Report Number 32

TIER II MUTAGENIC SCREENING OF 13 NIOSH PRIORITY COMPOUNDS

INDIVIDUAL COMPOUND REPORT METHYL BROMIDE

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May 30, 1981

Supported by

NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH
Division of Biomedical and Behavioural Science
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4676 Columbia Parkway, Cincinnati, Ohio 45226

Contract No. 210-78-0026

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AUTHENTICATION

"I, the undersigned, hereby declare that this work was performed under my supervision, according to the procedures herein described and that this report represents a true and accurate record of the results obtained."

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Project No. 409959

Report No. 1190

TABULATIONS

The table numbering system used informs the reader to what the table refers.

AT	-	Atmosphere Analysis
BW	-	Body Weights
UDS	-	Unscheduled DNA Synthesis
CA	-	Chromosomal Aberrations
DL	-	Dominant Lethal
SA	-	Sperm Abnormalities
RL	-	Recessive Lethal
MD	-	Multiple Dosing
M	-	Males
F	-	Females

Example:

CA-M24-1 = Chromosomal Aberrations, Males,
24 h Sampling Time-1

Abbreviations on Chromosomal Aberration Tables and Appendix Tables:

B w F	-	Break with fragment
B w/o F	-	Break without fragment

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LOCATION OF EXPERIMENT

All exposures of animals were conducted at the Elphinstone Research Centre site of Inveresk Research International Limited. *In vivo* studies and autopsies of mice and rats were also conducted at this site. *Drosophila* breeding was undertaken at the Institute of Animal Genetics, University of Edinburgh. Slide reading and the unscheduled DNA synthesis assay were performed at the Inveresk Gate Laboratories of Inveresk Research International Limited.

DISCLAIMER

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SUMMARY

Methyl bromide was subjected to a tier II mutagenic test screening programme. The assays used were the following:

1. Unscheduled DNA synthesis (UDS) assay in human diploid fibroblasts with exposures of 3 h duration and concentrations up to 70% in air over a minimal volume of culture medium.

2. Dominant lethal test in male rats with exposure to atmospheres containing 20 ppm or 70 ppm methyl bromide for 7 h/day for 5 consecutive days. Analysis of test atmospheres was by continuous infra-red absorption monitoring at a wavelength of 3.4 μm.

3. Sperm abnormality test in male mice using the same exposure conditions as in (2).

4. Cytogenetic test in male and female rat bone marrow cells using the same exposure conditions as in (2) or a single exposure of 7 h duration followed by sampling after 6 h, 24 h and 48 h.

5. Sex-linked recessive lethal (SLRL) test in Drosophila melanogaster with exposure to atmospheres of 20 ppm or 70 ppm for 5 h.

The results obtained were as follows:

1. There was no increase in UDS in cells treated with methyl bromide.

2. The frequency of aberrant cells was not increased by methyl bromide treatment.

3. There were no effects attributable to methyl bromide in the dominant lethal test on pregnancy frequency, numbers of corpora lutea graviditatis or implantations or the frequency of early deaths.

4. Sperm abnormality frequency was not affected by treatment.

5. Sex-linked recessive lethal mutation frequency was not increased as a result of methyl bromide exposure. Increases were seen, but these were neither reproduced nor dose related.

It was concluded that methyl bromide was devoid of genetic effects detectable in these experiments.

INTRODUCTION

Properties

Methyl bromide (bromomethane) (CAS No. 74-83-9) is a powerful fumigant gas which is one of the most toxic of the common alkylhalides. Industrial preparation is by condensation of hydrogen bromide with methanol or, alternatively, by the addition of sulphuric acid to sodium bromide and methanol, in which case the methyl bromide is removed by distillation.

It is a colourless and usually odourless gas, except at high concentrations when it has a sweetish, chloroform like odour a burning taste. A summary of its physical and chemical properties follows.

Formula	CH_3Br
Mol. wt.	94.94
B.P. (760 mm Hg)	3.6°C
M.P.	-94°C
Refractive index (20°C)	1.421
Vapour density (air = 1)	1.68
Vapour pressure (20°C)	1420 mm Hg

It is insoluble in water, but soluble in most organic solvents and it is non-inflammable in air, but does burn in oxygen.

Use

Methyl bromide is used in the fumigation of stored grain, particularly in corn weevil control and the destruction of eggs and larvae from other insects. It is also effective against fungi, but less so against bacteria and actinomycetes (McKeen, 1954). During fumigation inorganic bromide is formed along with a series of methylated products. Less than 10% is hydrolysed to methanol. Furthermore, methyl bromide decomposes faster at lower moisture contents, illustrating the relative unimportance of hydrolysis. In the glutin or protein fractions of wheat flour the methyl bromide derivatives found are N-methyl (50%), dimethyl sulphonium (30%) and methoxyl (20%). N-Methylation is highly specific, the target being largely the imidazole ring of histidine. In wheat flour, 3 compounds account for 75% of the total N-methylation products: 1- and 3-N-methyl histidines and 1,3-dimethyl histidine bromide (Winteringham et al, 1955).

Toxicology

When methyl bromide is inhaled, rapid pulmonary absorption occurs followed by elimination, partly as unchanged gas from the lungs and partly as bromide in the urine. It tends to

accumulate in fatty tissues. There is extensive hydrolytic fissions of the C-Br bond, resulting in non-volatile Br−, but the simultaneous formation of methanol is questionable (Williford et al, 1974). Reaction with thiol groups may be particularly important in methyl bromide poisoning and cysteine has proved to be efficacious in the prevention of methyl bromide poisoning (Mizyukova and Bakhishev, 1971). Acute inhalation studies have indicated that methyl bromide casuses hepatic glycogen depletion, along with a reduction in blood lactate and pyruvate levels (Bakhishev, 1970); there may also be a distinct hypertriglyceridaemia in animals including man (Hasegawa, 1969).

Methyl bromide poisoning has resulted in a deficiency of clotting factor χ (Graham, 1959), which, along with Ca++ and factors V (proaccelerin) and III (thromboplatin) forms the prothrombin activator.

Systemically, methyl bromide is cumulative and damaging to the nervous system, kidneys and lung. CNS effects include blurred vision, confusion, numbness, tremors and speech defects. Death following acute poisoning is usually caused by its irritant effects upon lung, while in chronic poisoning death is due to CNS injury. Locally, methyl bromide is extremely irritant to skin and may produce severe burns.

Studies on the genetic toxicology of methyl bromide appear to be very limited. When tested in desiccators, Simmon et al, (1977) showed that it was mutagenic in bacteria and in strain Salmonella typhimurium TA 100 the order of mutagenic response in the methyl halide series was bromide> chloride>iodide.

The objective of the work described in this report is to extend the genetic toxicology to mammalian systems in order to evaluate better the hazards of exposure to this chemical.

Exposure conditions used were:

Human fibroblasts:	up to 70% in air for 3 h.
Mice and rats:	20 ppm or 70 ppm for 7 h/day for one or 5 days.
Drosophila:	20 ppm or 70 ppm for 5 h.

MATERIALS AND METHODS

CHEMICALS

Test Substance

Two cylinders of methyl bromide gas, Batch No. 77371, were received from BDH Limited, Poole, Dorset, England in June 1979. The test material was a colourless gas and was retained under ambient conditions in the company dispensary until used.

Positive Control Substance

Ethyl methanesulphonate (EMS) was obtained from Koch-Light Laboratories, Colnbrook, Bucks and retained in a refrigerator in the company dispensary until used.

ANIMALS AND ANIMAL MANAGEMENT

Animals

CD rats (a remote Sprague-Dawley derived strain) were obtained from Charles River (U.K.) Limited, Manston, Kent.

B6C3F$_1$ hybrid mice were obtained from Charles River (U.S.A.).

These animals were obtained on the following dates.

Species	Date of Receipt	Age (Weeks)	Quarantine (Days)	Number (Sex)	Dates of Exposure	Comment
Rat	23 May 1979	10-11	11	220♂ 176♀	4-8 June 1979 11 June 1979	Multiple exposure. Single exposure.
Mouse	24 May 1979 8 June 1979	10-12 8-10	10 None	44♂ 80♀ x 10	4-8 June 1979 None	DL matings.

Pre-experiment Acceptance Tests

All animals were examined on arrival for signs of ill health. Twenty rats (10♂ and 10♀) and 4 mice were selected at random, then autopsied and subjected to a microbial examination together with a histopathological evaluation of main organs.

The organs which were taken for histopathology were: liver, kidney, heart, lung, thymus and a portion of ileum. Caecal contents were examined for pin worms. Bacteriology of certain samples was performed. The procedure adopted, in outline, is as follows.

1. Ileal contents are incubated in selenite broth.
2. Lung, liver and kidney samples are incubated on blood agar plates.
3. Lung sample is plated on McConkey's medium.
4. Liver sample which was plated onto blood agar is then taken into a selenite tube.
5. All samples in selenite broth are incubated for 24 h, then plated on McConkey's medium for 24 h.
6. Smears are prepared and stained. Any Gram-negative bacteria are then put through Enterotubes for identification.

Animal Management

Protective clothing, including laboratory gowns, over-shoes, rubber gloves and masks were worn at all times that personnel were involved in handling or husbandry of the test animals.

All the animals were located in a room which was separate from but adjacent to the area where the exposures were conducted.

They were housed individually in cages in a room with a light intensity of approximately 200 lux, a 12 h light-dark cycle, approximately 10 air changes per hour, temperature maintained at ca 22°C with extreme limits of 18.5°C and 26.5°C, and relative humidity ca 50%, with extreme limits of 40% and 61%.

Floors were swept and disinfected with a mop impregnated with Tego (A. & J. Beveridge, Edinburgh), an ampholytic detergent, during the experiment.

Walls, cage racks and floors were washed with Tego once a week during this study.

The rats designated for cytogenetic analysis were housed in suspended polycarbonate cages measuring 24 x 18 x 41 cm with steel mesh tops and bottoms. The cages were suspended over trays lined with absorbent paper. Rats designated for the dominant lethal study and mice for the sperm abnormality test were housed in polycarbonate cages measuring 24 x 11.5 x 30.5 cm and 11.5 x 12 x 46 cm respectively. Sterilised, white wood shavings were used as bedding material. Cages, trays and papers were changed each week of the experiment, or more frequently if considered necessary.

Diet

Food and water were freely available to the rats at all times. The diet was Spratts-Spillers No. 1. This was constituted as follows:-

	Stock Diet (%)
White fish meal	10.9
Maize meal	36.8
Wheat meal	30.9
Extracted soya meal	11.9
Wheat germ	4.0
Dried yeast	2.0
Spratts-Spillers salts and vitamins*	6.0

*Commercial mixture used for many years in laboratories throughout the U.K., but the detailed composition was not revealed to Inveresk Research International Limited.

Diet analysis was conducted and the results are presented in Appendix Diet.

Allocation of Rats and Mice to Cages and Treatment Groups

Empty cages were placed on racks and, upon receipt of the animals, starting with the male rats, a transporting box was opened and a rat placed in the first cage. A second rat was removed from the same transport box and placed in the second cage and so on until all the cages designated for the male rats each contained one animal.

This complete process was repeated for the female rats and male $B6C3F_1$ mice. The mice were kept on a separate rack from the rats.

Male and female rats were located at separate sides of the animal holding room (Appendix Loc-1).

Each cage was allocated to a specific treatment group using a series of random number permutations. Each permutation consisted of a random set of numbers from 1-4, corresponding to the number of dose groups in the study.

Treatment groups were colour coded as follows:

Green	-	Air Control
Blue	-	Low Dose
Red	-	High Dose
Brown	-	Positive Control

Animal Identification

The animals to be dosed were individually identified using brass ear tags bearing the animal number and suffix letter showing the compound designation. Each rat and mouse was ascribed a cage card which identified that animal by project number, animal number, sex and treatment group.

Female rats used in the dominant lethal test were identified by the cage card number of the male with which they were mated and their assessment week number.

Animal Positioning in the Exposure Chambers

Although homogeneity data were obtained which showed that there were no test compound concentration differences of any significance in the exposure chambers, animal positions were

rotated on a daily basis to minimise any possible exposure location variations. Animal location charts for each day were drawn up, as shown in Appendix Loc-2.

