ETHYLENE DIBROMIDE (EDB)

"ROBUST SUMMARIES"
For
HUMAN HEALTH EFFECTS TESTS

PREPARED FOR
THE GREAT LAKES CHEMICAL COMPANY

SUBMITTED BY:
HEALTH & ENVIRONMENTAL HORIZONS, LTD

DATE: December 4, 2001

Put NOAELs LOAELs in obvious place like results.

ETHYLENE DIBROMIDE: TOXICOLOGY SUMMARY

ACUTE TOXICITY:

ORAL Toxicity: The acute oral toxicity (LD50) was examined in rats, mice, rabbits and guinea pigs. The LD50s were 0.117-146 grams/kg (rat), 0.42 grams/kg (mice), 0.055 grams/kg (rabbit) and 0.110 grams/kg (g. pig). There were no adverse effects on body weight gain in any species. In a separate acute oral study in rats the LD50 for both sexes was 0.14 grams/kg.

INHALATION Toxicity: Rats and guinea pigs were examined in an acute inhalation stud, and the LC50s were 300 ppm (2.3 mg/L) in both species following a 3 hour exposure. The LC50 after 1 hour exposure was 700 ppm (5.39 mg/L).

DERMAL Toxicity: Acute dermal toxicity in rabbits was produced mortality in 2/5 at 0.3 grams/kg and 4/5 at 0.65 grams/kg, generally within 4 days. An LD50 was not calculated in this study. EDB produced moderate to severe erythema, edema and necrosis. There was also CNS depression and hypothermia at high doses (0.65-1.1 grams/kg).

IRRITATION: An eye irritation study conducted in rabbits produced pain, conjunctival irritation, and corneal necrosis that cleared in 48 hours.

REPEATED DOSE TESTING:

Ethylene dibromide was evaluated in repeat dose inhalation studies with varying lengths of exposure. In one study, rats, rabbits, guinea pigs and monkeys were examined for 9 to 220 days, with exposures lasting 7 hours per day. Total exposures ranged from 3 to 156, and concentrations varied from 25 to 100 ppm. Generally, 100 ppm for short duration produced mortality, weight loss, and adverse effects in the liver and lungs of rats and rabbits. There was mortality in rats exposed to 50 ppm for 91 days (10/20), as well as pneumonic consolidation in the lungs of survivors. The guinea pigs, rabbits and monkeys exposed to 50 ppm for 70-84 days showed some body weight depression, nervousness, and fatty degeneration of the liver. All species exposed to 25 ppm for 205-220 days showed no adverse effects.

In a separate 13 week inhalation study conducted in rats, animals were exposed to 3, 10, or 40 ppm 6hrs/day 5 days per week. Relative liver weights were increased at 10 and 40 ppm, with some pale livers in females at 40 ppm. Histopathological examination revealed primarily adverse effects of the lung (i.e. squamous metaplasia and hyperplasia of respiratory epithelium) and liver (fatty). These same parameters were examined in a recovery group and all adaverse effects had reversed except for focal epithelial hyperplasia in 1/10 females. An NOAEL of 3 ppm was demonstrated.

The carcinogenic potential of EDB was examined in mice and rats during 2 year inhalation exposures of 10 and 40 ppm, 6 hrs/day 5 days/week. Increased mortality was observed for high dose rats and mice of both sexes. EDB was oncogenic in both sexes of both species at all dose levels. Tumors observed were primarily adenomas/carcinomas in nasal passage and alveolar/bronchiolar adenomas/carcinomas.

In another carcnogenicity, rats were exposed to 20 ppm of EDB alone or with disulfiram (which was administered in the diet). Exposures were 7 hrs/day 5 days/week for 18 months. Mortality was increased in EDB (58% in males, 83% in females by 12 months) and EDB plus disulfiram (77-90% at 18 months) test groups. Body weight was significantly decreased, with effects in the EDB plus disulfiram groups more pronounced. Gross necropsy revealed hemosiderosis in the spleens and testicular atrophy in EDB/disulfiram test group. Tumor incidences were increased in the EDB groups above control and control/disulfiram test groups.

MUTAGENICITY ASSAYS:

Gene Mutation: EDB was examined for gene mutations in an in vitro mammalian cell assay using human lymphoblastoid cell lines. EDB was mutagenic at 5 ug/ml in AHH-1 cells and 20 ug/ml in TK-6 cells. Dose levels tested were 1-100 ug/ml.

EDB was examined along several other pesticides in an Ames Salmonella typhimurium assay using Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538, with and without activation. EDB was positive in TA98, TA100 and TA1535, as well as E. coli WP2 her. ~~Negative results were obtained in Typhimurium strains w/frame shift mutations (1537+1538)~~ Lithus also been concluded that EDB is transformed to a more reactive ~~thiiranium~~ ion by glutathione activation.

Chromosomal Aberration: Mice were injected with a single i.p. dose of 100 mg EDB/kg body weight. It did not affect mating performance, the number of implants, live embryos, or early and late embryonic deaths. EDB was not mutagenic in this dominant lethal assay. In a separate reproduction study in rats, dominant lethal effects were examined at 20-80 ppm via inhalation and no dominant lethal effects were observed.

REPRODUCTIVE TOXICITY STUDIES:

EDB was examined for reproductive effects in rats exposed to 19-89 ppm via inhalation, 7 hrs/day 5 days/week for 10 weeks (males) or 3 weeks (females). After exposure males were mated with females. EDB produced adverse effects in males at 89 ppm demonstrated by reduced testicular weights and serum testosterone levels, atrophy of reproductive organs and failed to impregnate females. Females had abnormal estrus cycles at 80 ppm.

Male and female rabbits exposed orally to 15-45 mg EDB/kg displayed mortality and other adverse effects at 45 mg/kg. Male sperm was analyzed and 3/13 of the parameters measured were adversely affected: motion, ALH and percentage of motile sperm. These effects were observed at doses which caused 30% mortality and liver damage in 43% of the survivors. Male fertility was not affected. No increase in anomalies was observed. There was liver and bile duct damage at 45 mg/kg. The effect on sperm were indicated to be at the level of the epididymis.

DEVELOPMENTAL TOXICITY STUDIES:

Developmental effects were examined in rats and mice via inhalation. Doses were 20-80 ppm administered during gestation for 23 hrs/day for 10 days. There were no adverse effects in rats and no abnormalities or anomalies. Mice displayed adverse effects on maternal welfare such as decreased body weight, feed consumption and survival (no mice alive in the 80 ppm group). The number of skeletal anomalies increased at 20 and 38 ppm, observed as incomplete ossifications to unossified skeletal tissue.

ROBUST SUMMARIES:

A. ACUTE TOXICITY:

1. REPORT NUMBER: AO-1

STUDY TYPE: Acute Oral Toxicity to Rats, Mice, Rabbits and Guinea Pigs

TEST MATERIAL: Ethylene dibromide; purity 99%; colorless liquid; specific gravity 2.17; boiling point 131.6 degrees C; solubility: water 0.26 gm/100 gm; soluble in alcohol; freely miscible in ether; melting point minimum 8.8 degrees C.

TESTING FACILITY: Biochemical Research Department, DOW Chemical Co.

STUDY NUMBER(S): Published report.

SPONSOR: Dow Chemical Company.

REPORT ISSUED or STUDY COMPLETION DATE: 1952; A.M.A. Archives of Industrial Hygiene and Occupational Medicine, Vol. 6.

TITLE OF REPORT: Toxicity of Ethylene Dibromide Determined in Experimental Animals

AUTHORS: V.K. Rowe, H.C. Spencer, D.D. McCollister, R.L. Hollingsworth and E.M. Adams.

RECOGNIZED METHOD, i.e. OECD: Pre-dates OECD and US EPA Test Guidelines.

GLP: Study was pre-GLP.

SPECIES/SEX: Rat, guinea pigs and rabbits were mature adults from Dow Chemical stock colonies; mice were young adults from Carworth Farms. Sexes tested as follows: Rats (m/f), mice (f), rabbits (f) and guinea pigs (m/f).

DOSE LEVEL(S) and NUMBER OF DOSES: Oral doses were given via gastric intubation. The number of doses was not reported but indicated it followed the Litchfield-Wilcoxon simplified method, which generally employs groups of 6-10 animals in each group; doses were spaced in geometric progression based upon results achieved with the previous dose. Aliquots of a 10% solution of EDB in olive oil were emulsified in 2 ml of a 5-10% acacia solution for rats, rabbits and guinea pigs. Aliquots of 1% solution in olive oil were used for mice.

NUMBER OF ANIMALS/DOSE: A total of 60 male rats, 40 female rats, 20 female mice, 55 female rabbits and 40 guinea pigs (mixed sex) were used.

MEASURED ENDPOINT/INDEX (i.e. LD50, symptoms): Lethality in the species tested were determined as follows: LD50s (rat/m) = 0.146 grams/kg; (rat/f) = 0.117 grams/kg; (mice/f) = 0.420 grams/kg; (rabbits/f) = 0.055 grams/kg; and (guinea pigs/mixed) = 0.110 grams/kg .

STUDY METHOD: Groups of white rats, rabbits, mice and guinea pigs were administered single oral doses via gastric intubation according to their individual body weight. All surviving animals were observed until they had recovered fully from any loss of weight and were gaining normally. This period was typically 2 weeks.

RESULTS/OBSERVATIONS: LD50s calculated according to the method of Litchfield-Wilcoxon, and are reported above. The rabbits appear to be the most sensitive and mice the least sensitive species tested.

take out all data quality

DATA QUALITY: Study was performed using adequate numbers of animals and fully characterized test material. It was conducted in accordance with contemporary scientific procedures for analyzing the acute oral lethality of a test material in experimental animals. Although full description of the test procedures was not reported, the results were

statistically analyzed and sufficiently describe the potential lethality in the laboratory species tested. The study therefore provides sufficient information to support the conclusions regarding the Oral LD50s determined in the species tested; demonstrating a range from 0.055 to 0.420 grams/kg body weight.

RELIABILITY:

1. w/o restriction [ ]
2. w restriction [X]
3. not reliable [ ]
4. not assignable [ ]

Reason: There was an incomplete description of the number of doses, animals per dose, and cageside observation.

2. REPORT NUMBER: AD-1

STUDY TYPE: Acute Dermal Toxicity in Rabbits

TEST MATERIAL: TEST MATERIAL: Ethylene dibromide; purity 99%; colorless liquid; specific gravity 2.17; boiling point 131.6 degrees C; solubility: water 0.26 gm/100 gm; soluble in alcohol; freely miscible in ether; melting point minimum 8.8 degrees C.

TESTING FACILITY: Biochemical Research Department, DOW Chemical Co.

STUDY NUMBER(S): Published report.

SPONSOR: Dow Chemical Company.

REPORT ISSUED or STUDY COMPLETION DATE: 1952; A.M.A. Archives of Industrial Hygiene and Occupational Medicine, Vol. 6.

TITLE OF REPORT: Toxicity of Ethylene Dibromide Determined in Experimental Animals

AUTHORS: V.K. Rowe, H.C. Spencer, D.D. McCollister, R.L. Hollingsworth and E.M. Adams.

RECOGNIZED METHOD, i.e. OECD: Pre-dates OECD and US EPA Test Guidelines.

GLP: Study was pre-GLP.

SPECIES/SEX: White rabbits; sex not specified.

DOSE LEVEL(s) and NUMBER OF DOSES: 4 doses: 0.21, 0.30, 0.65 and 1.1 grams/kg

NUMBER OF ANIMALS/DOSE: There were 15 rabbits in the low dose (0.21 grams/kg) and 5 rabbits in each of the remaining 3 dose levels.

STUDY METHOD: The method followed that of Draize, Woodard and Calvery (1944) except the impervious sleeves were covered with heavy cloth bandages. Animals were not restrained. The occluded exposures lasted 24 hours after which the bandages were removed and exposed areas were cleansed with soap and water. Animals that survived were observed for 14 days.

MEASURED ENDPOINT/INDEX (i.e. LD50, PII): An LD50 was not calculated. Deaths occurred as follows: 0.21 gm/kg (1/15), 0.30 gm/kg (2/5), 0.65 gm/kg (4/5) and 1.1 gm/kg (5/5).

RESULTS/OBSERVATIONS: EDB produced moderate to severe erythema, edema and necrosis. These usually healed with scarification. There was marked CNS depression and hypothermia at high doses. All deaths that occurred were experienced within 4 days of exposure.

DATA QUALITY: Study was performed using adequate numbers of animals and fully characterized test material. It was conducted in accordance with recognized standard scientific procedures for analyzing the acute dermal lethality of a test material in experimental animals. The study provides sufficient information to support the conclusions regarding the acute dermal toxicity of the test material in rabbits.

RELIABILITY:

1. w/o restriction [ ]
2. w restriction [X]
3. not reliable [ ]
4. not assignable [ ]

Reason: The acute dermal LD50 and dermal irritation scores were not reported. However, they do not detract from the scientific usefulness of the study since mortality and qualitative information were provided.

3. REPORT NUMBER: EI-1

STUDY TYPE: Eye Contact in Rabbits

TEST MATERIAL: TEST MATERIAL: Ethylene dibromide; purity 99%; colorless liquid; specific gravity 2.17; boiling point 131.6 degrees C; solubility: water 0.26 gm/100 gm; soluble in alcohol; freely miscible in ether; melting point minimum 8.8 degrees C.

TESTING FACILITY: Biochemical Research Department, DOW Chemical Co.

STUDY NUMBER(S): Published report.

SPONSOR: Dow Chemical Company.

REPORT ISSUED or STUDY COMPLETION DATE: 1952; A.M.A. Archives of Industrial Hygiene and Occupational Medicine, Vol. 6.

TITLE OF REPORT: Toxicity of Ethylene Dibromide Determined in Experimental Animals

AUTHORS: V.K. Rowe, H.C. Spencer, D.D. McCollister, R.L. Hollingsworth and E.M. Adams.

