneys. Inhalation studies in animals also indicate that methyl bromide may damage testicular tissue but decrements in reproductive performance are not observed unless concentrations are high enough to induce overt toxicity. Epidemiological survey studies have confirmed CNS damage in workers chronically exposed to methyl bromide.

Methyl bromide has been shown to be genotoxic in a variety of assays but has not been shown to be carcinogenic in animals or human epidemiology studies. Currently, IARC categorizes methyl bromide as a Class 3 substance (not classifiable as to carcinogenicity in humans) (1999).

4. EFFECTS ON THE ENVIRONMENT

Aquatic Effects

Acute toxicity: A number of studies in several fish species indicate that methyl bromide causes acute lethality at concentrations of 0.7 to 20 mg/liter (Canton et al., 1980; Dawson et al., 1975; Segers et al., 1984; Wildlife International, 1993a). Data from the Canton et al. (1980) study are considered relevant however; this study was not selected as the key study as limited data were available to complete a robust summary. The key fish study (Wildlife International, 1993a) reports a 96-hour LC50 in rainbow trout of 3.9 mg/l, a NOEC of 1.9 mg/l and a no mortality level of 2.9 mg/l. This study was conducted in a sealed system with no headspace over the water column to assure the methyl bromide remained in the water column. Analytical verification of methyl bromide concentrations in the water column was performed, and the study was conducted in compliance with USEPA Good Laboratory Practice regulations.

Similarly, two studies in daphnia with methyl bromide show the 48-hour EC50's for immobilization and mortality were 2.2 and 2.6 mg/L (Canton et al., 1980; Wildlife International, 1993b). Data from the Canton et al, 1980 study are considered relevant however; this study was not selected as the key study as limited data were available to complete a robust summary. In a well-conducted study (Wildlife International, 1993b) the NOEC for immobilization and mortality was 1.2 mg/L. The results from this study should be considered the key study for this endpoint.
The literature references 2 studies in aquatic plants (algae) (Canton et al, 1980) that show a calculated 48 hour EC50 of 5 and 3.2 mg/L, as the exposure duration for the calculated EC50's was unspecified. Because of the physicochemical properties of methyl bromide further testing in aquatic plants is not considered practical, due to the high evaporation rate of methyl bromide. In order to achieve and maintain appropriate methyl bromide concentrations in water, testing would need to be conducted in sealed vessels with no headspace above the water column, which would interfere with algal respiration and growth.

Chronic toxicity: Chronic studies were available for both fish (guppies and Medaka) (Wester et al. 1988) and daphnia (Canton et al 1980.) Studies were not considered reliable to provide definitive results and should not be considered relevant for the purposes of hazard classification. The chronic fish studies indicated that concentrations of 1 mg/L and greater were lethal to embryos of these fish within days to weeks and a concentration of 0.32 mg/L reduced weight gain (Wester et al., 1988). The chronic fish studies (Wester) do not follow OECD guideline in that the reproductive parameters critical to long-term fish studies were not evaluated. The end-point of concern in the Wester studies was the development of histologic lesions in fish that received toxic to lethal doses of methyl bromide. No such lesions were seen. In addition, based on available data, it is unclear whether headspace was present. If headspace were present, given the physical chemical properties of Methyl bromide, analytical monitoring results would be questionable.
In a chronic (12-day) study in daphnia (Canton et al., 1980) reproduction was inhibited at 0.1mg/L. The author concluded that the reason for the high mortality was unclear and could have been due to a transient decrease in the overall viability of the daphnia magna population. In addition, the author recommended a repeat of the study based on the questionable results.

Acute and chronic studies are summarized in Annex 2, Table 6.

Terrestrial Effects

Terrestrial non-mammalian species: Campbell and Beavers (1994) reported an oral LD50 of 73 mg/kg for methyl bromide in northern bobwhite. The body weight, egg weight, and number of eggs

were not affected in hens fed a diet that had been fumigated with methyl bromide (800 and 2,000 mg. Hr/liter) but sexual maturity was slightly affected (Cooper et al., 1978; Griffiths et al., 1978).

Terrestrial plants: Rice germination was not slowed by methyl bromide concentrations of 4 mg/liter if rice moisture content was 11% or less. Corn was more resistant, tolerating concentrations of 5 mg/L but germination was slowed by concentrations of 10 mg/L (Sittisuang and Nakakita, 1985). Methyl bromide yellowed lettuce leaves after 72 hours of exposure to methyl bromide at concentrations of 400 mg/m^3. Concentrations as high as 1,400 mg/m^3 for 72 hours did not affect water cress (Reichmuth and Noack, 1983).

Studies are summarized in Annex 2, Table 6.

Other

Because of its long half-life in the lower atmosphere and consequent eventual dispersion to the upper atmosphere, methyl bromide may be dissociated to form activated bromine species that may have a depleting affect on ozone.

Initial Assessment for the Environment

Due to its very high vapor pressure (it is a gas above 4°C), methyl bromide will tend to rapidly partition to the atmosphere from other environmental compartments.

Acute and chronic ecotoxicological tests indicate that methyl bromide is highly toxic to aquatic species. However, its rapid partitioning from water to air will mitigate the hazard to aquatic species from water-borne methyl bromide. Methyl bromide, as a soil and structural fumigant, is highly toxic to terrestrial species such as insects, nematodes, fungi, and other pests.

5. RECOMMENDATIONS

Methyl bromide is highly regulated in the United States based on its hazard data and use information. Currently the United States Environmental Protection Agency has authority to regulate methyl bromide under the Clean Air Act and Montreal Protocol. Methyl bromide is considered to be a Class 1 ozone depleting substance (ODS) and it has been agreed that a phase out of the chemical is to occur by the year 2005. Currently, there are two exemptions to the phase out, they are: quarantine and preshipment exemption; and critical and emergency exemptions. It is anticipated that methyl bromide will further be investigated by individual OECD member countries participating in the Montreal Protocol. As a result, it does not appear that further work will be necessary in the SIDS Program regarding the collection of exposure or release data from use, as the need for this information is required to be investigated for "exemptions" from the phase out.

It is recommended that this chemical is of low priority for further work.

6. REFERENCES

Alexeeff, G.V., Kilgore, W.W., Munoz, P., Watt, D., 1985. Determination of acute toxic effects in mice following exposure to methyl bromide. J. Toxicol. Environ. Health 15:109-123.

American Biogenics Corporation, 1986. Two-generation reproduction study in albino rats with methyl bromide - results of both generations (Study No. 4500-1525) (Unpublished final report).

Anger, W.K., Setzer, J.V., Russo, J.M., Brightwell, W.S., Wait, R.G., Johnson, B.L., 1981. Neurobehavioral effects of methyl bromide inhalation exposures. Scan. J. Work Environ. Health 7:40-47.

Anger, W.K., Moody, L., Burg, J., Brightwell, W.S., Taylor, B.J., Russo, J.M., Dickerson, N. Setzer, J.W., Johnson, B.L., Hicks, K., 1986. Neurobehavioral evaluation of soil and structural fumigators using methyl bromide and sulfuryl fluoride. Neurotoxicology 7:137-156.

ATSDR (Agency for Toxic Substances and Disease Registry), 1992. Bromomethane. Prepared by Life Systems, Inc. under contract to Clement International Corporation for ATSDR, U.S. Public Health Service.

Balander, P.A., Polyak, M.G., 1962. [Toxicological characteristics of methyl bromide.] J. Gig. I. Tokskol. 60:412-419 (in Russian).

Bentley, K.S., (I.E. DuPont de Nemours and Company), 1994. Detection of single strand breaks in rat testicular DNA by alkaline elution following in vivo inhalation exposure to methyl bromide. Haskell Laboratory Report N0. 54-94. Chemical Manufacturers Association DPR Vol. 123-188 # 162362.

Boorman, G.A., Hong, H.L., Jameson, C.W., Yoshitomi, K., Maronpot, R.R., 1986. Regression of methyl bromide-induced forestomach lesions in the rat. Toxicol. Appl. Pharmacol., 86:131-139.

Breslin, W.J., Zablotny, C.L., Bradley, G.J., Nitschke, K.D., Lomax, L.G., 1990a. Methyl bromide inhalation teratology probe study in New Zealand white rabbits. Midland, Michigan, The Dow Chemical Company (Unpublished final report).

Breslin, W.J., Zablotny, C.L., Bradley, G.F., Lomax, L.G., 1990b. Methyl bromide inhalation teratology study in New Zealand white rabbits. Midland, Michigan, The Dow Chemical Company (Unpublished final report).

Calvert, G.M., Mueller, C.A., Fajen, J.M., Chrislip, D.W., Russo, J., Briggle, T., Fleming, L.E., Suruda, A.J., Steenland, K., 1998. Health effects associated with sulfuryl fluoride and methyl bromide exposure among structural fumigation workers. Am. J. Public Health, 88(12):1774-1780.

Calvert, G.M., Talaska, G., Mueller, C.A., Ammenheuser, M.M., Au, W.W., Fajen, J.M., Fleming, L.E., Briggle, T., Ward, E., 1998. Genotoxicity in workers exposed to methyl bromide. Mutat. Res. 417(2-3):115-128.

Campbell, S.M., Beavers, J.B., 1994. Methyl Bromide: An acute oral toxicity study with the northern bobwhite. Wildlife International Ltd., Maryland, project no. 264-110.

Canton, J.H., 1980. Hydrobiological toxicological research with methyl bromide. National Institute of Public Health and Environmental Hygiene, Report No. 105/80, 4 p.
CDPR, 2000 Unpublished Studies

Cooper, D.M., Griffiths, N.M., Hobson-Frohock, A, Land, D.G., Rowell, J.G., 1978. Fumigation of poultry food with methyl bromide: effects on egg flavour, number, and weight. Br. Poult. Sci., 19: 537-542.

Danse, L.H.J.C., Van elsen, F.L., Van der Heijden, C.A., 1984 Methyl bromide: carcinogenic effects in the rat forestomach. Toxicol. Appl. Pharmacol., 72: 262-271.

Davenport, C.J., Ali, S.F., Miller, F.J., Lipe, G.W., Morgan, K.T., Bonnefoi, M.S., 1992. Effect of methyl bromide on regional brain glutathione, glutathione-S-transferases, monoamines, and amino acids in F344 rats. Toxicol. Appl. Pharmacol. 112:120-127.

Dawson, G.W., Jennings, A.L., Drozdowski, D., Rider, E., 1975. The acute toxicity of 47 industrial chemicals to fresh and saltwater fishes. J. Hazard. Mater., 1: 303-318.

Devaney, J.A., Beerwinkle, K.R., 1982. Control of the northern fowl mite on inanimate objects by fumigation: field studies. Poult. Sci. 62:43-46.

Driscoll, C.D., Hurley, J.M., 1993. Methyl bromide: single exposure vapor inhalation neurotoxicity study in rats. Unpublished report from Bushy Run Research Center Project no. 923N1197.

Djalali-Bbehzad, G., Hussain, S., Ostermann,-Golker, S., Segerbaeck, D., 1981. Estimation of genetic risks of alkylating agents. VI. Exposure of mice and bacteria to methyl bromide. Mutat. Res. 84:1-9.

Dudley, H.C., Miller, J.W., Neal, P.A., Sayers, R.R., 1940. Studies on foodstuffs fumigated with methyl bromide. Public Health Report, 55:2251-2282.

Ellenhorn, 1988. Medical Toxicology: Diagnosis and Treatment of Human Poisoning. (M.J. Elenhorn and D.G. Barceloux; eds) pp. 981-982, Elsevier, New York.

Eustis, S.L., Haber, S.B., Drew, R.T., Yang, R.S.H. 1988. Toxicology and pathology of methyl bromide in F344 rats and B6C3F1 mice following repeated inhalation exposure, Fund. Appl. Toxicol. 11:594-610.

Furuta, A., Hyakudo, T., Ohnishi, A., Hori, H., Tanaka, E., 1993. Neurotoxicity of methyl bromide – neuropathologic evaluation, preliminary study. Sangyo Ika Daigaku Zasshi 15: 21-27.

Gansewendt, B., Foest, U., Xu, D., Hallier, E., Bolt, H.M., Peter, H., 1991. Formation of DNA adducts in F-344 rats after oral administration of inhalation of [14C]methyl bromide. Food Chem. Toxicol. 29:557-563.

Gary, V.F., Nelson, R.L., Griffith, J., Harkins, M., 1990. Preparation for human study of pesticide applicators: sister chromatid exchanges and chromosome aberrations in cultured human lymphocytes exposed to selected fumigants. Teratog. Carcinog. Mutagen. 10:21-29.

Gosselin, 1984. Clinical Toxicology of Commercial Products, Fifth Edition (R.E. Gosselin, R.P. Smith, H.C. Hodge; eds), Williams and Wilkins, Baltimore.

Grant, W.M., Schuman, J.S., (1993). Toxicology of the Eye, Fourth Edition. Charles C. Thomas, Springfield.

Griffiths, N.M., Hobson-Frohock, A., Land, D.G., Levett, J.M., Cooper, D.M., Rowell, J.G. 1978. Fumigation of poultry food with methyl bromide: effects on flavour and acceptability of broiler meat. Br. Poul. Sci., 19: 529-535.

Hallier, E., Langhof, T., Dannappel, d., Leutbecher, M., Schroder, K., Goergens, H.W., Muller, A., Bolt, H.M., 1993. Polymorphism of glutathione conjugation of methyl bromide, ethylene oxide and dichloromethane in human blood: influence on the induction of sister chromatid exchanges (SCE) in lymphocytes. Arch. Toxicol. 67:173-178.

Hardin, B.D., Bond, G.P., Sikov, M.R., Andrew, F.D., Beliles, R.P., Niemeier, R.W., 1981. Testing of selected workplace chemicals for teratogenic potential. Scand. J. Work Environ. Health 7:66-75.

Hastings, L., Andringa, A., Miller, M.A., 1994. Exposure of the olfactory system to toxic compounds: structural and functional consequences. Inhal. Toxicol. 6 (suppl):437-440.

Hatch, G.G., Mamay, P.D., Ayer, M.L., Casto, B.C., Nesnow, S., 1983. Chemical enhancement of viral transformation in Syrian hamster embryo cells by gaseous and volatile chlorinated methanes and ethanes. Cancer Res. 43: 1945-1950.

Hayes, 1991. Handbook of Pesticide Toxicology, Vol 2 (W.J. Hayes, E.R. Laws, Jr.; eds), Academic Press, Inc., New York.

Honma, T., Sudo, A., Miyagawa, M., Sato, M., 1982. Significant changes in monoamines in rat brain induced by exposure to methyl bromide, Neurobehavioral Toxicol. Teratol. 4:521-524.

Honma, T., Miyagawa, M., Sato, M., Hasegawa, H. 1985. Neurotoxicity and metabolism of methyl bromide in rats. Toxicol. Appl. Pharmacol., 81: 183-191.

Honma, T., 1987a. Alteration of catcholamine metabolism in rat brain produced by inhalation exposure to methyl bromide. Jpn .J. Ind. Health, 29:218-219.

Honma, T., Muneyuki, M., Sato, M., 1987b. Methyl bromide alters catecholamine and metabolite concentrations in rat brain. Neurotoxicol. Teratol. 9:369-375.

Honma, T., Miyagawa, M., Sato, M., 1991. Inhibition of tyrosine hydroxylase activity by methyl bromide exposure. Neurotoxicol. Teratol. 13:1-4.

Honma, T., 1992. Brain microdialysis study of the effects of hazardous chemicals on the central nervous system. 1. Changes in monoamine metabolites induced by cerebral methyl bromide administration measured by two-probe microdialysis (TPMD) method. Ind. Health 30:47-60.

Howard, P.H., (1989). Handbook of Environmental Fate and Exposure Data for Organic Chemicals, Volume I: Large Production and Priority Pollutants. Pp 386-393, Lewis Publishers, Chelsea, Michigan.

HSDB, (1999). Hazardous Substances Data Bank. Database generated and maintained by the National Institute of Occupational Safety and Health (NIOSH).

Hubbs, A.F., Harrington, D.D., 1986. Further evaluation of the potential gastric carcinogenic effects of subchronic methyl bromide administration. In: Proceedings of the 36th Meeting of the American College of Veterinary Pathology and the Annual Meeting of the American Society of Veterinary and

Clinical Pathology, Denver, Colorado, December 1985. Denver, Colorado, American Society of Veterinary and Clinical Pathology, p 92.

Hurtt, M.E., Morgan, K.T., Working, P.K. 1987. Histopathology of acute toxic responses in selected tissues from rats exposed by inhalation to methyl bromide. Fundam. Appl. Toxicol., 9: 352-365.

Hurtt, M.E., Working, P.K., 1988. Evaluation of spermatogenesis and sperm quality in the rat following acute inhalation exposure to methyl bromide. Fundam. Appl. Toxicol. 10:490-498.

Hurtt, M.E., Thomas, D.A., Working, P.K., Monticello, T.M., Morgan K.T., 1988. Degeneration and regeneration of the olfactory epithelium following inhalation exposure to methyl bromide: pathology, cell kinetics and olfactory function. Fundam. Appl. Pharmacol. 94:311-328.

IARC (International Agency for Research on Cancer). (1986) Monographs on the evaluation of the carcinogenic risk of chemical to man. Geneva: World Health Organization, Methyl Bromide. p. 187-212, Lyon, France.

IARC (International Agency for Research on Cancer). (1999) Monographs on the evaluation of the carcinogenic risk of chemical to man. Volume 71, Part 2. Geneva: World Health Organization, Methyl Bromide. p. 721- 735, Lyon, France.

Ikawa, N., Araki, A., Nozaki, K., Matsushima, T., 1986. Micronucleus test of methyl bromide by the inhalation method. Mutat. Res. 164:269 (abstract).
Ikeda, T., Kishi, R., Yamamura, K., Miyake, H., Sato, M. 1980. Behavioral effects in rats following repeated exposure to methyl bromide, Toxicol. Lett. 6:317-321.

IPCS (International Programme on Chemical Safety), 1995. Environmental Health Criteria 166. Methyl Bromide. World Health Organization, Geneva.

IRIS (Integrated Risk Information System), 2000. Database generated by the U.S. Environmental Protection Agency.

Irish, D.D., Adams, E.M., Spencer, H.C., Rowe, V.K., 1940. The response attending exposure of laboratory animals to vapors of methyl bromide. J. Ind. Hyg. Toxicol., 22: 218-230.

JETOC. 1997. Mutagenicity test data of existing chemical substances (Based on the toxicity investigation system of the industrial safety and health law), Supplement, Tokyo, Japan Chemical Industry Ecology-Toxicology and Information Center, pp 253-255.

JML (Japanese Ministry of Labour), 1992. Toxicology and carcinogenesis studies of methyl bromide in F344 rat and BDF mice (inhalation studies). Tokyo, Industrial Safety and Health Association, Japanese Bioassay Laboratory, 197 pp (Unpublished report).

Kato, N., Morinobu, S., Ishizu, S. 1986. Subacute inhalation experiment for methyl bromide in rats. Ind. Health 24:87-103.

Kipplinger, G.A., 1994. Methyl Bromide: Acute Oral Toxicity Comparison Study of Microencapsulated Methyl Bromide and Liquid Methyl Bromide in Albino Rats. Unpublished report from WIL Research Laboratories, Project No. WIL-49011.

Kishi, R., Itoh, I., Ishizu, S., Harabuchi, I, Miyake, H., 1991. Symptoms among workers with long-term exposure to methyl bromide. An epidemiological study. Jpn. J. Ind. Health, 33:241-250.

Kramers, P.G.N., Voogd, C.E., Knaap, A.G.A.C., Van der Heijden, C.A., 1985a. Mutagenicity of methyl bromide in a series of short-term tests. Mutat. Res. 155:41-47.

Kramers, P.G.N., Bissumbher, B., Mout, H.C.A., 1985b. Studies with gaseous mutagens in Drosophila melanogaster. In: Short-term bioassays in the analysis of complex environmental mixtures IV., (Waters, M.D., Sandhu, S.S., Lewtas, J., Claxton, L., Straus, G., Nesnow, S., eds). Pp 65-73, Plenum Press, New York..

McGregor, D.B., 1981.Tier II mutagenic screening of 13 NIOSH priority compounds, Report No. 32 – Individual compound report: methyl bromide. Cincinnati, Ohio, National Institute of Occupational Safety and Health, 190 pp (PB83-130211).

MBIP, 2000 . Methyl Bromide Industry Panel Unpublishd Report.
Mertens, J.J.W.M., 1997. A 24-month chronic dietary study of methyl bromide in rats. Unpublished report from WIL Research Laboratories, Project No. WIL-49014.

Mitsumori, K., Maita, K., Kosaka, T., Miyaoka, T., Shirasu, Y., 1990. Two-year chronic toxicity and carcinogenicity study in rats of diets fumigated with methyl bromide. Food Chem. Toxicol. 28(2):109-119.

Miyagawa, M., 1982. Conditioned taste aversion induced by inhalation exposure to methyl bromide in rats. Toxicol. Lett. 10:411-416.
Moriya, M., Ohta, T., Watanabe, K., Miyazawa, T., Kato, K., Shirasu, Y., 1983. Further mutagenicity studies on pesticides in bacterial reversion assay systems. Mutat. Res. 116:185-216.

Morrissey, R.E., Schwetz, B.A., Lamb, J.C.IV, Ross, M.D., Teague, J.L., Morris, R.W., 1988. Evaluation of rodent sperm, vaginal cytology, and reproductive organ weight data from National Toxicology Program 13-Week studies. Fundam. Appl. Toxicol. 11:343-358.

Naas, D.J. 1990. Acute Oral Toxicity Study in Beagle Dogs with Methyl Bromide. Unpublished report from WIL Research Laboratories, Inc., Project No. WIL-49006.

Newton P.E. (1994a). An up-and-down acute inhalation toxicity study of methyl bromide in the dog. Unpublished report from Pharmaco-LSR Project number 93-6067.

Newton P.E. (1994b). A four-week inhalation toxicity study of methyl bromide in the dog. Unpublished report from Pharmaco LSR Project number 93-6068.

Newton, P.E., 1995. A chronic (12-month) toxicity study of methyl bromide fumigated feed in the dog. Unpublished report from Pharmaco LSR, Project Number 93-6068.

Norris, J.C., Driscoll, C.D., Hurley, J.M., 1993. Methyl Bromide: Ninety-Day Vapor Inhalation Neurotoxicity Study in CD Rats. Unpublished report from Bushy Run Research Center Project No. 92N1172.

NTP (1992). Toxicology and carcinogenesis studies of methyl bromide (CAS No. 74-83-9) in B6C3F1 mice (inhalation studies). Research Triangle Park, North Carolina, National Toxicology Program, 212 pp (Technical Report Series No. 385).

Ong, J.M., Stewart, J., Wen, Y., Whong, W., 1987. Application of SOS umu-test for the detection of genotoxic volatile chemicals and air pollutants. Environ. Mutagen. 9:171-176.

Peters, P.W.J., Verhoef, a., De liefde, A., Van Velsen, F.L., Van Soolingen, J., De Geus, D., Danse, L.H.J., Van Logten, M.J., (1982). Teratogenicity study of methyl bromide by oral administration. Bilthoven, National Institute for Public Health and Environmental Protection (RIVM report No. 618102 002) (in Dutch).

Pletsa, V., Steenwinkel, M.J., van Delft, J.H., Baan, R.A., Kyrtopoulos, S.A., 1999. Methyl bromide causes DNA methylation in rats and mice but fails to induce somatic mutations in lambda lacZ transgenic mice. Cancer Lett. 135(1):21-27.

Reichmuth, C., Noack, S. 1983. [Environmental effects of the fumigation of commodities.] Technol. Z. Getreide Mehl. Backwaren, 37: 139-144 (in German).

Reuzel, P.G.J., Dreef-Van der Meulen, H.C., Hollanders, V.M.H., Kuper, C.F., Feron, V.J. Van der Heijden, C.A. 1991. Chronic inhalation toxicity and carcinogenicity study of methyl bromide in Wistar rats. Food Chem. Toxicol., 29: 31-39

Russo, J.M., Anger, W.K., Setzer, J.V., Brightwell, W.S., 1984. Neurobehavioural assessment of chronic low-level methyl-bromide exposure in the rabbit. J. Toxicol. Environ. Health 14:247-255.

Segers, J.H.L., Temmink, J.H.M., Van den Berg, J.H.J., Wegman, R.C.C., 1984. Morphological changes in the gill of carp (*Cyprinus carpio* L.) exposed to acutely toxic concentrations of methyl bromide. Water Res., 18: 1437-1441.
Sikov, M.R., Cannon, W.C., Carr, D.B., Miller, R.A., Montgomery, L.F., Phelps, D.W. 1981. Teratologic assessment of buthylene oxide, stryene oxide and methyl bromide (Contract-No. 210-78-0025). Cincinnati, Ohio, US Department of Health and Human Services, 84 pp.

Simmon, V.F., Kauhanen, K., Tardiff, R.G., 1977. Mutagenic activity of chemicals identified in drinking water. In: Progress in genetic toxicology (Scott, D., Bridges, B.A., Sobels, F.H., eds), Elsevier/North-Holland Biomedical Press, Amsterdam, pp 249-258.

Sittisuang, P. Nakakita, H. 1985. The effect of phosphine and methyl bromide on germination of rice and corn seeds. J. Pestic. Sci., 10: 461-468.

Starratt, A.N., Bond, E.J., 1988. In vitro methylation of DNA by the fumigant methyl bromide. J. Environ. Sci. Health, 23(5):513-524.

Tanaka, S., Abuku, S.I., Seki, Y., Imamiya, S.Il, 1991. Evaluation of methyl bromide exposure on the plant quarantine fumigators by environmental and biological monitoring. Ind. Health 29:11-22.

Torkelson, T.R., 1994. Halogenated Aliphatic Hydrocarbons containing Chlorine, Bromine, and Iodine, Chapter 38 (pp. 4022-4030) in Patty's Industrial Hygiene and Toxicology: Toxicology Volume II Part E., Fourth Edition (G.D. Clayton, F.E. Clayton, eds), John Wiley & Sons, New York.

Tucker, J.D., Xu, J., Stewart, J., Ong, T., 1985. Development of a method to detect volatile genotoxins using sister chromatid exchanges. Environ. Mutagen 7:48 (abstract).

Tucker, J.D., Xu, J., Stewart, J., Baciu, P.C., Ong, T., 1986. Detection of sister chromatid exchanges induced by volatile genotoxicant. Teratog. Carcinog. Mutag. 6:14-21.

Verberk, M.M., Rooyakkers-Beemster, T., De Vlieger, M., Van Vliet, A.G.M., 1979. Bromine in blood, EEG and transaminases in methyl bromide workers. Br. J. Ind. Med. 36: 59-62.

Yamano, Y., 1991. [Experimental study on methyl bromide poisoning in mice. Acute inhalation study and the effects of glutathione as an antidote.] Jpn. J. Ind. Health 33:23-30 (in Japanese)

Wester, P.W., Canton, J.H., Dormans, J.A.M.A., 1988. Pathological effects in freshwater fish *Poecilia reticulata* (guppy) and *Oryzias latipes* (medaka) following methyl bromide and sodium bromide exposure. Aquat. Toxicol. 12:323-343.

WHO (World Health Organization). 1991. International programme on chemical safety – Environmental health criteria for methyl bromide. First draft, 173-194.

Wildlife International Report. (1993a). Methyl Bromide: 96-Hour Static Acute Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*), Final Report. Wildlife International, LTD. Project Number: 264A-105A. Conducted for Methyl Bromide Industry Panel, Chemical Manufacturers Association, MRID # 4306670.

Wildlife International Report. (1993b). Methyl Bromide: A 48-Hour Static Acute Toxicity Test with the Cladoceran (Daphnia magna), Final Report. Wildlife International, LTD. Project Number: 264A-102B. Conducted for Methyl Bromide Industry Panel, Chemical Manufacturers Association, MRID # 42932900.

Wilson, N.H., Newton, P.E., Rahi, M., Bolte, H.F., Suber, R.L., 1998. Methyl bromide: 1-year dietary study in dogs. Food Chem. Toxicol. 36(7):575-584.

ANNEXES

Annex 1

Regulations and Guidelines Applicable to Methyl Bromide (CAS# 74-83-9)

Agency	Description	Additional Information	References
EPA	CERCLA		40 CFR 302.4
	Reportable quantity	1 pound	Table
	Final reportable quantity	1,000 pounds	
EPA	Criteria for Priority Toxic Pollutants in the State of California; Human health (10^{-6} risk for carcinogens) for consumption of:		40 CFR 131.38
	Water & organism	48 µg/L	
	Organism only	4,000 µg/L	
EPA	Effluent guidelines and standards; toxic and pretreatment effluent standards	Designated toxic substance	40 CFR 401.15
EPA	Effluent guidelines and standards; electroplating point source category	Total toxic organics, which is the summation of all quantifiable values greater than 0.01 mg/L	40 CFR 413.01(i)
EPA	Effluent guidelines and standards	Priority pollutant	40 CFR 423 Appendix A
EPA	Groundwater monitoring		40 CFR 264
	Suggested method	8270 8010	Appendix IX
	Practical quantitation limits (µg/L)	10 20	
EPA	Health and safety data reporting; subject to all the provisions of Part 716		40 CFR 716.120(a)
	Effective date	06/01/87	
	Sunset date	06/01/97	
EPA	Identification and listing of hazardous waste; discarded commercial chemical products, off-specification species, container residues, and spill residues		40 CFR 261.33(f)
	Hazard waste number	U029	
EPA	Protection of stratospheric ozone; Class 1 controlled substance		40 CFR 82 Subpart A
	Ozone depletion potential[1]	0.7	Appendix A
EPA	Protection standards at inactive uranium processing sites	Listed constituent	40 CFR 192 Appendix I
EPA	Reference air concentration	0.8 $\mu g/m^3$	40 CFR 266 Appendix IV

[1]Used to determine production allowance and consumption allowance

Annex 1

Regulations and Guidelines Applicable to Methyl Bromide (CAS# 74-83-9)

Agency	Description	Additional Information	References
EPA	Residue analysis; organic compounds for which residues must be analyzed		40 CFR 266 Appendix VIII
EPA	Residue tolerances; agricultural commodities; where tolerances for inorganic bromide in or on the same raw agricultural commodity are set in two or more sections in this part, the overall quantity of inorganic bromide to be tolerated from use of two or more pesticide chemicals for which tolerances are established is the highest of the separate applicable tolerances		40 CFR 180.3(c)
EPA	Residue tolerances; agricultural commodities		40 CFR 180.123
EPA	Residue tolerances; food		40 CFR 180.521
EPA	Residue tolerances; food		40 CFR 180.522
EPA	Superfund; Extremely hazardous Reportable quantity Threshold planning quantity	 1,000 pounds 1,000 pounds	40 CFR 355 Appendix A
EPA	Toxic chemical release reporting; Community Right-to-Know Effective date	 01/01/87	40 CFR 372.65
OSHA	PEL (8 hour TWA)	20 ppm (ceiling)	29 CFR 1910.1000 Table Z-1
OSHA	Highly hazardous chemical Threshold quantity	 2,500 pounds	29 CFR 1910.119 Appendix A
USC	Hazardous air pollutant		42 USC 7412

Annex 2
Tables of Toxicity Results

Table 1 - Summary of critical subchronic toxicity studies in animals

Test Animal (No/sex/group; frequency/dur.)	Exposure (Route, Dose/ Concenc.)	Critical Effects (non-neoplastic)	Study Reference
Inhalation			
Rat (Wistar)	Inhalation	Parameters monitored: Body weights, clinical signs, neurobehavioral (12 & 28 days post-exposure), neurohistopathology	Ikeda, 1980
N.S./sex/group	0 ppm		
	200 ppm	Decreased body weight gain; neurobehavioral deficits (rotarod, spontaneous activity) at 12 and 28 days post-exposure; no histopathological changes in CNS or PNS.	
4 hr/d; 5 d/wk; 3 weeks	300 ppm	Decreased body weight gain; neurobehavioral deficits (rotarod, spontaneous activity) at 12 and 28 days post-exposure; no histopathological changes in CNS or PNS.	
Rat (Wistar)	Inhalation	Parameters monitored: Neurotransmitter levels in CNS.	Honma, 1982
N.S./sex/group	0 ppm		
	1 ppm	NOAEL	
Continuous, 3 weeks	5 ppm	Increased dopamine in striatum.	
	10 ppm	Decreased norepinephrine in hypothalamus, decreased serotonin in cortex.	
Rat (Wistar)	Inhalation	Parameters monitored: Body weights, clinical signs, selected histopathology.	WHO, 1991
N.S./sex/group	0 ppm		
	18 ppm	LOAEL; Tremors, unusual gait.	
	51 ppm	Tremors, unusual gait; micropathology in heart and lungs.	
6 hr/d, 5 d/wk for 3 weeks, continuous for last week	154 ppm	Tremors, unusual gait; micropathology in heart and lungs.	
Rat (Wistar)	Inhalation	Convulsions and pulmonary damage reported (concentrations not specified).	WHO, 1991
N.S./sex/group	0 ppm		
	33 ppm		
6 hr/d; 5 d/wk; 6 months	64 ppm		
	108 ppm		
	218 ppm		
Rat (F 344)	Inhalation		Eustis, 1988
N.S./sex/group	0 ppm		
6 hr/d, 5 d/wk for up to 6 weeks	160 ppm	Males affected more severely. Decreased survival. Target organs included: brain, nasal cavity, heart, adrenal gland, liver and testis. In CNS, neuronal necrosis in cerebral cortex, hipocampus, and thalamus.	
Rat (Sprague-Dawley)	Inhalation		Kato et al., 1986

10-12/sex/group	0 ppm		
	200 ppm	Focal necrosis and fibrosis of coronary ventricles and papillary muscle.	
4 hr/d; 5 d/wk; 6 weeks & 150 ppm for 11 weeks.	300 ppm	Focal necrosis and fibrosis of coronary ventricles and papillary muscle. Ataxia & paralysis.	
	400 ppm	Focal necrosis and fibrosis of coronary ventricles and papillary muscle. Ataxia & paralysis. Bilateral necrosis of dorso -external cortex. Testicular atrophy and sperm suppression.	
	150 ppm (11 wks)	Focal necrosis and fibrosis of coronary ventricles and papillary muscle.	
Rat (F344)	Inhalation	Sacrifice intervals at 1, 3, 5, & 8 days (10 rats/sac) and 6, 10, 17, 24, 38, 52, & 73 days (5 rats/sac)	Hurtt & Working, 1988
5 - 10/sacrifice interval	0 ppm		
6 hr/d for 5 days	200 ppm	No change in testis weights, sperm production, cauda epididymal sperm count, sperm morphology, percentage motile sperm, linear sperm velocity, or epididymal and testicular histology. Plasma testosterone reduced during exposures and immediately after but returned to normal by day 8 (3 days postexposure).	
Rat (F344)	Inhalation	Parameters monitored: Body weights, clinical signs, hematology, clinical chemistry, urinalysis, organ weights, histopathology.	NTP, 1992
10/sex/group	0 ppm		
	10 ppm	No effects reported.	
6 hr/d, 5 d/wk for 13 weeks	20 ppm	No effects reported. NOAEL.	
	40 ppm	Slight neurobehavioral and hematological changes.	
	80 ppm	Neurobehavioral and hematological changes.	
	120 ppm	Significant weight loss. Neurobehavioral and hematological changes.	
Mouse (B6C3F1)	Inhalation	Parameters monitored: Body weights, clinical signs, hematology, clinical chemistry, urinalysis, organ weights, histopathology.	NTP, 1992
10/sex/group	0 ppm		
	10 ppm	No effects reported.	
6 hr/d, 5 d/wk for 13 weeks	20 ppm	No effects reported. NOAEL.	
	40 ppm	Slight neurobehavioral and hematological changes.	
	80 ppm	Neurobehavioral and hematological changes.	
	120 ppm	Significant weight loss in males. Reduced survival in males. Neurobehavioral and hematological changes.	
Rats & mice from NTP 1992	Inhalation	Testicular damage found in both rats and mice, although levels causing lesions were not specified.	Morrissey et al., 1988
Mouse (B6C3F1)	Inhalation		Eustis, 1988
N.S./sex/group	0 ppm		
6 hr/d, 5 d/wk for up to 6 weeks	160 ppm	Males affected more severely. Decreased survival. Target organs included: brain, nasal cavity, heart, kidney, adrenal gland, liver and testis. In CNS, neuronal necrosis in internal granular layer of cerebellum.	
Oral			
Rat (Male Wistar)	Gavage in arachis oil		Danse et al., 1984
10/sex/group	0 mg/kg		
5 d/wk for 13 and 25 weeks	50 mg/kg	Forestomach: inflammation, acanthosis, fibrosis, hyperplasia (severity correlated with length of exposure). In a 10-12 week post-exposure recovery group, fibrosis and acanthosis persisted.	

Table 2 - Summary of Genotoxicity Studies for Methyl Bromide

Test Type	Test System	Dose	Results	Study Reference
In Vitro Assays for Gene Mutation – Bacterial				
Bacterial reverse mutation (Ames)	*Salmonella typhimurium*, strains TA98, TA100, TA1535, TA1537, TA1538 with & without metabolic activation	As a gas, 0.5 to 5 grams/m3 in a closed container	Mutagenic to strains TA100 and TA1535 but not others without metabolic activation.	Moriya et al., 1983
Bacterial reverse mutation (Ames)	*Salmonella typhimurium*, strain TA100 with & without metabolic activation	As a liquid, 10 to 10,000 mg/l; As a gas, 0.5 to 50 grams/m3 in a closed container	Positive in both liquid and gas assays. As liquid, mutagenic at 285 mg/l and higher. As gas, mutagenic at 1900 mg/m3 (plate assay) with or without metabolic activation. Negative with TA98 (with or w/o activation).	Kramers et al., 1985a
Bacterial reverse mutation (Ames)	*Salmonella typhimurium*, strain TA1535. Modified Ames using impingement (in situ) system with SOS umu-test.		Significant SOS response after 20 minutes impingement. SOS function detected by colorimetry of beta-galactosidase activity encoded by the lacZ gene regulated by the Umu operon.	Ong et al., 1987
Bacterial mutation	*Salmonella typhimurium*, strains TA98, TA100, TA1535 with and without metabolic activation	As a gas at concentrations of 0.1 to 0.5% atm.	Positive response at concentrations of 0.1% atm in strains TA100 &TA1535 with and without metabolic activation.	JETOC, 1997
Bacterial mutation	*Salmonella typhimurium*, strain TA100 without metabolic activation.	Tested as a liquid.	Positive response in TA100 without activation at concentrations of 0.4 ug/ml and higher.	Simmon et al., 1977
Bacterial reverse mutation (Ames)	*Escherichia coli* WP2hcr with and without metabolic activation.	As a gas, 0.5 to 5 gram/m3	Slight positive mutagenic response.	Djalali-Behzad et al., 1981
Bacterial mutation	*Escherichia coli* WP2 hcr.	0.5 to 6 umole/l	Positive response	Moriya et al., 1983
Bacterial mutation	*Klebsiella pneumoniae* mutation to streptococcus resistance, fluctuation test	As a gas, 0.95 to 19 gram/m3	Positive response at concentrations of 4.75 gram/m3 and higher with activation (not tested without activation).	Kramers et al., 1985a
Bacterial mutation	*Escherichia coli* WP2 uvrA reverse mutation with or without metabolic activation.	As a gas at concentrations of 0.1 to 0.5% atm.	Positive response at concentrations of 0.2% atm with or without metabolic activation.	JETOC, 1997
In Vitro Assays for Gene Mutation - Mammalian Cells				
Mouse Lymphoma Assay	L5178Y-TK+/- cells - gaseous methyl bromide in air-tight bottles.	30 to 30,000 mg/m3	Positive response. Dose-related increase in 6-thioguanine- and bromodeoxyuridine resistant mutants.	Kramers et al., 1985a
Unscheduled DNA Synthesis	Primary cultures of rat hepatocytes - gaseous methyl bromide in air-tight bottles - autoradiography.	10,000 to 30,000 mg/m3	No unscheduled DNA synthesis found.	Kramers et al., 1985a
Unscheduled DNA Synthesis	Primary cultures of rat hepatocytes - gaseous methyl bromide over culture media	70% MeBr in air for 3 hr (700,000 ppm)	No unscheduled DNA synthesis found.	McGregor, 1981
Sister Chromatid Exchange	Human lymphocytes in culture - gaseous exposure for 100 seconds.	As a gas at 4.3%	SCE increased from 10 to 16.8 per cell.	Tucker et al., 1981
Sister Chromatid Exchange	Human Go lymphocytes in culture - aqueous solution exposure	Aqueous solutions of 0 - 24 ul MeBr per liter.	Increased SCEs and chromosome aberrations with metabolic activation.	Garry et al., 1990

Sister Chromatid Exchange	Human lymphocytes in culture - aqueous solution exposure	Aqueous solutions of 19.5 ug MeBr per milliliter.	Increased SCEs without metabolic activation in glutathione non-conjugators. Negative response in lymphocytes from glutathione conjugators.	Hallier et al., 1993
DNA Binding	Binding to calf thymus DNA *in vitro*	Aqueous solutions of 48 ug MeBr per milliliter.	Increased covalent binding without metabolic activation (not tested with metabolic activation).	Starratt and Bond, 1988.
In Vitro Assays for Transformation				
Cell Transformation	Syrian hamster embryo cells - transformation by AS/adenovirus in sealed chambers - exposure to methyl bromide as a gas.	4,000 to 16,000 mg/m3 for 2 or 20 hours.	No enhanced transformation.	Hatch et al., 1983
In Vivo Assays for Genetic Toxicity				
Bone marrow cytogenetics	Rat (Sprague-Dawley)	0, 20, 70, ppm 7 hr/d for 1 to 5 days.	No significant increases in chromosomal aberrations.	McGregor, 1981
Micronucleus Assay	Mice (B6C3F1) exposed to gaseous MeBr.	10 to 200 ppm 6 h/d, 5 d/wk for 14 days or 10 to 120 ppm for 13 weeks.	At 200 ppm for 14 days, SCEs and micronuclei were increased in bone marrow cells of male and female mice (more marked in females). No increases in SCEs or micronuclei at 120 ppm for 13 weeks.	NTP, 1992.
Micronucleus Assay	Mice (BDF1) exposed to gaseous MeBr.	0, 154, 200, 260, 338, or 440 ppm 6 hr/d, 5 d/wk for 14 days	At 200 ppm for 14 days, SCEs and micronuclei were increased in bone marrow cells of male and female mice (more marked in females). No increases in SCEs or micronuclei at 120 ppm for 13 weeks.	Ikawa et al., 1986.
Micronucleus Assay	F-344 rats exposed to gaseous MeBr.	0, 154, 200, 260, 338, or 440 ppm 6 hr/d, 5 d/wk for 14 days	At 200 ppm for 14 days, SCEs and micronuclei were increased in bone marrow cells of male and female mice (more marked in females). No increases in SCEs or micronuclei at 120 ppm for 13 weeks.	Ikawa et al., 1986.
Micronucleus Assay	Humans: 32 MeBr fumigation workers (28 non-exposed referents) - Micronuclei measured in lymphocytes and oropharyngeal cells.	Exposure: 4 or more hr fumigation work w/MeBr during previous 2 wks (assumed conc. 1-5 ppm).	Trend observed toward increased micronuclei in peripheral lymphocytes that was not statistically significant and which did not show a dose - response.	Calvert et al., 1998
Hprt frequency	Humans: 32 MeBr fumigation workers (28 non-exposed referents) - hprt measured in peripheral lymphocytes.	Exposure: 4 or more hr fumigation work w/MeBr during previous 2 wks (assumed conc. 1-5 ppm).	In non-smokers, trend toward increased hprt variant frequencies found in peripheral lymphocytes that was not statistically significant and which did not show a dose-response.	Calvert et al., 1998
Dominant Lethal Assay	Sprague-Dawley (CD) male rats exposed to gaseous MeBr.	0, 20, 70 ppm 7 hr/d, 5 d/wk for 5 days.	Negative - no effect on 1) frequency of pregnancy, 2) number of corpora lutea or implantations per pregnancy, or 3) frequency of early deaths in offspring.	McGregor, 1981
Sex-linked Recessive Lethal Assay	*Drosophila melanogaster*	70 - 750 mg/m3, 5 d/wk, 3 wks	Negative.	Kramers et al., 1985b
Drosophila Somatic Wing-spot Assay	*Drosophila melanogaster* third instar larvae	0 - 20,000 mg/m3 for 1 hr.	Small & large, single & twin spot alterations found. Single spot alterations included several types and twin spot alterations occurred from mitotic recombination.	Katz, 1985/87

DNA damage (testicular DNA alkaline elution)	Rat (Sprague-Dawley)	0, 75, 150, 250 ppm 6 hr/d for 5 days	Damage to testicular DNA at 250 ppm; negative at 75 and 150 ppm.	Bentley, 1994
DNA alkylation	Mouse (CBA) - both intraperitoneal and inhalation	14 C-radiolabeled MeBr for 4 hr.	Positive response. Covalent binding of DNA in spleen and liver cells and hemoglobin alkylation.	Djalali-Behzad et al., 1981
DNA alkylation	F-344 rat oral or inhalation	14 C-radiolabeled MeBr orally or by inhal. for 6 hr.	Positive response. Covalent binding of DNA in liver, lung, stomach, and forestomach cells.	Gansewendt et al., 1991
DNA alkylation	Rat	14 C-radiolabeled MeBr orally single or repeated doses	Methylated DNA adducts (N7- and/or O6-methylguanine) found in glandular stomach, forestomach, liver, and other tissues at comparable levels. Repeated dosing caused marked decrease in O6-alkylguanine-DNA alkyltransferase (repair enzyme).	Plesta et al., 1999
DNA alkylation	Mouse (lambda lacZ transgenic)	14 C-radiolabeled MeBr orally single or repeated doses	Methylated DNA adducts (N7- and/or O6-methylguanine) found in glandular stomach, forestomach, liver, and other tissues at comparable levels. No mutagenesis found in lacZ transgene in any tissue up to 14 days post-treatment with up to 50 mg/kg (single dose) or 25 mg/kg MeBr (up to 10 daily doses).	Plesta et al., 1999

Table 3 - Summary of critical chronic toxicity/carcinogenicity studies in animals

Test Animal (No/sex/group; frequency/dur.)	Exposure (Route, Dose/ Concenc.)	Critical Effects (non-neoplastic)	Tumor Incidence (in target organs)	Study Reference
Inhalation				
Rat (Wistar)	Inhalation			Reuzel et al., 1991
50-60/sex/group	0 ppm			
	3 ppm	Hyperplasia of nasal epithelium.	No increased tumor incidence.	
	30 ppm	Hyperplasia of nasal epithelium.	No increased tumor incidence.	
6 hr/d; 5 d/wk; 29 months	90 ppm	In both sexes: Decreased body weights & survival; Myocardial degeneration; Hyperplasia of nasal epithelium; Esophageal hyperkeratosis (males only); forestomach lesions.	No increased tumor incidence.	
Rat (F344/DuCrj)	Inhalation			JML, 1992
86/sex/group	0 ppm			
	4 ppm	Inflammation of nasal epithelium.	No increased tumor incidence.	
	20 ppm	Inflammation of nasal epithelium.	No increased tumor incidence.	
6 hr/d; 5 d/wk; 2 years	100 ppm	In both sexes: No effect on survival; Decreased body weights; Altered hematological parameters; Inflammation, necrosis, metaplasia in nasal epithelium (more pronounced in males).	No increased tumor incidence.	
Mouse (Crj:BDF1)	Inhalation			JML, 1992
50/sex/group	0 ppm			
	4 ppm	No effects reported.	No increased tumor incidence.	
	16 ppm	No effects reported.	No increased tumor incidence.	
6 hr/d; 5 d/wk; 2 years	64 ppm	In both sexes: No effect on survival; Reduced body weight gain; Slight atrophy of granular layer of cerebellum; altered blood chemistries.	No increased tumor incidence.	
Mouse (B6C3F1)	Inhalation			NTP, 1992
50/sex/group	0 ppm			
	10 ppm	Hyperplasia of nasal epithelium.	No increased tumor incidence.	
	33 ppm	Hyperplasia of nasal epithelium.	No increased tumor incidence.	
6 hr/d; 5 d/wk; 2 years	100 ppm	In both sexes: Decreased body weights & survival; Increased thromi and myocardial degeneration; Hyperplasia, necrosis & metaplasia of nasal epithelium; tremors & limb paralysis; decrements in neurobehavioral tests, cerebral degeneration.	No increased tumor incidence.	

Oral - Dietary

Rat (Sprague-Dawley)	Microencapsulated MeBr in diet			Mertens, 1997
(?/sex/dose)	0 ppm basal diet			
Study Duration: Two years	0 ppm (microcapsules w/o MeBr)			
	0.5 ppm (0.02 mg/kg-d males; 0.03 mg/kg-d females)	No toxicological effects noted.	No increased tumor incidence.	
	2.5 ppm (0.11 mg/kg-d males; 0.15 mg/kg-d females)	No toxicological effects noted.	No increased tumor incidence.	
	50 ppm (2.20 mg/kg-d males; 2.92 mg/kg-d females)	No toxicological effects noted.	No increased tumor incidence.	
	250 ppm (11.1 mg/kg-d males; 15.1 mg/kg-d females)	Reduced food consumption, body weight gain, mean body weights in 1st 18 months of study in males and females.	No increased tumor incidence.	
Rat (Fischer 344)	Diet fumigated w/MeBr			Mitsumori et al., 1990
60/sex/dose	0 ppm (basal diet)			
Study Duration: Two years	500 ppm total Br as KBr			
	80 ppm Br from MeBr	No toxicological effects noted.	No increased tumor incidence.	
	200 ppm Br from MeBr	No toxicological effects noted.	No increased tumor incidence.	
	500 ppm Br from MeBr	Males only: slightly depressed body weight	No increased tumor incidence.	
Dogs (Beagle)	Diet fumigated w/MeBr			Newton, 1995
?/sex/dose	0 ppm (basal diet)			
Dogs were fed diets fumigated with MeBr 5d/wk	0.5 ppm (0.06 mg/kg-d males; 0.07 mg/kg-d females)	No toxicological effects noted.	No increased tumor incidence.	
Study Duration: 12 months	1.5 ppm (0.13 mg/kg-d males; 0.12 mg/kg-d females)	No toxicological effects noted.	No increased tumor incidence.	
	5 ppm (0.28 mg/kg-d males; 0.27 mg/kg-d females)	No toxicological effects noted.	No increased tumor incidence.	

Oral - Gavage

Rat (Wistar)	Daily gavage in arachis oil (5 d/wk)			Danse et al., 1984
10/sex/dose	0 mg/kg			
Study Duration: 90 days	0.4 mg/kg	No effects reported.	No increased tumor incidence.	
	2 mg/kg	Marked inflammation, hyperplasia, and hyperkeratosis.	No increased tumor incidence.	
	10 mg/kg	Marked inflammation, hyperplasia, and hyperkeratosis.	No increased tumor incidence.	
	50 mg/kg	Marked inflammation, hyperplasia, and hyperkeratosis.	Initially reported squamous cell carcinoma in forestomach of 13 rats (7 males; 6 females) and two papillomas of forestomach of two males. In a subsequent re-examination of slides, previously considered malignant lesions were inflammation and hyperplasia.	
Rat (Wistar)	Daily gavage in arachis oil (5 d/wk)			Boorman et al., 1986
15/sex/dose	0 mg/kg			
Study Duration: 13, 17, 21, or 25 weeks	50 mg/kg	Inflammation, acanthosis, fibrosis, and pseudoepitheliomatous hyperplasia in forestomach. By wk 25, more severe hyperplastic lesions.	Early squamous carcinoma in one rat.	
Rat (Wistar)	Daily gavage in arachis oil (5 d/wk)			Boorman et al., 1986
15/sex/dose	0 mg/kg			
Study Duration: 13, 17, or 21 wks followed by up to 25 wks observ.	50 mg/kg	Lesions similar to Danse et al. Recovery animals showed regression of hyperplasia in forestomach.	No tumors reported.	
Rat	Daily gavage in peanut oil (5 d/wk)	Some 90 day animals observed for 30-60 thereafter to assess reversibility.		Hubbs & Harrington, 1986
?/sex/dose	0 mg/kg			
Study duration: 30, 60, 90, or 120 days	25 mg/kg	Gross & microscopic lesions including, ulcers, fibrosis, pseudoepitheliomatous hyperplasia (hyperkeratosis, acanthosis, and epithelial peg formation). Changes were reversible.	No tumors reported.	
	50 mg/kg	Gross & microscopic lesions including, ulcers, fibrosis, pseudoepitheliomatous hyperplasia (hyperkeratosis, acanthosis, and epithelial peg formation). Changes were reversible.	No tumors reported.	

