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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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Casweell # 555

NOV 15 1988

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTAN

SUBJECT: Review of Data on Methyl Bromide (CAS 555)
Record 222234
EPA ID #53201-1
Project 80927

FROM: William L. Burnam, Acting Division Director
Health Effects Division (TS-769C)

11/15/88

TO: Jeff Kempter PM-32
Disinfectant Branch
Registration Division (TS-767C)

The attached review completes this action on Methyl Bromide. Mr. Chitlik's comments on the reproduction study and Bill Sette's comments on a neurotoxicity study have previously been forwarded to you.

The subchronic inhalation study was supplementary but basisally designed as a dose-setting study prior to NTP's long term oncogenicity study in mice by the inhalation route. This oncogenicity study has not been completely finished. The present study lacks some details and protocols which our EPA Guidelines recommend but it does provide useful information concerning Methyl Bromide. The raw data are needed prior to a determination of the acceptability of the study to satisfy data requirement.

The five mutagenicity screening studies were unacceptable and do not fulfill the mutagenicity battery data requirement.

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EPA: 68D80056
DYNAMAC No.: 111-A
October 17, 1988

DATA EVALUATION RECORD

Methyl Bromide

Subchronic Inhalation Study in Mice

STUDY IDENTIFICATION: R. T. Drew. A 90-day inhalation study of methyl bromide toxicity in mice. (Unpublished study No. BNL 34506 conducted by Brookhaven National Laboratory, Upton, NY, for the National Toxicology Program, Research Triangle Park, NC; dated June 1, 1984.) Accession No. 405784-01.

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: [signature]

Date: 10/17/88

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CHEMICAL: Methyl bromide.

TEST MATERIAL: Neat methyl bromide gas (99.8% pure).

STUDY/ACTION TYPE: Subchronic inhalation toxicity study in mice.

STUDY IDENTIFICATION: R. T. Drew. A 90-day inhalation study of methyl bromide toxicity in mice. (Unpublished study No. BNL 34506 conducted by Brookhaven National Laboratory, Upton, NY for the National Toxicology Program, Research Triangle Park, NC; dated June 1, 1984.) Accession No. 405784-01.

REVIEWED BY:

Finis Cavender, Ph.D., DABT
Principal Reviewer
Dynamac Corporation

Signature: Margaret E. Brower (
Date: Oct. 17, 1988

Margaret E. Brower, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: Margaret E. Brower
Date: Oct 17, 1988

APPROVED BY:

I. Cecil Felkner, Ph.D.
Acute & Subchronic Studies
Technical Quality Control
Dynamac Corporation

Signature: William L. McLellan (for
Date: Oct 17, 1988

William Burnam, M.S.
EPA Deputy Chief,
Toxicology Branch

Signature: _____
Date: _____

CONCLUSIONS: Under the conditions of the study, increased mortality, depressed body weight gain, and reduced sperm density were found to occur in male mice after repeated exposure by inhalation to methyl bromide at a dose level of 120 ppm for 13 weeks. Neurologically, severe curling and crossing of the hindlimbs and twitching of forelimbs were exhibited in these same animals. Neurological effects were less severe in females and in males exposed to 80 ppm and were not evident in mice exposed to 10, 20, and 40 ppm. Liver weights of males exposed to 40 and 120 ppm were found to be decreased while those of females exposed to 120 ppm were found to be increased. Kidney weights of high-dose males and brain weights of high-dose females were also found to be decreased. Histopathologic findings were not reported to be compound related.

Classification: Core Supplementary.

MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1. Neat methyl bromide gas, lot No. E21-1012-00, was 99.8% pure.

2. Five-week-old B6C3F1 mice were obtained from NCI Frederick Cancer Research Center; females weighed between 15.5 and 18.4 g and males between 19.0 and 22.9 g.

3. The neat methyl bromide was metered into the supply air of the inhalation exposure chambers at target concentrations of 0, 10, 20, 40, 80, and 120 ppm. Exposures were 6 hours/day, 5 days/week, for 13 weeks.

4. Analysis for covariance was used to statistically analyze body weight, behavioral measurement, and cytogenetic analyses. All other variables were analyzed using analysis of variance; t-tests were performed when significance between variables was indicated. Bonferroni's correction was applied to multiple t-tests.

5. Exposure atmospheres were monitored using a Miram 80 infrared spectrophotometer for 10 minutes of each hour.

B. Protocol: See Appendix A.

REPORTED RESULTS:

A. Exposure Atmospheres: The actual time-weighted average chamber concentrations were all within 6% of the target value. The daily mean of the 10-ppm exposure was greater than the target plus 20% for a total of four measurements; the daily means of the 20- ppm exposure exceeded the target plus 20% only once. At the higher concentrations, the daily mean never exceeded target plus 20%.

B. Mortality: Four male mice exposed to 120 ppm died or were sacrificed moribund prior to study termination. Three of these four deaths were considered to be compound related. Six additional mice escaped, were injured, or were found dead of unknown causes; these deaths were not considered to be compound related.

Clinical Signs: Evidence of fighting was occasionally noted among males; all mice exhibited facial hair loss due to the shape of the feeders. Neurobehavioral assessment is described below.

Neurobehavioral Assessment: Severe curling and crossing of the hindlimbs and twitching of the forelimbs were exhibited by male mice exposed to 120 ppm; maximum intensity of these symptoms was reported to have been exhibited 6 weeks following study initiation. However, quantitative behavioral testing was not reported to reveal any significant compound-related change. These neurobehavioral changes were reported to have been exhibited to a lesser degree in males exposed to 80 ppm and in females (exposure group not reported). The neurobehavioral assessment report was not included in the report.

Body Weight: The body weights of male mice exposed to 120 ppm were significantly decreased ($p \leq 0.05$) from controls beginning at week 7 until study termination. The body weights of females were found to be similar to controls. Graphic data but not tabular data were provided.

Hematology: Erythrocyte counts were found to be significantly increased ($p \leq 0.01$) in males exposed to 40 and 120 ppm when compared to concurrent controls; however, these increased values were within the range of erythrocyte counts for historical laboratory controls (Table 1). This trend was not found among females exposed to 40 and 120 ppm

TABLE 1. Erythrocyte Counts of Mice Exposed to Methyl Bromide for 90 Days[a]

Dose Group (ppm)	Erythrocyte Counts (x 10^6/mm^3)
Males	
0	7.72±0.31
10	7.44±0.17
20	7.66±0.20
40	9.19±0.26**
80	8.78±0.23
120	10.34±0.21**
Females	
0	9.80±0.09
10	8.07±0.26**
20	8.72±0.16**
40	10.31±0.14
80	9.54±0.20
120	10.07±0.18

[a]Based on 10 animals/sex/group with the exception of the groups exposed to 80 ppm, which contained 8 animals.

**Significantly different from controls ($p \leq 0.01$) with Bonferroni's correction for multiple tests.

since the mean erythrocyte counts for control females were increased when compared to that of control males. The erythrocyte counts of females exposed to 10 and 20 ppm were found to be slightly but significantly decreased ($p \leq 0.01$) when compared to concurrent controls. The study author did not consider these changes to be of any toxicological significance since they did not provide a consistent pattern of response. Other changes in hematological parameters were not dose related and did not provide a consistent pattern of response.

Pseudocholinesterase: No remarkable differences in pseudocholinesterase values were noted.

Organ Weights: The absolute and relative liver weights of male mice exposed to 40 ppm were found to be significantly decreased ($p \leq 0.01$) when compared to controls; absolute liver weights of males exposed to 120 ppm were slightly decreased (Table 2). The absolute and relative liver weights of females exposed to 120 ppm were found to be significantly increased when compared to concurrent controls ($p < 0.01$, $p < 0.05$). Absolute kidney weights were found to be slightly but significantly decreased ($p \leq 0.05$) in males exposed to 120 ppm; absolute brain weights were found to be slightly but significantly decreased ($p \leq 0.01$) in females exposed to this same test concentration.

Gross Pathology: Mottled and/or congested lungs were noted in at least one mouse from each group. Enlarged or accessory spleens were exhibited sporadically in dosed and control mice.

Histopathology: There were no compound-related histopathological findings reported. A histology report was not submitted with the study.

Cytogenetic Analysis: No remarkable differences were found in sister chromatid exchanges (SCEs) or the average generation time of cell cycle completion for male mice exposed to methyl bromide. In females, an increase in SCEs was noted in mice exposed to 10 ppm. The frequency of micronuclei in peripheral red blood cells was significantly different at 4 weeks for male and female mice. However, the differences were reported to be slight and the trend was toward lower micronuclei values at higher exposures. No differences were seen at 8 weeks and slight differences were seen at 12 weeks. The complete cytogenetics report was not included in the report.