RECOGNIZED METHOD, i.e. OECD: Pre-dates OECD and US EPA Test Guidelines.

GLP: Study was pre-GLP.

SPECIES/SEX: White rabbits; sex not specified.

DOSE LEVEL(s) and NUMBER OF DOSES: 0.1 ml

NUMBER OF ANIMALS/DOSE: The number of rabbits was not specified.

STUDY METHOD: The method followed that of Draize, Woodard and Calvery (1944). All animal eyes were initially examined with fluorescein. 4 hours later the test material was introduced into one eye of each rabbit. After 30 seconds of contact the eye was flushed for about 3 minutes to remove the test material. Eyes were examined at 2, 24 and 48 hours, or until eye irritation effects had subsided.

MEASURED ENDPOINT/INDEX (i.e. LD50, PII): Contact irritation effects on the eye.

RESULTS/OBSERVATIONS: EDB produced pain and conjunctival irritation that cleared in 48 hours. A very slight corneal necrosis was observed, but healing was prompt and complete. A 10% solution in propylene glycol produced much more severe response with corneal injury lasting 12 days, but no adverse effects on the iris or lens.

DATA QUALITY: Study was performed following a recognized standard scientific procedure for analyzing the eye irritation effects in rabbits. However, the number of rabbits tested, and any scores observed (i.e. degree of injury) were not reported. Thus, no primary irritation score could be calculated, nor the severity of the injury to eyes determined with accuracy. The report provides minimum information regarding the qualitative nature of potential eye injury, but not the quantitative injury that the test material produced in rabbits.

RELIABILITY:

1. w/o restriction [ ]
2. w restriction [ ]
3. not reliable [X]
4. not assignable [ ]

Reason: Specific details regarding the conduct of the study and scoring detract from its scientific usefulness as a definitive evaluation of the eye irritation potential of the test material.

4. REPORT NUMBER: AI-1

STUDY TYPE: Acute Inhalation Toxicity in Rats and Guinea Pigs

TEST MATERIAL: Ethylene dibromide; purity 99%; colorless liquid; specific gravity 2.17; boiling point 131.6 degrees C; solubility: water 0.26 gm/100 gm; soluble in alcohol; freely miscible in ether; melting point minimum 8.8 degrees C.

TESTING FACILITY: Biochemical Research Department, DOW Chemical Co.

STUDY NUMBER(S): Published report.

SPONSOR: Dow Chemical Company.

REPORT ISSUED or STUDY COMPLETION DATE: 1952; A.M.A. Archives of Industrial Hygiene and Occupational Medicine, Vol. 6.

TITLE OF REPORT: Toxicity of Ethylene Dibromide Determined in Experimental Animals

AUTHORS: V.K. Rowe, H.C. Spencer, D.D. McCollister, R.L. Hollingsworth and E.M. Adams.

RECOGNIZED METHOD, i.e. OECD: Pre-dates OECD and US EPA Test Guidelines.

GLP: Study was pre-GLP.

SPECIES/SEX: Rats and guinea pigs and rabbits were mature adults from Dow Chemical stock colonies. Both sexes were tested.

DOSE LEVEL(S) and NUMBER OF DOSES: Rats: 8 dose levels: 100, 200, 400, 800, 1000, 3000, 5000 and 10000 ppm. These doses are equivalent to: 0.77, 1.54, 3.08, 6.16, 12.3, 23.1, 38.5 and 77 mg/l, respectively. Guinea pigs: 2 dose: 200 and 400 ppm. Two separate groups of control animals, "air-exposed" control and "unexposed" control, were

used throughout the study. The upper dose levels of 5000 and 10,000 were nominal values, while the lower concentrations were determined analytically.

NUMBER OF ANIMALS/DOSE: The number of animals varied per dose and exposure period. For example, a single dose of 1000 ppm had 4 separate exposure durations with 15-30 animals per exposure period. The 10000 ppm dose had 5 exposure durations and 4-20 animals/exposure period. The 100 ppm dose had 3 exposure durations and 20 animals/exposure. The same pattern was true for both species. See table below for details.

MEASURED ENDPOINT/INDEX (i.e. LC50, symptoms): The lethality at various doses and exposures were plotted and the LC99.99, LC50, and LC0.01 determined for each duration and dose. Mortality determined by the method of Litchfield and Wilcoxon (1949).

STUDY METHOD: Groups of mature adult rats and guinea pigs were exposed to volatized concentrations of test material over varying lengths of time. The liquid material was metered into a 154 liter glass exposure chamber at a constant rate. Air flow in the chamber was regulated and vapor concentrations were checked by combustion and determination of total halogen. Body weights were measured. All surviving animals were observed until they had recovered fully from any loss of weight and were gaining normally. This period was typically 2 weeks. Selected animals were killed at 16-24 hours, their lungs, liver, heart, spleen, testes and kidneys weighed, and examined grossly and histologically.

RESULTS/OBSERVATIONS: LC50s were calculated according to the method of Litchfield-Wilcoxon, and plotted for each duration and dose. For example, from the graph provided, an LC50 of 700 ppm can be estimated from a 1 hr exposure, 300 ppm for 3 hrs and 200 ppm for 10 hours of exposure. EDB had a slight anesthetic action but depression of the CNS occurred only at the higher doses. At high doses, deaths generally occurred within 24 hours from respiratory or cardiac failure; while at lower concentrations, deaths were delayed sometimes for 12 days resulting from pneumonia. Animals lost weight, coats appeared rough, they were irritable, discharged blood-tinged fluid from the nose and died. Examination of rats necropsied showed increased weight of lungs, livers and kidneys with numerous histologic changes. These consisted of congestion, edema, hemorrhage, and inflammation in the lungs. In the kidneys, slight interstitial congestion, edema and slight cloudy swelling of the tubular epithelium were observed. The livers displayed cloudy swelling, centrilobular fatty degeneration and necrosis.

Mortality to Rats exposed to EDB:

Concentration		Period of Exposure	Total Number of Rats	Number that Died
PPM	Mg/l	Hr		
10,000	77	0.1	20	20
		0.07	10	7
		0.05	4	2
		0.03	20	1
		0.02	20	0
5,000	38.5	0.14	20	20
		0.10	10	9
		0.07	15	5
		0.05	30	3
		0.04	20	0
3,000	23.1	0.2	10	5
		0.1	20	0
1,000	12.3	0.5	20	20
		0.4	15	12
		0.3	15	4
		0.2	30	0
800	6.16	0.8	20	13
		0.63	20	10
		0.5	20	4
		0.4	20	4
400	3.08	5.0	20	20
		3.0	20	17
		2.5	20	19
		2	25	16
		1.4	25	5
		1	20	2
		0.8	20	1
		0.6	20	0
200	1.54	16	20	19
		12	20	10
		8.5	20	9
		7	11	4
		5	10	3
		4	5	0
		3	11	1
		2	5	0
		1.4	20	0
100	0.77	16	20	0
		12	20	0
		8.5	20	0

DATA QUALITY: Study was performed using adequate numbers of animals and fully characterized test material. It was conducted in accordance with contemporary scientific procedures for analyzing the acute inhalation lethality of a test material in experimental animals. The results were statistically analyzed and sufficiently describe the potential lethality in the laboratory species tested. The study therefore provides sufficient information to support the conclusions regarding the inhalation LC50s determined in the species tested

RELIABILITY:

1. w/o restriction [ ]
2. w restriction [X]
3. not reliable [ ]
4. not assignable [ ]

Reason: Study was conducted prior to GLP and before standard toxicology testing protocols were available, but nonetheless adequately describes the acute inhalation toxicity of the test material.

5. REPORT NUMBER: AO-2

STUDY TYPE: Acute Oral Toxicity to Rats

TEST MATERIAL: Ethylene dibromide; purity 99%; colorless liquid; specific gravity 2.17; boiling point 131.6 degrees C; solubility: water 0.26 gm/100 gm; soluble in alcohol; freely miscible in ether; melting point minimum 8.8 degrees C.

TESTING FACILITY: Biochemical Research Department, DOW Chemical Co.

STUDY NUMBER(S): Published report.

SPONSOR: Dow Chemical Company.

REPORT ISSUED or STUDY COMPLETION DATE: 1956; A.M.A. Archives of Industrial Hygiene and Occupational Medicine, Vol. 13; pages 1-7.

TITLE OF REPORT: Comparative Inhalation Toxicity of Fumigant Mixtures

AUTHORS: V.K. Rowe, F. Oyen, D.D. McCollister, and R.L. Hollingsworth.

RECOGNIZED METHOD, i.e. OECD: Pre-dates OECD and US EPA Test Guidelines.

GLP: Study was pre-GLP.

SPECIES/SEX: Male and female albino rats, from Dow Chemical stock colonies.

DOSE LEVEL(S) and NUMBER OF DOSES: Oral doses were given via gastric intubation, suspended in corn oil. There were 6 doses. It followed the Litchfield-Wilcoxon simplified method, using 10-20 animals in each group; doses spaced in geometric progression based upon results achieved with the previous dose. Aliquots of a 10% solution of EDB in olive oil were emulsified in 2 ml of a 5-10% acacia solution.

NUMBER OF ANIMALS/DOSE: A total of 100 rats (mixed sex) were used.

MEASURED ENDPOINT/INDEX (i.e. LD50, symptoms): LD50 = 0.14 grams/kg (mixed sexes).

STUDY METHOD: Groups of young adult albino rats were administered single oral doses via gastric intubation according to their individual body weight. All surviving animals were observed until they had recovered fully from any loss of weight and were gaining normally. This period was typically 2 weeks.

RESULTS/OBSERVATIONS: LD50 was calculated according to the method of Litchfield-Wilcoxon, as reported above.

DATA QUALITY: Study was performed using adequate numbers of animals. The test material was a pure sample. It was conducted in accordance with contemporary scientific procedures for analyzing the acute oral lethality of a test

material in experimental animals. Although full description of the test procedures was not reported, the results were statistically analyzed and sufficiently describe the potential lethality in the laboratory species tested. The study therefore provides sufficient information to support the conclusions regarding the Oral LD50 rats.

RELIABILITY:

1. w/o restriction [ ]
2. w restriction [X]
3. not reliable [ ]
4. not assignable [ ]

Reason: There was an incomplete description of the test method and cageside observation, although these same authors had published their results from a similar experiment in the same lab in.1952. Much of this information was used, as it was referenced in this article.

B. REPEATED DOSE (Subchronic) TOXICITY:

1. REPORT NUMBER: SC-1

STUDY TYPE: Repeated Dose Inhalation Exposures in Rats, Rabbits and Guinea Pigs

TEST MATERIAL: Ethylene dibromide; purity 99%; colorless liquid; specific gravity 2.17; boiling point 131.6 degrees C; solubility: water 0.26 gm/100 gm; soluble in alcohol; freely miscible in ether; melting point minimum 8.8 degrees C.

TESTING FACILITY: Biochemical Research Department, DOW Chemical Co.

STUDY NUMBER(S): Published report.

SPONSOR: Dow Chemical Company.

REPORT ISSUED or STUDY COMPLETION DATE: 1952; A.M.A. Archives of Industrial Hygiene and Occupational Medicine, Vol. 6.

TITLE OF REPORT: Toxicity of Ethylene Dibromide Determined in Experimental Animals

AUTHORS: V.K. Rowe, H.C. Spencer, D.D. McCollister, R.L. Hollingsworth and E.M. Adams.

RECOGNIZED METHOD, i.e. OECD: Pre-dates OECD and US EPA Test Guidelines.

GLP: Study was pre-GLP.

SPECIES/SEX: Rats, guinea pigs and rabbits were mature adults from Dow Chemical stock colonies. Both sexes were tested.

DOSE LEVEL(S) and NUMBER OF DOSES: The number of animals/dose varied with each protocol; e.g. some were exposed 7hr/day for 9 days, some for 91 days and some for 213 days. See below for details. In all cases, however, two separate groups of control animals, "air-exposed" control and "unexposed" control, were used throughout the study.

NUMBER OF ANIMALS/DOSE:

Species	Number/Sex M/F	Daily Exposure Hrs	Total Number of Exposures	Duration Days	Concentration PPM
Rat	10F	7	<7	9	100
Rabbit	4F	7	<3	4	100
Rat	20M/20F	7	63	91	50
Guinea Pig	4M/4F	7	57	80	50
Rabbit	1M/3F	7	59	84	50
Monkey	1M/1F	7	49	70	50
Rat	20M/20F	7	151	213	25
Guinea Pig	8M/8F	7	145	205	25
Rabbit	3M/1F	7	152	214	25
Monkey	1M/1F	7	156	220	25

STUDY METHOD: Groups of mature adult rats, guinea pigs, rabbits and monkeys were exposed to volatized concentrations of test material over varying lengths of time. Animals were generally exposed 7 hrs/day 5 days/week. The liquid material was metered into a 154 liter glass exposure chamber at a constant rate. Air flow in the chamber was regulated and vapor concentrations were checked by combustion and determination of total halogen. Body weights were measured. Animals were weighed twice/week and observed for appearance and behavior. Growth curves and mortality records were kept for each group. Representative hematological parameters were determined in rats and monkeys. All moribund animals were killed and examined for evidence of any organ damage.

MEASURED ENDPOINT/INDEX (i.e. LD50, PII): Studies conducted to determine vapor exposures that produce adverse effects at varying concentrations and duration of exposure. Levels below which such effects were observed or measured were estimated.

HEMATOLOGY: Blood that was collected was obtained at the time of autopsy and plasma-prothrombin-clotting time determined on animals exposed for 100 ppm.

CLINICAL CHEMISTRY: BUN determined from blood obtained at necropsy. Some liver sections obtained at necropsy were frozen in dry ice for subsequent total lipid analyses.

URINALYSIS: Not performed.

ORGAN WEIGHTS: The following organs were weighed: spleen, liver, kidneys, testes, heart, and lungs. Absolute and relative organ weights were determined.