Table 4 - Summary of critical reproductive/developmental toxicity studies in animals

Test Type	Test Animal (#/sex/group; freq/dur.)	Exposure (Route, Dose/ Concenc.)	Critical Effects	Study Reference
Reproductive Toxicity				
Two-Generation Reproduction	Rat (Sprague-Dawley); 25/sex/dose	Inhalation; 0, 3, 30, 90 ppm 6 hr/d, 5 d/wk over premating, gestation, & lactation periods		American Biogenics Corporation, 1986
		0 ppm		
		3 ppm	No effects noted. NOAEL for F2 generation.	
		30 ppm	Decreased body weight gain in F2 generation. LOAEL for F2's. NOAEL for F0's and F1's.	
		90 ppm	Decreased body and some organ weight gains (no associated histopathology) in F0 & F1 generations. No effect on reproductive organ appearance, weights, or histopathology in any generations. No effect on reproductive performance. F2 showed reduced growth rates but no developmental abnormalities. LOAEL for F0 and F1 generations.	
Reproductive Organ Toxicity	Rat (F344); No./sex/group not specified; part of 13- week NTP study.	Inhalation; 0, 30, 60, 120 ppm; 6 hr/d, 5 d/wk for 13 weeks.	Effects were not reported by exposure level in this comprehensive review of 50 NTP studies. Decrease in epididymis and cauda epididymis weights, increase in testes weights, decrease in sperm motility. No effects on estrus cycle length.	Morrissey et al., 1988.
Reproductive Organ Toxicity	Mouse (B6C3F1); No./sex/group not specified; part of 13-week NTP study.	Inhalation; 0, 30, 60, 120 ppm; 6 hr/d, 5 d/wk for 13 weeks.	Effects were not reported by exposure level in this comprehensive review of many NTP studies. Increase in epididymis and testes weights, decrease in sperm density, increase in abnormal sperm.	Morrissey et al., 1988.
Reproductive Organ Toxicity	Male Wistar rat, No./sex/group not specified.	Inhalation; 0, 70 ppm, 7 hr/d, 5 d/wk for up to 40 days.	Reproductive performance was not impaired.	Hardin et al., 1981; Sikov et al., 1981
Reproductive Organ Toxicity	Male Rat (F 344), No./group not specified.	Inhalation; 0, 160 ppm, 6 hr/d, 5 d/wk for up to six weeks.	Degeneration of spermatocytes and late stage spermatids characterized by separation and sloughing as well as formation of intratubular multinucleated giant cells. Degeneration of spermatogenic epithelium included moderate to severe loss of spermatogenic epithelial components.	Eustis et al., 1988
Reproductive Organ Toxicity	Male mice (B6C3F1), No./ group not specified.	Inhalation; 0, 160 ppm, 6 hr/d, 5 d/wk for up to six weeks.	Slight testicular degeneration occurred at high frequency.	Eustis et al., 1988
Reproductive Organ Toxicity	Male rat (Sprague Dawley), 10-12/group	Inhalation; 0, 200, 300, or 400 ppm, 4 hr/d, 5 d/wk for up to 6 weeks.	Adverse testicular effects in 1 of 10 rats exposed to 200 ppm, 6 of 10 at 300 ppm and 6 of 8 at 400 ppm. Unilateral atrophy of seminal epithelium, incomplete spermatogenesis, giant cells in seminal tubules. Necrotic spermatocytes occurred in seminal fluid without spermatozoa in tubules of epididymis adjacent to atrophied testes.	Kato et al., 1986.
Reproductive Organ Toxicity	Male rat (F344), 10/group during exposure period, 5/group during recovery period.	Inhalation; 0 or 200 ppm, 6 hr/d, 5 d/wk for 5 days. Followed by recovery up to 68 days.	No effect on testis weight, daily sperm production, cauda epididymal sperm count, sperm morphology, percent motile sperm, linear sperm velocity, and epididymal and testicular histology. Transient decrease in plasma testosterone and testicular nonprotein sulfhydryl concentrations during exposure.	Hurtt and Working, 1988
Reproductive Organ Toxicity	Male mouse (B6C3F1), 10/group.	Inhalation; 0, 20, or 70 ppm, 7 hr/d, 5 d/wk for 5 days.	No effect on spermatozoa found.	McGregor, 1981
Developmental Toxicity				
Terato-genicity	Female rat (Wistar); 39-45/group.	Inhalation; Pregestational/post-gestational: 0/0, 0/20, 0/70, 20/0, 20/20, 70/0, 70/70 ppm 7 hr/d, 5 d/wk for 3 weeks (sacrificed on gestational day 19).	No clinical evidence of maternal toxicity. No embryo- or fetotoxicity. No developmental abnormalities.	Hardin et al., 1981; Sikov et al., 1981

Terato-genicity	Female rabbit (New Zealand); 25/group.	Inhalation; Pregentational/post-gestational: 0/0, 0/20, 0/70, 20/0, 20/20, 70/0, 70/70 ppm 7 hr/d, 5 d/wk for 3 weeks (sacrificed on gestational day 19).	No clinical evidence of maternal toxicity. No embryo- or fetotoxicity. No developmental abnormalities. Severe neurotoxicity with excessive mortality observed in 70 ppm dams characterized by convulsions and paresis in hindlimbs after one week of exposure.	Hardin et al., 1981; Sikov et al., 1981
Terato-genicity (probe study)	Female rabbit (New Zealand); No/group unspecified.	Inhalation; Gestational days 7 - 19: 0, 10, 30, 50, 70, or 140 ppm 6 hr/d, 5 d/wk for 3 weeks (sacrificed on gestational day 28).	Maternal toxicity at 140 ppm including lethargy, right-sided head tilt, ataxia, and lateral recumbency. The neurobehavioral symptoms were associated with multifocal areas of inflammation of the meninges of the brain and/or bilaterally symmetrical necrosis or spongiosis in the midbrain. No embryotoxicity reported.	Breslin et al., 1990a
Terato-genicity	Female rabbit (New Zealand); 25/group.	Inhalation; Gestational days 7 - 19: 0, 20, 40, or 80 ppm 6 hr/d, 5 d/wk for 3 weeks (sacrificed on gestational day 28).	No embryo, fetal, or maternal effects noted at 20 or 40 ppm. Moderate to severe maternal toxicity at 80 ppm including decreased weight gain or absolute body weights, lethargy, right-sided head tilt, ataxia, and lateral recumbency (brain histopathology not reported). At 80 ppm, effects on fetuses included decreased weights, increased incidence of fused sternebrae, and increased incidence of anomalies including, predominantly, missing gallbladder or missing caudal lobe of lung.	Breslin et al., 1990b
Terato-genicity	Female rabbit; No/group unspecified.	Gavage in peanut oil; Gestational days 5 - 20: 0, 0.5, 5, 25, or 50 mg/kg.	Maternal toxicity at 25 and 50 mg/kg . Total resorption of embryos at 50 mg/kg. No skeletal or soft tissue abnormalities noted at 25 mg/kg or lower doses.	Peters et al., 1982

Table 5 - Summary of critical neurotoxicity studies in animals

Test Animal (No/sex/group; frequency/dur.)	Exposure (Route, Dose/ Concenc.)	Critical Effects	Study Reference
Inhalation			
Rat (Sprague-Dawley)	Inhalation		Norris et al., 1993
N.S./sex/group	0 ppm		
	30 ppm	Mean (but not relative) brain weights lower in females than controls. No histolopathological findings in brain.	
6 hr/d; 5 d/wk; 13 weeks	70 ppm	Lower body weights in females. Slightly decreased forelimb grip strength in males; decreased motor activity in females. See section 3.9 for other findings.	
	140 ppm	Two male rats died exhibiting convulsions, tremors, hyperactivity, rapid respiration and salivation. Body weights were significantly lower than controls. See section 3.9 for other findings.	
Rat (Sprague-Dawley)	Inhalation	Neurobehavioral parameters evaluated 3 hours, 7 days, and 14 days post-exposure. Neurobehavioral parameters included functional observational battery and motor activity.	Driscoll & Hurley, 1993
N.S./sex/group	0 ppm		
	30 ppm	No changes found.	
Single 6 hour exposure	350 ppm	Three hours post-exposure, decreased arousal, increased drooping of eyes, piloerection, decreased rearing, depressed body temperature, markedly decreased motor activity. No changes at later time points.	
Rat (Wistar)	Inhalation	Parameters monitored: Body weights, clinical signs, neurobehavioral (12 & 28 days post-exposure), neurohistopathology	Ikeda, 1980
N.S./sex/group	0 ppm		
	200 ppm	Decreased body weight gain; neurobehavioral deficits (rotarod, spontaneous activity) at 12 and 28 days post-exposure; no histopathological changes in CNS or PNS.	
4 hr/d; 5 d/wk; 3 weeks	300 ppm	Decreased body weight gain; neurobehavioral deficits (rotarod, spontaneous activity) at 12 and 28 days post-exposure; no histopathological changes in CNS or PNS.	
Rat	Inhalation	Parameters monitored: Saccharin water taste aversion.	Miyagawa, 1982
N.S./sex/group	0 ppm		
	25 ppm		
4 hours	50 ppm		
	100 ppm	Aversion to saccharin water increased with methyl bromide exposure in a concentration dependent manner.	
Mice (male Swiss-Webster)	Inhalation - nose only	Parameters monitored: Behavioral tests including single-task passive avoidance, rotarod	Alexeeff et al., 1985
N.S./sex/group	0 ppm		
	220 ppm	No behavioral effects.	
1 hour	440 ppm	No behavioral effects.	
	700 ppm	No behavioral effects.	
	900 ppm	No behavioral effects.	
	1000 ppm	Abnormal clinical signs. No effect on recall ability to perform a single-task passive-avoidance test at this concentration or less.	
	1200 ppm	Decreased recall ability to perform a single-task passive-avoidance test.	
	1500 ppm	Decreased recall ability to perform a single-task passive-avoidance test. Decreased motor coordination (rotarod test).	
Mouse (B6C3F1)	Inhalation		NTP, 1992
50/sex/group	0 ppm		
	10 ppm	Hyperplasia of nasal epithelium. No increased tumor incidence.	
	33 ppm	Hyperplasia of nasal epithelium. No increased tumor incidence.	
6 hr/d; 5 d/wk; 2 years	100 ppm	In both sexes: Decreased body weights & survival; Increased thromi and myocardial degeneration; Hyperplasia, necrosis & metaplasia of nasal epithelium; tremors & limb paralysis; decrements in neurobehavioral tests, cerebral degeneration. No increased tumor incidence.	
Rabbit (New Zealand)	Inhalation	Parameters monitored: Nerve conduction velocity of ulnar and sciatic nerves, eye-blink reflex.	Anger et al., 1981
2/sex/group	0 ppm		
7.5 hr/d, 4 d/wk for 4 weeks	65 ppm	Decreased eye-blink reflex magnitude. Decreased nerve conduction velocity. Hind limb paralysis. Decreased body weight gain.	

		weight gain.	
Rabbit (New Zealand)	Inhalation	Parameters monitored: Nerve conduction velocity of ulnar and sciatic nerves, eye-blink reflex.	Russo et al., 1984
2/sex/group	0 ppm		
7.5 hr/d, 4 d/wk for 8 months	27 ppm	No effects noted.	
	65 ppm	After a 6 - 8 week recovery of Anger et al. (1981) group, partial recovery was observed.	
Rat (Male Sprague-Dawley)	Inhalation	Parameters monitored: Neurological parameters in addition to other general toxicological parameters.	Kato et al., 1986
10-12/group	0 ppm		
	150 ppm -11 wks		
4 hr/d; 5 d/wk; 6 to 11 weeks	200 ppm - 6 wks		
	300 ppm - 6 wks	Ataxia and paralysis.	
	400 ppm - 6 wks	Ataxia and paralysis. Necrosis in bilateral regions of dorso-external cortex of cerebral hemisphere.	
Rat	Single subcu. Injection	Parameters monitored: Electroencephalographic activity and sleep-wakefulness	Tanaka et al., 1988
N.S./sex/group	0 mg/kg		
	5 mg/kg	Changes in wakefulness, non-REM and REM sleep, and circadian rhythms in dose-related manner.	
single injection	15 mg/kg	Changes in wakefulness, non-REM and REM sleep, and circadian rhythms in dose-related manner.	
	45 mg/kg	Changes in wakefulness, non-REM and REM sleep, and circadian rhythms in dose-related manner.	
	135 mg/kg	Slowing of EEG frequency in wakefulness stage and spike-wave activity. Not seen at lower doses.	
Rat (Sprague-Dawley)	Inhalation	Parameters monitored: Neurotransmitter levels in CNS.	Honma, 1982
5-6/sex/group	0 ppm		
	10 ppm	NOAEL	
24 hr	??? ppm		
	100 ppm	Decreased norepinephrine in hypothalamus & cortex/hippocampus, little effect on dopamine, serotonin or acetylcholine.	
	120 ppm	Decreased norepinephrine in hypothalamus & cortex/hippocampus, little effect on dopamine, serotonin or acetylcholine.	
Rat (Sprague-Dawley)	Inhalation	Parameters monitored: Neurotransmitter levels in CNS.	Honma, 1982
5-6/sex/group	0 ppm		
	1 ppm	NOAEL	
Continuous, 3 weeks	5 ppm	Increased dopamine in striatum.	
	10 ppm	Decreased norepinephrine in hypothalamus & cortex/hippocampus, little effect on dopamine, serotonin or acetylcholine.	
Rat (Sprague-Dawley)	Inhalation	Parameters monitored: Locomotor activity in activity cages.	Honma et al., 1985
3/group	0 ppm		
	63 ppm	No effect on locomotor activity.	
8 hours	125 ppm	No effect on locomotor activity.	
	188 ppm	Decreased locomotor activity.	
	250 ppm	Strongly decreased locomotor activity.	
Rat (Sprague-Dawley)	Inhalation	Parameters monitored: Thiopental induced reduction of righting reflex.	Honma et al., 1985
8/group	0 ppm	Only 2 of 8 rats had increased righting reflex times after thiopental injection (60 mg/kg)	
	63 ppm	All had increased righting reflex times, which were longer than controls.	
8 hours	125 ppm	All had increased righting reflex times, which were longer than controls.	
Rat (Sprague-Dawley)	Inhalation	Parameters monitored: Neurotransmitter levels in CNS.	Honma, 1987a, Honma et al., 1987b
N.S./sex/group	0 ppm		
	31 ppm		
8 hours	82 ppm	Decreased dopamine, norepinephrine & increased metabolites: homovanillic acid, 3-methoxy-4-hydroxyphenyl glycol in various regions of CNS. Largest changes in striatum; maximum effect time was 0 to 2 hours post exposure.	

	125 ppm	Decreased dopamine, norepinephrine & increased metabolites: homovanillic acid, 3-methoxy-4-hydroxyphenyl glycol in various regions of CNS. Largest changes in striatum; maximum effect time was 0 to 2 hours post exposure.	
	250 ppm	Decreased dopamine, norepinephrine & increased metabolites: homovanillic acid, 3-methoxy-4-hydroxyphenyl glycol in various regions of CNS. Largest changes in striatum; maximum effect time was 0 to 2 hours post exposure.	
Rat (Sprague-Dawley)	Inhalation	Parameters monitored: Tyrosine hydroxylase levels in striatum, hypothalamus, frontal cortex, midbrain, and medulla oblongata.	Honma, 1991
N.S./sex/group	0 ppm		
	16 ppm		
8 hours	??? ppm		
	??? ppm		
	250 ppm	Concentration dependent decrease in tyrosine hydroxylase activity. Hypothalamus most affected.	
Rat (Sprague-Dawley)	CNS perfusion (MeBr in 10% ethanol/90% artificial cerebrospinal fluid)	Parameters monitored: Dopamine and serotonin metabolites in various CNS regions.	Honma, 1992
N.S./sex/group	0 ppm		
	0.1 ug MeBr/ul vehicle	Reduced 5-hydroxyindoleacetic acid (serotonin metabolite).	
Perfusion via ventricle probe at rate of 2.5 ul/min	0.5 ug MeBr/ul vehicle	Striatum perfusate: Increased 3,4-dihydroxyphenyl acetic acid and homovanillic acid (dopamine metabolites) that persisted after perfusion stopped; decreased 5-hydroxyindoleacetic acid (serotonin metabolite) that returned to normal.	
	1 ug MeBr/ul vehicle	Striatum perfusate: Increased 3,4-dihydroxyphenyl acetic acid and homovanillic acid (dopamine metabolites) that persisted after perfusion stopped; decreased 5-hydroxyindoleacetic acid (serotonin metabolite) that returned to normal.	
Rat (Wistar)	Inhalation	Parameters monitored: Neurohistopathology of CNS & PNS.	Furuta et al., 1993
N.S./sex/group	0 ppm		
	290 ppm	No neurohistopathology reported in rats exposed for 8 weeks.	
6 hr/d, 3 d/wk for 3-8 weeks	500 ppm	Axonal degeneration of myelinated fibers at cervical level of fasciculus gracilis; necrosis/atrophy of neurons in caudate-putamen, thalamus, cingulate cortex after exposure of 10 to 18 days.	
Rat (F344)	Inhalation	Parameters monitored: regional brain glutathione-S-transferase activity, glutathione levels, monoamine and amino acid levels, neurohistopathology.	Davenport et al., 1992
10/sex/group	0 ppm		
6 hr/d for 5 days	150 ppm	Glutathione was depleted and glutathione transferase activity was decreased in frontal cortex, caudate nucleus, hippocampus, brain stem, and cerebellum. No changes in monoamines but aspartic acid and glycine were increased in frontal cortex and aspartic acid in the cerebellum. No histopathology found.	
Dog (Beagle) Pilot Study - Phase 1	Inhalation	Exposure-level range finding study. Six to seven hour single exposure to methyl bromide by inhalation.	Newton, 1994a
1/sex/group	0 ppm		
6-7 hours	233 ppm (1 male)	Ataxia, depression	
	314 pm (1 male, 1 female)	Ataxia, depression	
	345/350 ppm (1 male, 1 female)	Ataxia, depression	
	394 ppm (1 female)	Ataxia, depression	
Dog (Beagle) Pilot Study - Phase 2	Inhalation	Exposure-level range finding study. Six to seven hour single exposure to methyl bromide by inhalation.	Newton, 1994a
1/sex/group	0 ppm		
7 hr/day up to 4 days	55 ppm (1 male, 1 female)	No effects.	
	156 ppm (1 male, 1 female)	Decreased activity on day 3 and irregular gait on day 4.	

	268 ppm (1 male, 1 female)	Ataxia, depression	
	283 ppm (2 males, 1 female)	Ataxia, depression	
Dog (Beagle) 4-Week Inhalation Study	Inhalation	Subchronic inhalation for 4 weeks.	Newton, 1994b
4/sex/group	0 ppm		
7 hr/day, 5 d/wk for 4 weeks	5 ppm	No effects.	
	10 ppm	No effects.	
	25 ppm	No effects.	
	50 ppm	No effects.	
	100 ppm	No effects.	
Dog (Beagle) 4-Week Inhalation Study	Inhalation	Subchronic inhalation for 2 weeks. Dogs from above study used in this study. After four weeks, four of the controls (two females and two males) and all dogs in the 5 ppm group continued the test for an additional two weeks and the exposure concentration for the 10 ppm group was increased to 150 ppm.	Newton, 1994b
4/sex/group	0 ppm		
7 hr/day, 5 d/wk for 4 weeks	5 ppm	Neurological evaluation revealed no treatment related effects.	
	150 ppm	Severe body weight loss over the first few days of exposure. After five or six exposures, evaluation by a veterinary neurologist revealed ataxia, a base-wide stance, intention tremor, nystagmus, marked depression, and inability (unwillingness) to stand and perform postural responses. Due to the severity of these effects, the dogs were sacrificed. Significant neurological microscopic findings were found in this group consisting of vacuoles in the granular layer of the cerebellum.	
Mouse (B6C3F1) 16/sex/group	Inhalation 0 ppm		Eustis, 1988
	160 ppm	Males affected more severely. Decreased survival. In CNS, neuronal necrosis in internal granular layer of cerebellum.	
Rat	Inhalation		Irish, 1940
135 total	0 ppm		
	17 ppm	No effects reported.	
6 hr/d, 5 d/wk for up to 6 months	33 ppm	No effects reported.	
	65 ppm	No effects reported. NO AEL.	
	100 ppm	Paralysis, lung injury, and death.	
	200 ppm	Paralysis, lung injury, and death.	
Rabbit (New Zealand White)	Inhalation		Irish, 1940
104 total	0 ppm		
	17 ppm	No effects reported. NOAEL.	
6 hr/d, 5 d/wk for up to 6 months	33 ppm	Paralysis, lung injury, and death.	
	65 ppm	Paralysis, lung injury, and death.	
	100 ppm	Paralysis, lung injury, and death.	
	200 ppm	Paralysis, lung injury, and death.	
Guinea Pig	Inhalation		Irish, 1940
98 total	0 ppm	No effects reported.	
	17 ppm	No effects reported.	
6 hr/d, 5 d/wk for up to 6 months	33 ppm	No effects reported.	
	65 ppm	No effects reported.	
	100 ppm	No effects reported. NOAEL.	
	200 ppm	Paralysis, lung injury, and death.	
Monkey	Inhalation		Irish, 1940
13 total	0 ppm	No effects reported.	
	17 ppm	No effects reported.	
6 hr/d, 5 d/wk for up to 6 months	33 ppm	No effects reported.	
	65 ppm	Limb paralysis.	
	100 ppm	Paralysis and convulsions	
	200 ppm	Paralysis, lung injury, and death.	

Table 6 - Summary of critical aquatic and terrestrial toxicity studies

Type of Test	Test Subject (No/sex/group; frequency/dur.)	Parameters, concentr.	Critical Effects (non-neoplastic)	Study Reference
Acute Aquatic Toxicity – Invertebrates				
Acute toxicity (50% immobilization)	*Daphnia magna*	Exposed in static system to concentrations of 1.2, 2.2, 3.5, 5,8 and 9.8 mg/l for 48 hours.	EC50 (48 hr) = 2.6 mg/l; EC50 (24 hr) = 4.5 mg/l. NOEC = 1.2 mg/l. Toxic endpoint: immobilization.	Wildlife International, 1993b
Acute toxicity (50% immobilization)	*Daphnia magna*	Exposed in static system for 48 hours.	EC50 (48 hr) = 2.2 mg/l; Delayed swimming at 1.7 mg/l. Toxic endpoint: immobilization.	Canton et al., 1980
Acute Aquatic Toxicity - Vertebrates (fish)				
LC50	*Oncorhynchus mykiss* (Rainbow trout)	Exposed in static system for 96 hours.	LC50 (96 hr) = 3.9 mg/l; LC50 (48 hr) = 5.6 mg/l; LC50 (24 hr) = 7.7 mg/l; LOEC = 4.8 mg/l; NOEC = 2.9 mg/l	Wildlife International, 1993a
LC50	*Lepomis macrochirus* (Bluegill sunfish)	Exposed in static system for 96 hours.	LC50 (96 hr) = 11 mg/l	Dawson et al., 1975/77
LC50	*Menidia beryllina* (Tidewater silverside)	Exposed in static system for 96 hours.	LC50 (96 hr) = 12 mg/l	Dawson et al., 1975/77
LC50	*Cyprinus carpio* (Carp)	Exposed in static system for 4 hours.	LC50 (4 hr) = 17 mg/l; damage to gill epithelium.	Segers et al., 1984
LC50	*Oryzias latipes* (Medaka)	Exposed in static system for 96 hours.	LC50 (96 hr) = 0.7 mg/l; abnormal behavior at 0.4 mg/l.	Canton et al., 1980
LC50	*Poecilia reticulata* (guppy)	Exposed in static system for 96 hours.	LC50 (96 hr) = 0.8 mg/l; delayed swimming at 0.3 mg/l; immobilization at 0.6 mg/l.	Canton et al., 1980
LC50	*Poecilia reticulata* (guppy)	Exposed in semi-static system to concentrations of 0, 0.56, 1.0, and 1.8 mg/l for 96 hours.	Limited mortality at 1.0 and 1.8 mg/l. LCLo = 1.8 mg/l. NOEC = 0.56 mg/l.	Wester et al., 1988
LC50	*Oryzias latipes* (Medaka)	Exposed in semi-static system to concentrations of 0, 0.56, 1.0, and 1.8 mg/l for 96 hours.	Limited mortality at 1.8 mg/l. LCLo = 1.8 mg/l. NOEC = 1.0 mg/l.	Wester et al., 1988
Chronic Aquatic Toxicity - Invertebrates, including effects on reproduction, embryo and larva				
Effects on reproduction	*Daphnia magna*	Exposed in static system for 7 days.	Reproduction inhibited at 0.1 mg/l. *Data not considered reliable.*	Canton et al., 1980
Chronic Aquatic Toxicity - Vertebrates (fish), including effects on reproduction, embryo and larva				
Effects on reproduction and mortality	*Poecilia reticulata* (Guppy)	Exposed for 1 and 3 months to 0.032, 0.1, 0.32, 1.0, or 3.2 mg/l	100% mortality of embryos within 3 days at 3.2 mg/l; 100% mortality within 3 weeks at 1.0 mg/l. Significant wt loss at 0.32 mg/l. No histopathology noted. *Data not considered reliable*	Wester et al., 1988
Effects on reproduction and mortality	*Oryzias latipes* (Medaka)	Exposed for 1 and 3 months to 0.032, 0.1, 0.32, 1.0, or 3.2 mg/l	100% mortality of embryos before hatching at 1.8 or 3.2 mg/l; No histopathology noted. *Data not considered reliable.*	Wester et al., 1988
Terrestrial Toxicity - Non-mammalian species (including birds)				
Oral LD50	Northern Bobwhite	5/sex/dose exposed to 31.3, 62.5, 125, 250, 500, or 1000 mg/kg	LD50 = 73 mg/kg (95% CL: 62.5 to 125 mg/kg). No mortality at 31.3 mg/kg.	Campbell & Beavers, 1994
Effect of fumigation of diets	Hens (Rhode Island Reds)	Fed from hatchlings on diets fumigated with 800 mg.h/liter and 2000 mg.h/liter.	Body weight, egg weight & number not affected. Sexual maturity may have been slightly delayed. Meat tainted.	Cooper et al., 1978; Griffiths et al., 1978
Effect of fumigation on egg hatchability.	Hens (Rhode Island Reds)	Chicken eggs fumigated with 32 g/m3 MeBr (time unspecified)	No effects on fertility or hatchability of hens' eggs previously fumigated.	Devaney and Beerwinkle, 1982
Terrestrial Toxicity – Plants				
Effect on germination	*Oryza sativa* (Rice Japonica) and Zea mays (Corn) seeds	Fumigation at concentrations up to 4 mg/liter.	No effect on germination in rice with moisture content of 11% or less. Decreased germination in rice seeds with higher moisture content. Also methyl bromide adversely affected germination at higher temperatures. Corn was resistant to methyl bromide fumigation	Sittisuang and Nakakita, 1985

Effect on leaf appearance	*Lactuca sativa capitanta* (Lettuce) and *Nasturtium officinale* (Water Cress)	Fumigation at concentrations between 4 and 1400 mg/m3 for 72 hours.	Lettuce leaves yellowed but water cress was resistant even to the highest concentration.	Richmuth and Noack, 1983

SIDS DOSSIER
METHYL BROMIDE
CAS No. 74-83-9

Sponsor Country: USA

DATE: February 2001
Updated: February 2002

SIDS PROFILE

1.01 A.	**CAS No.**	74-83-9
1.01 C.	**CHEMICAL NAME (OECD Name)**	Methyl Bromide
1.01 D.	**CAS DESCRIPTOR**	Bromomethane
1.01 G.	**STRUCTURAL FORMULA**	CH_3Br
	OTHER CHEMICAL IDENTITY INFORMATION	
1.5	**QUANTITY**	
1.7	**USE PATTERN**	(a) Soil fumigation (b) Quarantine and commodity fumigation (c) Structural fumigation (d) Chemical intermediate (e) Organic synthesis, usually as a methylating agent (f) A low-boiling solvent, e.g. for extracting oils from nuts, seeds, and flowers
1.9	**SOURCES AND LEVELS OF EXPOSURE**	1. World consumption in 1990 was over 67 million kg
ISSUES FOR DISCUSSION		

SIDS SUMMARY

CAS NO: 74-83-9 Methyl Bromide		Information	OECD Study	GLP	Other Study	Estimation Method	Acceptable	SIDS Testing Required
STUDY		**Y/N**	**Y/N**	**Y/N**	**Y/N**	**Y/N**	**Y/N**	**Y/N**
PHYSICAL-CHEMICAL DATA								
2.1	Melting Point	Y	N	?	Y	Y	Y	N
2.2	Boiling Point	Y	N	?	Y	Y	Y	N
2.3	Density	Y	N	?	Y	Y	Y	N
2.4	Vapour Pressure	Y	N	?	Y	Y	Y	N
2.5	Partition Coefficient	Y	N	?	Y	Y	Y	N
2.6	Water Solubility	Y	N	Y	Y	Y	Y	N
	pH and pKa values	N	N	-	-	-	-	N
2.12	Oxidation: Reduction potential	N	N	-	-	-	-	N
OTHER P/C STUDIES RECEIVED		Y	Y	?	Y	N	Y	N
ENVIRONMENTAL FATE and PATHWAY								
3.1.1	Photodegradation	Y	N	?	Y	N	Y	N
3.1.2	Stability in water	Y	N	?	Y	N	Y	N
3.2	Monitoring data	Y	N	?	Y	N	Y	N
3.3	Transport and Distribution	Y	N	?	Y	N	Y	N
3.5	Biodegradation	Y	Y	Y	Y	N	Y	N
OTHER ENV FATE STUDIES RECEIVED		Y	Y	?	Y	N	Y	N
ECOTOXICITY								
4.1	Acute toxicity to Fish	Y	Y	Y	Y	N	Y	N
4.2	Acute toxicity to Daphnia	Y	Y	Y	Y	N	Y	N
4.3	Toxicity to Algae	Y	N	Y	Y	N	Y	N
4.5.2	Chronic toxicity to Daphnia	Y	N	?	N	N	Y	N
4.6.1	Toxicity to Soil dwelling organisms	N	N	?	-	-	-	N
4.6.2	Toxicity to Terrestrial plants	Y	Y	?	Y	N	Y	N
4.6.3	Toxicity to Birds	Y	Y	?	Y	N	Y	N
OTHER ECOTOXICITY STUDIES RECEIVED		Y	Y	?	Y	N	Y	N
TOXICITY								
5.1.1	Acute Oral	Y	Y	Y	Y	N	Y	N
5.1.2	Acute Inhalation	Y	Y	?	Y	N	Y	N
5.1.3	Acute Dermal	N	N	N	N	N	-	N
5.4	Repeated Dose	Y	Y	Y	Y	N	Y	N
5.5	Genetic Toxicity *in vitro*							
	. Gene mutation	Y	Y	Y	Y	N	Y	N
5.6	. Chromosomal aberration	Y	Y	Y	Y	N	Y	N
5.8	Genetic Toxicity *in vivo*	Y	Y	?	Y	N	Y	N
5.9	Reproduction Toxicity	Y	Y	?	Y	N	Y	N
5.11	Development / Teratogenicity	Y	Y	?	Y	N	Y	N
	Human experience	Y	Y	?	Y	N	Y	N
OTHER TOXICITY STUDIES RECEIVED		Y	Y	Y	Y	N	Y	N

1. GENERAL INFORMATION

1.01 SUBSTANCE INFORMATION

A. CAS-Number

74-83-9

B. Name *(IUPAC name)*

Methyl Bromide; bromomethane

C. Name *(OECD name)*

Methyl Bromide; bromomethane

D. CAS Descriptor

Bromomethane

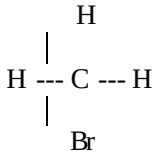
E. EINECS-Number

200-813-2

F. Molecular Formula

$CH_3 Br$

G. Structural Formula



H. Substance Group

Not applicable

I. Substance Remark

J. Molecular Weight

94.94

1.02 OECD INFORMATION

A. Sponsor Country: OECD

B. Lead Organization:

Name of Lead Organization: United States
Contact person: Oscar Hernandez (7403M)
Address: 1300 Pennsylvania Ave, NW

Washington DC 20460

C. Name of responder

Name: Susan Lewis, American Chemistry Council
Address: Arlington, Va

1.1 GENERAL SUBSTANCE INFORMATION

A. Type of Substance

element [ ]; inorganic [ ]; natural substance [ ]; organic [X]; organometallic [ ]; petroleum product [ ]

B. Physical State

gaseous [X]; liquid [ ]; solid **[ ]**

C. Purity

> 99.5%

1.2 SYNONYMS

monobromomethane

1.3 IMPURITIES

traces of chloromethane

1.4 ADDITIVES

Chloropicrin (2%)
Amyl acetate (0.3%)

1.5 QUANTITY

Quantity 100,000 to 500,000 tonnes
Remarks: Marking is non-confidential
Reference: Chemical News, 1990

1.6 LABELLING AND CLASSIFICATION

Labelling
Type:
Specific limits:
Symbols: T, Xi, N, Mut3 and Xn
Nota:
R-phrases: (23/25) Toxic by inhalation and if swallowed
(36/37/38) Irritating to eyes, respiratory system and skin
(40)

(48/20) Harmful: danger of serious damage to health by prolonged exposure in contact with skin.
(50)
(R50/53/59) Dangerous for the ozone layer Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment; dangerous for the ozone layer.

S-phrases: (1/2) Keep locked up and out of reach of children
(15) Keep away from heat
(27) Take off immediately all contaminated clothing
(36/37/38) Wear suitable protective clothing, gloves and eye/face protection
(38) In case of insufficient ventilation, wear suitable respiratory equipment
(45) In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)
(59) Dangerous for the ozone layer
(61) May cause harm to the unborn child

Remarks:

Classification

Type: As in directive 67/548/EEC
Category of danger: Toxic (T)
R-phrases: (23) Toxic by inhalation
Remarks: Non-confidential marking

Type: As in directive 67/548/EEC
Category of danger: Irritant (Xi)
R-phrases: (36/37/38) Irritating to eyes, respiratory system and skin
Remarks: Non-confidential marking

Type: As in directive 67/548/EEC
Category of danger: Dangerous to the environment (N)
R-phrases: (50) Dangerous for the ozone layer
(50/53) Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment
Remarks: Non-confidential marking

Type: Carcinogenicity
Remarks: IARC classification: Not classifiable as to its carcinogenicity to humans (Group 3): *inadequate evidence* in humans; *limited evidence* in experimental animals.

Type: Carcinogenicity

Remarks: US EPA classification: Group D: Not classifiable as to human carcinogenicity.

1.7 USE PATTERN

A. General

	Type of Use:	**Category:**
(a)	main industrial use	Non dispersive use Chemical Industry: used in organic synthesis
(b)	main	Non dispersive use

industrial use Low-boiling solvent use

Remarks: Presence in the Swedish Product Register (2001)

Products/Consumer Porducts	Main Use
2	Pesticide

Reference:

1.8 OCCUPATIONAL EXPOSURE LIMIT VALUE

Exposure limit value
Type: TLV – 8 hr TWA (US)
Value: 1 ppm (3.9 mg/m3)
Length of exposure period: 8 hr Time Weighted Average
Remark: Can be absorbed through skin
Reference: US ACGIH

Short term exposure limit value
Type: PEL Ceiling (US)
Value: 20 ppm (80 mg/m3)
Length of exposure period: Ceiling
Frequency: Single
Remark: Can be absorbed through skin
Reference: US OSHA

Exposure limit value
Type: limit value; ambient air
Value: (1 mg/m3)
Length of exposure period: ?
Remark:
Reference: Denmark

1.9 SOURCES OF EXPOSURE

(a)
Source: Media of release: Water from a production site
Remarks: The methyl bromide concentration in a sample of surface seawater has been given as 140 ng/liter. The average value of bromide ion concentrations in samples of coastal water near the North Sea was 18.4 mg/liter; the level of bromide ion in inland rivers was much lower except in regions where fumigation with methyl bromide was practiced, or, in areas of industrial pollution. There could be a risk of increased methyl bromide or bromide ion content in water in shallow wells near methyl bromide fumigation operations.
Reference:

(b)
Source: Media of release: Air from a production site
Remarks: Methyl bromide concentrations, measured in the air in unpopulated areas, range from 40 to 100 ng/m^3 in the Northern hemisphere being higher than those in the Southern hemisphere. Seasonal differences have been found in some studies. In urban and industrial areas, the levels are much higher, with average values up to 800 ng/m^3 and with some readings as high as 4µg methyl bromide/m^3. In the proximity of fields and greenhouses, during fumigation and aeration, the concentrations of methyl bromide are

considerably higher, values of 1-4 mg/m^3 being measured in one study at distances of up to 20 m from a greenhouse, a few hours after injection; a tenth of this value was found 4 days later.

References:

(c)
Source: Foods and crops
Remarks: Levels of methyl bromide or bromide may be elevated in foods that have either grown on soils previously treated with methyl bromide or have been fumigated post-harvest. On rare occasions, bromide levels in fresh vegetables, grown on previously fumigated with methyl bromide, have been observed to exceed the permitted residue level. Leafy vegetables can take up relatively large amounts of bromide ion without phytotoxic symptoms. Other crops, such as carnations, citrus seedlings, cotton, celery, peppers, and onions are sensitive to methyl bromide fumigation.

(d)
Source: Emission from motor car exhausts
Remarks: Engines operating on "leaded" petrol, containing ethylene dibromide as an additive contribute a much larger amount of methyl bromide to urban atmospheres than engines with catalytic converters burning "unleaded" fuel. About 45 tonnes of methyl bromide is produced from car exhaust in the United Kingdom annually. Methyl bromide concentrations in the range of 90-190 µg/m^3 have been measured in the exhaust emissions of motor vehicles using leaded petrol with EDB. Between 7000 and 18,000 tonnes of methyl bromide may be emitted annually from car exhaust.
References: Bell, 1988; Baumann & Heumann, 1987

(e)
Source: Emission from the ocean
Remarks: Perhaps the greatest source of methyl bromide is that naturally emitted from the oceans.
References: Howard, 1989.

1.10 ADDITIONAL REMARKS

A. Options for disposal

(a)
Remarks: Methyl bromide may be disposed by controlled incineration with scrubbing and ash disposal facilities. Rotary kiln incinerators operating at a temperature of 820 to 1600°C and residence times of seconds or more for liquids and gases are sufficient to dispose of methyl bromide. Fluidized bed incinerators operating at temperatures of 450 to 980°C with residence times of seconds or longer also may be used to dispose of methyl bromide.
References: HSDB, 1999

B. Other remarks

Remarks: In Sweden the Product Register (2001) indicated two (2) consumer products in which the main use was in Pesticide. No other specific information was given as to the % of methyl bromide in the product.
Reference: Sweden Product Register (2001).

2. PHYSICAL-CHEMICAL DATA

2.1 MELTING POINT

-93.66 ^{o} C
Reference: Merck Index (12th Ed), 1996

2.2 BOILING POINT

(a)
Value: 3.56°C
Pressure: at 1 atm
Decomposition: Yes **[ ]** No **[ ]** Ambiguous **[ ]**
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Remarks:
Reference: Matheson Gas Data Book, 1980; Windholtz, 1983

2.3 DENSITY (Relative density)

(a)
Type: Bulk density **[ ]**; Density **[ ]**; Relative Density **[X];** Vapor Density **[ ]**
Value: 3.974 kg/m^3
Temperature: 20°C
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Remarks:
Reference: Windholz, 1983

(b)
Type: Bulk density **[ ]**; Density **[ ]**; Relative Density **[X];** Vapor Density **[ ]**
Value: 1730 kg/m^3
Temperature: 0°C
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Remarks:
Reference: Matheson Gas Data Book, 1980; Windholz, 1983; Hommel, 1984

(c)
Type: Bulk density **[ ]**; Density **[ ]**; Relative Density **[ ]**; Vapor Density **[X]**
Value: 3.27
Temperature: 20°C
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Remarks: rel,; air = 1
Reference: Hommel, 1984

2.4 VAPOUR PRESSURE

(a) **Preferred result**
Value: 1893 kPa (1420 mmHg)
Temperature: 20 °C
Method: calculated **[ ]**; measured [ ]

GLP: Yes **[ ]** No **[ ]** ? **[X]**
Remarks:
Reference: Windholz, 1983; Stenger, 1978

(b)
Value: 1250 mmHg
Temperature: 20 °C
Method: calculated **[ ]**; measured [ ]

GLP: Yes **[ ]** No **[ ]** ? **[X]**
Remarks:
Reference: Hawley, 1987

(c)
Value: 1420 mmHg
Temperature: 20 °C
Method: calculated **[ ]**; measured [ ]

GLP: Yes **[ ]** No **[ ]** ? **[X]**
Remarks:
Reference: Merck Index (12th Edition), 1996

2.5 PARTITION COEFFICIENT $\log_{10}P_{ow}$

(a)
Log Kow: = 1.94 ± 0.31
Temperature: 25°C
Method: calculated **[ ]**; measured **[X]**
FIFRA Guideline 63-11
GLP: Yes **[ ]** No **[X]** ? **[ ]**
Remarks: Fulfilled California data requirements, not GLP audited
Reference: Bolsa Research Laboratory, 1990

(b)
Log Pow (Log Kow): 1.19
Temperature:
Method: calculated **[**X**]**; measured **[ ]**
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Remarks:
Reference: Hansch & Leo, 1979; Sangster, 1989

2.6 WATER SOLUBILITY *(if more than one, identify the recommended value)*

A. Solubility

(a) **Preferred result**
Value: 16.1 g/l
Temperature: 25°C
Description: Miscible**[ ]**; Of very high solubility **[ ]**;
Of high solubility **[ ]**; Soluble **[ ]**; Slightly soluble **[ ]**;
Of low solubility **[ ]**; Of very low solubility **[ ]**; Not soluble **[ ]**
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Remarks: Apparent solubility
Reference: Great Lakes Corporation, 1992

(b)
Value: 12 g/l
Temperature: 20°C
Description: Miscible **[ ]**; Of very high solubility **[ ]**;
Of high solubility **[ ]**; Soluble **[ ]**; Slightly soluble **[ ]**;
Of low solubility **[ ]**; Of very low solubility **[ ]**; Not soluble **[ ]**
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Remarks: Apparent solubility
Reference: Dow Chemical Co, 1992

(c)
Value: 15.4 g/l
Temperature: 25°C
Description: Miscible **[ ]**; Of very high solubility **[ ]**;
Of high solubility **[ ]**; Soluble **[ ]**; Slightly soluble **[ ]**;
Of low solubility **[ ]**; Of very low solubility **[ ]**; Not soluble **[ ]**
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Remarks: Forms a voluminous crystalline hydrate $CH_3Br.2OH_2O$ below 4°C
Reference: Wilhelm et al., 1977; Windholz, 1983

(d)
Value: 18.5 g/l
Temperature: 20 °C
Method:
GLP: Yes **[ ]** No **[X]** ? **[ ]**
Remarks:
Reference: Wilhelm, et al., 1977

(e)
Value: 18.00 g/l
Temperature: 20 °C
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Remarks:
Reference: Mackay & Shiu, 1981

(f)
Value: 16 g/l
Temperature: 20 °C
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Remarks:
Reference: Atochem, 1987

(g)
Value: 0.15 g/100 ml, 1.0 g/100 ml, 1.5 g/100 ml, 1.8 g/100 ml
Temperature: 25 ° C
Method: FIFRA Guideline 63-8
GLP: Yes **[X]** No **[ ]** ? **[ ]**
Remarks: Experimental design has encountered technical difficulties due to the gaseous state of methyl bromide and additional efforts may not produce results that improve existing data.
Reference: Literature Search; Great Lakes Corporation & The Dow Chemical Company, 1992

B. pH Value, pKa Value
Not applicable

2.7 FLASH POINT *(liquids)*

Value:	194 °C
Type of test:	Closed cup **[ ]**; Open cup **[ ]**; Other **[ ]**
Method:	
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Remarks:	Burns with difficulty
Reference:	Hommel, 1984

2.8 AUTO FLAMMABILITY *(solid/gases)*

Value:	537 ^{o} C
Pressure:	. . . hPa
Method:	
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Remarks:	
Reference:	Elf Atochem, Material Safety Data Sheet, 1993

2.9 FLAMMABILITY

(a)

Results:	Extremely flammable **[ ]**; Extremely flammable - liquified gas **[ ]**; Highly Flammable **[ ]**; Flammable **[ ]**; Non flammable **[X]**; Flammable in air **[ ]**; Contact with water liberates highly flammable gases **[ ]**; Other **[ ]**
Method:	
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Remarks:	13.5 – 14.5 % by volume
Reference:	Matheson Gas Data Book, 1980

(b)

Results:	Extremely flammable **[ ]**; Extremely flammable - liquified gas **[ ]**; Highly Flammable **[ ]**; Flammable **[ ]**; Non flammable **[X]**; Spontaneously flammable in air **[ ]**; Contact with water liberates highly flammable gases **[ ]**; Other **[ ]**
Method:	
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Remarks:	10 – 16 % by volume. It burns in oxygen.
Reference:	NFPA, 1984; Windholz, 1983

2.10 EXPLOSIVE PROPERTIES

(a)

Results:	Explosive under influence of a flame**[ ]**; More sensitive to friction than m-dinitrobenzene **[ ]**; More sensitive to shock than m-dinitrobenzene **[ ]**; Not explosive **[ ]**; Other **[X]**

Method:

GLP: Yes **[ ]** No **[ ]** ? **[X]**
Remarks: Explosions upon contact with aluminum and dimethyl sulfoxide
Reference: NFPA, 1984

(b)
Results: Explosive under influence of a flame**[ ]**;
More sensitive to friction than m-dinitrobenzene **[ ]**;
More sensitive to shock than m-dinitrobenzene **[ ]**; Not explosive **[ ]**;
Other **[X]**
Method:

GLP: Yes **[ ]** No **[ ]** ? **[X]**
Remarks: May form explosive mixture with air at concentrations of 10.1 to15.4% v/v
Reference: Elf Atochem, Material Safety Data Sheet, 1993.

2.11 OXIDIZING PROPERTIES

No Data Found.

2.12 OXIDATION: REDUCTION POTENTIAL

No Data Found.

2.13 ADDITIONAL DATA

A. Partition co-efficient between soil/sediment and water (Kd)

(a)
Value: Koc = 172, 174, 164 for Naaldwijk loamy sand, Aalsmeer loam, and Boskoop peaty clay, respectively
Method:

GLP: Yes **[ ]** No **[ ]** ? **[X]**
Remarks: For gaseous methyl bromide
Reference: Howard, 1989

(b)
Value: Log Koc = 2.1
Method:

GLP: Yes **[ ]** No **[ ]** ? **[X]**
Remarks: For gaseous methyl bromide and based on a measured water solubility of 9.5×10^{-3}.
Reference: Howard, 1989

B. Other data

(a)
Type: Soil absorption of methyl bromide from water
Exposure time: 24 hours
Method: This study was conducted in two phases. In the first phase, methyl bromide was absorbed onto soil from aqueous solutions of varying concentrations of

methyl bromide. In a second phase, desorption was measured. Results indicated that 89 to 97% of absorbed methyl bromide was desorbed by a single mixing with water.

GLP: Yes **[X]** No **[ ]** ?**[ ]**
Test Substance: 99.5% purity
Remarks: Conducted according to FIFRA Guideline 163-1
Reference: WIL Research Laboratory, 1986

(b)
Type of value: Henry's Law Constant
Results: 6.24×10^{-3} atm m3/mol
Remarks: Calculated using atmospheric pressure
Reference: Howard, 1989

(c)
Type of value: Henry's Law Constant
Results: .0533 kPa m^3/mol
Remarks: Calculated using atmospheric pressure
Reference: Windholz, 1983

(d)
Type of value: UV absorption
Results: max. 202 nm
Remarks:
Reference: Robbins, 1976b; Molina et al., 1982; Gillotay et al., 1989

(e)
Type of value: Solubility in petroleum based solvents
Results: Mean weight solubility: 228.1 g/l in K1 kerosene at 23°C and 239.5 g/l in Shell SOL 340HT at 20°C
GC analyzed mean solubility: 224.8 g/l for K1 kerosene and 251.7 g/l for Shell SOL 340HT
Remarks: Conducted according to 40 CFR 158.190
Reference: WIL Research Laboratory, 1994

(f)
Type of value: Solubility in other solvents
Results: Freely soluble in alcohol, chloroform, ether, carbon disulfide, carbon tetrachloride, and benzene
Remarks:
Reference: Windholz, 1983

(g)
Type: Corrosivity to Metal - Average corrosion rate data
Exposure time: 168 hours
Method:
GLP: Yes **[X]** No **[ ]** ?**[ ]**
Test Substance: Three prepared coupons were subjected to the conditions possible within a storage container; one receiving sub-surface liquid exposure, one suspended at the liquid/gas interface, one exposed to vapor only. A volume of 850 ml of test material was added.
Remarks: Corrosion rate was determined by the ASTM method G31-72. General surface corrosion was witnessed on all coupons with no signs of pitting or localized attack, even at engraved identifying marks. This test showed that methyl bromide can be safely stored in the containers that are currently specified.
Reference: Spires & Coffey, 1993

3. ENVIRONMENTAL FATE AND PATHWAYS

3.1 STABILITY

3.1.1 PHOTODEGRADATION

(a)
Type: Air **[X]**; Water **[ ]**; Soil **[ ]**; Other **[ ]**
Light source: Sun light **[ ]**; Xenon lamp **[ ]**; Other **[ ]**
Light spectrum: . . . . . nm
Relative intensity: . . . . . .
Spectrum of substance:
Concentration of Substance:
Temperature: -8 & 25 °C
Indirect Photolysis:
Type of sensitizer: Hydroxy radicals
Concentration of sensitizer: 5×10^5, 1×10^6, and 2×10^6 molecules/cm^3
Rate constant (radical): not reported (cm3/molecule*sec)
Degradation:
Half life: at OH concentrations of 5×10^5 molecules/cm^3
1.6 years at –8°C
1.1 years at 25°C
at OH concentrations of 1×10^6 molecules/cm^3
0.79 years at –8°C
0.57 years at 25°C
at OH concentrations of 2×10^6 molecules/cm^3
0.40 years at –8°C
0.29 years at 25°C
Method: calculated **[ ];** measured **[X]**
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance: Methyl bromide
Remarks: Direct photolysis is not expected to be significant in the troposphere since methyl bromide does not absorb appreciably at wavelengths <290 nm. Upward diffusion of methyl bromide to the stratosphere is expected to be the primary removal mechanism from the troposphere. In the stratosphere, photolysis is expected to be the major removal mechanism.
Reference: Howard, 1989

(b)
Type: Air **[ ]**; Water **[ ]**; Soil **[X]**; Other **[ ]**
Light source: Sun light **[ ]**; Xenon lamp **[ ]**; Other **[ ]**
Light spectrum: . . . . . nm
Relative intensity: . . . . . .
Spectrum of substance:
Concentration of Substance:
Temperature: 20 °C
Direct photolysis:
Half life: 10 days
Degradation: 6-14% (weight/weight) per day
Quantum yield: . . . . . .
Indirect Photolysis:
Type of sensitizer:
Concentration of sensitizer: . . . . . .
Rate constant (radical): cm3/molecule*sec
Degradation:
Method: calculated **[ ];** measured **[ ]**

GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks: This study evaluated direct photolyis on soil.
Reference: Herzel & Schmidt, 1984; Daelemans, 1978

(c)
Type: Air **[ ]**; Surface Water **[X]**; Soil **[ ]**; Other **[ ]**
Light source: Sun light **[ ]**; Xenon lamp **[ ]**; Other **[ ]**
Light spectrum: simulated light conditions
Relative intensity:
Spectrum of substance;
Concentration of Substance: 0.001 Molar
Temperature: 25 °C
Direct photolysis:
Half life: 258.6 hours (pH 5.0), 255.8 hours (pH 7.0), 361.0 hours (pH 9.0)
Degradation: . . . . . % (weight/weight) after . . . . . .(exposure time)
Quantum yield:
Indirect Photolysis:
Type of sensitizer:
Concentration of sensitizer:
Rate constant (radical): cm3/molecule*sec
Degradation: . . . . . % after . . . . . (exposure period)
Method: calculated **[ ]** measured **[ ]**
GLP: Yes **[X]** No **[ ]** ? **[ ]**
Test substance:
Remarks: This study evaluated direct photolyis on soil
Reference: Bolsa Research, 1993

3.1.2 STABILITY IN WATER

(a)
Type: Abiotic (hydrolysis) **[X]**; biotic (sediment) **[ ]**
Half life: 20 days
Degradation: Hydrolysis rate constant = 4.09 x 10-7 at pH 7 at 25°C after an unreported exposure time
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance: Methyl bromide
Remarks:
Reference: Howard, 1989

(b)
Type: Abiotic (hydrolysis) **[X]**; biotic (sediment) **[ ]**
Half life: 26.7 days
Degradation: Hydrolysis rate constant = 3.09 x 10-7 at an unreported pH at 25°C after an unreported exposure time
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance: Methyl bromide
Remarks:
Reference: Howard, 1989

3.1.3 STABILITY IN SOIL

(a)
Remarks: Koc values for various soil types indicate that methyl bromide will

not bind strongly to soil, although dry soils absorb more strongly relative to moist soils. Thus, methyl bromide may readily evaporate into the air or leach into ground water.

Reference: Howard, 1989

(b)

Remarks: Data submission from CMA Methyl Bromide Industry Panel (conducted by Great Lakes Corporation) to EPA. Data indicate that methyl bromide is not appreciably aerobically or anaerobically degraded in soil, but that loss is due to evaporation into the atmosphere. This study was submitted to US EPA to support FIFRA DCI. It is a collection of field dissipation data and secondary references.

Reference: Great Lakes Corporation, 1986

3.2 MONITORING DATA (ENVIRONMENT)

(a)

Type of Measurement: Background **[X]**; At contaminated site **[ ]**; Other **[ ]**
Media: Air
Results: 41 to 256 ppt (159 to 1005 ng/m^3) at 10 urban sites in the U.S. circa 1975-1980
Remarks:
Reference: HSDB, 1999

(b)

Type of Measurement: Background **[X]**; At contaminated site **[ ]**; Other **[ ]**
Media: Sea water
Results: 1.5 to 3.9 ug/liter
Remarks: Atlantic Ocean water off Dorchester, England, 1975
Reference: HSDB, 1999

(c)

Type of Measurement: Background **[X]**; At contaminated site **[ ]**; Other **[ ]**
Media: Sea water
Results: Mean of 1.2 ng/liter (ug/liter?)
Remarks: Eastern Pacific Ocean water (40 deg N to 30 deg S latitude)
Reference: HSDB, 1999

(d)

Type of Measurement: Background **[X]**; At contaminated site **[ ]**; Other **[ ]**
Media: Shore water
Results: Mean of 0.14 ug/liter
Remarks: Near Point Reyes, CA
Reference: HSDB, 1999

(e)

Type of Measurement: Background **[X]**; At contaminated site **[ ]**; Other **[ ]**
Media: Effluent water
Results: < 10 ug/liter
Remarks: in 1.4% of 1317 Storet samples (U.S.)
Reference: Howard, 1989

(f)

Type of Measurement: Background **[X]**; At contaminated site **[ ]**; Other **[ ]**
Media: Air
Results: 159 – 1005 ng/m3 (41 – 259 ppt)

Remarks: 10 major U.S. urban centers
Reference: HSDB, 1999

(g)
Type of Measurement: Background **[X]**; At contaminated site **[ ]**; Other **[ ]**
Media: Air
Results: 19 ng/m^3 – rural Northwest
1.9 – 3.5 ng/m^3 - rural Washington State
35 – 57 ng/m^3 monthly average range – Barrows, Alaska
Remarks: Rural concentrations
Reference: Howard, 1989

(h)
Type of Measurement: Background **[ ]**; At contaminated site **[ ]**; Other **[X]**
Media: Food
Remarks: Residues were found in a variety of grains, vegetables, and fruits fumigated with methyl bromide, including: wheat, rice, flour, corn, raisins, sorgum, cottonseed meal, and peanut meal. Methyl bromide concentrations were < 1 mg/kg within a few days. No residues were found after fumigation in asparagus, avocados, peppers, or tomatoes. Traces of methyl bromide were found in wheat, flour and other products fumigated with 370 mg/m3 after 9 days aeration.
Reference: HSDB, 1999; Howard, 1989; IARC 1982

3.3 TRANSPORT AND DISTRIBUTION BETWEEN ENVIRONMENTAL COMPARTMENTS INCLUDING ESTIMATED ENVIRONMENTAL CONCENTRATIONS AND DISTRIBUTION PATHWAYS

3.3.1 TRANSPORT

(a)
Type: Adsorption **[ ]**: Desorption **[ ]**; Volatility **[X]**: Other **[ ]**
Media: Water-Air
Method: Evaporation
Results: Rapid transfer from water to air
Remarks: The average half-life for methyl bromide in surface water, under field conditions, was calculated to be 6.6 h at a water temperature of about 11 °C, the decline being attributed to degradation and volatilization processes.
References: Wegman et al., 1981

(b)
Type: Adsorption **[ ]**: Desorption **[ ]**; Volatility **[X]**: Other **[X]**
Media: Water-Soil-Air
Method:
Results: Degradation to bromide ion in soil; evaporation to air
Remarks: Methyl bromide is nearly four times heavier than air, and much of that used as a soil fumigant diffuses throughout the surface to depths of 60-20 cm, some of it being hydrolyzed to bromide ion or decomposed by microorganisms, the remainder (45-90%) eventually being dissipated into the atmosphere. The rate of degradation of methyl bromide in soil was about 6-14-% per day at 20 °C. A review of the mechanisms of breakdown of methyl bromide indicated the unimolecular nucleophilic substitution should be the major mechanism for the hydrolysis of methyl bromide in water. The reaction of dissolved methyl bromide with the soil organic matter involved the transference of the methyl group to carboxy groups and N- and S-containing groups of amino acids and proteins of soil organic matter. The

fumigant is degraded in shallow topsoils, although the fumigant is relatively persistent in the underlying strata, where its diffusion into the atmosphere is no longer possible.

References: Herzel and Schmidt, 1984; Moje, 1960; Maw & Kempton, 1973; Brown et al., 1979; Daelemans, 1978

3.3.2 THEORETICAL DISTRIBUTION (FUGACITY CALCULATION)

No data located.