The treatment groups were constituted as follows:-

Species	Test	Dose Group	Animal Numbers Males	Females
Rat	Single dose cytogenetics	Air Control	1-30	161-190
		Low	31-60	191-220
		High	61-90	221-250
		Positive Control	91-120	251-280
	Multiple dose cytogenetics	Air Control	121-130	281-290
		Low	131-140	291-300
		High	141-150	301-310
		Positive Control	151-160	311-320
	Dominant lethal	Air Control	361-370	
		Low	371-380	
		High	381-390	
		Positive Control	391-400	
Mouse	Sperm abnormality	Air Control	321-330	
		Low	331-340	
		High	341-350	
		Positive Control	351-360	

ATMOSPHERE GENERATION AND EXPOSURE

Exposure Chambers

The exposure chambers were located in a room, adjacent to the animal holding area, specifically set aside for the study. Entry was restricted to personnel directly involved in the generating and monitoring of the test atmosphere.

Exposures to methyl bromide were carried out in 1.5 m^3 capacity chambers constructed of stainless steel and glass. The animals occupied a volume of 0.02 m^3 and were confined to a single tier of cages of 0.4 m^3 in volume (the breathing zone). The breathing zone was ventilated at the rate of 12 and 16 air changes per hour respectively for the low and high chambers. An additional chamber of 0.84 m^3 capacity was used for exposure of the air control group; the breathing zone in this chamber also was ventilated at the rate of 8 air changes per hour.

Compressed air was supplied by means of 2 Broomwade compressors (Type CAR31) fitted with automatic pressure control switches. These supplied filtered, conditioned, oil-free compressed air for subsequent dilution of test atmospheres.

Test atmospheres were exhausted from the exposure chambers using a Gast extract pump. Contaminated air extracted from the exposure chamber was 'scrubbed' using methylated spirits/water treatment. It was then diluted in the building exhaust air before discharging to the external atmosphere. The exposure chambers were maintained under slight negative pressure (variable, but normally 2-3 cm water) to minimise any possible leakage of test material into the working environment.

The generating apparatus and exposure chambers (Figures 1a and 1b) were positioned behind a screen in a room with a high efficiency exhaust system designed to ensure a safe working environment for laboratory personnel. The monitoring equipment was located on the outside of the screen at the opposite end of the room. The laboratory atmosphere was continuously monitored for any traces of the test compound. Exposure personnel wore breathing apparatus until it was shown that the room environment was clear of any possible contamination by methyl bromide. Protective gloves and laboratory coats were worn and the test compound was handled in an extract hood at all times.

Monitoring Equipment

The atmospheres within the exposure chambers were analysed by infra-red spectroscopy using Miran-1A Portable Gas Analysers (Foxboro/Wilks Inc). This type of instrument is a single beam, variable wavelength spectrometer, scanning the infra-red spectrum between 2.5 and 14.5 μm. It is equipped with a gas cell having a variable pathlength of between 0.75 and 21.75 m. Samples of the chamber air were continuously pumped (4 l/min) through nylon sample lines of 1/8" ID, to the gas cell of the analyser. The concentration was measured and relayed to a chart recorder (Servoscribe RE 541) to provide a permanent record of the chamber concentrations.

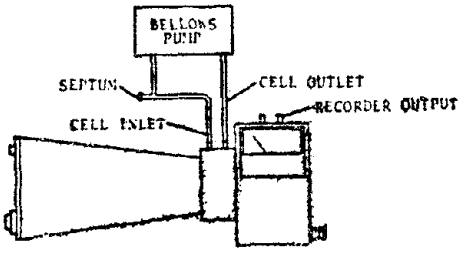
Calibration and Analytical Development

Most chemical compounds have characteristic infra-red spectra which can be used for identification and to quantify the amount present. The infra-red spectrum of methyl bromide was scanned using a 'closed loop calibration system' to generate a test atmosphere within the Miran gas cell. A strongly absorbing wavelength, free of interference from H_2O and CO_2, which provided suitable sensitivity was selected. Suitable pathlengths were chosen to provide optimal readings at the desired concentration levels. The gas analyser was zeroed by sampling laboratory air through a 'zero gas air' filter.

Calibration

The infra-red gas analysers used to monitor chamber atmospheres of methyl bromide were calibrated each day before vapour generation commenced.

The calibration was performed using a closed loop calibration system (see diagram below). Known volumes of methyl bromide gas were sequentially injected into the gas analyser via the closed loop calibration system through a rubber septum using a gas Pressure Lok glass micro syringe. After each injection the absorbence reading was allowed to stabilise as indicated on the chart recording.



SCHEMATIC DIAGRAM OF CLOSED LOOP CALIBRATION SYSTEM

The cumulative absorbence chart deflections for each injection were then measured and plotted against calculated concentrations to give a calibration graph used in subsequent determinations of chamber concentrations during atmospheric monitoring.

Analytical Conditions

Instrument Settings:

	Low Level	High Level
Wavelength :	3.4 µm	3.4 µm
Pathlength :	20.25 m	20.25 m
Absorbence Range :	0.1 A	0.1 A
Slit Width :	1.0 mm	1.0 mm
Meter Response :	10	4
Recorder Voltage :	0.5 V	1 V
Chart Speed :	300 mm/h	300 mm/h

Calibration Data

$$C\ (ppm) = \frac{V}{5.64}$$

Where:

C = Concentration (ppm)
V = Sample volume (µl)
5.64 = Volume of Miran sample chamber (l)

Example of the Calculation for V

Compound: Methyl bromide

C = 20 ppm
V = C x 5.64
= 20 x 5.64
= 112.8 µl

Therefore, to construct a calibration curve to cover the 20 ppm range, 50 µl samples of methyl bromide were injected into the analyser.

Atmosphere Generation

Schematic diagrams showing the vapour generating apparatus, exposure chambers and monitoring equipment is presented in Figures 1a and 1b. The test atmospheres were produced by appropriate dilution of the methyl bromide gas with filtered compressed air. The resulting mixture of methyl bromide/air was ducted through 7/8" stainless steel piping to the top of the exposure chamber.

The atmospheres in the exposure chambers were dynamic in that they were continuously generated for a single pass through the animal holding zone, before being extracted from the bottom and ducted away for 'scrubbing'.

The required atmospheric concentrations within the exposure chambers were maintained by finely regulating the flow of methyl bromide and diluting air into the mixing vessels, by means of adjustable flow meters.

Homogeneity Data

Before starting the animal exposures, chamber concentrations at both the high and low levels were determined by continuous monitoring for periods of up to 7 h. In addition, samples were measured from different areas (at least 9) of the animal holding zone to confirm uniformity of methyl bromide concentration.

Measurement of Chamber Concentrations

Atmospheric concentrations of methyl bromide were monitored continuously during the 7 h exposure period from the breathing zone of the animals. A separate monitoring system was used for each concentration level. Stainless steel sampling lines, fitted with a particulate filter (Whatman Mini-Filter, Grade 80) and positioned on a central reference point in each exposure chamber, were connected to the infra-red gas analysers via 1/8" ID nylon tubing. The sampling flow rate was approximately 4 l/min.

Photo-reduced traces showing exposure chamber concentrations along with the daily calibration are presented in Figure 3 and Tables AT-1 and 2.

Test Compound Utilisation

The volume of methyl bromide gas used to produce the test atmospheres was not measured.

Exposure Procedure

Exposures were conducted during the 7 h of between approximately 09.00 h and 16.00 h on each exposure day. Animals were not allowed access to food or water during the exposure period.

Each animal was removed from its housing cage, examined for any signs of ill health, the ear number checked, and then individually accommodated inside a stainless steel grid

compartment. The animals were then transferred to the exposure room and placed inside the exposure chamber according to the daily exposure location chart.

Animals exposed to methyl bromide were arranged in a single tier inside the exposure chamber. Air control animals were stacked in 2 tiers.

During the multiple exposure period, rats designated for the dominant lethal test, cytogenetic multi-dose test and the mice for the sperm abnormality test were exposed together for 7 h/day for 5 consecutive days. The single dose cytogenetic test rats were exposed on a different day. Animal positions within the exposure chambers were rotated on a daily basis to minimise any possible exposure location variations.

The chamber temperature and relative humidity were recorded at hourly intervals throughout the exposure period. The animals were also observed at regular intervals for the appearance of clinical signs or adverse reactions to treatment.

On completion of the exposure period and purging of the chamber of test compound (as observed on the chart recorder), the animals were removed from the exposure chamber and returned to the animal holding area.

The animals were then removed from their individual compartments, observed for clinical signs, ear numbers checked, body weights recorded and returned to their cages.

Positive Control Groups in Animal Tests

Preparation of Dosing Solutions

Dosing solutions were prepared daily 5 min before administration to the animals was started. The desired amount of ethyl methanesulphonate was weighed into a volumetric flask and diluted with distilled water to obtain the correct concentration.

Treatment of Rats and Mice with Ethyl methanesulphonate

Positive control animals were not allowed access to food or water whilst the remaining test groups were being exposed.

Ethyl methanesulphonate was administered orally by gavage to the rodents at a constant dose volume of 10 ml/kg at around 16.00 h on each day that dosing was required.

The dose levels received by each group of positive control animals were as follows:

Dominant lethal rats	100 mg/kg for 5 consecutive days.
Multi-dose cytogenetic rats	100 mg/kg for 5 consecutive days.
Single dose cytogenetic rats	250 mg/kg once only.
Sperm abnormality mice	200 mg/kg for 5 consecutive days.

UNSCHEDULED DNA SYNTHESIS ASSAY

Aseptic techniques were used throughout the preparation of materials and execution of the experimental methods.

Chemicals

The positive control substance, vinyl chloride, was obtained from BDH Limited, Poole, Dorset, England.

6-[^{3}H]-thymidine (21 Ci/mmol) and 8-[^{3}H]-deoxyguanosine (26.4 Ci/mmol) were obtained from the Radiochemical Centre, Amersham, England.

The polychlorinated biphenyl mixture, Aroclor 1254, was received from Analabs Incorporated, Newhaven, Connecticut, U.S.A.

Test Atmospheres

The methyl bromide and vinyl chloride were passed through gas flow meters and diluted with hydrocarbon-free air which had passed through another flow meter. Percentages of test gas in air were caluclated from the different flow rates recorded before the mixture passed into the culture flasks.

Cells

Unscheduled DNA synthesis, following treatment with test compound, was measured in human embryonic intestinal cells (Flow 11,000), passage 12-35 obtained from Flow Laboratories, Irvine, Scotland. This cell line was chosen because of its higher permeability to some substrates than certain other human cell lines tested.

Culture Maintenance and Growth Media

Cells in 175 cm^2 Nunc flasks were routinely maintained at 37^{o}C in Dulbecco's Minimum Essential Medium (DMEM) and in an atmosphere of 5% CO_2:95% air (v/v). The medium contained 2.0 g/l sodium bicarbonate and was supplemented with heat inactivated (65^{o}C, 30 min) foetal calf serum, (10% v/v), gentamycin (50 µg/ml) and glutamine (2 mM). DMEM (10x concentrated) and antibiotics were obtained from Gibco Europe Limited, Paisley, Scotland, and serum from Flow Laboratories, Irvine, Scotland.

Arginine-deficient medium contained 3.70 g/l sodium bicarbonate and was supplemented with heat inactivated foetal calf serum (5% v/v) and gentamycin (50 µg/ml). This medium was obtained from Flow Laboratories.

For sub-cultivation of confluent monolayers growing in complete DMEM, the medium was removed and the cells treated with a solution of 0.25% (w/v) trypsin in phosphate buffered balanced salt solution containing EDTA (0.0002% w/v). Excess trypsin was removed and the flasks incubated at 37°C until the cells began to detach from the plastic. 5 ml of fresh culture medium was then added and cells brought into suspension by repeated aspiration through a sterile 10 ml pipette. Samples of the cell suspension were added to medium in fresh culture flasks, the usual ratio for division of confluent monolayers being 1:4. If cells were to be frozen they were suspended in medium containing 10% v/v dimethylsulphoxide and stored in liquid nitrogen.

Animals

Male CD rats were obtained from Charles River (U.K.) Limited, Manston, Kent, England.

Male rats weighing 250-300 g were injected once i.p. with Aroclor 1254 (diluted in corn oil to a concentration of 200 mg/ml) at a dosage of 500 mg/kg 5 days before they were killed. The animals were allowed drinking water continuously but food was withheld 16 h before they were killed.