GROSS NECROPSY: All animals were fasted overnight, except guinea pigs. Each animal was weighed and killed. Gross appearance was recorded and its lungs, liver, heart, spleen, testes and kidneys weighed, and examined grossly.

HISTOPATHOLOGY: Tissues from these organs and the pancreas and adrenal glands were prepared and stained with hematoxylin and eosin. In some cases the liver and kidney were stained with Oil red O.

RESULTS/OBSERVATIONS: Results varied by species, concentration and duration, and are summarized below:
Rats (100 ppm for 7 days): 3/10 died after 1, 5 and 7 exposures; they lost weight and surviving animals were thin and unkempt; stomach contents were blood-tinged; lungs, liver and kidney weights were significantly increased; thickening of the alveolar walls and leucocytic infiltration in the lungs; cloudy swelling in the liver without fatty degeneration, and slight congestion and hemosiderosis of the spleen. BUN and other blood parameters were normal.
Rabbit (100 ppm for 3 days): 2/4 died after 2 exposures and another died after 3 exposures; necropsy revealed widespread central fatty degeneration of the liver, with necrosis. BUN and other blood parameters were normal.

Rat (50 ppm for 91 days): 10/20 males died due to pneumonia and upper respiratory tract infections; 4/20 females died. Animals displayed a reduced growth. In males there was increased liver, lung and kidney weights, while testes weights decreased. In females, liver and kidney weight increased, while spleen weights decreased. BUN and other blood parameters, and total lipid were normal. Pathology of the males showed pneumonic consolidation in the lungs. This was not evident in females. No effects observed in other organs.
Guinea Pig (50 ppm for 80 days): growth in both sexes was depressed, but no effect on mortality. Final body weights were significantly decreased. The absolute weights of lung, liver and kidneys were significantly increased. There was central fatty degeneration of the liver, and slight degenerative changes in the tubular epithelium of the kidney. All other parameters measured were normal.
Rabbit (50 ppm for 84 days): There was no evidence of any adverse effects in the parameters measured – growth, behavior, body weight, BUN or lipid. There were slight increases in liver and kidney weights.
Monkey (50 ppm for 70 days): Animals appeared ill and nervous throughout the study, and lost 5% of initial body weight. The only significant effect was slight liver weight increase with central fatty degeneration. Total lipid was slightly increased. All other parameters measured were normal.
Rat (25 ppm for 213 days): 10/20 males died due to pneumonia; 3/20 females died. Generally no adverse effects were observed, other than the mortality due to pneumonia.
Guinea Pig (25 ppm for 205 days): 4/8 males and 2/8 females died due to pneumonia. There were no other adverse effects observed.
Rabbit (25 ppm for 214 days): No adverse effects observed.
Monkey (25 ppm for 220 days): No adverse effects observed in any of the parameters measured.

DATA QUALITY: Study was performed using adequate numbers of animals and a fully characterized test material. It was conducted in accordance with contemporary scientific procedures for analyzing the inhalation toxicity to a test material in experimental animals and varying concentrations and duration of exposure. Sufficient parameters were measured in order to assess the adverse effects of a test material following repeated daily exposures. They included behavior, body weight gain/loss, hematology, clinical chemistry, organ weights, gross necropsy and histopathology. Although some of these parameters were limited in scope they nonetheless are considered to meet minimum standards and are adequate for the purposes of this study.

RELIABILITY:

1. w/o restriction [ ]
2. w restriction [X]
3. not reliable [ ]
4. not assignable [ ]

Reason: Study was conducted prior to GLP and before standard toxicology testing protocols were available, and therefore the parameters measured are considered limited in scope by today's standards. It nonetheless provides sufficient information to characterize potential adverse effects from limited repeat inhalation exposures.

2. REPORT NUMBER: SC-2

STUDY TYPE: 13 Week Inhalation Study in Rats

TEST MATERIAL: Ethylene dibromide; 99.6% pure.

TESTING FACILITY: Toxicology Research Laboratory, Health and Environmental Sciences, Dow Chemical, Midland, MI.

STUDY NUMBER(S): No number reported.

SPONSOR: Dow Chemical

REPORT ISSUED or STUDY COMPLETION DATE: February 11, 1980

TITLE OF REPORT: 13 Week Repeated Inhalation Study on Ethylene Dibromide (EDB) in Male and Female Rats

RECOGNIZED METHOD, i.e. OECD: Predates the OECD and US EPA Test guidelines.

GLP: Study pre-dates GLP; however, a Quality Assurance statement was provided.

SPECIES/SEX: Fischer 344 (CDF) rats obtained from Charles River Labs; males and females 9 weeks old.

ROUTE OF ADMINISTRATION: Inhalation.

DOSE LEVEL(s) and NUMBER OF DOSES: Dose levels of 0, 3, 10 and 40 ppm; administered 6 hours per day, 5 days/week for 13 weeks, for a total of 67 exposures. There were also 3 additional groups (10M/10F per group) for serial sacrifices: 1 week, 6 weeks and rats were held for a recovery period of 88-89 days and then necropsied to evaluate reversibility of effects.

NUMBER OF ANIMALS/DOSE: 10 male and 10 female rats per dose level. Animals were housed 2/cage during non-exposure, and 5 to 10/cage for females and males, respectively, during exposure periods.

STUDY METHOD: EDB was vaporized by metering the liquid at a calculated rate into a warmed vaporization flask (100 degrees C). The vapors were forced into the chamber with incoming and regulated. The nominal concentrations were calculated based upon the rate at which the liquid was dispensed and the total chamber airflow. Concentration was analyzed 3 times/day by GC with a flame ionization detector. Airflow was 300 ml/minute. The chamber was a 1 M^3 stainless steel and glass Rochester type chamber. Chamber temperature was 21-28 degrees C and relative humidity was 43-46%. Body weights of each animal were obtained prior to start of study, twice a week for the first 2 weeks and then weekly throughout the study. Animals were observed for signs of toxicity.

MEASURED ENDPOINT/INDEX (i.e. LD50, PII): An NOAEL of 3 ppm was determined. Study was preliminary to a longer term 2-year carcinogenicity study which was planned.

HEMATOLOGY: Conducted on 7 males/dose prior to sacrifice at 6 weeks, and on 7/sex/dose at necropsy after 13 weeks of exposure, and after the recovery period. Parameters measured included: total erythrocyte count, total WBC, differential leukocyte count, hemoglobin, and PCV.

CLINICAL CHEMISTRY: Conducted on 7 males/dose prior to sacrifice at 6 weeks, and on 7/sex/dose at necropsy after 13 weeks of exposure, and after the recovery period. Parameters measured included: BUN, SGPT, SAP, glucose and bilirubin. Serum bromide levels were measured in rats exposed for 6 weeks.

URINALYSIS: Conducted on 7 males/dose prior to sacrifice at 6 weeks, and on 7/sex/dose at necropsy after 13 weeks of exposure, and after the recovery period. Parameters measured included: specific gravity, pH, glucose, ketones, bilirubin, urobilinogen, occult blood and protein.

ORGAN WEIGHTS: The following organs were weighed from all groups: liver, kidneys, testes, heart, thymus and brain. Absolute and relative organ weights were determined. Fasting body weights were used for organ to body weight ratios.

GROSS NECROPSY: Gross necropsy was performed on all animals on study, with special attention to the upper respiratory tract. Rats were fasted overnight prior to necropsy. The nasal passageways were perfused with formalin fixative. A full compliment of tissues and organs were examined.

HISTOPATHOLOGY: Histopathological examinations were performed on a full compliment of tissues from all animals. It included the following reproductive organs: testes, ovaries, prostate, uterus, epididymides and accessory sex glands. In addition, transverse sections through the decalcified nasal cavity were prepared from 4 separate levels.

STATISTICAL ANALYSIS: Body weight, body weight gain, organ weight, urine specific gravity, hematology and clinical chemistry data were analyzed using ANOVA and Dunnett's test. The level of significance was $p<0.05$.

RESULTS/OBSERVATIONS: NOTE: information which follows is for 13 week exposure period only. No difference between low dose and control animals was observed for the following: morality, moribundity, appearance, body weight, organ weight, hematology, biochemistry, urinalysis, gross necropsy or microscopic comparisons. There were no deaths in any group related to exposure. Body weight was decreased at males 10 and 40 ppm, and in females at 40 ppm. Hematology: The only effect was a decreased hematocrit and hemoglobin in females at 40 ppm. Urinalysis: The only effect was a decrease in specific gravity in females at 40 ppm. Clinical chemistry: There were no treatment related changes. The serum bromide levels were elevated above controls in a dose-related manner in all groups of males. Organ-to-Body Weight Ratios: Relative liver weight increased in males at 40 ppm and in females at 10 and 40 ppm. Also, absolute liver weight increased in high dose females. Relative kidney weights increased in males only at 40 ppm. Gross Necropsy: The only observation was pale livers in 50% of the high dose females. Histopathology: Exposure related changes occurred primarily in the anterior portion of the nasal turbinates. At 40ppm there was slight diffuse or focal nonkeratinizing squamous metaplasia and hyperplasia of the respiratory epithelium in both sexes. At 10 ppm, there were slight degrees of isolated to multifocal hyperplasia of the respiratory epithelium, also in both sexes. Also, there was a slight increase in fatty livers in females at 40 ppm. There were no other significant histologic changes.

In the 88-89 day recovery group of rats, examination of the nasal turbinates demonstrated no discernible changes in comparison to controls, for males; while in females only 1/10 had a single focus of epithelial hyperplasia in the respiratory epithelium.

DATA QUALITY: Study was performed using adequate numbers of animals and a fully characterized test material. It was conducted in accordance with contemporary scientific procedures for analyzing the inhalation toxicity to a test material in experimental animals and varying concentrations and duration of exposure. Sufficient parameters were measured in order to assess the adverse effects of a test material following repeated daily exposures for up to 90 days. They included behavior, body weight gain/loss, hematology, clinical chemistry, urinalysis, organ weights, gross necropsy and histopathology. Special attention was paid to the respiratory tract and nasal passages. Although study was conducted prior to GLPs, there was a quality assurance statement from the laboratory. An NOAEL of 3 ppm was demonstrated.

RELIABILITY:

1. w/o restriction [X]
2. w restriction [ ]
3. not reliable [ ]
4. not assignable [ ]

Reason: Study was conducted prior to GLP and about the time that standard toxicology testing protocols were being developed, both nationally and internationally. It provides sufficient information to characterize potential adverse effects from repeat inhalation exposures, with an examination of progression and reversibility of effects.

3. REPORT NUMBER: CA-1

STUDY TYPE: Inhalation Carcinogenicity Study in Rats

TEST MATERIAL: Ethylene dibromide; purity >99%.

TESTING FACILITY: Midwest Research Institute, Biological Sciences Division, Kansas City, MO 64110.

STUDY NUMBER(S): Contract No. 210-76-0131.

SPONSOR: National Institute for Occupational Safety and Health.

REPORT ISSUED or STUDY COMPLETION DATE: February 1979.

TITLE OF REPORT: Study of Carcinogenicity and Toxicity of Inhaled 1,2-Dibromethane in Rats Treated with Disulfiram.

RECOGNIZED METHOD, i.e. OECD: Predates the OECD and US EPA Test guidelines; consistent with National Cancer Institute guidelines available at that time.

GLP: Study pre-dates GLP.

SPECIES/SEX: Weanling Sprague-Dawley CD rats; males weighing 131-134 grams, and females weighing 118-124 grams.

ROUTE OF ADMINISTRATION: Test material administered via inhalation, 7 hrs/day 5 days/week for 18 months. Disulfiram, 96% purity, was mixed in the diet at a concentration of 0.05%.

DOSE LEVEL(s) and NUMBER OF DOSES: Test groups were as follows; Control, Control plus 0.05% disulfiram in the diet, EDB 20ppm, and EDB plus 0.05% disulfiram in the diet.

NUMBER OF ANIMALS/DOSE: 48 male and 48 female rats in each of the four groups.

STUDY METHOD: Each group of Sprague-Dawley CD rats were placed in a 4.5 m^3 stainless steel, Rochester-type inhalation chamber and exposed to the test material vapors for 7 hrs/day, 5 days/week for 18 months. Rats were housed individually, and allowed free access to food and water except during exposure. The vapor was generated by bubbling nitrogen into a heated vessel containing EDB, maintained at 60 degrees C. EDB was mixed with incoming air and regulated at 736.3 l/minute. Airflow and EDB were both monitored. Concentration of EDB in the inhalation chamber during exposure was monitored via GC about 3 times per 7 hr exposure. Animals were rotated throughout the study so that by the 24th day they had been in every shelf and cage position. Chamber temperature and humidity were 70-80 degrees F and 35-55%, respectively. Animals observed twice daily throughout the each exposure period for clinical signs of toxicity. Individual body weights were recorded weekly for the first 14 weeks, then monthly thereafter. Food consumption was recorded from the 4th month to the end of the study. Complete gross necropsy and histopathological examination were performed on all animals.

MEASURED ENDPOINT/INDEX (i.e. LD50, PII): The study was conducted to determine whether ingestion of disulfiram during EDB exposures would alter the toxicity of EDB, since disulfiram inhibits acetaldehyde dehydrogenase and could inhibit the biotransformation of EDB.

HEMATOLOGY: No measurements were taken, except as follows: anemic appearing rats in the10th to12th month were selected for measurement of hematocrit, hemoglobin, rbc and wbc. This included the following groups: EDB plus disulfiram and EDB without disulfiram.

CLINICAL CHEMISTRY: No measurements were taken.

URINALYSIS: No measurements were taken.

ORGAN WEIGHTS: The following organs were weighed from all groups at necropsy: spleen, liver, kidneys and heart. Absolute and relative organ weights were determined.

GROSS NECROPSY: Gross necropsy was performed on all animals on study.