3.4 IDENTIFICATION OF MAIN MODE OF DEGRADABILITY IN ACTUAL USE

Media: Air

Remarks: There are three possible removal mechanisms for methyl bromide within the atmosphere: (a) reaction with the hydroxyl radical and other chemical species in the troposphere; (b) precipitation in the troposphere; and (c) transport and subsequent photolytic and chemical removal in the stratosphere. Removal by precipitation is thought to be unimportant. The most significant removal process is that of the reaction of methyl bromide with the hydroxyl radical in the troposphere (estimated removal time of 2 ± 0.5 years). A minor removal process is transport to the stratosphere followed by reaction with the hydroxyl radical and photodissociation with an estimated lifetime of about 30-40 years. Between 20 and 25 km above sea level, photodissociation is of equal importance to loss by diffusion and reaction with the hydroxyl radical. Above this altitude, photolysis plays the most important role.

Reference: UNEP, 1992; Robbins, 1976a

3.5 BIODEGRADATION

(a)

Type: aerobic **[X]**; anaerobic **[X]**

Inoculum: adapted **[ ]**; non-adapted **[X]**; sterilized **[X]**

Concentration: 200000 ppm methyl bromide

Medium: water **[ ];** water-sediment **[ ];** soil **[X];** sewage treatment **[ ]**

Degradation: See remarks

Results: See remarks

Kinetics: See remarks

Method: EPA Pesticide Assessment Guidelines, Subdivision N, Chemistry: Environmental Fate, (Guideline Nos. 162-1 & 162-2)

GLP: Yes **[ ]** No **[ ]** ? **[X]**

Test substance: Methyl Bromide

Remarks: Methyl bromide gas (200,000 ppm) was introduced into airtight stainless steel boxes containing two types of soil, sandy loam and clay loam, under 1) aerobic and anaerobic and 2) sterile and non-sterile conditions. For sandy loam soil, aerobic loss half-life was 35 hours (unsterile) and 47 hours (sterile); anaerobic loss half-life was 144 hr (unsterile) and 80 hr (sterile).

For clay loam soil, aerobic loss half-life was 3.8 hours (unsterile) and 2.5 hours (sterile); anaerobic loss half-life was 39 hr (unsterile) and 34 hr (sterile). Sterilization of soil was thought to introduce additional absorption sites, possibly accounting for shorter halflives in sterilized clay. First order differences were not seen between aerobic and anaerobic tests. Introduction of methyl bromide was considered to inhibit microbial activity in non-sterilized soils.

Reference: Radian Report, 1988.

(b)

Type:	aerobic **[X]**; anaerobic **[ ]**
Inoculum:	Activated sludge
Concentration:	5.01 mg/l
Medium:	water **[ ];** water-sediment **[ ];** soil **[X];** sewage treatment **[ ]**
Degradation:	17% after 28 days
Results:	
Method:	Closed bottle
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Test substance:	Methyl Bromide
Remarks:	Sludge 2 mg/liter
Reference:	IUCLID, 1994

(c)

Test substance:	Methyl Bromide
Remarks:	Methylotrophic bacteria may oxidize methyl bromide to formaldehyde.
Reference:	HSDB, 1999; Howard, 1989.

(d)

Test substance:	Methyl Bromide
Remarks:	"Decomposition of methyl bromide in soil results in the production of bromide ion. The rate of bromide production is influenced by soil type; it is greatest in peatey manure, intermediate in loam (clay soil), and least in sand."
Reference:	IARC, 1986

3.6 BOD_5,COD OR RATIO BOD_5/COD

No specific data found.

3.7 BIOACCUMULATION

Results:	BCF = 4.7 (estimated)
Remark:	Estimated using a measured log octanol/water partition coefficient of 1.10. This value indicates that methyl bromide accumulation will not be significant in aquatic species.
Reference:	Lyman, 1982.

3.8 ADDITIONAL REMARKS

A. Sewage treatment *(information on treatability of the substance)*

No specific data found. Sewage treatment would be difficult for this gaseous substance.

B. Other information *(information that will help to focus the exposure assessment (either qualitative or quantitative)*

No specific data found. Methyl bromide is expected to dissipate rapidly due to its gaseous nature.

4. ECOTOXICOLOGICAL DATA

4.1 ACUTE/PROLONGED TOXICITY TO FISH

(a)

Type of test:	static **[X]**; semi-static **[ ]**; flow-through **[ ]**; other *(e.g. field test)* **[ ]** open-system **[ ]**; closed-system **[ ]**
Species:	*Oncorhynchus mykiss* (Rainbow trout)
Exposure period:	96 hr
Results:	LC_{50} (24h) = > 7.7 mg/l LC_{50} (48h) = 5.6 mg/l LC_{50} (96h) = 3.9 mg/l NOEC = 2.9 mg/l (96 hr) - mortality LOEC = 4.6 mg/l (96 hr) - mortality NOEL = 1.9 mg/l (96 hr) - clinical signs LOEL = 2.9 mg/l (96 hr) - clinical signs
Analytical monitoring:	Yes **[X]** No **[ ]** ? **[ ]**
Method:	1) Series 72, Pesticide Assessment Guidelines, Subdivision E hazard Evaluation: Wildlife and Aquatic Organisms; 2) Standard Evaluation Procedures, Acute Toxicity Test for Freshwater Fish; 3) Standard Practice for Conducting Acute Toxicity Test with Fishes, Macroinvertebrates and Amphibians.
GLP:	Yes **[X]** No **[ ]** ? **[ ]**
Test substance:	Methyl bromide
Remarks:	
Reference:	Wildlife International Report, 1993a.

(b)

Type of test:	static **[X]**; semi-static **[ ]**; flow-through **[ ]**; other *(e.g. field test)* **[ ]** open-system **[ ]**; closed-system **[ ]**
Species:	*Lepomis macrochirus* (Bluegill sunfish)
Exposure period:	96 hr
Results:	LC_{50} (24h) = LC_{50} (48h) = LC_{50} (96h) = 11 mg/l NOEC = ... mg/l LOEC = ... mg/l
Analytical monitoring:	Yes **[ ]** No **[X]** ? **[ ]**
Method:	Static
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Test substance:	Methyl bromide
Remarks:	
Reference:	Dawson et al., 1975/77

(c)

Type of test:	static **[X]**; semi-static **[ ]**; flow-through **[ ]**; other *(e.g. field test)* **[ ]** open-system **[ ]**; closed-system **[ ]**
Species:	*Menidia beryllina* (Tidewater silverside)
Exposure period:	96 hr
Results:	LC_{50} (24h) = LC_{50} (48h) = LC_{50} (96h) = 12 mg/l NOEC = ... mg/l LOEC = ... mg/l
Analytical monitoring:	Yes **[X]** No **[ ]** ? **[ ]**
Method:	OECD TG 203 (1992)

GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance: Methyl bromide
Remarks:
Reference: Dawson et al., 1975/77

(d)
Type of test: static **[X]**; semi-static **[ ]**; flow-through **[ ]**; other *(e.g. field test)* **[ ]** open-system **[ ]**; closed-system **[ ]**
Species: *Cyprinus carpio* (Carp)
Exposure period: 4 hr
Results: LC_{50} (4h) = 17 mg/l
Analytical monitoring: Yes **[ ]** No **[ ]** ? **[X]**
Method:
GLP: Yes **[X]** No **[ ]** ? **[ ]**
Test substance: Methyl bromide
Remarks: Damage to the gill epithelium was the most pronounced morphological damage, which probably caused the death of the fish by suffocation.
Reference: Segers et al., 1984

(e)
Type of test: static **[ ]**; semi-static **[X]**; flow-through **[ ]**; other *(e.g. field test)* **[ ]** open-system **[ ]**; closed-system **[X]**
Species: *Oryzias latipes* (Medaka)
Exposure period: 96 hr
Results: LC_{50} (96h) = 0.7 mg/l
Analytical monitoring: Yes **[ ]** No **[ ]** ? **[X]**
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance: Methyl bromide > 99.9%
Remarks: Abnormal behavior at 0.4 mg/l. Studies conducted in closed glass bottles without aeration (pH 8.2, 23°C, O_2>6.5 mg/l, $CaCO_3$=209.4 mg/l). Data from this study are considered relevant however; this study was not selected as the key study as limited data were available to complete a robust summary. The Wildlife International Report, 1993a, had sufficient data to prepare a robust summary and values were consistent with the results from this study.
Reference: Canton et al., 1980.

(f)
Type of test: static **[ ]**; semi-static **[X]**; flow-through **[ ]**; other *(e.g. field test)* **[ ]** open-system **[ ]**; closed-system **[ ]**
Species: *Poecilia reticulata* (guppy)
Exposure period: 96 hr
Results: LC_{50} (96h) = 0.8 mg/l
Analytical monitoring: Yes **[ ]** No **[ ]** ? **[X]**
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance: Methyl bromide > 99.9%
Remarks: Delayed swimming at 0.3 mg/l; immobilization at 0.6 mg/l. Studies conducted in closed glass bottles without aeration (pH 8.2, 23°C, O_2> 6.5 mg/l, $CaCO_3$=209.4 mg/l). Data from this study are considered relevant however; this study was not selected as the key study as limited data were available to complete a robust summary. The Wildlife International Report, 1993a, had sufficient data to prepare a robust summary and values were consistent with the results from this study.
Reference: Canton et al., 1980.

(g)

Type of test:	static **[ ]**; semi-static **[X]**; flow-through **[ ]**; other *(e.g. field test)* **[ ]** open-system **[X]**; closed-system **[ ]**
Species:	*Poecilia reticulata* (guppy)
Exposure period:	Up to 4 days to concentrations of 0.56, 1.0, and 1.8 mg/l
Results:	limited mortality at 1.0 and 1.8 mg/l NOEC = 0.56 mg/l
Analytical monitoring:	Yes **[X]** No **[ ]** ? **[ ]**
Method:	
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Test substance:	Methyl bromide
Remarks:	Degenerative changes in epithelia, especially of gills at all concentrations. Necrosis of thymic cortex and testis.
Reference:	Wester et al., 1988.

(h)

Type of test:	static **[ ]**; semi-static **[X]**; flow-through **[ ]**; other *(e.g. field test)* **[ ]** open-system **[X]**; closed-system **[ ]**
Species:	*Oryzias latipes* (Medaka)
Exposure period:	Up to 4 days to concentrations of 0.56, 1.0, and 1.8 mg/l
Results:	limited mortality at 1.8 mg/l NOEC = 1.0 mg/l
Analytical monitoring:	Yes **[X]** No **[ ]** ? **[ ]**
Method:	
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Test substance:	Methyl bromide
Remarks:	Degenerative changes in epithelia, especially of gills at all concentrations. Necrosis of thymic cortex and testis.
Reference:	Wester et al., 1988

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

A. Daphnia

(a)

Type of test:	static **[X]**; semi-static **[ ]**; flow-through **[ ]**; other *(e.g. field test)* **[ ]**; open-system **[ ]**; closed-system **[** X **]**
Species:	Daphnia magna
Exposure period:	48 hours to concentrations of 1.2, 2.2, 3.5, 5.8, and 9.8 mg/l
Results:	EC_{50} (24h) = 4.5 mg/l EC_{50} (48h) = 2.6 mg/l NOEC = 1.2 mg/l
Analytical monitoring:	Yes **[X]** No **[ ]** ? **[ ]**
Method:	1) Series 72, Pesticide Assessment Guidelines, Subdivision E hazard Evaluation: Wildlife and Aquatic Organisms; 2) Standard Evaluation Procedures, Acute Toxicity Test for Freshwater Fish; 3) Standard Practice for Conducting Acute Toxicity Test with Fishes, Macroinvertebrates and Amphibians.
GLP:	Yes **[X]** No **[ ]** ? **[ ]**
Test substance:	Methyl bromide
Remarks:	
Reference:	Wildlife International Report, 1993b.

(b)

Type of test:	static **[X]**; semi-static **[ ]**; flow-through **[ ]**; other *(e.g. field test)* **[ ]**; open-system **[ ]**; closed-system **[** X **]**

Species:	Daphnia magna
Exposure period:	48 hours
Results:	$EC_{50\ (48h)}$ = 2.2 mg/l
Analytical monitoring:	Yes **[ ]** No **[ ]** ? **[X]**
Method:	
GLP:	Yes **[X]** No **[ ]** ? **[ ]**
Test substance:	Methyl bromide
Remarks:	Delayed swimming at 1.7 mg/l. Studies conducted in closed glass bottles without aeration (pH 8.2, 19°C, O_2>6.5 mg/l, $CaCO_3$=209.4 mg/l). Data from this study are considered relevant however; this study was not selected as the key study as limited data were available to complete a robust summary. The Wildlife International Report, 1993b, had sufficient data to prepare a robust summary and values were consistent with the results from this study.
Reference:	Canton, J.H., et al., 1980.

B. Other aquatic organisms

No data located.

4.3 TOXICITY TO AQUATIC PLANTS e.g. Algae

(a)

Species:	Chlorella pyrenoidosa (Algae)
End-point:	Biomass **[ ]**; Growth rate **[X]**; Other **[ ]**
Exposure period:	48 hours
Results:	Growth: EC_{50} = 5 mg/l (exposure duration not specified)
Analytical monitoring:	Yes **[X]** No **[ ]** ? **[ ]**
Method:	Protocol guideline not specified
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Test substance:	Methyl bromide
Remarks:	Regarding test conditions, temperature was 24°C, water hardness was 54.05 mg/l CaCO3, and pH was 7.7.
Reference:	Canton, JH et al,(1980)

(b)

Species:	Scenedesmus quadricauda (Algae)
End-point:	Biomass **[ ]**; Growth rate **[X]**; Other **[ ]**
Exposure period:	48 hours
Results:	Growth: EC_{50} = 3.2 mg/l (exposure duration not specified)
Analytical monitoring:	Yes **[X]** No **[ ]** ? **[ ]**
Method:	Protocol guideline not specified
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Test substance:	Methyl bromide
Remarks:	Regarding test conditions, temperature was 24°C, water hardness was 54.05 mg/l CaCO3, and pH was 7.7.
Reference:	Canton, JH et al (1980)

4.4 TOXICITY TO BACTERIA

No data located.

4..5 CHRONIC TOXICITY TO AQUATIC ORGANISMS

4.5.1. CHRONIC TOXICITY TO FISH (*effects on reproduction, embryo/larva etc.*)

(a)

Type of test:	static **[ ]**; semi-static **[X]**; flow-through **[ ]**; other *(e.g. field test)* **[ ]**; open-system **[ ]**; closed-system **[X]**
Species:	*Poecilia reticulata* (Guppy)
End-point:	Length of fish **[ ]**; Weight of fish **[ ]**; Reproduction rate **[ ]**; Other **[X]**
Exposure period:	1 and 3 months to 0.032 – 3.2 mg/l
Results:	after 3 months; NOEC = 0.1 mg/l (for behavior and appearance) NOLC = 0.32 mg/l
Analytical monitoring:	Yes **[X]** No **[ ]** ? **[ ]**
Method:	
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Test substance:	Methyl bromide
Remarks:	At 3.2 mg/liter, 100% mortality after 3 days; at 1.0 mg/liter, 100% mortality within 3 weeks. At 0.32 mg/liter, significantly decreased weight in both sexes. No histopathological damage to internal organs. The chronic fish studies do not follow OECD guideline in that the reproductive parameters critical to long-term fish studies were not evaluated. The end-point of concern in the Wester studies was the development of histologic lesions in fish that received toxic to lethal doses of methyl bromide. No such lesions were seen. In addition, based on available data, it is unclear whether headspace was present. If headspace was present, given the physical chemical properties of Methyl bromide, analytical monitoring results would be questionable.
Reference:	Wester et al., 1988

(b)

Type of test:	static **[ ]**; semi-static **[X]**; flow-through **[ ]**; other *(e.g. field test)* **[ ]**; open-system **[ ]**; closed-system **[X]**
Species:	*Oryzias latipes* (Medaka)
End-point:	Length of fish **[ ]**; Weight of fish **[ ]**; Reproduction rate **[ ]**; Other **[X]**
Exposure period:	1 and 3 months to 0.032 – 3.2 mg/l
Results:	after 1 month; NOEC = 0.56 mg/l (behavior and appearance) NOLC = 0.32 mg/l after 3 months; NOEC = 0.32 mg/l (behavior and appearance) NOLC = 0.32 mg/l
Analytical monitoring:	Yes **[ ]** No **[ ]** ? **[X]**
Method:	
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Test substance:	Methyl bromide
Remarks:	At 3.2 mg/liter, 100% mortality after 3 days; at 1.0 mg/liter, partial mortality within 3 weeks. At 0.32 mg/liter, significantly decreased weight in both sexes. No histopathological damage to internal organs. The chronic fish studies do not follow OECD guideline in that the reproductive parameters critical to long-term fish studies were not evaluated. The end-point of concern in the Wester studies was the development of histologic lesions in fish that received toxic to lethal doses of methyl bromide. No such lesions were seen. In addition, based on available data, it is unclear whether headspace was present. If headspace was present, given the physical chemical properties of Methyl bromide, analytical monitoring results would be questionable.

Reference: Wester et al., 1988

4.5.2. CHRONIC TOXICITY TO AQUATIC INVERTEBRATES *(e.g. daphnia reproduction. The need to conduct tests for this end-point will depend inter alia upon concern for long term effects.)*

Type of test:	static **[X]**; semi-static **[ ]**; flow-through **[ ]**; other *(e.g. field test)* **[ ]**; open-system **[ ]**; closed-system **[** X **]**
Species:	Daphnia magna
Exposure period:	12 days
Results:	LC_{50} =
Analytical monitoring:	Yes **[ ]** No **[ ]** ? **[X]**
Method:	
GLP:	Yes **[X]** No **[ ]** ? **[ ]**
Test substance:	Methyl bromide
Remarks:	Effect on reproduction at 0.1 mg/l. Studies conducted in closed glass bottles without aeration (pH 8.2, 19°C, O_2>6.5 mg/l, $CaCO_3$=209.4 mg/l). The study author concluded that "The reason for the high mortality is not clear: it could be a result of a transient decrease in the overall viability of the Daphnia Magna population." The authors recommended a repeat of this study. The results of this study are considered questionable at best.
Reference:	Canton, et al., 1980.

4.6 TOXICITY TO TERRESTRIAL ORGANISMS

4.6.1 TOXICITY TO SOIL DWELLING ORGANISMS

No data located.

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

(a)

Species:	*Oryza sativa* (Rice, Japicona) and *Zea mays* (Corn (Maize) Seeds)
End-point:	Emergence **[X]**; Growth **[X]**; Other **[ ]**
Exposure period:	
Results:	No detrimental effect up to 4 mg/liter was observed in rice seeds at a moisture content of 11%, but as the moisture content and temperature increased, methyl bromide had an increasing effect on germination. Maize seeds were much more tolerant. Exposure to 5 mg/l did not produce any harmful effects on the germination of maize seeds. At concentrations higher than 10 mg/l, the viability of maize seeds declined in a similar way to that of rice seeds.
Method:	
GLP:	Yes **[ ]** No **[X]** ? **[ ]**
Test substance:	
Remarks:	Rice seeds were found to absorb more methyl bromide than corn seeds. Seeds with higher moisture content absorbed more and seeds with the same moisture content absorbed more at higher temperatures than at lower temperatures.
Reference:	Sittisuang & Nakakita, 1985

(b)

Species:	*Lactuca sativa capitata* (Lettuce) and *Nasturtium officinale* (Water Cress)
End-point:	Emergence **[X]**; Growth **[X]**; Other **[ ]**
Exposure period:	72 hours at concentrations between 4 and 1400 mg/m^3

Results: At 400 mg/m^3, yellowing of lettuce leaves became apparent, while no apparent visible effects were observed on watercress up to the highest concentration.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks:
Reference: Reichmuth & Noack, 1983

4.6.3 TOXICITY TO OTHER NON MAMMALIAN TERRESTRIAL SPECIES (INCLUDING AVIAN)

(a)
Species: Rhode Island Red Hens
End-point: Mortality **[ ]**; Reproduction rate **[ ]**; Weight **[ ]**; Other **[X]**
Exposure period:
Results:
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:purity:
Remarks: Hens were fed, from hatching, on diets that had been fumigated with methyl bromide at the concentration recommended for the elimination of salmonellae (800 mg.h/litre and 2000 mg.h/litre). Body weight, egg weight, and egg number were not significantly affected, but sexual maturity may have been slightly affected. The same group had previously shown that the taste of meat from broiler chickens was similarly tainted.
Reference: Cooper et al., 1978; Griffiths et al., 1978

(b)
Species: Rhode Island Red Hens
End-point: Mortality **[ ]**; Reproduction rate **[X]**; Weight **[ ]**; Other **[ ]**
Exposure period: 24 hours
Results:
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:purity:
Remarks: No adverse effects on either the fertility or hatchability of hens' eggs, previously fumigated with methyl bromide at 32 g/m^3.
Reference: Devaney & Beerwinkle, 1982

(c)
Species: Northern Bobwhite
Type of Test: Oral LD50
End-point: Mortality **[X]**; Reproduction rate **[ ]**; Weight **[ ]**; Other **[ ]**
Exposure period: Range of single oral doses to determine oral LD50
Doses: 31.3, 62.5, 125, 250, 500, 1000 mg/kg (5/sex/dose)
Results: LD50 = 73 mg/kg (95% confidence limits: 62.5 to 125 mg/kg).
No mortality in the 31.3 mg/kg dosage group, 30% mortality at the 62.5 mg/kg test dosage, and 100% mortality at the 125, 250, 500, and 1000 mg/kg test dosages.
Method: FIFRA Guideline 71-1
GLP: Yes **[X]** No **[ ]** ? **[ ]**
Test substance purity: 99.87%
Remarks: The acute oral LD50 value for a northern bobwhite's exposure to methyl bromide as a single oral dose was approximately 73 mg/kg.
Reference: Campbell & Beavers, 1994

4.7 BIOLOGICAL EFFECTS MONITORING (INCLUDING BIOMAGNIFICATION)

Results	Application of methyl bromide as a soil fumigant resulted in the almost complete eradication of populations of a wide variety of microflora and fauna, as well as other soil organisms, thus altering, at least temporarily, the trophic structure of the soil environment. Treatment with 100% methyl bromide and other methyl bromide/chloropicrin formulations reduced populations of *Fusarium*, *Pythium*, and *Rhizooctonia* species in soil. Nine weeks after application, populations were still significantly lower. Seedlings grown in treated plots had the least amount of damping off and root rot. Methyl bromide dosed under plastic sheeting at a rate of 300 g/m^3 for 30 cm depth 100 g/m^3, killed all insects, though small numbers of soil nematodes and mites were collected during subsequent sampling.
Remarks:	
Reference	Sassaman et al, 1986; Enebak et al., 1988; Heungens & Roos, 1982

4.8 BIOTRANSFORMATION AND KINETICS

No data located.

4.9 ADDITIONAL REMARKS

Nematodes and insects are the target species for methyl bromide pesticidal activity. Methyl bromide has been shown through historical use to be efficacious for this purpose.

5. TOXICITY

5.1 ACUTE TOXICITY

5.1.1 ACUTE ORAL TOXICITY

(a) **Preferred Result**

Type:	LD_0 **[ ]**; LD_{100} **[ ]**; LD_{50} **[X]**; LDL_0 **[ ]**; Other **[ ]**
Species/strain:	Rat
Value:	= 214 (mg/kg): Discriminating dose: N/A
Method:	OECD 401. 1981
GLP:	Yes **[]** No **[ ]** ? **[X]**
Test substance:	
Reference:	Danse et al., 1984

(b)

Type:	LD_0 **[ ]**; LD_{100} **[ ]**; LD_{50} **[X]**; LDL_0 **[ ]**; Other **[ ]**
Species/strain:	Rat
Value:	104 and 133 mg/kg Discriminating dose: N/A
Method:	Other
GLP:	Yes **[X]** No **[ ]** ? **[ ]**
Test substance:	Liquid and microencapsulated form
Remarks:	The oral LD_{50} values were 104 mg/kg for liquid methyl bromide and 133 mg/kg for microencapsulated methyl bromide.
Reference:	Kipplinger, 1994

(c)

Type:	LD_0 **[ ]**; LD_{100} **[ ]**; LD_{50} **[X]**; LDL_0 **[ ]**; Other **[ ]**
Species/strain:	Beagle Dog
Value:	No deaths at dose of 3, 5, 50 mg/kg. Mortality at 500 mg/kg Discriminating dose: N/A.
Method:	Other
GLP:	Yes **[X]** No **[ ]** ? **[**X**]**
Test substance:	Commercial; purity
Remarks:	500 mg/kg produced severe signs of toxicity and vomiting followed by death within 24 hours of dosing. A 50 mg/kg dose elicited signs of toxicity and vomiting of reddish material but no deaths. At low doses of 5 and 3 mg/kg, dogs vomited shortly after dosing. An oral LD_{50} study could not be conducted since dogs vomited the dose.
Reference:	Naas, 1990

(d)

Species:	Northern Bobwhite
Type of Test:	Oral LD50
End-point:	Mortality **[X]**; Reproduction rate **[ ]**; Weight **[ ]**; Other **[ ]**
Exposure period:	Range of single oral doses to determine oral LD50
Doses:	31.3, 62.5, 125, 250, 500, 1000 mg/kg (5/sex/dose)
Results:	LD50 = 73 mg/kg (95% confidence limits: 62.5 to 125 mg/kg). No mortality in the 31.3 mg/kg dosage group, 30% mortality at the 62.5 mg/kg test dosage, and 100% mortality at the 125, 250, 500, and 1000 mg/kg test dosages.
Method:	FIFRA Guideline 71-1
GLP:	Yes **[X]** No **[ ]** ? **[ ]**
Test substance purity:	99.87%

Remarks: The acute oral LD50 value for a northern bobwhite's exposure to methyl bromide as a single oral dose was approximately 73 mg/kg.
Reference: Campbell & Beavers, 1994

5.1.2 ACUTE INHALATION TOXICITY

(a)
Type: LC_0 **[ ]**; LC_{100} **[ ]**; LC_{50} **[X]**; LCL_0 **[ ]**; Other **[ ]**
Species/strain: Rat
Exposure time: 30 minutes
Value: LC50 = 2833 ppm
Method:
GLP: Yes **[ ]** No **[X]** ? **[ ]**
Test substance: Methyl bromide
Remarks:
Reference: Bakhishev, 1973

(b)
Type: LC_0 **[ ]**; LC_{100} **[ ]**; LC_{50} **[X]**; LCL_0 **[ ]**; Other **[ ]**
Species/strain: Rat
Exposure time: 60 minutes
Value: LC50 = 1880 ppm
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance: Methyl bromide
Remarks:
Reference: Zwart, 1988;1992

(c) **Preferred Result**
Type: LC_0 **[ ]**; LC_{100} **[ ]**; LC_{50} **[X]**; LCL_0 **[ ]**; Other **[ ]**
Species/strain: Rat
Exposure time: 4 hours
Value: LC50 = 781 ppm
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance: Methyl bromide
Remarks:
Reference: Kato, 1986

(d)
Type: LC_0 **[ ]**; LC_{100} **[ ]**; LC_{50} **[X]**; LCL_0 **[ ]**; Other **[ ]**
Species/strain: Rat
Exposure time: 8 hours
Value: LC50 = 302 ppm
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance: Methyl bromide
Remarks:
Reference: Honma, 1985

(e)
Type: LC_0 **[ ]**; LC_{100} **[ ]**; LC_{50} **[X]**; LCL_0 **[ ]**; Other **[ ]**
Species/strain: Mouse
Exposure time: 30 minutes
Value: LC50 = 1700 ppm
Method:
GLP: Yes **[ ]** No **[X]** ? **[ ]**

Test substance: Commercial, purity: 96.5%
Remarks:
Reference: Bakhishev, 1973

(f)
Type: LC_0 **[ ]**; LC_{100} **[ ]**; LC_{50} **[X]**; LCL_0 **[ ]**; Other **[ ]**
Species/strain: Mouse
Exposure time: 60 minutes
Value: LC50 = 1200 ppm
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance: Methyl bromide
Remarks: Based on all signs of neurotoxicity, the NOEL was 338 ppm.
Reference: Alexeeff et al., 1985

(g)
Type: LC_0 **[ ]**; LC_{100} **[ ]**; LC_{50} **[X]**; LCL_0 **[ ]**; Other **[ ]**
Species/strain: Mouse
Exposure time: 120 minutes
Value: LC50 = 397 ppm
Method:
GLP: Yes **[ ]** No **[X]** ? **[ ]**
Test substance: Methyl bromide
Remarks:
Reference: Balander and Polyak, 1962

(h)
Type: LC_0 **[ ]**; LC_{100} **[ ]**; LC_{50} **[X]**; LCL_0 **[ ]**; Other **[ ]**
Species/strain: Mouse
Exposure time: 4 hours
Value: LC50 = 405 ppm
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance: Methyl bromide
Remarks:
Reference: Yamano, 1991

Non-LC50 acute inhalation studies

(i)
Type: LC_0 **[ ]**; LC_{100} **[ ]**; LC_{50} **[ ]**; LCL_0 **[ ]**; Other **[X]**
Species/strain: F344 Rat
Exposure time: 4 hours
Exposure Levels: 150, 225, 338, 506, 760, or 1140 ppm
Method: Other
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance: Grade and purity not stated.
Remarks: Single exposure resulted in decreased locomotor activity, ataxia, nasal discharge, lacrimation, diarrhea, irregular breathing and bradypnea in rats exposed to 338 ppm and greater. No clinical signs of toxicity were evident in animals exposed to 225 ppm methyl bromide or less. Histologic evaluations revealed metaplasia of the olfactory epithelium for rats exposed to 225, 338, and 506 ppm methyl bromide.
Reference: Japanese Ministry of Labor, 1992

(j)
Type: LC_0 **[ ]**; LC_{100} **[ ]**; LC_{50} **[ ]**; LCL_0 **[ ]**; Other **[X]**

Species/strain: Rat
Exposure time: 8 hours
Exposure Levels: 63, 125, 188, 250 ppm
Method: Other
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance: Grade and purity not stated.
Remarks: Thiopental sleep was potentiated at 63 ppm and greater, measured by time to loss of righting reflex upon thiopental injection. Body weight gain and body temperature were decreased in rats exposed to methyl bromide concentrations of 125 ppm and greater. Neurotoxicity, indicated by reduced locomotor activty, was seen at concentrations of 188 ppm and 250 ppm methyl bromide. These effects were reversible within 24 hours of exposure.
Reference: Honma et al., 1985

(k)
Type: LC_0 **[ ]**; LC_{100} **[ ]**; LC_{50} **[ ]**; LCL_0 **[ ]**; Other **[X]**
Species/strain: F344 Rat
Exposure time: 6 hours/day, 5 consecutive day regimen
Exposure Levels: 0, 90, 175, 250 and 325 ppm
Method: Other
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance: Grade and purity not stated.
Remarks: Diarrhea was noted by the end of the second day of exposure for 250 ppm and 325 ppm animals. By the end of the third exposure, animals from these groups showed ataxia. Two of the 325 ppm rats exhibited tremors and/or convulsions during the fourth exposure. Subsequently, three animals from this group died after the fourth exposure. Clinical signs of neurotoxicity were not observed in the 250 ppm and 325 ppm animals after a single or second exposure to methyl bromide.
Reference: Hurtt et al., 1987

(l)
Type: LC_0 **[ ]**; LC_{100} **[ ]**; LC_{50} **[ ]**; LCL_0 **[ ]**; Other **[X]**
Species/strain: Rat and Rabbit
Exposure time: 0 – 32 hours
Exposure levels: 108 - 12850 ppm
Method: Other
GLP: Yes **[ ]** No **[X]** ? **[ ]**
Test substance: Grade and purity not stated.
Remarks: Rabbits tolerated exposure to 220 ppm for 20 hours but exposure at this concentration for 32 hours resulted in 100% mortality. At 2570 ppm, rabbits survived a one hour exposure while 100% mortality was observed after 2.2 hours.
Reference: Irish, 1940

(m)
Type: LC_0 **[ ]**; LC_{100} **[ ]**; LC_{50} **[ ]**; LCL_0 **[ ]**; Other **[X]**
Species/strain: Beagle Dog
Exposure time: 1 – 4 days, 6-7 hr/day
Exposure Levels: Phase 1: 233 ppm (1 male), 324 ppm (1 male, 1 female), 345 ppm (1 male, 1 female), 394 ppm (1 female);
Phase 2: 55 ppm (1 male, 1 female), 156 ppm (1 male, 1 female), 268 ppm (1 male, 2 females), or 283 ppm (2 males, 1 female)
Method: Other
GLP: Yes **[X]** No **[ ]** ? **[ ]**
Test substance: Commercial, purity: 96.5%

Remarks: Phase 1: Signs of toxicity were observed at all concentrations; therefore the one-day NOAEL was <233 ppm. In the second phase of the study, dogs were exposed to 55 ppm (1 male, 1 female), 156 (1 male, 1 female), 268 ppm (1 male, 2 females), or 283 ppm (2 males, 1 female) for 7 hours/day for up to four days. The 268 ppm and 283 ppm dogs were exposed for two days and developed clinical signs of toxicity. Therefore, the 2-day NOAEL was <268 ppm. The 55 ppm and 156 ppm dogs were exposed for 7 hours/day for 4 consecutive days. No effects were seen in either the 55 ppm or 156 ppm dogs during Days 1 and 2 of exposure. However, the 156 ppm animals showed decreased activity during exposure of Days 3 and 4 and irregular gait during the post exposure period on Day 4.
Reference: Newton, 1994a

5.1.3 ACUTE DERMAL TOXICITY

No studies have been reported.

5.1.4 ACUTE TOXICITY, OTHER ROUTES OF ADMINISTRATION

Type: LC_0 **[ ]**; LC_{100} **[ ]**; LC_{50} **[ ]**; LCL_0 **[ ]**; Other **[ ]**
LD_0 **[ ]**; LD_{100} **[ ]**; LD_{50} **[X]**; LDL_0 **[ ]**; Other **[ ]**
Species/strain: Rats (Sprague-Dawley, Male, 9/group)
Route of Administration: i.m. **[ ]**; i.p. **[ ]**; i.v. **[ ]**; infusion **[ ]**; s.c. **[X]**; other **[ ]**
Exposure time: N.A.
Value: 135 mg/kg
Method: Other
GLP: Yes **[ ]** No **[X]** ? **[ ]**
Test substance: Substance grade not stated, purity: unknown
Remarks: For a single subcutaneous administration, an LD_{50} was found to be 135 mg/kg body weight (range 75-250 mg/kg)
Reference: Tanaka et al., 1988

5.2 CORROSIVENESS/IRRITATION

5.2.1 SKIN IRRITATION/CORROSION

There are no reports on skin effects in animals.

5.2.2 EYE IRRITATION/CORROSION

(a)
Remarks: Grant reports the results of experimental exposure of methyl bromide gas directly to rabbit eyes. This exposure resulted in severe irritation that began to clear within five days. In humans, splashes or direct exposure to vapors has not caused severe eye irritation. Severe acute exposure in humans has resulted in neurological sequelae that include visual impairment that is delayed in onset and usually reversible.
Reference: Grant, 1993.

(b)
Remarks: Lacrimation has been observed in rats during inhalation exposures at methyl bromide levels exceeding 2570 ppm. In mice, eye irritation was observed at concentrations of 823 ppm.
Reference: Irish, 1940; Balander & Polyak, 1962.

5.3 SKIN SENSITISATION

No data located.

5.4 REPEATED DOSE TOXICITY

(a)
Species/strain: Rat (Wistar)
Sex: Female **[ ]**; Male **[X]**; Male/Female **[ ]**; No data **[ ]**
Route of Administration: Inhalation
Exposure period: 3 weeks
Frequency of treatment: 4 hours/day; 5 days/week
Post exposure observation period: 15 to 28 days
Dose: 200 – 300 ppm (778 – 1167 mg/m3)
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[ ]**; Concurrent vehicle **[X]**; Historical **[ ]**
NOEL: none
LOEL: 200 ppm
Results: Decreased body weight gain at both exposure levels. Behavioral tests conducted 12 and 28 days after last exposure. Decrements in rotarod and spontaneous activity 12 and 28 days post exposure. No detectable histopathological changes in central or peripheral neurological tissues.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Reference: Ikeda, 1980.

(b)
Species/strain: Rat (Wistar)
Sex: Female **[ ]**; Male **[X]**; Male/Female **[ ]**; No data **[ ]**
Route of Administration: Inhalation
Exposure period: 3 weeks
Frequency of treatment: Continuous
Post exposure observation period: none
Dose: 1, 5 and 10 ppm
Control group: Yes **[ ]**; No **[ ]**; No data **[X]**;
Concurrent no treatment **[ ]**; Concurrent vehicle **[ ]**; Historical **[ ]**
NOEL:
LOEL: 5 ppm
Results: At 10 ppm: decreased norepinephrine in hypothalamus, decreased serotonin in cortex. At 5 ppm: increased dopamine in striatum.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Reference: Honma, T. 1982.

(c)
Species/strain: Rat
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: Inhalation
Exposure period: 6 months
Frequency of treatment: 7-8 hours/day; 5 days/week
Post exposure observation period:
Dose: 33, 64, 108, and 218 ppm (130, 250, 420 and 850 mg/m3)
Control group: Yes **[ ]**; No **[ ]**; No data **[X]**;
Concurrent no treatment **[ ]**; Concurrent vehicle **[ ]**; Historical **[ ]**

NOEL: 33 ppm
LOEL:
Results: Convulsions and pulmonary damage reported (exposure levels not specified).
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Reference: WHO, 1991.

(d)
Species/strain: Rat (Fischer 344)
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: Inhalation
Exposure period: 6 weeks
Frequency of treatment: 6 hours/day; 5 days/week
Post exposure observation period: none
Dose: 160 ppm (622 mg/m3)
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[ ]**; Concurrent vehicle **[X]**; Historical **[ ]**
NOEL: none
LOEL:
Results: Males were more susceptible than females, exhibiting 50% mortality after 14 days with reduced lung, kidney, spleen, liver, brain, and testis weights. Males showed more severe changes in neuronal tissues including necrosis with necrosis of olfactory tissue. Testicular degeneration was also observed.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Reference: Eustis, 1988.

(e)
Species/strain: Rat (Wistar)
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: Inhalation
Exposure period: 4 weeks
Frequency of treatment: 6 hours/day; 5 days/week for 3 weeks; continuous for last week
Post exposure observation period: none
Dose: 18, 51, 154 ppm (70, 200, 600 mg/m3)
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[ ]**; Concurrent vehicle **[X]**; Historical **[ ]**
NOEL: none
LOEL: 18 ppm
Results: Behavioral effects included temors and unusual gait. Micropathological changes noted in heart and lungs.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Reference: WHO, 1991

(f)
Species/strain: Rat (Wistar)
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: Inhalation
Exposure period: 13 weeks
Frequency of treatment: 6 hours/day; 5 days/week
Post exposure observation period: none
Dose: 1, 7, 43 ppm (4, 25, 166 mg/m3)
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;

Concurrent no treatment **[ ]**; Concurrent vehicle **[X]**; Historical **[ ]**
NOEL: 7 ppm
LOEL: 43 ppm
Results: Histopathology in livers rats from the high exposure group.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Reference: WHO, 1991

(g)
Species/strain: Rat (Wistar)
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: Inhalation
Exposure period: 29 months
Frequency of treatment: 6 hours/day; 5 days/week
Post exposure observation period: none
Dose: 3, 30 90 ppm (12, 117, 350 mg/m3)
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[ ]**; Concurrent vehicle **[X]**; Historical **[ ]**
NOEL: none
LOEL: 3 ppm
Results: Degeneration and hyperplastic lesions in nasal epithelium, characterized as mild, in all exposure groups. Increased mortality at 90 ppm with reduced body weight gain, reduced absolute brain weights, increased hemothorax, myocardial degeneration, and thrombi in heart, and increased mortality.
Method:
GLP: Yes **[X]** No **[ ]** ? **[ ]**
Test substance:
Reference: T.N.O., 1987; Reuzel, 1991

(h)
Species/strain: Rat (F344/DuCrj)
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: Inhalation
Exposure period: 2 years
Frequency of treatment: 6 hours/day; 5 days/week
Post exposure observation period: none
Dose: 0, 4, 20 100 ppm (0, 16, 78, 389 mg/m3); 50/sex/exposure level
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[ ]**; Concurrent vehicle **[X]**; Historical **[ ]**
NOEL: none
LOEL:
Results: No affects on mortality were observed at any exposure level. In the high exposure group, both sexes exhibited decreased body weight with increased RBCs, hematocrit and hemoglobin (males only) and altered blood chemistries. High dose subjects also showed inflammation, necrosis, and metaplasia in nasal epithelium with the effect more pronounced in males. Males from the lower exposure groups showed inflammation of nasal epithelia.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Reference: Japanese Ministry of Labor, 1992

(i)
Species/strain: Rat (Wistar)
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**

Route of Administration: gavage
Exposure period: 90 days
Frequency of treatment: 5 days/week
Post exposure observation period:
Dose: 0, 0.4, 2, 10, or 50 mg/kg/day in arachis oil
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[ ]**
NOEL: 0.4 mg/kg/day
LOEL: 2 mg/kg/day
Results: At 50 mg/kg/day, marked, diffuse hyperplasia of the epithelium of the forestomach was seen in all animals. Squamous cell carcinoma of the forestomach was diagnosed in 13 of 20 animals receiving 50 mg/kg/day. In a subsequent re-examination of slides by a panel of NTP pathologists, lesions previously considered malignant carcinomas were re-categorized as forestomach lesions, characterized by inflammation and hyperplasia. Inflammatory lesions of forestomach were also seen in animals treated with 2 and 10 mg/kg/day.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance: Commercial, purity: 96.5%
Reference: Danse et al., 1984

(j)
Species/strain: Rat (Wistar)
Sex: Female **[ ]**; Male **[X]**; Male/Female **[ ]**; No data **[ ]**
Route of Administration: Gavage
Exposure period: Phase 1: 13, 17, 21, or 25 weeks – Phase 2: 13, 17, or 21 weeks followed by up to 25 weeks observation.
Frequency of treatment: 5 days/week
Post exposure observation period: Up to 25 weeks
Dose: 50 mg/kg/day dissolved in arachis oil
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[ ]**; Concurrent vehicle **[X]**; Historical **[ ]**
NOEL: none
LOEL:
Results: Forestomach lesions included inflammation, acanthosis, fibrosis and hyerplasia, with severity depending on length of treatment. In 13 week group, partial recovery of these lesions was seen with persistence of fibrosis and acanthosis 10-12 weeks post-exposure. One rat from the 25 week treatment group showed evidence of an early malignant carcinoma in the forestomach.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Reference: Boorman, 1986

(k)
Species/strain: Mouse (Crj:BDF1)
Sex: Female **[ ]**; Male **[X]**; Male/Female **[ ]**; No data **[ ]**
Route of Administration: Inhalation
Exposure period: 104 weeks
Frequency of treatment: 6 hours/day, 5 days/week
Post exposure observation period: none
Dose: 0, 4, 16, 64 ppm (16, 62, 250 mg/m3) (50/sex/exposure level)
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[ ]**; Concurrent vehicle **[X]**; Historical **[ ]**
NOEL: none
LOEL:

Results: No effect on mortality at any exposure level. High exposure group: reduced body weight gain, increased incidence of slight atrophy of granular layer of cerebellum in both sexes, altered blood chemistries. No effect reported at lower exposures.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance: 99.9% purity
Reference: Japanese Ministry of Labor, 1992

(l)
Species/strain: Mouse (B6C3F1)
Sex: Female **[ ]**; Male **[X]**; Male/Female **[ ]**; No data **[ ]**
Route of Administration: Inhalation
Exposure period: 6 weeks
Frequency of treatment: 6 hours/day, 5 days/week
Post exposure observation period: none
Dose: 160 ppm (622 mg/m3)
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[ ]**; Concurrent vehicle **[X]**; Historical **[ ]**
NOEL: none
LOEL:
Results: High mortality; males sacrificed at 10 weeks, females at 8 weeks when mortality exceeded 50%. Decreased lung, heart, thymus, brain, and liver weights. Necrosis in CNS, nephrosis, atrophy of inner zone of adrenal cortex, testicular degeneration.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Reference: Eustis, 1988

(m)
Species/strain: Mouse (B6C3F1)
Sex: Female **[ ]**; Male **[X]**; Male/Female **[ ]**; No data **[ ]**
Route of Administration: Inhalation
Exposure period: 13 weeks
Frequency of treatment: 6 hours/day, 5 days/week
Post exposure observation period: none
Dose: 10, 20, 40, 80, 120 ppm (39, 78, 156, 312, 468 mg/m3)
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[ ]**; Concurrent vehicle **[X]**; Historical **[ ]**
NOEL: none
LOEL:
Results: Slight neurobehavioral changes, hematological changes at 40 ppm and above. No histopathological changes.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Reference: NTP, 1992

(n)
Species/strain: Mouse (B6C3F1)
Sex: Female **[ ]**; Male **[X]**; Male/Female **[ ]**; No data **[ ]**
Route of Administration: Inhalation
Exposure period: 2 years (exposure of 20 weeks only due to toxicity)
Frequency of treatment: 6 hours/day, 5 days/week
Post exposure observation period: remainder of lives after 20 weeks exposure
Dose: 0, 10, 33, 100 ppm (0, 39, 128, 389 mg/m3) (86/sex/exposure level)

Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[ ]**; Concurrent vehicle **[X]**; Historical **[ ]**
NOEL:
LOEL:
Results: High toxicity, including weight loss and excessive mortality, caused exposures to cease after 20 weeks. At termination after 2 years of life, survival and body weights were reduced in high exposure males. Both sexes exhibited reduced thymus weights (exposure level?) and neurological symptoms predominantly in the high exposure mice, the latter characterized by tremors, abnormal posture and limb paralysis that generally persisted throughout the observation period. High-exposure males (and later, high-exposure females) exhibited neurobehavioral deficits at three months, including reduced startle response. Predominantly in the high exposure group, histopathology revealed lesions of the cerebellum, cerebrum, heart (chronic myopathy), sternal dysplasia, and necrosis/metaplasia of the olfactory epithelium.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Reference: NTP, 1992

(o)
Species/strain: Beagle Dog
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: inhalation
Exposure period: 4 weeks
Frequency of treatment: 5 days/week, 7 hours/day
Postexposure observation period:
Dose: 0, 5, 10, 25, 50, or 100 ppm (4/sex/group)
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[ ]**
NOEL: No data
LOEL: No data
Results: No clinical evidence of neurotoxicity was seen in any group throughout the four weeks of exposure.
Method: OECD: 411 (1981)
GLP: Yes **[X]** No **[ ]** ? **[ ]**
Test substance: Commercial, purity: 96.5%
Remarks: After four weeks, four of the controls (two males and two females) and all dogs in the 5 ppm group continued the test for an additional two weeks and the exposure concentration for the 10 ppm group was increased to 150 ppm. Dogs exposed to 150 ppm showed severe body weight loss over the first few days of exposure. After five or six exposures, evaluation of the 150 ppm animals revealed ataxia, a base-wide stance, intention tremor, nystagmus, marked depression and inability (unwillingness) to stand and perform postural responses. Due to the severity of these effects, the dogs were sacrificed. Microscopic findings were limited to the 150 ppm group in which significant neurologic effects were seen and consisted of vacuoles in the granular layer of the cerebellum
Reference: Newton, 1994b.

5.5 GENETIC TOXICITY IN VITRO

A. BACTERIAL TESTS

(a)
Type: Bacterial reverse mutation assay
System of testing:
Species/strain: *Salmonella typhimurium* (strains TA98, TA100, TA1535, TA1537, and TA1538)
Concentration: 0.5 to 5 g/m3 in a closed container
Metabolic activation: With **[ ]**; Without **[ ]**; With and Without **[X]**; No data **[ ]**
Results: Mutagenic to strains TA100 and TA1535 but not others without metabolic activation.
Cytotoxicity conc: With metabolic activation: mg/plate
Without metabolic activation: mg/plate
Precipitation conc: mg/plate
Genotoxic effects: + ? -
With metabolic activation: **[ ] [ ] [X]**
Without metabolic activation: **[X] [ ] [ ]**
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks: Consistent with action of alkylating agents.
Reference: Moriya et al., 1983

(b)
Type: Bacterial reverse mutation assay
System of testing: Liquid assay (10 to 1000 mg/l) and gas plate assay (closed containers – 500 – 50000 mg/m3)
Species/strain: *Salmonella typhimurium* TA100
Concentration: see above
Metabolic activation: With **[ ]**; Without **[ ]**; With and Without **[X]**; No data **[ ]**
Results: Positive mutagenic response in both liquid and gaseous plate assays. In liquid assay, positive responses were seen at 285 mg/l and higher; in plate assay, postive responses were seen at 1900 mg/m3 and higher. Presence of Aroclor-induced rat metabolic activation system did not affect response in plate assay.
Cytotoxicity conc: With metabolic activation: mg/plate
Without metabolic activation: mg/plate
Precipitation conc: mg/plate
Genotoxic effects: + ? -
With metabolic activation: **[ ] [ ] [ ]**
Without metabolic activation: **[ ] [ ] [ ]**
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks: Consistent with action of alkylating agents.
Reference: Kramers et al., 1985a

(c)
Type: Bacterial reverse mutation assay
System of testing: Gas plate assay (closed containers – 1,000 5,0000 ppm)
Species/strain: *Salmonella typhimurium* TA98, TA100, TA 1535
Concentration: see above
Metabolic activation: With **[ ]**; Without **[ ]**; With and Without **[X]**; No data **[ ]**
Results: Positive mutagenic response at 1,000 ppm with strains TA 100 & TA 1535 with or without metabolic activation.
Cytotoxicity conc: With metabolic activation: mg/plate
Without metabolic activation: mg/plate
Precipitation conc: mg/plate

Genotoxic effects: + ? -
With metabolic activation: **[ ] [ ] [ ]**
Without metabolic activation: **[ ] [ ] [ ]**
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks: Consistent with action of alkylating agents.
Reference: JETOC, 1997

(d)
Type: Bacterial reverse mutation assay
System of testing: Liquid assay (10 to 1000 mg/l)
Species/strain: *Salmonella typhimurium* TA100
Concentration: see above
Metabolic activation: With **[ ]**; Without **[X]**; With and Without **[X]**; No data **[ ]**
Results: Positive mutagenic response without activation in TA 100 at 0.4 mg/l and higher.
Cytotoxicity conc: With metabolic activation: mg/plate
Without metabolic activation: mg/plate
Precipitation conc: mg/plate
Genotoxic effects: + ? -
With metabolic activation: **[ ] [ ] [ ]**
Without metabolic activation: **[ ] [ ] [ ]**
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks: Consistent with action of alkylating agents.
Reference: Simmon et al., 1977

(e)
Type: Bacterial reverse mutation assay
System of testing: Modified Ames using impingement (in situ) system with SOS umu-test
Species/strain: *Salmonella typhimurium*
Concentration:
Metabolic activation: With **[ ]**; Without **[ ]**; With and Without **[X]**; No data **[ ]**
Results: Negative in a modified Ames test using the impingement (*in situ*) test system. Significant SOS response after 30 minutes impingement with the SOS *umu*-test.
Cytotoxicity conc: With metabolic activation: mg/plate
Without metabolic activation: mg/plate
Precipitation conc: mg/plate
Genotoxic effects: + ? -
Without metabolic activation: **[X] [ ] [X]**
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks: SOS function was detected by colorometry of beta-galactosidase activity encoded by the IacZ gene regulated by the Umu operon.
Reference: Ong et al., 1987

(f)
Type: Mutation to streptomycin independence
System of testing: Aqueous solution of methyl bromide
Species/strain: *Escherichia coli* WP2 hcr/Salmonella TA100, 1535, 98, 1537, 1538
Concentration: 0.5 – 6 umol/l
Metabolic activation: With **[ ]**; Without **[ ]**; With and Without **[X]**; No data **[ ]**

Results: Postive response in *E. coli*, Salmonella TA100, 1535 in the absence of metabolic activation. Negative in *E. coli*, Salmonella TA100 and 1535 in the presence of metabolic activation.

Cytotoxicity conc: With metabolic activation: mg/plate
Without metabolic activation: mg/plate

Precipitation conc: mg/plate

Genotoxic effects:

	+	?	-
With metabolic activation:	**[ ]**	**[ ]**	**[X]**
Without metabolic activation:	**[ ]**	**[ ]**	**[X]**

Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks: Consistent with action of alkylating agents.
Reference: Moriya et al., 1983

(g)
Type: Bacterial reverse mutation assay
System of testing:
Species/strain: *Escherichia coli* Sd4
Concentration: 0.5 to 5 g/m3 in a closed container
Metabolic activation: With **[ ]**; Without **[ ]**; With and Without **[ ]**; No data **[X]**
Results: Slight positive mutagenic response

Cytotoxicity conc: With metabolic activation: mg/plate
Without metabolic activation: mg/plate

Precipitation conc: mg/plate

Genotoxic effects:

	+	?	-
With metabolic activation:	**[ ]**	**[X]**	**[ ]**
Without metabolic activation:	**[X]**	**[ ]**	**[ ]**

Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks: Consistent with action of alkylating agents. High toxicity observed.
Reference: Djalali-Behzad et al., 1981

(h)
Type: Mutation to streptococcus resistance
System of testing: Fluctuation test
Species/strain: *Klebsiella pneumoniae*
Concentration: 950 to 19000 mg/m3
Metabolic activation: With **[X]**; Without **[ ]**; With and Without **[ ]**; No data **[X]**
Results: Positive mutagenic response at concentrations of 4750 mg/m3 and higher.