Preparation of the 9,000 g Supernatant Fluid from Livers

Freshly killed animals were thoroughly swabbed with 70% alcohol, the abdomen opened and liver removed, taking care not to cut into the gastro-intestinal tract and thereby contaminating the sample. The liver was collected in ice-cold 0.15 M-KCl, which was also the solution used for homogenisation.

The liver was weighed and a volume of ice-cold 0.15 M-KCl equivalent to 3 times its weight was added. The liver was homogenised by 8 strokes of a glass tube vessel while the Teflon pestle (radial clearance 0.14-0.15 mm) was rotating at about 1,200 r.p.m. The homogenate was transferred to sterile polypropylene centrifuge tubes and spun at 9,000 g for 10 min at 0° to 2°C. The supernatant fluid was decanted leaving behind a thick pellet of (mainly) whole cells, nuclei and mitochondria. Post-mitochondrial supernatant fluids were freshly prepared in sufficient quantity for the experiment and stored in liquid nitrogen until required.

Ice-cold 0.05 M-phosphate buffer, pH 7.4, was added to pre-weighed NADP and glucose-6-phosphate, etc., as follows to give a final concentration in the S-9 mix of:

NADP-di-Na-salt	4 mM (= 3.366 mg/ml)
Glucose-6-phosphate-di-Na-salt	5 mM (= 1.521 mg/ml)
$MgCl_2.6H_2O$	8 mM (= 1.626 mg/ml)
KCl	33 mM (= 2.460 mg/ml)

This solution was immediately filter-sterilised by passage through an 0.45 µm Millipore filter and mixed with the liver 9,000 g supernatant fluid in the following proportion:

co-factor solution	9 parts
liver preparation	1 part

DNA Repair Assay

The cells were harvested, sedimented, suspended in fresh culture medium at a density of 5 x 10^4 cells/ml and 2 ml samples of this suspension were pipetted into 35 mm tissue culture Petri dishes containing 3 sterile coverslips (Lux Scientific Corporation, California, U.S.A.). These were then incubated at 37°C in a humid atmosphere of 5% CO_2 in air for 72 h. The medium from each of the dishes was then replaced with 2 ml of arginine-deficient DMEM supplemented with 5% heat inactivated foetal bovine serum and the plates incubated for 24 h. The medium was then replaced with a further 2 ml of arginine-deficient DMEM and the incubation continued for a further 48 h. At the end of this time the cultures were divided into 2 groups and 100 µl of S-9 mix added to one of them. Solutions of hydroxyurea (250 mM) in sterile distilled water and 6-[^{3}H]-thymidine (21 Ci/mmol) were added to each culture giving final concentrations of 2.5 mM and 10 µCi/ml respectively.

Methyl bromide or vinyl chloride in air was metered (see p. 15) in culture flasks from which the culture medium had been decanted.

After incubation for 3 h at 37°C the cultures were repeatedly rinsed in phosphate buffered saline (PBS) which removed loose cells and soluble [^{3}H]-thymidine. They were then incubated for 10 min in sodium citrate (1%) and, finally, fixed in methanol:acetic acid (3:1) for 18 h. For ease of handling during processing for autoradiography the coverslips were air dried and attached, cells uppermost, to clean microscope slides with a drop of mountant, DePeX. The cells were then processed for autoradiography and stained.

Autoradiography

The autoradiographic procedures were carried out in the darkroom at a temperature of 20°C ± 2°C. Illumination was by a safelight fitted with a Kodak filter No. 1 (red) lit by a 25 watt bulb some 4-6 feet away from the working area.

Stripping film (Kodak AR-10) was used to coat the cultures and the procedures recommended by Rogers (1973) were followed. Pieces of stripping film of suitable size were floated, emulsion side down, on the surface of the glass distilled water. After 2 min when the film had swollen, it was picked up in the surface of the slide bearing the cells.

The slide with the film on it was left to stand vertically in a gentle stream of cool air for 20 min and then placed in a large light-tight box containing a quantity of silica gel and allowed to dry slowly for 24 h at room temperature. After drying the slides were placed in a small light-tight box containing a few granules of silica gel, to keep them dry, and exposed at 4°C for 14 days. The autoradiographs were then developed in Kodak D19 developer for 7 min, washed in 2% acetic acid for 1 min and fixed in Kodak Unifix for 7 min. They were then rinsed in tap water and finally immersed in slowly running tap water and washed for 20-30 min. The excess film was trimmed away leaving only that covering the cell cultures.

Quantification of Repair Synthesis

The stained autoradiographs were examined with a Leitz Dialux 20 L microscope. Fifty nuclei were examined for each culture. The data are recorded as the average net grain counts for 3 coverslips ± the standard deviation.

CYTOGENETIC ANALYSIS OF RAT BONE MARROW CELLS

Metaphase Cell Preparations

Each rat was injected i.p. with 3 mg/kg colchicine dissolved in Hank's Balanced Salt Solution (HBSS) 4 h after the last dose was given. Two hours later the rats were killed by neck dislocation.

One femur from each animal was dissected out, cleaned of adherent tissue and the marrow aspirated into a 10 ml plastic blood sample tube containing 4 ml HBSS at ambient temperature and lithium heparin (250 IU). Each tube was labelled with the appropriate random number from a slide coding sheet. Hence, from this time until the completed result sheets were de-coded, the rat number and group were unknown to the scientists and technicians.

The cell suspension was centrifuged at 1,500 r.p.m. for 5 min, the supernatant fluid discarded and replaced with 4 ml fresh HBSS. The cells were suspended, then centrifuged again and the supernatant fluid discarded.

4-5 ml 0.075 M-KCl pre-heated to 37^{o}C was added to the cells while they were agitated on a vortex mixer. Following incubation for 20 min in a 37^{o}C water bath, the cells were centrifuged, the supernatant fluid decanted and the cells fixed in 4 ml freshly prepared fixative (methanol:glacial acetic acid; 3:1). The fixative was removed after centrifugation and replaced with 2 ml fresh fixative. Tubes containing fixed cells were stored in a 4^{o}C refrigerator overnight.

The following morning (or later, up to 3 days) the fixative was changed and cell suspensions dropped onto clean slides labelled with the same number as the tube and allowed to dry thoroughly.

Slides were stained in a bath of Giemsa R66 (Gurr) diluted with 10 parts distilled water for 30 min, rinsed briefly in distilled water, dehydrated in alcohol, cleared in xylene and mounted in DePeX.

Slide Reading

Leitz binocular microscopes were used for this purpose. Magnification was nominally x 1,000 using x 10 magnification eye pieces and x 100 objectives.

Wherever possible, for each animal 50 cells with a minimum of 41 well spread chromosomes were examined and scored. The location of all spreads examined was recorded using the microscope stage vernier. The slide number was always located on the right hand side.

The number of abnormalities was recorded on sheets of the design shown in Appendix Form-1. Abnormalities looked for were: gaps, breaks, fragments, dicentrics, translocations (within the limitations of the staining methods) and pulverisation.

DOMINANT LETHAL TESTING IN MALE RATS

Mating

1. Day 1: The male rats were transferred to the test or control treatments described above (10 rats per treatment) and maintained on these treatments until Day 5 (i.e., 5 days). The animals were caged individually during the treatment. All experimental treatments ceased on Day 5.

2. Day 5: Two virgin female rats were introduced to each of the 40 cages containing single, treated male rats.

3. Day 12: Male rats were transferred to fresh cages which did not contain rats.

4. Day 22: Female rats were killed and examined for pregnancy and dominant lethal effects.

5. Steps (2), (3) and (4) above were repeated on each of the next 9 consecutive weeks.

Assessment

It was assumed that most matings which led to fertilisation occurred either 2 or 3 days after introducing female rats to the cages containing the males. The female rats were killed by neck dislocation 14 days after the assumed dates of fertilisation, i.e., 17 days after caging females with males.

Ovaries and uteri of the killed rats were removed and the ovaries examined for corpora lutea graviditatis, which were counted and this result recorded. Uteri were then opened, examined for live implantations, early deaths and late deaths. These data and any observed abnormalities were recorded on sheets of the design shown in Appendix Form-2.

Live implantations were recognised as rat foetuses normally developed for approximately Day 14 of gestation and with a vasculature which had clearly been functioning until at least maternal death.

A late death was diagnosed as a foetus where organogenesis had occurred, but was now bloodless due to death of the foetus within the last 2 days of intra-uterine existence.

An early death was diagnosed as a point of uterine reaction to an implanting blastula. Since embryonic development had not proceeded, further placental development had stopped and, usually, regressed. The product was a small, raised, discrete spot along the line of implantations and apparently consisting mostly of deoxygenated and clotted blood.

SPERM ABNORMALITIES TEST IN MICE

Preparation

Mice were killed 5 weeks from the last day of dosing (i.e., Friday 13 July 1979) by neck dislocation.

The abdominal cavity was opened and the testes eased into it. The seminal ducts were exposed by gentle traction and the cauda epididymides were cut off. These were transferred to a small beaker containing 2 ml fixative (0.01% glutaraldehyde in 0.25 M-sucrose, 0.05 M-phosphate buffer, pH 7.4). The cauda epididymides were finely minced and the sperm dispersed using a fine bore Pasteur pipette. The sperm suspension was decanted into a centrifuge tube labelled with the randomised number, where it was left for at least 30 min.

After centrifugation at 500 r.p.m. for 3 min, a few drops of the supernatant fluid were spread along the length of a clean slide labelled with the randomised number. The slides were allowed to air dry overnight. The smears were stained in 1% eosin dissolved in distilled water:ethanol; 1:1 for 45 min. After rinsing briefly, slides were dried overnight on a hot plate, cleared in xylene for 5 min and mounted in DePeX.

Assessment

Slides were examined using a Leitz Dialux 20 microscope. Assessment techniques and criteria were guided by the work of Wyrobek and Bruce, (1975).

The following types of sperm were not scored:

(1) separated tails and heads.

(2) clumps of sperm.

(3) sperm orientated so that the hook could not be seen.

(4) sperm partially masked by any remaining stain droplets.

Otherwise, sperm were scored and placed in one of the following categories:

I Normal

II Abnormal

A. hook upturned or elongated.

B. banana-shaped head.

C. amorphous head.

D. abnormal tail (sharp, 180° angle or tight coiling only).

E. miscellaneous (these were specified in footnotes, could include multiple tails, double heads, twisted neck, filamentous mid-piece, enlarged mid-piece, plier type).

The data were recorded on score sheets of the type shown in Appendix Form-3.

SEX-LINKED RECESSIVE LETHAL TEST IN DROSOPHILA MELANOGASTER

The basc or Müller-5 test was used (Spencer and Stern, 1948; Würgler et al 1977). In this test, recessive lethal mutations induced in the X-chromosomes of treated male gametes are detected in the F_2 generation by the absence of wild-type males in the progeny of individual gametes. F_3 generation flies were also observed since this allows the detection of mosaics or delayed mutations which may not appear in the F_2 generation.

Strains

The wild-type flies were Oregon K (OrK). Two lines, designated A and B, were established in November 1978 and maintained by shaking over to fresh medium bottles every 2-3 weeks.

The Müller-5 (M-5) flies had the basc balancer X-chromosome, $ln(1)\ SC^{S1L}\ SC^{8R} + S\ SC^{S1}\ SC^{8}\ w^{aB}$.

Medium

Stocks were maintained in half-pint milk bottles containing approximately 100 ml medium. All flies on test were kept in 3" x 1" glass vials containing approximately 8 ml medium and stoppered with cotton wool. This medium contained:

maize meal	150 g
treacle	130 g
agar (Sigma)	20 g
yeast, flaked	22 g
propionic acid	5 ml
*Nipogen	1 g

which was added to one litre water and boiled before being dispersed to sterile maintenance bottles or glass vials.

Exposures

Three day old male OrK flies were used. They were exposed in a glass vessel through which the test atmospheres were passed at the required concentrations at a rate of ca 5 l/min before passing directly into the infra-red analyser. Transference of flies from feeding vials to exposure chamber was performed when they were lightly anaesthetised with carbon dioxide.

*Nipogen: bacteriostatic agent (BDH Limited).

The length of exposure in the main test was determined by running a toxicity test in the week prior to the main exposure. Groups of 100 flies were exposed for varying times, which were initially intended to be 1, 3 and 7 h. These times had to be modified, however, in view of the effects seen of the test compound on the flies.