HISTOPATHOLOGY: Histopathological examinations were performed on the following tissues from all groups: liver, heart, stomach, kidneys, pancreas, spleen, adrenals, thyroid, pituitary, urinary bladder, brain, skin, sternal bone marrow, lungs, mesenteric and tracheobronchial lymph nodes, salivary glands, zymbal glands, testes, prostate, mammary glands, ovaries, uterus, and abnormal appearing tissues. Nasal cavity was not examined owing the poor state of the preserved tissues.

STATISTICAL ANALYSIS: Differences between control and test values for body weight, food consumption and organ weights were compared by Tukey's omega procedure. Data on tumor incidence were anlyzed by Fisher's exact probability test.

RESULTS/OBSERVATIONS: Mortality was increased in the EDB and EDB plus disulfiram test groups. Mortality in the disulfiram plus EDB group at 12 months was 58% (M) and 83% (F). By the end of the 15th month all animals in this group had died or were terminated. Mortality in the EDB group at 18 months was 90% (M) and 77% (F). Body weight was also significantly decreased in both disulfiram groups, compared to controls. The effects in the EDB plus disulfiram group were more pronounced than the other groups. The EDB group gained weight comparable to controls, except for depressed body weights in the 15th and 18 months of exposure. Hematological measurements in the EDB and EDB/disulfiram groups demonstrated normal values for the EDB group, but EDB/disulfiram group had lower hematocrit, hemoglobin, and rbc counts. Meaningful organ weights comparisons could not be performed for the EDB groups because of the mortality that occurred in both groups. Two trends were, however, reported by the authors: increased absolute liver weight and decreased testes weight in the EDB/disulfiram group when compared to the EDB group only. All control organ weights were reportedly normal. Gross necropsy: Two significant findings were observed: (1) hemosiderosis in the spleens of EDB/disulfiram group (both sexes) and disulfiram alone (females), and (2) atrophy of the testes and prostate in the EDB/disulfiram group. Histopathololgy: Histopatholgical examination was essentially an elucidation of tumor incidences among groups. The control and control/disulfiram groups were comparable except for a marginal increase in mammary turmors for disulfiram females. EDB only: Males had increased tumors in spleen, adrenals and mesenchymal tissue; females had increased tumors in spleen, adrenals and mammary glands. EDB/disulfiram: tumor incidence was signficantly increased in both sexes as follows: males – liver, spleen, mesenchymal tissue, kidney, adrenal, thyroid, brain and lung; females - liver, spleen, mesenchymal tissue, kidney, adrenal, thyroid, brain and mammary. Tumors were both benign and malignant.

DATA QUALITY: Study was performed using adequate numbers of animals and a fully characterized test material. It was conducted in accordance with contemporary scientific procedures for analyzing the inhalation toxicity to a test material in experimental animals. Study was specifically designed to investigate the potential adverse effects of workers in an alcohol control program, using disulfiram, exposed to daily exposures of EDB in the work environment. Sufficient parameters were measured in order to assess the adverse effects of a test material following repeated daily exposures for 12 to 18 months. They included behavior, body weight gain/loss, limited hematology, gross necropsy and histopathology. An NOAEL was not demonstrated.

RELIABILITY:

1. w/o restriction [ ]
2. w restriction [X]
3. not reliable [ ]
4. not assignable [ ]

Reason: Study was conducted prior to GLP and about the time that standard toxicology testing protocols were being developed, both nationally and internationally. It provides sufficient information to characterize potential adverse effects from repeat inhalation exposures to a single level of EDB, when inhaled alone or in combination with disulfiram ingestion.

4. REPORT NUMBER: CA-2

STUDY TYPE: Inhalation Carcinogenicity Study in Rats

TEST MATERIAL: Ethylene dibromide; CAS#106-93-4; purity >99%; colorless, volatile liquid.

TESTING FACILITY: Hazleton Laboratories, America, Inc. (under contract to Tracor Jitco)

STUDY NUMBER(S): Technical Report Series No. 210; NIH Publication No. 82-1766.

SPONSOR: National Toxicology Program: National Cancer Institute and National Institute of Environmental Health Sciences from National Institutes of Health; National Center for Toxicological Research from FDA; and the National Institute for Occupational Safety and Health, Centers for Disease Control.

REPORT ISSUED or STUDY COMPLETION DATE: March 1982.

TITLE OF REPORT: Carcinogenicity Bioassay of 1,2-Dibromethane in F344 Rats and B6C3F1 Mice.

RECOGNIZED METHOD, i.e. OECD: OECD 451; consistent with National Cancer Institute guidelines available at that time.

GLP: Yes.

SPECIES/SEX: Fischer 344 Rats, males and females 6 weeks old at the start of exposure.

ROUTE OF ADMINISTRATION: Test material administered via inhalation, 6 hrs/day 5 days/week for 88-103 weeks. Animals lived in the inhalation chamber throughout the study, except when being weighed or observed.

DOSE LEVEL(s) and NUMBER OF DOSES: Test groups were as follows: 0, 10 and 40 ppm.

NUMBER OF ANIMALS/DOSE: 50 male and 50 female rats in each group. Males were housed 3 per cage and females 4 per cage.

STUDY METHOD: Each group of Fisher 344 rats was placed in a 6 m^3 stainless steel inhalation chamber and exposed to the test material vapors for 6 hrs/day, 5 days/week for 88-104 weeks. Rats were allowed access to food and water 1 hour after exposure and until it was removed the next morning prior to exposure. EDB was mixed with incoming air and regulated at 1000 l/minute. Airflow and EDB were both monitored. Concentration of EDB in the inhalation chamber during exposure was monitored via GC 4 times per 6 hr exposure. Chamber temperature and humidity were 22 degrees C and 50%, respectively. Animals observed twice daily throughout the each exposure period for clinical signs of toxicity. Individual body weights were recorded monthly for the first 79 weeks, then twice monthly thereafter beginning at week 80. Complete gross necropsy and histopathological examination were performed on all animals.

MEASURED ENDPOINT/INDEX (i.e. LD50, PII): There was no NOAEL as the purpose of the study was to examine the carcinogenic potential to laboratory animals.

HEMATOLOGY: No measurements were taken.

CLINICAL CHEMISTRY: No measurements were taken.

URINALYSIS: No measurements were taken.

ORGAN WEIGHTS: No organ weights were determined.

GROSS NECROPSY: Gross necropsy was performed on all animals on study.

HISTOPATHOLOGY: Histopathological examinations were performed on a full compliment of tissues, including but not limited to, the following tissues from all groups: adrenal, bile duct, brain, diaphragm, duodenum, epididymis, esophagus, eye, femur, heart, ileum, jejunum, kidney, large intestine, liver, lung, lymph nodes (cervical and mesenteric), ovary, pancreas, parathyroid, pituitary, preputial gland, prostate, salivary glands, seminal vesicleskin, spleen, stomach, testes, thyroid, trachea, urinary bladder, uterus and vagina. Nasal cavity and sinuses were fixed whole in neutral buffered 10 formalin and/or Bouin's solution, decalcified and sectioned.

STATISTICAL ANALYSIS: Differences between control and test groups for effects on survival (Kaplan and Meier; Cox; Tarone's extensions of Cox for trend; and one-tailed P test), and incidence of neoplastic and non-neoplastic lesions (one-tailed Fisher exact test; Bonferroni test for inequality; Cochran-Armitage test for linear trend).

RESULTS/OBSERVATIONS: Mean body weight of high dose males and females was lower than other groups. Mortality was increased in the high dose group. Survival in high dose decreased to 10% in males (wk 89) and 16% in females (wk 91), at which point all remaining animals in this group were sacrificed. Survival in control and low dose groups was comparable, with 70-78% survival to weeks 104-106. Non-neoplastic changes: High dose – increased incidence of hepatic centrilobular necrosis (M/F), toxic nephropathy (M/F), testicular degeneration, congestion and

suppurative bronchopneumonia (M/F), suppurative inflammation and epithelial hyperplasia of the trachea (M/F), and inflammation and epithelial hyperplasia of the nasal cavity (M/F). Low Dose - inflammation and epithelial hyperplasia of the nasal cavity (M/F), testicular degeneration, and retinal atrophy (F). Neoplastic changes: High dose – carcinoma and adenocarcinoma of the nasal cavity (M/F), hemangiosarcoma of the spleen (M/F), mesothelioma of the tunica vaginalis, bronchiolar carcinoma (F), mammary fibroadenoma (F), and invasive carcinoma in brain from nasal cavity (M/F). Low dose – adenoma, adenocarcinoma and adenomatous polyp in nasal cavity (M/F), mesothelioma of the tunica vaginalis, and mammary fibroadenoma (F).

DATA QUALITY: Study was performed using adequate numbers of animals and a fully characterized test material. It was conducted in accordance with contemporary scientific procedures for analyzing the inhalation toxicity to a test material in experimental animals. Study was specifically designed to investigate the carcinogenic potential of EDB in rats. Sufficient parameters were measured in order to assess the oncogenic effects of the test material following repeated daily exposures for 104 weeks. They included behavior, body weight gain/loss, gross necropsy and histopathology. The test material was oncogenic in both sexes of rats at doses of 10 and 40 ppm.

RELIABILITY:

1. w/o restriction [X]
2. w restriction [ ]
3. not reliable [ ]
4. not assignable [ ]

Reason: Study was conducted in accordance with scientifically sound principles and GLP procedures, and the data generated support the conclusions.

5. REPORT NUMBER: CA-3

STUDY TYPE: Inhalation Carcinogenicity Study in Mice

TEST MATERIAL: Ethylene dibromide; CAS#106-93-4; purity >99%; colorless, volatile liquid.

TESTING FACILITY: Hazleton Laboratories, America, Inc. (under contract to Tracor Jitco)

STUDY NUMBER(S): Technical Report Series No. 210; NIH Publication No. 82-1766.

SPONSOR: National Toxicology Program: National Cancer Institute and National Institute of Environmental Health Sciences from National Institutes of Health; National Center for Toxicological Research from FDA; and the National Institute for Occupational Safety and Health, Centers for Disease Control.

REPORT ISSUED or STUDY COMPLETION DATE: March 1982.

TITLE OF REPORT: Carcinogenicity Bioassay of 1,2-Dibromethane in F344 Rats and B6C3F1 Mice.

RECOGNIZED METHOD, i.e. OECD: OECD 451; consistent with National Cancer Institute guidelines available at that time.

GLP: Yes.

SPECIES/SEX: B6C3F1 mice, males and females 5 weeks old at the start of exposure.

ROUTE OF ADMINISTRATION: Test material administered via inhalation, 6 hrs/day 5 days/week for 78-103 weeks. Animals lived in the inhalation chamber throughout the study, except when being weighed or observed.

DOSE LEVEL(s) and NUMBER OF DOSES: Test groups were as follows: 0, 10 and 40 ppm.

NUMBER OF ANIMALS/DOSE: 50 male and 50 female mice in each group. Mice were housed 4 per cage, by sex.

STUDY METHOD: Each group of B6C3F1 mice was placed in a 6 m^3 stainless steel inhalation chamber and exposed to the test material vapors for 6 hrs/day, 5 days/week for 78-103 weeks. Mice were allowed access to food and water 1 hour after exposure and until it was removed the next morning prior to exposure. EDB was mixed with incoming air and regulated at 1000 l/minute. Airflow and EDB were both monitored. Concentration of EDB in the inhalation chamber during exposure was monitored via GC 4 times per 6 hr exposure. Chamber temperature and humidity were 22 degrees C and 50%, respectively. Animals observed twice daily throughout the each exposure period for clinical signs of toxicity. Individual body weights were recorded monthly for the first 79 weeks, then twice monthly thereafter beginning at week 80. Complete gross necropsy and histopathological examination were performed on all animals.

MEASURED ENDPOINT/INDEX (i.e. LD50, PII): There was no NOAEL as the purpose of the study was to examine the carcinogenic potential to laboratory animals.

HEMATOLOGY: No measurements were taken.

CLINICAL CHEMISTRY: No measurements were taken.

URINALYSIS: No measurements were taken.

ORGAN WEIGHTS: No organ weights were determined.

GROSS NECROPSY: Gross necropsy was performed on all animals on study.

HISTOPATHOLOGY: Histopathological examinations were performed on a full compliment of tissues, including but limited to, the following tissues from all groups: adrenal, bile duct, brain, diaphragm, duodenum, epididymis, eye, femur, heart, ileum, jejunum, kidney, large intestine, liver, lung, lymph nodes (cervical and mesenteric), ovary, pancreas, parathyroid, penis, pituitary, preputial gland, prostate, salivary glands, seminal vesicle, skin, spleen, stomach, testes, thyroid, trachea, urinary bladder, uterus and vagina. Nasal cavity and sinuses were fixed whole in neutral buffered 10 formalin and/or Bouin's solution, decalcified and sectioned.

STATISTICAL ANALYSIS: Differences between control and test groups for effects on survival (Kaplan and Meier; Cox; Tarone's extensions of Cox for trend; and one-tailed P test), and incidence of neoplastic and non-neoplastic lesions (one-tailed Fisher exact test; Bonferroni test for inequality; Cochran-Armitage test for linear trend).

RESULTS/OBSERVATIONS: Mean body weight of high dose males and females was lower than other groups. Survival was decreased in high dose females and low dose males. Survival in controls and low dose females and high dose male groups were comparable. The male portion of the study was run for 79 weeks and the females for 104 weeks. Non-neoplastic changes: High dose – increased incidence of serous inflammation of the nasal cavity (M/F), epthelial hyperplasia of the bronchus (M/F) and bronchiole (F), alveolar epithelial hyperplasia of the lung (F), and suppurative inflammation of the prostate. Low Dose - increased incidence of serous inflammation of the nasal cavity (M/F), epthelial hyperplasia of the bronchus and bronchiole (F), and suppurative inflammation of the prostate. Neoplastic changes: High dose – carcinoma and adenocarcinoma of the nasal cavity (F), bronchiolar adenomas and carcinomas (M/F), subcutaneous fibrosarcoma (F), mammary adenocarcinoma (F), and hemangiosarcoma of the ovary. Low dose – bronchiolar carcinomas (M/F), subcutaneous fibrosarcoma (F), and mammary adenocarcinoma (F).