TABLE 2. Body Weights and Selected Absolute and Relative Organ Weights (±S.E.) in Mice Exposed to Methyl Bromide for 90 Days[a]

Dose Group (ppm)	Body Weight (g)	Liver		Kidney[b]	Brain[c]	
		Absolute (g)	Relative (%)	Absolute (g)	Absolute (g)	Relative (%)
			Males			
0	29.0±0.44	1.82±0.04	6.3±0.1	0.28± 0.10	0.46± 0.14	--
10	30.1±0.32	1.83±0.05	6.1±0.1	0.28± 0.05	0.47± 0.08	--
20	28.3±0.70	1.66±0.08	5.9±0.2	0.26± 0.06	0.46± 0.08	--
40	28.7±0.69	1.42±0.06**	5.0±0.2**	0.27± 0.11	0.46± 0.06	--
80	27.9±0.85	1.62±0.10	5.8±0.2	0.27± 0.12	0.45± 0.03	--
120	25.5±0.66**	1.51±0.10	5.9±0.2	0.23± 0.09*	0.43± 0.06	--
			Females			
0	23.1±0.36	1.27±0.03	5.5±0.1	0.18± 0.04	0.48± 0.34	2.09±0.04
10	23.5±0.34	1.37±0.04	5.8±0.1	0.18± 0.08	0.47± 0.10	2.00±0.04
20	23.5±0.29	1.22±0.02	5.2±0.1	0.18± 0.05	0.49± 0.06	2.08±0.03
40	23.8±0.41	1.36±0.04	5.7±0.1	0.18± 0.04	0.47± 0.07	1.99±0.04
80	23.8±0.44	1.32±0.04	5.5±0.1	0.19± 0.04	0.48± 0.03	2.02±0.03
120	23.3±0.24	1.45±0.03**	6.2±0.2*	0.19± 0.07	0.44± 0.09**	1.90±0.03**

[a]Based on 10 animals/sex/group with the exception of the groups exposed to 80 ppm, which contained 8 animals.
[b]Relative kidney weights were not reported for males or females.
[c]Relative brain weights were not reported for males.
*Significantly different from controls ($p < 0.05$) with Bonferroni's correction for multiple tests.
**Significantly different from controls ($p < 0.01$) with Bonferroni's correction for multiple tests.

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Sperm Morphology and Vaginal Cytology Evaluation (SMVCE): In males exposed to 120 ppm, sperm density was reduced to 67% of the control values. Caudal, epididymal, and testicular weights were not affected by exposure to the test compound. In females exposed to 120 ppm, the estrous cycle was increased from 4.0 to 4.5 days. The complete SMVCE report was not included in the report.

STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: Exposure by inhalation to methyl bromide for 13 weeks at doses of 10, 20, 40, 80, or 120 ppm produced increased mortality (17%), depressed weight gain, and reduced spleen density in males exposed to 120 ppm. Neurologically, severe curling and crossing of the hind-limbs and twitching of the forelimbs were exhibited in these same animals. Neurological effects were less severe in females.

The quality assurance statement was signed and dated May 30, 1984.

REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS: The study design was adequate and the conduct of the study was acceptable. However, the following deficiencies were noted:

(1) The EPA Pesticide Assessment Guidelines, 1982, for 90-day subchronic inhalation studies designates that the rat is the preferred species for inhalation testing and that the study laboratory should provide justification for its selection of another species. In addition, at least 10 animals/sex/group were designated for testing with nonpregnant females. For this study, B6C3F1 mice were used as the test animals (no justification was provided); only eight mice/sex/group were used for the 80-ppm exposure groups and males were inadvertently included with females impregnating three mice (replaced with nonpregnant mice). Furthermore, clotting potential, designated to be included in the hematological parameters, was not measured. Blood chemistry determinations (with the exception of pseudocholinesterase) and ophthalmological examinations were not measured, as designated in the 1982 EPA Pesticide Assessment Guidelines.

(2) No individual data are included in the report. Reference is made throughout the study to appendices containing detailed information but these appendices were not submitted with the study report. Without the individual data, it was impossible to completely assess many parameters. These individual data should be provided by the study sponsor.

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(3) Other than the final body weights submitted with organ weight data, only graphic body weight data were included in the study for weights taken during the study.

(4) The exposure conditions of the concurrent controls were not specified.

(5) The study author reported the presence of numerous small clots and significant numbers of micronuclei in blood samples of doses males and females. The implication of these data and that of the discrepancy in erythrocyte counts in high-dose males and females cannot be assessed without the individual data. These data are also necessary to determine whether the decreased absolute and relative brain weights exhibited in high-dose females can be correlated with the neurobehavioral findings.

The study was used to recommend test concentrations for a 2-year carcinogenicity study. The study recommended these concentrations to be 10, 50, and 100 ppm.

APPENDIX A
Protocol

Methyl bromide toxicology review

Page _____ is not included in this copy.

Pages 12 through 17 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

X FIFRA registration data

___ The document is a duplicate of page(s) _____

___ The document is not responsive to the request

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

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EPA No.: 68080056
DYNAMAC No. 111-B5
October 14, 1988

DATA EVALUATION RECORD

Methyl Bromide

Mutagenicity--Sex-Linked Recessive Lethal Assay in Drosophila melanogaster

STUDY IDENTIFICATION: McGregor, D.B. Tier II mutagenic screening of 13 NIOSH priority compounds, individual compound reports, methyl bromide. (Unpublished study No. 210-78-0026, report No. 1190, prepared by Iveresk Research Int. Ltd., Musselburgh, Scotland, UK for the National Institute for Occupational Safety and Health, Cincinnati, OH; submitted by the Methyl Bromide Industry Panel; dated May 30, 1981.) MRID No. 405791-01.

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: Robert J. Weir for

Date: 10-14-88

1. CHEMICAL: Methyl bromide; bromomethane (CAS No. 74-83-9).

2. TEST MATERIAL: Methyl bromide, batch No. 77371, was described as a colorless gas; the purity was not reported.

3. STUDY/ACTION TYPE: Mutagenicity--Sex-linked recessive lethal assay in Drosophila melanogaster.

4. STUDY IDENTIFICATION: McGregor, D.B. Tier II mutagenic screening of 13 NIOSH priority compounds, individual compound reports, methyl bromide. (Unpublished study No. 210-78-0026, report No. 1190, prepared by Iveresk Research Int. Ltd., Musselburgh, Scotland, UK for the National Institute for Occupational Safety and Health, Cincinnati, OH; submitted by the Methyl Bromide Industry Panel; dated May 30, 1981.) MRID No. 405791-01.

5. REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 10-13-88

I. Cecil Felkner, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 10-14-88

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Genetic Toxicology Studies
Technical Quality Control
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 10-14-88

William Burnam, Ph.D.
EPA Reviewer and
Branch Chief,
Toxicology Branch for
Herbicide, Fungicide, and
Antimicrobial Support

Signature: _____
Date: _____

7. CONCLUSIONS:

A. Methyl bromide (CH_3Br) at test atmospheres of 20 and 70 ppm was evaluated in two independent studies for the ability to induce sex-linked recessive lethal (SLRL) mutations in pre- and postmeiotic germ cells of Drosophila melanogaster. Although higher than historical-control frequencies (combined) were observed in the majority of broods from both treatment groups, the assay was compromised for the following reasons:

1. No analytical data for test material concentrations during the exposure time (5 hours) were provided.

2. The highest assayed dose (70 ppm CH_3Br) was neither toxic nor induced a sterilizing effect.

3. The sample size for either the individual tests or combined experiments was too low to provide confidence in the results (See Reviewers' Discussion and Interpretation of Study Results, Section 14).

B. The study is unacceptable.

8. RECOMMENDATIONS: It is recommended that the study author provide analytical data for test material concentrations during the exposure intervals. The following additional suggestions are made in accordance with procedures recommended by Lee et al.:[1]

- o Assay CH_3Br to an appropriate level or provide justification for use of the high dose.
- o Use sufficient sample sizes within different cell stages to ensure that the total number of chromosomes tested is adequate.
- o Include a concurrent negative control group of equal sample size to ensure maximum statistical power of the assay.

Items 9 and 10--see footnote 2.

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[1] Lee, W.R., Abrahamson, S., Valencia, R., vonHalle, E.S., Wurgler, F. E., and Zimmering, S. The sex-linked recessive lethal test for mutagenesis in Drosophilia melanogaster. A report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutat. Res. 123(1983):183-279.

[2] Only items appropriate to this DER have been included.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material:

a. Description: Methyl bromide (CH_3Br) from batch No. 77371 was described as a colorless gas and was maintained in two cylinders under ambient conditions. Physical and chemical properties and safety procedures for handling the test material were reported.

b. Exposure Chamber: The exposure chamber was described as a glass vessel through which the required test atmospheres of CH_3Br were introduced at a flow rate of ~5 L/minute before passing directly into the infrared analyzer. No further information was provided. It was noted that while the exposure chamber, atmosphere generation, monitoring equipment, and analytical procedures for measurement of chamber concentrations in rodent studies conducted with CH_3Br were described in detail, little or no specific information was available for the Drosophila studies. A footnote to the analytical data table (CBI p 46) indicated that flies were treated in the 20-ppm. exposure chamber for 7 hours. This conflicts with data indicating a 5-hour exposure.

2. Test Animals: D. melanogaster, wild-type Oregon K. (OrK), designated stocks A and B, and Basc female cultures were maintained at Iveresk Research International Ltd. and the University of Edinburgh, respectively. Male stocks were maintained on medium containing corn meal, molasses, agar, yeast, proprionic acid, and Nipogen (bacteriostatic agent).

3. Toxicity Test: Groups of 100 3-day-old wild-type males were exposed to 20 or 70 ppm CH_3Br for 1, 3, or 5 hours. Following exposure, flies were held overnight at 26°C and transported to the University of Edinburgh. Upon arrival, flies were assessed for viability and four males/group/exposure time were mated with four virgin females. The remaining unmated males were observed for toxic effects. Mated females were allowed to lay eggs for 24 hours on medium darkened with charcoal. The number of eggs laid and the number hatched were recorded and a hatchability index (HI) was calculated.