Cytotoxicity conc: With metabolic activation: mg/plate
Without metabolic activation: mg/plate

Precipitation conc: mg/plate

Genotoxic effects:

	+	?	-
With metabolic activation:	**[ ]**	**[ ]**	**[ ]**
Without metabolic activation:	**[X]**	**[ ]**	**[ ]**

Method: OECD 471 (1983)
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks: Consistent with action of alkylating agents.
Reference: Kramers et al., 1985a

B. NON-BACTERIAL IN VITRO TEST

(a)
Type: Mouse lymphoma assay

System of testing: Gaseous methyl bromide in air-tight bottles
Species/strain: L5178Y-TK+/- cells
Concentration: 0.03 – 30 mg/l
Metabolic activation: With **[ ]**; Without **[ ]**; With and Without **[ ]**; No data **[X]**
Results: Dose-related increase in 6-thioguanine- and bromodeoxyuridine-resistant mutants.
Cytotoxicity conc: With metabolic activation: mg/plate
Without metabolic activation: mg/plate
Precipitation conc:
Genotoxic effects:

	+	?	-
With metabolic activation:	**[ ]**	**[ ]**	**[ ]**
Without metabolic activation:	**[ ]**	**[ ]**	**[ ]**

Method: OECD 473 (1983)
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks:
Reference: Kramers et al., 1985a

(b)
Type: Unscheduled DNA Synthesis
System of testing: Gaseous methyl bromide in air-tight bottles. Autoradiography
Species/strain: Primary cultures of rat hepatocytes
Concentration: 10 – 30 mg/l
Metabolic activation: With **[ ]**; Without **[ ]**; With and Without **[ ]**; No data **[X]**
Results: No unscheduled DNA synthesis found.
Cytotoxicity conc: With metabolic activation: mg/plate
Without metabolic activation: mg/plate
Precipitation conc:
Genotoxic effects:

	+	?	-
With metabolic activation:	**[ ]**	**[ ]**	**[ ]**
Without metabolic activation:	**[ ]**	**[ ]**	**[ ]**

Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks:
Reference: Kramers et al., 1985a

(c)
Type: Sister Chromatid Exchange
System of testing: Gaseous exposure for 100 seconds
Species/strain: Human lymphocytes in culture
Concentration: 4.3%
Metabolic activation: With **[ ]**; Without **[ ]**; With and Without **[ ]**; No data **[X]**
Results: SCE increased from 10 to 16.8 per cell.
Cytotoxicity conc: With metabolic activation: mg/plate
Without metabolic activation: mg/plate
Precipitation conc:
Genotoxic effects:

	+	?	-
With metabolic activation:	**[ ]**	**[ ]**	**[ ]**
Without metabolic activation:	**[ ]**	**[ ]**	**[ ]**

Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks:
Reference: Tucker et al., 1981

(d)

Type:	Sister Chromatid Exchange and Chromosome Aberrations
System of testing:	Exposure in aqueous solution
Species/strain:	Human G_o lymphocytes in culture
Concentration:	0 – 24 ug/ml
Metabolic activation:	With **[ ]**; Without **[ ]**; With and Without **[X]**; No data **[ ]**
Results:	SCE increased in dose-related manner. Chromosome aberrations increased in dose-related manner. Significant increase in chromosome aberrations with S-9 metabolic activation system.
Cytotoxicity conc:	With metabolic activation: mg/plate
	Without metabolic activation: mg/plate
Precipitation conc:	
Genotoxic effects:	+ ? -
	With metabolic activation: **[X] [ ] [ ]**
	Without metabolic activation: **[ ] [ ] [ ]**
Method:	
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Test substance:	
Remarks:	
Reference:	Garry et al., 1990

(e)

Type:	Sister Chromatid Exchange
System of testing:	Exposure in aqueous solution
Species/strain:	Human lymphocytes in culture
Concentration:	19.5 ug/ml
Metabolic activation:	With **[ ]**; Without **[ ]**; With and Without **[X]**; No data **[ ]**
Results:	Increased SCE without metabolic activation in glutathione non-conjugators. Negative response in lymphocytes from conjugators.
Cytotoxicity conc:	With metabolic activation: mg/plate
	Without metabolic activation: mg/plate
Precipitation conc:	
Genotoxic effects:	+ ? -
	With metabolic activation: **[X] [ ] [ ]**
	Without metabolic activation: **[ ] [ ] [ ]**
Method:	
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Test substance:	
Remarks:	
Reference:	Hallier et al., 1993

(f)

Type:	DNA Binding
System of testing:	Exposure in aqueous solution
Species/strain:	Calf DNA in vitro
Concentration:	48 ug/ml
Metabolic activation:	With **[ ]**; Without **[X]**; With and Without **[ ]**; No data **[ ]**
Results:	Increased covalent binding without metabolic activation. (not tested with activation).
Cytotoxicity conc:	With metabolic activation: mg/plate
	Without metabolic activation: mg/plate
Precipitation conc:	
Genotoxic effects:	+ ? -
	With metabolic activation: **[X] [ ] [ ]**
	Without metabolic activation: **[ ] [ ] [ ]**
Method:	
GLP:	Yes **[ ]** No **[ ]** ? **[X]**

Test substance:
Remarks:
Reference: Starratt & Bond, 1988

(g)
Type: Cell Transformation
System of testing: transformation by AS/adenovirus in sealed chambers
Species/strain: Syrian hamster embryo cells
Concentration: 4000 – 16000 mg/m^3 methyl bromide for 2 or 20 hours
Metabolic activation: With **[ ]**; Without **[ ]**; With and Without **[X]**; No data **[ ]**
Results: No enhanced transformation found.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks:
Reference: Hatch et al., 1983

(h)
Type: Unscheduled DNA Synthesis
System of testing: Gaseous methyl bromide over culture media.
Species/strain: Primary cultures of rat hepatocytes
Concentration: 70% methyl bromide in air for 3 hours exposure.
Metabolic activation: With **[ ]**; Without **[ ]**; With and Without **[ ]**; No data **[X]**
Results: No increase in unscheduled DNA synthesis.
Cytotoxicity conc: With metabolic activation: mg/plate
Without metabolic activation: mg/plate
Precipitation conc:
Genotoxic effects:

	+	?	-
With metabolic activation:	**[ ]**	**[ ]**	**[ ]**
Without metabolic activation:	**[ ]**	**[ ]**	**[ ]**

Method:
GLP: Yes **[X]** No **[ ]** ? **[ ]**
Test substance:
Remarks:
Reference: McGregor, 1981

5.6 GENETIC TOXICITY IN VIVO

(a)
Type: Combined Micronucleus and Sister Chromitid Exchange assay
Species/strain: B6C3F_1 Mice
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: Inhalation
Exposure period: 6 h/day, 5 days/week for 14 days (up to 778 mg/m^3 or 200 ppm), or 13 weeks (up to 467 mg/m^3 or 120 ppm)
Doses: 12 to 200 ppm (14 days) & 10 to 120 ppm for 13 weeks
Results: At 200 ppm for 14 days, SCE's and micronuclei were increased in bone marrow cells of male and female mice with increases more marked in females. No significant increases in either SCEs or micronuclei were observed in male and female mice to a concentration of 120 ppm for 13 weeks.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks: Increases were more pronounced in females.
Reference: NTP, 1992

(b)
Type: Micronucleus assay
Species/strain: Fischer rats (F344)
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: Inhalation
Exposure period: 6 h/day, 5 days/week for 14 days
Doses: 0, 154, 200, 260, 338, 440 ppm (0, 600, 778, 1011, 1314, 1712 mg/m^3)
Results: Increased polychromatic erythrocytes with micronuclei were observed males (10X) and females (3X) at 338 ppm.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks:
Reference: Ikawa et al., 1986

(c)
Type: Micronucleus assay
Species/strain: Mice BDF_1
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: Inhalation
Exposure period: 6 h/day, 5 days/week for 14 days
Doses: 0, 154, 200, 260, 338, 440 ppm (0, 600, 778, 1011, 1314, 1712 mg/m^3)
Results: In bone marrow, increased micronuclei occurred in 200 ppm male mice (10X increase) and 154 ppm female mice (6X increase). In peripheral lymphocytes, micronuclei were increased in 200 ppm male mice (32X) and 120 ppm female mice (3X).
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks:
Reference: Ikawa et al., 1986

(d)
Type: Micronucleus assay
Species/strain: Humans (Methyl Bromide Fumigation Workers – n = 32; nonexposed control cohort – n =28)
Sex: Female **[ ]**; Male **[X]**; Male/Female **[ ]**; No data **[ ]**
Route of Administration: Inhalation
Exposure period: 4 or more hours of fumigation work with methyl bromide within previous two weeks
Doses: Concentration was assumed to be 1 – 5 ppm during fumigation work.
Results: Trend toward increased micronuclei in peripheral lymphocytes (not statistically significant; no dose-response).
Method: Micronuclei measured in peripheral lymphocytes and oropharyngeal cells.
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks:
Reference: Calvert et al., 1998

(e)
Type: Hprt frequency
Species/strain: Humans (Methyl Bromide Fumigation Workers – n = 32; nonexposed control cohort – n =28)
Sex: Female **[ ]**; Male **[X]**; Male/Female **[ ]**; No data **[ ]**
Route of Administration: Inhalation
Exposure period: 4 or more hours of fumigation work with methyl bromide within previous two weeks

Doses: Concentration was assumed to be 1 – 5 ppm during fumigation work.
Results: In non-smokers, a trend toward increased hprt variant frequencies detected in peripheral lymphocytes (not statistically significant; no dose-response).
Method: Measured in peripheral lymphocytes cells.
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks:
Reference: Calvert et al., 1998

(f)
Type: Dominant Lethal assay
Species/strain: Rats (Sprague-Dawley, male CD)
Sex: Female **[ ]**; Male **[X]**; Male/Female **[ ]**; No data **[ ]**
Route of Administration: Inhalation
Exposure period: 7 h/day, 5 days/week for 5 days
Doses: 0, 20, 70 ppm (0, 78, 272 mg/m^3)
Results: Negative. No effect on: 1) frequency of pregnancy, 2) number of corpora lutea or implantations/pregnancy, or 3) frequency of early deaths.
Method:
GLP: Yes **[X]** No **[ ]** ? **[ ]**
Test substance:
Remarks:
Reference: McGregor, 1981

(g)
Type: Somatic wing-spot assay
System of testing: Inhalation exposure of Drosophila larvae
Species/strain: *Drosophila melanogaster* third instar larvae
Route of Administration: Inhalation
Concentration: 0 – 20000 mg/m3 methyl bromide for 1 hour
Metabolic activation: With **[ ]**; Without **[ ]**; With and Without **[X]**; No data **[ ]**
Results: Small and large, single as well as twin spot alterations found. Single spot alterations included several types and twin spot alterations occurred from mitotic recombination.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks:
Reference: Katz, 1985, 1987

(h)
Type: Sex-linked recessive lethal assay
Species/strain: *Drosophila melanogaster* (Berlin-K wild type)
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: Inhalation
Exposure period: 6 h/day, 5 days/week for 3 weeks
Doses: 70 – 750 mg/m^3
Results: Negative
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks:
Reference: Kramers et al., 1985b

(i)
Type: Sex-linked recessive lethal assay
Species/strain: *Drosophila melanogaste*r (Oregon-K wild type)

Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: Inhalation
Exposure period: 5 hours
Doses: 0, 20, 70 ppm (0, 78, 272 mg/m^3)
Results: Negative
Method:
GLP: Yes **[X]** No **[ ]** ? **[ ]**
Test substance:
Remarks:
Reference: McGregor, 1981

(j)
Type: DNA alkylation
Species/strain: Mouse (CBA)
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: Inhalation & intraperitoneal injection
Exposure period: 4 hours (for inhalation)
Doses:
Results: Found radiolabel in DNA from liver and spleen cells. Also, positive for hemoglobin alkylation.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance: 14C-radiolabeled methyl bromide
Remarks:
Reference: Djalali-Behzad et al., 1981

(k)
Type: DNA alkylation
Species/strain: Rat (F344)
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: Oral and Inhalation
Exposure period: 6 hours (for inhalation)
Doses:
Results: Found 14C-radiolabel in DNA from liver, lung, stomach, and forestomach cells.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance: 14C-radiolabeled methyl bromide
Remarks:
Reference: Gansewendt et al., 1991

(l)
Type: DNA alkylation
Species/strain: Rat
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: Gavage
Exposure period: single (50 mg/kg) or 10 repeated (25 mg/kg) oral doses
Doses:
Results: Methylated DNA adducts (N7- and/or O6-methyl guanine) found in glandular stomach, forestomach, liver, and other tissues at comparable levels. Repeated dosing caused marked decrease in O6-alkylguanine-DNA alkyltransferase (repair enzyme).
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance: 14C-radiolabeled methyl bromide
Remarks:
Reference: Pletsa et al., 1999

(m)
Type: DNA alkylation
Species/strain: Mouse (lambda lacZ transgenic)
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: Gavage
Exposure period: single or repeated oral doses
Doses:
Results: Methylated DNA adducts (N7- and/or O6-methyl guanine) found in glandular stomach, forestomach, liver, and other tissues at comparable levels. No mutagenesis found in lacZ trasgene in any tissue up to 14 days post-treatment with up to 50 mg/kg (single dose) or 25 mg/kg methyl bromide (up to 10 daily doses).
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance: 14C-radiolabeled methyl bromide
Remarks:
Reference: Pletsa et al., 1999

(n)
Type: Bone marrow cytogenetics
Species/strain: Sprague Dawley rats
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: Inhalation
Exposure period: 7 hours/day for 1 to 5 days.
Doses: 0, 20, 70 ppm (0, 78, 272 mg/m^3)
Results: Negative
Method:
GLP: Yes **[X]** No **[ ]** ? **[ ]**
Test substance:
Remarks: No significant increases in the frequency of chromosomal aberrations.
Reference: McGregor, 1981

(o)
Type: DNA damage assay
Species/strain: Sprague Dawley rats
Sex: Female **[ ]**; Male **[X]**; Male/Female **[ ]**; No data **[ ]**
Route of Administration: Inhalation
Exposure period: 6 hours/day for 5 consecutive days.
Doses: 0, 75, 150 and 250 ppm
Results: Damage to testicular DNA at 250 ppm; negative at 75 and 150 ppm.
Method: Testicular DNA Alkaline Elution
GLP: Yes **[X]** No **[ ]** ? **[ ]**
Test substance:
Remarks: The 250 ppm concentration induced severe clinical signs of toxicity.
Reference: Bentley, 1994

5.7 CARCINOGENICITY

(a)
Species/strain: Rat (Wistar)
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: inhalation
Exposure period: 29 months
Frequency of treatment: 6 hours/day, 5 days/week
Postexposure observation period:
Doses: 0, 3, 30 or 90 ppm

Control group: Yes **[ ]**; No **[ ]**; No data **[X]**;
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[ ]**
Results: NOEL was >90 ppm after 12 months of exposure, 3 ppm after 24 months of exposure, and <3 ppm after 29-months of exposure
Method: OECD 451 (1981)
GLP: Yes **[X]** No **[ ]** ? **[ ]**
Test substance:
Remarks: At the 90 ppm concentration, decreased survival was noted for both the males and females from the end of the second year through termination at 29 months. Also at this concentration, body weights, especially females, were lower than the control group from week 4 and throughout the remainder of the study, and decreased absolute brain weight was noted for females. No differences in hematology, clinical pathology or urinanalysis were seen at either the 3 month or one year intervals. No treatment related evidence of neoplasia was observed in the study. Treatment related nonneoplastic pathology consisted of an increased incidence of thrombi in the heart and myocardial degeneration for both sexes from the 90 ppm group. Irritation of the nasal cavity characterized by hyperplasia of the olfactory epithelium was seen in a time-related fashion for all methyl bromide treatment groups.
Reference: TNO, 1987; Dreef-van der Meulen et al., 1989; Reuzel et al., 1987/1991

(b)
Species/strain: Rat (F344/DuCrj)
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: Inhalation
Exposure period: 2 years
Frequency of treatment: 6 hours/day; 5 days/week
Post exposure observation period: none
Dose: 0, 4, 20 100 ppm (0, 16, 78, 389 mg/m3); 50/sex/exposure level
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[ ]**; Concurrent vehicle **[X]**; Historical **[ ]**
NOEL: none
LOEL:
Results: No affects on mortality were observed at any exposure level. In the high exposure group, both sexes exhibited decreased body weight with increased RBCs, hematocrit and hemoglobin (males only) and altered blood chemistries. High dose subjects also showed inflammation, necrosis, and metaplasia in nasal epithelium with the effect more pronounced in males. Males from the lower exposure groups showed inflammation of nasal epithelia.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Reference: Japanese Ministry of Labor, 1992

(c)
Species/strain: Mouse (Crj:BDF1)
Sex: Female **[ ]**; Male **[X]**; Male/Female **[ ]**; No data **[ ]**
Route of Administration: Inhalation
Exposure period: 104 weeks
Frequency of treatment: 6 hours/day, 5 days/week
Post exposure observation period: none
Dose: 0, 4, 16, 64 ppm (16, 62, 250 mg/m3) (50/sex/exposure level)
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[ ]**; Concurrent vehicle **[X]**; Historical **[ ]**
NOEL: none
LOEL:

Results: No effect on mortality at any exposure level. High exposure group: reduced body weight gain, increased incidence of slight atrophy of granular layer of cerebellum in both sexes, altered blood chemistries. No effect reported at lower exposures.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance: 99.9% purity
Reference: Japanese Ministry of Labor, 1992

(d)
Species/strain: Mouse ($B6C3F_1$)
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: inhalation
Exposure period: 2 year
Frequency of treatment: 6 hours/day, 5 days/week
Postexposure observation period: No data
Doses: 0, 10, 33, or 100 ppm
Control group: Yes **[ ]**; No **[ ]**; No data **[X]**;
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[ ]**
Results: The 100 ppm exposure concentration exceeded an acceptable Maximum Tolerated Dose for carcinogenicity testing. A NOAEL was set at less than 10 ppm due to neurobehavioral testing changes and sternal dysplasia observed in higher dose groups
Method: OECD 451 (1981)
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks: The 100 ppm exposure concentration was terminated after 20 weeks due to debilitating neurotoxicity and mortalities; these animals were exposed to untreated air for the remainder of the two-year study period. No evidence of carcinogenic activity. (See also Other Relevant Information A. Specific toxicities for neurotoxicity).
Reference: NTP, 1992
(e)
Species/strain: Rat (Wistar)
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: gavage in arachis oil
Exposure period: 90 days
Frequency of treatment:
Postexposure observation period:
Doses: 0.4, 2, 10, or 50 mg/kg (10 animals/sex/group)
Control group: Yes **[ ]**; No **[ ]**; No data **[X]**;
Concurrent no treatment **[ ]**; Concurrent vehicle **[ ]**; Historical **[ ]**
Results: At the highest dose level of 50 mg/kg, squamous cell carcinomas of the forestomach were initially reported for 13 out of 20 animals. However, in a subsequent re-examination of slides by a panel of NTP pathologists, lesions previously considered malignant carcinomas were re-categorized as forestomach lesions, characterized by inflammation and hyperplasia. Inflammatory lesions of forestomach were also seen in animals treated with 2 and 10 mg/kg/day. A marked diffuse hyperplasia of the epithelium of the forestomach was seen in all animals in this group.
Method: OECD 451 (1981)
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks: Forestomach lesions reported at 50 mg represented inflammation and hyperplasia rather than malignant lesions.
Reference: Danse et al, 1984; Pesticide & Toxic Chemical News; 1984

(f)
Species/strain: Rat (Wistar)
Sex: Female **[ ]**; Male **[X]**; Male/Female **[ ]**; No data **[ ]**
Route of Administration: gavage in peanut oil vehicle
Exposure period: 13, 17, 21, or 25 weeks
Frequency of treatment: 5 times per week
Postexposure observation period: 12 weeks
Doses: 50 mg/kg (15 animals /group)
Control group: Yes **[ ]**; No **[ ]**; No data **[X]**;
Concurrent no treatment **[ ]**; Concurrent vehicle **[ ]**; Historical **[ ]**
Results: Evidence of malignancy, seen in one rat, was considered to be a very early carcinoma.
Method: OECD 451 (1981)
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks: At week 13, inflammation, acanthosis, fibrosis, and pseudoepitheliomatous in the forestomach were observed. At week 25, all rats had hyperplastic lesions of the forestomach that were more severe than those at 13 weeks.
In the stop treatment group that had received methyl bromide for 13 weeks, there was a regression of the stomach lesions, but at the 12-week final sacrifice, adhesions, fibrosis, and mild acanthosis remained.
Reference: Boorman et al., 1986

(g)
Species/strain: Rat
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[ ]**; No data **[X]**
Route of Administration: Oral (gavage)
Exposure period: No data
Frequency of treatment: No data
Postexposure observation period: 30, 60, 90, or 120 days (some 90 day animals observed for 30 to 60 days
Doses: 25 and 50 mg/kg
Control group: Yes **[ ]**; No **[ ]**; No data **[X]**;
Concurrent no treatment **[ ]**; Concurrent vehicle **[ ]**; Historical **[ ]**
Results: No evidence of malignancy was seen in the stomachs of treated rats.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks: No tumors found. Gross and microscopic alterations found, predominantly in the non-glandular part of the stomach. Ulceration and pseudoepitheliomatous hyperplasia (hyperkeratosis, acanthosis, and epithelial peg formation) found in squamous epithelium of stomach. Fibrosis, foreign material (hair) and inflammation found in muscularis mucosa, submucosa, and tunica muscularis of some rats. Lesions regressed markedly in a 30-60 day recovery period.
Reference: Hubbs & Harrington, 1986

(h)
Species/strain: Rat (F-344)
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: oral feeding study (diets fumigated with methyl bromide)
Exposure period: 2 year
Frequency of treatment: No data
Postexposure observation period: No data
Doses: 80, 200, or 500 mg bromide/kg food (60/sex/dose)
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[ ]**; Concurrent vehicle **[X]**; Historical **[ ]**

Results: No carcinogenic effects were observed.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks: In this study, total bromide levels varied among the experimental exposure groups, apparently not methyl bromide. The experimental diets were fumigated with methyl bromide (presumably, the control diet was not). It is not clear what levels of methyl bromide were in the experimental group diets or that methyl bromide levels varied among the experimental group diets.
Reference: Mitsumori et al., 1990

(i)
Species/strain: Rat (Sprague-Dawley)
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: oral feeding study (microencapsulated methyl bromide)
Exposure period: 2 year
Frequency of treatment: Daily
Postexposure observation period: none
Doses: 0, 0.5, 2.5, 50, or 250 ppm (?/sex/dose)
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[ ]**; Concurrent vehicle **[X]**; Historical **[ ]**
Results: No carcinogenic effects were observed.
Method:
GLP: Yes **[X]** No **[ ]** ? **[ ]**
Test substance:
Remarks: No toxicological effects noted except at high exposure level. 250 ppm rats of both sex exhibited reduced food consumption, body weight gain, mean body weights in the first 18 months of exposure. No increased incidence of tumors was found at any exposure level.
Reference: Mertens, 1997

(j)
Species/strain: Dog (Beagle)
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: oral feeding study (diet fumigated with methyl bromide)
Exposure period: 12 Months
Frequency of treatment: 5 days/week
Postexposure observation period: none
Doses: 0, 0.5, 1.5, or 5 ppm (?/sex/dose)
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[ ]**; Concurrent vehicle **[X]**; Historical **[ ]**
Results: No carcinogenic effects were observed.
Method:
GLP: Yes **[X]** No **[ ]** ? **[ ]**
Test substance:
Remarks: No toxicological effects noted at any exposure level. No increased incidence of tumors was found at any exposure level.
Reference: Newton, 1995; Wilson, 1998

5.8 TOXICITY TO REPRODUCTION

(a)
Type: Fertility **[ ]**; One generation study **[ ]**; Two generation study **[X]**;
Other **[ ]**
Species/strain: Rat (Sprague-Dawley) (25/sex/exposure level)
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: whole body inhalation

Exposure period: approximately 8 months
Frequency of treatment: 6 hours/day, 5 days/week
Postexposure observation period:
Premating exposure period: male: . . . . . ., female:
Duration of the test:
Doses: 0, 3, 30, or 90 ppm
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[ ]**
NOEL Parental: N/A
NOEL F1 Offspring: N/A
NOEL F2 Offspring: N/A
Results: No deaths or noteworthy antemortem clinical findings were observed over the course of the study. The 90 ppm F0 males had significantly decreased body weights at five of the 10 pre-mating intervals and at final sacrifice. No other decreases in body weights were observed among the F0 generation or during the F1 generation prior to the gestational period for the F2 litter. A slight depression of body weight was noted during the gestation and lactation periods for the 90 ppm F1 dams. Reproductive performance was not altered and there were no significant differences in pup survival. No methyl bromide related anomalies were noted for the progeny. Gross pathologic examination revealed no treatment related lesions in either the parental animals or their progeny. Mean brain weight for the 90 ppm (F0 and F1) and females (F1) were decreased. Increased liver to body weight ratio for the 90 ppm F0 males and females and increased heart to brain weight ratios for the 90 ppm F1 females were noted. No other significant differences were seen in the parent organ weight data. No significant differences in the F1b progeny body and organ weights were noted but statistically significant decreases in final body weights were observed for the 90 ppm F2b males and females and the 30 ppm F2b females. F2b progeny organ weights were significantly reduced for the: 90 ppm female brain, heart, kidney and liver weights; the 30 ppm female liver weight; and the 30 and 90 ppm liver to brain weight ratio. The 30 and 90 ppm F2b female brain to body weight ratio was increased. There were no other significant differences noted for progeny. Microscopic examination of the reproductive organs and other tissues revealed no treatment related lesions.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks:
Reference: American Biogenics Corporation, 1986

(b)
Type: Fertility **[X]**; One generation study **[ ]**; Two generation study **[ ]**;
Other **[ ]**
Sperm morphology and vaginal cytology examinations (SMVCEs)
Species/strain: Rat (F344))
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: whole body inhalation
Exposure period: 13 weeks
Frequency of treatment: not specified
Duration of the test: 13 weeks
Doses: 30, 60, 120 ppm (117, 233, 467 mg/m^3)
Control group: Yes **[ ]**; No **[ ]**; No data **[ ]**;
Results: Decreased body weight, decreased cauda epididymis weight, increased relative testicular weight, decreased sperm density, increased percent abnormal sperm. No effects on estrous cycle of females.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**

Test substance:
Remarks:
Reference: Morrissey et al., 1988

(c)
Type: Fertility **[X]**; One generation study **[ ]**; Two generation study **[ ]**; Other **[ ]**
Sperm morphology and vaginal cytology examinations (SMVCEs)
Species/strain: Mouse (B6C3F1)
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: whole body inhalation
Exposure period: 13 weeks
Frequency of treatment: not specified
Postexposure observation period:
Premating exposure period: male: . . . . . ., female: .....
Duration of the test:
Doses: 10, 40, 120 ppm (39, 156, 467 mg/m^3)
Control group: Yes **[ ]**; No **[ ]**; No data **[ ]**;
Results: Decreased body weight. Increased relative epididymis and testicular weights, decreased sperm density, increased percent abnormal sperm. No effects reported for females.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks:
Reference: Morrissey et al., 1988

(d)
Type: Fertility **[X]**; One generation study **[ ]**; Two generation study **[ ]**; Other **[ ]**
Species/strain: Rat
Sex: Female **[ ]**; Male **[X]**; Male/Female **[ ]**; No data **[ ]**
Route of Administration: whole body inhalation
Exposure period: Up to 40 days
Frequency of treatment:
Postexposure observation period:
Premating exposure period: male: . . . . . ., female:.....
Duration of the test:
Doses: 70 ppm (272 mg/m^3)
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[ ]**
Results: Reproductive performance unimpaired.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks:
Reference: Hardin et al., 1981; Sikov et al., 1981

(e)
Type: Fertility **[X]**; One generation study **[ ]**; Two generation study **[ ]**; Other **[ ]**
Species/strain: Rat
Sex: Female **[ ]**; Male **[X]**; Male/Female **[ ]**; No data **[ ]**
Route of Administration: whole body inhalation
Exposure period: Up to 6 weeks
Frequency of treatment: 6 hours/day, 5 days/week
Postexposure observation period:

Premating exposure period: male: . . . . . ., female: .....
Duration of the test:
Doses: 160 ppm (622 mg/m^3)
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[ ]**
Results: Degeneration of spermatocytes and late stage spermatids characterized by separation and sloughing as well as formation of intratubular multinucleated giant cells. Degeneration of spermatogenic epithelium included more or less severe loss of spermatogenic epithelial components.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks:
Reference: Eustis et al., 1988

(f)
Type: Fertility **[X]**; One generation study **[ ]**; Two generation study **[ ]**;
Other **[ ]**
Species/strain: Mouse
Sex: Female **[ ]**; Male **[X]**; Male/Female **[ ]**; No data **[ ]**
Route of Administration: whole body inhalation
Exposure period: Up to 6 weeks
Frequency of treatment: 6 hours/day, 5 days/week
Postexposure observation period:
Premating exposure period: male: . . . . . ., female: .....
Duration of the test:
Doses: 160 ppm (622 mg/m^3)
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[ ]**
NOEL Parental: N/A
NOEL F1 Offspring: N/A
NOEL F2 Offspring: N/A
Results: Testicular degeneration seen with low severity but high frequency.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks:
Reference: Eustis et al., 1988

(g)
Type: Fertility **[X]**; One generation study **[ ]**; Two generation study **[ ]**;
Other **[ ]**
Species/strain: Rat (Sprague-Dawley) (10 – 12/group)
Sex: Female **[ ]**; Male **[X]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: whole body inhalation
Exposure period: 6 weeks
Frequency of treatment: 4 hours/day, 5 days/week
Postexposure observation period:
Premating exposure period: male: . . . . . ., female: .....
Duration of the test:
Doses: 0, 200, 300, or 400 ppm (10/dose)
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[ ]**; Concurrent vehicle **[ ]**; Historical **[ ]**
NOEL Parental: N/A
NOEL F1 Offspring: N/A
NOEL F2 Offspring: N/A

Results: In 1 of 10 rats at 200 ppm and 6 of 10 at 300 ppm, and 6 of 8 at 400 ppm, adverse effects on the testes were seen. These effects were unilateral and included: atrophy of seminal epithelium, incomplete spermatogenesis, giant cells in seminal tubules. Necrotic spermatocytes occurred in seminal fluid without spermatozoa in tubules of epididymis adjacent to atrophied testes.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks:
Reference: Kato et al., 1986

(h)
Type: Fertility **[X]**; One generation study **[ ]**; Two generation study **[ ]**; Other **[X]**
Species/strain: Rat (F344N)
Sex: Female **[ ]**; Male **[X]**; Male/Female **[ ]**; No data **[ ]**
Route of Administration: whole body inhalation
Exposure period: 5 days
Frequency of treatment: 6 hours/day, 5 days/week
Postexposure observation period:
Premating exposure period: male: . . . . . ., female: .....
Duration of the test:
Doses: 200 ppm (778 mg/m^3)
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**; Concurrent no treatment **[ ]**; Concurrent vehicle **[X]**; Historical **[ ]**
NOEL Parental: N/A
NOEL F1 Offspring: N/A
NOEL F2 Offspring: N/A
Results: No effect on testis weight, daily sperm production, cauda epididymal sperm count, sperm morphology, percent motile sperm, linear sperm velocity, and epididymal and testicular histology. Transient decrease in plasma testosterone and testicular nonprotein sulfhydryl concentrations during exposure.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks:
Reference: Hurtt and Working, 1988

(i)
Type: Fertility **[X]**; One generation study **[ ]**; Two generation study **[ ]**; Other **[ ]**
Species/strain: Mouse (B6C3F1)
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: whole body inhalation
Exposure period: 5 days
Frequency of treatment: 7 hours/day, 5 days/week
Postexposure observation period:
Premating exposure period: male: . . . . . ., female: .....
Duration of the test:
Doses: 0, 20, or 70 ppm (0, 78, 272 mg/m^3) (10/males/dose)
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**; Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[ ]**
NOEL Parental: N/A
NOEL F1 Offspring: N/A
NOEL F2 Offspring: N/A
Results: No effect on spermatozoa found.

Method:
GLP: Yes **[X]** No **[ ]** ? **[ ]**
Test substance:
Remarks: .....
Reference: McGregor, 1981

5.9 DEVELOPMENTAL TOXICITY/TERATOGENICITY

(a)
Species/strain: Rat (Wistar) (39-45/group)
Sex: Female **[X]**; Male **[ ]**; Male/Female **[ ]**; No data **[ ]**
Route of Administration: Inhalation
Duration of the test: Caesarean sacrifice on the 19th day
Exposure period: Days 1-19 of gestation or Pregestationally to 20 – 70 ppm for 3 weeks
Frequency of treatment: 7 hours/day or 5/week for 3 weeks
Doses: (Pregestational/Gestational) 0/0, 0/20, 0/70, 20/0, 20/20, 70/0, 70/70 ppm
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[ ]**
NOEL Maternal Toxicity: N/A
NOEL teratogenicity: N/A
Results: No clinical evidence of maternal toxicity, fetotoxicity, or developmental toxicity was observed in any exposure scenario.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks: Three weeks premating exposure.
Reference: Sikov et al., 1981; Hardin et al., 1981

(b)
Species/strain: Rabbit (New Zealand White) (25/group)
Sex: Female **[X]**; Male **[ ]**; Male/Female **[ ]**; No data **[ ]**
Route of Administration: Inhalation
Duration of the test: Sacrifice on the 30th day for control and low dose; After 1 week for high dose
Exposure period: Days 1-24 of gestation
Frequency of treatment:
Doses: 0, 20, or 70 ppm (24/group)
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[ ]**
NOEL Maternal Toxicity: N/A
NOEL teratogenicity: N/A
Results: No fetotoxicity, or developmental toxicity was observed for the 20 ppm group.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks: Rabbits exposed to 70 ppm were terminated due to excessive mortality and neurotoxicity characterized by convulsions and paresis in the hindlimbs seen after one week of treatment.
Reference: Sikov et al., 1981; Hardin et al., 1981

(c)
Species/strain: Rabbit (New Zealand White) – probe study
Sex: Female **[X]**; Male **[ ]**; Male/Female **[ ]**; No data **[ ]**
Route of Administration: Inhalation
Duration of the test: Sacrifice on gestation day 17
Exposure period: Days 7-19 of gestation

Frequency of treatment: 6 hours/day
Doses: 0, 10, 30, or 50 ppm in one study; 0, 70, or 140 ppm in the second
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[ ]**
NOEL Maternal Toxicity: N/A
NOEL teratogenicity: N/A
Results: Evidence of toxicity was observed only in the 140 ppm group does. All does exposed to methyl bromide at this concentration showed lethargy and decreased food consumption after 8 exposures. With continued exposure, signs of neurotoxicity were apparent and resulted in sacrifice of the does on gestation day 17. No apparent embryotoxicity was observed at any exposure level.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks:
Reference: Breslin et al., 1990a

(d)
Species/strain: Rabbit (New Zealand White) – (Phase I: 26/group - Phase II: 25/group)
Sex: Female **[X]**; Male **[ ]**; Male/Female **[ ]**; No data **[ ]**
Route of Administration: Inhalation
Duration of the test: Caesarean delivery was performed on day 28 of gestation
Exposure period: Days 7-19 of gestation
Frequency of treatment: 6 hours/day
Doses: 0, 20, 40, or 80 ppm in the initial phase; 0 or 80 ppm in the second phase
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[ ]**
NOEL Maternal Toxicity: N/A
NOEL teratogenicity: N/A
Results: In the first phase, maternal toxicity, evidenced by decreased body weight gain and clinical signs of neurotoxicity, was observed in three of the does from the 80 ppm group. The clinical signs consisted of right-sided head tilt, ataxia, slight lateral recumbency and lethargy. In the second study, a significant decrease in bodyweight during gestation was the only evidence of maternal toxic 80 ppm group only. In phase 1, fetal findings consisted of low incidences of omphalocele, hemorrhaging with or without hydrops (edema), retroesophogeal right subclavian artery, gall bladder agenesis, and fused sternebra. Fetal effects in phase 2 were limited to decreased fetal weight, hemorrhaging with or without hydrops and gall bladder agenesis.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks:
Reference: Breslin et al., 1990a, 1990b

(e)
Species/strain: Rats
Sex: Female **[X]**; Male **[ ]**; Male/Female **[ ]**; No data **[ ]**
Route of Administration: Gavage (peanut oil vehicle)
Duration of the test: Doses administered daily during organogenesis
Exposure period: Days 5-19 of gestation
Frequency of treatment: Single daily doses on days 5 through 20 of organogenesis
Doses: 0, 0.5, 5, 25, 50 mg/kg/day
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[ ]**; Concurrent vehicle **[X]**; Historical **[ ]**
NOEL Maternal Toxicity: N/A

NOEL teratogenicity: N/A
Results: Maternal toxicity at 25 and 50 mg/kg/day. No developmental effects at 25 mg/kg/day or below. Effects at 50 mg/kg/day considered to be related to maternal toxicity and not a direct effect of methyl bromide.
Method:
GLP: Yes **[ ]** No **[X]** ? **[ ]**
Test substance:
Remarks:
Reference: Peters et al., 1982

5.10 OTHER RELEVANT INFORMATION

A. Specific toxicities

(a)
Type: Acute neurotoxicity
Species/strain: Rat (CD Sprague-Dawley)
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: inhalation
Duration of the test: Sacrificed after 15 days
Exposure period: 6 hours
Frequency of treatment:
Post-exposure evaluations: 7 and 14 days
Doses: 0, 30, 100, or 350 ppm
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[ ]**
Results: All animals survived to study termination. No methyl bromide induced effects for body or brain weights. Neurobehavioral effects were observed only in the 350 ppm exposed group and were limited to the 3-hour post exposure assessment. Effects noted in male and female rats consisted of decreased arousal, increased incidences of drooping or half-shut eyelids, piloerection, decreased rearing, depressed body temperature, and markedly decreased motor activity. The 350 ppm males had a decreased tail pinch response while females from this group showed increased urination and abnormal air righting response. No treatment related histological finding were seen in nervous system or nasal tissues.
Method:
GLP: Yes **[X]** No **[ ]** ? **[ ]**
Test substance:
Remarks:
Reference: Driscoll & Hurley, 1993

(b)
Type: Acute neurotoxicity (aversion to saccharin taste)
Species/strain: Rat
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: inhalation
Duration of the test: 1 day
Exposure period: 4 hours
Frequency of treatment:
Post-exposure evaluations: Not applicable.
Doses: 0, 25, 50, or 100 ppm
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[ ]**
Results: Aversion to saccharin water increased with methyl bromide exposure in a concentration dependent manner.

Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks:
Reference: Miyagawa, 1982

(c)
Type: Acute neurotoxicity (neurotransmitter levels)
Species/strain: Rat
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: inhalation
Duration of the test: 1 day
Exposure period: 8 hours
Frequency of treatment:
Post-exposure evaluations: Not applicable.
Doses: 0, 31, 82, 125, or 250 ppm
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[ ]**
Results: NOAEL = 31 ppm. Subjects at higher exposure levels exhibited decreased dopamine, norepinephrine, and increased metabolites (homovanillic acid, 3-methoxy-4-hydroxyphenyl glycol) in various regions of CNS. Largest effects in striatum; maximum effect time was 0 – 2 hours post-exposure.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks: Monitored neurotransmitter levels in CNS.
Reference: Honma et al., 1987a & b

(d)
Type: Acute neurotoxicity (neurotransmitter levels)
Species/strain: Rat
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: inhalation
Duration of the test: 1 day
Exposure period: 8 hours
Frequency of treatment:
Post-exposure evaluations: Not applicable.
Doses: 0, 16, ??, ??, or 250 ppm
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[ ]**
Results: Throughout exposure range, subjects exhibited concentration-dependent decrease in tyrosine hydroxylase activity. Hypothalamus most effected.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks: Monitored neurotransmitter levels in CNS (tyrosine hydroxylase in striatum, hypothalamus, frontal cortex, midbrain, and medulla oblongata.
Reference: Honma, 1991

(e)
Type: Acute neurotoxicity (neurotransmitter levels)
Species/strain: Rat
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: CNS perfusion (MeBr in 10% ethanol/90% artificial cerebrospinal fluid)
Duration of the test: 1 day
Exposure period:
Frequency of treatment:

Post-exposure evaluations: Not applicable.
Doses: 0, 0.1, 0.5, or 1 ug MeBr/ul vehicle
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[ ]**
Results: At 0.1 ug/ul, reduced 5-hyrdoxyindoleacetic acid (serotonin metabolite). At 0.5 and 1.0 ug/ul in striatum perfusate, increased 3,4-dihydroxyphenyl acitic acid and homovanillic acid (dopamine metabolites) that persisted after perfusion stopped; decreased 5-hydroxyindoleacetic acid that returned to normal.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks: Monitored neurotransmitter levels in CNS (domamine and serotonin metabolites in various regions of the CNS).
Reference: Honma, 1992

(f)
Type: Subacute neurotoxicity
Species/strain: Rat (10/sex/group)
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: Inhalation
Duration of the test: 5 day
Exposure period: 6 hr/day for 5 days
Frequency of treatment:
Post-exposure evaluations: Not applicable.
Doses: 0 or 150 ppm
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[ ]**
Results: Glutathione depleted and glutathione transferase activity decreased in frontal cortex, caudate nucleus, hippocampus, brain stem, and cerebellum. No changes in monoamines but aspartic acid and glycine were increased in frontal cortex and aspartic acid in cerebellum. No histopathology found.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks: Monitored regional brain glutathione-S-transferase activity, glutathione levels, monoamine and amino acid levels, neurohistopathology.
Reference: Davenport et al., 1992

(g)
Type: Subchronic neurotoxicity
Species/strain: Rat
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: Inhalation
Duration of the test: 3-8 weeks
Exposure period: 6 hr/day, 3 day/week, for 3 – 8 weeks
Frequency of treatment:
Post-exposure evaluations: Not applicable.
Doses: 0, 290, or 500 ppm
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[ ]**
Results: No neurohistopathology reported at 290 ppm for 8 weeks. At 500 ppm, axonal degeneration of myelinated fibers at cervical levels of fasciculus gracilis; necrosis/atrophy of neurons in caudate-putamen, thalamus, cingulated cortex after expsure of 10 to 18 days.
Method:

GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks: Monitored neurohistology of CNS & PNS.
Reference: Davenport et al., 1992

(h)
Type: Subchronic neurotoxicity
Species/strain: Rat (CD Sprague-Dawley)
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: inhalation
Duration of the test:
Exposure period: 13 weeks
Frequency of treatment: 6 hours/day, 5 days/week
Doses: 0,30, 70, or 140 ppm
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[ ]**
Results: At the 140 ppm concentration, two male rats died during the first month. Clinical signs observed for these rats included convulsions, tremors, hyperactivity, rapid respiration and salivation. Mean body weights were significantly lower than the controls. Neurologic evaluations for males revealed increased hind limb splay (Weeks 4, 8, 13), abnormal air righting reflex (Week 13), and decreased forelimb strength (Week 13). Female rats demonstrated lower arousal scores (Weeks 8, 13), decreased rearing (Weeks 4, 8, 13) and significantly decreased motor activity (Week 13). Mean absolute brain weights were significantly lower for both sexes; no differences were noted for the relative brain weights indicating that lower absolute brain weight was a reflection of the generally lower body weights for the treated animals. Gross lesions were limited to moderate to severe brain hemorrhage in the two 140 ppm male animals which died. Microscopic lesions in the brain were found in these two males and in one 140 ppm male that survived the 13-week exposure. Microscopic lesions in the brain were seen in these three males and consisted of neuronal necrosis in the hippocampus, necrosis and malacia in the cerebral cortex and basal ganglia, and malacia and/or necrosis in the thalamus and midbrain. The lesions were more severe for one of the males which was found dead and the male that survived the 13-week secondary effects of brain swelling related to the convulsions. One additional 140 ppm male had slight neuronal edema in the hippocampus. Other lesions in the 140 ppm group consisted of minimal regenerative dysplasia of the olfactory epithelium of the nasal cavity in three males and three females and minimal peripheral nerve degeneration in two males and two females. In the 70 ppm group, lower mean body weights and weight gain were seen for females from Week 9 onwards of the study. Neurologic findings were limited to slightly decreased (5% lower than the control group), no difference was seen for the relative brain weight and no microscopic pathology was seen. At 30 ppm, the mean absolute brain weight for females was statistically significantly lower than control (5% lower than the control group), however, no difference was seen in relative brain weight and no microscopic pathology in the brain was seen. Peripheral nerve degeneration was observed in one female rat. This finding was considered incidental since nerve degeneration was not seen in animals from the 70 ppm group.
Method:
GLP: Yes **[X]** No **[ ]** ? **[ ]**
Test substance:
Remarks:
Reference: Norris et al., 1993

(i)
Type: Subchronic neurotoxicity
Species/strain: Rat (CD Sprague-Dawley) (2/sex/group)
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: inhalation
Duration of the test:
Exposure period: 4 weeks (second phase at 55 ppm for 35 weeks)
Frequency of treatment: 7.5 hours/day, 4 days/week
Doses: 0, or 65 ppm (second phase at 55 ppm)
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[ ]**
Results: No effect found on any parameters. In an extended study at 55 ppm, rats showed no effects after 35 weeks.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks: Parameters monitored included nerve conduction velocity of ulnar and sciatic nerves, open field activity, grip coordination.
Reference: Anger et al., 1981

(j)
Type: Subchronic neurotoxicity
Species/strain: Rabbit (New Zealand) (2/sex/group)
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: inhalation
Duration of the test:
Exposure period: 4 weeks
Frequency of treatment: 7.5 hours/day, 4 days/week
Doses: 0, or 65 ppm
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[ ]**
Results: Decreased eye-blink reflex magnitude. Decreased nerve conduction velocity. Hind limb paralysis. Decreased body weight gain.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks: Parameters monitored included nerve conduction velocity of ulnar and sciatic nerves, and eye blink reflex.
Reference: Anger et al., 1981

(k)
Type: Subchronic neurotoxicity
Species/strain: Rabbit (New Zealand) (2/sex/group)
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: inhalation
Duration of the test:
Exposure period: 8 months
Frequency of treatment: 7.5 hours/day, 4 days/week
Doses: 0, 27, or 65 ppm
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[ ]**
Results: Decreased eye-blink reflex magnitude. Decreased nerve conduction velocity. Hind limb paralysis. Decreased body weight gain. After 6-8 week recovery, effects were partially reversible.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:

Remarks: Follow-up to Anger et al., 1981. Parameters monitored included nerve conduction velocity of ulnar and sciatic nerves, and eye blink reflex.
Reference: Russo et al., 1981

(l)
Species/strain: Mouse ($B6C3F_1$)
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: inhalation
Exposure period: 2 year
Frequency of treatment: 6 hours/day, 5 days/week
Postexposure observation period: No data
Doses: 0, 10, 33, or 100 ppm
Control group: Yes **[ ]**; No **[ ]**; No data **[X]**;
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[ ]**
Results: The 100 ppm exposure concentration exceeded an acceptable Maximum Tolerated Dose for carcinogenicity testing. A NOAEL was set at less than 10 ppm due to neurobehavioral testing changes and sternal dysplasia observed in higher dose groups
Method: OECD 451 (1981)
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks: The 100 ppm exposure concentration was terminated after 20 weeks due to debilitating neurotoxicity and mortalities; these animals were exposed to untreated air for the remainder of the two-year study period. Interim sacrifice of ten mice per sex/treatment level was performed after 6 months and 15 months. Clinical signs of neurotoxicity consisting of tremors, paralysis, gait disturbances, and abnormal posture were noted for 100 ppm males and females. Similar findings were seen for a few of the 33 ppm exposed animals. After 3 months of exposure, neurobehavioral changes were noted for the 10 ppm and 33 ppm groups after six months of exposure. Decreased body weights were observed in females dosed at 33 ppm and in both sexes dosed at 100 ppm. Exposure related histologic changes were generally limited to the 100 ppm animals and consisted of findings in the brain (degeneration of the cerebrum and cerebellum), heart (degeneration and cardiomyopathy), sternal dysplasia and either necrosis or metaplasia of the olfactory epithelium. (see also 5.7 Carcinogenicity)
Reference: NTP, 1992

(m)
Type: Subchronic neurotoxicity
Species/strain: Rat/Unknown (135 total)
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[ ]**; No data **[X]**
Route of Administration: Inhalation
Exposure period: Up to six months
Frequency of treatment: 6hr/day, 5 day/week
Doses: 0, 17 33,65, 100, or 200 ppm
Control group: Yes **[ ]**; No **[ ]**; No data **[X]**;
Concurrent no treatment **[ ]**; Concurrent vehicle **[ ]**; Historical **[ ]**
Results: No effects reported at 17, 33 and 65ppm (NOAEL) paralysis, lung injury and death at 100 and 200 ppm
Method:
GLP: Yes **[ ]** No **[X]** ? **[ ]**
Test substance: Methyl bromide
Remarks: These studies are a secondary reference with a Klimisch score of 4 but they are widely referenced and the results are considered relevant and consistent.

Reference: Irish, 1940

(n)
Type: Subchronic neurotoxicity
Species/strain: Rabbit/New Zealand White (104 total)
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[ ]**; No data **[X]**
Route of Administration: inhalation

Exposure period: Up to six months
Frequency of treatment: 6hr/day, 5 day/week
Doses: 0, 17 33,65, 100, or 200 ppm
Control group: Yes **[ ]**; No **[ ]**; No data **[X]**;
Concurrent no treatment **[ ]**; Concurrent vehicle **[ ]**; Historical **[ ]**
Results: No effects reported at 17, (NOAEL) paralysis, lung injury and death at 33 and 65ppm 100 and 200 ppm
Method:
GLP: Yes **[ ]** No **[X]** ? **[ ]**
Test substance: Methyl bromide
Remarks: These studies are a secondary reference with a Klimisch score of 4 but they are widely referenced and the results are considered relevant and consistent.
Reference: Irish, 1940

(o)
Type: Subchronic neurotoxicity
Species/strain: Guines pig (98 total)
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[ ]**; No data **[X]**
Route of Administration: inhalation
Exposure period: Up to six months
Frequency of treatment: 6hr/day, 5 day/week
Doses: 0, 17 33,65, 100, or 200 ppm
Control group: Yes **[ ]**; No **[ ]**; No data **[X]**;
Concurrent no treatment **[ ]**; Concurrent vehicle **[ ]**; Historical **[ ]**
Results: No effects reported at 17, 33 and 65ppm and 100 ppm (NOAEL) paralysis, lung injury and death at 200 ppm
Method:
GLP: Yes **[ ]** No **[X]** ? **[ ]**
Test substance: Methyl bromide
Remarks: These studies are a secondary reference with a Klimisch score of 4 but they are widely referenced and the results are considered relevant and consistent.
Reference: Irish, 1940

(p)
Type: Subchronic neurotoxicity
Species/strain: Monkey (13 total)
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[ ]**; No data **[X]**
Route of Administration: inhalation
Exposure period: Up to six months
Frequency of treatment: 6hr/day, 5 day/week
Doses: 0, 17 33,65, 100, or 200 ppm
Control group: Yes **[ ]**; No **[ ]**; No data **[X]**;
Concurrent no treatment **[ ]**; Concurrent vehicle **[ ]**; Historical **[ ]**
Results: No effects reported at 17, and 33 ppm limb paralysis at 65 ppm, paralysis and convulsions at 100 ppm, paralysis lung injury and death at 200 ppm ....
Method:
GLP: Yes **[ ]** No **[X]** ? **[ ]**

Test substance: Methyl bromide
Remarks: These studies are a secondary reference with a Klimisch score of 4 but they are widely referenced and the results are considered relevant and consistent.
Reference: Irish, 1940

Toxicodynamics, toxicokinetics

Type: Toxicokinetics
Results: The uptake of ^{14}C-methyl bromide shows rapid first order kinetics over a broad range of exposure concentrations. Peak concentrations of radiolabel were reached in the blood, liver, fat and brain within 1 hour of exposure and generally plateau at these concentrations during an 8 hour exposure. Highest tissue concentrations of ^{14}C were found in the lung, adrenal, kidney, liver and nasal turbinates. Very low concentrations of ^{14}C were found in the spinal cord and brain. Approximately 80% of radiolabel was eliminated by 65 hour post exposure. Tissue elimination half-lives varies from 1.5 to 8 hours with the exception of the liver (33 hours). In a study with non-radiolabelled methyl bromide, maximum concentrations of methyl bromide were reached in the liver, fat, brain, muscle, kidney, and blood after one hour of exposure and remained at this level through the eight hour exposure period. The highest methyl bromide concentration was found in fat. Methyl bromide was rapidly eliminated from these tissues with a half-life of about 30 minutes post exposure and no methyl bromide was detected in any tissue at 48 hours post exposure. The primary excretion of methyl bromide is as exhaled CO_2. Approximately 47% of total ^{14}C-methyl bromide dose was excreted in expired air as ^{14}C-CO_2; 1% of the expired radioactivity was identified as parent methyl bromide. Urine and fecal excretion of ^{14}C-methyl bromide were 22% and 2% respectively. No evidence of parent methyl bromide was found in excreta samples. The ^{14}C-CO_2 exhalation shows a biphasic pattern with 85% excreted with a half-life of approximately four hours and the remaining 15% with a half life of about 11 hours. Urine and fecal half-lives were 10 hours and 16 hours, respectively. The initial step in the metabolism of methyl bromide is considered to be the release of bromine ion. Peak concentrations of bromine in blood, kidneys and liver occurred from four to eight hours after methyl bromide exposure with tissue half-lives of approximately 5 days.
Remarks:
References: Medinsky et al., 1994, 1995; Anderson et al. 1980; Gargas and Anderson, 1982; Honma et al., 1985; Bond et al., 1985

5.11 EXPERIENCE WITH HUMAN EXPOSURE

(a)
Results: The potential routes of human exposure to methyl bromide are oral, dermal, or inhalation. Extensive studies have shown that residues of methyl bromide found in crops grown on fumigated soils are virtually non-detectable. In addition, concentrations in commodities treated post-harvest, decrease rapidly after required aeration and are non-detectable after relatively short period of time. Humans are not exposed to unsafe levels of methyl bromide's metabolite in food. There is no significant likelihood of oral exposure through consumption of treated food. People living in close proximity to fumigated fields, greenhouses or structures are protected from the risk of significant inhalation exposure through special notice requirements, safety precautions, and the use of buffer zones. The potential for dermal or inhalation exposure is highest for applicators, and other

personnel who are involved in manufacturers, filling, handling or application of methyl bromide. Strictly applied safety measure in manufacturing and filling installations limit the potential risk of exposure to plant personnel. In addition, fumigator/applicators are protected from dermal and inhalation exposures through adherence to strict safety procedures and the use of protective equipment

Remarks: Tolerance levels for the metabolite of methyl bromide in treated foods have been established by the EPA.

Reference:

(b)

Subjects: Fumigation workers

Route of Administration: Inhalation

Exposure period: Chronic

Frequency of treatment: 6 hours/day, 5 days/week

Postexposure observation period: No data

Doses: 1 – 5 ppm assumed

Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[ ]**

Results: All fumigation workers performed less well in dexterity tests and had a higher incidence of tunnel carpel syndrome than non-exposed subjects. Although sulfuryl fluoride workers showed CNS effects including pattern memory deficits and olfactory thresholds, methyl bromide workers did not. Other parameters evaluated in this study were not affected by exposure to fumigants. The authors ascribed decrements in dexterity and the increased incidence of carpel tunnel syndrome to ergonomic stresses rather than to exposure to fumigants.

Method:

GLP: Yes **[ ]** No **[ ]** ? **[X]**

Test substance:

Remarks: Study was conducted with 123 structural fumigation workers predominantly exposed either to methyl bromide or sulfuryl fluoride for an average of 1.20 and 2.85 years, respectively. A reference cohort of 120 workers had no pesticide exposure. All participants were evaluated for 1) nerve conduction velocity and amplitude, 2) vibrotactile threshold, 3) neurobehavioral parameters (hand-eye coordination, reaction time, continuous performance, symbol digit, pattern memory, serial digit learning, and mood scales), 4) visual acuity, 5) olfactory function, and 6) renal function.

Reference: Calvert, 1998

(c)

Subjects: Fumigation workers (greenhouse fumigators)

Route of Administration: Inhalation

Exposure period: Chronic

Frequency of treatment: 6 hours/day, 5 days/week

Postexposure observation period: No data

Doses: 1 – 5 ppm assumed.

Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[ ]**

Results: EEGs were normal except that some workers showed a slight diffuse increase in beta and theat activity. Affected workers, when compared to unaffected workers, showed statistically elevated bromide levels in blood (10.9 vs 8.2 mg Br/l).