DATA QUALITY: Study was performed using adequate numbers of animals and a fully characterized test material. It was conducted in accordance with contemporary scientific procedures for analyzing the inhalation toxicity to a test material in experimental animals. Study was specifically designed to investigate the carcinogenic potential of EDB in mice. Sufficient parameters were measured in order to assess the oncogenic effects of the test material following repeated daily exposures for 78-104 weeks. They included behavior, body weight gain/loss, gross necropsy and histopathology. The test material was oncogenic in both sexes of mice at doses of 10 and 40 ppm.

RELIABILITY:

1. w/o restriction [X]
2. w restriction [ ]
3. not reliable [ ]
4. not assignable [ ]

Reason: Study was conducted in accordance with scientifically sound principles and GLP procedures, and the data generated support the conclusions.

C. GENETIC TOXICITY

1. REPORT NUMBER: MU-1

STUDY TYPE: In Vitro Mammalian Cell Gene Mutation Test

TEST MATERIAL: 1,2-Dibromoethane obtained from Aldrich Chemical Company.

STUDY NUMBER(S): None reported.

SPONSOR: Gentest Ltd. Partnership

TESTING FACILITY: Gentest Ltd. Partnership, Woburn, MA.

TITLE OF REPORT: Mutagenicity of 1,2-dichloroethane and 1,2-dibromoethane in two human lymphoblastoid cell lines.

REPORT ISSUED or STUDY COMPLETION DATE: Mutation Research, 142 (1985), pg 133-140.

RECOGNIZED METHOD, i.e. OECD: Not stated; but consistent with OECD 476.

GLP: Not stated.

TEST ORGANISM USED: Human lymphoblastoid cell lines, AHH-1 and TK-6. Cell line AHH-1 contains mixed-function oxygenase activity ; gene mutations are measured at the hgprt locus (hypoxanthine guanine phosphoribosyl transferase) via resistance to 6-thioguanine. TK-6 cell line does not contain mixed-function oxygenase activity; they are heterozygous at the tk (thymidine kinase) locus and gene mutations are measured there via resistance to trifluorothymidine.

TEST COMPOUND CONCENTRATIONS USED: 5-6 concentrations, with the highest doses causing cytotoxicity. In AHH-1 the concentrations were: 1, 2, 5, 10, 50 and 100 ug/ml. In TK-6 the concentrations were: 2, 5, 20, 40 and 60 ug/ml.. All test chemical and negative controls were tested in triplicate, and all positive controls were tested in duplicate cultures.

CONTROL MATERIALS: The following control materials were employed.

Positive Control:

Non-Activation: 4-Nitroquinoline-N-oxide (70 ng/ml) used with TK-6
Benzo[a]pyrene (3.1 ug/ml) used with AHH-1

Negative control: DMSO

TEST PERFORMANCE: Background mutation frequency was reduced by treating TK6 and AHH-1 lymphoblasts for 48 and 72 hours, respectively, with CHAT (deoxycytidine, hypoxanthine, aminopterin and thymidine). Cells were centrifuged and resuspended in medium containing THC (CHAT without aminopterin). Cultures counted daily and exposed to test

material 2-3 days after resuspension in THC. All cell manipulations after this point were conducted in medium containing penicillin and streptomycin. Cytotoxicity was determined by cell growth survival: treated response divided by negative control response to yield relative survival.

PROTOCOL: TK-6: Each culture of 4-5 x 10^7 TK6 cells was exposed to test material for 20 hours (@1.5 cell generations). During phenotypic expression (3 days for tk locus), cell concentration was determined daily. Cultures were plated on the 3rd day after treatment in medium containing trifluorothymidine. It was heated at 56 degrees C for 1 hour and incubated for 12 days, then scored for the presence or absence of colonies.

AHH-1: Each culture of 4-5 x 10^7 AHH-1 cells was exposed to test material for 28 hours (@1.5 cell generations). During phenotypic expression (6 days for hgprt locus), cell concentration was determined on days 0, 1, 3 and 5 after exposure. Cultures were plated on the 6th and 7th days after treatment in medium containing 6-thioguanine. It was incubated for 14 days, then scored for the presence or absence of colonies.

REPORT RESULTS: 1,2-dibromoethane was mutagenic in both human cell lines; at 5 ug/ml in AHH-1 cells, and at 20 ug/ml in Tk-6 cells. It demonstrated equal mutagenic potential in both cell lines. Although AHH-1 contains five times more glutathione S-transferase activity than TK-6 cells, there was no apparent increase in the mutagenic activity of 1,2-dibromoethane between the two cell lines.

CONCLUSION: Test material was mutagenic in the in vitro mammalian cell gene mutation assay using human lymphoblasts.

DATA QUALITY: Study was conducted in accordance with a recognized published scientific procedure for examining the mutagenic potential of a test compound in these cell lines and is consistent with OECD test guideline 476. Test method utilized recognized positive controls that gave the expected positive responses, confirming the sensitivity of the method. The purity of the test material was not stated.

RELIABILITY:

1. w/o restriction [ ]
2. w restriction [X]
3. not reliable [ ]
4. not assignable [ ]

Reason: Although the purity of the test material was not reported, the source was Aldrich Chemical Company, and samples of EDB produced by Aldrich Chemical Company are known to be 96-99% pure (see Robust summary CA-1).

2. REPORT NUMBER: MU-2

STUDY TYPE: In Vivo Sex-Linked Recessive Lethal Test in Drosophila Melanogaster (Gene Mutation)

TEST MATERIAL: 1,2-Dibromoethane

STUDY NUMBER(S): None reported.

SPONSOR: Not stated.

TESTING FACILITY: Central Laboratory for Mutagenicity Testing (Germany).

TITLE OF REPORT: Mutagenicity Testing of Cyclamate and Some Pesticides in Drosophila Melanogaster.

REPORT ISSUED or STUDY COMPLETION DATE: Experientia, 30(6), (1974), pg 621-623.

RECOGNIZED METHOD, i.e. OECD: Not stated; pre-dates OECD 477.

GLP: Pre-dates GLP.

TEST ORGANISM USED: Berlin K Drosophila melanogaster, male and female adults.

TEST COMPOUND CONCENTRATIONS USED: A single dose of 0.3 mM, administered in solution.

CONTROL MATERIALS: Positive control agent(s) were not reported. The negative control used (when necessary was DMSO or ethanol).

TEST PERFORMANCE: The test material was administered as a solution to 2 adult males for 3 days, after which they were mated to two females for 3 days. A sequence of two 3-day breeding periods was initiated followed by one 4-day brood. The male was transferred to a new vial and remated with two new females after each breeding period.

REPORT RESULTS: 1,2-dibromoethane was mutagenic in Drosophila producing the following lethals per number of chromosomes: 6/1201 (Brood 1), 18/1209 (Brood 2), and 16/1234 (Brood 3). Broods 2 and 3 represent the effect on spermatids and spermatocytes, respectively.

CONCLUSION: Test material was mutagenic in Drosophila melanogaster.

DATA QUALITY: Study was conducted prior to recognized standardized test methods or GLP. The purity of the test material was not stated, and sufficient information regarding the control materials used was not provided.

RELIABILITY:

1. w/o restriction [ ]
2. w restriction [ ]
3. not reliable [X]
4. not assignable [ ]

Reason: Insufficient information was provided regarding the purity of the test material and the protocol and procedures followed. Also, DMSO, used as a vehicle in this study, is recommended to be avoided as a vehicle for this particular test.

3. REPORT NUMBER: MU-3

STUDY TYPE: Dominant Lethal Assay in Rats and Mice

TEST MATERIAL: 1,2-Dibromoethane obtained from Tokyo Kasei Kogyo Company.

STUDY NUMBER(S): None reported.

SPONSOR: Ministry of Agriculture, Forestry and Fisheries (Japan).

TESTING FACILITY: Toxicology Division, Institute of Environmental Toxicology, Tokyo, Japan.

TITLE OF REPORT: Dominant Lethal Mutation Induced in Male Rats by 1,2-Dibromo-3-Chloropropane (DBCP)

REPORT ISSUED or STUDY COMPLETION DATE: Mutation Research, 77 (1980), pages 71-78.

RECOGNIZED METHOD, i.e. OECD: Not stated; pre-dates OECD 478.

GLP: Pre-dates GLP.

TEST ANIMALS USED: Male and female SD rats (12 weeks old) and BDF1 mice (10 weeks old).

TEST COMPOUND CONCENTRATIONS USED: Rat – 10 or 30 mg/kg; mice – 100 or 150 mg/kg. All doses administered via gastric intubation, dissolved in olive oil. All doses given once a day for 5 days.

CONTROL MATERIALS: Positive control agent was ethyl methanesulfonate (EMS) given i.p.. The negative control used was olive oil given orally.

TEST PERFORMANCE: Rat: 15 male rats were given the test material once a day for 5 days, then mated overnight with an untreated virgin female in the pro-estrus stage. Females were checked in the morning for the presence of vaginal plug or sperm, and separated from males. Females were mated one per male and continued for 10 successive weeks.
Mice: Groups of 7-9 male mice were given the test material once a day for 5 days, then mated with 2 untreated virgin females for 7 days. Females were examined every morning for the presence of a vaginal plug and caged separately when observed. Any females remaining in the male cage after one week were removed and replace with 2 new females, and continued for 6 weeks. The mouse experiment was repeated twice.
All pregnant rats and mice were killed 12-14 days after conception and the numbers of C.L., implants, live embryos and early and late embryonic deaths counted.

STATISTICS: The Mann-Whitney U Test was used for all results.

REPORT RESULTS: The test material did not affect mating performance in either species, and no adverse effects on number of implants, corpora lutea, live embryos or early and late embryonic deaths. The positive control, EMS, yielded the expected positive dominant lethal effects in both species.

CONCLUSION: 1,2-dibromoethane was not mutagenic in the dominant lethal assay in rats and mice.

DATA QUALITY: Study was conducted prior to recognized standardized test methods or GLP. Nonetheless, the method appears to follow recognized contemporary test methods for analyzing dominant lethal effects in rats and mice. The purity of the test material was not stated and the mating efficiency was not reported

RELIABILITY:

1. w/o restriction [ ]
2. w restriction [ ]
3. not reliable [X]
4. not assignable [ ]

Reason: Insufficient information was provided regarding the purity of the test material and the mating efficiency was not reported in the tables provided.

4. REPORT NUMBER: MU-4

STUDY TYPE: Dominant Lethal Assay in Mice

TEST MATERIAL: 1,2-Dibromoethane obtained from the National Institute of Environmental Health Sciences' chemical repository at Radian Corporation, Austin, TX..

STUDY NUMBER(S): None reported.

SPONSOR: Supported by NIEHS Contract No. NO1-ES-55078.

TESTING FACILITY: NIEHS, RTP, N. Carolina

TITLE OF REPORT: Ethylene dibromide: negative results with the mouse dominant lethal assay and the electrophoretic specific-locus test.

REPORT ISSUED or STUDY COMPLETION DATE: Mutation Research, 282 (1992), pages 127-133.

RECOGNIZED METHOD, i.e. OECD: Consistent with OECD 478, and Epstein et. al. (1972).

GLP:Yes.

TEST ANIMALS USED: Male DBA/2J mice (9 weeks old) and C57BL/6J female mice (12-16 weeks old).

TEST COMPOUND CONCENTRATIONS USED: 40 males received a single i.p. injection of 100 mg/kg of test material in physiologic saline.

CONTROL MATERIALS: Positive control agent was procarbazine given as single i.p. injection of 400 mg/kg to 10 males. The solvent control used was physiologic saline.

TEST PERFORMANCE: Male mice were sequentially mated at 4-day intervals to 12-16 week old virgin females. Females were not examined for vaginal plugs. They were killed 12 days following removal from the breeding cage and examined. Each female was checked for pregnancy, total number of implants, early fetal deaths.

STATISTICS: The Mann-Whitney U Test was used for all results. Comparisons of pregnancy rates in treated groups and solvent control were made using the one-sided Fisher Exact test.

REPORT RESULTS: The test material did not affect mating performance, the number of implants, live embryos or early and late embryonic deaths. The positive control, procarbazine, yielded the expected positive dominant lethal effects in both species.

CONCLUSION: 1,2-dibromoethane was not mutagenic in the dominant lethal assay in mice.

DATA QUALITY: Study was conducted at NIEHS, a facility known to follow recognized standardized contemporary test methods GLP regulations. The method appears to follow recognized contemporary test methods for analyzing dominant lethal effects in mice. The purity of the test material was not stated but came from their chemical repository demonstrated to use a material that is.>96% pure.

RELIABILITY:

1. w/o restriction [ ]
2. w restriction [X]
3. not reliable [ ]
4. not assignable [ ]

Reason: Although the purity of the test material was not reported, the source was the chemical repository for NIEHS. They had previously reported using a sample of EDB which was 96% pure (see Robust summary CA-1).

5. REPORT NUMBER: MU-5

STUDY TYPE: In Vitro Mammalian Cell Gene Mutation Assay

TEST MATERIAL: 1,2-Dibromoethane obtained from Matheson, Coleman & Bell.

STUDY NUMBER(S): None reported.

SPONSOR: Supported byUS EPA under Interagency Agreements 40-516-75 and 40-732-78, and the Office of Health and Environmental Research, US Dept. of Energy under contract W-7405-eng-26 with the Union Carbide Corporation..

TESTING FACILITY: Oak Ridge National Laboratory, Oak Ridge, TN

TITLE OF REPORT: Effect of metabolic activation on the cytotoxicity and mutegenicity of 1,2-dibromoethane in the CHO/HGPRT system.

REPORT ISSUED or STUDY COMPLETION DATE: Mutation Research, 85 (1982), pages 377-388.

RECOGNIZED METHOD, i.e. OECD: OECD 476; EPA Health Effects Testing Guidelines Subpart 798.5300.

GLP: Pre-dates GLP.