4. Sex-Linked Recessive Lethal (SLRL) assay

a. Exposure: The methods described above were used to expose 80 to 100 P_1 males of each stock (A and B) to 20 and 70 ppm of CH_3Br for 5 hours; the length of exposure was selected from the results of the toxicity test. An additional group of 60 P_1 males from stock B were fed 0.4% ethylmethanesulfonate (EMS) in sucrose for 5 hours; this group served as the positive control. A negative control group was not included; however, historical F_2 generation control data from nine experiments and from several experiments with F_3 crosses were included in the report.

b. Mating:

1) Brood I. On the morning following exposure, individual P_1 males from stocks A and B were mated with two virgin untreated females; the length of the mating interval was not reported. This constituted brood I and represented the sampling of germ cells that were primarily mature sperm at the time of treatment.

2) Brood II. The individual P_1 males were remated with two virgin females for a 3-day mating period. This constituted brood II and represented the sampling of germ cells that were primarily late or middle spermatids at the time of treatment.

3) Brood III. The individual P_1 males were remated with two virgin females 3 days after the first mating. This constituted brood III and represented the sampling of germ cells that were primarily early spermatids and spermatocytes at the time of treatment.

c. F_2 Generation: One to 4 days after emergence of the adult flies, 400 cultures/brood/P_1 stock/treatment group of the F_1 generation were permitted a round of brother-sister mating, and the progeny (F_2) was scored. For the positive control group, 100 cultures from brood I were crossed.

d. F_3 Generation: F_3 crosses were performed as described with F_2 progeny arising from stock A-treated P_1 males; the EMS-treated group was not mated to yield F_3 offspring.

e. Scoring the F_2 and F_3: The F_2 and F_3 progeny from each brood of the treatment groups and F_2 progeny from the positive control group were examined for the presence of lethal or nonlethal cultures and the frequency of X-linked recessive lethals was calculated.

6. Evaluation Criteria:

a. Negative Response: The assay was considered negative if the lethal frequency of the treated groups in duplicate experiments was <0.05% of the historical control value.

b. Positive Response: The compound was considered positive if the lethal frequency in the same brood of duplicate experiments was >1.0% of the historical control value.

7. Statistical Analysis: The data were not analyzed statistically.

B. Protocol: A protocol was not provided.

12. REPORTED RESULTS:

A. Toxicity Test: Exposure of P_1 males to 20 and 70 ppm CH_3Br for 1, 3, or 5 hours had little or no effect on survival; ≥ 94% of all treated flies survived. Although the number of eggs laid by females mated to P_1 males in the low-dose groups was low, fertility was not affected in the high-dose groups at any exposure interval. Based on the results, the author stated that males for the SLRL assay were exposed to 20 and 70 ppm CH_3Br for 5 hours.

B. SLRL Assay: Representative results from both stocks of P_1 males are presented in Table 1. As shown, higher than control percent lethal mutations were seen in all broods of the A stock exposed to 20 ppm. In the duplicate assay, percent lethal mutations in brood II of the 20-ppm exposure group was also higher than the historical control; no mutations were seen in the F_3 crosses. With the exception of brood I F_2 offspring of stock B males and brood I F_3 offspring of stock A males, no lethal mutations were scored in the high-dose group. The data for individual P_1 stocks provided no indication of a reproducible or dose-related increase in mutation. However, when these data were combined (Table 2), the results suggest a dose-related increase in the percent lethals for brood I. Similarly, increases in the percent lethals for brood II of both treatment groups and low-dose brood III were calculated. Combined percent lethals for all brood in the 20-ppm group were also higher than the control values.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

1. The author concluded that there was no compelling evidence for the assumption that the observed lethals were induced by CH_3Br.

TABLE I. Representative Results of the Sex-Linked Recessive Lethal Assay in D. melanogaster Exposed to Methyl Bromide

	Dose	Brood[a] I	Percent[b] Lethals	Brood[a] II	Percent[b] Lethals	Brood[a] III	Percent[b] Lethals	Combined[c]	Combined[d] Percent Lethals
					F_2 Generation				
Test Material									
Methyl bromide	20 ppm	1/317[e]	0.32	3/369	0.81	1/365	0.27	5/1051	0.48
		0/319[f]	0	1/374	0.27	0/368	0	1/1061	0.09
	70 ppm	0/332[e]	0	1/357	0.28	0/338	0	1/1027	0.10
		2/330[f]	0.61	0/352	0	0/339	0	2/1021	0.20
Positive Control									
Ethylmethane-sulfonate	0.4%	19/56[f]	33.9	--	-	-	-	19/56	33.9
					F_3 Generation				
Methyl bromide	20 ppm	0/387[e]	0	0/282	0	0/283	0	0/952	0
	70 ppm	1/390[e]	0.26	0/290	0	0/285	0	1/965	0.10
					F_2 Generation (9 experiments)				
Historical Background Control Data		6/5319[e]	0.12	2/5309	0.04	5/5339	0.09	13/15967	0.08
		6/5264[f]	0.11	2/5088	0.03	0/4713	0	8/15065	0.05
					F_3 Generation (0-4 experiments)				
		-	--	0/1200[e]	0	0/989	0		0

[a] Number of Lethals/Number of Tests Scored.

[b] $\frac{\text{Number of Lethals}}{\text{Number of Tests Scored}} \times 100.$

[c] Total Number of Lethals/Total Number of Tests Scored.

[d] $\frac{\text{Total Number of Lethals}}{\text{Total Number of Tests scored}} \times 100.$

[e] Stock A, P_1 male offspring.

[f] Stock B, P_1 male offspring.

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TABLE 2. Combined Results for P_1 Male Stocks A and B in the Sex-Linked Recessive Lethal Assay in *D. melanogaster* Exposed to Methyl Bromide (F_2 Generation)

Treatment	Dose	Brood[a] I	Percent[b] Lethals	Brood[a] II	Percent[b] Lethals	Brood[a] III	Percent[b] Lethals	Combined[c]	Combined[d] Percent Lethals
Test Material									
Methyl bromide	20 ppm	1/636	0.16	4/743	0.54	1/733	0.14	6/2112	0.28
	70 ppm	2/662	0.30	1/709	0.14	0/677	0	3/2048	0.10
Historical Background Control Data	--	12/10583	0.11	4/10397	0.04	5/10052	0.05	21/31032	0.07

[a] Number of Lethals/Number of Tests Scored.

[b] $\frac{\text{Number of Lethals}}{\text{Number of Tests Scored}} \times 100.$

[c] Total Number of Lethals/Total Number of Tests Scored.

[d] $\frac{\text{Total Number of Lethals}}{\text{Total Number of Tests Scored}} \times 100.$

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B. Quality assurance personnel were identified, but no quality assurance statement was provided.

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14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We assess that CH_3Br was not adequately tested in the SLRL assay for the following reasons:

1. Analytical data to support the study author's claim that 20 and 70 ppm CH_3Br were generated and maintained throughout the 5-hour exposure period were not provided. A footnote to the tabular 20-ppm analytical data would suggest that these data apply to this study; however, the exposure time is inconsistent (7 hours) with the reported 5-hour exposure.

2. No toxicity was seen at either exposure concentration; therefore, the data are insufficient to ensure that a maximum tolerated dose was achieved.

3. The sample size either for independent experiments or for the combined results were insufficient to draw meaningful conclusions. For example, in order to prove statistically, using the Kastenbaum-Bowman tables as recommended by the U.S. EPA Gene-Tox work group on D. melanogaster,[3] that the 0.28% combined lethal frequency of the low-dose group (Table 2) was a significant effect, the number of tests required (given the performing laboratory's historical frequency of 0.07%) would be at least 5000 total tests or 3000 tests/experiment.

4. The author included historical control data from in-house experiments; however, the treated males were transported to another facility (30 minutes in transit) for mating and completion of the assay. Therefore, the historical control insects were not maintained under the same environmental conditions for the entire experiment. Additionally, Lee et al.[4] recommended the use of concurrent negative control groups of equal sample size to ensure maximum statistical power of the assay.

Although the lethal mutation frequencies for brood I (20 and 70 ppm CH_3Br) and, particularly, brood II of the low-dose group are suggestive of a compound effect, the lack of adequate sample size precludes a determination as to whether the induced frequencies are compound related or artifactual. It is concluded, therefore, that the study is unacceptable and should be repeated.

Item 15--see footnote 2.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 27-29.

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[3] Lee et al. Mutat. Res. 123(1983): 183-279.

[4] Ibid.

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APPENDIX A

Material and Methods

Methyl bromide toxicology review

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007006

EPA No.: 68D80056
DYNAMAC No. 111-B4
October 14, 1988

DATA EVALUATION RECORD

METHYL BROMIDE

Mutagenicity--Sperm Morphology Assay in Mice

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: Robert J. Weir for

Date: 10-14-88

007006

EPA No.: 68D80056
DYNAMAC No. 111-B4
October 14, 1988

DATA EVALUATION RECORD

METHYL BROMIDE

Mutagenicity--Sperm Morphology Assay in Mice

REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E McCarroll
Date: 10-13-88

I. Cecil Felkner, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: I Cecil Felkner
Date: 10-14-88

APPROVED BY:

I. Cecil Felkner, Ph.D.
Genetic Toxicology Studies
Technical Quality Control
Dynamac Corporation

Signature: I Cecil Felkner
Date: 10-14-88

William Burnam, Ph.D.
EPA Reviewer and
Branch Chief,
Toxicology Branch for
Herbicide, Fungicide, and
Antimicrobial Support

Signature: _____
Date: _____

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity--Sperm morphology assay in mice.

ACCESSION NUMBER: 405791-01.

TEST MATERIAL: Methyl bromide.