Method:

GLP: Yes **[ ]** No **[ ]** ? **[X]**

Test substance:

Remarks: Results were gathered by questionnaire (symptom incidence), by EEG, and by a general medical and neurological evaluation.
Reference: Verberk et al., 1979

(d)
Subjects: Fumigation workers (soil & structural fumigators)
Route of Administration: Inhalation
Exposure period: Chronic
Frequency of treatment: 6 hours/day, 5 days/week
Postexposure observation period: No data
Doses: 1 – 5 ppm assumed.
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[ ]**
Results: Methyl bromide cohorts performed less well in tests of cognitive function, reflexes, and sensory and visual performance. Results were confounded by co-exposures to other fumigants or other chemicals, poor age-matched controls, lack of knowledge of exposure levels, industrial hygiene practices, medication use, alcohol consumption, educational background, ethnicity, and other factors. Particularly regarding ethnicity, the Wechsler Memory Scale test was administered in English even though the methyl bromide cohorts were comprised of a higher proportion of Mexican Americans than the control cohort.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks: Four groups evaluated included 1) structural fumigators using methyl bromide >80% of the time and soil fumigators using a methyl bromide/chloropicrin combination (total n=32), 2) structural fumigators using sulfuryl fluoride >80% of the time (n=24), 3) fumigators using both methyl bromide and sulfuryl fluoride (n=18), and 4) a control group of workers in the fumigation industry but not applying fumigants (n=29). Exposed workers had been fumigators for more than one year and engaged in fumigation operations within 50 days of neurological evaluation.
Reference: Anger et al., 1986

(e)
Subjects: Methyl bromide manufacturing workers
Route of Administration: Inhalation
Exposure period: Chronic
Frequency of treatment: 6 hours/day, 5 days/week
Postexposure observation period: No data
Doses: Less than 4 mg/m3 (<1 ppm) with occasional excursions up to 20 mg/m3 (5-6 ppm).
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[ ]**
Results: The incidence of symptoms was greater in methyl bromide workers compared to controls. Bromide ion concentration in urine did not correlate with symptom incidence.
Method:
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance:
Remarks: Workers were comprised of 75 males employed from 1 to 25 years in a methyl bromide manufacturing plant. The incidence of acute and general symptoms were gathered by means of a questionnaire and compared to a group of non-exposed control (railway) workers. Methyl bromide were monitored every six months.
Reference: Kishi et al., 1991

6. REFERENCES

Alexeeff, G.V., Kilgore, W.W., Munoz, P., Watt, D. 1985. Determination of acute toxic effects in mice following exposure to methyl bromide. J. Toxicol. Environ. Health, 15: 109-123.

Andersen, M., Gargas, M., Jones, R., Jenkins, L. 1980. Determination of the kinetic constants for metabolism of inhaled toxicants in vivo using gas uptake measurements. Toxicol. Appl. Pharmacol., 54: 100-116

Anger, W.K., Setzer, J.V., Russo, J.M., Brightwell, W.S., Wait, R.G., Johnson, B.L., 1981. Neurobehavioral effects of methyl bromide inhalation exposures. Scan. J. Work Environ. Health 7:40-47.

Anger, W.K., Moody, L., Burg, J., Brightwell, W.S., Taylor, B.J., Russo, J.M., Dickerson, N. Setzer, J.W., Johnson, B.L., Hicks, K., 1986. Neurobehavioral evaluation of soil and structural fumigators using methyl bromide and sulfuryl fluoride. Neurotoxicology 7:137-156.

American Biogenics Corporation, 1986. Two-generation reproduction study in albino rats with methyl bromide - results of both generations (Study No. 4500-1525) (Unpublished final report).

Atochem, 1987. Fumyl-o-gas. Fiche de données de sécurité. Paris, Groupe Elf Aquitaine, 4 pp.

Balander, P.A., Polyak, M.G., 1962. Toxicological characteristics of methyl bromide. J. Gig. I. Tokskol. 60:412-419.

Bakhishev, G.N., 1973. Relative toxicity of aliphatic halohydrocarbons to rats. Farmakol. Toksikol., 8:140-142 (in Russian).

Baumann, H., Heumann K.G., 1987. Analysis of organobromine compounds and hydrogen bromide in motor car exhaust gases with a GC/microwave plasma system. Fresenius Z. Anal. Chem., 327: 186-192.

Bell, C.H., 1988. Minimum concentration levels of methyl bromide required for full efficacy against seven species of stored-product beetle at two temperatures.

Bentley, K.S., (1994). Detection of single strand breaks in rat testicular DNA by alkaline elution following in vivo inhalation exposure to methyl bromide. Unpublished report from E.I. DuPont Haskell Laboratories Project no. 9714-001: MBIP/21/ALK/HASK:999.

Bolsa Research, 1990. Methyl bromide – octanol/water partition coefficient. Report No. BR 172:90. Sponsor: CMA Methyl Bromide Industry Panel.

Bolsa Research, 1993. Photohydrolysis of methyl bromide. Report No. BR 289.1:93. Sponsor: CMA Methyl Bromide Industry Panel.

Bond, J.A., Dutcher, J.S., Medinsky, M.A., Henderson, R.F., Birnbaum, L.S. 1985. Disposition of ^{14}C methyl bromide in rats after inhalation. Toxicol. Appl. Pharmacol., 78: 259-267

Boorman, G.A., Hong, H.L., Jamieson, C.W., Yoshitomi, K., Maronpot, R.R., 1986. Regression of methyl bromide induced forestomach lesions in the rat. Toxicol. Appl. Pharmacol., 86: 131-139.

Breslin, W.J., Zablotny, C.L., Bradley, G.J., Nitschke, K.D., Lomax, L.G., 1990a. Methyl bromide inhalation teratology probe study in New Zealand white rabbits. Unpublished study from Dow Chemicals Company Toxicology Laboratory.

Breslin, W.J., Zablotny, C.L., Bradley, G.F., Lomax, L.G., 1990b. Methyl bromide inhalation teratology study in New Zealand white rabbits. Midland, Michigan, The Dow Chemical Company (Unpublished final report).

Brown, A.L., Burau, R.G., Meyer, R.D., Raski, D.J., Wilhelm, S., Quick, J., 1979. Plant uptake of bromide following soil fumigation with methyl bromide. Calif. Agric., 33: 11-13.

Calvert, G.M., Mueller, C.A., Fajen, J.M., Chrislip, D.W., Russo, J., Briggle, T., Fleming, L.E., Suruda, A.J., Steenland, K., 1998. Health effects associated with sulfuryl fluoride and methyl bromide exposure among structural fumigation workers. Am. J. Public Health, 88(12):1774-1780.

Calvert, G.M., Talaska, G., Mueller, C.A., Ammenheuser, M.M., Au, W.W., Fajen, J.M., Fleming, L.E., Briggle, T., Ward, E., 1998. Genotoxicity in workers exposed to methyl bromide. Mutat. Res. 417(2-3):115-128.

Campbell, S.M., Beavers, J.B., 1994. Methyl Bromide: An acute oral toxicity study with the northern bobwhite. Wildlife International Ltd., Maryland, project no. 264-110.

Canton, J.H., 1980. Hydrobiological toxicological research with methyl bromide. National Institute of Public Health and Environmental Hygiene, Report No. 105/80, 4 p.

Cooper, D.M., Griffiths, N.M., Hobson-Frohock, A., Land, D.G., Rowell, J.G., 1978. Fumigation of poultry food with methyl bromide: effects on egg flavour, number, and weight. Br. Poult. Sci., 19: 537-542.

Daelemans, A. 1978. Uptake of methyl bromide by plants and uptake of bromide from decontaminated soils. Leuwen, Belgium, Catholic University (Dissertation) (in Flemish).

Danse, L.H.J.C., Van elsen, F.L., Van der Heijden, C.A., 1984. Methyl bromide: carcinogenic effects in the rat forestomach. Toxicol. Appl. Pharmacol., 72: 262-271.

Davenport, C.J., Ali, S.F., Miller, F.J., Lipe, G.W., Morgan, K.T., Bonnefoi, M.S., 1992. Effect of methyl bromide on regional brain glutathione, glutathione-S-transferases, monoamines, and amino acids in F344 rats. Toxicol. Appl. Pharmacol. 112:120-127.

Dawson, G.W., Jennings, A.L., Drozdowski, D., Rider, E. 1975/77. The acute toxicity of 47 industrial chemicals to fresh and saltwater fishes. J. Hazard. Mater., 1: 303-318.

Determination of water solubility of methyl bromide, 1992. Great Lakes Corporation and The Dow Chemical Company submitted to Chemical Manufacturers Association.

Devaney, J.A. Beerwinkle, K.R., 1982. Control of the northern fowl mite on inanimate objects by fumigation: field studies. Poult. Sci., 62: 43-46.

Djalali-Behzad, G., Hussain, S., Ostermann-Golker, S., Segerbaeck, D. 1981. Estimation of genetic risks of alkylating agents. VI. Exposure of mice and bacteria to methyl bromide. Mutat. Res., 84: 1-9.

Dow Chemical Corporation, 1992. Determination of water solubility of methyl bromide (FIFRA Guideline 63-8) submitted to Chemical Manufacturers Association.

Dreef-Van der Meulen, H.C., Reuzel, P.G.J., Kuper, C.F., Hollanders, V.M.H., Feron, V.J., 1989. Chronic inhalation toxicity of methyl bromide in rats. Third International Symposium on Soil Disinfestation, Leuwen, Belgium, September 26-30, 1988. Acta Hortic., 255: 313-315.

Driscoll, C.D., Hurley, J.M., 1993. Methyl Bromide: single exposure vapor inhalation neurotoxicity study in rats. Unpublished report from Bushy Run Research Center Project No. 92N1197.

Elf Atochem, Material Safety Data Sheet, 1993.

Enebak, S.A., Palmer, M.A., Blanchette, R.A. 1988. Effect of soil-borne pathogen control methods on the production of white pine nursery seedlings. Phytopathology, 78: 1532-1533.

Eustis, S.L., 1988. Toxicology and pathology of methyl bromide in F344 rats and B6C3F1 mice following repeated inhalation exposure. Fund. Appl. Toxicol. 11:594-610.

Furuta, A., Hyakudo, T., Ohnishi, A., Hori, H., Tanaka, E., 1993. Neurotixocity of methyl bromide – neuropathologic evaluation, preliminary study. Sangyo Ika Daigaku Zasshi 15: 21-27.

Gansewendt, B., Foest, U., Xu, D., Hallier, E., Bolt, H.M., Peter, H., 1991. Formation of DNA adducts in F-344 rats after oral administration of inhalation of [14C]methyl bromide. Food Chem. Toxicol. 29:557-563.

Garry, V.F., Nelson, R.L., Griffith, J., Harkins, M., 1990. Preparation for human study of pesticide applicators: Sister chromatid exchanges and chromosome aberrations in cultured human lymphocytes exposed to selected fumigants, Teratog. Carcinog. Mutag. 10:21-29.

Gargas, M., Andersen, M., 1982. Metabolism of inhaled brominated hydrocarbons: validation of gas uptake results by determination of stable metabolite. Toxicol. Appl. Pharmacol., 66: 55-68.

Gillotay, D., Simon, P.C. Dierickx, L., 1989. Temperatures dependence of ultraviolet absorption cross-sections of brominated methanes and ethanes. In: Bojkov RD & Fabian P, ed. Ozone in the atmosphere. Proceedings of the Quadrennial Ozone Symposium 1988 and Tropospheric Ozone Workshop, Göttingen, Germany, 4-13 August 1988. Hampton, Virginia, Deepak Publishing, pp. 706-709.

Grant, W.M., Schuman, J.S., 1993. Toxicology of the Eye, Fourth Edition. Charles C. Thomas, Springfield.

Great Lakes Corporation, 1986. Data submission from Chemical Manufacturers Association to EPA for methyl bromide. Methyl bromide Field Dissipation Study conducted by Great Lakes Corporation according to EPA-negotiated protocol.

Great Lakes Corporation, 1992. Determination of water solubility of methyl bromide (FIFRA Guideline 63-8) submitted to Chemical Manufacturers Association.

Griffiths, N.M., Hobson-Frohock, A., Land, D.G., Levett, J.M., Cooper, D.M., Rowell, J.G. 1978. Fumigation of poultry food with methyl bromide: effects on flavour and acceptability of broiler meat. Br. Poul. Sci., 19:529-535.

Hallier, E., Langhof, T., Dannappel, d., Leutbecher, M., Schroder, K., Goergens, H.W., Muller, A., Bolt, H.M., 1993. Polymorphism of glutathione conjugation of methyl bromide, ethylene oxide and dichloromethane in human blood: influence on the induction of sister chromatid exchanges (SCE) in lymphocytes. Arch. Toxicol. 67:173-178.

Hansch, C. Leo, A. 1979. Substituent constants for correlation in chemistry and biology. New York, Chichester, Brisbane, Toronto, John Wiley & Sons, p.173.

Hardin, B.D., Bond, G.P., Sikov, M.R., Andrew, F.D., Beliles, R.P., Neimeier, R.W., 1981. Testing of selected workplace chemicals for teratogenic potential. Scand .J. Work Environ. Health 7:66-75.

Hatch, G.G., Mamay, P.D., Ayer, M.L., Casto, B.C., Nesnow, S., 1983. Chemical enhancement of viral transormation in Syrian hamster embryo cells by gaseous and volatile chlorinated methanes and ethanes. Cancer Res. 43:1945-50.

Hawley's Condensed Chemical Dictionary, 11th Ed., 1987. N.I. Sax and R.J. Lewis, eds., Van Nostrand Reinhold Co., New York.

Heungens, A., Roos, W.J., 1982. L'influence du dazomet et du bromure de méthyle sur la faune et la flore de la litière de pin. Rev. Agric., 35(2):2005-2050.

Hommel, G., 1984. [Handbook of dangerous goods (Explanatory leaflet 127).] Berlin, Heidelberg, New York, Springer-Verlag (in German).

Honma, T., 1982. Significant changes in monoamines in rat brain induced by exposure to methyl bromide, Neurobehavioral. Toxicol. Teratol. 4:521-524.

Honma, T., Miyagawa, M., Sato, M., Hasegawa, H. 1985. Neurotoxicity and metabolism of methyl bromide in rats. Toxicol. Appl. Pharmacol., 81:183-191.

Honma, T., 1987a. Alteration of catcholamine metabolism in rat brain produced by inhalatin exposure to methyl bromide. Jpn .J. Ind. Health, 29:218-219.

Honma, T., Muneyuki, M., Sato, M., 1987b. Methyl bromide alters catecholamine and metabolite concentrations in rat brain. Neurotoxicol. Teratol. 9:369-375.

Honma, T., Miyagawa, M., Sato, M., 1991. Inhibition of tyronsie hydroxylase activity by methyl bromide exposure. Neurotoxicol. Teratol. 13:1-4.

Honma, T., 1992. Brain microdialysis study of the effects of hazardous chemicals on the central nervous system. 1. Changes in monoamine metabolites induced by cerebral methyl bromide administration measured by two-probe microdialysis (TPMD) method. Ind. Health 30:47-60.

Howard, P.H., 1989. Handbook of Environmental Fate and Exposure Data for Organic Chemicals, Volume I: Large Production and Priority Pollutants. Pp 386-393, Lewis Publishers, Chelsea, Michigan.

HSDB, 1999. Hazardous Substances Data Bank. Database generated and maintained by the National Institude of Occupational Safety and Health (NIOSH).

Hubbs, A.F., Harrington, D.D., 1986. Further evaluation of the potential gastric carcinogenic effects of subchronic methyl bromide administration. In: Proceedings of the 36th Meeting of the American College of Veterinary Pathology and the Annual Meeting of the American Society for Veterinary and Clinical Pathology, Denver, Colorado, December 1985. Denver, Colorado, American Society for Veterinary and Clinical Pathology, p. 92.

Hurtt, M.E., Morgan, K.T., Working, P.K., 1987. Histopathology of acute toxic responses in selected tissues from rats exposed by inhalation to methyl bromide. Fundam. Appl. Toxicol., 9:352-365.

IARC (International Agency for Research on Cancer). 1986. Monographs on the evaluation of the carcinogenic risk of chemical to man. Geneva: World Health Organization, Methyl Bromide. p. 187- 212, Lyon, France.

Ikeda, T., 1980. Behavioral effects in rats following repeated exposure to methyl bromide, Toxicol. Lett. 6:293-299.

Irish, D.D., Adams, E.M., Spencer, H.C., Rowe, V.K., 1940. The response attending exposure of laboratory animals to vapors of methyl bromide. J. Ind. Hyg. Toxicol., 22:218-230.

IUCLID, 1996. International Uniform Chemical Information Database. Database generated and maintained by the European Chemicals Bureau for Existing Chemicals, Edition 1 – 1996, European Commission.

Japanese Ministry of Labour, 1992. Toxicology and carcinogenesis studies of methyl bromide in F344 rat and BDF mice (inhalation studies). Tokyo, Industrial Safety and Health Association, Japanese Bioassay Laboratory, 197 pp (Unpublished report).

Kato, N., Morinobu, S., Ishizu, S. 1986. Subacute inhalation experiment for methyl bromide in rats. Ind. Health 24:87-103.

Katz, A.J., 1985. Genotoxicity of methyl bromide in somatic cells of Drosophila larvae. Environ. Mutagen., 7:13 (abstract)

Katz, A.J., 1987. Inhalation of methyl bromide gas induces mitotic recombination in somatic cells of Drosophila melanogaster. Mutat. Res. 192:131-135.

Kipplinger, G.A., 1994. Methyl Bromide: Acute Oral Toxicity Comparison Study of Microencapsulated Methyl Bromide and Liquid Methyl Bromide in Albino Rats. Unpublished report from WIL Research Laboratories, Project No. WIL-49011.

Kishi, R., Itoh, I., Ishizu, S., Harabuchi, I, Miyake, H., 1991. Symptoms among workers with long-term exposure to methyl bromide. An epidemiological study. Jpn. J. Ind. Health, 33:241-250.

Kramers, P.G.N., Voogd, C.E., Knaap, A.G.A.C., Van der Heijden, C.A., 1985a. Mutagenicity of methyl bromide in a series of short-term tests. Mutat. Res., 155: 41-47.

Kramers, P.G.N., Bissumbhar, B., Mout, H.C.A., 1985b. Studies with gaseous mutagens in Drosophila melanogaster. In: Waters M.D., Sandhu, S.S., Lewtas, J., Claxton, ;L., Straus, G., Nesnow, S., eds. Short-term bioassays in the analysis of complex environmental mixtures IV., New York, London, Plenum Press, pp 65-73.

Lyman, W.J., Reehl, W.F., Rosenblatt, D.H, 1982. Handbook of Chemical Property Estimation Methods, Environmental Behavior of Organic Compounds, McGraw -Hill, New York.

Mackay, D., Shui, W.K., 1981. A critical review of Henry's Law Constants for chemicals of environmental interest. J. Phys. Chem. Ref. Data, 10: 1175-1199.

Matheson gas data book, 1980. The Matheson gas data book. East Rutherford, New Jersey, Matheson Gas products (A Division of Will Ross), vol 6, pp 361-363.

Maw, G.A., Kempton, R.J., 1973. Methyl bromide as a soil fumigant. Soils Fertil., 36:41-47.

McGregor, D.B., 1981. Tier II mutagenic screening of 13 NIOSH priority compounds, Report No. 32 – Individual compound report: methyl bromide. Cincinnati, Ohio, National Institute of Occupational Safety and Health, 190 pp (PB83-130211).

Medinsky, M., Bond, J., Dutcher, J., Birnbaum, L., 1984. Disposition of ^{14}C-methyl bromide in rats after inhalation. Toxicology 32:187-196.

Medinsky, M., Dutcher, J., Bond, J., Henderson, R., Mauderly, J., Snipes, M., Mewhinney, J., Cheng, Y., Birnbaum, L., 1985. Uptake and excretion of ^{14}C-methyl bromide as influenced by exposure concentration. Toxicol. Appl. Pharmacol., 78:215-225.

Merck Index (12th Edition), 1996. Merck & Co., Budavari et al., editors, Whitehouse Station, New Jersey.

Mertens, J.J.W.M., 1997. A 24-month chronic dietary study of methyl bromide in rats. Unpublished report from WIL Research Laboratories, Project No. WIL-49014.

Mitsumori, K., Maita, K., Kosaka, T., Miyaoka, T., Shirasu, Y., 1990. Two-year oral chronic toxicity and carcinogenicity study in rats of diets fumigated by methyl bromide. Food Chem. Toxicol., 28:109-119.

Miyagawa, M., 1982. Conditioned taste aversion induced by inhalatin exposure to methyl bromide in rats. Toxicol. Lett. 10:411-416.

Moje, W. 1960. The chemistry and nematocidal activity of organic halicides. In: Metcalf RL, ed. Volume III. Advances in pest control research. New York, Interscience Publishers, pp. 181-217.

Molina, L.T., Molina, M.J., Rowland, F.S., 1982. Ultraviolet absorption cross sections of several brominated methanes and ethanes of atmospheric interest. J. Phys. Chem., 86:2672-2676.

Moriya, M., Ohta, T., Watanabe, K., Miyazawa, T., Kato, K., Shirasu, Y. 1983. Further mutagenicity studies on pesticides in bacterial reversion assay systems. Mutat. Res., 116: 185-216.

Naas, D.J., 1990. Acute Oral Toxicity Study in Beagle Dogs with Methyl Bromide. Unpublished report from WIL Research Laboratories, Inc., Project No. WIL-49006.

Newton, P.E. 1994a. An up-and-down acute inhalation toxicity study of methyl bromide in the dog. Unpublished report from Pharmaco-LSR Project number 93-6067.

Newton, P.E. 1994b. A four-week inhalation toxicity study of methyl bromide in the dog. Unpublished report from Pharmaco LSR Project number 93-6068.

Newton, P.E., 1995. A chronic (12-month) toxicity study of methyl bromide fumigated feed in the dog. Unpublished report from Pharmaco LSR, Project Number 93-6068.

NFPA, 1984. Fire protection guide on hazardous materials, 9th ed. Quincy, Maryland, National Fire Protection Agency, 5 pp. (NFPA No. SPP-1E)

Norris, J.C., Driscoll, C.D., Hurley, J.M., 1993. Methyl Bromide: Ninety-Day Vapor Inhalation Neurotoxicity Study in CD Rats. Unpublished report from Bushy Run Research Center Project No. 92N1172.

NTP, 1992. Toxicology and carcinogenesis studies of methyl bromide (CAS No. 74-83-9) in B6C3F1 mice (inhalation studies). Research Triangle Park, North Carolina, National Toxicology Program, 212 pp (Technical Report Series No. 385).

Ong, J.M., Stewart, J., Wen, Y., Whong, W. 1987. Application of SOS umu-test for the detection of genotoxic volatile chemicals and air pollutants. Environ. Mutagen., 9: 171-176.

Pesticide & Toxic Chemical News, 1984. No evidence of methyl bromide carcinogenicity found by NTP-Panel. Pestic. Toxic Chem. News, 13: 9-10.

Peters, P.W.J., Verhoef, A., De Liefde, A., Van Velsen, F.L., Van Soolingen, J., De Geus, D., Danse, L.H.J., Van Logten, M.J., 1982. Teratogenicity study of methyl bromide by oral administration. Bilthoven, National Institute for Public Health and Environmental Protection (RIVM report No. 618102 002) (in Dutch).

Pletsa, V., Steenwinkel, M.J., van Delft, J.H., Baan, R.A., Kyrtopoulos, S.A., 1999. Methyl bromide causes DNA methylation in rats and mice but fails to induce somatic mutations in lambda lacZ transgenic mice. Cancer Lett. 135(1):21-27.

Radian Report, 1988. Final Report for the Environmental Fate Studies of Methyl Bromide. Conducted by Radian Corporation, Austin, TX, for the Methyl Bromide Industry Panel of the Chemical Manufacturers Association, Arlington, VA, Report DCN: 88-266-040-03, RC No.: 266 – 040-04-01.

Reichmuth, C. Noack, S. 1983. [Environmental effects of the fumigation of commodities.] Technol. Z. Getreide Mehl Backwaren, 37:139-144 (in German).

Reuzel, P.G.J., Dreef-Van der Meulen, H.C., Hollanders, V.M.H., Kuper, C.F., Feron, V.J. Van der Heijden, C.A., 1991. Chronic inhalation toxicity and carcinogenicity study of methyl bromide in Wistar rats. Food Chem. Toxicol., 29:31-39

Robbins, D.E., 1976a. Photodissociation of methyl chloride and methyl bromide in the atmosphere. Geophys, Res, Lett,, 3: 213-216
Robbins, D.E., 1976b. UV absorption cross sections for methyl bromide and methyl chloride. Geophys, Res, Lett,, 3:757-758.

Sangster, J. 1989. Octanol-water partition coefficients of simple organic compounds. J. Phys. Chem. Ref. Data, 18:1185.

Sassaman, J.F., Jacobs, M.M., Chin, P.H., Hsia, S., Pienta, R.J., Kelly, J.M., 1986. Pesticide background statements: methyl bromide and chloropicin. In: Volume II. Fungicides and fumigants. Washington, DC, US Department of Agriculture, Forest Service (MB/C-1-156)(Agriculture Handbook No. 661).

Segers, J.H.L., Temmink, J.H.M., Van den Berg, J.H.J., Wegman, R.C.C., 1984. Morphological changes in the gill of carp (*Cyprinus carpio* L.) exposed to acutely toxic concentrations of methyl bromide. Water Res,, 18:1437-1441.

Sikov, M.R., Cannon, W.C., Carr, D.B., Miller, R.A., Montgomery, L.F., Phelps, D.W., 1981. Teratologic assessment of buthylene oxide, stryene oxide and methyl bromide (Contract-No. 210-78-0025). Cincinnati, Ohio, US Department of Health and Human Services, 84 pp.

Simmon, V.F., Kauhanen, K., Tardiff, R.G. 1977. Mutagenic activity of chemicals identified in drinking water. In: Scott D, Bridges BA, & Sobels FH, ed. Progress in genetic toxicology. Amsterdam, Elsevier/ North-Holland Biomedical Press, pp 249-258.

Sittisuang, P. Nakakita, H., 1985. The effect of phosphine and methyl bromide on germination of rice and corn seeds. J. Pestic. Sci., 10:461-468.

Spires, P.E., Coffee, R.K., 1993. Methyl bromide formulation corrosion studies. CMA Reference No.: MBIP-19.0-CORROS-GLC. Methyl Bromide Industry Panel, Washington DC.

Starratt, A.N., Bond, E.J., 1988. In vitro methylation of DNA by the fumigant methyl bromide. J. Environ. Sci. Health, 23(5):513-524.

Stenger, V.A., 1978. Bromine compounds. In: Kirk-Othmer encyclopedia of chemical technology, 3rd ed. New York, Chichester, Brisbane, Toronto, John Wiley and Son, vol 4, pp 243-263.

Tanaka, S., Arito, H., Abuku, S., Imamiya, S., 1988. Acute effects of methyl bromide on electroencephalographic activity and sleep-wakefulness in rats. Ind. Health, 29, 101-114.

T.N.O., 1987. Chronic (29 month) inhalation toxicity and carcinogenic study of methyl bromide in rats, Report V86.469/221044.

Tucker, J.D., Xu, J., Stewart, J., Ong, T., 1985. Development of a method to detect volatile genotoxins using sister chromatid exchanges. Environ Mutagenesis 7:48-49 (Abstract).

UNEP, 1992. Methyl bromide and the ozone layer: a summary of current understanding; Montreal Protocol Assessment Supplement; Synthesis report of the methyl bromide interim scientific assessment and methyl bromide interim technology and economic assessment requested by the United Nations Environment Programme on behalf of the Contracting Parties to the Montreal Protocol, June 1992. Nairobi, United Nations Environment Programme, pp 33.

Verberk, M.M., Rooyakkers-Beemster, T., De Vlieger, M., Van Vliet, A.G.M., 1979. Bromine in blood, EEG and transaminases in methyl bromide workers. Br. J. Ind. Med. 36: 59-62.

Yamano, Y., 1991. Experimental study on methyl bromide poisoning in mice. Acute inhalation study and the effects of glutathione as an antidote. Jpn. J. Ind. Health 33:23-30 (in Japanese)

Wegman, R.C.C., Greve, P.A., De Heer, H., Hamaker, P.H., 1981. Methyl bromide and bromide-ion in drainage water after leaching of glasshouse soils. Water Air Soil Pollut., 16:3-11.

Wester, P.W., Canton, J.H., Dormans, J.A.M.A., 1988. Pathologicals effects in freshwater fish *Poecilia reticulata (*guppy) and *Oryzias latipes* (medaka) following methyl bromide and sodium bromide exposure. Aquat. Toxicol., 12:323-343.

WHO (World Health Organization). 1991. International programme on chemical safety – Environmental health criteria for methyl bromide. First draft, 173-194.

Wildlife International Report, 1993a. Methyl Bromide: 96-Hour Static Acute Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*), Final Report. Wildlife International, LTD. Project Number: 264A-105A. Conducted for Methyl Bromide Industry Panel, Chemical Manufacturers Association, MRID # 4306670.

Wildlife International Report, 1993b. Methyl Bromide: A 48-Hour Static Acute Toxicity Test with the Cladoceran (Daphnia magna), Final Report. Wildlife International, LTD. Project Number: 264A-102B. Conducted for Methyl Bromide Industry Panel, Chemical Manufacturers Association, MRID # 42932900.

Wilhelm, E., Battino, R., Wilcock, R.J., 1977. Low pressure solubility of gases in liquid water. Chem. Rev., 77:219-262.

WIL Research Laboratories, 1986. An absorption study with soil and methyl bromide. Study conducted by WIL Research Laboratory sponsored by the Chemical Manufacturers Association, Washington, D.C., Report No. WIL 49002.

WIL Research Laboratories, 1994. Determination of methyl bromide solubility in petroleum-based solvents, Final Report, Study number WIL-49012.

Wilson, N.H., Newton, P.E., Rahi, M., Bolte, H.F., Suber, R.L., 1998. Methyl bromide: 1-year dietary study in dogs. Food Chem. Toxicol. 36(7):575-584.

Windholtz, M., 1983. The Merck index, 10th ed. Rahway, New Jersey, Merck & Co., Inc., p. 865.

Zwart, A., 1988. acute inhalation study of methyl bromide in rats. Zeist, The Netherlands, CIVO Institutes, TNO, 17 pp. CIVO Report No. V88. 127/27.

Zwart, A., Arts, J.H.E., Ten Berge, W.F., Appelman, L.M., 1992. Alternative acute inhalation toxicity testing by determination of the concentration-time-mortality relationship: experimental comparison with standard LC50 testing. Reg. Toxicol. Pharmacol. 15:278-290.

ROBUST STUDY SUMMARIES
METHYL BROMIDE
CAS No. 74-83-9

Sponsor Country: USA

DATE: September 2001
Updated: February 2002

INDEX OF ROBUST SUMMARIES FOR METHYL BROMIDE

ROBUST SUMMARY – MeBr RS1 01

Study Type:	**Water Solubility**
Title:	Determination of water solubility of methyl bromide at 25°C and specified partial pressures of methyl bromide
Laboratory:	Great Lakes Corporation
Laboratory Study ID#:	Not specified.
Study director(s):	Not specified.
Report author(s):	Not specified.
Study Initiation Date:	Not specified.
Study Completion Date:	Not specified. Report Date: Not specified.

Remark: This report was part of a literature search submitted to EPA as MRID# 42537801 and represents Great Lakes Corporation data. Whether the study was conducted in house or at an outside analytical laboratory is not specified.

Protocol Guideline

No specific protocol guideline number was included with the report although methodology is described. In addition, reference is made to "Chemical Fate Testing Guidelines: Water Solubility, CG-1500" (no specific reference included).

GLP Compliance

None specified. This may be an old report prior to GLPs.

Test Substance

Identity:	Methyl Bromide (a.k.a., bromomethane – CAS# 74-83-9)
Purity:	99.9%
Supplied as:	Not specified; presumed as liquefied gas (under pressure) in cylinders.

Method

Known quantities (0.15, 1.0, 1.5, and 1.8 grams) of liquid methyl bromide were introduced into air-tight vials containing 100 ml water and allowed to come to equilibrium at 25°C for 24 hours. After equilibrium was reached, the amount of methyl bromide in water was measured by gas chromatography.

Results

The apparent solubility was 1.61 gram of methyl bromide per 100 milliliters of water or 16.1 g/L at 25°C at a partial pressure of one atmosphere. The pH and the pKa were not reported. Methyl bromide would probably not change the pH of the distilled deionized water used in the study from neutral.

Conclusions

Methyl bromide is slightly soluble in water under experimental conditions.

References

MRID# 42537801. Determination of Water Solubility of Methyl Bromide. Literature Search Submission containing 3 studies. October 30, 1992.

Data Quality

The data quality from this study is considered marginal. The report included adequate documentation for method and results. This study reaches Klimisch level 2.

General Remarks

Not only are water solubility values difficult to determine for gases like methyl bromide, they may be of questionable value when airtight containers are used (at least in terms of directly calculating concentrations likely to be encountered in surface or ground water). This is because, under test conditions using small airtight containers, the partial pressure of the gas above the water is extremely high compared to environmental conditions. This causes a backpressure that does not permit the gas to leave the water, leaving an artificially high concentration in the water under experimental conditions. This would not occur in the environment because the gas above the water would diffuse away rapidly, thereby allowing more gas to leave the water. Water solubility is not always determined using airtight containers.

ROBUST SUMMARY – MeBr RS1 02

Study Type:	**Water Solubility**
Title:	Solubility of methyl bromide in Water at 20°C.
Laboratory:	Dow Chemical Co. Analytical Laboratories
Laboratory Study ID#:	40-844
Study director(s):	D. A. Mapes.
Report author(s):	D. A. Mapes.
Study Initiation Date:	Not specified.
Study Completion Date:	Not specified. Report Date: April 11, 1972

Remark: This report was part of a literature search submitted to EPA as MRID# 42537801 and represents Dow Chemical Company data.

Protocol Guideline

No specific protocol guideline number was included with the report although methodology is described.

GLP Compliance

None specified. This may be an old report prior to GLPs.

Test Substance

Identity:	Methyl Bromide (a.k.a., bromomethane – CAS# 74-83-9)
Purity:	Not specified.
Supplied as:	Not specified; presumed as liquefied gas (under pressure) in cylinders.

Method

Methyl bromide gas was bubbled through water for 45 minutes at 20°C to achieve saturation. The concentration of methyl bromide in saturated water was measured immediately by gas chromatography (Varian 1400 with flame detector).

Results

The apparent solubility was 1.2 grams of methyl bromide per 100 milliliters of water or 12 g/L at 20°C. The pH and the pKa were not reported. Methyl bromide would probably not change the pH of the distilled deionized water used in the study.

Conclusions

Methyl bromide is slightly soluble in water under experimental conditions.

References

Mapes, D.A., 1972. Solubility of Methyl Bromide in Water at 20ºC. Unpublished report from Dow Analytical Laboratories. Report 40-844.

US EPA MRID# 42537801. Determination of Water Solubility of Methyl Bromide. Literature Search Submission containing 3 studies. October 30, 1992.

Data Quality

The data quality from this study is considered marginal. The report included adequate documentation for method and results. This study reaches Klimisch level 2.

General Remarks

This study differs from that conducted by Great Lakes Corporation in that equilibrium was reached in open-air vials rather than airtight, sealed vials. Otherwise, the partial pressure of methyl bromide gas above the water would have been greater in this study, leading to higher concentrations in the water. Interestingly, this Dow study followed the concentration of methyl bromide in water over the course of 7 hours when the vials were open to air. The concentration in the water dropped only to 0.6 g/100 ml, indicating slow transfer from water to air.

ROBUST SUMMARY – MeBr RS1 03

Study Type:	**Solubility in Organic Solvents**
Title:	Determination of Methyl Bromide Solubility in Petroleum-Based Solvents.
Laboratory:	WIL Research Laboratories, Inc.
Laboratory Study ID#:	WIL-49012
Study director(s):	Loren W. Severs, M.S.
Report author(s):	Loren W. Severs, M.S.
Study Initiation Date:	March 7, 1994.
Study Completion Date:	June 2, 1994 Report Date: April 11, 1972

Remark: This report was part of a literature submission (MRID# 43257101).

Protocol Guideline

US EPA FIFRA Pesticide Assessment Guidelines, Subdivision D, October 1982, Section 63-8 and 40 CFR, 158.190.

GLP Compliance

The report contains a statement page indicating that the study was conducted according to "Good Laboratory Practice Regulations" (no specific references), which was signed by Deborah L. Little, manager of WIL Quality Assurance. Dates of study/data inspection were included.

Test Substance

Identity:	Methyl Bromide (a.k.a., bromomethane – CAS# 74-83-9)
Purity:	99.5% +.
Supplied as:	Presumed as liquefied gas (under pressure) in cylinders. Supplier: Aldrich Chemical Co., Lot number 00122DZ.

Method

Methyl bromide gas was bubbled through K-1 Kerosene and Shell SOL 340HT for 5 minutes to achieve supersaturation. The time required to reach supersaturation was determined by weighing the solutions at several time points. Solutions were allowed to off-gas to saturation, which took no more than one hour (again, weights were determined at several time points).

Solubility was determined in two ways: by weight and by gas chromatographic analysis. By weighing the saturated solution and subtracting the tared weight of the container and solvent, methyl bromide solubility was determined.

Solubility was also determined using gas chromatography where dilutions of both saturated solutions were made in acetone. These were injected into a Hewlett-Packard 5890 gas chromatograph equipped with at flame ionization detector (7673A Autosampler).

Results

Using the weight method, methyl bromide was determined to have a solubility in kerosene of 22.81 grams/100 ml (± 1.7 S.D.) at 23ºC. In Shell SOL 340HT, methyl bromide solubility was 23.95 grams/100 ml (± 0.99 S.D.) at 20ºC.

By gas chromatography, methyl bromide was determined to have a solubility in kerosene of 22.48 grams/100 ml (± 1.9 S.D.) at 23ºC. In Shell SOL 340HT, methyl bromide solubility was 25.17 grams/100 ml (± 0.99 S.D.) at 20ºC.

Conclusions

Methyl bromide is soluble in K-1 Kerosene and Shell 340HT. Whether determined by weight or GC analysis, the solubility values of methyl bromide in K-1 kerosene or Shell SOL 340HT agreed well. No explanation was given as to why solubilities were determined at two different temperatures for the two different solvents.

References

Severes, L. W., 1994. Determination of Methyl Bromide Solubility in Petroleum-Based Solvents. Unpublished report from WIL Research Laboratories. WIL-49012.

US EPA MRID# 43257101.

Data Quality

The data quality from this study is considered high. The report included adequate documentation for method and results. This study reaches Klimisch level 1.

General Remarks

This study was conducted in containers that were open to air. In airtight containers, methyl bromide may have been more soluble since a high partial pressure of methyl bromide would have built-up over the surface of the solvent. Although not necessarily representative of the natural environment, the latter experimental conditions might have resulted in higher solubility values.

ROBUST SUMMARY – MeBr RS1 04

Study Type:	**Octanol/Water Partition Coefficient Determination**
Title:	Methyl Bromide – Octanol/Water Partition Coefficient.
Laboratory:	Bolsa Research Associates, Inc.
Laboratory Study ID#:	BR 172:90
Study director(s):	Steven R. Secara
Report author(s):	Steven R. Secara
Study Initiation Date:	Not specified.
Study Completion Date:	August 23, 1990 Report Date: August 23, 1990

Remark: This report was part of a literature submission (MRID# 42541301).

Protocol Guideline

40 CFR, 796.1550 and California Notice 87-6.

GLP Compliance

The report contains a statement page indicating that the study "Does not meet the requirements of 40 CFR Part 160" because it was not GLP audited by laboratory QA unit personnel and because it was designed to meet California data requirements.

Test Substance

Identity:	Methyl Bromide (a.k.a., bromomethane – CAS# 74-83-9)
Purity:	"100% pure"
Supplied as:	Presumed as liquefied gas (under pressure) in cylinders. Supplier: Trical Inc., No Lot number provided.

Method

Methyl bromide gas was bubbled through chilled, water-saturated octanol for an unspecified period of time to obtain a stock solution. The concentration was not determined. Two sequential 10-fold dilutions of this solution were made and known volumes of these solutions were mixed with known volumes of octanol-saturated water in sealed vials. This step was conducted in triplicate. The vials were agitated at 25ºC for 1 hour to achieve equilibrium. Both phases were then subjected to GC analysis (each vial in triplicate), using a gas chromatograph equipped with a mass-selective detector (selective ion monitoring mode). The log of the ratio in the octanol phase divided by that in the aqueous phase was determined to be the log octanol water partition coefficient.

Results

The log K_{ow} for methyl bromide was determined to be 1.94 ± 0.31. The log K_{ow} of the three vial replicates ranged from 1.61 to 2.20.

Conclusions

Although determination of the log K_{ow} for a gas is somewhat problematic, this study was able to establish a reproducible value within an adequate variability range. Methyl bromide does not have a high K_{ow} and consequently would not tend to bioaccumulate.

References

Secara, S.R., 1990. Methyl Bromide – Octanol/Water Partition Coefficient. Unpublished report from Bolsa Research Associates, Inc. BR 172:90.

US EPA MRID# 42541301.

Data Quality

The data quality from this study is considered acceptable. The report included adequate documentation for method and results. This study reaches Klimisch level 2.

General Remarks

Under the conditions of this experiment, the log K_{ow} determined for methyl bromide does not indicate a high propensity for bioaccumulation.

ROBUST SUMMARY – MeBr RS1 05

Study Type:	**Corrosivity to inert materials**
Title:	Methyl Bromide Formulation Corrosion Studies
Laboratory:	Great Lakes Chemical Corporation
Laboratory Study ID#:	MBIP-19.0-CORROS-GLC
Study director(s):	Preston E. Spires
Report author(s):	Preston E. Spires and Richard K. Coffey
Study Initiation Date:	March 1, 1993
Study Completion Date:	August 1, 1993 Report Date: August 1, 1993

Remark: This report was part of a literature submission (MRID# 42933201).

Protocol Guideline

ASTM Method G31-72 and EPA guideline 63-20 are referenced. A protocol is included in the study report, as is a reprint of ASTM method G31-72 which is entitled "Laboratory Immersion Corrosion Testing of Metals."

GLP Compliance

The report contains a statement page, signed by the study director, indicating that this study was conducted in compliance with EPA FIFRA Good Laboratory Practices Standards (CFR Part 160; 50 FR 34052, August 17, 1989).

Test Substance

Identity:	Methyl Bromide (a.k.a., bromomethane – CAS# 74-83-9)
Purity:	99.9% for pure methyl bromide; other mixtures containing primarily methyl bromide but also 2.1-2.3% and 33% chloropicrin were also tested.
Supplied as:	Presumed as liquefied gas (under pressure) in cylinders. Supplier: Great Lakes Chemical, Lot numbers S39, S3I, and S3P (for pure methyl bromide), S55, S4R, and S42 (for methyl bromide with 2.1-2.3% chloropicrin), S6T, S6S, and S6U (for methyl bromide with 33% chloropicrin).

Method

The corrosive effect of methyl bromide liquid (and 2 formulations containing 2 different concentrations of chloropicrin) upon 1018 mild steel "coupons" was evaluated by exposing the coupons to the methyl bromide for 168 hours at 37ºC under pressure. Coupons (in triplicate) were exposed by 1) immersion under the liquid methyl bromide sample ("liquid sample), 2) suspension at the interface between the liquid and headspace gas ("interface sample"), and 3) suspension above the liquid in the headspace gas ("vapor sample"). The weight difference before and after exposure was used to calculate the rate of corrosivity, which was defined in "mils" per year.

Results

The rate of corrosivity was not much greater than that for the blank coupon that underwent only cleaning preparation. Whether immersed, suspended at the interface, or above in the vapor, corrosion was similar for all three methyl bromide preparations. The presence of chloropicrin increased the rate of corrosion slightly. Corrosivity was 0.07, 0.20, and 1.0 mils per year for pure methyl bromide, MeBr with 2.1-2.3% chloropicrin, and MeBr with 33% chloropicrin, respectively.

Conclusions

All three samples of methyl bromide showed low rates of corrosivity toward 1018 mild steel, including the two containing chloropicrin up to 33%. The authors concluded that 1018 mild steel was an appropriate material for containing methyl bromide for all preparations tested.

References

Spires, P.E., Coffey, R.K., 1993. Methyl Bromide Formulation Corrosion Studies, Final Report. Unpublished report from Great Lakes Chemical Corporation, MBIP-19.0-CORROS-GLC. US EPA MRID# 42933201.

ASTM Designation: G 31-72 (Reapproved 1985). Standard Practice for Laboratory Immersion Corrosion Testing of Metals.

Data Quality

The data quality from this study is considered acceptable. The report included adequate documentation for method and results. This study reaches Klimisch level 1.

General Remarks

Under the conditions of this study, methyl bromide shows a low degree of corrosivity toward mild steel.

ROBUST SUMMARY – MeBr RS3 01 (Ecotoxicity)

Study Type:	**Acute Toxicity in Daphnia**
Title:	Methyl Bromide: A 48-Hour Static Acute Toxicity Test with the Cladoceran (*Daphnia magna*), Final Report
Laboratory:	Wildlife International Ltd.
Laboratory Study ID#:	264A-102B
Study director(s):	Kurt R. Drottar
Report author(s):	Drottar, K.R., Swigert, J.P.
Study Initiation Date:	June 29, 1993
Study Completion Date:	July 31, 1993 Report Date: September 16, 1993

Protocol Guideline

Series 72-2 (FIFRA Guideline, Subdivision E). Study was conducted in accordance with EPA Pesticide Assessment Guidelines, Subdivision E Hazard Evaluation: Wildlife and Aquatic Organisms (EPA 540/9-82-024, 1982). Other references used to generate the protocol included US EPA "Standard Evaluation procedures, Acute Toxicity Test for Freshwater Invertebrates" (EPA540/9-85-005) and ASTM "Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians" (ASTM E 729-88).

GLP Compliance

According to a statement that is part of the study report, signed by the Study Director, this study was conducted in accordance with Good Laboratory Practices (40 CFR Part 160, EPA/FIFRA Good Laboratory Practice Regulations and OECD, ISBN 92-84-12367-9, Paris 1982). Study was observed and audited by laboratory Quality Assurance personnel.

Test Substance

Identity:	Methyl Bromide (a.k.a., bromomethane – CAS# 74-83-9)
Purity:	99.87%
Supplied as:	Liquefied gas (under pressure) in five 10-pound cylinders, Lot 6RL4, from Great Lakes Chemical Corporation.

Study Design

Daphnia were exposed to methyl bromide in water for 48 hours in a static system to determine toxicity and/or lethality. Toxicity was determined by immobilization and expressed as the "EC50" (effective concentration causing immobilization of half the Daphnia). Based on a prior dose range-finding test, Daphnia were exposed to the following concentrations. Daphnia were exposed to each concentration in 4 replicates (5 Daphnia per replicate) with a total of 20 Daphnia per concentration level.

Group	Methyl Bromide Concentration (mg/L) Target (Nominal)	Methyl Bromide Concentration (mg/L) Actual (Measured)*	No/Concentration
Negative Control	0	0	20
1	1.3	1.2	20
2	2.2	2.2	20
3	3.6	3.5	20
4	6.0	5.8	20
5	10	9.8	20

* No standard deviation provided.

Test subjects (species, strain & sex):	*Daphnia magna* from in-house cultures.
Route of administration:	Respiration, whole -body.
# Exposure levels (# of Groups):	Six (including an water-only control group).
Control group(s):	Water-only (0 mg/L).
Exposure duration:	48 hours.
Exposure regimen:	24 hours/day for 2 consecutive days.
Post-exposure duration:	None.
# Animals/sex/dose:	20/concentration (4 replicates x 5 daphnids/replicate).

Other Study Parameters

Age at start of study:	Neonates (less than 24 hrs old).
Assignment to groups:	Randomized basis (progeny from at least three adults per test concentration).
Exposure chamber description:	Sealed plastic serum bottles filled with water and having no headspace.
Chamber volume:	125 ml.
Chamber water flow rate:	Not applicable (static exposures).
Chamber temperature:	20 +- 1 ^{o}C.
Dissolved oxygen:	>60% (8.0 - 8.8 mg/L).
Water pH:	8.5 – 8.6.
Water hardness:	140 – 186 mg/L as $CaCO_3$.
Water conductivity:	340 µmohms/cm.
Exposure solutions:	Prepared by injecting a known volume of methyl bromide gas through a septum into the chamber containing the test water.
Analytical monitoring:	Sampled at 0 and 48 hours using a gas chromatograph equipped with a flame ionization detector. Two ml of test water containing methyl bromide was removed by volumetric syringe and introduced to autosampler vials for immediate analysis.
Light cycle:	16 hrs light; 8 hrs dark.
Food:	Not fed during exposure.

Toxicity Endpoints Monitored

Clinical signs:	Monitored at 2, 24, and 48 hours. Signs were limited to a diagnosis of immobilization.
Mortality:	Monitored at 2, 24, and 48 hours.

Statistical Methods

Probit analysis using the binomial method of Thompson (1947) to calculate EC50s and LC50s with 95% confidence limits at 24 and 48 hours.

Results

Clinical signs: No immobilization was apparent at the low-exposure concentration of 1.2 mg/L at any time point. After 24 hours, no immobilization was apparent at the next two higher exposure levels of 2.2 and 3.5 mg/L. Immobilization was apparent after 24 hours in about half the daphnia exposed to 5.8 mg/L and in all the daphnia at 9.8 mg/L. These incidences roughly corresponded to the incidence of mortality. A methyl bromide concentration of 2.2 mg/L for 48 hours caused immobilization in 2 of 20 daphnids and concentrations higher than this caused immobilization or death in all subjects. 48-hr Immobilization NOAEL = 1.2 mg/L. Immobilization LOAEL = 2.2 mg/L.

Morbidity/mortality: No mortality was apparent at the low-exposure concentration of 1.2 mg/L at any time point. At 24 hours, no mortality was apparent at the next two higher exposure levels of 2.2 and 3.5 mg/L; however, one daphnid was dead after 48 hours in the 2.2 mg/L group and all daphnids were dead after 48 hours exposure to 3.5 mg/L. At 5.8 mg/L, mortality was 75% after 24 hours and 100% after 48 hours. At 9.8 mg/L, all daphnids were dead by 24 hours. Mortality NOAEL = 1.2 mg/L. Mortality LOAEL = 2.2 mg/L.

Conclusions

The 48-hour EC50 value for *Daphnia magna* exposed to methyl bromide was 2.6 mg/L (2.2 mg/L < 95% Confidence Limit < 3.5 mg/L). The 48-hour NOAEL for either immobilization or mortality was 1.2 mg/L. The 48-hour LOAEL for either immobilization or mortality was 2.2 mg/L.

References

Drottar, K.R., Swigert, J.P., 1993. Methyl Bromide: A 48-Hour Static Acute Toxicity Test with the Cladoceran (*Daphnia magna*), Final Report. Unpublished report from Wildlife International, Ltd. Project No. 264A-102B.

Thompson, W.R., 1947. Bacteriological Reviews Vol. II, No. 2:115-145.

Data Quality

The data quality from this study is considered high. The report included comprehensive documentation for method and results. The conducting laboratory is reputable. This study reaches Klimisch level 1.

General Remarks

The 48-hour EC50 was 2.6 mg/L.

ROBUST SUMMARY – MeBr RS3 02 (Ecotoxicity)

Study Type:	**Acute Toxicity in Rainbow Trout**
Title:	Methyl Bromide: A 96-Hour Static Acute Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*), Final Report
Laboratory:	Wildlife International Ltd.
Laboratory Study ID#:	264A-105A
Study director(s):	Kurt R. Drottar
Report author(s):	Drottar, K.R., Swigert, J.P.
Study Initiation Date:	September 17, 1993
Study Completion Date:	December 16, 1993 Report Date: December 16, 1993

Protocol Guideline

Series 72-2 (FIFRA Guideline, Subdivision E). Study was conducted in accordance with EPA Pesticide Assessment Guidelines, Subdivision E Hazard Evaluation: Wildlife and Aquatic Organisms (EPA 540/9-82-024, 1982). Other references used to generate the protocol included US EPA "Standard Evaluation procedures, Acute Toxicity Test for Freshwater Fish" (EPA540/9-85-006) and ASTM "Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians" (ASTM E 729-88).

GLP Compliance

According to a statement that is part of the study report, signed by the Study Director, this study was conducted in accordance with Good Laboratory Practices (40 CFR Part 160, EPA/FIFRA Good Laboratory Practice Regulations and OECD, ISBN 92-84-12367-9, Paris 1982). This study was observed and audited by laboratory Quality Assurance personnel.

Test Substance

Identity:	Methyl Bromide (a.k.a., bromomethane – CAS# 74-83-9)
Purity:	99.87%
Supplied as:	Liquefied gas (under pressure) in five 10-pound cylinders, Lot 6RL4, from Great Lakes Chemical Corporation.

Study Design

Juvenile rainbow trout were exposed to methyl bromide in water for 96 hours in a static system to determine toxicity and/or lethality. Toxicity was determined by a diagnosis of 1) lethargy, 2) discoloration, 3) loss of equilibrium, or 4) lying on side with only gill movement. Trout were exposed to the concentrations shown in the table below in 4 replicates (5 trout per replicate) with a total of 20 trout per concentration. Toxicity and mortality were recorded at 14, 24, 48, 72, and 96 hours.

Group	Methyl Bromide Concentration (mg/L) Target (Nominal)	Methyl Bromide Concentration (mg/L) Actual (Measured)*	No/Concentration
Negative Control	0	0	20
1	1.0	1.3	20
2	1.7	1.9	20
3	2.9	2.9	20
4	4.8	4.6	20
5	8.0	7.7	20

* No standard deviation provided.

Test subjects (species, strain & sex): Rainbow trout (Mt. Lassen Trout Farm, Red Bluff, CA).
Route of administration: Respiration, whole -body.
Exposure levels (# of Groups): Six (including a water-only control group).
Control group(s): Water-only (0 mg/L).
Exposure duration: 96 hours.
Interim observation periods: 14, 24, 48, and 72 hours.
Exposure regimen: 24 hours/day for 4 consecutive days.
Post-exposure duration: None.
Animals/sex/dose: 20/concentration (4 replicates x 5 trout/replicate).

Other Study Parameters

Age at start of study: Juveniles.
Average length (control group): 23 mm (ranging from 21 to 24 mm).
Average weight (control group): 0.13 g/fish (ranging from 0.10 to 0.16 grams).
Acclimation period: ~53 hours prior to test initiation.
Assignment to groups: Randomized basis.
Exposure chamber description: Sealed plastic serum bottles filled with water and having no headspace.
Average loading rate: 0.16 gram fish per liter of water.
Chamber volume: Approximately 4 liters.
Chamber water flow rate: Not applicable (static exposures).
Chamber temperature: 12 +- 1 ^{o}C.
Dissolved oxygen: >60% (8.0 - 8.8 mg/L).
Water pH: 8.3.
Water hardness: 136 as $CaCO_3$.
Water conductivity: 333 µmohms/cm.
Exposure solutions: Prepared by injecting a known volume of methyl bromide gas through a septum into the chamber containing the test water.
Analytical monitoring: Sampled at 0 and 96 hours using a gas chromatograph equipped with a flame ionization detector. Two ml of test water containing methyl bromide was removed by volumetric syringe and introduced to autosampler vials for immediate analysis.
Light cycle: 16 hrs light; 8 hrs dark.
Food: Not fed during acclimation or exposure periods.

Toxicity Endpoints Monitored

Clinical signs:	Monitored at 14, 24, 48, 72 and 96 hours. Signs included 1) lethargy, 2) discoloration, 3) loss of equilibrium, or 4) lying on side with only gill movement.
Mortality:	Monitored at 14, 24, 48, 72 and 96 hours.

Statistical Methods

Probit analysis using the binomial method of Stephan (1977) to calculate LC50s with 95% confidence limits at 48, 72, and 96 hours.