TEST CELLS USED: Chinese hamster ovary (CHO) cells, subline CHO-K_1-BH_4.

Properly maintained: Yes, in Ham's F-12 medium with newborn calf serum (10%)
Periodically checked for mycoplasm: Not stated.
Periodically checked for karyotype stability: Not stated.
Periodically "cleansed" against high spontaneous background: Not stated.

LOCUS EXAMINED: hypoxanthine-guanine phosphoribosyl transferase locus (HGPRT).

TEST COMPOUND CONCENTRATIONS USED: 0-5 mM

CONTROL MATERIALS:
Ethyl Methanesulfonate (EMS)
n-nitrosodimethylamine (DMN)
Solvent Control: DMSO
Metabolic Activation System:
S9 from rats treated with Aroclor 1254, with three separate fractions
(Mg-S9/Ca, Mg-S9/Ca-Mg-microsomes)

ACTIVATION: Aroclor 1254-induced rat liver S9 prepared from male Sprague-Dawley. Immediately prior to use, three variations of S9 were mixed as follows: Mg-S9: with 10 mM $MgCl_2$, 4 mM NADP, 5 mM G-6-P, 30 mM KCl, 10 mM $MgCl_2$ and 50 mM NaP buffer, pH 8.0. Ca, Mg-S9: same as above with $CaCl_2$ included. Ca-Mg-Microsome: 1 ml of microsome preparation added to 9 ml of buffered salt solution above and 10mM $CaCl_2$ and 0.5 units of G-6-P.

TEST PERFORMANCE: CHO/HGPRT Mutation Assay: Cells were seeded in F12CM5 and Ham's F12 medium at a density 5 x 10^5 cells/25 cm^2 flask, and incubated at 37°C in 5% CO_2 for 16 hrs. Cells to be treated in the presence of a metabolic activation system were washed once in saline G and the medium replaced with 4 ml of serum-free Ham's medium F12 and 1 ml of the appropriate activation system. Duplicate flasks were exposed to varying concentrations of test article for 5 hrs at 37°C. Afterward, cells were washed with saline G, F12CM5 added, and incubated overnight. The next day an estimation of cytotoxicity by mutagen treatment was determined by measuring the cloning efficiency of 200 cells plated in triplicate. Cells were subcultured to determine phenotypic expression. At the end of the subculture period (1-8 days), mutation frequency expressed as the number of mutant colonies per $1x10^6$ cells in 5 plates.

REPORT RESULTS: Several experiments within the test, using different activation systems and differing concentrations of EDB demonstrated that EDB is more cytotoxic in the presence of Ca, Mg-S9 than without it; however, the mutagenic activity remained unchanged. Resulsts supported the findings that metabolic activation does not increase the mutagenic activity of EDB, just the cytotoxicity. Further they concluded that the increase cytotoxicity was NADP dependent

CONCLUSION: It was not the intention to demonstrate whether or not EDB was mutagenic, but rather to examine its mutagenic potential under varying activation systems and exploration of detoxifying mechanisms.

DATA QUALITY: Study was conducted at Oak Ridge National Laboratory, a facility known to follow recognized standardized contemporary test methods and GLP regulations. However, this study was not a standard mutagenic assay, but an investigation into the causative factors, i.e. metabolic activating systems, that can alter EDB's cytoxicity and mutagenicity. Nonetheless, the test method does confirm the mutagenic potential for EDB in the CHO/HGPRT system. The purity of the test material was provided.

RELIABILITY:

1. w/o restriction [ ]
2. w restriction [ ]
3. not reliable [X]
4. not assignable [ ]

Reason: The purity of the test material was not provided and cannot be ascertained from information available in the report.

6. REPORT NUMBER: MU-6

STUDY TYPE: Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells In Vivo

TEST MATERIAL: 1,2-Dibromoethane obtained from Matheson, Coleman & Bell; redistilled before use.

STUDY NUMBER(S): None reported.

SPONSOR: Supported NIH grants CA 21820 and T-32-ES07091.

TESTING FACILITY: Arizona Health Sciences Center, University of Arizona, Tucson, AZ

AUTHORS: R.D. White, I.G. Sipes, A.J. Gandolfi and G.T. Bowden

TITLE OF REPORT: Characterization of the hepatic DNA damage caused by 1,2-dibromoethane using the alkaline elution technique.

REPORT ISSUED or STUDY COMPLETION DATE: Carcinogenesis, Vol2, No..9 (1981), pg839-844.

RECOGNIZED METHOD, i.e. OECD: Pre-dates OECD 486. Technique developed by Kohn, Erickson and Ewig (1976 and 1981). At present there is no international standardized test method for this assay.

GLP: Pre-dates GLP.

TEST CELLS USED: Male Swiss Webster mice (CFW) weighing 25-35 grams. EDB, dissolved in DMSO, was administered via i.p. injection 3 hours prior to sacrifice in order to facilitate rapid absorption into the liver.

PRINCIPLE OF THE TEST METHOD: This method was developed to evaluate DNA strand breaks. The alkaline elution assay uses isolated hepatic nuclei and separates single-stranded DNA on the basis of it length. The small pore size filter eliminates the passage of long-stranded DNA and allows the examination of single-stranded DNA breaks caused by xenobiotics. The elution rate is directly related to the number of single-strand breaks, and increases as the number of breaks increase.

TEST COMPOUND CONCENTRATIONS USED: 0, 25, 50 and 75 mg/kg in DMSO. The volume was kept constant.

CONTROL MATERIALS: DMSO used as vehicle control, and X-irradiation of hepatic nuclei (DNA cross-link assay).

TEST PERFORMANCE: Hepatic nuclei (treated and untreated) were loaded onto filters, lysed with buffer (sodium lauryl sulfate, and sodium EDTA, pH7.4); pH was adjusted to 10 with tetraethylammonium hydroxide. A pump was hooked to the filter, the pH of the buffer adjusted to 12.3 and the DNA elution was pulled through the filter. Six 3 ml fractions were collected and amount of DNA quantitated. Results (elution rate constants) were analyzed using the Student's t-test.

REPORT RESULTS: EDB caused a dose-dependent increase in the amount of damage to hepatic DNA, demonstrating the presence of alkali-labile sites in DNA.

CONCLUSION: It was not the intention to demonstrate whether or not EDB was mutagenic, but rather to examine whether bioactivation of EDB damages hepatic DNA by creating unstable adducts. The authors concluded that these results confirm previous efforts using sucrose density gradients that demonstrated the presence of DNA strand breaks after oral administration of EDB (75-220 mg/kg).

DATA QUALITY: This study was not a standard mutagenic assay for which an international standard method exists. It does conform to recognized published papers in peer-reviewed journals and is an investigation into the mechanisms for carcinogenesis in mammals. The purity of the test material was not provided, but was indicated to be redistilled and therefore presume it was reasonably pure (e.g. >96%).

RELIABILITY:

1. w/o restriction [ ]
2. w restriction [X]
3. not reliable [ ]
4. not assignable [ ]

7. REPORT NUMBER: MU-7

STUDY TYPE: Unscheduled DNA Synthesis (UDS) in Mammalian Cells In Vitro and In Vivo

TEST MATERIAL: 1,2-Dibromoethane obtained from Aldrich Chemical Company.

STUDY NUMBER(S): None reported.

SPONSOR: Not stated.

TESTING FACILITY: Chemical Industry Institute of Toxicology, RTP, NC.

AUTHORS: T. Smith-Oliver, B. E. Butterworth, R. D. White and P.K. Working

TITLE OF REPORT: Induction of DNA Repair in rat spermatocytes and hepatocytes by 1,2-dibormoethane: role of glutathione conjugation.

REPORT ISSUED or STUDY COMPLETION DATE: Carcinogenesis, Vol7, No..3 (1986), pg467-472.

RECOGNIZED METHOD, i.e. OECD: Pre-dates OECD 482.

GLP: Not stated. However, it is known that studies conducted at CIIT followed GLP regulations when they were promulgated.

TEST CELLS USED: CDF (F344)/CrlBr male rats weighing 250-290 grams.

PRINCIPLE OF THE TEST METHOD: This method was developed to evaluate the effects of xenobiotics on DNA damage, quantitated as unscheduled DNA synthesis.

TEST COMPOUND CONCENTRATIONS USED: 10, 50 and 100 uM in DMSO (1%) in vitro; and 10, 50 and 100 mg/kg in corn oil in vivo (given i.p. and p.o).

CONTROL MATERIALS: DMSO used as vehicle control, and DMN (hepatocytes) and Methylmethanesulfonate (MMS) (spermatocytes) as positive controls.

TEST PERFORMANCE: In vitro: Hepatocytes and Spermatocytes were isolated and exposed for 18 hours to EDB and 10 uCi/ml ^{3}H-thymidine. In vivo: Rats were treated by gavage or i.p. injection with EDB in corn oil. Exposure times were based on previous experience to maximize positive results in both spermatocytes and hepatocytes. Hepatocytes were

isolated and incubated for 4 hours in Williams Medium and 10 uCi/ml ^{3}H-thymidine. Spermatocytes were isolated and incubated for 24 hours in Williams Medium and 10 uCi/ml ^{3}H-thymidine. DNA repair was measured as unscheduled DNA synthesis (UDS) by quantitative autoradiography as net grains per nucleus (NG). A positive response in hepatocytes was an average >5 NG, and in spermatocytes it was >2NG in >10% of cells responding. The effects of pretreatment with metyrapone or diethylmaleate, both in vitro and in vivo, were included to evaluate the influence of hepatic mixed function oxidases (e.g. cytochrome P450) and glutathione, respectively, on EDB's genotoxicity.

REPORT RESULTS: In vitro exposure to EDB caused a dose-dependent increase in DNA repair in both spermatocytes and hepatocytes. Following in vivo exposure to EDB, only hepatocytes exhibited UDS and then only when EDB was given by i.p. injection and only at the high dose. Spermatocytes did not show UDS after in vivo exposures.

CONCLUSION: The results demonstrated that the liver mixed function oxidases do not play a role in the production of genotoxic metabolites of EDB, in vitro or in vivo. EDB induction of UDS was decreased when liver glutathione was depleted. Thus, the authors conclude that the conjugation of EDB to glutathione may be the first step in the production of genotoxic metabolite in hepatocytes both in vitro and in vivo. The same pathway was suggested to be functioning in spermatocytes as well..

DATA QUALITY: This study conforms to recognized UDS assays that have been standardized nationally and internationally. It also conforms to recognized published papers in peer-reviewed journals and is an investigation into the mechanisms for carcinogenesis in mammals. The purity of the test material was not provided.

RELIABILITY:

1. w/o restriction [ ]
2. w restriction [X]
3. not reliable [ ]
4. not assignable [ ]

Reason: Although the purity of the test material was not reported, the source was Aldrich Chemical Company, and samples of EDB produced by Aldrich Chemical Company are known to be 96-99% pure (see Robust summary CA-1).

8. REPORT NUMBER: MU-8

STUDY TYPE: *Salmonella typhimurium* Reversion Assay

TEST MATERIAL: 1,2-Dibromoethane obtained from Aldrich Chemical Company.

STUDY NUMBER(S): None reported.

SPONSOR: Supported in part by grants from the Ministry of Agriculture, Forestry and Fisheries and the Ministry of Education, Science and Culture, Tokyo, Japan.

TESTING FACILITY: Institute of Environmental Toxicology, Tokyo, Japan

TITLE OF REPORT: Further Mutagenicity Studies on Pesticides in Bacterial Reversion Assay Systems

REPORT ISSUED or STUDY COMPLETION DATE: Published in Mutation Research, 116, pg. 185-216, 1983.

RECOGNIZED METHOD, i.e. OECD: OECD test guideline 471. Procedure followed Ames et al. (1975).

GLP: Not stated.

TEST ORGANISM USED: *Salmonella typhimurium* strains: TA98, TA100, TA1535, TA1537 and TA1538, and Escheria coli WP2 her. Strains were cultured overnight in liquid nutrient broth and stored at –80 degrees C. Their genetic markers and response to positive controls and number of spontaneous revertants were checked.

TEST COMPOUND CONCENTRATIONS USED: 5 concentrations were evaluated with appropriate vehicle and positive controls. Doses used: 50, 100, 500, 1000 and 5000 ug/plate.

CONTROL MATERIALS: The positive control materials that were used were not identified in the published report. The negative control was Distilled water or DMSO.

ACTIVATION: Source and preparation of the S-9 fraction was not identified.

TEST PERFORMANCE: Salmonella typhimurium reversion assay as described by Ames et al. (1975).

PRELIMINARY CYTOTOXICITY ASSAY: The report did not indicate whether these had been done. Based upon the number of pesticides examined it is most likely this information was derived from the manufacturer or literature. The results were not expressed as the number of revertants per survivors, but as revertants per plate. Therefore, toxicity was not examined in this study.

PROTOCOL: Tests were carried out according to Ames et al. (1975). Each dose level of the compound was tested with and without the S9 mix with each strain of *S. typhimurium*. When E. coli was used, histidine and biotin in top agar were replaced by tryptophan at the same concentration. 0.1 ml of bacterial culture of each indicator strain were added to test tubes containing 2 ml of molten agar supplemented with biotin and a trace of histidine, 0.1 ml of the appropriate dilution/suspension of the test product and 0.5 ml of the S9 mix, if any. The ingredients were mixed and immediately poured onto the minimal agar plates with modified Vogel-Bonner E medium. After the top agar has set, the plates are incubated at 37 degrees C for 2 days. Plates were scored for number of revertants/plate.

REPORT RESULTS: Test compound did induce a significant increase in the number of revertant colonies for *Salmonella typhimurium* strains TA1535, TA100, TA98 (weak) and in E. coli, with and without S9 activation, at doses up to and including 5000 ug/plate.

CONCLUSION: Test material was mutagenic in *S. typhimurium* TA 98, TA100 and TA1535 and E. coli WP2 her with and without S9 activation.