Exposed flies were kept overnight in their feeding vials in a 26°C water bath, then transported from the exposure laboratory to the assessment laboratory at the Institute of Animal Genetics, University of Edinburgh. This journey took ca 30 min, the vials being packed in cotton inside an expanded polystyrene case.

Toxicity Test

Upon arrival at the assessment laboratory, the vials were examined and the numbers of survivors recorded. From these survivors 4 males were picked and mated with 4 virgin females. These females were allowed to lay their eggs on medium darkened with charcoal for 24 h, then removed. The number of eggs laid was recorded. After a further 24 h, the eggs remaining unhatched were counted and recorded. From these figures a hatchability index could be calculated and compared with the untreated control.

$$\text{Hatchability index} = \frac{\text{No. of eggs hatched}}{\text{No. of eggs laid}} \times 100$$

Recessive Lethal Test

Each treated male was given a number which was retained throughout the brood analysis and which his progeny retained through to the F_2 generation and, where appropriate, the F_3 generation. Any clusters of mutants could, therefore, be seen readily.

Treated males were mated individually to virgin Müller-5 females in the ratio 1♂:2♀ on the morning following the day of exposure. Each male was re-mated to 2 more virgin females 3 days and, again, 8 days after the first mating. All matings ceased on Day 11. The 3 broods obtained in this way ensured that sperm treated at all stages of spermatogenesis were tested.

Emergence for F_1 generation flies from the pupae began about 10 days after mating.

Matings for the F_2 generation were set up 1-4 days later by mating brother with sisters.

Assessment of effects in the F_3 generation was undertaken in the same way as for the F_2 generation.

Experiments were normally scored 11-14 days after setting up the F_2 or F_3 crosses. Vials were examined by eye and scored as non-lethal if 2 or more wild-type males were seen. If these were not seen the flies were shaken out onto a carbon monoxide permeated pad and examined under the microscope. Vials in which there were no wild-type males and 8 or more M-5 males were checked for the presence of heterozygous (M-5/OrK) females and scored as recessive lethals if these were present. If a vial could not be unambiguously scored, it was returned to the incubator room to be rescored the next day, when more flies had hatched.

Vials which could not be scored after all the flies had hatched were an indication for re-assessment of the F_1 females, e.g. if only one OrK male was present or no OrK male and less than 8 Müller-5 males. This was done by taking 2 heterozygous females and crossing with Müller-5 males. Vials in which there was no F_2 generation were scored sterile.

STATISTICAL EVALUATION

Cytogenetics Tests

The data were transformed using the Freeman-Tukey transformation for proportions:

$$y = \sin^{-1}\left(\sqrt{\frac{x}{n+1}}\right) + \sin^{-1}\left(\sqrt{\frac{x+1}{n+1}}\right)$$

where, x = number of cells with abnormalities
n = number of cells
y = transformed cells

A one-sided Student's t test was used on the transformed values.

This analysis was performed (a) including all abnormalities and (b) excluding cells only exhibiting gaps.

Dominant Lethal Assay

The variates analysed were:

Corpora lutea graviditatis (eliminating cases with zero total implantations)
Total implantations
Live implantations
Live implantations + early deaths
Early deaths, Freeman-Tukey Poisson transformation
Early deaths, Freeman-Tukey binomial transformation

Each female was regarded as an independent replicate and the negative control, low dose and high dose groups were analysed together, the positive control group being analysed separately.

The proportion of females with one or more, or 2 or more, early deaths was calculated, after which treatment and control groups were compared using the chi-square test.

The fertility index (or pregnancy frequency) was treated in a way similar to the last statistic: the number of pregnant females per number of mated females was computed and the chi-square test used to compare each treatment group with its concurrent control. In these calculations, pregnancy was defined as (a) females with corpora lutea graviditatis and (b) females with implantations.

In addition to the above calculations, which were as originally required by protocol, the statistician applied his own analysis of the proportions of early deaths. The treatment means were expressed on a logistic scale. One

analysis assumed pure binomial variation, but, since this is often false, a second analysis assuming between litter variation was also applied. A third analysis allowed for linear dependence of the proportion of early deaths on total implantations.

The analysis assumed that the probability of an early death varies between females in the ith treatment group with mean θ_i and variance $\phi\ \theta_i(1-\theta_i)$ and, given this probability, the individual early deaths within a female occur independently. These assumptions imply that if r_{ij} and n_{ij} denote respectively the numbers of early deaths and total implantations in the jth female in the ith treatment group, then

$$E(r_{ij}/n_{ij}) = \theta_i$$

$$Var(r_{ij}/n_{ij}) = n_{ij}^{-1}\ \theta_i(1-\theta_i)[1 + \phi(n_{ij}-1)]$$

The θ_i values for the different treatment groups were compared. The value of ϕ, a dispersion parameter, is of less interest and may be assumed to have the same (unknown) value for each treatment. The beta binomial model described by Williams (1975) is a special case of the more general model assumed here. A different special case is the correlated binomial model of Kupper and Haseman (1978) or, equivalently, the additive model of Altham (1978), in which ϕ is regarded as an intra-family correlation coefficient.

For the beta binomial model, Williams (1975) suggested the use of maximum likelihood estimation and likelihood ratio tests. The more general model now assumed specifies only the first 2 moments of the distribution, consequently, likelihood methods cannot be applied. Instead, θ_i terms are estimated by weighted least squares, given the value of ϕ, by minimising.

$$S(\theta) = \sum_{ij} \frac{(r_{ij}-n_{ij}\theta_i)^2}{n_{ij}\theta_i(1-\theta_i)(1 + \phi(n_{ij}-1))}$$

The value of ϕ is estimated iteratively by equating the minimised value of $S(\theta)$ to its degrees of freedom (total number of females minus the number of treatments).

The advantages of this method of analysis over the approaches of Williams (1975) or Kupper and Haseman (1978) are twofold. Firstly, the analysis can be accomplished without any special programming by exploiting the ideas of Wedderburn (1974) and using the GLIM package. Secondly, the method does not rest on strong distributional assumptions and may be expected to be more robust, while the results of Kleinman

(1973) encourage the hope that little efficiency is lost by using weighted least squares when the beta binomial in fact holds.

These data were analysed using the GLIM programme package interactively. The value of ϕ was generally assumed to be independent of treatment effects, except for the positive control which was analysed using a separate ϕ estimate. The GLIM programme provided the estimates $\hat{\mu}_i$ of $\mu_i = \log [\theta(1-\theta_i)^{-1}]$ and the standard errors of these estimators, which are given in the table. Also given are the corresponding estimates of θ_i obtained from the back transformation $\theta_i = \exp(\hat{\mu}_i)/(1 + \exp(\hat{\mu}_i))$.

Sperm Abnormalities Test

The data were transformed using the Freeman-Tukey transformation for proportions:

$$y = \sin^{-1}\left(\sqrt{\frac{x}{n+1}}\right) + \sin^{-1}\left(\sqrt{\frac{x+1}{n+1}}\right)$$

where, x = number of abnormal sperm
n = number of sperm examined

A one-sided t test was used on the transformed values. This analysis was performed on (a) total abnormal cells and (b) each of the abnormal categories A-E.

Sex-linked Recessive Lethal Test

The untreated control frequency of lethals in the flies used was about 0.2%. True mutation frequencies can only be determined within certain limits because only integral numbers of mutations can be recorded (Würgler et al 1975). These frequencies strongly depend on the sizes of the test groups studied (i.e. the size of individual broods), which are relatively small.

Based upon previous experiences with this test, which is meaningful but insensitive (Rinehart, 1969), it is considered that, in place of a test for statistical significance, it is better to look for a reproducible increase in the frequency of lethals over the historical control value of about 0.1%. There is, of course, no opportunity for lethals to accumulate. Control values accumulated over the past 1.5 years are as follows:

F_2 Generation

	Stock A			Stock B			Total
	Brood			Brood			
	1	2	3	1	2	3	
No. of experiments	9	9	9	9	9	9	54
No. of gametes	5319	5309	5339	5264	5088	4713	31026
% Lethals	0.12	0.04	0.09	0.11	0.03	0.00	0.07

F_3 Generation

	Stock A			Stock B			Total
	Brood			Brood			
	1	2	3	1	2	3	
No. of experiments	0	2	2	1	1	4	10
No. of gametes	0	1200	989	400	300	2000	4889
% Lethals	0	0.00	0.00	0.30	0.00	0.10	0.08

Against this background, the criteria for result assessment were:

(a) a compound giving frequencies below 0.5% in duplicate experiments is considered to show no evidence of mutagenic activity.

(b) a compound giving frequencies greater than 1.0% in the same brood in duplicate experiments is considered to show mutagenic potential.

(c) a compound giving frequencies between 0.5% and 1.0% shows evidence of possibly being mutagenic. Although this evidence is not conclusive, the compound clearly would deserve further study.

RESULTS

Instrument Calibration

Calibration of the IR spectrometers was performed daily when atmosphere generation work was undertaken during the development phase and when animals were being exposed to test vapours. An example of a calibration curve is given in Figure 2. Data for the construction of such curves are given for various exposure dates in Tables AT-1 and 2. The reproducibility of the calibration curve data from day to day is good.

The wavelength chosen for monitoring methyl bromide was 3.4 μm. At this wavelength there is minimal interference from water: thus, there is weak stretching of O-H at 2.7 mμ, a medium bonding of O-H at 6.2 mμ and intermolecular H-bonding over the range 2.76-2.86 mμ. Pathlength was standardised at 20.25 m.

Calibration ranges adopted were 8.85-35.4 ppm (20 ppm target concentration) and 17.7-88.5 ppm (70 ppm target concentration).

Chamber Atmospheres - Homogeneity

Prior to exposure of the animals, the chamber atmospheres were sampled at different positions to establish that adequate mixing of methyl bromide was occurring. The results are shown in Table AT-3, where it can be seen that the maximum deviations encountered were -4.6% at the 20 ppm target concentration and -1% at the 70 ppm target concentration.

Chamber Atmospheres - Achieved Concentrations

A sample chart record taken during a day on which animals were exposed is shown in Figure 3. From charts such as this, deviations from the target concentrations of 20 ppm and 70 ppm were obtained and recorded in Tables AT-4 to 6.

Deviations from the target concentrations of more than $\pm$ 10% were encountered. The maximum concentrations recorded were 31 ppm and 116 ppm for the 20 ppm and 70 ppm target concentrations respectively. Overall, the exposures were considered to be acceptable with the time averaged concentrations approximating very closely to the desired target concentrations.

Animal Location

In Appendix Loc-1 and Appendix Loc-2 are shown respectively the locations of the cage racks in the holding room and typical examples of exposure location sheets as used during the study.

Pre-experimental Acceptance Tests (PEAT)

23 May 1979 Delivery: Ten male and 10 female rats were randomly selected for PEAT. There were no significant clinical observations and the microbiological/parasitological assays did not reveal any infections. At autopsy 2 male rats showed irregular congestion of both kidneys. Histopathological examination showed vascular congestion in the kidneys of one of those rats only. Subcapsular foci of lymphocytes were observed in the kidneys of one female rat. Lung histopathology revealed peribronchial foci of lymphocytes in 8/10 male and 5/10 female rats examined. Perivascular oedema was observed in 2 female rats whilst alveolar oedema was observed in one female rat.

It was considered that the lesions observed did not warrant rejection of the batch of animals received.

24 May 1979 Delivery: Four male mice were randomly selected for PEAT. There were no significant clinical observations and the microbiological/parasitological assays did not reveal any infections. Histopathological examination of the lungs revealed peribronchial infiltration by lymphocytes in one male mouse whilst foci of alveolitis, peribronchial mononuclear cells, alveolar haemorrhage and oedema were observed in another male mouse. In the view of the pathologist these animals were suitable for use for the sperm abnormality test.

Clinical Observations and Body Weights

Six mice were found dead on Day 2 of dosing as a result of being wrongly dosed with 400 mg/kg EMS instead of 200 mg/kg. Ten fresh mice were selected and dosed with 200 mg/kg/day EMS for 5 days. The 4 surviving mice given the wrong dose of EMS were kept, although sperm samples from them were not assessed. No clinical signs were observed in mice exposed to 20 ppm methyl bromide. Mice exposed to 70 ppm methyl bromide appeared subdued during the 7 h exposure period on Day 2. Two male mice were tremulous at the end of exposure on Day 5. One male mouse died 5 days after exposure. In the rats traces of blood around the nostrils, in some of the animals exposed to 70 ppm was the only clinical sign observed during or after exposure to atmospheres containing 20 ppm or 70 ppm methyl bromide. Body weights were apparently not affected during the 5 day exposure period (Tables BW-1 to 4 and Appendix Tables BW-1 to 4). Dosing with EMS for 5 days adversely affected body weights of the male and female rats and a slight reduction was also observed in the male mice used in the sperm abnormalities test.