Results

Clinical signs: No signs of toxicity were apparent at the two lowest exposure concentrations of 1.3 and 1.9 mg/L at any time point. For the mid-exposure group (2.9 mg/L), some toxicity occurred that included lethargy and discoloration in a low percentage of the fish (4 of 20) at 48 hours that increased in severity and frequency with time and also included loss of equilibrium in some subjects by the end of the exposure period. (No mortality occurred at this exposure level). At the next dose level of 4.6 mg/L, adverse signs also began to occur at 48 hours and were similar in nature but were more severe and occurred in more subjects. At the highest concentration of 7.7 mg/L, adverse signs began to occur after only 24 hours and quickly were lethal (see below). Clinical signs NOAEL = 1.9 mg/L. Clinical signs LOAEL = 2.9 mg/L.

Morbidity/mortality: No mortality occurred at the three lowest exposure concentrations of 1.3, 1.9, and 2.9 mg/L. In the 4.6 mg/L group, two of 20 trout were dead after 48 hours, 7 were dead after 72 hours, and 17 after 96 hours. At the highest exposure concentration of 7.7 mg/L, mortality was 10% (2 of 20) after 24 hours and 100% after 48 hours. Mortality NOAEL = 2.9 mg/L. Mortality LOAEL = 4.6 mg/L.

Conclusions

The LC50 values for trout exposed to methyl bromide at various time points are listed below.

	MeBr Conc. (mg/L)	Lower 95% Confidence Limit	Upper 95% Confidence Limit
24-hr LC50	> 7.7	N/A	N/A
48-hr LC50	5.6	4.6	7.7
72-hr LC50	5.0	2.9	7.7
96-hr LC50	3.9	2.9	4.6

The Mortality NOAEL is 2.9 mg/L. The clinical signs NOAEL is 1.9.

References

Drottar, K.R., Swigert, J.P., 1993. Methyl Bromide: A 96-Hour Static Acute Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*), Final Report. Unpublished report from Wildlife International, Ltd. Project No. 264A-105A.

Stephan, C.E., 1977. Methods for Calculating an LC50, Pages 65-81 in "Aquatic Toxicology and Hazard Evaluations," American Society for Test and Materials. Publication Number STP 634. Philadelphia, PA.

Data Quality

The data quality from this study is considered high. The report included comprehensive documentation for method and results. The conducting laboratory is reputable. This study reaches Klimisch level 1.

General Remarks

The 96-hour LC50 of methyl bromide in rainbow trout was3.9 mg/L.

ROBUST SUMMARY – MeBr RS3 03

Study Type:	**Acute Toxicity in Quail**
Title:	Methyl Bromide: An Acute Oral Toxicity Study with the Northern Bobwhite.
Laboratory:	Wildlife International Ltd.
Laboratory Study ID#:	264A-110
Study director(s):	Susan M. Campbell
Report author(s):	Campbell, S.M., Beavers, J.B.
Study Initiation Date:	November 9, 1993
Study Completion Date:	January 12, 1994 Report Date: January 12, 1994

Protocol Guideline

Series 71-1 (FIFRA Guideline, Subdivision E). Study was conducted in accordance with EPA Pesticide Assessment Guidelines, Subdivision E Hazard Evaluation: Wildlife and Aquatic Organisms (EPA 540/9-82-024, 1982).

GLP Compliance

According to a statement that is part of the study report, signed by the Study Director, this study was conducted in accordance with Good Laboratory Practices (40 CFR Part 160, EPA/FIFRA Good Laboratory Practice Regulations, OECD, ISBN 92-84-12367-9, Paris 1982, and Japan's MAFF, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 1984). Different phases of this study were observed and audited by laboratory Quality Assurance personnel.

Test Substance

Identity:	Methyl Bromide (a.k.a., bromomethane – CAS# 74-83-9)
Purity:	99.87%
Supplied as:	Liquefied gas (under pressure) in five 10-pound cylinders, Lot 6RL4, from Great Lakes Chemical Corporation.

Study Design

Young, fasted bobwhite were gavaged with single doses of methyl bromide dissolved in peanut oil and observed thereafter for up to 14 days. During the observation period, subjects were monitored twice daily for signs of toxicity and mortality. Bobwhite were exposed to the doses shown in the table below in groups of 5 per sex. Food consumption was recorded for days 0-3, 4-7, and 8-14.

Group	Methyl Bromide Dose Nominal (mg/kg)	Methyl Bromide Dose Actual (mg/kg)	No/Sex/Dose
Negative Control	0	0	5
1	31.3	20.9	5
2	62.5	75	5
3	125	108	5
4	250	170	5
5	500	460	5
6	1,000	960	5

* No standard deviation provided.

Test subjects (species, strain & sex):	Northern Bobwhite (*Colinus virginianus*) from Top Flight Quail Farm, Belvidere, NJ).
Route of administration:	Oral (gavage).
# Exposure levels (# of Groups):	Seven (including an peanut oil-only control group).
Control group(s):	Peanut oil-only (0 mg/kg).
Exposure duration:	Single oral dose by gavage.
Observation intervals:	Twice daily for 14 days post-exposure.
Post-exposure duration:	Up to 14 days.
# Animals/sex/dose:	5/sex/dose.

Other Study Parameters

Age at start of study:	21 weeks.
Body weight:	168 to 218 grams at test initiation.
Acclimation period:	Three weeks.
Assignment to groups:	Not specified.
Dosage solutions:	Liquid methyl bromide was dissolved in peanut oil to concentrations that would deliver the desired dose in a volume of approximately 6 ml/kg.
Analytical monitoring:	Dosing solutions were analyzed using a gas chromatograph equipped with a flame ionization detector. These measurements determined actual dosages.
Housing:	Galvanized wire pens (78 x 51 with roofs 20 – 25 cm high).
Temperature:	18.2ºC ±1.2 ºC.
Humidity:	45% ± 14%.
Light cycle:	Reported to be 8 hrs light per day.
Food:	Grain-based game bird ration (details specified in report).

Toxicity Endpoints Monitored

Body weights:	Measured immediately prior to dosing, and by group on days 3, 7, and 14
Food consumption:	Measured by group over days 0-3, 4-7, and 8-14.
Clinical signs:	Monitored twice daily.
Mortality:	Monitored twice daily.

Statistical Methods

Probit analysis, using the binomialmethod of Stephan (1977), was employed to calculate LC50s with 95% confidence limits.

Results

Body weights: Body weights were reduced in the lowest dose group (31.3 mg/kg). This weight loss was slight in males and more marked in females. Both sexes showed weight loss at the next dose level of 62.5 mg/kg. Mortality was too great to assess the affect on body weights in the higher dose groups. Body weight NOAEL not established. Body weight LOAEL = 31.3 mg/kg.

Food consumption: Food consumption was reduced in females from the 31.3 mg/kg test group. Food consumption NOAEL not established. Food consumption LOAEL = 31.3 mg/kg.

Clinical signs: No signs of toxicity were apparent in the peanut oil control group. Subjects from all methyl bromide dose groups exhibited clinical signs of toxicity. These signs included: "loss of coordination, lower limb weakness, a ruffled appearance, hyperexcitability, reduced reaction to external stimuli (sound and movement), depression, prostrate posture, loss of righting reflex, wing droop, lethargy, and shallow and rapid respiration." Signs began to appear within minutes at the two higher doses and within hours at the lower doses. In surviving subjects, signs usually disappeared by day 7 post-dosing. Clinical signs NOAEL not established. Clinical signs LOAEL = 31.3 mg/kg.

Morbidity/mortality: No deaths occurred in the peanut oil control group or in the lowest dose group of 31.3 mg/kg. 30% mortality occurred at 62.5 mg/kg and mortality was 100% at all higher dose levels (i.e., 125, 250, 500, and 1,000 mg/kg). When it occurred, mortality usually took place within hours (especially at the higher dose levels) and almost always within the first 24 hours after dosing. Mortality NOAEL = 31.3 mg/kg. Mortality LOAEL = 62.5 mg/kg.

Conclusions

The oral LD50 values for Northern Bobwhite dosed with methyl bromide was found to be 73 mg/kg with 95% confidence limits of 62.5 and 125 mg/kg. The mortality NOAEL is 31.3 mg/kg and the mortality LOAEL is 62.5 mg/kg. No NOAEL for clinical signs was established since they were observed in the lowest dose group.

References

Campbell, S. M., Beavers, J.B., 1994. Methyl Bromide: An Acute Oral Toxicity Study with the Northern Bobwhite. Unpublished report from Wildlife International, Ltd. Project No. 264A-110.

Stephan, C.E., 1977. Methods for Calculating an LC50, Pages 65-81 in "Aquatic Toxicology and Hazard Evaluations," American Society for Test and Materials. Publication Number STP 634. Philadelphia, PA.

Data Quality

The data quality from this study is considered high. The report included comprehensive documentation for method and results. The conducting laboratory is reputable. This study reaches Klimisch level 1.

General Remarks

An LD50 of 73 mg/kg is considered to be in the toxic range.

ROBUST SUMMARY – MeBr RS3 05 (Ecotoxicity)

Study Type:	**Toxicity to Aquatic Plants, e.g., Algae**
Title:	Hydrobiological Toxicological Program with Methyl Bromide
Laboratory:	National Institute of Public Health , Bilthoven- The Netherlands
Laboratory Study ID#:	Not given
Study director(s):	Not given
Report author(s):	Canton, J.H., Wegman, R.C.C., Mathijssen-Spickman, E.A.M. and Wammes, J.Y.
Study Initiation Date:	Not given
Study Completion Date:	Not Given Report Date: August, 1980

Protocol Guideline

Concept-standards NEN 6501, 6502, 6504 and 6506 (1 to 4) of Dutch Institute of Normalization

GLP Compliance

Not known but likely not compliant with Good Laboratory Practices Regulations.

Test Substance

Identity:	Methyl Bromide (a.k.a., bromomethane – CAS# 74-83-9)
Purity:	>99.9%
Supplied as:	Unknown but received from Britisch Drug House

Study Design

Chlorella pyrenoidosa and *Scenedesmus quadricauda* were exposed to methyl bromide in water for 48 hours in a closed, static system. Toxicity was determined by evaluation of inhibition of cell growth. Cell growth was determined by counting the number of cells/mL using a Coulter counter with aperture 4, attenuation 0.5, tube 70 micron for *Chlorella* and aperature 16, attenuation 1, tube 100 micron for *Scenedesmus*.

Test species, strain:	*Chlorella pyrenoidosa* and *Scenedesmus quadricauda.*
Route of administration:	Addition to K_1 medium.
# Exposure levels (# of Groups):	Not given.
Control group(s):	K_1 medium
Exposure duration:	48 hours.
Exposure regimen:	24 hours/day for 2 consecutive days.
Post-exposure duration:	None.
Organisms/dose:	10^4 cells/mL

Other Study Parameters

Age at start of study:	Not applicable.
Assignment to groups:	Not applicable
Exposure chamber description:	Enclosed chambers.
Chamber volume:	300 ml.

Chamber water flow rate:	Not applicable (static exposures).
Chamber temperature:	24 +- 1 ^{o}C.
Dissolved oxygen:	
Water pH:	7.7
Water hardness:	54.05 mg/L $CaCO_3$
Water conductivity:	Not given
Exposure solutions:	Not given
Analytical monitoring:	Sampled at 0 and 48 hours from highest and lowest concentration. Analysis was performed by a gas chromatographic method. Correction factor derived from these measurements applied to nominal concentrations to determine "corrected" concentration.
Light cycle:	Continuous (>5000 lux)
Food:	Not applicable.

Toxicity Endpoints Monitored

Toxicity Endpoint:	Growth inhibition monitored at 24 and 48 hours.

Statistical Methods

Not given

Results

Methyl bromide caused a concentration dependent inhibition of algal growth.

Conclusions

The 24-hour and 48-hour EC50 values for *Chlorella pyrenoidosa* were 2.1 to 6.7 mg/L and 5.0 mg/L, respectively, when corrected for analytical measurements performed on the highest and lowest concentrations. Similarly, the 24-hour and 48-hour EC50 values for *Scenedesmus quadricauda* were 2.2 mg/L and 3.2 mg/L, respectively, when analytical correction was made.

References

Canton, J.H., Wegman, R.C.C., Mathijssen-Spickman, E.A.M. and Wammes, J.Y., (1980). Hydrobiological toxicological research with methyl bromide. National Institute of Public Health and Environmental Hygiene, Report No. 105/80

Data Quality

The report was translated from the original report that was written in Dutch and contains minimal data.

General Remarks

The EC50 values for methyl bromide in algae were >2 mg/L.

ROBUST SUMMARY – MeBr RS4 01

Study Type:	**Acute Oral Toxicity (Rat Oral LD50)**
Title:	Acute Oral Toxicity Comparison of Microencapsulated Methyl Bromide and Liquid Methyl Bromide in Albino Rats
Laboratory:	WIL Research Laboratories, Inc.
Laboratory Study ID#:	WIL-49011
Study director(s):	Gary R. Kiplinger
Report author(s):	Gary R. Kiplinger
Study Initiation Date:	March 2, 1994
Study Completion Date:	September 22, 1994 Report Date: September 22, 1994

Protocol Guideline

81-1 (EPA FIFRA Pesticide Assessment Guidelines, Subdivision F: Hazard Evaluation: Human and Domestic Animals, Section 81-1) and the TSCA Health Effects Test Guidelines, 40 CFR 798.1175.

GLP Compliance

According to a statement that is part of the study report, signed by the Study Director, this study was conducted in accordance with EPA Good Laboratory Practices (Parts 160 and 792 of 40 CFR). A second page in the report lists the data inspected and dates of inspection, which is signed by the manager of the laboratory Quality Assurance Unit.

Test Substance

Identity:	Methyl Bromide (a.k.a., bromomethane – CAS# 74-83-9)
Purity:	1) As liquefied gas: 99.5% 2) As microencapsulated form: 6.10%
Supplied as:	1) Liquified gas (under pressure) in cylinders, Lot 00122DZ, from Aldrich Chemical Co. (Milwaukee, WI). 2) Microencapsulated methyl bromide (white powder), Lot 9234-45-A, Pharmaco: LSR, Inc. (East Millstone, NJ).
Administered as:	Dilution of either form in corn oil vehicle.

Phase 1 Study Design ("Reconfirmation" Study)

Group	Methyl Bromide Dose (mg/kg) (Nominal)	Methyl Bromide Dose (mg/kg) (Actual)	No/Sex/Concentration
Negative Control	0	0	5
Low (liquid MeBr)	80	80	5
Mid (liquid MeBr)	120	120	5
High (liquid MeBr)	160	160	5

Phase 2 Main Study Design ("Comparison" Study)

Group	Methyl Bromide Dose (mg/kg) (Nominal)	Methyl Bromide Dose (mg/kg) (Actual)	No/Sex/Concentration
Negative Control	0	0	5
Low (liquid MeBr)	80	80	5
Mid (liquid MeBr)	120	120	5
High (liquid MeBr)	160	160	5
Low (microencap MeBr)	80	98	5
Mid (microencap MeBr)	120	146	5
High (microencap MeBr)	160	195	5

Test subjects (species, strain & sex): Rat, albino (Crl:CD® BR), males and females.
Route of administration: Oral (gavage).
Exposure levels (# of Groups): Seven (including control group).
Control group(s): Corn oil.
Exposure duration: Single.
Exposure regimen: Gavage administration.
Post-exposure duration: 14 days.
Animals/sex/dose: 5/sex/dose (see table above).
Terminal sacrifice: 14 days post-dosing.
Interim sacrifice intervals: None.

Other Study Parameters

Age at receipt: Approximately 4 weeks.
Acclimation period: Approximately 3 weeks.
Age at start of study: Approximately 7 weeks.
Average weight at start of study: Males: 190-240 grams; Females: 145-180 grams.
Assignment to groups: Randomized weight basis (body weight stratification in a block design).
Housing: Individually in wire mesh cages suspended above cage-board.
Inhalation chamber description: Steel and glass, rectangular
Inhalation chamber volume: 0.9 m^3.
Chamber air flow rate: 0.2 m^3/min (13 air changes/hr)
Chamber temperature & humidity: 22 +- 2 ^oC; 40-60%, both monitored continuously.
Chamber atmosphere generation: Dilution with air of methyl bromide gas from cylinders.
Chamber atmosphere monitoring: Sampled every 30 minutes during the exposure period with a gas chromatograph equipped with a flame ionization detector.
Light cycle: 12 hrs light; 12 hrs dark.
Temperature: Recorded twice hourly during exposure. 66 - 77 ^oF.
Humidity: Recorded twice hourly during exposure. 40-70%
Food: Pelleted, certified AGWAY PROLAB Animals Diet 3000 (Agway, Inc.)
Access to food: Available *ad libitum* except during inhalation exposures and behavioral tests.
Access to water: Available *ad libitum* except during inhalation exposures.

Remarks: Prior to the Phase 1 & 2 studies, two probe studies were conducted with 1 male and 1 females per dose level.

In the initial probe, all rats died that were gavaged with 100, 200, 300, 400, and 500 mg/kg liquid methyl bromide (in corn oil) (rats survived a dose of 50 mg/kg). When a second probe was undertaken at doses of 25, 50, 75, 100 and 175 mg/kg, all rats survived 25 and 50 mg/kg and males survived 75 and 100 mg/kg. Based on these results, a "reconfirmation" phase was conducted prior to the definitive study where liquid methyl bromide in corn oil was administered to more rats per group to establish doses for the definitive phase 2 study and to reconfirm previously published values. In this phase 1 reconfirmation study, liquid methyl bromide in corn oil was administered to 5 rat/sex at doses of 0, 100, and 150 mg/kg. This resulted in an oral LD50 of 122 mg/kg (combined sexes). In the probe phases, the "reconfirmation" phase, and the following definitive phase study, all rats were fasted 18-20 hours prior to dosing. For the definitive study ("comparative" study), dose volumes were 5.03, 7.55, and 10.06 ml/kg for the low, mid, and high liquid and microencapsulated MeBr groups, respectively

Toxicity Endpoints Monitored

Clinical signs:	1, 3, 4 hours immediately post-dosing and twice daily for 14 days.
Morbidity/mortality:	1, 3, 4 hours immediately post-dosing and twice daily for 14 days.
Body weights:	Taken on dose days –1, 0 (immediately prior to dosing), and on post-dosing days 7 and 14.
Food consumption:	Recorded daily.
Necropsy:	Performed on all animals including examination of the cranial, thoracic and abdominal cavities. Stomach, duodenum, jejunum, and ileum were preserved in 10% neutral buffered formalin (definitive phase only).
Histopathology:	Preserved tissues were sectioned, stained and processed into slides, then examined microscopically for signs of toxicity.

Statistical Methods

LD50's were calculated by the method of Litchfield and Wilcoxon

Results

Phase 1 "Reconfirmation" Study (corn oil only)

	LD50 (mg/kg)	95% Confidence Limits (mg/kg)	Slope	95% Confidence Limits
Males	139	125-155	1.09	1.07 – 1.12
Females	107	97-119	1.09	1.06 - 1.11
Combined	122	79-190	1.17	0.99 - 1.38

Clinical signs: At all dose levels, the following signs were noted: hypoactivity, staining of urogenital area, abnormal feces (mucoid, soft, decreased quantity). At 100 and 150 mg/kg, six or more animals exhibited dry yellow staining of mouth area, dry red staining around eyes, nose, mouth, and forelimbs, ataxia, salivation, and/or ocular discharge. At 150 mg/kg, additional findings were hypothermia and brown urogenital staining in 4 animals, as well as abdominal distension in all animals. All signs did not persist beyond day 7 post-dosing except for abdominal distension in the 150 mg/kg animals, which persisted in some animals until sacrifice on day 14 post-dosing.

Morbidity/Mortality: For the 50, 100, and 150 mg/kg groups, mortality was 0/10, 1/10, and 9/10, respectively. Nine of 10 deaths occurred on day one post-dosing.

Body weights: Body weights were reduced on days 7 and 14 post-dosing for the rats treated with 100 and 150 mg/kg methyl bromide.

Macroscopic Examinations: All non-survivors showed gastric abnormalities including distended stomach (usually yellow or clear fluid filled). Nine of 10 non-survivors had a thickened lining of the non-glandular portion of the stomach; half had dark red patches in the glandular stomach and one rat from the 150 mg/kg group had red fluid in the jejunum. One male non-survivor from the 150 mg/kg group had reddened kidneys, white patches on the liver, and a hemorrhagic thymus. Another was found with a reddened mesenteric lymph node.

Of the animals surviving the 2-week observation period, approximately 50% of the animals from all dose levels exhibited adhesions of the stomach to the abdominal wall. The non-glandular part of the stomach was thickened in two animals from the 100 mg/kg group and one animal from the 150 mg/kg group. Other single occurrences of gross pathological lesions included: mettled lungs, splenic cysts, white foamy contents in the trachea, and necrotic adipose tissue in the abdomin.

Phase 2 "Comparison" Study (microencapsulated MeBr vs "liquid" MeBr in corn oil)

	LD50 (mg/kg)	95% Confidence Limits (mg/kg)	Slope	95% Confidence Limits
Males (MeBr in corn oil)	>120 but <160*	Not calculated	N/A	N/A
Females (MeBr in corn oil)	86	77 – 95	1.09	1.05 – 1.12
Combined (MeBr in corn oil)	104	83 – 130	1.30	1.03 – 1.63
Males (microencapsulated MeBr)	159	131 – 192	1.24	1.06 – 1.46
Females (microencapsulated MeBr	105	95 – 116	1.09	1.09 – 1.12
Combined (microencapsulated MeBr	133	106 – 167	1.30	1.03 – 1.63

* Unable to determine LD50 from data.

Clinical signs: Clinical signs were similar between microencapsulated and liquid methyl bromide. In all animals that did not survive (35 total), almost all exhibited hypoactivity and most showed ataxia. Approximately one third of non-survivors had hypothermia and labored breathing. About 1 in five non-survivors suffered prostration and ocular discharge. Two non-survivors were observed to have tremors. In animals that survived to day 14, of the above described symptoms, only hypoactivity and ataxia were observed in some animals. In addition, all survivors exhibited distended stomach by observation days 2 – 6, which usually persisted until the scheduled necropsy, 14 days post-dosing. Most of the survivors also had red staining around the nose that usually occurred only on days 1 or 2 post-dosing. Finally, in the 160 mg/kg liquid MeBr group, some survivors were noted with mucoid feces and yellow urogenital staining.

Morbidity/Mortality: Mortality was similar between microencapsulated and liquid methyl bromide. 34 of 35 non-scheduled deaths from MeBr occurred by day 2 post-dosing. For the 80, 120, and 160 mg/kg liquid MeBr groups, mortality was 2/10, 6/10, and 10/10, respectively. For the microencapsulated groups, mortality was 1/10, 7/10, and 9/10, for the low, mid, and high dose groups, respectively.

Body weights: Body weights were reduced on days 7 and 14 post-dosing for the rats treated with either form of methyl bromide.

Macroscopic Examinations: All non-survivors showed gastric abnormalities that, for most subjects, included: reddened mucosal lining, edematous glandular stomach, and stomach distension. Single

animals exhibited dark read areas in the non-glandular stomach, reduced mucosal lining, and red streaks in the stomach. Four animals had clear fluid in the thoracic cavity. Other findings were considered post-mortem changes in non-survivors.

Of the animals surviving the 2-week observation period (including 12 liquid MeBr animals and 13 microencapsulated MeBr animals), all showed gastric abnormalities. All had adhesions attaching the stomach to other internal organs or the abdominal wall, diaphragm, or cecum. About half of the animals (equally for the two forms of MeBr) showed white material adhering to the mucosal lining of the nonglandular stomach and a lower proportion had a thickening of the stomach lining. Four survivors had a necrotic area on the liver.

Microscopic examination: Lesions were consistent and occurred with a similar frequency between the liquid and microencapsulated methyl bromide groups non-survivors and survivors. In non-survivors, no specific histopathology was evident to which death could be attributed. Non-survivors showed hemorrhages in the glandular mucosa of the stomach. Submucosal edema was also evident in a majority of non-survivors. One liquid MeBr subject showed cytoplasmic vacuolation of the gastric submucosa.

Most survivors exhibited squamous cell hyperplasia, ulceration, multiple areas of adhesion of the stomach. A lower incidence was found of granulomatous inflammation, lymphoplasmacytic cell infiltration, chronic active inflammation, and granulocyte infiltration of the stomach.

Conclusions

For "liquid" methyl bromide (i.e. methyl bromide dissolved in corn oil), the rat oral LD50 (combined sexes) in the confirmation phase was 122 mg/kg (79 mg/kg < 95% CL > 180 mg/kg) and, in the "comparison phase, was 104 mg/kg (83 mg/kg < 95% CL > 130 mg/kg). The rat oral LD50 for microencapsulated form of methyl bromide was 133 mg/kg (106 mg/kg < 95% CL > 167 mg/kg). All three rat oral LD50's from both phases of study were consistent and corroborated published LD50 values. Most deaths occurred by day 2 post-dosing and marked gastric lesions were present in most subjects. No differences could be attributed to the form of the methyl bromide administered, whether dissolved directly in corn oil or microencapsulated and suspended in corn oil.

References

Kiplinger, G.R., 1994. Acute Oral Toxicity Comparison Study of Microencapsulated Methyl Bromide and Liquid Methyl Bromide in Albino Rats. Unpublished report from WIL Research Laboratories, Project No. WIL-49011.

Data Quality

The data quality from this study is considered high. The report included comprehensive documentation for method and results. The conducting laboratory is reputable. This study reaches Klimisch level 1.

General Remarks

The LD50s found in this study tend to agree with other published values and the form of the methyl bromide (liquid dissolved in corn oil or microencapsulated methyl bromide suspended in corn oil) does not seem to change the lethality dosages.

ROBUST SUMMARY – MeBr RS4 02

Study Type: Acute Inhalation Neurotoxicity

Title: Methyl Bromide: Single Exposure Vapor Inhalation Neurotoxicity Study in Rats.

Laboratory: Bushy Run Research Center (Export, PA)

Laboratory Study ID#: 92N1197
Study director(s): Cynthia D. Driscoll
Report author(s): C. D. Driscoll, and J. M. Hurley

Study Initiation Date: Not specified.
Study Completion Date: May 27, 1993 Report Date: May 27, 1993

Protocol Guideline

81-8 (FIFRA Guideline, Subdivision F: Protocol 81-8). Study was conducted in accordance with EPA Pesticide Assessment Guidelines (Subdivision F, Addendum 10 – Neurotoxicity Series 81, March 1991).

GLP Compliance

According to a statement that is part of the study report, signed by the Study Director, this study was conducted in accordance with Good Laboratory Practices (Part 160 of 40 CFR [EPA/FIFRA Good Laboratory Practice Regulations and OECD, C(81)30(final)]).

Test Substance

Identity: Methyl Bromide (a.k.a., bromomethane – CAS# 74-83-9)
Purity: >99%
Supplied as: Liquified gas (under pressure) in five 10-pound cylinders, Lot RL4, from Great Lakes Chemical Corporation (El Dorado, AR).

Study Design

Group	Methyl Bromide Concentration (ppm) Target (Nominal)	Methyl Bromide Concentration (ppm) Actual (Measured)*	No/Sex/Concentration
Negative Control	0	0	15
Low	30	33.1 ± 0.68	15
Mid	100	100 ± 1.9	15
High	350	344 ± 2.4	15

* Plus or minus one standard deviation.

Test subjects (species, strain & sex): Rat (CD®), males and females.
Route of administration: Inhalation, whole-body.
Exposure levels (# of Groups): Four (including an air-only control group).
Control group(s): Air-only (0 ppm).
Exposure duration: Single, 6-hour exposure.
Exposure regimen: Single exposure.

Post-exposure duration:	None.
# Animals/sex/dose:	15/sex/concentration (see table above).
Terminal sacrifice:	After behavioral tests.
Interim sacrifice intervals:	None.

Other Study Parameters

Age at receipt:	Approximately 4 weeks.
Acclimation period:	Approximately 3 weeks.
Age at start of study:	Approximately 7 weeks.
Average weight at start of study:	Males: 190-240 grams; Females: 145-180 grams.
Assignment to groups:	Randomized weight basis (body weight stratification in a block design).
Housing:	Individually in wire mesh cages suspended above cage-board.
Inhalation chamber description:	Steel and glass, rectangular
Inhalation chamber volume:	0.9 m^3.
Chamber air flow rate:	0.2 m^3/min (13 air changes/hr)
Chamber temperature & humidity:	22 +- 2 ^oC; 40-60%, both monitored continuously.
Chamber atmosphere generation:	Dilution with air of methyl bromide gas from cylinders.
Chamber atmosphere monitoring:	Sampled every 30 minutes during the exposure period with a gas chromatograph equipped with a flame ionization detector.
Light cycle:	12 hrs light; 12 hrs dark.
Temperature:	Recorded twice hourly during exposure. 66 - 77 ^oF.
Humidity:	Recorded twice hourly during exposure. 40-70%
Food:	Pelleted, certified AGWAY PROLAB Animals Diet 3000 (Agway, Inc.)
Access to food:	Available *ad libitum* except during inhalation exposures and behavioral tests.
Access to water:	Available *ad libitum* except during inhalation exposures.

Toxicity Endpoints Monitored

Clinical signs:	Twice daily (detailed physical examination conducted once weekly).
Morbidity/mortality:	Twice daily.
Physical examinations:	Pretest and weekly including examination for palpable masses.
Ophthalmic exam:	None.
Body weights:	Pretest, weekly, at neurobehavioral evaluations, and at termination.
Food consumption:	Recorded weekly.
Hematology:	None.
Clinical chemistries:	None.
Urinalyses:	None.
Interim sacrifices:	None
Organ weights:	None except brain.

Functional Observational Battery (FOB) tests:

FOB's were conducted prior to exposure, within 3 hours following the single 6 hour exposure, and 7 and 14 days following exposure. Ten animals/sex from each group were evaluated. Behavioral parameters included: cage posture, tremors, unusual behavior, breathing pattern, urination, startle response, muscle tone, salivation, dehydration, visual placing, body weight,

convulsions, vocalization, gait, arousal, rears, tail pinch response, piloerection, exophthalmus, fur appearance, grip strength, air righting, handling reaction, palpebral closure, body position, defecation, approach response, pupil size lacrimation, emaciation, crusts, core body temperature, and hind leg splay.

Motor activity test intervals:
Motor activity was evaluated at the same time intervals as the FOB battery and immediately after FOB evaluation. Activity was monitored for 180 minutes. All 15 rats/sex from each group were evaluated for ambulatory activity, fine motor activity, rearing, and the sum of these activities.

Gross pathology: Abbreviated necropsy was performed on all animals including examination of the thoracic and peritoneal cavities.

Neurohistopathology: After fixation in neutral buffered formalin, brains, spinal cords, heads, and peripheral nerves were collected for microscopic examination. These tissues were collected from six subjects/sex from the control and high exposure group. Because neurohistopathology revealed changes in the high exposure group (see results), microscopic examinations subsequently was performed on subjects from the 30 and 70 ppm groups. Slides were prepared via paraffin embedding (and processed with hematoxylin and eosin, luxol fast blue, and Bielschowsky's stains) of the brain, spinal cord, Gasserian ganglia, nerve roots, and dorsal root ganglia. Peripheral nerves (sciatic, peroneal, sural, and tibial) were embedded in methyl methacrylate, sliced, and also processed with the above stains. Heads were decalcified and 4 sections of the nasal cavity were paraffin embedded, sectioned, and stained.

Statistical Methods

For clinical observations, body weights, organ weights, food consumption, Levene's test for equality of variance was conducted followed by appropriate analysis of variance test (parametric or nonparametric), followed (when "F" statistic was significant) by "t" tests for pairwise comparisons. Nested ANOVA was used for motor activity data and FOB data.

Results

Clinical signs: During the 6-hour exposure, rats from the high exposure group (350 ppm) were observed to have dropping eyelids and to be generally less active than controls. Clinical signs NOAEL = 100 ppm. LOAEL = 350 ppm.

Morbidity/Mortality: No deaths resulted from acute methyl bromide exposure at any concentration. Mortality NOAEL = 350 ppm. No LOAEL established.

Body weights: No changes in body weights or body weight gain were associated with methyl bromide exposure at any concentration. Body weight NOAEL = 350 ppm. No LOAEL established.

Organ weights: No differences were found among exposure groups for brain weights. Brain weight NOAEL = 350 ppm. Brain weight LOAEL not established.

Macroscopic Examinations: No exposure-related gross lesions were evident that could be attributed to methyl bromide exposure. NOAEL = 350 ppm. No LOAEL established.

Histopathology: No exposure-related histopathological changes were found that could be attributed to methyl bromide exposure. NOAEL = 350 ppm. No LOAEL established

Functional Observations: When measure within 3 hours of exposure, rats of both sexes from the 350 ppm group showed decreased activity as measured by arousal and number of rearings when compared to air-treated controls. These rats also showed a greater incidence of eyelid drop and/or closure as well as a decrease in core body temperature. 350 ppm rats also showed increased autonomic signs including: increased urination, piloerection, and decreased core body temperature. A few rats in the high exposure group also showed abnormal gait and posture as well as decreased reaction to tail pinch. These findings in the high exposure subjects were considered to be related to methyl bromide exposure. Rats from the lower exposure groups did not exhibit different behavior from controls. When tested 7 and 14 days later, no differences from controls were noted in any of the rats from any exposure concentration. Thus, in the high exposure group that did show differences from controls, these behavioral effects were transient in nature. FOB NOAEL = 100 ppm. FOB LOAEL = 350 ppm immediately following a single 6-hr exposure.

Activity: Activity was decreased significantly in the 350 ppm group rats when evaluated 3 hours post-exposure. This effect was transient in that it did not re-occur in when measured 1 and 2 weeks post-exposure. Activity NOAEL = 100 ppm. Activity LOAEL = 350 ppm immediately following a single 6-hr exposure.

Conclusions

In an acute neurotoxicity study (Driscoll and Hurley, 1993), male and female CD (Sprague-Dawley) rats were exposed via inhalation for six hours to methyl bromide at concentrations of 0, 30, 100, or 350 ppm. Animals were assessed for clinical signs and changes in body weights. Neurobehavioral evaluations (functional observation battery and motor activity) were performed within three hours of exposure and at seven and 14 days post-exposure. After 15 days, gross pathological evaluation was performed and brains were weighed. Microscopic evaluations were performed on central and peripheral nervous tissue. All animals survived to study termination. No methyl bromide induced effects were noted for body or brain weights. Neurobehavioral effects were observed only in the 350 ppm exposed group at the 3-hour post exposure assessment only. Effects noted in male and female rats consisted of decreased arousal, increased incidences of drooping or half-shut eyelids, piloerection, decreased rearing, depressed body temperature, and markedly decreased motor activity. The 350 ppm males had a decreased tail pinch response while females from this group showed increased urination and abnormal air righting response. These effects were transient in that they occurred in animals evaluated 3 hours following the single exposure and did not occur 7 or 14 days later. No treatment related histological findings were seen in nervous system or nasal tissues. The NOAEL for transient neurotoxic effects is 100 ppm and the LOAEL is 350 ppm.

References

Driscoll, C.D., Hurley, J.M., 1993. Methyl bromide: single exposure vapor inhalation neurotoxicity study in rats. Unpublished report from Bushy Run Research Center Project no. 923N1197.

Data Quality

The data quality from this study is considered high. The report included comprehensive documentation for method and results. The conducting laboratory is reputable. This study reaches Klimisch level 1.

General Remarks

The effects of methyl bromide from an acute (6 hour) inhalation exposure appear to be transient at the highest exposure level tested (350 ppm).

ROBUST SUMMARY – MeBr RS4 03

Study Type:	**Subchronic Inhalation Neurotoxicity**
Title:	Methyl Bromide: Ninety-Day Inhalation Neurotoxicity Study in CD® Rats
Laboratory:	Bushy Run Research Center (Export, PA)
Laboratory Study ID#:	92N1172
Study director(s):	James C. Norris
Report author(s):	J. C. Norris, C. D. Driscoll, and J. M. Hurley
Study Initiation Date:	Not specified.
Study Completion Date:	September 29, 1993 Report Date: September 29, 1993

Protocol Guideline

82-5b (FIFRA Guideline, Subdivision F: Protocol 82-5b). Study was conducted in accordance with EPA Pesticide Assessment Guidelines (Subdivision F, Addendum 10 – Neurotoxicity Series 81, March 1991).

GLP Compliance

According to a statement that is part of the study report, signed by the Study director, this study was conducted in accordance with Good Laboratory Practices (Part 160 of 40 CFR [EPA/FIFRA Good Laboratory Practice Regulations and OECD, C(81)30(final)]).

Test Substance

Identity:	Methyl Bromide (a.k.a., bromomethane – CAS# 74-83-9)
Purity:	>99%
Supplied as:	Liquified gas (under pressure) in five 10-pound cylinders, Lot RL4, from Great Lakes Chemical Corporation (El Dorado, AR).

Study Design

Group	Methyl Bromide Concentration (ppm) Target (Nominal)	Methyl Bromide Concentration (ppm) Actual (Measured)*	No/Sex/ Concentration
Negative Control	0	0	15
Low	30	29.9 ± 1.02	15
Mid	70	70.7 ± 2.80	15
High	140	137 ± 6.47	15

* Plus or minus one standard deviation.

Test subjects (species, strain & sex):	Rat (CD®), males and females.
Route of administration:	Inhalation, whole-body.
# Exposure levels (# of Groups):	Four (including an air-only control group).
Control group(s):	Air-only (0 ppm).
Exposure duration:	13 weeks
Exposure regimen:	6 hours/day, 5 days/week (no exposure on weekends).
Post-exposure duration:	15 days.

# Animals/sex/dose:	15/sex/concentration (see table above).
Terminal sacrifice:	15 days after exposure.
Interim sacrifice intervals:	None.

Other Study Parameters

Age at receipt:	Approximately 4 weeks.
Acclimation period:	Four weeks.
Age at start of study:	Approximately 8 weeks.
Average weight at start of study:	Males: 265-350 grams; Females: 173-222 grams.
Assignment to groups:	Randomized weight basis (body weight stratification in a block design).
Housing:	Individually in wire mesh cages suspended above cage-board.
Inhalation chamber description:	Steel and glass, rectangular
Inhalation chamber volume:	0.9 m^3.
Chamber air flow rate:	For 0, 70 & 140 ppm groups, 0.2 m^3/min (13 air changes/hr); for 30 ppm group (0.3 m^3/hr (13 air changes/hr)
Chamber temperature & humidity:	22 +- 2 ^oC; 40-60%
Chamber atmosphere generation:	Dilution with air of methyl bromide gas from cylinders.
Chamber atmosphere monitoring:	Sampled every 30 minutes during exposure periods with a gas chromatograph equipped with a flame ionization detector.
Light cycle:	12 hrs light; 12 hrs dark.
Temperature:	Recorded twice hourly. 66 - 77 ^oF.
Humidity:	Recorded twice hourly. 40-70%
Food:	Meal form of chow. Diet No. 5002 (meal), (PMI Feed, Inc., St. Louis, MO).
Access to food:	Available *ad libitum* except during inhalation exposures and behavioral tests.
Access to water:	Available *ad libitum* except during inhalation exposures.

Toxicity Endpoints Monitored

Clinical signs:	Twice daily (detailed physical examination conducted once weekly).
Morbidity/mortality:	Twice daily.
Physical examinations:	Pretest and weekly including examination for palpable masses.
Ophthalmic exam:	None.
Body weights:	Pretest, weekly, at neurobehavioral evaluations, and at termination.
Food consumption:	Not monitored.
Hematology:	None.
Clinical chemistries:	None.
Urinalyses:	None.
Interim sacrifices:	None
Organ weights:	None except brain.

Functional Observational Battery (FOB) tests:

FOB's were conducted prior to exposure and during the fourth, eighth, and thirteenth weeksof exposure. Ten animals/sex from each group were evaluated. Behavioral parameters included: cage posture, tremors, unusual behavior, breathing pattern, urination, startle response, muscle tone, salivation, dehydration, visual placing, body weight, convulsions, vocalization, gait, arousal, rears, tail pinch response, piloerection,

exophthalmus, fur appearance, grip strength, air righting, handling reaction, palpebral closure, body position, defecation, approach response, pupil size lacrimation, emaciation, crusts, core body temperature, and hind leg splay.

Motor activity test intervals: Motor activity was evaluated prior to exposure and the weekend following the fourth, eighth, and thirteenth weeks of exposure. All 15 rats/sex from each group were evaluated for ambulatory activity, fine motor activity, rearing, and the sum of these activities.

Gross pathology: An abbreviated necropsy was performed on all animals including examination of the thoracic and peritoneal cavities.

Neurohistopathology: After fixation in neutral buffered formalin, brains, spinal cords, heads, and peripheral nerves were collected for microscopic examination. These tissues were collected from six subjects/sex from the control and high exposure group. Slides were prepared via paraffin embedding (and processed with hematoxylin and eosin, luxol fast blue, and Bielschowsky's stains) of the brain, spinal cord, Gasserian ganglia, nerve roots, and dorsal root ganglia. Peripheral nerves (sciatic, peroneal, sural, and tibial) were embedded in methyl methacrylate, sliced, and also processed with the above stains. Heads were decalcified and 4 sections of the nasal cavity were paraffin embedded, sectioned, and stained.

Statistical Methods

For clinical observations, body weights, organ weights, food consumption, Levene's test for equality of variance was conducted followed by appropriate analysis of variance test (parametric or nonparametric), followed (when "F" statistic was significant) by "t" tests for pairwise comparisons. Nested ANOVA was used for motor activity data and FOB data.

Results

Clinical signs: Two males from the high exposure group exhibited salivation, rapid breathing, hyperactivity, and convulsions, which occurred on three separate occasions for a surviving rat and A second animal that did not survive until final sacrifice, had convulsions one day prior to death. Clinical signs NOAEL = 70 ppm. LOAEL = 140 ppm.

Morbidity/mortality: Two males from the high exposure group died. One had convulsions and the other showed no clinical signs. Mortality NOAEL = 70 ppm. LOAEL = 140 ppm.

Body weights: Body weights and body weight gains of rats from the 140 ppm group was significantly decreased in both sexes compared to the control group. For the 70 ppm group, males showed a significant decrease in body weight gain only during the first exposure week. However, female body weights were significantly below controls during weeks 9 and 11-13 of exposure and female body weight gain was below controls for weeks 5 through 13 of exposure. Females but not males from the 30 ppm group showed a trend toward lower body weights and body weight gains that was not statistically significant. Body weight NOAEL = 30 ppm (males) and none for females. LOAEL = 70 ppm for males and 30 ppm for females.

Food consumption: Food consumption of rats from the 140 ppm males and females were occasionally significantly decreased compared to either control group over the exposure period. Females from the 70 ppm group showed occasional weekly decreased consumption. Food consumption NOAEL = 70 ppm (males) and 30 ppm (females). LOAEL = 140 ppm (males) and 70 ppm (females).

Organ weights: Absolute brain weights were significantly decreased in males and females (6% and 10 %, respectively) from the 140 ppm group and in females from the 30 ppm (4%) and 70 ppm (5%) groups, compared to controls. These changes paralleled decreased body weights and body weight gains. Brain weights relative to total body weight, which could be expected to be less divergent from controls in growing animals, were not significantly different from controls in any methyl bromide exposure group. Absolute brain weight NOAEL = 70 ppm (males) and none (females). Absolute brain weight LOAEL = 140 ppm (males) and 30 ppm (females). Relative brain weight NOAEL = 140 ppm (both sexes). Relative brain weight LOAEL not established.

Macroscopic examinations: Two males from the 140 ppm group that died during exposure exhibited moderate to severe brain hemorrhage. No other visible lesions were found in any animals at necropsy. NOAEL = 70 ppm (males) and 140 ppm (females). LOAEL = 140 ppm (males) and none for females.

Histopathology: The two non-surviving males from the high exposure group sustained neuronal necrosis in the hippocampus, necrosis and malacia in the cerebral cortex and basal ganglia, and malacia/necrosis in the thalamus and midbrain. Another male from this group showed edema of the hippocampus. No brain lesions were found in females from this group or animals of either sex from the mid and low dose groups. No cerebellar lesions were found in any animals. In the peripheral nervous system, slight vacuolation of nerve fibers and/or axonal degeneration were found in the tibial nerve of 2 females and tibial or sciatic nerves of 2 males from the 140 ppm group. One female in the 30 ppm group showed similar slight lesions. Since the incidence and severity of this lesion was slight, the report authors did not conclude that it definitely resulted from methyl bromide exposure (but could not rule it out). Neurohistological NOAEL = 70 ppm (males) and 140 ppm (females). Neurohistological LOAEL = 140 ppm (males) and none for females.

Functional Observations: Males in the 140 ppm group showed increased mean hind leg splay that was statistically significantly increased over controls at weeks 4 and 13. At the week 8 test interval, this effect was also present but not statistically significant when compared to controls. Trends toward decreased forelimb grip strength that were not statistically significant also occurred in the 140 ppm group at most exposure intervals and in the 70 ppm males at the final evaluation (week 13) prior to sacrifice. Females from the 140 ppm group showed ataxic gait (3 at week 4, 2 at week 8, and 1 at week 13). Only one female showed this effect at more than one test interval. Females from the high exposure group also showed lower arousal scores and rearing events. FOB NOAEL = 70 ppm (both sexes). FOB LOAEL = 140 ppm (both sexes).

Activity: Total motor activity in males was unaffected in males but was significantly reduced in females from both the 70 and 140 ppm groups. Regarding individual activities, females showed decreased fine movements, rearings, and ambulation. Activity NOAEL = 140 ppm (males) and 30 ppm (females). Activity LOAEL = 70 ppm (females), none established for males.

Conclusions

In a subchronic inhalation neurotoxicity study (Norris et al., 1993), CD (Sprague-Dawley) rats (15/sex/dose) were exposed to methyl bromide concentrations of 0, 30, 70, or 140 ppm six hours/day, five days/week for 13 weeks. At the 140 ppm concentration, two male rats died during the first month. Clinical signs observed for these two rats included convulsions, tremors, hyperactivity, rapid respiration, and salivation. Mean body weights were significantly lower than the controls. Neurological evaluations for males revealed increased hind limb splay (weeks 4, 8, 13), abnormal air righting reflex (week 13), and decreased forelimb grip strength (week 13). Female rats demonstrated lower arousal scores (weeks 8 and 13), decreased rearing (weeks 4, 8, and 13), and significantly decreased motor activity (week 13). Mean absolute brain weights were

significantly lower for both sexes; no differences were noted for the relative brain weights indicating that lower absolute brain weights were a reflection of the generally lower body weights for the treated animals. Gross lesions were limited to moderate to severe brain hemorrhage in the two 140 ppm male animals that died. Microscopic lesions in the brain were found in these two males and in one 140 ppm male that survived the 13-week exposure. Microscopic lesions in the brain were seen in these three males and consisted of neuronal necrosis in the hippocampus, necrosis and malacia in the cerebral cortex and basal ganglia, and malacia and/or necrosis in the thalamus and midbrain. One additional 140 ppm male had slight neuronal edema in the hippocampus. Other lesions in the 140 ppm group consisted of minimal regenerative dysplasia of the olfactory epithelium of the nasal cavity in three males and three females and minimal peripheral nerve degeneration in two males and two females. In the 70 ppm group, lower mean body weights and weight gain were reported for females from week 9 onward. Neurological findings were limited to slightly decreased forelimb grip strength (week 13) in males and decreased motor activity (week 13) in females. Although the mean absolute brain weights for females were statistically significantly decreased (5% lower than the control group), no difference was seen for the relative brain weight and no microscopic pathology was observed. At 30 ppm, the mean absolute brain weight for females was statistically significantly lower than control (5% lower than the control group), however, no difference was seen in relative brain weight and no microscopic pathology in the brain was found in females. Since female body weights were significantly reduced in the 2 highest exposure groups and trended lower in the low exposure group, absolute brain weights would be expected to be lower in growing animals. Body weight NOAEL = 30 ppm for males and none for females. Body weight LOAEL = 70 ppm (males) and 30 ppm (females). Neurotoxicity NOAEL (based on histopathology and behavioral effects but not brain weights or motor activity) = 70 ppm and 140 for females. Neurotoxicity LOAEL = 140 ppm (males) and none for females.

References

Norris, J.C., Driscoll, C.D., Hurley, J.M., 1993. Methyl Bromide: Ninety-Day Vapor Inhalation Neurotoxicity Study in CD Rats. Unpublished report from Bushy Run Research Center Project No. 92N1172.

Data Quality

The data quality from this study is considered high. The report included comprehensive documentation for method and results. The conducting laboratory is reputable. This study reaches Klimisch level 1.

General Remarks

Subchronic (90-day) exposure to methyl bromide at 140 ppm results in weight loss and neurotoxicity. A statistically significant effect on absolute brain weights was observed in female rats at levels of exposure as low as 30 ppm (lowest tested). However, brain weights relative to total body weights were not decreased but were increased, although not significantly. Females also showed lower absolute body weights than controls at all exposure levels. Since subjects were young and still growing in this subchronic test, it could be expected that decreased body weights reflected reduced growth (i.e., not just a decreased proportion of adipose or muscular tissue where absolute brain weight could be expected to be unaffected, absent a direct affect on this organ). Since no neurohistopathology accompanied the lower absolute brain weight in females at any exposure level and FOB was affected only in the high exposure group, indicating little affect on the brain at 30 and 70 ppm in females, a more meaningful expression of brain weights would be on a basis relative to total body weight and this reveals no difference in brain weights.

ROBUST SUMMARY – MeBr RS4 04a

Study Type:	**In vitro Unscheduled DNA Synthesis (UDS) in human diploid fibroblasts.**
Title:	Tier II Mutagenic Screening of 13 NIOSH Priority Compounds: Individual Compound Report – Methyl Bromide
Laboratory:	Inveresk Research International Limited
Laboratory Study ID#:	409959 (Contract No. 210-78-0026) (Report No. 1190)
Study director(s):	Douglas B. McGregor, PhD
Report author(s):	Douglas B. McGregor, PhD
Study Initiation Date:	Not specified.
Study Completion Date:	Not specified. Report Date: May 30, 1981.

Protocol Guideline

None specified (this study predates published protocols). No references for methodology were given.

Remark: This report provides the results of five different genetic toxicology assays. It does not present methodology information separately for each assay but combines them all under a methodology section and some design information is in the summary table section. Thus, the presentation of information is not clear. For example, all the methyl bromide concentrations to which the fibroblasts are exposed are given only in a table (Summary Table UDS-1, page 50).

The Unscheduled DNA Synthesis assay is described on page 15 of the report. While some methods were adequately described (e.g., how S-9 was made), other methods used for this assay were poorly described or described under "DNA Repair Assay" (page 19). The precise method of exposure of methyl bromide gas is referred to in this section as being described on page 15; however, this page (and the entire section) deals only with animal exposures. Tritiated deoxyguanosine is reported as being obtained from a vendor but the use of this reagent is not described. The phase of the cell cycle is not described. No published references were provided in the narrative that pertains to methodology. The reference section contained citations without titles that may include some pertinent to this assay.

GLP Compliance

None specified.

Test Substance

Identity:	Methyl Bromide (a.k.a., bromomethane – CAS# 74-83-9)
Purity:	Not specified.
Supplied as:	Liquified gas (under pressure) in two cylinders, Batch No. 77371, from BDH Limited, (Dorset, England).

Study Design and Results

Group	Concentration (%) (Nominal)		Mean No. Grains/Nucleus ± S.D.	
	With S-9	Without S-9	With S-9	Without S-9
DMSO Vehicle (Negative) Control	1	1	5.2 ± 5.0	3.3 ± 2.7
Vinyl Chloride Positive Control	12.5	25	95.8 ± 42.9	4.4 ± 2.5
Methyl Bromide	5	5	4.4 ± 4.1	1.7 ± 1.6
	10	10	7.0 ± 5.4	3.3 ± 4.5
	20	20	4.1 ± 3.5	4.7 ± 3.6
	30	30	5.9 ± 4.0	4.4 ± 3.9
	40	40	7.3 ± 6.3	4.4 ± 3.2
	50	50	3.8 ± 4.4	6.0 ± 4.2
	60	60	12.1 ± 11.1	6.7 ± 5.2
	70	70	9.8 ± 6.8	6.6 ± 5.7

Test system (species, strain):	Human embryonic intestinal (diploid fibroblast) cells (Flow 11,000), passage 12-35 obtained from Flow Laboratories, Irvine Scotland.
Route of administration:	Cells exposed to gaseous methyl bromide.
# Exposure levels (# of Groups):	Eight including DMSO vehicle (negative) control.
Negative Control:	DMSO vehicle (0 ppm).
Positive Control:	Vinyl chloride.
Solvent:	DMSO (dimethylsulfoxide).
Number of replicates:	Three.
Number of metaphases analyzed:	50.
Exposure duration:	Three hours.
Exposure regimen:	Not specified.
# Cells/exposure level:	5,000/ml x 2 ml = 10,000 cells.
Method of detection:	Counting of grains on a photographic plate from 50 randomly selected cells, i.e., autoradiography of tritiated thymidine incorporated into nuclear DNA.

Statistical Methods

Not described.

Results

Cytotoxicity was not reported at methyl bromide concentrations up to 70% in air, with or without a metabolic activation system. The description of results (page 36) and Table UDS-1 (page 50) indicates no excess thymidine incorporation into non-S phase cells exposed to methyl bromide over a wide variety of concentrations (see table above), with or without an S-9 metabolic activation system, when compared to DMSO vehicle controls. The positive control, vinyl chloride, did produce excess thymidine incorporation, showing that the cells were responsive.

Conclusions

Exposure of human diploid fibroblasts to methyl bromide concentrations ranging from 5 to 70% for three hours did not increase unscheduled DNA repair.

References
None specified (none "called out") in the narrative portion of report.

Data Quality

While the procedures for this assay are poorly described, the data quality from this study is considered adequate. This study is assigned Klimisch level 2 (reliable with restrictions).

General Remarks

This study would have benefited from being reported separately from the four other studies described in the report.

ROBUST SUMMARY – MeBr RS4 04b

Study Type:	**In vivo Rat Bone Marrow Cytogenetics Assay.**
Title:	Tier II Mutagenic Screening of 13 NIOSH Priority Compounds: Individual Compound Report – Methyl Bromide
Laboratory:	Inveresk Research International Limited
Laboratory Study ID#:	409959 (Contract No. 210-78-0026) (Report No. 1190)
Study director(s):	Douglas B. McGregor, PhD
Report author(s):	Douglas B. McGregor, PhD
Study Initiation Date:	Not specified.
Study Completion Date:	Not specified. Report Date: May 30, 1981.

Protocol Guideline

None specified (this study predates published protocols). No references for methodology were given.

Remark: This report provides the results of five different genetic toxicology assays. It does not present methodology information separately for each assay but combines them all under a methodology section and some design information is in the summary table section. Thus, the presentation of information is not clear. For example, all the methyl bromide concentrations to which the fibroblasts are exposed are given only in a table (Summary Table UDS-1, page 50).

The rat bone marrow cytogenetics assay is described on page 21 of the report. While some methods were adequately described, other methods used for this assay were poorly described. No published references were provided in the narrative that pertains to methodology. The reference section contained citations without titles that may include some pertinent to this assay.

GLP Compliance

None specified.

Test Substance

Identity:	Methyl Bromide (a.k.a., bromomethane – CAS# 74-83-9)
Purity:	Not specified.
Supplied as:	Liquified gas (under pressure) in two cylinders, Batch No. 77371, from BDH Limited, (Dorset, England).