DATA QUALITY: Study was conducted in accordance with recognized published scientific procedure for examining the mutagenic potential of a test compound in *S. typhimurium* bacteria strains. Test method utilized did not state what positive controls were used. There were no results presented with respect to a "preliminary cytotoxicity assay" for determining the appropriate dose levels to use. It is not known whether the test was conducted in compliance with GLP regulations. The purity of the test material was identified.

RELIABILITY:

1. w/o restriction [ ]
2. w restriction [ ]
3. not reliable [x]
4. not assignable [ ]

REASON: The report lacked certain specific information such as purity of test material, cytotoxicity dose levels, the number of revertants/survivors, and other details.

Reference: Ames, B.N., McCann, J. and Yamasaki, E. (1975). Methods for detecting carcinogens and mutagens with Salmonella/Mammalian-Microsome Mutagenicity Test. Mutation Research, 31, 347-364.

D. DEVELOPMENTAL TOXICITY

1. REPORT NUMBER: DE-1

STUDY TYPE: Developmental Toxicity in Rats

TEST MATERIAL: Ethylene dibromide; purity 99%; obtained from Aldrich Chemical Company.

STUDY NUMBER(S): Contract No. 68-01-3242; EPA-560/6-77-028

SPONSOR: U.S. Environmental Protection Agency

TESTING FACILITY: Biological Sciences Division, Midwest Research Institute, Kansas City, Missouri

TITLE OF REPORT: Toxicity Studies of Selected Chemicals Task IV: The Developmental Toxicity of Ethylene Dibromide Inhaled by Rats and Mice During Organogenesis.

AUTHORS: R.D. Short, J.L. Minor, J.M. Winston, J. Seifter and Cheng-Chun Lee

REPORT ISSUED or STUDY COMPLETION DATE: June 1977

RECOGNIZED METHOD, i.e. OECD: Pre-dates OECD and US EPA Test Guidelines.

GLP: Study pre-dates GLP.

SPECIES/SEX: Charles River CD rats; females were nulliparous; males of the same strain and source were used for mating.

AGE at Start of Test: Sexually mature females, age not specified.

ROUTE: Inhalation.

DURATION OF TEST: All females were sacrificed on day 20 of gestation.

DOSE LEVEL(s) and NUMBER OF DOSES: 0, 20, 38 and 80 ppm (based upon time weighted average), administered on days 6 thru 15 of gestation; 23 hrs per day for 10 consecutive days. Two control were used, one with unrestricted food and one with restricted access to food.

NUMBER OF ANIMALS/DOSE: 15-17F per dose. Pregnancy determined by daily inspection by vaginal smear for sperm.

VEHICLE: None.

INHALATION CHAMBER DESIGN: Rochester type stainless steel chamber with a volume of 3.5 m^3. Clean air flow rate of 10-12 changes per hour. EDB vapor was generated by bubbling nitrogen into a glass vessel that was maintained at 30 degrees C. EDB was mixed in the air stream in a plenum at the top of the chamber. EDB concentration measured every 2 hours during exposure. During the 10 day inhalation period, EDB-treated rats were housed in the inhalation chamber. The feed was changed daily to prevent possible accumulation of EDB in the feed.

CAGING/HOUSING: Each female was paired with one male for mating. After confirmation of mating, females were returned to their cage.

CAGESIDE OBSERVATIONS: Observations were made of maternal welfare and fetal development. Body weight and feed consumption were measured during and after the exposure period.

STATISTICAL METHODS: The Fisher exact test and Mann-Whitney 2-tailed rank test were used to analyze the data.

GROSS PATHOLOGY: Females were sacrificed on day 20 and the abdominal and thoracic cavities were examined. The umbilical cord was clamped and severed distally in order to prevent blood loss. Fetuses were removed from the uterine horn, weighed and examined for external anomalies. One half of the viable fetuses were fixed in Bouin's solution and examined for soft tissue anomalies by free-hand slicing method. The remaining viable fetuses were fixed in 70% alcohol, eviscerated, stored in 1% KOH, stained in alizarin red, and the skeletons examined for anomalies.

FERTILITY AND REPRODUCTIVE PERFORMANCE: The following data were recorded for each group.

- o numbers of resorptions (early and late), and viable and dead fetuses.
- o number of pregnant females (based upon presence of implantation sites at autopsy.
- o number of live fetuses/dam
- o fetal weight

FINDINGS/MEASURED ENDPOINT/INDEX (i.e. LOEL, NOAEL): Deaths were reported at 80 ppm. Weight loss was evident at 38 and 80 ppm. Feed consumption was reduced at all EDB concentrations and remained depressed at 80 ppm after termination of exposure. There were a reduced number of implants and increased resorptions at 80 ppm. Body weight of fetuses was reduced at 38 ppm. Since there were no fetuses at 80 ppm, this endpoint could not be determined. There were no external soft tissue or skeletal anomalies observed that were of concern. A Maternal NOAEL in this developmental toxicity test was not demonstrated, owing to the decreased body weight gain and feed consumption during exposure at 20 ppm. An NOAEL for fetal effects was demonstrated at 20 ppm, with decreased number of viable fetuses and increased resorptions at 38 ppm.

Summary of effects of EDB on rats during organogenesis:

	EDB (ppm)				
	0	20	38	80	0 (feed restrict)
Number of Rats Exposed	17	15	16	16	16
Pregnant	17	11	15	16	15
Alive	17	11	15	8*	15
Non-pregnant	0	4	1	0	1
Alive	0	4	1	0	1
Body Weight Change					
During Exposure	39	28	-30*	-77*	-47*
After Exposure	59	69	67	11*	92*
Feed Consumption					
During Exposure	19	15*	9*	3*	4*
After Exposure	22	21	22	7*	25
Pregnant Survivors	17	11	15	8*	15
Implants/Dam	14.5	13.7	12.7	11.3*	13.1
Viable Fetuses (%)	96	98	98	0*	97
Dead Fetuses (%)	0	0	0	13	0
Early resorptions (%)	4	2	2	88	3
Late resorptions (%)	0	0	0	0	0
Dams with Complete resorptions	0	0	0	7*	0
Live Litters	17	11	15	0	15
Fetuses/dam	13.9	13.4	12.5	-	12.5
Males (%)	45	53	43	-	48
Fetal Weight (gm)	4	3.9	3.6*	-	3.4*

* significantly different from control.

CONCLUSION: EDB, via inhalation, had an adverse effect on maternal welfare, measured by decreased body weight gain, feed consumption, and survival. There were no anomalies observed in the soft tissue or skeletal tissue.

DATA QUALITY: Study was conducted at Midwest Research Institute, under contract to US EPA, and a facility known to follow recognized standardized contemporary test methods and GLP regulations. This study was conducted prior to GLP regulations and standard international test methods. The study, however, meets recognized contemporary standards for analyzing the developmental toxicity of a test material in laboratory animals. The purity of the test material was provided. Certain details of the protocol were not provided, such as: the body weight of the pregnant females (only the weight change), the number of females mated (only the number pregnant), the number of males used, the number of corpora lutea, and temperature and humidity of the inhalation chamber (only the temperature and humidity of the facility). These omissions do not detract from the overall scientific quality of the study conducted and reported.

RELIABILITY:

1. w/o restriction [ ]
2. w restriction [X]
3. not reliable [ ]
4. not assignable [ ]

2. REPORT NUMBER: DE-2

STUDY TYPE: Developmental Toxicity in Mice

TEST MATERIAL: Ethylene dibromide; purity 99%; obtained from Aldrich Chemical Company.

STUDY NUMBER(S): Contract No. 68-01-3242; EPA-560/6-77-028

SPONSOR: U.S. Environmental Protection Agency

TESTING FACILITY: Biological Sciences Division, Midwest Research Institute, Kansas City, Missouri

TITLE OF REPORT: Toxicity Studies of Selected Chemicals Task IV: The Developmental Toxicity of Ethylene Dibromide Inhaled by Rats and Mice During Organogenesis.

AUTHORS: R.D. Short, J.L. Minor, J.M. Winston, J. Seifter and Cheng-Chun Lee

REPORT ISSUED or STUDY COMPLETION DATE: June 1977

RECOGNIZED METHOD, i.e. OECD: Pre-dates OECD and US EPA Test Guidelines.

GLP: Study pre-dates GLP.

SPECIES/SEX: CD-1 mice; females were nulliparous; males of the same strain and source were used for mating.

AGE at Start of Test: Sexually mature females, age not specified.

ROUTE: Inhalation.

DURATION OF TEST: All females were sacrificed on day 18 of gestation.

DOSE LEVEL(s) and NUMBER OF DOSES: 0, 20, 38 and 80 ppm (based upon time weighted average), administered on days 6 thru 15 of gestation; 23 hrs per day for 10 consecutive days. Two control groups were used, one with unrestricted food and one with restricted access to food.

NUMBER OF ANIMALS/DOSE: 18-22F per dose. Pregnancy determined by daily inspection for presence of copulation plugs.

VEHICLE: None.

INHALATION CHAMBER DESIGN: Rochester type stainless steel chamber with a volume of 3.5 m^3. Clean air flow rate of 10-12 changes per hour. EDB vapor was generated by bubbling nitrogen into a glass vessel that was maintained at 30 degrees C. EDB was mixed in the air stream in a plenum at the top of the chamber. EDB concentration measured every 2 hours during exposure. During the 10 day inhalation period, EDB-treated mice were housed in the inhalation chamber. The feed was changed daily to prevent possible accumulation of EDB in the feed.

CAGING/HOUSING: Each female was exposed to proven male breeders. After confirmation of mating, females were returned to their cage.

CAGESIDE OBSERVATIONS: Observations were made of maternal welfare and fetal development. Body weight and feed consumption were measured during and after the exposure period.

STATISTICAL METHODS: The Fisher exact test and Mann-Whitney 2-tailed rank test were used to analyze the data.

GROSS PATHOLOGY: Females were sacrificed on day 18 and the abdominal and thoracic cavities were examined. The umbilical cord was clamped and severed distally in order to prevent blood loss. Fetuses were removed from the uterine horn, weighed and examined for external anomalies. One half of the viable fetuses were fixed in Bouin's solution and examined for soft tissue anomalies by free-hand slicing method. The remaining viable fetuses were fixed in 70% alcohol, eviscerated, stored in 1% KOH, stained in alizarin red, and the skeletons examined for anomalies.

FERTILITY AND REPRODUCTIVE PERFORMANCE: The following data were recorded for each group.

- o numbers of resorptions (early and late), and viable and dead fetuses.
- o number of pregnant females (based upon presence of implantation sites at autopsy.
- o number of live fetuses/dam
- o fetal weight

FINDINGS/MEASURED ENDPOINT/INDEX (i.e. LOEL, NOAEL): Deaths were reported at 38 and 80 ppm, and also in the control group on a restricted diet. It was reported the control deaths were due to cannibalism, which means they were grouped housed. Weight loss was evident at 20 and 38 ppm (there were no animals alive in the 80 ppm group). There were reduced fetal body weights and increased late resorptions at 20 ppm. At 38 ppm there were reduced numbers of viable fetuses, increased resorptions and reduced fetal body weight. In the restricted feed control groups there were reduced viable fetuses and increased resorptions. Since there were no fetuses at 80 ppm, these endpoints could not be determined. There were 3/218 fetuses with exencephally at 20 ppm. The number of runts was increased at 38 ppm. The number of skeletal anomalies observed was increased in the 20 and 38 ppm groups. These ranged from incomplete ossifications to unossified skeletal tissue. A NOAEL in this developmental toxicity test was not demonstrated.

Summary of effects of EDB on mice during organogenesis (reported as percentages, unless otherwise indicated):

	EDB (ppm)				
	0	20	38	80	0 (feed restrict)
Number of Mice Exposed	18	20	20	22	18
Pregnant	18	19	17	19	9
Alive	18	19	10*	0*	6*
Non-pregnant	0	1	3	3	4
Alive	0	1	3	0	4
Body Weight Change					
During Exposure	16.2	7.5*	-3.8*	-	-8.8*
After Exposure	5.6	6.5	0.5	-	4.6
Feed Consumption					
During Exposure	6.3	3.9*	2.3*	0.8*	0.9*
After Exposure	6.4	5.6	2.9	-	5.8

Pregnant Survivors	18	19	10	0	6
Implants/Dam	12.3	12.8	12.3	-	9.2
Viable Fetuses (%)	94	90	35*	-	2*
Dead Fetuses (%)	0	0	1	-	0
Early resorptions (%)	4	4	51	-	88*
Late resorptions (%)	2	6*	13	-	10
Dams with Complete resorptions	0	0	6*	-	5*
Live Litters	18	19	4	0	1
Fetuses/dam	11.5	11.5	10.8	-	1
Males (%)	53	58	52	-	100
Fetal Weight (gm)	1.35	1.09*	0.63*	-	0.98

* significantly different from control.

Some of the more significant skeletal anomalies observed in mice are summarized below (reported as percentages, unless otherwise indicated):

	EDB (ppm)		
	0	20	38
Litters inspected (number)	18	19	4
Fetuses inspected (number)	110	115	24
Nasal bones: curved medially	1.9	16.1	20.8
Occipital fontanel enlarged	0	15.7	88.1
Supraoccipital: unossified	0	1.8	41.4
Incomplete ossification	0	15.7	54.8
Incus unossified	1.9	28.5	95.8
Hyoid bone: unossified	0	4.6	35.5
Incomplete ossification	0	0.8	21.7
Sternebrae: Ossified normally	75.2	64.5	0
Unossified	4.7	7.5	91.7
Incompletely ossified	8	19.3	54.3
Split	0	13	36.3
Paws: unossified	0	0	25
Phalanges unossified	0	11.4	70.8

CONCLUSION: EDB, via inhalation, had an adverse effect on maternal welfare, measured by decreased body weight gain, feed consumption, and survival. There were increased incidences of skeletal anomalies observed in the mice exposed to EDB.