Body weights of the rats dosed once in the test for chromosomal aberrations are shown in Table BW-2 and Appendix Table BW-2.

UNSCHEDULED DNA SYNTHESIS ASSAY

In the assay involving tritiated thymidine incorporation into non-S phase cells, there was no indiction of any increase in the number of silver grains per nucleus at any concentration of methyl bromide (Table UDS-1). The highest concentration used, in air, was 70%.

Vinyl chloride was used as a concurrent positive control substance. It tended to be cytotoxic at the concentrations of 12.5%, 25% and 50% in air which were used. However, sufficient cells remained at the end of the exposure period for silver grains to be counted in the 12.5% group (with S-9 mix) and in the 25% group (without S-9 mix). There was no increase over the DMSO treated control group in the absence of S-9 mix, but there was a large increase in the number of silver grains per nucleus induced by 12.5% vinyl chloride in the presence of S-9 mix.

It was concluded that the cells were able to respond to treatment with a gaseous mutagen (vinyl chloride) but methyl-bromide did not induce any increase in UDS.

CYTOGENETIC ANALYSIS OF RAT BONE MARROW CELLS

Data are presented in Table CA-MD-M-1 to CA-F48-2 and Appendix Tables CA-MD-M to CA-F48.

In the multiple exposure cytogenetic test, there was a significant increase in the number of aberrant cells from the male rats exposed to the 70 ppm methyl bromide atmosphere ($P<0.05$). The increase over the air control value lost its statistical significance if cells containing only gaps were excluded. In female rats, on the other hand, there was no significant difference from the air control group in the frequencies of aberrant cells. Hence, the biological significance of the difference seen in the male rats is open to doubt, particularly as sex differences are a more remote possibility in the metabolism of and response to a simple methylating agent.

In the single exposure test neither male nor female rats showed increases in the frequencies of aberrant cells at the 6 h, 24 h or 48 h sampling times. With reference to the air control group, the female rats at the 6 h sampling time showed statistically significant decreases in total aberrant cell frequency following exposure to both 20 ppm and 70 ppm methyl bromide atmospheres. The greater reduction was in rats exposed to 20 ppm atmosphere, so, it seems unlikely that methyl bromide was protecting cells from damage or selectively killing aberrant cells.

The groups treated with EMS over 5 days showed increases in the frequencies of aberrant cells which were statistically significant in the males, but not in the females. After a single dose of EMS total aberrant cell frequencies were significantly increased in male rats at the 6 h ($P<0.05$) and 24 h ($P<0.001$) sampling times and in female rats at the 24 h ($P<0.001$) and 48 h ($P<0.05$) sampling times. If cells other than those containing only gaps are analysed statistically, significant increases were seen in males only at the 24 h ($P<0.001$) sampling time and in females at the 24 h ($P<0.001$) and 48 h ($P<0.05$) sampling times.

DOMINANT LETHAL TEST

Data are given in Tables DL-1 to 9 and Appendix Table DL.

Pregnancy frequency was calculated in 2 ways: firstly, by considering as pregnant females with corpora lutea graviditatis (Table DL-1) and secondly and more reliably by considering as pregnant only females with implantations (Table DL-2). With nether method was there any effect upon pregnancy frequency due to methyl bromide treatment, but there were reductions in Weeks 2 and 3 in the positive control groups. In Week 1 there were generally low pregnancy frequencies which were clearly unrelated to treatment.

Corpora lutea graviditatis counts (Table DL-3) were not reduced in either of the methyl bromide treated groups except in Week 7 of the 20 ppm methyl bromide treated group ($P<0.01$). There was not a significant or greater reduction in the 70 ppm methyl bromide treated group of this week. In the EMS treated group, significant reductions were observed in Weeks 1, 3 and 9 ($P<0.05$ in all cases).

Implantations per pregnancy (Table DL-4) were unaffected by methyl bromide treatment, but were reduced in Weeks 1-4 of the positive control group. The greatest effect was seen in Week 3 ($P<0.001$).

The frequencies of live implantations (Table DL-5) and live implantations and late deaths (Table DL-6) followed very closely the pattern of total implantations per pregnancy. There were no significant changes in the methyl bromide treated groups and changes in the positive control group were restricted to Weeks 1-4.

A review of the data showing pregnancies with either (1) one or more early deaths or (2) two or more early deaths (Table DL-7) did not indicate any increase in these frequencies in the methyl bromide treated groups, when compared with the air control group.

Analysis of the proportions of early deaths following Freeman-Tukey poisson or binomial transformation (Tables DL-8 and 9) did not indicate any increases attributable to methyl bromide. There was a significant decrease in Week 3 of the 20 ppm exposure group ($P<0.05$), but this was not also seen in the 70 ppm exposure group. In the EMS treated group there were significant increases in early death frequency in Week 1, 5 and 7, following Freeman-Tukey poisson transformation and in Week 1, 5, 7 and 9, following Freeman-Tukey binomial transformation. There was also a significant reduction in Week 3, following Freeman-Tukey poisson transformation.

SPERM ABNORMALITY TEST

There were no increases in the frequencies of abnormal sperm in any of the categories examined (Table SA-1 and 2 and Appendix Table SA). In the EMS treated group there was a significant increase in the frequency of abnormal sperm ($P<0.05$) which was due in particular to an increase in Category C, amorphous head ($P<0.05$).

SEX-LINKED RECESSIVE LETHAL TEST IN DROSOPHILA

There was no information on the toxicity of methyl bromide to flies, so, a preliminary study was made (Table RL-1).

A dose ranging experiment was undertaken on 1 June 1979 in which flies were exposed to 20 ppm or 70 ppm methyl bromide for 1, 3 or 5 h. No signs of toxicity were seen at either concentration or during any of the exposure times. Very few eggs were laid by females mated with flies exposed to 20 ppm methyl bromide, but fertility was not affected by 70 ppm. It was concluded, therefore, that the reduction in fertility observed in the low concentration group was not as a result of exposure.

Exposure conditions for the main test on 11 June 1979 were 20 ppm or 70 ppm methyl bromide atmospheres for 5 h. Two breeding stocks (A and B) were exposed (Table RL-2) in the main test. No signs of toxicity were observed and fertility was acceptable. The frequencies of lethals in the F_2 generation were high for Stock A flies exposed to 20 ppm methyl bromide, i.e. 0.32%, 0.81% and 0.27% in Broods 1, 2 and 3 respectively. Such high frequencies were not also seen in Stock B flies exposed to the 20 ppm atmosphere or in Stock A flies exposed to the 70 ppm atmosphere. Stock B flies in the 70 ppm atmosphere generated 0.61% lethals in the first brood, but none in either Brood 2 or 3.

In the F_3 generation a single lethal (0.26%) was observed in Brood 1 from Stock A flies. The integral nature of these data was certainly responsible for some of the high frequencies (it is not possible to have less than one recessive lethal event, unless there are none). Had these frequencies been reproducible (e.g. the very high frequency observed in Brood 2, Stock A, 20 ppm atmosphere) they would have given rise to the suspicion that these lethals had been induced by the treatment. It is concluded, however, that there is no compelling evidence for the assumption that the observed lethals were induced by methyl bromide treatment, although it was recognised that group sizes were rather small.

EMS treatment (0.4% v/v in sucrose for 5 h) of Stock B flies induced a large increase in the frequency of lethals (33.9%).

CONCLUSIONS

It is concluded that, in these assays and under the adopted exposure conditions, there was no evidence for a mutagenic effect of methyl bromide. This is quite different from the reported activity in Ames' test where relatively high activity is seen.

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TABLE AT-1

Methyl Bromide
Calibration Data for Low Level

Dose Level: 20 ppm v/v

Batch No. 77371

Volume µl	Conc., ppm,(v/v)	Cumulative Chart Deflection, mm					
		4 June 1979	5 June 1979	6 June 1979	7 June 1979	8 June 1979	11 June 1979
0	0	0	0	0	0	0	0
50	8.85	44.0	32.0	33.0	35.0	35.0	36.0
100	17.7	88.0	66.0	66.0	69.0	70.0	72.5
150	26.6	131.0	99.0	99.0	104.0	105.0	112.0
200	35.4	171.0	133.0	132.0	140.0	139.0	148.0
Chart deflection (mm) for 20 ppm		99.0	75.0	74.0	78.0	79.0	84.0

Instrument Setting

Pathlength: 20.25 m
Wavelength: 3.4 µm
Absorbence Range: 0.1 AUFS
Slit Width: 1 mm
Meter Response: 10
Recorder Voltage: 0.5 V
Chart Speed: 300 mm/h

Calibration

Syringe: Gas - Pressure Lok
Injection Volume: 50 µl
No. of Repeat Injections: 4

TABLE AT-2

Methyl Bromide
Calibration Data for High Level

Dose Level: 70 ppm v/v Batch No. 77371

Volume µl	Conc., ppm, (v/v)	Cumulative Chart Deflection, mm					
		4 June 1979	5 June 1979	6 June 1979	7 June 1979	8 June 1979	11 June 1979
0	0	0	0	0	0	0	0
100	17.7	27.0	24.0	23.5	25.0	25.0	26.0
200	35.4	54.0	47.0	48.0	50.0	50.0	52.0
300	53.1	80.0	70.0	72.0	74.0	74.0	77.0
400	70.8	107.0	92.0	94.0	98.0	99.0	102.0
500	88.5	132.0	114.0	117.0	121.5	123.0	127.0
Chart deflection (mm) for 70 ppm		106.0	91.0	94.0	97.0	98.0	101.0

Instrument Setting

Pathlength: 20.25 m
Wavelength: 3.4 µm
Absorbence Range: 0.1 AUFS
Slit Width: 1.0 mm
Meter Response: 4
Recorder Voltage: 1.0 V
Chart Speed: 300 mm/h

Calibration

Syringe: Gas - Pressure Lok
Injection Volume: 100 µl
No. of Repeat Injections: 5

TABLE AT-3

Methyl Bromide
Chamber Atmosphere
Homogeneity Data

Dose Level: 20 ppm and 70 ppm

Sample Location	% Deviation from Reference Sampling Point	
	Low	High
Reference Point (R)	0	0
Right Centre (RC)	+1.5	0
Right Front (RF)	-1.5	-1
Centre Front (CF)	-4.6	-1
Left Front (LF)	-1.5	0
Left Centre (LC)	0	0
Left Back (LB)	0	0
Centre Back (CB)	0	0
Right Back (RB)	0	-1

Top view of exposure chamber

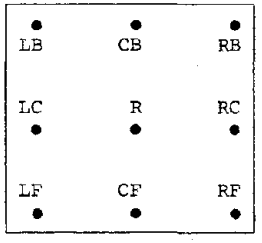


TABLE AT-4

Methyl Bromide
Atmospheric Analysis by Infra-red Spectroscopy
Target Concentration 20 ppm

Exposure Day	% Deviation from Target Concentration in Minutes												Time Averaged Concentration for 7 h (ppm)
	-15	-12.5	-10	-7.5	-5	-2.5	0	+2.5	+5	+7.5	+10	+12.5	
Single ®	-	-	10	5	60	-	25	45	45	20	160	50[a]	21.4
Multiple 1	-	50	135	35	50	135	15	-	-	-	-	-	18.7
Multiple 2	-	-	-	20	-	5	120	75	65	15	120	-	20.8
Multiple 3	-	-	-	-	45	-	65	20	65	110	65	50	21.1
Multiple 4	-	-	5	10	55	95	220	35	-	-	-	-	19.7
Multiple 5	25	-	5	10	5	50	135	95	95	-	-	-	20.0