Study Design

Phase 1: Single 7-Hour Exposure

Group	Methyl Bromide Concentration (ppm) Target (Nominal)	Methyl Bromide Conc. Range (ppm) Actual (Measured)	No/Sex/Conc. (6-hr post-exposure)	No/Sex/Conc. (24-hr post-exposure)	No/Sex/Conc. (48-hr post-exposure)
Neg. (Air-only) Control	0	0	10	10	10
Low	20	18.7 – 21.4	10	10	10
Mid	70	69.4 – 73.4	10	10	10
Positive Control*	250 mg/kg EMS oral	250 mg/kg EMS oral	10	10	10

* Ethyl methanesulfonate (EMS); single oral gavage dose.

Phase 2: Five Consecutive Daily 7-Hour Exposures

Group	Methyl Bromide Concentration (ppm) Target (Nominal)	Methyl Bromide Conc. Range (ppm) Actual (Measured)	No/Sex/Conc. (6-hr post-exposure)
Neg. (Air-only) Control	0	0	10
Low	20	18.7 – 21.4	10
Mid	70	69.4 – 73.4	10
Positive Control*	100 mg/kg EMS oral	100 mg/kg EMS oral	10

* Ethyl methanesulfonate (EMS); 5 consecutive daily gavage doses.

Test subjects (species, strain & sex): Rat (CD® Sprague-Dawley derived), males and females from Charles River (UK) Limited, Manston, Kent.
Route of administration: Inhalation, whole-body.
Exposure levels (# of Groups): Four (including air-only and positive control groups).
Control group(s): Air-only (0 ppm).
Positive control group(s): Ethyl methanesulfonate (orally administered at 100 mg/kg for 5 consecutive days).
Exposure duration: Phase 1: single, 7-hour exposure.
Phase 2: 7 hr/day, for 5 consecutive days.
Exposure regimen: Phase 1: single 7-hour exposure.
Phase 2: five consecutive daily 7-hour exposures.
Post-exposure duration: None.
Animals/sex/dose/sacrifice interval: 10/sex/concentration/sacrifice (see table above).
Terminal sacrifice: Phase 1: 6 hr, 24 hr, and 48 hr post-exposure.
Phase 2: 6 hr post-exposure.

Remark: No mention is made in the report of colchicine injection to arrest cells in S-phase.

Other Study Parameters

Age at receipt: 10 - 11 weeks.
Acclimation period: Not specified.
Age at start of study: Not specified.
Weight ranges at start of study: Males: 340 - 450 grams; Females: 180-280 grams.

Assignment to groups:	Randomized weight basis.
Housing:	Individually, in polycarbonate cages w/steel mesh tops and bottoms, suspended above cage-board.
Inhalation chamber description:	Steel and glass, rectangular
Inhalation chamber volume:	1.5 m^3.
Chamber air flow rate:	12 - 16 air changes/hr.
Chamber temperature & humidity:	22 +- 4 ^oC; 40-61%.
Chamber atmosphere generation:	Dilution with air of methyl bromide gas from cylinders.
Chamber atmosphere monitoring:	Sampled continuously during the exposure period with a Miran-1A Portable Gas Analyzer (single beam infrared between 2.5 & 14.5 um).
Light cycle:	12 hrs light; 12 hrs dark.
Temperature:	22 +- 4 ^oC; 40-61%.
Humidity:	Recorded twice hourly during exposure. 40-70%
Food:	Spratts-Spillers No. 1.
Access to food:	Available *ad libitum* except during inhalation exposures and behavioral tests.
Access to water:	Available *ad libitum* except during inhalation exposures.

Toxicity Endpoints Monitored

Although not mentioned in the methodology section, presumably, the following were monitored: chromatid/chromosome breaks, gaps, deletions, fragments, pulverized chromosomes, acentric fragments, translocations, triradials, quadradial, rings, dicentrics, minute chromosomes, dicentrics, polypoids, hyperdipoids, etc.

Only chromosome and chromatid gaps and breaks are reported in the results tables. These endpoints were evaluated in 50 spreads (cells) per subject.

Statistical Methods

After transforming data to the Freeman-Tukey Binomial distribution, means were compared by "t" test.

Results

The author of the report concluded that no biologically significant increase in the incidence of chromosomal aberrations occurred in methyl bromide exposed rats in either phase; i.e., from either single or multiple exposure. In the multiple exposure (but not the single exposure) assay, the 70 ppm group had a statistically increased incidence over controls of aberrant cells. However, when cells containing only gaps were excluded, this group did not have an incidence that was increased over controls. This raises that question of biological significance according to the author, especially since females showed no effect and a sex difference would not be expected with a simple alkylating agent.

Conclusions

Inhalation exposure to methyl bromide concentrations of 20 or 70 ppm did not increase chromosomal aberrations, whether exposed for a single 7-hour period or after five consecutive daily 7-hour periods.

References

None specified (none "called out") in the narrative portion of report.

Data Quality

While the procedures for this assay are poorly described and disorganized, the data quality from this study is considered adequate. This study is assigned Klimisch level 2 (reliable with restrictions).

General Remarks

This study would have benefited from being reported separately from the four other studies described in the report. It is noteworthy that, in the multiple dose study, the EMS positive control females did not show a statistically increased incidence of aberrations compared to controls.

ROBUST SUMMARY – MeBr RS4 04c

Study Type:	**Rat Dominant Lethal Assay.**
Title:	Tier II Mutagenic Screening of 13 NIOSH Priority Compounds: Individual Compound Report – Methyl Bromide
Laboratory:	Inveresk Research International Limited
Laboratory Study ID#:	409959 (Contract No. 210-78-0026) (Report No. 1190)
Study director(s):	Douglas B. McGregor, PhD
Report author(s):	Douglas B. McGregor, PhD
Study Initiation Date:	Not specified.
Study Completion Date:	Not specified. Report Date: May 30, 1981.

Protocol Guideline

None specified (this study predates published protocols). No references for methodology were given.
Remark: This report provides the results of five different genetic toxicology assays. It does not present methodology information separately for each assay but combines them all under a methodology section and some design information is in the summary table section. Thus, the presentation of information is not clear.

The Dominant lethal assay is described on page 23 of the report. While some methods were adequately described, other methods used for this assay were poorly described. No published references were provided in the narrative that pertains to methodology. The reference section contained citations without titles that may include some pertinent to this assay.

GLP Compliance

None specified.

Test Substance

Identity:	Methyl Bromide (a.k.a., bromomethane – CAS# 74-83-9)
Purity:	Not specified.
Supplied as:	Liquified gas (under pressure) in two cylinders, Batch No. 77371, from BDH Limited, (Dorset, England).

Study Design

Group	Methyl Bromide Concentration (ppm) Target (Nominal)	Methyl Bromide Conc. Range (ppm) Actual (Measured)	No/males/conc.	No/females/conc. /week for 10 weeks
Neg. (Air-only) Control	0	0	10	20
Low	20	18.7 – 21.4	10	20
Mid	70	69.4 – 73.4	10	20
Positive Control*	100 mg/kg EMS oral	100 mg/kg EMS oral	10	20

* Ethyl methanesulfonate (EMS); 5 consecutive daily gavage.

Males only were exposed for 7 hours per day on five consecutive days. Immediately after exposure, males were paired with two virgin females for 1 week. Females were removed at the end of one week and two new virgin females were introduced. This process was repeated for 10 weeks to cover the period of spermatogenesis. Mated females were sacrificed 14 days after presumed fertilization (days 2 or 3 after introduction) and ovaries and uteri were removed. Ovaries were examined for corpora lutea graviditatis, which were counted to assess pre-implantation loss. Ovaries were examined for live implantations, early deaths, and late deaths.

Test subjects (species, strain & sex):	Rat (CD® Sprague-Dawley derived), males and females from Charles River (UK) Limited, Manston, Kent.
Route of administration:	Inhalation, whole-body.
# MeBr Exposure levels (# of Groups):	Three (including an air-only control group).
Negative Control group(s):	Air-only (0 ppm).
Positive control group(s):	Ethyl methanesulfonate (administered orally at 100 mg/kg for 5 consecutive days).
Exposure duration:	7 hr/day, for 5 consecutive days.
Exposure regimen:	Five consecutive daily 7-hour exposures.
Post-exposure duration:	None.
# Animals/sex/group:	10 males and 2 females/male x 10 consecutive weeks or 200 females per group (each male was mated with 2 females/week for 10 consecutive weeks post-exposure).
Terminal sacrifice:	Males: 10 weeks post-exposure; females: 14 days post mating.

Other Study Parameters

Age at receipt:	10 - 12 weeks for males; approximately 8 weeks for females.
Acclimation period:	10 days for males; none for females.
Age at start of study:	Not specified.
Weight ranges at start of study:	Males: 340 - 450 grams; Females: 180-280 grams.
Assignment to groups:	Randomized weight basis.
Housing:	Individually, in polycarbonate cages w/steel mesh tops and bottoms, suspended above cage-board.
Inhalation chamber description:	Steel and glass, rectangular
Inhalation chamber volume:	1.5 m^3.
Chamber air flow rate:	12 - 16 air changes/hr.
Chamber temperature & humidity:	22 +- 4 ^oC; 40-61%.
Chamber atmosphere generation:	Dilution with air of methyl bromide gas from cylinders.
Chamber atmosphere monitoring:	Sampled continuously during the exposure period with a Miran-1A Portable Gas Analyzer (single beam infrared between 2.5 & 14.5 um).
Light cycle:	12 hrs light; 12 hrs dark.
Temperature:	22 +- 4 ^oC; 40-61%.
Humidity:	Recorded twice hourly during exposure. 40-70%
Food:	Spratts-Spillers No. 1.
Access to food:	Available *ad libitum* except during inhalation exposures and behavioral tests.
Access to water:	Available *ad libitum* except during inhalation exposures.

Toxicity Endpoints Monitored

Corpora lutea were counted in the ovary to assess pre-implantation loss. Live implantations, early and late deaths were counted in the uterus to determine fertility indices. Total implantations, live

implantations, live implantations plus early deaths, early deaths (Freeman-Tukey Poisson transformation), and early deaths (Freeman-Tukey binomial transformation).

Statistical Methods

A very technical discussion of statistical distributions is included in the report, which also discusses various data transformation procedures required to render the data into analyzable form. However, the tests used to compare control and treated groups, once the data were transformed, are not described (e.g., ANOVA or "t" test?). Reference is made to a "GLIM" program (presumably a statistical software package) but no further details were given.

Results

Implantations per pregnancy, frequencies of live implantations, and live implantations were not decreased and corpora lutea and early and late deaths were not increased in offspring of male rats exposed to methyl bromide concentrations of 30 or 70 ppm compared to controls. The positive control agent, ethyl methanesulfonate, did increase early deaths, indicating a dominant lethal effect.

Conclusions

Methyl bromide did not have a dominant lethal effect on rats exposed to methyl bromide concentrations of 0, 20, or 70 ppm for 7 hours per day, for 5 consecutive days.

References

None specified (none "called out") in the narrative portion of report.

Data Quality

While the procedures for this assay are poorly described and disorganized, the data quality from this study is considered adequate. This study is assigned Klimisch level 2 (reliable with restrictions).

General Remarks

This study would have benefited from being reported separately from the four other studies described in the report.

ROBUST SUMMARY – MeBr RS4 04d

Study Type:	**Sperm Abnormality Test in Mice.**
Title:	Tier II Mutagenic Screening of 13 NIOSH Priority Compounds: Individual Compound Report – Methyl Bromide
Laboratory:	Inveresk Research International Limited
Laboratory Study ID#:	409959 (Contract No. 210-78-0026) (Report No. 1190)
Study director(s):	Douglas B. McGregor, PhD
Report author(s):	Douglas B. McGregor, PhD
Study Initiation Date:	Not specified.
Study Completion Date:	Not specified. Report Date: May 30, 1981.

Protocol Guideline

None specified (this study predates published protocols). No references for methodology were given.
Remark: This report provides the results of five different genetic toxicology assays. It does not present methodology information separately for each assay but combines them all under a methodology section and some design information is in the summary table section. Thus, the presentation of information is not clear.

The Sperm Abnormality assay is described on page 25 of the report. While some methods were adequately described, other methods used for this assay were poorly described. Only one published reference was provided in the narrative that pertains to methodology. The reference section contained citations without titles that may include some pertinent to this assay.

GLP Compliance

None specified.

Test Substance

Identity:	Methyl Bromide (a.k.a., bromomethane – CAS# 74-83-9)
Purity:	Not specified.
Supplied as:	Liquified gas (under pressure) in two cylinders, Batch No. 77371, from BDH Limited, (Dorset, England).

Study Design

Group	Methyl Bromide Concentration (ppm) Target (Nominal)	Methyl Bromide Conc. Range (ppm) Actual (Measured)	No/males/conc.
Neg. (Air-only) Control	0	0	10
Low	20	18.7 – 21.4	10
Mid	70	69.4 – 73.4	10
Positive Control*	100 mg/kg EMS oral	100 mg/kg EMS oral	10

* Ethyl methanesulfonate (EMS); 5 consecutive daily gavage doses.

Males only were exposed for 7 hours per day on five consecutive days. Five weeks after the last exposure, mice were sacrificed and sperm collected, plated on slides, dried, stained, and sperm were examined microscopically for abnormalities. Sperm with the following characteristics were considered abnormal: hook upturn or elongated, banana-shaped head, amorphous head, abnormal tail (sharp, 180^{o} angle or tight coiling only), miscellaneous (e.g., multiple tails, double heads, twisted neck, filamentous mid-piece, enlarged mid-piece, plier types).

Test subjects (species, strain & sex):	Mouse (B6C3F1), males from Charles River (UK) Limited, Manston, Kent.
Route of administration:	Inhalation, whole-body.
# MeBr Exposure levels (# of Groups):	Three (including an air-only control group).
Negative Control group(s):	Air-only (0 ppm).
Positive control group(s):	Ethyl methanesulfonate (orally administered at 100 mg/kg for 5 consecutive days).
Exposure duration:	7 hr/day, for 5 consecutive days.
Exposure regimen:	Five consecutive daily 7-hour exposures.
Post-exposure duration:	None.
# Animals/sex/group:	10 males per group.
Terminal sacrifice:	5 weeks post-exposure.

Other Study Parameters

Age at receipt:	10 - 12 weeks.
Acclimation period:	10 days.
Age at start of study:	Not specified.
Weight ranges at start of study:	18 – 22 gram range for MeBr mice; slightly heavier for EMS positive control mice.
Assignment to groups:	Randomized weight basis.
Housing:	Individually, in polycarbonate cages w/steel mesh tops and bottoms, suspended above cage-board.
Inhalation chamber description:	Steel and glass, rectangular
Inhalation chamber volume:	1.5 m^3.
Chamber air flow rate:	12 - 16 air changes/hr.
Chamber temperature & humidity:	22 +- 4 ^{o}C; 40-61%.
Chamber atmosphere generation:	Dilution with air of methyl bromide gas from cylinders.
Chamber atmosphere monitoring:	Sampled continuously during the exposure period with a Miran-1A Portable Gas Analyzer (single beam infrared between 2.5 & 14.5 um).
Light cycle:	12 hrs light; 12 hrs dark.
Temperature:	22 +- 4 ^{o}C; 40-61%.
Humidity:	Recorded twice hourly during exposure. 40-70%
Food:	Not specified.
Access to food:	Available *ad libitum* except during inhalation exposures and behavioral tests.
Access to water:	Available *ad libitum* except during inhalation exposures.

Toxicity Endpoints Monitored

Sperm with 1) hook upturned or that is elongated, 2) banana-shaped head, 3) amorphous head, 4) abnormal tail (sharp, 180o angle or tight coiling only), or 5) miscellaneous (e.g., multiple tails, double heads, twisted neck, filamentous mid-piece, enlarged mid-piece, plier types).

Statistical Methods

Observational count data was transformed using the Freeman-Tukey transformation for proportions (presumably to convert it to a normal distribution). Once transformed, a one-sided "t" test was used to distinguish statistically significant differences of treated compared to controls.

Results

No increased frequencies in abnormal sperm were found in methyl bromide exposed mice when compared to mice exposed to air.

Conclusions

Methyl bromide did not cause abnormal sperm in mice exposed to methyl bromide concentrations of 0, 20, or 70 ppm for 7 hours per day, for 5 consecutive days.

References

Sperm were scored according to the method of Wyobek and Bruce (1975).

Wyobeck, A.J., Bruce, W.R., Proc. Natl. Acad. Sci. 72:4425. (title not reported).

Data Quality

While the procedures for this assay are poorly described and disorganized, the data quality from this study is considered adequate. This study is assigned Klimisch level 2 (reliable with restrictions).

General Remarks

This study would have benefited from being reported separately from the four other studies described in the report.

ROBUST SUMMARY – MeBr RS4 04e

Study Type: **Sex-Linked Recessive Lethal Test in Drosophila.**

Title: Tier II Mutagenic Screening of 13 NIOSH Priority Compounds: Individual Compound Report – Methyl Bromide

Laboratory: Inveresk Research International Limited

Laboratory Study ID#: 409959 (Contract No. 210-78-0026) (Report No. 1190)
Study director(s): Douglas B. McGregor, PhD
Report author(s): Douglas B. McGregor, PhD

Study Initiation Date: Not specified.
Study Completion Date: Not specified. Report Date: May 30, 1981.

Protocol Guideline

None specified (this study predates published protocols). No references for methodology were given.

Remark: This report provides the results of five different genetic toxicology assays. It does not present methodology information separately for each assay but combines them all under a methodology section and some design information is in the summary table section. Thus, the presentation of information is not clear.

The Sperm Abnormality assay is described on page 25 of the report. While some methods were adequately described, other methods used for this assay were poorly described. No published references were provided in the narrative that pertains to methodology. The reference section contained citations without titles that may include some pertinent to this assay.

GLP Compliance

None specified.

Test Substance

Identity: Methyl Bromide (a.k.a., bromomethane – CAS# 74-83-9)
Purity: Not specified.
Supplied as: Liquified gas (under pressure) in two cylinders, Batch No. 77371, from BDH Limited, (Dorset, England).

Study Design

Group	Methyl Bromide Concentration (ppm) Target (Nominal)	Methyl Bromide Conc. Range (ppm) Actual (Measured)	Number of males /conc	Number of females /conc	Number of broods /conc.	Number of /generations /conc
Low	20	18.7 – 21.4	2	4	3	3
Mid	70	69.4 – 73.4	2	4	3	3
Positive Control*	EMS oral*	EMS oral*	2	4	3	3

* Ethyl methanesulfonate (EMS); 0.4% in sucrose food for 5 hours.

In a preliminary toxicity test, 100 flies were exposed to the above concentrations for 1, 3, and 5 hours. Toxicity was measured by the hatchability index. Results indicated that a 5-hour exposure was appropriate (i.e., no toxicity occurred after 5 hours). In the recessive lethal study, only Wild-type males were exposed to methyl bromide at concentrations of 0, 20, or 70 ppm for 5 hours. These males were then mated with two separate unexposed virgin females carrying a heterozygous trait that produce two male phenotypes (wild and inbred types) on days 1, 3 and 8 following exposure (covering the period of spermatogenesis). These matings produced three F1 broods (each the combined offspring of the two females). Males from the F1 generation were mated with sibling females and scored for wild type males (the absence of which would indicate a recessive lethal effect). Apparently, this was repeated for a third generation although the methodology narrative is unclear (results section narrative and summary tables report results for an F3 generation). A positive control, ethyl methanesulfonate, was administered in food (0.4% v/v in sucrose for 5 hr).

Test subjects (species, strain & sex):	Drosophila melanogaster (males: wild-type Oregon K, females: Muller-5 with basc balancer X-chromosome).
Route of administration:	Inhalation, whole-body.
# MeBr Exposure levels (# of Groups):	Two (no air-only control group).
Negative Control group(s):	None. Historical control rates were used.
Positive control group(s):	Ethyl methanesulfonate (orally administered at 0.4% mg/kg in sucrose for 5 hours).
Exposure duration:	5 hours.
Exposure regimen:	Single 5-hour exposure.
Post-exposure duration:	None.
# Animals/sex/group:	2 males per group mated with 2 unexposed virgin females separately on days 1, 3, and 8 following exposure. Three broods per generation (over three generations) constituted the basic statistical unit.

Toxicity Endpoints Monitored

The lack of appearance of wild-type males in subsequent-generation males from the males originally exposed to the test agent, constituted evidence for a sex-linked recessive lethal mutation.

Statistical Methods

Results were judged for biological significance based upon whether a reproducible increase above historical controls was found. This is because the rates of recessive lethality are low in the number of Drosophila broods that can reasonably comprise a group. Thus, no statistics were applied. Rather, lethality frequencies were compared to historical and concurrent controls. A compound showing a rate below 0.5% was considered negative, greater than 1.0% was positive and between 0.5 and 1.0% was considered equivocal for recessive lethality.

Results

Spurious increases in lethality rates were seen but they 1) did not follow a dose-response, 2) were not consistent across generations, or 3) were not consistent among broods within a given generation.

Conclusions

Methyl bromide did not cause an increase in the rate of sex-linked recessive lethality in Drosophila melanogaster exposed to methyl bromide concentrations of 0, 20, or 70 ppm for a single 5-hour exposure.

References

Spencer, W.P., Stern, C., 1948, Genetics 33:43.

Wurgler, F.E., Sobels, F.H., Vogel, E., 1977. In "Handbook of Mutagenicity Test Procedures." (B.J. Kilbey, M. Legator, W. Nichols, C. Ramel, eds.) Elsevier, Amsterdam.

Data Quality

While the procedures for this assay are poorly described and disorganized, the data quality from this study is considered adequate. This study is assigned Klimisch level 2 (reliable with restrictions).

General Remarks

This study would have benefited from being reported separately from the four other studies described in the report.

ROBUST SUMMARY – MeBr RS4 05

Study Type: **Chronic Toxicity (12-month dietary study in dogs)**

Title: A Chronic (12-Month) Toxicity Study of Methyl Bromide Fumigated Feed in the Dog (Final Report)

Laboratory: Huntingdon Life Sciences (Pharmaco-LSR – East Millstone, N.J., USA)

Laboratory Study ID#: 94-3186

Study Initiation Date:	April 24, 1994	Necropsy Date:	May 8 & 9, 1995
Study Completion Date:	January 4, 1996	Report Date:	January 4, 1996

Protocol Guideline 83-1 (FIFRA Guideline, Subdivision F: Protocol 83-1 "Chronic Toxicity Studies," revised November 1984)

GLP Compliance

According to a statement that is part of the study report, signed by the manager of the Pharmaco-LSR Quality Assurance Unit, this study was conducted in accordance with Good Laboratory Practices (Part 160 of 40 CFR [EPA/FIFRA Good Laboratory Practice Regulations]).

Test Substance

Identity: Methyl Bromide (a.k.a., bromomethane – CAS# 74-83-9)
Purity: "100% Active Ingredient"
Supplied as: Gas, Lot No. "SLV"; supplier: Great Lakes Chemical

Study Design

Test Subjects (species, strain & sex): Dog (Beagle), males and females.
Route of Administration: Diet; feed fumigated with methyl bromide gas.
Exposure Levels (# of Groups): Four (including an air-only control group).
Exposure Level Concentrations: Average target methyl bromide residue levels: 0, 0.5, 1.5, and 5 ppm. Average target dose for males and females: 0.0, 0.013, 0.038, and 0.125 mg/kg-d). Actual time weighted average (TWA) dose (over feeding period of 1 hour) for males: 0.0, 0.06, 0.13, and 0.27 mg/kg-d; for females: 0.0, 0.07, 0.12, and 0.26 mg/kg-d.
Control Groups: Feed-only (0 ppm methyl bromide).
Exposure regimen: Five days/week (no exposure on weekends).
Exposure Duration: 12 months.
Post-exposure Duration: None.
Animals/Sex/Dose: 4/sex/dose at doses of 0, 0.5, and 1.5 ppm; 8/sex/dose at the high dose of 5 ppm.
Terminal Sacrifice: After 12 months of exposure.
Interim Sacrifice Intervals: None.
Interim Test Intervals: Three and six months for hematology and clinical chemistries. Six months for urinalysis.
Remarks: Prestudy trials were used to determine the fumigation regimen that would result in the desired methyl bromide

concentrations in the feed. Feed was presented daily to the subjects 2 hours after fumigation with methyl bromide when degassing had slowed. Feed concentrations (and doses) of methyl bromide were calculated on a time weighted average basis because methyl bromide degassing, although slowed after 2 hours, continued to evaporate from the feed over the course of the 60 minute feeding interval. The food fumigation regimen was designed to result in concentrations at 2 hours (initiation of feeding) that would result in doses based on the empirical observation that, on average, the dogs consumed 75% of the feed during the first 30 minutes of the 60 minute feeding period and 25% during the last 30 minutes. Chow was in meal form for easier fumigation. Dogs received untreated feed on weekends.

Other Study Parameters

Age at receipt:	Approximately 6 months.
Acclimation period:	Five weeks.
Age at start of study:	Approximately 7 months.
Target survival at terminal sacrifice:	100%.
Average weight at start of study:	Males: 10 kg; Females: 8.1 kg.
Assignment to groups:	Randomized weight basis; littermates not assigned to same group.
Housing:	Individual elevated metal grid cages.
Light cycle:	12 hrs light; 12 hrs dark.
Temperature:	16 to 26 °C monitored and recorded twice daily.
Humidity:	24 to 78% monitored and recorded once daily.
Food:	Meal form of chow. Certified Canine Diet, No. 5007 (meal), (PMI Feed, Inc., St. Louis, MO).
Access to water:	*Ad libitum* via automated watering system.

Toxicity Endpoints Monitored

Clinical signs:	Twice daily.
Morbidity/mortality:	Twice daily.
Physical examinations:	Pretest and weekly.
Ophthalmic exam:	Pretest and termination.
Body weights:	Pretest, weekly, and at termination.
Food consumption:	Measured and recorded daily.
Hematology:	Three times prior to exposure, 3, 6, and 12 months.
Clinical chemistries:	Three times prior to exposure, 3, 6, and 12 months.
Urinalyses:	Once, prior to exposure, and at 3, 6, and 12 months.
Interim sacrifices:	None.
Organ weights:	Brain, heart, kidneys, liver, lungs, ovaries, spleen, testes (with epididymides), and thyroid/parathyroid.
Gross pathology:	Conducted on all animals.
Histopathology:	Tissues from all animals were preserved in formalin. These included: adrenal glands, aorta, bone (femur, sternebra), bone marrow (femur, sternum), brain (3 sections), esophagus, eyes with optic nerve, gall bladder, heart, intestine (cecum, colon, duodenum, ileum, jejunum, rectum), kidneys, larynx, liver, lungs lymph nodes, mammary gland, nasopharyngeal tissues, nerve (sciatic),

ovaries, pancreas, pituitary, prostate, salivary (submandibular), skeletal muscle, skin, spinal cord, (cervical, thoracic, lumbar), spleen, stomach, testes (w/epididymides), thymic region, thyroid/parathyroid, trachea, urinary bladder, uterus (body, horns, cervix), and tissues with macroscopic lesions. Tissues were examined for damage from chronic toxicity and for neoplastic lesions. All of the these tissues were examined microscopically from all animals.

Statistical Methods

Body weights:	Analysis of variance followed by Dunnet's test.
Organ weights:	Analysis of variance followed by Dunnet's test.
Hematology data:	Analysis of variance followed by Dunnet's test.
Biochemistry data:	Analysis of variance followed by Dunnet's test.

Results

Clinical signs: No differences were noted between control and methyl bromide-treated animals regarding behavioral or clinical signs. Clinical signs NOAEL = 0.27 mg/kg-d (males) and 0.26 mg/kg-d (females). No LOAEL established.

Morbidity/Mortality: No unscheduled deaths occurred during the course of the study. Mortality NOAEL = 0.27 mg/kg-d (males) and 0.26 mg/kg-d (females). No LOAEL established.

Body weights: There was no effect from methyl bromide exposure on body weights. Body weight NOAEL = 0.27 mg/kg-d (males) and 0.26 mg/kg-d (females). No LOAEL established.

Organ weights: Methyl bromide exposure produced no effect upon absolute or relative organ weights. A statistically significant difference in absolute kidney weights was found in mid and high dose females. When compared on a basis relative either to total body weight or to brain weight, however, no difference was detected. Because of this, and because a dose response was not exhibited, kidney weights were not considered to be affected by methyl bromide exposure. Organ weight NOAEL = 0.27 mg/kg-d (males) and 0.26 mg/kg-d (females). No LOAEL established.

Food consumption: Methyl bromide did not affect food consumption. NOAEL = 0.27 mg/kg-d (males) and 0.26 mg/kg-d (females). No LOAEL established.

Ophthalmic Effects: Ophthalmic examinations revealed no effect from methyl bromide exposure. Body weight NOAEL = 0.27 mg/kg-d (males) and 0.26 mg/kg-d (females). No LOAEL established.

Hematology: Sporadic changes were noted in some hematology parameters at different collection points. Specifically, high dose males showed a significant decrease in hematocrit values compared to controls at 3 and 6 months. Hemoglobin was decreased in these males at 6 and 12 months. Finally, mid-dose females exhibited elevated hematocrits, hemoglobin, and RBC counts at 12 months. These changes were not considered related to methyl bromide exposure because they varied widely in the baseline assessments prior to treatment, were not consistent among time points or between genders, and did not correlate with other, related parameters (e.g., reticulocyte counts did not change when RBC's were elevated). Hematology NOAEL = 0.27 mg/kg-d (males) and 0.26 mg/kg-d (females). No LOAEL established.

Clinical chemistries: Random differences were found that reached statistical significance when compared to controls at the 3 collection times. At 3 months, total protein and globulin was slightly

increased high dose males and A/G ratio was decreased. High dose females exhibited elevated potassium. At 6 months, mid and high dose males had decreased calcium. At study termination, the A/G ratio was decreased in high dose males and calcium was decreased in mid and high dose males. The authors of the study did not attribute these changes to methyl bromide exposure because they were small, within normal ranges, not seen in both sexes, not repeated consistently at different collection points, and were not generally dose-related. Clinical chemistry NOAEL = 0.27 mg/kg-d (males) and 0.26 mg/kg-day (females). No LOAEL established.

Urinalyses: No changes were found that could be attributed to methyl bromide exposure. Urinalysis NOAEL = 0.27 mg/kg-d (males) and 0.26 (females). No LOAEL established.

Gross pathology: Examination at necropsy revealed no effect from methyl bromide exposure. NOAEL = 0.27 mg/kg-d (males) and 0.26 mg/kg-d (females). No LOAEL established.

Histopathology: Although incidental lesions were found, none were considered related to methyl bromide exposure. NOAEL = 0.27 mg/kg-d (males) and 0.26 (females). No LOAEL established.

Conclusions

No toxicologically significant effects from methyl bromide exposure were seen on clinical observations, body weight, body weight gain, food consumption, clinical pathology, urinalysis, ophthalmology, absolute or relative organ weights, or macroscopic or microscopic pathology. Based on the results of this study, the NOEL for methyl bromide when administered via fumigated feed to beagle dogs was 5 ppm (> 0.27 mg/kg/day for males and > 0.26 mg/kg/day for females).

References

Newton, P.E., 1995. A chronic (12-month) toxicity study of methyl bromide fumigated feed in the dog. Unpublished report from Pharmaco LSR, Project Number 93-6068.

Wilson, N.H., Newton, P.E., Rahi, M., Bolte, H.F., Suber, R.L., 1998. Methyl bromide: 1-year dietary study in dogs. Food Chem. Toxicol. 36(7):575-584.

Data Quality

The data quality from this study is considered high. The report included comprehensive documentation for method and results. The conducting laboratory is reputable. This study reaches Klimisch level 1.

General Remarks

This study was reliably conducted with exposure levels chosen so as to reveal the intrinsic toxicity of methyl bromide, and usually bracketing adverse effects so that NOAELs could be identified for each effect (LOAELs generally were not established).

ROBUST SUMMARY – MeBr RS4 06

Study Type:	**Chronic Toxicity/Carcinogenicity**
Title:	Chronic (29-month) Inhalation Toxicity and Carcinogenicity Study of Methyl Bromide in Rats
Laboratory:	Netherlands Organization of Applied Scientific Research (T.N.O.)
Laboratory Study ID#:	V86.469/221044
Study Completion Date:	March 7, 1985 Report Date: January 1987
Protocol Guideline	Not specified.

GLP Compliance

According to a statement that is part of the study report, signed by the manager of the TNO Quality Assurance Unit, this study was conducted in accordance with Good Laboratory Practices (Specific guidelines not reported).

Test Substance

Identity:	Methyl Bromide (a.k.a., bromomethane – CAS# 74-83-9).
Purity:	> 98.8% (< 50 ppm water; < 10 ppm HBr).
Supplied as:	Liquefied, compressed methyl bromide gas in pressurized cylinders (45 kg/cylinder, supplied by Air Products).

Study Design

Test subjects (species, strain & sex):	Rat (Wistar; Cpb:WU strain), males and females.
Route of administration:	Inhalation, whole-body.
# Exposure levels (# of Groups):	Four (including an air-only control group).
Exposure level concentrations:	Nominal concentrations: 0, 3, 30, and 90 ppm. Actual conc (+-SD): 3.1(+-0.4), 29.6(+-1.9), and 89.1(+-3.4) ppm.
Control group(s):	Air-only (0 ppm).
Exposure duration:	Up to 29 months of age.
Exposure regimen:	6 hours/day, 5 days/week (no exposure on weekends).
Post-exposure duration:	None.
# Animals/sex/concentration:	90/sex/conc. total; 50/sex/conc. for lifetime exposure duration (remainder split 10/sex among three sacrifice and one behavioral test intervals).
Interim sacrifice intervals:	13, 52, and 104 weeks (10/sex/conc.).
Interim test intervals:	41 weeks for behavioral test battery (10/sex/conc.).
Terminal sacrifice:	At 29 months of age.

Other Study Parameters

Age at receipt:	Four weeks.
Acclimation period:	Two weeks.
Age at start of study:	Six weeks.
Target survival at terminal sacrifice:	30% (this resulted in ages at final sacrifice of 107 weeks for males and 109 weeks for females).

Inhalation chamber volume:	2.5 m^3.
Chamber air flow rate:	40-60 m^3/hr.
Chamber temperature:	21 +- 2 ^oC.
Chamber atmosphere generation:	Computer controlled dilution with air of methyl bromide gas from cylinders.
Chamber atmosphere monitoring:	Sampled in triplicate every 30 minutes during exposure periods with an Intersmat GC 120 gas chromatograph equipped with a flame ionization detector (calibrated monthly).
Housing:	Suspended wire mesh cages in inhalation chambers.
Light cycle:	12 hrs light; 12 hrs dark.
Access to food and water:	None during exposures; *ad libitum* during non-exposure periods.

Toxicity Endpoints Monitored

Clinical Signs:	Daily
Morbidity/Mortality:	Daily
Body Weights:	Weekly, for first three months; monthly thereafter
Hematology:	Week 13 and week 52 (10/sex/group)
Clinical Chemistries:	Week 14 and week 53 (10/sex/group)
Urinalyses:	Week 13 and week 52 (10/sex/group)
Interim Sacrifices:	Week 13, week 52, and week 104 (10/sex/group)
Organ Weights:	Adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, spleen, testes, and thymus
Gross pathology:	Conducted on all animals
Histopathology:	Tissues from all animals were preserved in formalin. Tissues were examined for damage from chronic toxicity and for neoplastic lesions. Tissues examined microscopically from the 0 and 90 ppm group included: Adrenals, aorta, axillary lymph nodes, brain (stem, cerebrum, cerebellum), caecum, coagulating glands, colon, duodenum, epididymides, extraorbital lachrymal glands, eyes, harderian glands, heart, ileum, jejunum, kidneys, liver, lungs (with mediastinal lymph nodes, trachea and larynx), mammary glands, mesenteric lymph nodes, nose (cross sections at 4 levels), esophagus, ovaries, pancreas, parotid salivary glands, pharynx, pituitary, prostate, sciatic nerve, seminal vesicles, skeletal muscle (thigh), skin (flank), spinal cord, spleen, sternum (with bone marrow) stomach, sub-maxillary salivary glands, sublingual salivary glands, testes, thymus, thyroid with parathyroid, urinary bladder, uterus (with cervix), zymball glands, bone marrow smears, all gross lesions. Tissues examined microscopically from the 3 and 30 ppm included: all gross lesions found at autopsy, heart, and the nasal cavity.

Statistical Methods

Body weights:	Analysis of co-variance followed by Dunnet's test.
Organ weights:	Analysis of co-variance followed by Dunnet's test.
Hematology data:	Analysis of co-variance followed by Dunnet's test.
Biochemistry data:	Analysis of co-variance followed by Dunnet's test.
Mortality incidence:	Fisher exact probability test
Histopathology incidence:	Fisher exact probability test

Results

Clinical signs: No differences were noted between control and methyl bromide-treated animals regarding behavioral or clinical signs. Clinical signs NOAEL = 90 ppm (males and females). No LOAEL established.

Morbidity/Mortality: Increased in 90 ppm group toward end of second year in both males and females. Increased mortality was associated with increased incidence of heart thrombi, which the study authors speculated might be causative. Mortality NOAEL = 30 ppm (males and females); Mortality LOAEL = 90 ppm (males and females).

Body weights: In the 90 ppm group, male and female body weights were slightly less than controls after four weeks of exposure. Differences were not always statistically significant in males but were always so in females. Females exposed to 3 and 30 ppm showed weight loss from exposure days 309 to 337. Females from these two groups resumed weight gain thereafter. Males, which were housed in the same chambers at 3 and 30 ppm, did not show weight loss or decreased weight gain during the same period. Nor did females at 90 ppm exhibit these effects over this period. No reason was apparent that explained the weight loss in the 3 and 30 ppm females. Much earlier in the study (exposure week 5), a transient lower body weight (not frank weight loss) was found in the 30 ppm males that did not occur at the next weighing. Because of the inconsistencies, no treatment related effect was ascribed to the body weight effects in subjects exposed to 3 or 30 ppm. However, methyl bromide exposure did appear related to the effects found in the 90 ppm group. Body weight NOAEL = 30 ppm (males and females); Body weight LOAEL = 90 ppm (males and females).

Organ weights: At 53 weeks, average absolute kidney weights were significantly lower in 90 ppm males and 30 and 90 ppm females. Expressed relative to total body weight, kidney weights were significantly lower for 30 and 90 ppm males and these decreases exhibited a dose-response relationship. Brain weights were decreased compared to controls on an absolute but not relative basis in 90 ppm females at 53 and 104 weeks. While not statistically significant for males the same pattern held for absolute brain weights (also no difference relative to body weight). Relative but not absolute thyroid weights were decreased in females exposed to 30 ppm (not considered treatment related). Absolute organ weight NOAEL = 3 ppm (females) and 30 ppm (males). Relative organ weight NOAEL = 3 ppm (males) and 30 ppm (females).

Hematology: Random differences were found at week 13 and week 52 that reached statistical significance in some groups. Specifically, mean packed cell volume was slightly decreased in 90 ppm females at 13 weeks (but not 52 weeks and erythrocyte count was normal). Also, neutrophil counts were increased in the 3 ppm females at week 13, erythrocyte counts were decreased in 30 ppm males at 13 weeks. At week 52, white cell counts were higher in 3 and 90 ppm males when compared statistically to an unusually low control value but were within the normal range.

Because these changes 1) were all slight in magnitude, 2) did not co-occur at the two test intervals of 13 and 52 weeks, 3) did not show a coherent pattern with other parameters (i.e., decreased mean packed cell volume did not correlate with a decreased red blood cell count), 4) did not exhibit a concentration-response relationship, 5) were within normal ranges or 6) were significant because of an unusual control value, they were not considered treatment-related. Hematology NOAEL = 90 ppm (males and females). No LOAEL was found for hematology.

Clinical chemistries: BUN was decreased in a dose related manner in 30 and 90 ppm males at 53 weeks. Although not seen with females, this finding may be related to methyl bromide exposure. Total protein was higher (63.4 g/l) in 90-ppm males at week 53 than controls (60.9 g/l). Because the control value was considered unusually low, the study authors did not consider increased protein to be treatment-related. Females exposed to 30 ppm methyl bromide exhibited an increase in alkaline

phosphatase that did not re-occur at week 53. Because of the lack of re-occurrence and lack of dose-response, the alkaline phosphatase findings were not considered treatment related. Alanine amino transferase and aspartate amino transferase were both decreased in 90 ppm females at 53 weeks. Because the control was unusually high and this effect did not occur at 13 weeks, the authors did not attribute the effect to methyl bromide exposure. Decreased BUN NOAEL = 3 ppm (males), 90 ppm (females). Decreased BUN LOAEL = 30 ppm (males), none for females.

Urinalyses: No changes were found that could be attributed to methyl bromide exposure. Urinalysis NOAEL = 90 ppm (males and females). No LOAEL established.

Gross pathology: Increased incidence of hemothorax in males and females from the 90 ppm group. NOAEL = 30 ppm (males and females); LOAEL = 90 ppm (males and females).

Histopathology: Regarding non-neoplastic lesions, a concentration-related increase in irritation of the nasal epithelia was found in methyl bromide exposed rats of both sexes at all exposure levels and all four sacrifice times. These lesions occurred in the dorso-medial part of the nasal cavity and were characterized by basal cell hyperplasia and degeneration of the overlying epithelium. While concentration-related, these lesions did not increase appreciably with exposure time. An increased incidence also was seen of thrombi in the heart at various exposure times after 53 weeks and was most pronounced in the 90 ppm group for both males and females. Controls did not exhibit this heart lesion. Hyperkeratosis of the esophagus and stomach was found in both sexes exposed to 90 ppm methyl bromide but was statistically higher only in males. No higher incidence of neoplastic lesions occurred that could be attributed to methyl bromide exposure.

Conclusions

The major findings were: 1) degeneration and slight to moderate hyperplasia in the nasal olfactory epithelium that increased with dose, 2) damage to heart tissue (significant at the 90 ppm level), 3) esophageal hyperkeratosis at 90 ppm in males only at 29 months, and 4) forestomach lesions that were not statistically significant. No treatment-related gross or microscopic changes were observed in the brains or lungs of exposed animals.

Irritation of the nasal olfactory epithelium was characterized by degeneration and hyperplasia. This lesion increased in severity with concentration, ranging from very slight to moderate. Irritation also increased somewhat with time, even in controls, suggesting an age-related effect. A statistically significant increase was found between controls and the low-exposure group (3 ppm) at the end of the 29-month exposure period but not before (as with the higher exposure groups). The frequency of this lesion also increased with age in the controls from 12 through 24 and 29 months. All but one of the lesions in the 3-ppm group was described as slight or very slight. One moderate lesion of the nasal mucosa also was observed in a control animal at the 24-month sacrifice interval. The NOAEL for this lesion was 90 ppm after 12 months of exposure, 3 ppm after 24 months, and < 3 ppm after 29 months.

References

Reuzel, P.G.J., Dreef-Van der Meulen, H.C., Hollanders, V.M.H., Kuper, C.F., Feron, V.J. Van der Heijden, C.A. 1991. Chronic inhalation toxicity and carcinogenicity study of methyl bromide in Wistar rats. Food Chem. Toxicol., 29: 31-39

T.N.O., 1987. Chronic (29 month) inhalation toxicity and carcinogenic study of methyl bromide in rats, Report V86.469/221044.

Data Quality

The data quality from this study is considered high. The report included comprehensive documentation for method and results. The conducting laboratory is reputable. This study reaches Klimisch level 1.

General Remarks

This study was reliably conducted with exposure levels chosen so as to reveal the intrinsic toxicity of methyl bromide, and usually bracketing adverse effects so that NOAELs and LOAELs could be identified for each effect.

ROBUST SUMMARY – MeBr RS4 07

Study Type: **Chronic Toxicity/Carcinogenicity**

Title: A 24-Month Chronic Dietary Study of Methyl Bromide in Rats

Laboratory: WIL Research Laboratories, Inc. (Ashland, OH, USA)

Laboratory Study ID#: WIL-49014

Study Initiation Date: December 16, 1994
Study Completion Date: December 9, 1997 Report Date: December 9, 1997

Protocol Guideline 83-5 (FIFRA Guideline, Subdivision F: Protocol 83-5)

GLP Compliance

According to a statement that is part of the study report, signed by the WIL Study director, this study was conducted in accordance with Good Laboratory Practices (Part 160 of 40 CFR [EPA/FIFRA Good Laboratory Practice Regulations]).

Test Substance

Identity: Methyl Bromide (a.k.a., bromomethane – CAS# 74-83-9)
Purity: Two batches of 0.48% and 3.44% microencapsulated methyl bromide
Supplied as: Microencapsulated methyl bromide

Study Design

Dietary Methyl Bromide Concentration (ppm)	Description	Dose (mg/kg/day) Males	Dose (mg/kg/day) Females	No./Sex/Dose
0	Basal diet only	0	0	70*
0	Diet with empty microcapsules	0	0	70*
0.5	Diet w/microencapsulated methyl bromide	0.02	0.03	50
2.5	Diet w/microencapsulated methyl bromide	0.11	0.15	50
50	Diet w/microencapsulated methyl bromide	2.20	2.92	70*
250	Diet w/microencapsulated methyl bromide	11.10	15.12	70*

* The twenty lowest numbered subjects/sex (numbers assigned to subjects at random by computer) were allocated to the 12-month chronic toxicity portion of the study.

Test subjects (species, strain & sex): Rat (Crl:CD®(SD)BR), males and females.
Route of Administration: Diet containing microencapsulated methyl bromide.
# of exposure levels: Five (0, 0.5, 2.5, 50, & 250 ppm)
Control Groups: Feed-only (0 ppm methyl bromide).
Microcapsules in feed.
Exposure regimen: Seven days/week.

Exposure Duration:	12 months for chronic toxicity portion; 24 months for oncogenicity portion of study.
Post-exposure duration:	None.
# Animals/sex/dose:	See table above.
Terminal sacrifice:	Twelve months for chronic toxicity portion of study; 24 months for oncogenicity portion.
Interim sacrifice intervals:	12 months (chronic toxicity subjects).
Interim test intervals:	Hematology: 3, 6, 12, 18, & 24 months from 10 lowest numbered rats/sex from each group. Clinical chemistries: 6, 12, 18, & 24 months from 10 lowest numbered rats/sex from each group. Urinalysis: 3, 6, 12, 18, & 24 months from 10 lowest numbered rats/sex from each group.
Remarks:	The diets for the lower dose levels (0.5 and 2.5 ppm) were prepared using the 0.48% microencapsulated methyl bromide. The higher dose level diets were prepared using the 3.44% microencapsulated methyl bromide preparation.

Other Study Parameters

Age at receipt:	Approximately 4 weeks.
Acclimation period:	Three weeks.
Age at start of study:	Approximately 7 weeks.
Target survival at terminal sacrifice:	Not specified; time of 24 months was target.
Average weight at start of study:	Males: ~200 grams; Females: ~150 grams.
Assignment to groups:	Randomized weight basis (body weight stratification in a block design).
Housing:	Individually in wire mesh cages suspended above cage-board.
Light cycle:	12 hrs light; 12 hrs dark.
Temperature:	70 - 74 ^{o}F monitored and recorded once daily.
Humidity:	15.6 – 82.8% monitored and recorded once daily.
Food:	Meal form of chow. Diet No. 5002 (meal), (PMI Feed, Inc., St. Louis, MO).
Access to food:	Weeks 0 – 65: Males: 30 grams; Females: 23 grams. After 65 weeks: Males: 35 grams; Females: 30 grams.
Access to water:	*Ad libitum*.

Toxicity Endpoints Monitored

Clinical signs:	Once daily (detailed physical examination conducted once weekly).
Morbidity/mortality:	Twice daily.
Physical examinations:	Pretest and weekly including examination for palpable masses.
Ophthalmic exam:	Exams performed prior to treatment and at 51 weeks on all animals.
Body weights:	Pretest, weekly, and at termination.
Food consumption:	Measured daily and recorded weekly.
Hematology:	3, 6, 12, 18, & 24 months.
Clinical chemistries:	6, 12, 18, & 24 months.
Urinalyses:	3, 6, 12, 18, & 24 months.
Interim sacrifices:	12 months (this was actually the final sacrifice for the chronic toxicity portion of the study)
Organ weights:	Brain, kidneys, liver, ovaries, and testes.
Gross pathology:	Complete necropsy performed on all animals.

Histopathology: Tissues from all animals were preserved in formalin. These included: adrenal glands, aorta, bone (femur, sternebra), bone marrow (femur, sternum), brain (3 sections), eyes with optic nerve, gastrointestinal tract (esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum), heart, kidneys, liver, lungs lymph nodes, mammary gland, ovaries, pancreas, peripheral nerve (sciatic), pituitary, prostate, salivary gland (submaxillary), skeletal muscle, skin, spinal cord, (cervical, thoracic, lumbar), spleen, testes (w/epididymides), thymus, thyroid/parathyroid, trachea, urinary bladder, uterus (body, horns, cervix), and tissues with macroscopic lesions. Tissues were examined for damage from chronic toxicity and for neoplastic lesions. All of these tissues were examined microscopically from animals in both control groups and the high dose group. Microscopic examination of tissues from the remaining groups was limited to lungs, brain, eyes (with optic nerve), stomach, liver, kidneys, masses, and all gross lesions suspected of being treatment related.

Statistical Methods

Body weights:	Analysis of variance followed by Dunnett's test.
Organ weights:	Analysis of variance followed by Dunnett's test.
Food consumption data:	Analysis of variance followed by Dunnett's test.
Hematology data:	Analysis of variance followed by Dunnett's test.
Biochemistry data:	Analysis of variance followed by Dunnett's test.
Clinical pathology data:	Analysis of variance followed by Dunnett's test.
Survival:	RXC Chi-square test.
Palpable masses:	Fisher's Exact Test

Results

Clinical signs: No differences were noted between control and methyl bromide-treated animals regarding behavioral or clinical signs. Clinical signs NOAEL = 250 ppm or 11.10 mg/kg-d (males) and 15.12 mg/kg-d (females). No LOAEL established.

Morbidity/Mortality: No differences in survival was attributable to methyl bromide administration. Mortality NOAEL = 250 ppm or 11.10 mg/kg-d (males) and 15.12 mg/kg-d (females). No LOAEL established.

Palpable masses: No differences in the incidence of palpable masses was attributable to methyl bromide administration. NOAEL = 250 ppm or 11.10 mg/kg-d (males) and 15.12 mg/kg-d (females). No LOAEL established.

Body weights: Body weights and body weight gains of rats from the 250 ppm group were significantly decreased in both sexes compared to either control group. These decreases tended to disappear during the second year of study. Body weight NOAEL = 50 ppm or 2.20 mg/kg-d (males) and 2.92 mg/kg-d (females). LOAEL = 250 ppm.

Food consumption: Food consumption of rats from the 250 ppm group were significantly decreased in both sexes compared to either control group. These decreases tended to disappear during the second year of study. Food consumption NOAEL = 50 ppm or 2.20 mg/kg-d (males) and 2.92 mg/kg-d (females). LOAEL = 250 ppm.

Organ weights: Methyl bromide exposure produced no effect upon absolute or relative organ weights. A statistically significant difference in absolute kidney, liver, and testes weights was found in high dose males. However, when compared on a basis relative to total body weight, no difference was detected. Because of this, and because no accompanying microscopic changes were observed in these organs, organ weight changes were not considered to be the result of methyl bromide exposure. Organ weight NOAEL = 250 ppm or 11.10 mg/kg-d (males) and 15.12 mg/kg-d (females). No LOAEL established.

Ophthalmic Effects: Ophthalmic examinations revealed no effect from methyl bromide exposure. Body weight NOAEL = 250 ppm or 11.10 mg/kg-d (males) and 15.12 mg/kg-d (females). No LOAEL established.

Macroscopic Examinations: No lesions were found at necropsy from either the 12-month chronic toxicity subjects or the 24-month oncogenicity subjects that could be attributed to methyl bromide exposure. NOAEL = 250 ppm or 11.10 mg/kg-d (males) and 15.12 mg/kg-d (females). No LOAEL established.

Hematology: Other than spurious changes considered unrelated to exposure, no hematological parameters were affected by methyl bromide exposure. Hematology NOAEL = 250 ppm or 11.10 mg/kg-d (males) and 15.12 mg/kg-d (females). No LOAEL established.

Clinical chemistries: Other than spurious changes considered unrelated to exposure, no clinical chemistry parameters were affected by methyl bromide exposure. Clinical chemistry NOAEL = 250 ppm or 11.10 mg/kg-d (males) and 15.12 mg/kg-d (females). No LOAEL established.

Urinalyses: Other than spurious changes considered unrelated to exposure, no urinalysis parameters were affected by methyl bromide exposure. Urinalysis NOAEL = 250 ppm or 11.10 mg/kg-d (males) and 15.12 mg/kg-d (females). No LOAEL established.

Gross pathology: Examination at necropsy revealed no effect from methyl bromide exposure. NOAEL = 250 ppm or 11.10 mg/kg-d (males) and 15.12 mg/kg-d (females).

Histopathology: Although incidental non-neoplastic were found, none were considered related to methyl bromide exposure. No affect on tumor incidence was found. NOAEL = 250 ppm or 11.10 mg/kg-d (males) and 15.12 mg/kg-d (females).

Conclusions

No toxicologically significant effects from methyl bromide exposure were seen on clinical observations, body weight, body weight gain, food consumption, clinical pathology, urinalysis, ophthalmology, absolute or relative organ weights, or macroscopic or microscopic pathology, including tumor incidence. Based on the results of this study, the NOEL for methyl bromide when administered in the diet in microcapsules was 50 ppm or 2.20 mg/kg-d (males) and 2.92 mg/kg-d (females) and the LOAEL was 250 ppm or 11.10 mg/kg-d (males) and 15.12 mg/kg-d (females).

References

Mertens, J.J.W.M., 1997. A 24-month chronic dietary study of methyl bromide in rats. Unpublished report from WIL Research Laboratories, Project No. WIL-49014.

Data Quality

The data quality from this study is considered high. The report included comprehensive documentation for method and results. The conducting laboratory is reputable. This study reaches Klimisch level 1.

General Remarks

This study was reliably conducted with exposure levels chosen so as to reveal the intrinsic toxicity of methyl bromide, and usually bracketing adverse effects so that NOAELs and LOAELs could be identified for each effect.

ROBUST SUMMARY – MeBr RS4 08

Study Type: **Chronic Toxicity/Carcinogenicity**

Title: Toxicology and Carcinogenesis Studies of Methyl Bromide (CAS No. 74-83-9) in B6C3F1 Mice (Inhalation Studies)

Sponsoring Organization: U.S. National Toxicology Program (NTP)

Conducting Laboratory: Brookhaven National Laboratories
Laboratory Study ID#: Not specified.
NTP Report #: NTP TR 385 (NIH Publication No. 92-2840)
Report Date: March 1992.

Study Completion Date: Not specified. Last day of exposure: 15 September 1986.

Protocol Guideline

Not specified.

Remark: This robust summary was written from an NTP study report. As such, protocol guidelines were not specified. However, all NTP studies from this period were conducted using modern protocols.

GLP Compliance

FDA Good Laboratory Practices were followed (21 CFR Part 58). An independent quality assurance auditor evaluated the study data, including histopathology.

Test Substance

Identity: Methyl Bromide (a.k.a., bromomethane – CAS# 74-83-9).
Purity: 99.8% (report page 176) (purity remained stable over the course of the study). Purity was analyzed at the Midwest Research Institute analytical chemistry laboratory, Kansas City, MO.
Supplied as: Liquefied, compressed methyl bromide gas in five pressurized cylinders supplied by Matheson Gas Products, Joliet, IL. Lot No. E21-1012-00.