SYNONYM(S): Bromomethane.

STUDY NUMBER(S): 210-78-0026.

SPONSOR: National Institute for Occupational Health, Cincinnati, OH.

TESTING FACILITY: Iversek Research Int. Ltd., Musselburgh, Scotland, UK.

TITLE OF REPORT: Tier II Mutagenic Screening of NIOSH Priority Compounds, Individual Compound Reports, Methyl Bromide.

AUTHOR(S): McGregor, D.B.

REPORT ISSUED: May 20, 1981.

CONCLUSIONS: 007006

The inhalation exposure of male mice to 20 and 70 ppm methyl bromide (7 hours/day for 5 consecutive days) did not show clear evidence of toxicity, induce cytotoxicity in the target organ (testis), or increase the frequency of morphological alterations in sperm. The inability to demonstrate test material's interaction with target cells in the tissue precludes acceptance of these results as valid evidence that methyl bromide is not a mutagen in male mouse germinal cells.

Classification: The study is unacceptable.

A. MATERIALS:

1. Test Compound: Methyl bromide (CH_3Br) from batch No. 77371 was described as a colorless gas and was maintained in two cylinders under ambient conditions. Physical and chemical properties and methods for safe handling were reported.

2. Test Animals: Species: mouse; strain: B6C3F1 males; age: 10 to 12 weeks; source: Charles River (USA).

3. Inhalation Chambers:

   a. Description: The test exposure chambers were constructed of stainless steel and glass and had a capacity of 1.5 m^3. The animals occupied a volume of 0.02 m^3 and were confined to a single tier of cages (volume occupied = 0.4 m^3). This area was ventilated at a flow rate of 12 to 16 air changes/hour. Test chambers were maintained under a slight negative pressure. Air control animals were held in a 0.84-m^3 chamber; the chamber was ventilated at a rate of 8 air changes/hour.

   b. Aerosol Generation/Exhaust: Test atmospheres were produced by appropriate dilution of CH_3Br with filtered compressed air and were introduced into the top of the exposure chambers through a 7/8-inch stainless steel pipe. The test atmospheres were continuously generated for a single pass through the chamber; CH_3Br/air mixtures were regulated in the mixing vessel by adjustable flow meters. Test atmospheres were exhausted with a Gast extraction pump, passed through a scrubber containing methylated spirits/water, diluted with building exhaust air, and discharged to the environment.

   c. Chamber Concentrations/Monitoring Equipment: Prior to initiation of the study, homogeneity of test atmospheres in the exposure chambers was determined for periods up to 7 hours. Test atmospheres were continuously monitored during exposure. All determinations were performed with daily precalibrated infrared gas analyzers; the sample flow rate was 4 L/minutes. Chamber temperatures and humidity were recorded hourly during the exposures; these data were not reported.

B. STUDY DESIGN:

1. Animal Assignment: Males were identified by ear tag and cage card number (randomization procedures, if any, were not reported) and assigned to the following test groups:

Test group	Dose	Males/ Group
Negative Control[a]		
Air	--	10
Test Group[a]		
Low	20 ppm	10
High	70 ppm	10
Positive Control[b]		
Ethylmethane-sulfonate (EMS)	200 mg/kg	10

[a]Negative control (air) and test doses were administered by inhalation for 7 hours/day for 5 consecutive days.

[b]EMS was administered by oral gavage for 5 consecutive days.

2. Animal Maintenance: Animals were housed individually and were maintained in an environment controlled for temperature (18.5-26.5°C), humidity (40-61%), and light (12 hours/day). Spratts-Spillers No. 1 rodent feed and water were provided ad libitum except during the aerosol exposure periods.

3. Preliminary Toxicity Study: A preliminary range-finding study was not performed and the rationale for dose selection was not reported.

4. Sperm Morphology Assay:

a. Compound Exposure: Ten males per group were exposed to 20 or 70 ppm CH_3Br for 7 hours/day for 5 consecutive days. The 10 males in the positive control group were administered 200 mg/kg EMS by oral gavage for 5 consecutive days.

b. Animal Observations: Males were observed during and after the exposure periods for signs of toxicity. Body weights were recorded daily following exposures.

c. Animal Sacrifice/Sperm Preparation: Animals were sacrificed 5 weeks after the final dosing by cervical dislocation. The cauda epididymides were excised and minced in small beakers containing fixative (0.01% glutaraldehyde in 0.25 M sucrose and 0.05 M phosphate buffer). Sperm suspensions were centrifuged and placed on slides; slides were stained with 1% eosin:ethanol (1:1) and coded.

d. Sperm Analysis: One thousand sperm per animal were scored for the frequency of up-turned or elongated hooks, banana-shaped heads, amorphous heads, folded tails, or other miscellaneous abnormalities.

e. Statistical Evaluation: The data were transformed using the Freeman-Tukey transformation and analyzed by a one-sided t-test at $p<0.05$.

f. Quality Assurance: Quality assurance personnel were identified, but a quality assurance statement was not provided.

C. REPORTED RESULTS:

1. Test Material Analyses:

a. Homogeneity: Homogeneity analysis of chamber concentrations was performed prior to initiation of the study. Samples taken from eight areas of each chamber indicated that, in general, the test atmospheres were uniformly distributed throughout the exposure chambers and were within 5% of the target concentrations.

b. Achieved Concentrations: Atmospheric analysis of test concentrations were conducted continuously throughout each daily 7-hour exposure. Based on the reported data, the average achieved daily concentrations in the low dose ranged from 18.7 to 21.4 ppm; achieved concentrations in the high-dose chamber ranged from 68.7 to 73.4 ppm. The author stated, however, that $\geq$10% deviations from the target concentration occurred; the maximum concentrations recorded for 20 and 70 ppm were 31 and 116 ppm, respectively. Extremes for values lower than the desired target concentrations were not reported.

2. Animal Observations: One high-dose male died 5 days after exposure; deaths in the positive control group (6) resulted from technical error. Other reported clinical signs were mice in the high-dose group that appeared subdued during the exposure period on day 2 and two high-dose mice were tremulous at the end of exposure on day 5. No other toxic signs were observed.

   Body weights for test and air control groups showed no compound-related effects; body weight gains for the CH_3Br treatment groups were comparable or slightly higher than the negative-control value.

3. Sperm Morphology Assay: As shown in Table 1, the average percent abnormal sperm in the 20- and 70-ppm CH_3Br exposure groups were lower than the air control group. By contrast, a significant increase in the percent abnormal sperm was found in the mice exposed to the positive control, EMS (200 mg/kg X 5 days).

D. STUDY AUTHOR'S CONCLUSIONS:

The study author concluded that, under the adopted exposure conditions, there was no evidence for a mutagenic effect of methyl bromide.

E. REVIEWER'S DISCUSSION AND INTERPRETATION OF RESULTS:

We assess that while the results of the sperm morphology assay with CH_3Br were negative, the lack of clear compound-related toxicity or cytotoxicity to the target organ (testes) precludes acceptance of these findings as valid evidence that CH_3Br is not mutagenic in male mice germinal cells. Unless the author can justify the use of 70 ppm, under the described conditions as the high dose, the assay should be repeated. It is acknowledged, however, that the sperm morphology assay is not required to support EPA registration of pesticides.[1]

F. CBI APPENDIX: Appendix A, Materials and Methods, Animals and Animal Management, CBI pp. 7-10; Atmosphere Generation and Exposure, CBI pp. 11-15; Sperm Morphology Assay, CBI pp. 25-26.

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[1]EPA FIFRA Guideline Subdivision F: Pesticide Assessment Guidelines: Hazard Evaluation--Human and Domestic Animals, dated 11-30-82.

TABLE 1. Representative Results of the Sperm Morphology Assay in Mice Exposed to Methyl Bromide

007006

Substance	Dose	No. of Males Scored	No. of Sperm Examined	Average No. of Abnormal Sperm	Average Percent Abnormal Sperm
Negative Control[a]					
Air	--	10	9000[c]	287	3.19
Positive Control[b]					
Ethylmethanesulfonate	200 mg/kg	10	10000	936	9.36*
Test Substance[a]					
Methyl bromide[a]	20 ppm	9	9000[c]	264	2.93
	70 ppm	9	8000[c,d]	188	2.35

[a] Inhalation exposure of 7 hours/day X 5 days.

[b] Oral gavage administration for 5 days.

[c] No sperm were observed in single animals.

[d] One high-dose male died.

* Significantly different from the control values at $p<0.05$.

007006

APPENDIX A

Materials and Methods

Methyl bromide toxicology review

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087006

EPA No.: 68080056
Dynamac No.: 111-B3
October 14, 1988

DATA EVALUATION RECORD

METHYL BROMIDE

Mutagenicity - Dominant Lethal Assay in Rats

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: [signature]

Date: 10-14-88

51

007006

DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity--Dominant lethal assay in rats.

ACCESSION/MRID NUMBER: 405791-01.

TEST MATERIAL: Methyl bromide.

SYNONYM(S): Bromomethane.

STUDY NUMBER(S): 210-78-0026.

SPONSOR: National Institute for Occupational Health, Cincinnati, OH.

TESTING FACILITY: Iveresk Research Int. Ltd., Musselburg, Scotland, UK.

TITLE OF REPORT: Tier II Mutagenic Screening of 13 NIOSH Priority Compounds, Individual Compound Reports, Methyl Bromide.

AUTHOR(S): McGregor, D.B.

REPORT ISSUED: May 30, 1981.