Target Concentration 70 ppm

Exposure Day	% Deviation from Target Concentration in Minutes											Time Averaged Concentration for 7 h (ppm)
	<-10	-10	-7.5	-5	-2.5	0	+2.5	+5	+7.5	+10	>+10	
Single	20[a]	10	-	25	45	170	85	-	25	5	35[a]	69.4
Multiple 1	-	-	-	15	20	50	70	135	85	45	-	73.0
Multiple 2	-	-	-	5	-	25	175	80	60	75	-	73.4
Multiple 3	-	-	5	25	50	5	30	130	50	125	-	73.4
Multiple 4	-	-	-	105	135	145	35	-	-	-	-	68.7
Multiple 5	30[b]	-	5	25	5	45	290	20	-	-	-	69.5

(a) Back pressure from primary dilution air and methyl bromide meters causing oscillations in flow of undiluted methyl bromide. This gave rapid increases and decreases in concentration and lack of stability

(b) Sticking valve on Methyl Bromide cylinder caused a gradual decrease in chamber concentration down to ~40 ppm. This was quickly rectified and concentration returned to within limits after 30 min

® Drosophila also exposed for 7 h

TABLE BW-1

Methyl Bromide
Multiple Exposure Cytogenetics Test
Group Mean Body Weights (g) for the Dosing Period of Male and Female CD Rats

Sex	Day	Air Control (0 ppm)	20 ppm	70 ppm	5 x 100 mg/kg EMS
Male	1	375.0 ± 19.1	364.5 ± 20.0	369.6 ± 15.1	361.9 ± 21.9
	2	378.8 ± 20.2	364.4 ± 18.0	370.5 ± 16.1	358.8 ± 20.7
	3	382.9 ± 20.1	369.9 ± 18.2	373.7 ± 17.1	347.2 ± 18.8
	4	384.7 ± 20.1	372.8 ± 20.5	376.4 ± 17.9	334.2 ± 16.4
	5	388.6 ± 20.7	373.4 ± 18.5	378.9 ± 17.4	323.6 ± 14.0
	Weight gain /loss	13.6	8.9	9.3	-38.3
Female	1	219.5 ± 17.5	225.2 ± 19.8	212.8 ± 24.7	217.7 ± 15.8
	2	220.7 ± 17.2	224.3 ± 19.7	212.5 ± 25.5	217.8 ± 17.1
	3	223.4 ± 18.5	228.3 ± 19.2	214.4 ± 25.4	211.0 ± 17.8
	4	222.8 ± 19.5	227.2 ± 18.8	214.5 ± 25.9	205.2 ± 19.9
	5	223.4 ± 18.6	228.6 ± 18.0	215.8 ± 25.3	199.6 ± 20.3
	Weight gain /loss	3.9	3.4	3.0	-18.1

TABLE BW-2

Methyl Bromide
Single Exposure Cytogenetics Test
Group Mean Body Weights (g) for Male and Female CD Rats

Sex	Sampling Time (Hours Post Exposure)	Air Control (0 ppm)	20 ppm	70 ppm	250 mg/kg EMS
Male	6	405.7 ± 24.8	391.9 ± 23.2	393.9 ± 34.9	406.2 ± 30.6
	24	404.8 ± 22.2	410.2 ± 19.2	387.2 ± 17.0	416.0 ± 20.6
	48	393.2 ± 23.8	396.5 ± 15.7	394.7 ± 22.6	416.4 ± 18.8
Female	6	241.1 ± 20.0	236.8 ± 19.9	235.0 ± 15.4	232.9 ± 11.6
	24	242.8 ± 15.1	235.1 ± 15.3	257.6 ± 24.3	241.7 ± 13.2
	48	243.4 ± 16.7	235.4 ± 21.4	241.1 ± 21.7	231.0 ± 24.2

TABLE BW-3

Methyl Bromide
Dominant Lethal Assay
Group Mean Body Weights (g) for the Dosing Period of Male CD Rats

Day	Air Control (0 ppm)	20 ppm	70 ppm	5 x 100 mg/kg EMS
1	366.9 ± 19.5	367.2 ± 20.7	372.6 ± 20.0	375.0 ± 21.1
2	372.7 ± 20.9	369.7 ± 22.3	378.2 ± 21.2	372.2 ± 19.8
3	376.5 ± 22.4	376.6 ± 22.8	387.6 ± 21.0	360.3 ± 18.3
4	377.4 ± 23.3	374.6 ± 21.3	382.5 ± 22.8	347.4 ± 18.6
5	382.1 ± 24.2	378.8 ± 20.8	385.1 ± 21.8	339.0 ± 17.7
Weight gain/loss	15.2	11.6	12.5	-36.0

TABLE BW-4

Methyl Bromide
Sperm Abnormalities Test
Group Mean Body Weights (g) for Dosing Period of Male B6C3F$_1$ Mice

Day	Air Control (0 ppm)	20 ppm	70 ppm	1 x 400 mg/kg + 4 x 200 mg/kg EMS	5 x 200 mg/kg EMS
1	20.4 ± 1.3	20.0 ± 1.7	19.7 ± 1.1	20.3 ± 1.5	28.8 ± 1.7
2	20.7 ± 1.2	20.3 ± 1.8	20.1 ± 1.3	19.0 ± 2.2	28.6 ± 1.7
3	21.1 ± 1.0	20.6 ± 2.0	20.6 ± 1.4	18.8 ± 1.7	28.2 ± 2.4
4	21.3 ± 0.9	21.1 ± 2.0	20.4 ± 1.4	19.5 ± 1.3	29.5 ± 1.8
5	21.2 ± 1.1	21.3 ± 1.8	20.4 ± 1.6	20.3 ± 1.5	27.8 ± 1.8
Weight gain/loss	0.8	1.3	0.7	0.0*	-1.0

*n = 4

TABLE UDS-1

Methyl Bromide
Unscheduled DNA Synthesis

Group	Concentration (%)		Mean Number of Grains/Nucleus ± S.D.	
	With S-9	Without S-9	With S-9	Without S-9
Dimethylsulphoxide	.1	1	5.2 ± 5.0	3.3 ± 2.7
Vinyl Chloride	12.5	25.0	95.8 ± 42.9	4.4 ± 2.5
Methyl Bromide	5	5	4.4 ± 4.1	1.7 ± 1.6
	10	10	7.0 ± 5.4	3.3 ± 4.5
	20	20	4.1 ± 3.5	4.7 ± 3.6
	30	30	5.9 ± 4.0	4.4 ± 3.9
	40	40	7.3 ± 6.3	4.4 ± 3.2
	50	50	3.8 ± 4.4	6.0 ± 4.2
	60	60	12.1 ± 11.1	6.7 ± 5.2
	70	70	9.8 ± 6.8	6.6 ± 5.7

TABLE CA-MD-M-1

Methyl Bromide
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Males

Multiple Dosing

Sampling Time: 6 h

Group	Number of Spreads Observed	Observed Aberrations						Miscellaneous
		Chromatid			Chromosome			
		Gap	B w F	B w/o F	Gap	B w F	B w/o F	
Air Control, 7 h/day	500	6	-	-	-	-	-	-
20 ppm, 7 h/day	500	5	1	-	-	-	-	-
70 ppm, 7 h/day	500	10	5	-	2	-	-	-
EMS, 100 mg/kg/day	500	8	9	-	3	-	-	3 Chromatid Fragments

TABLE CA-MD-M-2

Methyl Bromide
Cytogenetic Analysis of Rat Bone Marrow Cells
Summary of Observed Aberrations
Males

Multiple Dosing — Sampling Time: 6 h

Treatment Group	Spreads with Aberrations					
	Total			Excluding Gaps		
	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t
Air Control	0.211	0.0431		0.141	0.0354	
20 ppm	0.251	0.0431	0.654	0.160	0.0354	0.398
70 ppm	0.362	0.0431	2.485*	0.210	0.0354	1.383
EMS, 100 mg/kg	0.391	0.0431	2.963**	0.247	0.0354	2.117*

S.E. of mean = Standard error of Freeman-Tukey binomial transformation mean
*P<0.05
**P<0.01

TABLE CA-MD-F-1

Methyl Bromide
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Females

Multiple Dosing

Sampling Time: 6 h

Group	Number of Spreads Observed	Observed Aberrations						Miscellaneous
		Chromatid			Chromosome			
		Gap	B w F	B w/o F	Gap	B w F	B w/o F	
Air Control, 7 h/day	500	7	2	-	1	-	-	-
20 ppm, 7 h/day	500	6	1	-	-	-	-	-
70 ppm, 7 h/day	500	1	-	1	1	-	-	1 Chromatid Fragment
EMS, 100 mg/kg/day	500	8	5	1	2	-	-	2 Chromatid Fragments

TABLE CA-MD-F-2

Methyl Bromide
Cytogenetic Analysis of Rat Bone Marrow Cells
Summary of Observed Aberrations
Females

Multiple Dosing — Sampling Time: 6 h

Treatment Group	Spreads with Aberrations					
	Total			Excluding Gaps		
	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t
Air Control	0.271	0.0453		0.180	0.0308	
20 ppm	0.260	0.0453	-0.178	0.160	0.0308	-0.458
70 ppm	0.179	0.0453	-1.438	0.171	0.0308	-0.218
EMS, 100 mg/kg	0.333	0.0453	0.955	0.261	0.0308	1.854

S.E. of mean = Standard error of Freeman-Tukey binomial transformation mean

TABLE CA-M6-1

Methyl Bromide
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Males

Single Dosing — Sampling Time: 6 h

Group	Number of Spreads Observed	Observed Aberrations						Miscellaneous
		Chromatid			Chromosome			
		Gap	B w F	B w/o F	Gap	B w F	B w/o F	
Air Control, 7 h/day	500	14	1	-	4	1	-	-
20 ppm, 7 h/day	450	6	-	-	2	-	-	-
70 ppm, 7 h/day	500	9	-	-	1	-	-	-
EMS, 250 mg/kg/day	500	33	7	-	-	-	-	-

TABLE CA-M6-2

Methyl Bromide
Cytogenetic Analysis of Rat Bone Marrow Cells
Summary of Observed Aberrations
Males

Single Dosing — Sampling Time: 6 h

Treatment Group	Spreads with Aberrations					
	Total			Excluding Gaps		
	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t
Air Control	0.396	0.0489		0.180	0.0276	
20 ppm	0.258	0.0516	-1.932	0.141	0.0291	-0.993
70 ppm	0.302	0.0489	-1.354	0.141	0.0276	-1.020
EMS, 250 mg/kg	0.558	0.0489	2.346*	0.250	0.0276	1.771

S.E. of mean = Standard error of Freeman-Tukey binomial transformation mean
*P<0.05

TABLE CA-M24-1

Methyl Bromide
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Males

Single Dosing

Sampling Time: 24 h

Group	Number of Spreads Observed	Observed Aberrations						Miscellaneous
		Chromatid			Chromosome			
		Gap	B w F	B w/o F	Gap	B w F	B w/o F	
Air Control, 7 h/day	500	3	-	1	-	-	-	1 Chromatid Fragment
20 ppm, 7 h/day	500	5	2	-	-	-	-	1 Dicentric
70 ppm, 7 h/day	500	2	1	-	-	-	-	-
EMS, 250 mg/kg/day	500	77	202	7	-	4	-	11 Multiple Aberrations 5 Chromatid Fragments

TABLE CA-M24-2

Methyl Bromide
Cytogenetic Analysis of Rat Bone Marrow Cells
Summary of Observed Aberrations
Males

Single Dosing — Sampling Time: 24 h

Treatment Group	Spreads with Aberrations					
	Total			Excluding Gaps		
	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t
Air Control	0.219	0.0506		0.180	0.0516	
20 ppm	0.271	0.0506	0.717	0.200	0.0516	0.273
70 ppm	0.200	0.0506	-0.264	0.160	0.0516	-0.273
EMS, 250 mg/kg	1.127	0.0506	12.67***	0.915	0.0516	10.06***

S.E. of mean = Standard error of Freeman-Tukey binomial transformation mean
***P<0.001

TABLE CA-M48-1

Methyl Bromide
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Males

Single Dosing — Sampling Time: 48 h

Group	Number of Spreads Observed	Observed Aberrations						Miscellaneous
		Chromatid			Chromosome			
		Gap	B w F	B w/o F	Gap	B w F	B w/o F	
Air Control, 7 h/day	500	7	2	-	-	-	-	-
20 ppm, 7 h/day	450	2	3	-	-	-	-	-
70 ppm, 7 h/day	450	7	8	-	-	-	-	-
EMS, 250 mg/kg/day	500	18	7	3	2	-	-	2 Multiple Aberrations