Study Design

Group	Target MeBr Conc. (ppm) (Nominal)	Actual MeBr Conc. (ppm) (range)*	Total No per Sex per Group	6 Mo. Sac. No per Sex per Group	15 Mo. Sac. No per Sex per Group	Final Sac. No per Sex per Group	Behavioral No per Sex per Group
Air-only Control	0	0	86	10	10	50	16
Low	10	±20% target	86	10	10	50	16
Mid	33	±10% target	86	10	10	50	16
High	100	±10% target	86	10†	10†	50†	16†

† Because of excessive mortality after 20 weeks in this group, these target numbers changed. See "Remark" below.

Remark: Excessive mortality occurred in mice exposed to 100 ppm methyl bromide after 20 weeks (27/86 males; 7/86 females). As a result, methyl bromide exposures were discontinued thereafter for this group and animals from this group were subsequently exposed to air for the remainder of the scheduled 24-month exposure period. This precluded the interim sacrifices of 100ppm males at 6 and 15 months as well as the females at 6 months. This left 70 male mice and 60 female (nominal) mice for the final sacrifice in the 100 ppm group. Finally, also because of the excessive mortality, males from the 100 ppm group did not undergo neurobehavioral testing subsequent to the 3-month interim test. All other mice, including females from the 100 ppm group, were tested at 3-month intervals as scheduled.

Test subjects (species, strain & sex):	Rat (Wistar; Cpb:WU strain), males and females.
Route of administration:	Inhalation, whole-body.
# Exposure levels (# of Groups):	Four (including an air-only control group).
Exposure level concentrations:	Nominal concentrations: 0, 3, 33, and 100 ppm. Actuals were within ±10%.
Control group(s):	Air-only (0 ppm).
Exposure duration:	Up to 29 months of age.
Exposure regimen:	6 hours/day, 5 days/week (no exposure on weekends).
Post-exposure duration:	None.

Animals/sex/concentration: 86/sex/group total; 50/sex/group for lifetime exposure duration; 10/sex/group for interim toxicity sacrifices (@ 6 and 15 mo); 16/sex/group for tri-monthly behavioral tests.

Interim sacrifice intervals:	6 and 15 months (10/sex/conc.).
Interim test intervals:	3-month intervals for behavioral test battery (16/sex/conc).
Terminal sacrifice:	After 24 months of exposure.

Other Study Parameters

Age at receipt:	Four weeks.
Acclimation period:	Two weeks.
Age at start of study:	Six weeks.
Inhalation chamber volume:	1.4 m^3.
Chamber airflow rate:	21 m^3/hr.
Chamber air changes:	15 changes per hour.
Chamber temperature:	Not specified.
Chamber atmosphere generation:	Computer controlled dilution with air of methyl bromide gas from cylinders.
Chamber atmosphere monitoring:	Each chamber sampled hourly for 10 minutes during exposure periods with a Miran 80 infrared spectrophotometer (at 3.327 microns).
Housing:	Suspended wire mesh cages in inhalation chambers.
Animal room temperature:	65 - 82 ^oF.
Animal room humidity:	11 – 85%.
Light cycle:	12 hrs light; 12 hrs dark.
Access to food and water:	Water: *ad libitum* at all times. Food (NIH-07): none during exposures - *ad libitum* during non-exposure periods.

Toxicity Endpoints Monitored

Clinical Signs:	Twice daily (once daily on weekends).
Morbidity/Mortality:	Twice daily (once daily on weekends).

Body Weights:	Weekly, for first three months, monthly for next 18 months, bi-weekly thereafter.
Hematology:	Month 6, 15 and at terminal sacrifice.
Clinical Chemistries:	None.
Urinalyses:	None.
Interim Sacrifices:	Months 6, 15, and 24 (terminal).
Organ Weights:	Brain, heart, right kidney, liver, lungs, spleen, right testis, and thymus.
Gross pathology:	Conducted on all animals not used in neurobehavioral phase.
Histopathology:	Tissues from all animals were preserved in formalin. Tissues were examined for damage from chronic toxicity and for neoplastic lesions. Tissues examined microscopically from the control and high exposure groups included: Adrenals, brain, bronchial lymph nodes, cecum, colon, costochondral junction, duodenum, epididymis/seminal vesicles/prostate/testes or ovaries/uterus, esophagus, femur including marrow, gall bladder, gross lesions and masses, heart, ileum, jejunum, kidneys, larynx, liver, lungs (with mainstem bronchi), mammary gland, mandibular lymph nodes, mediastinal lymph nodes, mesenteric lymph nodes, nasal cavity and turbinates, oral cavity, pancreas, parathyroid glands, pituitary gland, rectum, salivary glands, sciatic nerve, skin, spleen, spinal cord, sternebrae with marrow, vertebra, stomach, thymus, thyroid gland, trachea, and urinary bladder.
Neurobehavioral Tests:	Locomotor activity, exploratory behavior, startle response, grip strength, analgesia response, and foot splay.
Neurohistopathology:	At 20 weeks (100 ppm group), 6, 15, and 24 months, microscopic examinations were conducted of the brain, spinal cord and nerves.

Statistical Methods

Continuous variables:	Jonckheere's test for trends in significance of dose-response followed by nonparametric multiple comparison test of either Dunn or Shirley for comparing exposed to control groups.
Mortality incidence:	Probability of survival: Kaplan Meier product-limit procedure. Dose-Response Effect: Cox for difference between two groups and Tarone's life table test for dose-related trend.
Neoplastic incidence:	Ratio of animals found with lesion in a tissue to number examined or autopsied. Logistic regression for non-lethal tumors. Dinse and Lagakos for lethal tumors.

Results

Clinical signs: No differences were noted between control and methyl bromide-treated animals regarding behavioral or clinical signs. Clinical signs NOAEL = 100 ppm (males and females). No LOAEL established.

Morbidity/Mortality: Increased in 100 ppm group toward end of second year in both males and females. Increased mortality was associated with increased incidence of heart thrombi, which the study authors speculated might be causative. Mortality NOAEL = 33 ppm (males and females); Mortality LOAEL = 100 ppm (males and females).

Body weights: In the 100 ppm group, male and female body weights were slightly less than controls after four weeks of exposure. Differences were not always statistically significant in males but were

always so in females. Females exposed to 10 and 33 ppm showed weight loss from exposure days 309 to 337. Females from these two groups resumed weight gain thereafter. Males, which were housed in the same chambers at 10 and 33 ppm, did not show weight loss or decreased weight gain during the same period. Nor did females at 100 ppm exhibit these effects over this period. No reason was apparent that explained the weight loss in the 10 and 33 ppm females. Much earlier in the study (exposure week 5), a transient lower body weight (not frank weight loss) was found in the 33 ppm males that did not occur at the next weighing. Because of the inconsistencies, no treatment related effect was ascribed to the body weight effects in subjects exposed to 10 or 33 ppm. However, methyl bromide exposure did appear related to the effects found in the 100 ppm group. Body weight NOAEL = 33 ppm (males and females); Body weight LOAEL = 100 ppm (males and females).

Organ weights: At 53 weeks, average absolute kidney weights were significantly lower in 100 ppm males and 33 and 100 ppm females. Expressed relative to total body weight, kidney weights were significantly lower for 33 and 100 ppm males and these decreases exhibited a dose-response relationship. Brain weights were decreased compared to controls on an absolute but not relative basis in 100 ppm females at 53 and 104 weeks. While not statistically significant for males the same pattern held for absolute brain weights (also no difference relative to body weight). Relative but not absolute thyroid weights were decreased in females exposed to 33 ppm (not considered treatment related). Absolute organ weight NOAEL = 10 ppm (females) and 33 ppm (males). Relative organ weight NOAEL = 10 ppm (males) and 33 ppm (females).

Hematology: Random differences were found at week 13 and week 52 that reached statistical significance in some groups. Specifically, mean packed cell volume was slightly decreased in 100 ppm females at 13 weeks (but not 52 weeks and erythrocyte count was normal). Also, neutrophil counts were increased in the 10 ppm females at week 13, erythrocyte counts were decreased in 30 ppm males at 13 weeks. At week 52, white cell counts were higher in 10 and 100 ppm males when compared statistically to an unusually low control value but were within the normal range.

Because these changes 1) were all slight in magnitude, 2) did not co-occur at the two test intervals of 13 and 52 weeks, 3) did not show a coherent pattern with other parameters (i.e., decreased mean packed cell volume did not correlate with a decreased red blood cell count), 4) did not exhibit a concentration-response relationship, 5) were within normal ranges or 6) were significant because of an unusual control value, they were not considered treatment-related. Hematology NOAEL = 100 ppm (males and females). No LOAEL was found for hematology.

Histopathology: In the high exposure group only, an increased incidence over controls was observed of lesions in the central nervous system that was largely associated with early deaths in this group. Lesions of the cerebrum included focal, cortical neuronal necrosis occasionally with mild edema, congestion and gliosis. Cerebellar lesions included focal to diffuse nuclear pyknosis of cells from the internal granular layer without involvement of Purkinje cells. NOAEL = 33 ppm. LOAEL = 100 ppm.

A concentration-related increase in irritation of the nasal epithelia was found in methyl bromide exposed rats. Olfactory epithelial necrosis and metaplasia were statistically increased in both sexes from the high but not lower exposure groups. Necrosis consisted of focal cell death and loss of olfactory epithelium (nerve cells and sustentacular cells) “resulting in a sculptured outline of the mucosal surface.” This finding was associated solely with subjects dying within the first 20 weeks of exposure in the 100 ppm group. Metaplastic changes were associated more with surviving mice and was characterized by focal areas were olfactory epithelium was replaced by ciliated columnar epithelium, similar to respiratory epithelium. NOAEL = 33 ppm. LOAEL = 100 ppm.

Chronic cardiomyopathy occurred in a dose-response manner, although this finding was statistically significant only in the high exposure group when compared to controls. Myocardial degeneration was also seen but only in the high exposure group. Myocardial degeneration, seen in 30 of 33 male mice dying prematurely from exposure to 100 ppm methyl bromide, was characterized by "myofiber sarcoplasmic hyalinization and/or vacuolization and by variation in nuclear size accompanied by mild interstitial hypercellularity." Myocardial degeneration was similarly more highly associated in female mice dying early in the 100 ppm methyl bromide group. Chronic cardiomyopathy included "focal myofiber atrophy, fibrosis, and focal to diffuse mononuclear cell infiltrates" and was a lesion seen with far greater frequency in mice surviving exposure to the 24-month final sacrifice. Chronic cardiomyopathy NOAEL (none established). LOAEL = 10 ppm.

Sternal dysplasia was detected at a statistically increased incidence in the 100 ppm group and was observed in lower dose groups, suggesting a dose-reponse relationship. This non-lethal lesion may be described as "ventral to ventrolateral deviation of the manubrium with subluxatin of other sternebrae. Irregular proliferative protuberances composed of well-differentiated mature cartilage and bone were often present along the sternebral articular surfaces causing a 'lipping' effect." NOAEL = 10 ppm. LOAEL = 33 ppm.

No increase in tumor incidence was associated with methyl bromide exposure.

Histopathology (cardiomyopathy) NOAEL = none established; Histopathology LOAEL = 10 ppm (both sexes).

Neurobehavioral testing: Decrements were found in behavioral test parameters in the high exposure group males after 3 months exposure. Because of high mortality in this group, males were not tested thereafter for behavioral changes. Findings at 3 months in high-dose males and females included: lower activity, higher startle response, higher hot plate latency, and lower hindlimb grip strength. At six months in the female high exposure group (males not tested), activity scores were reduced and increased startle response had disappeared. The female 100 ppm subjects showed no differences from controls at 9 months or thereafter until final sacrifice. At the 24-month final sacrifice, females from the 100 ppm exposure group again exhibited lower activity and heightened startle response. No changes from controls were evident in the lower exposure groups regarding behavior. Behavioral NOAEL = 33 ppm (both sexes); Behavioral LOAEL = 100 ppm (both sexes).

Conclusions

Before scheduled sacrifices, significantly early mortality was observed in the 100 ppm high exposure group. After 20 weeks of exposure, 31% of males and 8% of females from this group had died. Consequently, exposures were discontinued and this group was observed for the remainder of the study. Due to the high mortality at 100 ppm, mice from this group were not evaluated at the 6-month interim sacrifice. Survival in groups exposed to 10 and 30 ppm methyl bromide was similar to controls. Mice from the 100 ppm exposure group showed concomitant reduced body weights and reduced absolute and relative thymus weights, as well as clinical signs that included tremors, abnormal posture, and limb paralysis. These clinical signs also were observed in 1 control, 5 low-exposure, and 9 mid-exposure animals, indicating a dose-response relationship. No changes in hematology related to methyl bromide exposure were noted in the study.

No adverse effects were found at the 6-month interim sacrifice in 10 and 33 ppm mice of either sex (10/sex/group were evaluated). At the 15-month interim sacrifice, females but not males from the high exposure group were evaluated and showed lesions of the brain, sternum, and heart. Specifically, cerebellar and cardiac degeneration (1 of 8 incidence for both lesions) and sternum dysplasia (2 of 8) occurred in the 100 ppm females. At 33 ppm, 1 of 10 males and 1 of 10 females

showed sternum dysplasia. Tumors were found at 15 months that occurred randomly in control and treated groups and showing no dose-response relationship.

At the final sacrifice following 24 months of exposure, a methyl bromide related increase in cerebellar and cerebral degeneration was found in high-exposure animals of both sexes. Cerebellar lesions were characterized by focal to diffuse nuclear pyknosis of the internal granular layer cells. Cerebral degeneration consisted of focal, cortical neuronal necrosis sometimes with mild edema, congestion and gliosis. The incidence of chronic cardiomyopathy and myocardial degeneration were increased in high exposure animals. Both the brain and heart effects occurred with higher frequency in subjects not surviving exposures. The occurrence of sternal dysplasia was statistically increased in both sexes from the high exposure group. This lesion was not associated with premature death. Olfactory epithelial necrosis and metaplasia were statistically increased in both sexes from the high exposure group.

Behavioral parameters affected by methyl bromide exposure included significant changes in startle response, grip strength, and activity levels in high exposure group animals of both sexes.

The overall Histological NOAEL was not established due to chronic cardiomyopathy effects. Cardiomyopathy was not significantly increased in the low or mid-exposure groups but a dose-response trend was evident suggesting biological significance for this finding in the lower exposure groups. Ignoring cardiomyopathy, a NOAEL of 33 ppm and a LOAEL of 100 ppm was evident for non-cancer effects from this study.

No carcinogenic effect was associated from methyl bromide exposure at any exposure level.

References

Eustis, S.L., Haber, S.B., Drew, R.T., Yang, R.S.H. 1988. Toxicology and pathology of methyl bromide in F344 rats and B6C3F1 mice following repeated inhalation exposure, Fund. Appl. Toxicol. 11:594-610.

NTP, 1992. Toxicology and Carcinogenesis Studies of Methyl Bromide (CAS No. 74-83-9) in B6C3F1 Mice (Inhalation Studies). National Toxicology Program, Research Triangle Park, N.C. March 1992. NTIS Publication Number: PB92189257.

Data Quality

The data quality from this study is considered high. The report included comprehensive documentation for method and results. The conducting laboratory is reputable. This study reaches Klimisch level 1.

General Remarks

This study was reliably conducted with exposure levels chosen so as to reveal the intrinsic toxicity of methyl bromide, and usually bracketing adverse effects so that NOAELs and LOAELs could be identified for each effect.

ROBUST SUMMARY – MeBr4 RS4 09

Study Type: Two-Generation Reproduction

Title: Two-Generation Reproduction Study Via Inhalation in Albino Rats Using Methyl Bromide (Final Report)

Laboratory: American Biogenics Corporation

Laboratory Study ID#: 450-1525
Study director(s): Dale A. Mayhew
Report author(s): Not specified.

Study Initiation Date: May 14, 1984
Study Completion Date: June 19, 1985 Report Date: February 19, 1986

Protocol Guideline

This study probably predates published protocols.

GLP Compliance

According to a statement that is part of the study report, signed by the American Biogenics Study Director, this study was conducted in accordance with EPA Good Laboratory Practices.

Test Substance

Identity: Methyl Bromide (a.k.a., bromomethane – CAS# 74-83-9)
Purity: Not specified (presumed on-file with the sponsor); but Great Lakes "Meth-O-Gas" is high purity (>99%).
Supplied as: "Meth-O-Gas" from Great Lakes Chemical in 200 lb. pressurized cylinders (Lots 3/84-702-2 through 3/84-702-4).

Study Design

First generation (FO) rats (25/sex/group) were exposed to methyl bromide gas over the period of at least one cycle of spermatogenesis for males and 2 estrous cycles for females prior to mating. For both sexes, this included daily (6 hr/d, 5 d/wk) exposures: 1) eight weeks prior to mating, 2) three weeks for mating to produce the F1a litter, 3) an intervening period of lactation of four weeks or more for this first litter, and 4) three weeks for mating for the subsequent F1b litter. During gestation for the first litter, exposure of females was temporarily suspended from day 21 of gestation until day 5 of lactation whereupon exposures were resumed. Females, but not pups, continued to be exposed until pups were weaned (day 28 of lactation) and continued thereafter for production of a second litter (F1b) were a similar suspension and resumption of exposure took place.

The F1 generation was selected from the F1b litter (25/sex/group). Pre-mating exposures began on days 28 - 33 of life and continued for 11 weeks until sexual maturity. Thereafter, a similar exposure and breeding regime (as for the F0 generation) was conducted to produce two litters (F2a and F2b). Parental generations were monitored for reproductive performance and offspring for toxicity and developmental effects (see toxicity endpoints below).

F0 Exposure Regime

Group	Target Methyl Bromide Concentration (ppm)	Actual Methyl Bromide Concentration (ppm)*	No./Sex/Concentration
1	0	0	25
2	3	3	25
3	30	30	25
4	90	90.2	25

* Standard deviations not reported.

F1 Exposure Regime

Group	Target Methyl Bromide Concentration (ppm)	Actual Methyl Bromide Concentration (ppm)*	No./Sex/Concentration
1	0	0	25
2	3	3	25
3	30	30.1	25
4	90	90	25

* Standard deviations not reported.

Test subjects (species, strain & sex):	Rat (albino Charle s River CD Sprague-Dawley), total of 114 males and 115 females.
Age at receipt:	Six weeks.
Acclimation period:	Approximately 3 weeks.
Age at start of study:	F0 generation: 62 days old. F1 generation: 29 to 33 days old.
Average weight at start of study:	F0 Males: ~315 grams; F0 Females: ~205 grams. F1 Males: ~70 grams; F1 Females: ~70 grams.
Assignment to groups:	Randomized weight basis.
Housing:	Individually in wire mesh cages suspended above cage-board.
Light cycle:	12 hrs light; 12 hrs dark.
Temperature:	Not reported in information available (see remark).
Humidity:	Not reported in information available (see remark).
Food:	Not reported in information available (see remark).
Route of administration:	Inhalation, whole-body.
# Exposure levels (# of Groups):	Four (including an air-only control group).
Exposure level concentrations:	Nominal concentrations: 0, 3, 30, and 90 ppm. Actual concentrations: see tables above.
Control group(s):	Air-only (0 ppm).
Exposure duration:	F0 males: ~26 weeks (133-134 exposures). F0 females: ~28 weeks (132-135 exposures). F1 males: ~28 weeks (139-140 exposures). F1 females: ~31 weeks (143-145 exposures).
Exposure regimen:	6 hours/day, 5 days/week (no exposure on weekends).
Post-exposure duration:	None.
# Animals/sex/concentration:	25/sex/conc. total.
Interim sacrifice intervals:	None.
Interim test intervals:	None.
Terminal sacrifice:	After production of the second litter for both parental generations.

Remark: Only the narrative portion of this very large report was available to the reviewer as well as an EPA Data Evaluation Record (DER), which reviewed this study. No summary tables or appendices were available. Information on airflow rates, chamber temperature and humidity, as well as more detail about methyl bromide analysis may have been contained in unavailable summary tables and appendices.

Exposure Chamber Parameters

Inhalation chamber volume:	8 m^3 glass and stainless steel.
Chamber air flow rate:	Not reported in information available.
Chamber temperature:	Not reported in information available.
Chamber atmosphere generation:	Computer controlled dilution with air of methyl bromide gas from cylinders.
Chamber atmosphere monitoring:	Sampled hourly during exposure periods with a gas chromatograph.
Housing:	Suspended wire mesh cages in inhalation chambers.
Light cycle:	12 hrs light; 12 hrs dark.
Access to food and water:	None during exposures.
Remark:	Only the narrative portion of this very large report was available to the reviewer. Information on airflow rates, chamber temperature and humidity, as well as more detail about methyl bromide analysis may have been contained in unavailable summary tables and appendices.

Toxicity Endpoints Monitored

Clinical signs:	Observed twice daily (physicalexamination conducted once weekly).
Morbidity/mortality:	Monitored twice daily.
Food consumption:	Measured daily and recorded weekly.
Body weights:	Pretest, weekly, and at termination.
Organ weights:	Brain (including brain stem), heart, kidneys, liver, ovaries, and testes.
Reproductive performance:	Percentage of pairs copulating, females pregnant, and females delivering were recorded, as were the number of viable litters, number of pups per litter (viable & non-viable).
Offspring:	Pup weight, viability, & sex ratio, morphological & behavioral characteristics.
Gross pathology:	Complete necropsy performed on all animals.
Histopathology:	Reproductive organs were examined microscopically from the control and high dose groups. These included: vagina, uterus, ovaries, testes with epididymides, seminal vesicles, and prostate gland. In addition, grossly observable abnormal lesions were also examined microscopically from all groups. The above and following tissues were retained from all groups: nasal turbinates, lung, thymus, sternum, mesenteric lymph nodes, and stomach/forestomach.

Statistical Methods

Mortality:	ANOVA followed by Tukey's or Scheffe's comparisons.
Body weights:	ANOVA followed by Tukey's or Scheffe's comparisons.
Absolute Organ weights:	ANOVA followed by Tukey's or Scheffe's comparisons.
Organ weight ratios:	Kruskal-Wallis analysis.
Food consumption data:	ANOVA followed by Tukey's or Scheffe's comparisons.
Reproductive data:	Chi-Square analysis.

Progeny anomaly data: Chi-Square analysis.

Results

Clinical signs: No differences were noted between control and methyl bromide-treated animals regarding behavioral or clinical signs. Clinical signs NOAEL = 90 ppm. No LOAEL established.

Morbidity/Mortality: Methyl bromide did not affect survival. Mortality NOAEL = 90 ppm. No LOAEL established.

Body weights: In the F0 generation, males from the 90 ppm group showed decreased body weights and body weight gains compared to the controls. No differences were noted in the F1 generation. No pup weight differences were noted in the F2a or F2b litters from the F1 generation. Body weight NOAEL = 30 ppm (males), 90 ppm (females). LOAEL = 90 ppm (males), none established for females.

Food consumption: Food consumption of female rats from F0 generation was significantly decreased in all treatment groups, but only during the first week of exposure. No other differences were noted for this or any other generation. Because the decrease occurred only during the first week of exposure of only one generation (not showing a dose-response), and because body weights were not affected, this may have represented a measurement error or a spurious result. Thus, the biological significance of this finding is in question. Food consumption NOAEL and LOAEL were not established.

Organ weights: Methyl bromide exposure produced some effects upon absolute or relative organ weights. A statistically significant decrease in absolute brain weight was detected in the 90 ppm males from the F0 parental generation and the males and females from the F1 parental generation. In females from the F1 generation, this effect upon brain weight was also apparent when expressed as a ratio of heart to brain weights. No accompanying microscopic changes were reported. Organ weight NOAEL = 30 ppm (males) and 90 ppm (females). No LOAEL established.

Reproductive performance: Reproductive performance was not altered in either parental generation by methyl bromide exposures as measured by fertility rates. NOAEL = 90 ppm. No LOAEL established.

Remark regarding reproductive performance: Dynamac Inc. performed an EPA "Data Evaluation Record" (DER) for this study in 1987. In this review, Dynamac regarded the "slightly decreased pregnancy rates in the F2b interval. . .to indicate reproductive toxicity at 30 and 90 ppm." Such a trend is apparent only in the F1b litters and is not statistically significant. The other three litters did not show any tendency for reduced pregnancy rates. The original report authors did not attribute reduced survival to methyl bromide exposure and this review concurs with those of the original authors.

Offspring: Viability and sex ratios were not affected by methyl bromide exposure. No grossly observable abnormalities occurred as a result of methyl bromide exposure. Body weights were decreased compared to controls in the mid and high exposure groups. This was reflected in some absolute but not relative organ weights.

Pup body weight NOAEL = 3 ppm. LOAEL = 30 ppm.
Pup survival, sex ratio NOAEL = 90 ppm. No LOAEL established.
Pup abnormality NOAEL = 90 ppm. No LOAEL established.

Remark regarding offspring effects: Dynamac Inc. performed an EPA "Data Evaluation Record" (DER) for this study in 1987. In this review, Dynamac concluded that pups from the 30 and 90 ppm groups had lower survivability as evidenced by lower calculated percent survival over the period of lactation. Such an effect, if real, was slight, did not reach statistical significance, and was apparent only in the F1b litter for the 90 ppm group and the F2a litter for both the 30 and 90 ppm groups. Most importantly, the F1a and F2b litters did not show a similar tendency for reduced survival. The original report authors did not attribute reduced survival to methyl bromide exposure and this review concurs with those of the original authors.

Gross pathology: Examination at necropsy revealed no effect from methyl bromide exposure. NOAEL = 90 ppm. No LOAEL established.

Histopathology: No histopathology was associated with methyl bromide exposure in any generation. NOAEL = 90 ppm. No LOAEL established.

Conclusions

Neither reproductive performance nor reproductive organs were affected in F0 or F1 parental generations in a two-generation reproductive study where rats were exposed by whole -body inhalation to 0, 3, 30, or 90 ppm methyl bromide for 6 hr/day, 5 days per week, for at least 8 weeks prior to mating and over the period for production of two litters. Body weights in males were significantly reduced compared to control in the 30 and 90 ppm groups. Brain weights were also decreased in F0 males from the high exposure group and F1 males and females from the high exposure group. No other toxicological parameters were altered in parental animals.

Pup body weights were reduced compared to controls in the 30 and 90 ppm groups in litters from the F1 parental generation. Absolute organ weights were also decreased but not relative organ weights (which may have reflected smaller body sizes). Other sporatic organ weights differences were found in offspring, which were not consistent among exposure levels, litters, or generations. Methyl bromide did not affect litter size, sex ratio, survival through lactation, or grossly observable abnormalities.

References

American Biogenics Corporation, 1986. Two-generation reproduction study in albino rats with methyl bromide - results of both generations (Study No. 4500-1525) (Unpublished final report).

Data Quality

The procedures for this assay are described adequately with sufficient documentation. The data quality from this study is considered adequate. This study is assigned Klimisch level 2 (reliable with restrictions).

General Remarks

This older study was conducted in accordance with modern protocols.

ROBUST SUMMARY – MeBr RS4 10

Study Type: Developmental Toxicity (Teratology) in Rabbits

Title: Teratologic Assessment of Butylene Oxide, Styrene Oxide, and Methyl Bromide.

Laboratory: Battelle, Pacific Northwest Laboratory

Laboratory Study ID#: None (Contract No. 210-78-0025)
Report Date: July 1981

Protocol Guideline

No specific protocol guideline specified. Study was conducted in 1981, prior to standardized protocols.

GLP Compliance

Not specified.

Test Substance

Identity: Methyl Bromide (a.k.a., bromomethane – CAS# 74-83-9).
Purity: 99.5% minimum purity.
Supplied as: Liquefied, compressed methyl bromide gas in pressurized cylinders, supplied by Matheson Gas Products).

Study Design

Group	Methyl Bromide Concentration (ppm) Target (Nominal)	Methyl Bromide Concentration (ppm) Actual (Measured)*	No/Dams/ Concentration
Negative Control	0	0	24
Low	20	19.3 ± 0.19	24
High	70	68.7 ± 2.18	24

* Plus or minus one standard deviation.

Test subjects (species, strain & sex): Rabbit (New Zealand White), virgin females.
Route of administration: Inhalation, whole-body.
Exposure levels (# of Groups): Three
Exposure level concentrations: Nominal concentrations: 0, 20 and 70 ppm. Actual conc (+-SD): see table above.
Control group(s): Air-only (0 ppm).
Exposure duration: Days 1 through 24 of gestation for days 0 and 20 ppm groups, exposures terminated at day 15 for 70 ppm group because of toxicity.
Exposure regimen: 7 hours/day, 7 days/week.
Post-exposure duration: None.
Dams/concentration: 25 dams/concentration.
Terminal sacrifice: Day 30 of gestation.

Other Study Parameters

Age at receipt:	4.5-6 months.
Acclimation period:	Approximately 3 weeks.
Age at start of study:	5 – 7 months.
Weight at start of study:	3.0 to 5.5 kg.
Inhalation chamber volume:	2.3 m^3.
Chamber air flow rate:	Not specified.
Air changes/hr:	Not specified.
Chamber temperature:	~22 ^oC.
Chamber atmosphere generation:	Dilution with air of methyl bromide gas from cylinders.
Chamber atmosphere monitoring:	Monitored 4 to 12 times per day from at least 2 points in the chambers. Methyl bromide concentrations were measured with a photo ionization detector (model/manufacturer not specified).
Housing:	Suspended wire mesh cages in inhalation chambers.
Light cycle:	12 hrs light; 12 hrs dark.
Access to food and water:	Food and water was available *ad libitum* during non-exposure periods. Food consisted of Wayne Rabbit Diet.

Toxicity Endpoints Monitored

Clinical Signs:	Daily
Morbidity/Mortality:	Daily
Body Weights:	Recorded prior to exposure and on days 1, 9, 16, 23 and 30 of gestation.
Organ Weights:	Liver, lungs, kidneys, and placenta.
Teratologic Evaluation:	Corpora lutea, implantations, pre-implantation loss, resorptions, and litter size were enumerated. In addition, the following were recorded: number of live and dead fetuses, number and positions of resorption sites, number of corpora lutea, sex and body weight of each fetus, crown-rump length, any external fetal alteration. Fetuses were sacrificed and dissected to determine visceral alterations by the method of Staples. Fetuses were preserved in alcohol, eviscerated, and cleared and stained with alizarin red-S for skeletal examination.

Statistical Methods

For continuous data, ANOVA was used to evaluate differences among groups. This was followed by either Dunnett's test for equal variance or unequal variance. Fisher's exact probability test was used in maternal and fetal data for tests of differences between proportions with Bonferronni's correction for multiple comparisons.

Results

Maternal clinical signs: Rabbits from the 70 ppm methyl bromide group began to show generalized signs of distress following one week of exposure. Signs progressed to convulsive movements, and later, to hind-limb paresis. Termination of exposure after 15 days resulted in improvement in some of the subjects. However, only one subject survived until the 30 days gestation period was

completed (see morbidity/mortality). No clinical signs of toxicity were noted in the lower exposure group. Clinical signs NOAEL = 20 ppm. LOAEL = 70 ppm.

Maternal morbidity/mortality: 24 of 25 dams did not survive exposure of 70 ppm methyl bromide. This occurred despite the fact that exposures were discontinued on day 15 of gestation (scheduled to continue through day 24 of gestation). No deaths occurred in the 20 ppm group. Maternal mortality NOAEL = 20 ppm. Maternal mortality LOAEL = 70 ppm.

Maternal body weights: In the 70 ppm group, severe body weight loss was observed after approximately 10 days of exposure severe clinical signs, weight loss and deaths began to occur. No significant differences were observed in dams from the 20 ppm group when compared to controls. Maternal body weight NOAEL = 20 ppm. Maternal body weight LOAEL = 70 ppm.

Maternal organ weights: No affect on organ weights was observed in the 20 ppm group other than a tendency for increased lung weights that was not statistically significant. In the 70 ppm group, where significant toxicity occurred, the liver weight of the single surviving dam was markedly less than the control mean. Maternal absolute and relative organ weight NOAEL = 20 ppm. LOAEL = 70 ppm.

Food consumption: In the 70 ppm group, food consumption was markedly reduced after approximately 10 days of exposure when severe clinical signs, weight loss and deaths began to occur. No significant differences were observed in dams from the 20 ppm group when compared to controls. Maternal food consumption NOAEL = 20 ppm. Maternal food consumption LOAEL = 70 ppm.

Maternal histopathology: No statistically significant increases of pathological lesions were noted in the organs examined when methyl bromide exposed dams were compared to controls. NOAEL = 20 ppm. LOAEL not established due to poor survival.

Maternal reproductive effects: No differences in pregnancy rates. NOAEL = 70. LOAEL not established.

Fetal Effects: No effect was found on fecundity, embryotoxicity, or fetal viability from methyl bromide exposure. Since only one dam survived in the 70 ppm group, an conclusion about the effect of methyl bromide exposure on these parameters cannot be reached for this high exposure group. No effect from methyl bromide exposure was noted on soft-tissue or skeletal anomalies. NOAEL = 20 ppm; LOAEL not established.

Conclusions

Severe toxicity was observed in the rabbit dams exposed to 70 ppm methyl bromide. Neurotoxicity and mortality began to occur after one week of exposure in dams from this group and was sufficiently severe so that exposure was terminated prematurely on day 15 (rather than scheduled day 24) of gestation. Only one dam survived until scheduled sacrifice on day 30 of gestation. Fetuses from this dam and dams from the 20 ppm group incurred no higher incidence of embryotoxicity, fetotoxicity, or birth defects. The NOAEL for maternal toxicity was 20 ppm and the LOAEL was 70 ppm. Because survival was insufficient to draw conclusions about embryotoxicity, fetotoxicity, or birth defects at the high exposure level, the NOAEL for these endpoints is 20 ppm and a LOAEL was not observed.

References

Hardin, B.D., Bond, G.P., Sikov, M.R., Andrew, F.D., Beliles, R.P., Niemeier, R.W., 1981. Testing of selected workplace chemicals for teratogenic potential. Scand. J. Work Environ. Health 7:66-75.

Sikov, M.R., Cannon, W.C., Carr, D.B., Miller, R.A., Montgomery, L.F., Phelps, D.W. 1981. Teratologic assessment of buthylene oxide, stryene oxide and methyl bromide (Contract-No. 210-78-0025). Cincinnati, Ohio, US Department of Health and Human Services, 84 pp.

Data Quality

GLP's were just being standardized and published in the early 1980s. No quality assurance statement was included in the report that refers to published GLPs and no standardized protocol guidelines were reported (none of the latter were established in the early 1980s). The study seems to have been carefully conducted but the report was not comprehensive in its documentation. One section in the report that discusses methodology for the three test substances in general indicates that 24 dams were used per exposure level but another section that discusses methyl bromide specifically indicates that the number was 25. This study would reach Klimisch level 2 "Reliable with Restrictions."

General Remarks

Report was confusing and difficult in that it lacked clarity and was overly complex. This was due in large part to the fact that results of three chemicals and two species were reported. Only two exposure levels were used and the high level caused such severe toxicity and mortality (only 1 of 25 dams survived a truncated exposure) so as to render it almost useless for evaluating developmental toxicity. The exposures were not conducted only over the period of organogenesis but were started earlier, on day 1 of gestation. No rationale for this was given. This is a non-standard approach for teratology studies but would tend to err on the side of detecting an effect, if such a potential existed.

ROBUST SUMMARY – MeBr RS4 11

Study Type: **Developmental Toxicity (Teratology) in Rats**

Title: Teratologic Assessment of Butylene Oxide, Styrene Oxide, and Methyl Bromide.

Laboratory: Battelle, Pacific Northwest Laboratory

Laboratory Study ID#: None (Contract No. 210-78-0025)
Report Date: July 1981

Protocol Guideline

No specific protocol guideline specified. Study was conducted in 1981, prior to standardized protocols.

GLP Compliance

Not specified.

Test Substance

Identity: Methyl Bromide (a.k.a., bromomethane – CAS# 74-83-9).
Purity: 99.5% minimum purity.
Supplied as: Liquefied, compressed methyl bromide gas in pressurized cylinders, supplied by Matheson Gas Products).

Study Design

Group	Methyl Bromide Concentration (ppm) Target (Nominal)[1]	Methyl Bromide Concentration (ppm) Actual (Measured)[1] [2]	No. Dams exposed/ Concentration
Air-Air	0 - 0	0 - 0	42
Air-Low	0 - 20	0 - 19.6 ± 0.9	40
Air-High	0 - 70	0 - 68.5 ± 1.7	40
Low-Air	20 - 0	19.6 ± 0.9 – 0	38
Low-Low	20 - 20	19.6 ± 0.9 - 19.6 ± 0.9	40
High-Air	70 - 0	68.5 ± 1.7 - 0	39
High-High	70 - 70	68.5 ± 1.7 - 68.5 ± 1.7	40

[1] The first concentration shows the pre-gestational period exposure concentration; the second shows the gestational period exposure concentration.
[2] Plus or minus one standard deviation.

Test subjects (species, strain & sex): Rat (Wistar), virgin females.
Route of administration: Inhalation, whole-body.
Exposure levels (# of Groups): Three
Exposure level concentrations: Nominal concentrations: 0, 20 and 70 ppm. Actual conc (+-SD): see table above.
Control group(s): Air-only (0 ppm).
Pre-gestational exposure: 3 weeks prior to insemination, 7 hours/day, 5 days/week.
Gestational Exposure: Days 1 through 19 of gestation.

Exposure regimen (pre-gestation):	6 hours/day, 5 days/week (3 weeks immediately prior to mating).
Exposure regimen (gestation):	6 hours/day, 7 days/week, (21 consecutive days).
Post-exposure duration:	None.
# Dams/concentration:	~ 40 dams/concentration (see table above for exact number).
Terminal sacrifice:	Day 21 of gestation.

Other Study Parameters

Age at receipt:	4-5 weeks.
Acclimation period:	Minimum of 10 days.
Age at start of study:	~ 6 weeks.
Weight at start of study:	~ 240 grams.
Inhalation chamber volume:	2.3 m^3.
Chamber air flow rate:	Not specified.
Air changes/hr:	Not specified.
Chamber temperature:	~22 ^oC.
Chamber atmosphere generation:	Dilution with air of methyl bromide gas from cylinders.
Chamber atmosphere monitoring:	Monitored 4 to 12 times per day from at least 2 points in the chambers. Methyl bromide concentrations were measured with a photo ionization detector (model/manufacturer not specified).
Housing:	Suspended wire mesh cages in inhalation chambers.
Light cycle:	12 hrs light; 12 hrs dark.
Humidity:	Not controlled.
Access to food and water:	Food and water was available *ad libitum* during non-exposure periods. Food consisted of Wayne "Lab-Blox" Diet.

Toxicity Endpoints Monitored

Clinical Signs:	Daily
Morbidity/Mortality:	Daily
Body Weights:	Recorded prior to exposure and on days 1, 9, 16, 23 and 30 of gestation.
Organ Weights:	Liver, lungs, kidneys, and placenta.
Food consumption:	Two days prior to exposures; 3X weekly during the three week pre-gestational period; 2 day intervals during gestational exposure.
Teratologic Evaluation:	Corpora lutea, implantations, pre-implantation loss, resorptions, and litter size were enumerated. In addition, the following were recorded: number of live and dead fetuses, number and positions of resorption sites, number of corpora lutea, sex and body weight of each fetus, crown-rump length, any external fetal alteration. Fetuses were sacrificed and dissected to determine visceral alterations by the method of Staples. Fetuses were preserved in alcohol, eviscerated, and cleared and stained with alizarin red-S for skeletal examination.

Statistical Methods

For continuous data, ANOVA was used to evaluate differences among groups. This was followed by either Dunnett's test for equal variance or unequal variance. Fisher's exact probability test was used in maternal and fetal data for tests of differences between proportions with Bonferronni's correction for multiple comparisons.

Results

Maternal clinical signs: No clinical signs were reported indicating toxicity from methyl bromide exposure at either exposure level. Clinical signs NOAEL = 70 ppm. No LOAEL established.

Maternal morbidity/mortality: No deaths occurred that were related to methyl bromide exposure. Two dams died on the first night of breeding that was attributed to fighting as a result of the unfamiliar multiple -subject per cage housing. Mortality/morbidity NOAEL = 70 ppm. LOAEL not established.

Maternal body weights: In the 70 ppm (high-high) group, slight, but statistically significant body weight differences were observed compared to controls after during the gestational period exposures. This difference was not statistically significant by the end of the gestational exposure period. In addition, in the 70 ppm (air-high) group, a statistically significant lower body weight was observed compared to controls after the second week of gestational period exposure. This was the only time period where a statistical difference was observed for the 70 ppm (air-high) group. No weight differences were noted for the 20 ppm groups compared to controls. Maternal body weight NOAEL = 20 ppm. Maternal body weight LOAEL = 70 ppm.

Maternal organ weights: No affect on organ weights was observed at either exposure level with either exposure regimen (pre-gestational exposure or no pre-gestational exposure). Maternal absolute and relative organ weight NOAEL = 70 ppm. No LOAEL established.

Food consumption: No affect was noted from methyl bromide exposure on food consumption. Maternal food consumption NOAEL = 70 ppm. No LOAEL established.

Maternal histopathology: Four of 5 instances of hydronephrosis occurred in the three groups that had a 70 ppm exposure during one of the two exposure periods (i.e., 1 in the air-high group, 1 in the high-air group, and 2 in the high-high group). These instances were not statistically elevated over controls although a pooled analysis was not conducted. NOAEL = 20 ppm. LOAEL = 70 ppm.

Maternal reproductive effects: No differences were noted in pregnancy rates when methyl bromide exposed dams were compared to controls. NOAEL = 70. LOAEL not established.

Fetal Effects: No effect was found on fecundity, embryotoxicity, or fetal viability from methyl bromide exposure. No effect from methyl bromide exposure was noted on soft-tissue or skeletal anomalies. NOAEL = 70 ppm; LOAEL not established.

Conclusions

No significant maternal toxicity was noted other than a transient lower body weight compared to controls. Fetuses exposed to either 20 or 70 ppm methyl bromide incurred no higher incidence of embryotoxicity, fetotoxicity, or birth defects than air controls. A three-week pre-gestational exposure, conducted with a subset of dams, did not influence the outcome of the study. The NOAEL for maternal toxicity was 20 ppm (body weight) and the LOAEL was 70 ppm. The NOAEL for fetal effects was 70 ppm and no LOAEL was established.

References

Hardin, B.D., Bond, G.P., Sikov, M.R., Andrew, F.D., Beliles, R.P., Niemeier, R.W., 1981. Testing of selected workplace chemicals for teratogenic potential. Scand. J. Work Environ. Health 7:66-75.

Sikov, M.R., Cannon, W.C., Carr, D.B., Miller, R.A., Montgomery, L.F., Phelps, D.W. 1981. Teratologic assessment of buthylene oxide, stryene oxide and methyl bromide (Contract-No. 210-78-0025). Cincinnati, Ohio, US Department of Health and Human Services, 84 pp.

Data Quality

GLP's were just being standardized and published in the early 1980s. No quality assurance statement was included in the report that refers to published GLPs and no standardized protocol guidelines were reported (none of the latter were established in the early 1980s). The study seems to have been carefully conducted but the report was not comprehensive in its documentation. Only two exposure levels were evaluated. The number of dams per exposure level in various tables changes from table to table. For example, for mortality results (Table 2), the number of dams exposed is 39 for the "high-air" group yet the table reporting results of sperm positive rats (Table 17) is 37 for this same group and for Table 18 reporting statistics for pups, the number of dams exposed (evaluated?) is 36. No clear explanation for these differences is given. This study would reach Klimisch level 2 "Reliable with Restrictions."

General Remarks

Report was confusing and difficult in that it lacked clarity and was overly complex. In large part, this was due to the fact that results of three chemicals and two species were reported in this single report. The exposures were not conducted only over the period of organogenesis but were started earlier, on day 1 of gestation. Moreover, an unusual pre-gestational exposure was included that is not typically part of a developmental toxicity study. No rationale for this study design was given. While non-standard for such studies, these features would tend to err on the side of detecting an effect, if such a potential existed.

ROBUST SUMMARY – MeBr RS4 12

Study Type: Developmental Toxicity (Teratology)

Title: Methyl Bromide Inhalation Teratology Study in New Zealand White Rabbits.

Laboratory: Toxicology Research Laboratory, Dow Chemical Company
Laboratory Study ID#: K-000681-033
Study Initiation Date: August 8, 1989 Study Completion Date: June 18, 1990
Report Date: June 18, 1990

Protocol Guideline

No specific protocol guideline specified.

GLP Compliance

According to a signed statement that is part of the study report, signed by the manager of the Laboratory Quality Assurance Unit, this study was conducted in accordance with Good Laboratory Practices published by the US FDA (GLP Procedures for Non-clinical studies, 1988), the US EPA (FIFRA GLP Standards, 1989), the Japanese Ministry of Agriculture, Forests, and Fisheries (1984), and the Organization for European Cooperation and Development (1982).

Test Substance

Identity: Methyl Bromide (a.k.a., bromomethane – CAS# 74-83-9).
Purity: 99.6% (0.127% air, 0.101% oxybismethane, 0089% water, 0.054% methyl chloride).
Supplied as: Liquefied, compressed methyl bromide gas in pressurized cylinders; Lot No. 041889, supplied by Hyyield-Bromide, Rocky Point, NC).

Study Design

Part I

Group	Methyl Bromide Concentration (ppm) Target (Nominal)	Methyl Bromide Concentration (ppm) Actual (Measured)*	No/Dams/ Concentration
Negative Control	0	0	26
Low	20	20.4 ± 0.9	26
Mid	40	40.0 ± 1.4	26
High	80	79.8 ± 2.5	26

* Plus or minus one standard deviation.

Part II

Group	Methyl Bromide Concentration (ppm) Target (Nominal)	Methyl Bromide Concentration (ppm) Actual (Measured)*	No/Dams/ Concentration
Negative Control	0	0	17
Naïve Control (Buck 73)	No inhalation chamber exposure	No inhalation chamber exposure	16
High	80	79.8 ± 2.5	17

* Plus or minus one standard deviation.

Part 2 was undertaken to determine whether results from Part 1 were reproducible and to determine whether some fetal alterations (lack of gall bladder) might be attributable to a single buck (No. 73).

Test subjects (species, strain & sex):	Rabbit (New Zealand White), males and females.
Route of administration:	Inhalation, whole-body.
# Exposure levels (# of Groups):	Part I: Four (including an air-only control group). Part II: Three (including 2 air-only controls)
Exposure level concentrations:	Part I: Nominal concentrations: 0, 20, 40, and 80 ppm. Actual conc (+-SD): See table above. Part II: Nominal concentrations were 0 & 80 ppm. See table above for actual concentrations +-SD.
Control group(s):	Air-only (0 ppm). Also a naïve control for Part 2
Exposure duration:	Days 7 through 19 of gestation.
Exposure regimen:	6 hours/day, 7 days/week.
Post-exposure duration:	None.
# Dams/concentration:	Part 1: 26 dams/concentration; Part 2: 17 dams/concentration
Terminal sacrifice:	Day 28 of gestation.

Other Study Parameters

Age at receipt:	Not specified.
Acclimation period:	Not specified.
Age at start of study:	Not specified.
Weight at start of study:	3.5 to 4.5 kg.
Inhalation chamber volume:	4.1 m^3.
Chamber air flow rate:	800 liters/minute (48 m^3/hr).
Air changes/hr:	11.7
Chamber temperature:	~22 ^oC.
Chamber atmosphere generation:	Computer controlled dilution with air of methyl bromide gas from cylinders.
Chamber atmosphere monitoring:	Sampled 14 times/exposure periods with a Varian 1400 gas chromatograph equipped with a flame ionization detector (calibrated monthly).
Housing:	Suspended wire mesh cages in inhalation chambers.
Light cycle:	12 hrs light; 12 hrs dark.
Access to food and water:	Food access was restricted to 8 oz/day (none during exposures) of Certified Rabbit Chow No. 5322 (RPI, St Louis); access to water was *ad libitum* during non-exposure periods.

Toxicity Endpoints Monitored

Clinical Signs:	Daily
Morbidity/Mortality:	Daily
Body Weights:	Weekly (every 3-4 days during gestational period)
Organ Weights:	Brain, kidneys, liver, and lungs (Part 1 only); and gravid uterus Parts 1 & 2).
Gross pathology:	Corpora lutea, implantations, Pre-implantation loss, resorptions, and litter size were enumerated. In addition, the following were recorded: number and position of fetuses in utero, number of live and dead fetuses, number and positions of resorption sites, number of corpera lutea, sex and body weight of each fetus, any external fetal alteration. Fetuses were sacrificed and dissected to determine visceral alterations by the method of Staples. Fetuses were preserved in alcohol, eviscerated, and cleared and stained with alizarin red-S for skeletal examination.

Statistical Methods

For maternal body weights, body weight gain, fetal body weights, gravid uterine weights and absolute and relative organ weights, Bartett's test for equality of variance was applied. Based upon the outcome, a parametric or nonparametric ANOVA was performed. If ANOVA "F" statistic was significant for homogeneous variances, Dunnett's test was applied. If significant for nonhomogeneous variances, a Wilcoxon Rank Sum test was applied with Bonferroni's correction to determine statistical significance between an individual group and its control.

For pre-implantation loss, resorptions, and fetal alterations among litters and the fetal population, data were analyzed using the Wilcoxon test with bonferroni's correction. Corpora lutea, implants, and litter sizes were subjected to a nonparametric ANOVA followed by the Wilcoxon Rank-Sum test with bonferroni's correction. Pregnancy rates were evaluated with the Fisher exact probability test.

Results

Part 1

Maternal clinical signs: One dam from the high exposure group delivered early on day 27 of gestation. This animal showed signs of maternal toxicity including decreased body fat and ingesta, decreased feces, lethargy, ataxia, perineal soiling, rightk-sided head tilt, lateral recumbency and severe weight loss (464 grams). All other dams survived exposures. Other dams from the high exposure (80 ppm) group exhibited decreased feces (not eating), lethargy, right-sided head tilt, ataxia, and lateral recumbency. Most of these signs occurred in three rabbits from the high exposure group and were consistent with an earlier probe study at 140 ppm wherein these same signs correlated with morphological changes within the nervous system including multifocal areas of inflammation of the meninges and bilateral symmetrical necrosis or spongiosis in the midbrain. No clinical signs were noted in the lower exposure groups. Clinical signs NOAEL = 40 ppm (males and females). LOAEL = 80 ppm.

Maternal morbidity/mortality: No deaths in the dams occurred as a result of methyl bromide exposure. Maternal mortality NOAEL = 80 ppm. Maternal mortality LOAEL not established.

Maternal body weights: In the 80 ppm group, maternal body weights were less than controls after four weeks of exposure. Two of the 26 dams were severely affected with weight losses of 464 and 604 grams. No weight losses or decreased weight gains were reported for the 20 and 40 ppm group dams. Maternal body weight NOAEL = 40 ppm. Maternal body weight LOAEL = 80 ppm.

Maternal organ weights: No affect on organ weights was observed at any exposure level. Maternal absolute and relative organ weight NOAEL = 80 ppm. No LOAEL established.

Fetal Effects: No increased malformations were found in fetuses from the 20 or 40 ppm exposure groups. However, fetuses from dams exposed to 80 ppm exhibited a high incidence of malformations (14.5% vs. 2.1% in controls). Of 23 malformations, 18 were either missing gall bladders or missing caudal lobe of the lung (i.e., agenesis of these structures). Fetuses from this group also had lower body weights. The incidence of missing gall bladder was statistically significant when compared to controls. The incidence in the control group was unusually high and was correlated with a single sire that also had a missing gall bladder. The laboratory historical control showed no instance of this anomaly in 3597 fetuses (from 939 litter). The incidence of a missing caudal lobe of the lung also was unusually high in the control group. An increased incidence of fused sternebrae was also observed in fetuses from the 80 ppm group that the authors concluded may be related to maternal toxicity. NOAEL = 40 ppm; LOAEL = 80 ppm.

Part 2

Maternal clinical signs: Three dams exhibited decreased feces and one showed perineal soiling. The lethargy, right-sided headd tilt, ataxia, and lateral recumbency observed in Part 1 were not seen in Part 2.

Maternal morbidity/mortality: One dam died on day 19 of gestation. This animal had edematous lungs and death was considered treatment related.

Maternal Body weights: No significant differences in absolute body weights were observed then the 80 ppm group was compared either to chamber or naïve controls. Body weight gain was lower in treated dams than chamber controls that sporadically reached statistical significance over the course of treatment.

Fetal Effects: The increased incidence of malformations found in Part 1 was not reproduced in Part 2. No statistically significant increases in malformations were found when the 80 ppm group was compared to either control. The incidence of missing gall bladder, although not statistically significant, was elevated in the 80 ppm group (4.3%) compared to chamber controls (0.9%) or naïve controls (0%). The sire for the naïve controls, which had a missing gall bladder, did not appear to genetically predispose its offspring to this anomaly. The incidence of missing caudal lobe of the lung was similar between fetuses from the 80 ppm group and chamber controls. However, both groups had a high incidence that was elevated above the naïve controls as well as the chamber controls and 80 ppm subjects from Part 1. No other anomalies were detected.

Conclusions

In part A, dams from the high methyl bromide exposure group (80 ppm) exhibited moderate to severe toxicity. In this group, clinical signs of maternal toxicity included lethargy, right-sided head tilt, ataxia, and lateral recumbency. In addition, body weights and body weight gains were

decreased. Fetuses from the 80 ppm group showed decreased weights and an increased incidence of fused sternebrae, which the authors concluded was related to maternal toxicity. Fetuses also had a higher incidence of missing gall bladder and missing caudal lobe of the lung. This finding was associated with a sire that also had a missing gall bladder. Consequently, a second phase of study (Part 2) was conducted wherein dams were exposed to 80 ppm and compared to both a chamber and naïve control. The same male that had the missing gall bladder sired all the naïve control fetuses. Part 2 results did not show the same severity of maternal toxicity. Fetuses did not exhibit significantly elevated incidences of missing gall bladder or lung lobe, although the rates were elevated and higher than the naïve control, which showed no agenesis. This indicated that the genetic anomaly in the sire did not contribute to the elevated incidences in Parts 1 and 2 although a genetic predisposition, triggered by stress or toxicity, cannot be ruled out.

According to the report, which referenced Palmer (1968), the occurrence of a missing gall bladder or a missing caudal lobe of the lung is considered a minor variation rather than a frank birth defect. Fused sternebrae are associated with maternal toxicity (Khera, 1985). Thus, the changes found in fetuses from dams exposed to 80 ppm methyl bromide may be characterized as anomalies rather than frank birth defects and are likely the result of stress from toxicity. The NOAEL for maternal and developmental toxicity is 40 ppm and the LOAEL is 80 ppm.

References

Breslin, W.J., Zablotny, C.L., Bradley, G.J., Nitschke, K.D., Lomax, L.G., 1990a. Methyl bromide inhalation teratology probe study in New Zealand white rabbits. Midland, Michigan, The Dow Chemical Company (Unpublished final report).

Breslin, W.J., Zablotny, C.L., Bradley, G.F., Lomax, L.G., 1990b. Methyl bromide inhalation teratology study in New Zealand white rabbits. Midland, Michigan, The Dow Chemical Company (Unpublished final report).

Khera, K.S., 1985. Maternal toxicity: A possible etiological factor in embryo-fetal deaths and fetal malformations of rodent-rabbit species. Teratology 31:129-153.

Palmer, A.K., 1968. Spontaneous malformations of the New Zealand White Rabbit: The background to safety evaluation tests. Lab. Anim. 2:195-206.