CONCLUSIONS:

Male rats were continuously exposed by inhalation to 20 and 70 ppm methyl bromide 7 hours/day for 5 consecutive days and sequentially mated with virgin females for 10 weeks. Although nonsignificant, two-fold increases were seen in the frequency of early deaths for the low-dose rats at week 1 and for the high-dose rats only at week 3, no definitive conclusions can be reached for the following reasons:

1. No clear evidence of compound related toxic effects were seen; therefore, the data were insufficient to establish that the maximum tolerated dose (MTD) was tested.

2. The number of mated females (20) and the number of successful pregnancies were below the number recommended (30 females/group/mating)[1] to provide an adequate sample size and also to provide assurance that the statistical power was sufficient to detect a doubling of the spontaneous background frequency of dominant lethal mutations.

Classification: The study is unacceptable and should be repeated in accordance with recommended procedures for the dominant lethal assay.[2]

A. MATERIALS:

1. Test Compound: Methyl bromide (CH_3Br) from batch No. 77371 was described as a colorless gas and was maintained in two cylinders under ambient conditions. Physical and chemical properties and methods for safe handling were reported.

2. Test Animals: Species: rat; strain: CD Sprague-Dawley; age: 10-11 weeks; source: Charles River (UK) Ltd., Manston, Kent, UK.

3. Inhalation Chambers:

   a. Description: The test exposure chambers were constructed of stainless steel and glass and had a capacity of 1.5 m^3. The animals occupied a volume of 0.02 m^3 and were confined to a single tier of cages (volume occupied = 0.4 m^3). This area was ventilated at a flow rate of 12 to 16 air changes/hour. Test chambers were maintained under a slight negative pressure. Air control animals were held in a 0.84-m^3 chamber; the chamber was ventilated at a rate of 8 air changes/hour.

---

[1] Green, S., Auletta, A., Fabricant, J., Kapp, R., Manandhar, M., Shea, C., Springer, J., and Whitfield, B. Current status of bioassays in genetic toxicology--The dominant lethal assay. Mutat. Res. 54 (1985): 49-67.

[2] Ibid.

b. Aerosol Generation/Exhaust: Test atmospheres were produced by appropriate dilution of the CH_3Br with filtered compressed air and introduced into the top of the exposure chambers through a 7/8-inch stainless steel pipe. The test atmospheres were continuously generated for a single pass through the chamber; CH_3Br/air mixtures were regulated in the mixing vessel by adjustable flow meters. Test atmospheres were exhausted with a Gast extraction pump, passed through a scrubber containing methylated spirits/water, diluted with building exhaust air, and discharged to the environment.

c. Chamber Concentrations/Monitoring Equipment: Prior to the initiation of the study, homogeneity of test atmospheres in the exposure chambers was determined for periods up to 7 hours. Test atmospheres were continuously monitored during exposure. All determinations were performed with daily precalibrated infrared gas analyzers; sample flow rate was 4 L/minutes. Chamber temperatures and humidity were recorded hourly during the exposures; these data were not reported.

B. STUDY DESIGN:

1. Animal Assignment: Females were identified with the cage card number of the male with which they were mated and the week of access; males were identified by ear tag and cage card number (randomization procedures, if any, were not reported) and assigned to the following test groups:

Test group	Dose	Males/ Group	Females/ Mating Interval
Negative Control[a]			
Air	--	10	20
Test Group[a]			
Low	20 ppm	10	20
High	70 ppm	10	20
Positive Control[b]			
Ethylmethane-- sulfonate (EMS)	100 mg/kg	10	20

[a]Negative control and test doses were administered by inhalation for 7 hours/day for 5 consecutive days.

[b]EMS was administered by oral gavage for 5 consecutive days.

2. Animal Maintenance: Animals were housed individually except during mating and were maintained in an environment controlled for temperature (18.5-26.5°C), humidity (40-61%), and light (12 hours/day). Spratts-Spillers No. 1 rodent feed and water were provided ad libitum except during the aerosol exposure periods.

3. Preliminary Toxicity Study: A preliminary range-finding study was not performed and the rationale for dose selection was not reported.

4. Dominant Lethal Assay:

   a. Compound Exposure: Ten males per group were exposed to 20 or 70 ppm CH_3Br for 7 hours/day for 5 consecutive days. The 10 males in the positive control group were administered 100 mg/kg EMS by oral gavage for 5 consecutive days.

   b. Mating/Sacrifice of Females: At the completion of treatment, males were mated with two virgin females for 7 days. Females were replaced with two virgin females and the mating sequence was repeated for a total of 10 weeks. Females were sacrificed 14 days after mating; ovaries and uteri were removed and examined.

   c. Animal Observations: Males were observed during and after the exposures for signs of toxicity. Body weights were record daily following the exposures.

   d. Examination and Scoring of Uteri: Corpora lutea were counted, pregnancy rates were determined, and the uterine contents were examined for live implants and early and late deaths.

   e. Statistical Methods: Pregnancy rate, total implants, live implants, live implants plus early deaths, and early deaths were analyzed at p values of 0.05, 0.001, and 0.0001 by Chi-square test, Freeman-Tukey Poisson transformation, Freeman-Tukey binomial transformation, or by additional analyses developed by the reporting laboratory's statistician.

   f. Quality Assurance: Quality assurance personnel were identified, but a quality assurance statement was not provided.

C. REPORTED RESULTS:

1. Test Material Analyses:

a. Homogeneity: Homogeneity analysis of chamber concentrations was performed prior to initiation of the study. Samples taken from eight areas of each chamber indicated that, in general, the test atmospheres were uniformly distributed throughout the exposure chambers and that doses administered were within 5% of the target concentrations.

b. Achieved Concentration: Atmospheric analysis of test concentrations were conducted continuously throughout each daily 7-hour exposure. Based on the reported data, the average achieved daily concentrations in the low dose ranged from 18.7 to 21.4 ppm; achieved concentrations in the high-dose chamber ranged from 68.7 to 73.4 ppm. The author stated, however, that $\geq$ 10% deviations from the target concentrations occurred; the maximum concentrations recorded for 20 and 70 ppm were 31 and 116 ppm, respectively. Extremes for values lower than the desired target concentrations, however, were not reported.

2. Animal Observations: The authors stated that traces of blood around the nostrils of some animals exposed to 70 ppm CH_3Br were observed. No other toxic signs were reported for male rats exposed by inhalation to 20 and 70 ppm CH_3Br for 7 hours/day for 5 consecutive days. From the body weight determinations performed daily following the exposures, there was no indication of a compound-related effect, and body weight gains for the CH_3Br-dosed groups were comparable to the air control group.

3. Dominant Lethal Assay: As shown in Table 1, the pregnancy rate for all groups was low following the first week of mating. Pregnancy rates lower than the negative control groups were recorded for the 20-ppm groups at weeks 2 and 3 and for the 70-ppm group at week 3. Beyond week 3, all dosed group pregnancy rates were comparable to the negative control group. Total implants for the 20 ppm dose group were lower than the control value for the first three mating weeks; however, only findings from the second week were significant. Early implantation loss and the mutation index (MI) for early deaths were higher than the control group for the week-1 low-dose matings. Although the MI (early deaths) was approximately two-fold higher than the control value, statistical evaluation of transformed early death frequencies did not indicate a significant effect. For the remaining mating intervals, exposure to 20-ppm CH_3Br did not adversely affect relevant dominant lethal parameters. Results for the high-dose group matings at week 3 indicated that live implants were low, early implantation loss was high, and the MI (early deaths) was ~2-fold higher

TABLE 1. Representative Results from Selected Mating Weeks of the Dominant Lethal Assay in Rats Exposed to Methyl Bromide

Mating Week	Substance	Dose	Pregnancy Rate[a]	Corpora Lutea	Total Implants	Live Implants	Late Implantation Loss[b]	Early Implantation Loss[b]	Proportion of Females with >0 Early Death	Proportion of Females with >1 Early Death	Mutation Index (Early Deaths)[b,c]	Mutation Index (All Deaths)[b]
1	Air	0 ppm	75	12.6	11.5	10.6	0.2	0.7	6/15	3/15	0.06	0.08
	EMS[e]	100 mg/kg	65	7.9*	7.1*	4.0***	0.2	2.9[f]	8/13	7/13	0.41	0.44
	CH_3Br	20 ppm	65	12.7	10.5	9.0	0.1	1.4	8/13	5/13	0.13	0.14
		70 ppm	75	14.0	12.7	11.6	0.5	0.6	8/15	1/15	0.05	0.09
2	Air	0 ppm	90	14.8	13.7	12.2	0.4	1.1	10/18	6/18	0.08	0.11
	EMS	100 mg/kg	25	9.0	4.4*	1.8***	0	2.6	1/5	1/5	0.59	0.59
	CH_3Br	20 ppm	80	13.8	11.9*	11.3	0.2	0.4	5/16	1/16	0.03	0.05
		70 ppm	90	14.6	13.9	12.7	0	1.2	10/18	4/18	0.09	0.09
3	Air	0 ppm	95	12.7	13.2	11.9	0.7	0.6	9/19	3/19	0.05	0.10
	EMS	100 mg/kg	25	5.6*	1.8***	1.8***	1.8	0.0[f]	0/5	0/5	0.0	1.00
	CH_3Br	20 ppm	80	13.4[g]	12.8	11.9	0.8	0.1[f]	2/16	0/16	0.01	0.07
		70 ppm	80	13.4[g]	13.1	10.8[g]	0.9	1.4	8/16	2/16	0.11	0.18
4	Air	0 ppm	85	12.1	13.2	11.0	0.3	1.9	9/17	6/17	0.14	0.17
	EMS	100 mg/kg	85	11.4	10.4*	5.5***	3.2	1.7	13/17	9/17	0.16	0.47
	CH_3Cr	20 ppm	95	13.8[g]	13.0	11.2[g]	0.9	0.8	11/19	5/19	0.06	0.13
		70 ppm	100	12.4	11.8	8.9	1.5	1.4	11/20	6/20	0.12	0.25

[a] Pregnancy Rate = $\frac{\text{No. of Females with Implants}}{\text{No. of Mated Females}} \times 100$.