TABLE CA-M48-2

Methyl Bromide
Cytogenetic Analysis of Rat Bone Marrow Cells
Summary of Observed Aberrations
Males

Single Dosing — Sampling Time: 48 h

Treatment Group	Spreads with Aberrations					
	Total			Excluding Gaps		
	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t
Air Control	0.286	0.0537		0.180	0.0382	
20 ppm	0.251	0.0567	-0.450	0.207	0.0403	0.479
70 ppm	0.330	0.0567	0.564	0.207	0.0403	0.479
EMS, 250 mg/kg	0.423	0.0537	1.799	0.285	0.0382	1.943

S.E. of mean = Standard error of Freeman-Tukey binomial transformation mean

TABLE CA-F6-1

Methyl Bromide
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Females

Single Dosing — Sampling Time: 6 h

Group	Number of Spreads Observed	Observed Aberrations						Miscellaneous
		Chromatid			Chromosome			
		Gap	B w F	B w/o F	Gap	B w F	B w/o F	
Air Control, 7 h/day	450	23	2	-	4	-	-	1 Exchange 1 Pair of Minutes
20 ppm, 7 h/day	474	8	2	-	2	-	-	1 Pair of Minutes
70 ppm, 7 h/day	443	12	-	-	3	-	-	-
EMS, 250 mg/kg/day	358	26	1	-	8	-	-	-

TABLE CA-F6-2

Methyl Bromide
Cytogenetic Analysis of Rat Bone Marrow Cells
Summary of Observed Aberrations
Females

Single Dosing — Sampling Time: 6 h

Treatment Group	Spreads with Aberrations					
	Total			Excluding Gaps		
	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t
Air Control	0.540	0.0460		0.238	0.0303	
20 ppm	0.338	0.0460	-3.112**	0.206	0.0303	-0.735
70 ppm	0.397	0.0460	-2.200*	0.165	0.0303	-1.701
EMS, 250 mg/kg	0.606	0.0514	0.960	0.240	0.0338	0.052

S.E. of mean = Standard error of Freeman-Tukey binomial transformation mean

*P<0.05

**P<0.01

TABLE CA-F24-1

Methyl Bromide
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Females

Single Dosing — Sampling Time: 24 h

Group	Number of Spreads Observed	Observed Aberrations						Miscellaneous
		Chromatid			Chromosome			
		Gap	B w F	B w/o F	Gap	B w F	B w/o F	
Air Control, 7 h/day	500	8	3	-	-	-	-	-
20 ppm, 7 h/day	450	4	1	-	-	-	-	-
70 ppm, 7 h/day	500	2	1	1	-	1	-	1 Chromatid Fragment
EMS, 250 mg/kg/day	500	85	231	3	3	15	-	31 Multiple Aberrations 6 Chromatid Fragments 2 Chromosomal Fragments 1 Ring Chromosome

TABLE CA-F24-2

Methyl Bromide
Cytogenetic Analysis of Rat Bone Marrow Cells
Summary of Observed Aberrations
Females

Single Dosing — Sampling Time: 24 h

Treatment Group	Spreads with Aberrations					
	Total			Excluding Gaps		
	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t
Air Control	0.288	0.0476		0.191	0.0370	
20 ppm	0.230	0.0501	-0.084	0.163	0.0390	-0.525
70 ppm	0.291	0.0476	0.031	0.211	0.0370	0.381
EMS, 250 mg/kg	1.276	0.0476	14.69***	1.137	0.0370	18.11***

S.E. of mean = Standard error of Freeman-Tukey binomial transformation mean
***P<0.001

TABLE CA-F48-1

Methyl Bromide
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Females

Single Dosing

Sampling Time: 48 h

Group	Number of Spreads Observed	Observed Aberrations						Miscellaneous
		Chromatid			Chromosome			
		Gap	B w F	B w/o F	Gap	B w F	B w/o F	
Air Control, 7 h/day	450	5	-	-	-	1	-	-
20 ppm, 7 h/day	500	8	-	-	-	-	-	-
70 ppm, 7 h/day	500	12	4	1	-	-	-	-
EMS, 250 mg/kg/day	500	9	7	2	-	-	-	-

TABLE CA-F48-2

Methyl Bromide
Cytogenetic Analysis of Rat Bone Marrow Cells
Summary of Observed Aberrations
Females

Single Dosing — Sampling Time: 48 h

Treatment Group	Spreads with Aberrations					
	Total			Excluding Gaps		
	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t
Air Control	0.263	0.0501		0.163	0.0349	
20 ppm	0.238	0.0475	-0.358	0.141	0.0331	-0.460
70 ppm	0.355	0.0475	1.330	0.219	0.0331	1.175
EMS, 250 mg/kg	0.405	0.0475	2.056*	0.289	0.0331	2.635*

S.E. of mean = Standard error of Freeman-Tukey binomial transformation mean
*P<0.05

TABLE DL-1

Methyl Bromide

Dominant Lethal Test in Rats

Pregnancy Frequency (Females with Corpora Lutea Graviditatis)

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	20 ppm	70 ppm	5 x 100 mg/kg EMS
1	75%	65%	75%	85%
2	90%	84%	90%	75%
3	100%	85%	90%	55%
4	85%	100%	100%	90%
5	95%	95%	90%	95%
6	100%	95%	85%	95%
7	95%	95%	95%	100%
8	95%	100%	85%	95%
9	100%	95%	100%	100%
10	95%	95%	95%	95%

TABLE DL-2

Methyl Bromide
Dominant Lethal Test in Rats
Pregnancy Frequency (Females with Implantations)

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)		20 ppm		70 ppm		5 x 100 mg/kg EMS	
1	15/20	75%	13/20	65%	15/20	75%	13/20	65%
2	18/20	90%	16/19	80%	18/20	90%	5/20	25%
3	19/20	95%	16/20	80%	16/20	80%	5/20	25%
4	17/20	85%	19/20	95%	20/20	100%	17/20	85%
5	19/20	95%	19/20	95%	17/20	85%	18/20	90%
6	18/20	90%	19/20	95%	16/20	80%	18/20	90%
7	18/20	90%	17/19	89%	19/20	95%	20/20	100%
8	18/20	90%	19/20	95%	17/20	85%	19/20	95%
9	20/20	100%	18/19	95%	19/20	95%	20/20	100%
10	19/20	95%	19/20	95%	18/20	90%	19/20	95%

TABLE DL-3

Methyl Bromide
Dominant Lethal Test in Rats
Total Number of Corpora Lutea per Pregnancy

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	20 ppm	70 ppm	5 x 100 mg/kg EMS
1	[1] 12.6 ± 0.61	12.7 ± 0.65	14.0 ± 0.61	7.9 ± 1.44*
2	14.8 ± 0.63	13.8 ± 0.66	14.6 ± 0.63	9.0 ± 2.26
3	12.7 ± 0.50	13.0 ± 0.54	12.9 ± 0.54	5.6 ± 1.86*
4	12.1 ± 0.54	13.4 ± 0.51	12.4 ± 0.50	11.4 ± 0.73
5	12.3 ± 0.52	12.4 ± 0.52	12.6 ± 0.54	12.3 ± 0.47
6	13.4 ± 0.52	13.0 ± 0.51	13.1 ± 0.56	12.5 ± 0.39
7	13.9 ± 0.62	11.4 ± 0.64**	12.5 ± 0.60	13.3 ± 0.68
8	13.4 ± 0.72	12.8 ± 0.70	13.2 ± 0.74	13.4 ± 0.35
9	13.3 ± 0.44	12.2 ± 0.45	12.8 ± 0.44	11.8 ± 0.60*
10	13.6 ± 0.47	12.8 ± 0.47	12.9 ± 0.48	12.9 ± 0.70

1 = Mean ± standard error of mean
*P<0.05
**P<0.01

TABLE DL-4

Methyl Bromide
Dominant Lethal Test in Rats
Total Implantations per Pregnancy

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	20 ppm	70 ppm	5 x 100 mg/kg EMS
1	[1] 11.5 ± 0.99	10.5 ± 1.06	12.7 ± 0.99	7.1 ± 1.48*
2	13.7 ± 0.63	11.9 ± 0.67	13.9 ± 0.63	4.4 ± 2.23*
3	13.2 ± 0.45	12.8 ± 0.49	13.1 ± 0.49	1.8 ± 0.37***
4	13.2 ± 0.60	13.0 ± 0.57	11.8 ± 0.56	10.4 ± 0.80*
5	12.2 ± 0.61	12.2 ± 0.61	12.7 ± 0.64	11.6 ± 0.70
6	13.1 ± 0.51	13.3 ± 0.50	12.7 ± 0.54	12.4 ± 0.43
7	12.8 ± 0.63	12.2 ± 0.65	12.3 ± 0.61	12.4 ± 0.71
8	12.5 ± 0.65	13.6 ± 0.63	13.1 ± 0.67	13.4 ± 0.43
9	12.9 ± 0.52	12.2 ± 0.55	12.2 ± 0.53	11.7 ± 0.72
10	13.0 ± 0.53	13.0 ± 0.53	12.1 ± 0.54	12.0 ± 0.95

1 = Mean ± Standard error of mean

*P<0.05

***P<0.001

TABLE DL-5

Methyl Bromide
Dominant Lethal Test in Rats
Live Implantations per Pregnancy

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	20 ppm	70 ppm	5 x 100 mg/kg EMS
1	[1] 10.6 + 1.18	9.0 ± 1.27	11.6 ± 1.18	4.0 ± 0.95***
2	12.2 ± 0.64	11.3 ± 0.68	12.7 ± 0.64	1.8 ± 0.73***
3	11.9 ± 0.79	11.9 ± 0.86	11.5 ± 0.86	1.8 ± 0.37***
4	11.0 ± 0.90	11.6 ± 0.88	8.9 ± 0.83	5.5 ± 0.84***
5	10.5 ± 0.75	11.5 ± 0.75	11.8 ± 0.79	9.2 ± 0.93
6	11.2 ± 0.75	10.9 ± 0.73	11.1 ± 0.80	11.4 ± 0.45
7	12.1 ± 0.68	11.2 ± 0.70	10.6 ± 0.66	10.8 ± 0.76
8	11.5 ± 0.96	11.2 ± 0.94	11.2 ± 0.99	11.1 ± 0.81
9	11.4 ± 0.94	9.7 ± 0.99	9.8 ± 0.97	9.6 ± 0.94
10	10.7 ± 0.97	10.0 ± 0.97	11.1 ± 1.00	10.5 ± 1.04

1 = Mean ± standard error of mean

***P<0.001

TABLE DL-6

Methyl Bromide
Dominant Lethal Test in Rats
Live Implantations and Late Deaths per Pregnancy

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	20 ppm	70 ppm	5 x 100 mg/kg EMS
1	[1] 10.8 ± 1.07	9.1 ± 1.15	12.1 ± 1.07	4.2 ± 0.98***
2	12.6 ± 0.62	11.5 ± 0.65	12.7 ± 0.62	1.8 ± 0.73***
3	12.6 ± 0.46	12.6 ± 0.51	12.4 ± 0.51	1.8 ± 0.37***
4	11.3 ± 0.71	12.4 ± 0.71	10.4 ± 0.66	8.7 ± 0.77*
5	11.9 ± 0.60	11.6 ± 0.60	12.2 ± 0.64	10.7 ± 0.65
6	12.2 ± 0.59	11.6 ± 0.58	11.7 ± 0.63	11.6 ± 0.44
7	12.5 ± 0.59	11.5 ± 0.61	11.6 ± 0.58	11.5 ± 0.71
8	11.8 ± 0.77	12.8 ± 0.75	11.8 ± 0.79	12.2 ± 0.56
9	12.2 ± 0.69	11.3 ± 0.73	10.7 ± 0.71	9.8 ± 0.93
10	11.3 ± 0.74	11.6 ± 0.74	11.1 ± 0.76	10.7 ± 0.97