DATA QUALITY: Study was conducted at Midwest Research Institute, under contract to US EPA, and a facility known to follow recognized standardized contemporary test methods and GLP regulations. This study was conducted prior to GLP regulations and standard international test methods. The study, however, meets recognized contemporary standards for analyzing the developmental toxicity of a test material in laboratory animals. The purity of the test material was provided. Certain details of the protocol were not provided, such as: the body weight of the pregnant females (only the weight change), the number of females mated (only the number pregnant), the number of males used, the number of corpora lutea, and temperature and humidity of the inhalation chamber (only the temperature and humidity of the facility). These omissions do not detract from the overall scientific quality of the study conducted and reported.

RELIABILITY:

1. w/o restriction [ ]
2. w restriction [X]
3. not reliable [ ]
4. not assignable [ ]

E. REPRODUCTIVE TOXICITY

1. REPORT NUMBER: RP-1

STUDY TYPE: Reproductive Toxicity in Rats

TEST MATERIAL: Ethylene dibromide; purity 99%; obtained from Aldrich Chemical Company.

STUDY NUMBER(S): Contract No. 68-01-3242

SPONSOR: U.S. Environmental Protection Agency

TESTING FACILITY: Pharmacology and Toxicology, Midwest Research Institute, Kansas City, Missouri

TITLE OF REPORT: Effects of Ethylene Dibromide on Reproduction in Male and Female Rats

AUTHORS: R.D. Short, J.L. Minor, J.M. Winston, J. Seifter, Chuen-Bin Hong and Cheng-Chun Lee

REPORT ISSUED or STUDY COMPLETION DATE: Toxicology and Applied Toxicology, 49, 97-105 (1979).

RECOGNIZED METHOD, i.e. OECD: Pre-dates OECD and US EPA Test Guidelines.

GLP: Study pre-dates GLP.

SPECIES/SEX: Charles River CD rats; females were nulliparous; males of the same strain and source were used for mating.

AGE at Start of Test: Sexually mature males and females, age not specified.

ROUTE: Inhalation.

DURATION OF TEST: All females were sacrificed mid-gestation, calculated from the midweek of their presumptive mating. One group of males was killed after the 10 week exposure period. Another group was mated with females for 2 weeks and then killed.

DOSE LEVEL(s) and NUMBER OF DOSES: Four groups of males were exposed to 0, 19, 39 and 89 ppm (based upon time weighted average), administered 7 hr/day, 5 days/week for 10 weeks. Afterwards, 9-10 males/group were killed. An additional 9-10 males were housed individually with 2 females for mating for two weeks and then killed. In a separate part of the study, 4 groups of females were exposed to 0, 20, 39, or 80 ppm 7 hr/day, 7 days/week for 3 weeks, mated with males, and then killed at mid-gestation.

NUMBER OF ANIMALS/DOSE: The number of animals varied with each protocol. Generally, there were 30-33 males and 20 females/dose group. Pregnancy determined by daily inspection of vaginal smear for sperm.

VEHICLE: None.

INHALATION CHAMBER DESIGN: Rochester type stainless steel chamber with a volume of 3.5 m^3. Clean air flow rate of 10-12 changes per hour. EDB vapor was generated by bubbling nitrogen into a glass vessel that was maintained at 60 degrees C. EDB was mixed in the air stream in a plenum at the top of the chamber. EDB concentration measured 3 times/day and a time-weighted average calculated. Rats removed from inhalation chamber after each daily exposure. Rats had free access to food and water except during the 7 hour exposure period.

CAGING/HOUSING: Males exposed: Males mated with females were caged one male to two virgin females, and each male was mated with 4 females. Females exposed: After exposure, two females mated to one male and then females killed at mid-gestation.

CAGESIDE OBSERVATIONS: Animals were observed for mortality and body weight change and food consumption were measured.

STATISTICAL METHODS: Comparisons of analysis of variance, the Fisher's exact test and Mann-Whitney 2-tailed rank test were used to analyze the data.

GROSS PATHOLOGY: Males exposed for 10 weeks were killed, testes weighed and blood from the aorta obtained to measure serum testosterone. Males exposed and then mated with females for two weeks were killed and their reproductive organs removed and examined histologically. This included testes, epididymides, seminal vesicle, and prostate. Females mated to these males were killed at mid-gestation, and the number of implants, viable implants and resorptions determined. Females exposed and then mated with untreated males, were killed and the number of implants, viable implants, and resorptions determined. Also, their uterus and ovaries were examined histologically.

FINDINGS/MEASURED ENDPOINT/INDEX (i.e. LOEL, NOAEL):
Observations: Males exposed to 39 and 89 ppm EDB had reduced body weight gain, and mortality was increased at 89 ppm (7/33). No adverse effects at 19 ppm. Females exposed to 80 ppm had reduced body weight gain and food consumption. Mortality was also increased at 80 ppm (10/50). No adverse effects at 20 and 39 ppm.
Reproductive performance: Males exposed to 89 ppm failed to impregnate any females. Females exposed to 80 ppm showed less frequent matings and pregnancies, with only 40% of the exposed rats becoming pregnant. There was no apparent effect on implants, resorptions or viable implants/dam. Females exposed to 80 ppm were in diestrus and did not begin to cycle until 3-4 days after exposure ended. This resulted in fewer females mating during the 10 week mating period.
Histological examination: Males exposed to 89 ppm displayed atrophy of the testes (10/10), epididymides (10/10), seminal vesicles (9/9) and prostate (10/10). There was also calcification of the testes and sperm granuloma in the epididymides. Marginala effects in the uterus and ovaries were observed in females exposed to 80 ppm (i.e. mild vacuolated degeneration of the epithelium of the uterus and ovarian cysts)

CONCLUSION: EDB had an adverse effect on reproductive performance at inhalation exposures of 89 ppm in males and 80 ppm in females. Males had reduced testicular weights and serum testosterone levels, atrophy of the reproductive organs and failed to impregnate any female rats. Females exposed to 80 ppm had abnormal estrus cycles during exposure. This returned to normal when exposure was terminated. Authors noted that the male protocol of 10 weeks exposure and 2 weeks mating was similar to a dominant lethal assay by Green et al. (1977), and did not cause dominant lethal mutations at 19 and 39 ppm.

DATA QUALITY: Study was conducted at Midwest Research Institute, under contract to US EPA, and a facility known to follow recognized standardized contemporary test methods and GLP regulations. This study was conducted prior to GLP regulations and standard international test methods. However, the study did not extend the observations to the rearing, weaning, lactation and other indices incorporated in single or multi-generation reproduction study. It is a study of the potential effects of inhalation exposure on the reproductive performance in males and females, but does not measure the effects in their progeny. The purity of the test material was provided. The present study would also be considered an acceptable dominant lethal assay for germinal mutations.

RELIABILITY:

1. w/o restriction [ ]
2. w restriction [X]
3. not reliable [ ]
4. not assignable [ ]

2. REPORT NUMBER: RP-2

STUDY TYPE: Examination of Effects on Sperm and Fertility in Rabbits

TEST MATERIAL: Ethylene dibromide; purity 99%; obtained from Aldrich Chemical Company.

STUDY NUMBER(S): Contract No. IR920859

SPONSOR: National Toxicology Program, NIEHS, NC

TESTING FACILITY: Research Triangle Institute, RTP, NC

TITLE OF REPORT: The Effects of Ethylene Dibromide on Semen Quality and Fertility in the Rabbit: Evaluation of a Model for Human Seminal Characteristics.

AUTHORS: J. Williams, B. C. Gladen, T.W. Turner, S.M. Schrader and R.E. Chapin

REPORT ISSUED or STUDY COMPLETION DATE: Fundamental and Applied Toxicology, 16, 687-700 (1991).

RECOGNIZED METHOD, i.e. OECD: There are no international test standards for this type of study.

GLP: Yes.

SPECIES/SEX: Sexually mature noninbred New Zealand White rabbits. Mean age of 9 months when received and trained for 1-2 months prior to exposure. Mean weight was 4.3 kg. Virgin female rabbits were 5-7 months old.

ROUTE: Subcutaneous.

DURATION OF TEST: Dosed for 5 days, and then evaluated for up to 12 weeks post-dosing.

DOSE LEVEL(s) and NUMBER OF DOSES: EDB given in corn oil at 0, 15, 30, or 45 mg/kg body weight.

NUMBER OF ANIMALS/DOSE: 8-10 males/group for semen examination; 3 females/male/time point in the fertility study.

VEHICLE: Corn oil.

CAGING/HOUSING: Housed individually.

CAGESIDE OBSERVATIONS: Animals were observed for mortality, clinical signs and body weight change and food consumption.

STATISTICAL METHODS: Mixed-model analysis of variance used for all parameters.

TEST PROTOCOL: Sexually mature male rabbits were dosed subcutaneously. Weekly semen samples for 6 weeks pre-exposure, during the week of exposure and 12 weeks post-exposure were collected and analyzed. Analysis included: sperm concentration, number, morphology, viability, motion parameters, pH, osmolality, volume, fructose, citric acid, carnitine, protein, and acid phosphatase. Male fertility was assessed pre-exposure, at 4 weeks and 12 weeks post-exposure by artificial insemination of 3 females/male/time point. Time points were (1) pre-exposure, (2) 4 weeks post-exposure (for effects on spermatid) and 12 weeks post-exposure (for effects on fertility following complete spermatogenesis).There were 6 males per dose group. The percent pregnant, litter size, fetal body weight and structural development were examined.

CLINICAL CHEMISTRY: Blood samples were collected pre-exposure, 3 hrs, 3, 7 and 14 days post-exposure. Blood analyzed for: sorbitol dehydrogenase (SDH), alanine transaminase (ALT), total bile acids, cholesterol, protein, alkaline phosphatase (ALK), 5'-nucleotidase (NUC), creatine kinase (CK), albumin, urea nitrogen, and creatine.

GROSS PATHOLOGY: At termination of the study, rabbits were killed and target organs weighed and fixed in formalin (liver and kidney) or Bouin's fluid (testis and epididymides). Tissues were examined histologically.

FINDINGS/MEASURED ENDPOINT/INDEX (i.e. LOEL, NOAEL): 3 rabbits in the high dose group were killed in extremis on the last day of dosing. Rabbits in the high dose exhibited hunched posture, lethargy, rough coat and hypothermia. Food consumption was decreased in a dose-related manner in all dose groups, but there were not body weight

reductions. Changes in clinical chemistry measurements were evident in the high dose group only, in practically all endpoints measured (most were elevated above normal values). Of the 13 parameters measured to assess the effects on sperm, only 3 were adversely effected by treatment with EDB: motion characteristics (curvilinear and straight line velocities), ALH (amplitude of lateral head displacement), and percentage of motile sperm. All three parameters were decreased in comparison to controls. Accessory gland function was examined in 11 parameters which were measured in rabbit semen. Of these, 4 were adversely effected: pH, ejaculate volume, total and tartrate-resistant AP activities. Male fertility was not effected by treatment as determined by the percentage of pregnant females or mean litter size. The only apparent effect was a decrease in the mean fetal body weight when adjusted for litter size at 4 weeks post-dosing in high dose males rabbits. No increase in anomalies were determined. Histologically there was liver and bile duct damage (hepatocyte necrosis and bile duct proliferation/hyperplasia) in high dose males. The authors concluded that EDB has an adverse effect on sperm which is evident at doses which also caused 30% mortality and liver damage in 43% of the survivors. The authors suggest that the effects on sperm are at the level of the epididymis (epididymal spermatozoa, late spermatids, and increased AP activity in seminal plasma).

CONCLUSION: This study was conducted to assess the male reproductive effects of EDB in the rabbit in an effort to evaluate the rabbit as suitable animal model for human response. The rabbit was not as sensitive as humans, based upon other experimental evidence, but is considered an acceptable animal model for human response to EDB exposure since semen quality is altered in both species.

DATA QUALITY: There are no international standards for performance of these types of studies. It was an examination of the adverse effects of EDB on the reproductive organs, performance, and sperm/semen quality in an animal species selected to be a suitable surrogate for humans. It was conducted by recognized experts in this field and in a laboratory known to be at the forefront of performing such reproductive experiments in animals. It is also known that this laboratory follows GLP procedures. This study examined one aspect of the effects of xenobiotics on reproductive performance in laboratory animals, and its limitations are based upon that premise.

RELIABILITY:

1. w/o restriction [ ]
2. w restriction [X]
3. not reliable [ ]
4. not assignable [ ]

TABLE 1: DATA MATRIX : HUMAN HEALTH EFFECTS STUDIES FOR: **ETHYLENE DIBROMIDE**

CAS #	ACUTE LD50/LC50			IRRITATION		SKIN	REPEAT DOSE	REPRO	DEVELOP -MENTAL	GENETIC TOXICOLOGY	
	Oral	Dermal	Inhalation	Eye	Skin	Sensitize				Gene Mutation	Chromosome Aberration
106-93-4	AO-1 (2)	AD-1 (2)	AI-1 (2)	EI-1 (3)			SC-1 (2)	RP-1 (2)	DE-1 (2)	MU-1 (2)	MU-3 (3)
	AO-2 (2)						SC-2 (1)	RP-2 (2)	DE-2 (2)	MU-2 (3)	MU-4 (2)
										MU-8 (3)	MU-5 (3)
							CA-1 (2)				MU-9 (2)**
							CA-2 (1)			OTHER GENETIC TOXICOLOGY ENDPOINTS	
							CA-3 (1)			UDS	MU-6 (2)
										UDS	MU-7 (2)

Each entry denotes a study reference number, for example: AO-2, EI-1, SC-1, etc., followed by Klimisch Rating in ( )

Klimisch Ratings are ranked as follows: (1) = reliable without restriction; (2) = reliable with restriction; (3) = not reliable; and (4) = not assignable.

*SC are Subchronic studies and CA-1 and CA-2 are Carcinogenicity studies.

**Note there is no separate Robust summary for MU-9, since it is part of the reproduction study, RP-1.

[Health & Environmental Horizons, Ltd., 12/4/01]