[b] Values calculated by our reviewers.

[c] Mutation Index (early deaths) = $\frac{\text{Average No. of Early Deaths}}{\text{Total Implants}}$.

[d] Mutation Index (all deaths) = $\frac{\text{Average No. of Early and Late Deaths}}{\text{Total Implants}}$.

[e] EMS = Ethylmethanesulfonate.

[f] Transformed data for early death frequency were reported to be significantly different from the control value ($p < 0.05$).

[g] Calculation errors uncovered in the summary tables; recalculation of the raw data indicated the values presented by our reviewers.

* Significantly different from the control value at $p < 0.05$.
** Significantly different from the control value at $p < 0.01$.
*** Significantly different from the control value at $p < 0.001$.

007006

than the control frequency. The remaining high-dose matings showed no trends that would be suggestive of a positive response. The findings with the positive control group (EMS) are of interest because parameters that indicate a positive response (i.e., reductions in total implants and live implants with accompanying increases in early fetal deaths) were severely affected by EMS during the first three mating weeks. However, only the transformed data for the early-death frequency of week 1 was significantly increased.

D. STUDY AUTHOR'S CONCLUSIONS

The study author concluded that, under the adopted exposure conditions, there was no evidence for a mutagenic effect of methyl bromide.

E. REVIEWER'S DISCUSSION AND INTERPRETATION OF RESULTS:

We assess that CH_3Br was not adequately tested for the potential to induce dominant lethal mutations in male rat germinal cells for the following reasons:

1. The highest assayed dose was not clearly toxic to the male rats nor did it induce a cytotoxic effect on the target cell tissue; therefore, the data are insufficient to establish that the MTD was investigated.

2. The number of mated females (20) and the number of successful pregnancies were below the number recommended (30 females per group per mating)[3] to provide an adequate sample size. Therefore, the statistical power required to detect a doubling of the spontaneous frequency was compromised. The loss of statistical power was highlighted by the lack of significance in early death frequencies for the positive control group where obvious differences in early implantation loss occurred. We are, therefore, unable to assess the relevance of the ~2-fold increase in the early-death MIs for the low-dose group at week 1 and for the high-dose group at week 3. These increased MIs appear to be isolated events relative to dose and the timing of stages in the spermatogenic cycle. It was, never the less, noted that the increased MIs occurred in early mating weeks; most mutagens that are positive in the dominant lethal assay generally affect postmeiotic cells (detectable in the first 3 weeks).[4]

---

[3] Ibid.

[4] Bateman, A. J. The dominant lethal assay in the male mouse. In: Handbook of Mutagenicity Test Procedures, B. J. Kilbey, M. Legator, W. Nicols, C. Ramel, eds., Amsterdam: Elsevier Scientific Publishing Co. (1977), pp. 235-334.

Several calculation errors were found in the summary tables, and, although we do not believe that the uncovered calculation errors (particularly for high-dose week-3 live implants) would have altered the author's conclusion, it did contribute to our interpretation of the results. We assess, from the above considerations, that no definitive conclusions can be reached; the study is unacceptable and should be repeated.

F. CBI APPENDIX: Appendix A, Materials and Methods, Animals and Animal Management, CBI pp. 7-10; Atmospheric Generation and Exposure, CBI pp. 11-15; Dominant Lethal Assay, CBI pp. 23-24.

007086

APPENDIX A

Material and Methods

Methyl bromide toxicology review

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EPA No.: 68080056
Dynamac No.: 111-B2
October 14, 1988

DATA EVALUATION RECORD

METHYL BROMIDE

Mutagenicity - In Vivo Cytogenetic Assay in Rats

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: Daniel Fellmann for

Date: 10-14-88

72

007006

EPA No.: 68080056
Dynamac No.: 111-83
October 14, 1988

DATA EVALUATION RECORD

METHYL BROMIDE

Mutagenicity - Dominant Lethal Assay in Rats

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Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 10-13-88

I. Cecil Felkner, Ph.D.
Independent Reviewer
Dynamac Corporation

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APPROVED BY:

I. Cecil Felkner, Ph.D.
Genetic Toxicology Studies
Technical Quality Control
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 10-14-88

William Burnam, Ph.D.
EPA Reviewer and
Branch Chief,
Toxicology Branch for
Herbicide, Fungicide, and
Antimicrobial Support

Signature: _____
Date: _____

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DATA EVALUATION RECORD

METHYL BROMIDE

Mutagenicity - In Vivo Cytogenetic Assay in Rats

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Genetic Toxicology Studies
Technical Quality Control
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 10-14-88

William Burnam, Ph.D.
EPA Reviewer and
Branch Chief, Toxicology Branch for Herbicide, Fungicide, and Antimicrobial Support

Signature: _____
Date: _____

14

DATA EVALUATION RECORD 007006

STUDY TYPE: Mutagenicity--In vivo cytogenetic assay in rats.

ACCESSION/MRID NUMBER: 405791-01.

TEST MATERIAL: Methyl bromide.

SYNONYM(S): Bromomethane.

STUDY NUMBER(S): 210-78-0026.

SPONSOR: National Institute for Occupational Health, Cincinnati, OH.

TESTING FACILITY: Iveresk Research Int. Ltd., Musselburg, Scotland, UK.

TITLE OF REPORT: Tier II Mutagenic Screening of 13 NIOSH Priority Compounds, Individual Compound Reports, Methyl Bromide.

AUTHOR(S): McGregor, D.B.

REPORT ISSUED: May 30, 1981.

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CONCLUSIONS: Under the conditions of this assay, neither the acute (one 7-hour exposure) nor subacute (7 hour/day x 5 days) inhalation exposure of male and female rats to 20 and 70 ppm methyl bromide (CH_3Br) caused a significant increase in chromosome aberrations in bone marrow cells sampled over the entire mitotic cycle. However, the test material was not clearly toxic to the animals and did not induce a cytotoxic effect in the target organ. The study, therefore, provides no assurance that the maximum tolerated dose (MTD) was achieved.

Classification: The study is unacceptable; unless the study author can justify the use of 70 ppm CH_3Br as the high dose, the study should be repeated.

A. MATERIALS:

1. Test Compound: CH_3Br from batch No. 77371 was described as a colorless gas and was maintained in two cylinders under ambient conditions. Physical and chemical properties and methods for safe handling were reported.

2. Test Animals: Species: rat; strain: CD Sprague-Dawley; age: 10-11 weeks; source: Charles River (UK) Ltd., Manston, Kent, UK.

3. Inhalation Chambers:

   a. Description: The test exposure chambers were constructed of stainless steel and glass and had a capacity of 1.5 m^3. The animals occupied a volume of 0.02 m^3 and were confined to a single tier of cages (volume occupied = 0.4 m^3). This area was ventilated at a flow rate of 12 to 16 air changes/hour. Test chambers were maintained under a slight negative pressure. Air control animals were held in a 0.84-m^3 chamber; the chamber was ventilated at a rate of 8 air changes/hour.

   b. Aerosol Generation/Exhaust: Test atmospheres were produced by appropriate dilution of CH_3Br with filtered compressed air and introduced into the top of the exposure chambers through a 7/8-inch stainless steel pipe. The test atmospheres were continuously generated for a single pass through the chamber; CH_3Br/air mixes were regulated in the mixing vessel by adjustable flow meters. Test atmospheres were exhausted with a Gast extraction pump, passed through a scrubber containing methylated spirits/water, diluted with building exhaust air, and discharged to the environment.

   c. Chamber Concentrations/Monitoring Equipment: Prior to the initiation of the study, homogeneity of test atmospheres in the exposure chambers was determined for periods

up to 7 hours. Test atmospheres were continuously monitored during exposure. All determinations were performed with daily precalibrated infrared gas analyzers; sample flow rate was 4L/minutes. Chamber temperatures and humidity were recorded hourly during the exposures; these data were not reported.

B. STUDY DESIGN:

1. Animal Assignment: Animals were identified by ear tag and cage card number (randomization procedures, if any, were not reported) and assigned to the following test groups:

Test group	Dose	Males/ Group	Females/ Group
Acute Study			
Negative Control[a]			
Air	--	30	30
Test Group[a]			
Low	20 ppm	30	30
High	70 ppm	30	30
Positive Control[b]			
Ethylmethane-sulfonate (EMS)	250 mg/kg	30	30
Subacute Study			
Negative Control[a]			
Air	--	10	10
Test Group[a]			
Low	20 ppm	10	10
High	70 ppm	10	10
Positive Control[b]			
EMS	100 mg/kg	10	10

[a]Negative control and test doses were administered by inhalation for 7 hours/day for 1 day in the acute study or for 5 consecutive days in the subacute study.

[b]EMS was administered by oral gavage either once (acute) or for 5 consecutive days (subacute).

2. Animal Maintenance: Animals were housed individually and were maintained in an environment controlled for temperature (18.5-26.5°C), humidity (40-61%), and light (12 hours/day). Spratts-Spillers No. 1 rodent feed and water were provided ad libitum except during the aerosol exposure periods.

3. Preliminary Toxicity Study: A preliminary range-finding study was not performed and the rationale for dose selection was not reported.

4. Cytogenetic Assay:

   a. Compound Administration

      1. Acute Cytogenetic Assay: Thirty animals per group per sex were exposed via inhalation to 0, 20, or 70 ppm CH_3Br for 7 hours. The positive control ethylmethanesulfonate (EMS; 250 mg/kg) was administered once by oral gavage.

         Representative males and females from each group were sacrificed by cervical dislocation at 6, 24, and 48 hours after compound exposure; body weights were determined prior to sacrifice.

      2. Subacute Cytogenetic Assay: The animals in the subacute study (10/sex/group) were exposed as described to 0, 20, or 70 ppm CH_3Br for 7 hours/day for 5 consecutive days. Rats in the positive control group were given 100 mg/kg EMS by oral gavage for 5 days. Body weight was measured daily following the exposures. Rats were sacrificed 6 hours after the final dose was administered.

   b. Animal Sacrifice/Bone Marrow Harvest: Colchicine (3 mg/kg, ip) was injected 4 hours prior to the appropriate sacrifice interval. Bone marrow cells were collected from one femur by aspiration into Hanks' balanced salt solution; tubes containing the cell suspensions were coded. Cell suspensions were centrifuged, incubated in 0.075 M KCl for 20 minutes, and recentrifuged; supernants were discarded. The cell pellets were fixed in methanol:glacial acetic acid (3:1), refrigerated overnight, resuspended in fresh fixative, and dropped onto slides and stained.

   c. Slide Analysis: A maximum of 50 well-spread metaphases per animal were scored for the presence of cytogenetic abnormalities. Chromosome aberrations were characterized as gaps, breaks, fragments, dicentrics, translocations, and pulverized cells. Data were evaluated both with and without gaps.

d. Statistical Evaluation: Data were transformed using the Freeman-Tukey transformation for preparations and transformed data were analyzed by a one-sided Student's t-test at p values of 0.05, 0.01, and 0.001.

e. Quality Assurance: Quality assurance personnel were identified but a quality assurance statement was not provided.

C. REPORTED RESULTS:

1. Test Material Analyses:

a. Homogeneity: Homogeneity analysis of chamber concentrations was performed prior to initiation of the study. Samples taken from eight areas of each chamber indicated that, in general, the test atmospheres were uniformly distributed throughout the exposure chambers and were within 5% of the target concentrations.

b. Achieved Concentration: Atmospheric analysis of test concentrations were conducted continuously throughout each daily 7-hour exposure. Based on the reported data, the average achieved daily concentrations in the low dose ranged from 18.7 to 21.4 ppm; achieved concentrations in the high-dose chamber ranged from 68.7 to 73.4 ppm. The author stated, however, that $\geq$10% deviations from the target concentrations occurred; the maximum concentrations recorded for 20 and 70 ppm were 31 and 116 ppm, respectively. Extremes for values lower than the desired target concentrations were not reported.

2. Animal Observations: No animals died while on study and the only reported clinical sign was blood around the nostrils of "some" animals exposed to 70 ppm CH_3Br. Body weight determination for the acute and subacute exposures showed no significant differences among treatment and negative control groups. Females in the subacute exposure groups did, however, show a slight dose-related decrease in body weight gain and non-dose-related reductions in body weight gain were seen in the males.

3. Cytogenetic Assay: Representative results for the acute exposure phase of the cytogenetic assay are presented in Table 1. No significant increase in chromosomal aberrations occurred after acute exposure to 20 or 70 ppm CH_3Br. Although females exposed to the high dose showed a slight increase in the percent aberrant cells and number of aberrations per cell following the 48-hour harvest, when compared to the overall aberration frequencies for the control females, the increase was negligible. Results from the 24-hour harvest (this is considered the optimum time for detection of EMS-induced chromosome aberrations) of animals exposed to the

TABLE 1. Representative Results of the Acute In Vivo Cytogenetic Study in Rats Exposed to Methyl Bromide

Substance	Dose	Exposure Time[a]	Sex	No. of Animals Analyzed per Group	No. of Metaphases Examined	% Aberrant Cells per Group[bc]	Total No. of Aberrations per Group[b]	No. of Aberrations per Cell[d]
Negative Control								
Air	—	6	M	10	500	0.4	2	0.004
			F	10	450[e]	0.9	4	0.009
		24	M	10	500	0.2	1	0.002
			F	10	500	0.6	3	0.006
		48	M	10	500	0.2	2	0.002
			F	10	450[g]	0.2	1	0.002
Positive Control								
Ethylmethane-sulfonate	250 mg/kg	24	M	10	500	28.6	222*	0.4
			F	10	500	35.2	289*	0.6
Test Material								
Methyl bromide	20 ppm	6	M	10	450[g]	0.0	0	0.0
			F	10	474[e]	0.6	3	0.006
		24	M	10	500	0.4	2	0.004
			F	10	450[h]	0.2	1	0.002
		48	M	10	450[g]	0.7	3	0.007
			F	10	500	0.0	0	0.0
	70 ppm	6	M	10	500	0.0	0	0.0
			F	10	443[e]	0.0	0	0.0
		24	M	10	500	0.2	1	0.002
			F	10	500	0.8	4	0.008
		48	M	10	450[g]	0.7	3[f]	0.007
			F	10	500	1.0	5	0.01

[a] Time after compound administration.

[b] Excluding gaps.

[c] Percent aberrant cells = $\frac{\text{No. of cells with aberrations}}{\text{No. of metaphases examined}} \times 100$; calculated by our reviewers.

[d] No. of aberrations per cell = $\frac{\text{Total no. of aberrations}}{\text{No. of metaphases examined}}$; calculated by our reviewers.

[e] Several animals had < 50 metaphase cells.

[f] Calculation errors found in summary tables; recalculation of raw data indicates numbers presented by our reviewers.

[g] Single animals had no metaphase cells.

[h] No raw data were found.

* Significantly different from the control value at $p<0.001$.

positive control are shown in Table 1. Following multiple exposures of the test animals to 20 and 70 ppm CH_3Br, no evidence of a clastogenic effect was observed (Table 2). The author stated that there was a significant increase in the number of aberrant cells from male rats exposed to 70 ppm CH_3Br. The significance, however, resulted from the inclusion of gaps in the statistical analysis and was lost when the data were analyzed without gaps.

D. STUDY AUTHOR'S CONCLUSIONS:

The study author concluded that under the adopted exposure conditions, there was no evidence for a mutagenic effect of methyl bromide.

E. REVIEWER'S DISCUSSION AND INTERPRETATION OF RESULTS:

We assess that while the results of the acute and subacute cytogenetic assays conducted with methyl bromide provided no indication of a clastogenic response, the lack of clear compound-related toxicity or cytotoxicity to the target cells gives no assurance that CH_3Br was assayed to the MTD. Therefore, unless the study author can justify the use of 70 ppm CH_3Br as the high dose, the assay should be repeated. Additionally, calculation errors were uncovered in the summarized results presented by the study author. The recalculated values did not, however, affect the outcome of the study.

F. CBI APPENDIX: Appendix A, Materials and Methods, Animals and Animal Management, CBI pp. 7-10; Atmospheric Generation and Exposure, CBI pp. 11-15; Cytogenetic Assay, CBI pp. 21-22.

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TABLE 2. Representative Results of the Subacute In Vivo Cytogenetic Study in Rats Exposed to Methyl Bromide

Substance	Dose	Exposure Time[a] (days)	Sex	No. of Animals Analyzed per Group	No. of Metaphases Examined	% Aberrant Cells per Group[b,c]	Total No. of Aberrations per Group[b]	No. of Aberrations per Cell[d]
Negative Control								
Air		5	M	10	500	0	0	0
			F	10	500	0.4	2	0.004
Positive Control								
Ethylmethane-sulfonate	100 mg/kg	5	M	10	500	1.6	11	0.022
			F	10	500	1.4	8	0.016
Test Material								
Methyl bromide	20 ppm	5	M	10	500	0.2	1	0.002
			F	10	500	0.2	1	0.002
	70 ppm		M	10	500	1.0	5	0.01
			F	10	500	0.4	2	0.004

[a] Time after compound administration.
[b] Excluding gaps.

[c] Percent aberrant cells = $\frac{\text{No. of cells with aberrations}}{\text{No. of metaphases examined}} \times 100$; calculated by our reviewers.

[d] No. of aberrations per cell = $\frac{\text{Total no. of aberrations}}{\text{No. of metaphases examined}}$; calculated by our reviewers.

Significantly different from the control value at $p<0.001$.

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APPENDIX A

Materials and Methods

Methyl bromide toxicology review

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EPA: 68D80056
DYNAMAC No.: 111-B1
October 13, 1988

DATA EVALUATION RECORD

Methyl Bromide

Mutagenicity--Unscheduled DNA Synthesis Assay
in Human Embryonic Intestinal Cells

STUDY IDENTIFICATION: McGregor, D. B. Tier II mutagenic screening of 13 NIOSH priority compounds, individual compound reports, methyl bromide. (Unpublished study No. 210-78-0026, report No. 1190, prepared by Iversek Research Int. Ltd., Musselburgh, Scotland, UK, for the National Institute for Occupational Safety and Health, Cincinnati, OH; submitted by the Methyl Bromide Industry Panel; dated May 30, 1981). MRID No. 405791-01.

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: [signature]

Date: 10-14-88

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1. CHEMICAL: Methyl bromide; bromomethane (CAS No. 74-83-9).

2. TEST MATERIAL: Methyl bromide, batch No. 77371, was described as a colorless gas; the purity was not reported.

3. STUDY/ACTION TYPE: Mutagenicity--Unscheduled DNA synthesis assay in human embryonic intestinal cells.

4. STUDY IDENTIFICATION: McGregor, D. B. Tier II mutagenic screening of 13 NIOSH priority compounds, individual compound reports, methyl bromide. (Unpublished study No. 210-78-0026, report No. 1190, prepared by Iversek Research Int. Ltd., Musselburgh, Scotland, UK, for the National Institute for Occupational Safety and Health, Cincinnati, OH; submitted by the Methyl Bromide Industry Panel; dated May 30, 1981). MRID No. 405791-01.

5. REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 10-13-88

I. Cecil Felkner, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 10-14-88

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Genetic Toxicology Studies
Technical Quality Control
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 10-14-88

William Burnam, Ph.D.
EPA Reviewer and
Branch Chief,
Toxicology Branch for
Herbicide, Fungicide, and
Antimicrobial Support

Signature: _____
Date: _____

7. CONCLUSIONS:

A. Human embryonic intestinal cells were exposed to eight nonactivated and S9-activated test atmospheres ranging from 5 to 70% methyl bromide (CH_3Br) and evaluated for the induction of unscheduled DNA synthesis (UDS). CH_3Br was neither cytotoxic nor genotoxic; however, the assay was compromised for the following reasons:

1. The lack of cytotoxicity suggests that the 3-hour exposure did not allow sufficient time for the test vapors to interact with the target cells; a minimum of 4 hours is recommended for exposure of human embryonic lung cells (WI-38) to test materials in solution.[1]

2. The ability of this diploid human cell line to detect genotoxic activity by weak genotoxins has not been established previously or by this assay.

3. The use of dimethylsulfoxide (DMSO) as the negative control is not justified. Cells were exposed to varying concentrations of CH_3Br diluted in air; therefore, air is the appropriate negative control.

B. The study is unacceptable.

8. RECOMMENDATIONS: If use of this cell line can be justified and if validated data can be presented, the repeat assay should be performed using doses that show cytotoxicity or the authors should indicate that 70% CH_3Br is the maximum concentration that can be achieved. Otherwise, it is recommended that the assays be repeated using recommended procedures[2] and either a well-characterized human diploid cell line (i.e., WI-38) or another appropriate mammalian test system.

Items 9 and 10--see footnote 3.

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[1]Mitchell, A. D., Casciano, D. A., Meltz, M. L.. Robinson, D. E., San, R. H. C., William, G. M., and Von Halle, E. S. Unscheduled DNA synthesis tests, a report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutat. Res. 123(1983):363-410.

[2]Ibid.

[3]Only items appropriate to this DER have been included.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: CH_3Br from batch No. 77371 was described as a colorless gas and was maintained in two cylinders under ambient conditions. Physical and chemical properties and safety procedures for handling the test material were reported.

2. Cell Line: Human embryonic intestinal cells (FLOW 11,000, passage 12-35) were obtained from Flow Laboratories, Irvine, Scotland. Cells were maintained as monolayers at 37°C in Dulbecco's Minimum Essential Medium (DMEM) supplemented with fetal calf serum (10%), glutamine (2 mM), and antibiotics. The characteristics of the cell line were not reported. Prior to use, confluent monolayers were trypsinized, sedimented, suspended at a density of 5×10^4 cells/mL in DMEM, dispensed into 35-mm culture dishes containing three sterile coverslips, and incubated for 72 hours.

3. Metabolic Activation: The S9 fraction was prepared from the livers of male CD rats induced with Aroclor 1254. The S9 mix contained 10% S9 fraction and the appropriate cofactors.

4. UDS Assay:

a. Cell Preparation: Cells attached to the coverslips were refed arginine-deficient DMEM and the incubation was continued for 24 hours. Cells were refed and reincubated for an additional 48 hours. At the conclusion of the final incubation, hydroxyurea and tritiated thymidine were added to each culture at a final concentration of 2.5 mM and 10 μCi/mL, respectively. The S9 mix (100 μL) was added to half the cultures.

b. Exposure: Exposure of attached cells to eight test atmospheres ranging from 5 to 70% CH_3Br and three concentrations of the positive control, vinyl chloride (12.5, 25, and 50%), both with and without S9 activation, was accomplished in culture flasks. Prior to exposure, the culture medium was removed. The test material and positive control were passed through gas flow meters, diluted to the appropriate concentration with flow-meter controlled hydrogen-free air, and introduced into the appropriate flasks. The flow rates for test gases and air were recorded before the mixtures were passed into the culture flasks and the percentages of test gas in

air were calculated. The negative control consisted of cells exposed to nonactivated and S9-activated 1% DMSO. Following a 3-hour exposure, coverslips were repeatedly rinsed, incubated for 10 minutes in 1% sodium citrate, fixed in methanol:acetic acid (3:1), and mounted for autoradiography.

c. Preparation of Autoradiography: Slides were coated with Kodak-AR-10 emulsion at 20°C, air dried, and held in light-tight boxes at 4°C for 14 days. The autoradiographs were developed in Kodak D19, rinsed, and fixed with Kodak Unifix; the labeled nuclear grains were counted. The authors did not indicate whether the slides were coded.

d. Grain Counting: The report stated that 50 nuclei were examined for each culture and the data were recorded as the average net grain counts for the coverslips ± the standard deviation. Since no raw data were presented, we can only assume that 150 nuclei from each experimental point were evaluated.

5. Evaluation Criteria: No criteria either to establish the validity of the study or the biological significance of the findings were presented.

6. Statistical Analysis: The data were not analyzed statistically.

B. Protocol: A protocol was not provided.

12. REPORTED RESULTS: There was no indication from the tabular results or the author's discussion that CH_3Br, at any dose either with or without S9 activation, was cytotoxic. As shown in Table 1, the eight nonactivated concentrations of CH_3Br did not appreciably increase the nuclear grain counts as compared to the DMSO-control group. In the presence of S9 activation, slight but nondose-related increases in UDS were seen in cultures exposed to the 60 and 70% test atmospheres of CH_3Br. Results for the positive control indicated that 12.5% vinyl chloride in the presence of S9 activation induced a marked increase in UDS.

TABLE 1. Representative Results of the Human Embryonic Intestinal Cell Unscheduled DNA Synthesis Assay with Methyl Bromide (CH_3Br)

Substance	Dose Percent	S9 Activation	No. of Scored Nuclei	Mean Number of Grains/ Nucleus ± S.D.
Negative Control				
Dimethylsulfoxide	1	-	150	3.3±2.7
		+	150	5.2±5.0
Positive Control				
Vinyl Chloride	25	-	150	4.4±2.5
	12.5	+	150	95.8±42.9
Test Material				
Methyl Bromide	50[a]	-	150	6.0±4.2
	60	-	150	6.7±5.2
	70	-	150	6.6±5.7
	50[a]	+	150	3.8±4.4
	60	+	150	12.1±11.1
	70	+	150	9.8±6.8

[a] Results for lower test atmospheres (5, 10, 20, 30, and 40% CH_3Br) were generally comparable to the dimethylsulfoxide-treated cultures.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The author concluded that the cells were able to respond to treatment with a gaseous mutagen (vinyl chloride) but CH_3Br did not induce any increase in UDS.

B. Quality assurance personnel were identified, but no quality assurance statement was provided.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. We assess that the results of this study, as performed, cannot be evaluated for the following reasons:

1. Although the highest tested atmospheric concentration of CH_3Br probably approached the maximum that could be assayed, no cytotoxicity was seen. The lack of cytotoxicity suggested to us that the length of exposure (3 hours) should have been increased; recommended time for exposure of human embryonic lung cells (WI-38) to chemicals in solution is 4 hours.[4]

2. The author stated that the human embryonic intestinal cell line used in this assay was selected because of higher permeability than other human cell lines to some substrates. However, no information was furnished to support this claim. We are not aware of any published studies indicating the suitability of these fibroblasts for the UDS assay.

Without historical data on the background frequencies of UDS, response of the cell line to known nonactivated and S9-activated genotoxins, and the adequacy of a 3-hour exposure, the sensitivity of these cells to detect weak genotoxic activity cannot be established.

Similarly, no information was provided on the upper limit of cell line age. This is an important consideration because diploid human cell lines lose their repair capacity with increasing passages. The cell line used in this assay was from passage 12-35; 25 passages is considered the maximum for WI-38.

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[4] Mitchell et al. Mutat. Res. 123(1983): 363-410.

3. Since the test atmospheres for both CH_3Br and vinyl chloride were prepared by dilution with air, it was unclear why the test results were compared to cultures treated with DMSO rather than cells exposed only to air.

4. The methods used to report the final results were equally unclear. The report indicated that average net grain counts were calculated while the tabulated results listed mean number of grains/nucleus. In general, net grain counts refer to the counts derived from subtracting cytoplasmic grain counts from nucleus grain counts. However, fibroblasts generally have negligible cytoplasmic background counts; therefore, we assume that the reported results were not adjusted and that the tabulated data were correctly identified.

5. The lack of individual data precluded an independent statistical evaluation of the results; however, the variation in data, as indicated by the high standard deviations for the majority of experimental points, probably eliminated any potential significant findings.

Item 15--see footnote 3.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 17-20.

APPENDIX A

Materials and Methods

Methyl bromide toxicology review

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