1 = Mean ± standard error of mean

*P<0.05

***P<0.001

TABLE DL-7

Methyl Bromide

Dominant Lethal Test in Rats

Frequency of Pregnancies with One or More or Two or More Early Deaths

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)		20 ppm		70 ppm		5 x 100 mg/kg EMS	
	>0	>1	>0	>1	>0	>1	>0	>1
1	6/15	3/15	8/13	5/13	8/15	1/15	8/13	7/13
2	10/18	6/18	5/16	1/16	10/18	4/18	1/5	1/5
3	9/19	3/19	2/16	0/16	8/16	2/16	0/5	0/5
4	9/17	6/17	11/19	5/19	11/20	6/20	13/17	9/17
5	4/19	2/19	9/19	2/19	7/17	1/17	13/18	3/18
6	8/18	4/18	15/19	7/19	4/16	4/16	10/18	4/18
7	4/18	1/18	7/17	3/17	9/19	3/19	13/20	4/20
8	7/18	4/18	5/19	2/19	9/17	4/17	10/19	5/19
9	7/20	2/20	8/19	1/19	13/19	6/19	11/20	10/20
10	13/19	7/19	9/19	4/19	6/18	5/18	9/19	6/19

TABLE DL-8

Methyl Bromide
Dominant Lethal Test in Rats
Early Death Frequency, Freeman-Tukey Poisson Transformation

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	20 ppm	70 ppm	5 x 100 mg/kg EMS
1	[1] 1.751 ± 0.2671	2.360 ± 0.2869	1.803 ± 0.2671	3.125 ± 0.5401*
2	2.148 ± 0.2639	1.488 ± 0.2799	2.184 ± 0.2639	2.269 ± 1.2690
3	1.785 ± 0.1829	1.177 ± 0.1993*	1.835 ± 0.1993	1.000 ± 0.0000*
4	2.475 ± 0.3386	2.011 ± 0.3203	2.270 ± 0.3122	2.705 ± 0.2723
5	1.375 ± 0.1831	1.747 ± 0.1831	1.625 ± 0.1935	2.176 ± 0.1963**
6	1.884 ± 0.2928	2.648 ± 0.2850	1.777 ± 0.3106	1.981 ± 0.2284
7	1.355 ± 0.2076	1.746 ± 0.2136	1.816 ± 0.2021	2.095 ± 0.2007*
8	1.745 ± 0.3054	1.655 ± 0.2973	2.167 ± 0.3143	2.133 ± 0.2930
9	1.666 ± 0.2708	1.837 ± 0.2855	2.402 ± 0.2779	2.555 ± 0.3540
10	2.556 ± 0.3258	2.116 ± 0.3258	1.837 ± 0.3348	2.143 ± 0.3237

1 = Mean ± standard error of mean

*P<0.05

**P<0.01

TABLE DL-9

Methyl Bromide
Dominant Lethal Test in Rats
Early Death Frequency, Freeman-Tukey Binomial Transformation

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	20 ppm	70 ppm	5 x 100 mg/kg EMS
1	[1] 0.550 ± 0.0998	0.808 ± 0.1072	0.514 ± 0.0998	1.354 ± 0.2117**
2	0.576 ± 0.0702	0.441 ± 0.0744	0.569 ± 0.0702	1.084 ± 0.4495
3	0.483 ± 0.0510	0.328 ± 0.0556*	0.501 ± 0.0556	0.665 ± 0.0519
4	0.703 ± 0.1079	0.546 ± 0.1021	0.697 ± 0.0995	0.856 ± 0.0910
5	0.413 ± 0.0577	0.492 ± 0.0577	0.447 ± 0.0610	0.634 ± 0.0547*
6	0.521 ± 0.0850	0.735 ± 0.0828	0.497 ± 0.0902	0.556 ± 0.0651
7	0.373 ± 0.0547	0.486 ± 0.0563	0.512 ± 0.0532	0.608 ± 0.0636*
8	0.563 ± 0.1121	0.467 ± 0.1091	0.601 ± 0.1153	0.587 ± 0.0857
9	0.481 ± 0.0927	0.536 ± 0.0978	0.723 ± 0.0951	0.853 ± 0.1472*
10	0.726 ± 0.1043	0.615 ± 0.1043	0.536 ± 0.1072	0.657 ± 0.1025

1 = Mean ± standard error of mean

*P<0.05

**P<0.01

TABLE SA-1

Methyl Bromide
Sperm Abnormality Test in Mice
Numbers and Proportions of Abnormalities

Multiple Dosing

Dose Group	Number Normal	Number Abnormal*						Percent Abnormal					
		A	B	C	D	E	Total	A	B	C	D	E	Total
Air Control, 7 h/day	8713	14	24	115	47	87	287	0.16	0.27	1.28	0.52	0.97	3.19
20 ppm, 7 h/day	8732	24	21	107	42	70	264	0.27	0.23	1.19	0.47	0.78	2.93
70 ppm, 7 h/day	7814	11	22	72	25	58	188	0.14	0.28	0.90	0.31	0.73	2.35
EMS, 200 mg/kg/day	9064	48	30	509	166	183	936	0.48	0.30	5.09	1.66	1.83	9.36

* A = Hood up-turned or hook elongated
  B - Banana-shaped head
  C = Amorphous head
  D = Folded tail
  E = Miscellaneous (double head, double tail, twisted neck, filamentous mid-piece, enlarged mid-piece, plier type)

TABLE SA-2

Methyl Bromide

Sperm Abnormality Test in Mice

Means of Freeman-Tukey Binomial Transformation

± Standard Error

Multiple Dosing

Dose Group	Abnormality Category					
	A	B	C	D	E	Total
Air Control, 7 h/day	7.86	10.80	22.92	14.74	19.75	35.87
	± 1.295	± 0.878	± 0.988	± 1.297	± 1.533	± 1.552
20 ppm, 7 h/day	10.46	10.29	22.21	14.09	17.73	34.50
	± 1.295	± 0.878	± 0.988	± 1.297	± 1.533	± 1.552
70 ppm, 7 h/day	8.23	11.22	19.23	11.03	16.96	30.75
	± 1.374	± 0.932	± 1.048	± 1.376	± 1.626	± 1.647
EMS, 200 mg/kg/day	11.88	13.40	31.90*	13.04	24.83	45.98*
	± 2.748	± 1.863	± 2.096	± 2.752	± 3.252	± 3.293

A = Hook up-turned or hook elongated

B = Banana-shaped head

C = Amorphous head

D = Folded tail

E = Miscellaneous (double head, double tail, twisted neck, filamentous mid-piece, enlarged mid-piece, plier type)

* = $P<0.05$

TABLE RL-1

Methyl Bromide
Drosophila Dose Ranging Experiment

Day		20 ppm						Control
		1 h		3 h		5 h		
0	No. of males exposed	100		100		100		
1	No. and % survival	100	100	100	100	100	100	
2	No. of eggs laid by 4 females	36		13		7		176
3	No. and % hatched	32	%8%	9	69%	7	100%	159/90.3

TABLE RL-1 (continued)

Methyl Bromide

Day		70 ppm						Control
		1 h		3 h		5 h		
0	No. of males exposed	100		100		100		
1	No. and % survival	100	100	94	94	99	99	
2	No. of eggs laid by 10 females	386		438		387		217
3	No. and % hatched	340	88%	318	72%	319	82%	190/87.5

TABLE RL-2

Methyl Bromide

Drosophila SLRL Procedure and Results

Compound: Methyl Bromide **Concentration:** 20 ppm **Stock:** A
Length of Exposure: 5 hrs **Test exposure given:** 11.6.79

	Brood 1	Brood 2	Brood 3
F_1 set up	12.6.79	15.6.79	20.6.79
F_2 set up	27.6.79	28.6.79	3.7.79
F_2 scored	11.7.79	13.7.79	16.7.79
F_2 repeats scored			
F_3 set up	11.7.79	13.7.79	16.7.79
F_3 scored	24.7.79	26.7.79	27.7.79
F_3 repeats scored			

RESULTS

	Brood 1	Brood 2	Brood 3	All Broods
No. of F_1 vials	96	94	84	274
No. of sterile F_1 vials	12	16	9	37
No. of F_1 vials used in F_2	84	77	72	233
No. of F_2 vials set up	400	400	400	1200
No. of F_2 vials scored	317	369	365	1051
No. of F_2 vials containing lethals	1	3	1	5
Frequency of F_2 lethals	0.315%	0.813%	0.274%	0.476%
No. of F_3 vials set up	400	300	295	995
No. of F_3 vials scored	387	282	283	952
No. of F_3 vials containing lethals	0	0	0	0
Frequency of F_3 lethals	0	0	0	0

TABLE RL-2 (continued)

Methyl Bromide

Drosophila SLRL Procedure and Results

Compound: Methyl Bromide **Concentration:** 20 ppm **Stock:** B
Length of Exposure: 5 hrs **Test exposure given:** 11.6.79

	Brood 1	Brood 2	Brood 3
F_1 set up	12.6.79	15.6.79	20.6.79
F_2 set up	27.6.79	29.6.79	3.7.79
F_2 scored	10.7.79	10.7.79	17.7.79
F_2 repeats scored			
F_3 set up			
F_3 scored			
F_3 repeats scored			

RESULTS

	Brood 1	Brood 2	Brood 3	All Broods
No. of F_1 vials	99	99	86	284
No. of sterile F_1 vials	7	3	13	23
No. of F_1 vials used in F_2	92	96	73	261
No. of F_2 vials set up	400	400	400	1200
No. of F_2 vials scored	319	374	368	1061
No. of F_2 vials containing lethals	0	1	0	1
Frequency of F_2 lethals	0	0.267%	0	0.094%
No. of F_3 vials set up				
No. of F_3 vials scored				
No. of F_3 vials containing lethals				
Frequency of F_3 lethals				

TABLE RL-2 (continued)

Methyl Bromide

Drosophila SLRL Procedure and Results

Compound: Methyl Bromide Concentration: 70 ppm Stock: A

Length of Exposure: 5 hrs Test exposure given: 11.6.79

	Brood 1	Brood 2	Brood 3
F_1 set up	12.6.79	15.6.79	20.6.79
F_2 set up	26.6.79	28.6.79	2.7.79
F_2 scored	9.7.79	12.7.79	17.7.79
F_2 repeats scored	27.7.79		
F_3 set up	9.7.79	12.7.79	17.7.79
F_3 scored	23.7.79	26.7.79	30.7.79
F_3 repeats scored			

RESULTS

	Brood 1	Brood 2	Brood 3	All Broods
No. of F_1 vials	97	89	82	268
No. of sterile F_1 vials	11	9	8	28
No. of F_1 vials used in F_2	86	80	74	240
No. of F_2 vials set up	400	400	400	1200
No. of F_2 vials scored	332	357	338	1027
No. of F_2 vials containing lethals	0	1	0	1
Frequency of F_2 lethals	0	0.280%	0	0.097%
No. of F_3 vials set up	401	300	300	1001
No. of F_3 vials scored	390	290	285	965
No. of F_3 vials containing lethals	1	0	0	1
Frequency of F_3 lethals	0.256%	0	0	0.104%

TABLE RL-2 (continued)

Methyl Bromide

Drosophila SLRL Procedure and Results

Compound: Methyl Bromide Concentration: 70 ppm Stock: B

Length of Exposure: 5 hrs Test exposure given: 11.6.79

	Brood 1	Brood 2	Brood 3
F_1 set up	12.6.79	15.6.79	20.6.79
F_2 set up	26.6.79	29.6.79	2.7.79
F_2 scored	9.7.79	13.7.79	16.7.79
F_2 repeats scored			
F_3 set up			
F_3 scored			
F_3 repeats scored			

RESULTS

	Brood 1	Brood 2	Brood 3	All Broods
No. of F_1 vials	99	95	88	282
No. of sterile F_1 vials	4	6	10	20
No. of F_1 vials used in F_2	95	89	78	262
No. of F_2 vials set up	400	400	400	1200
No. of F_2 vials scored	330	352	339	1021
No. of F_2 vials containing lethals	2	0	0	2
Frequency of F_2 lethals	0.606%	0	0	0.196%
No. of F_3 vials set up				
No. of F_3 vials scored				
No. of F_3 vials containing lethals				
Frequency of F_3 lethals				

TABLE RL-2 (continued)

Methyl Bromide

Drosophila SLRL Procedure and Results

Compound: EMS Concentration: 0.4% v/v Stock: B

Length of Exposure: 5 hrs Test exposure given: 11.6.79

	Brood 1	Brood 2	Brood 3
F_1 set up	12.6.79		
F_2 set up	26.6.79		
F_2 scored	11.7.79		
F_2 repeats scored			
F_3 set up			
F_3 scored			
F_3 repeats scored			

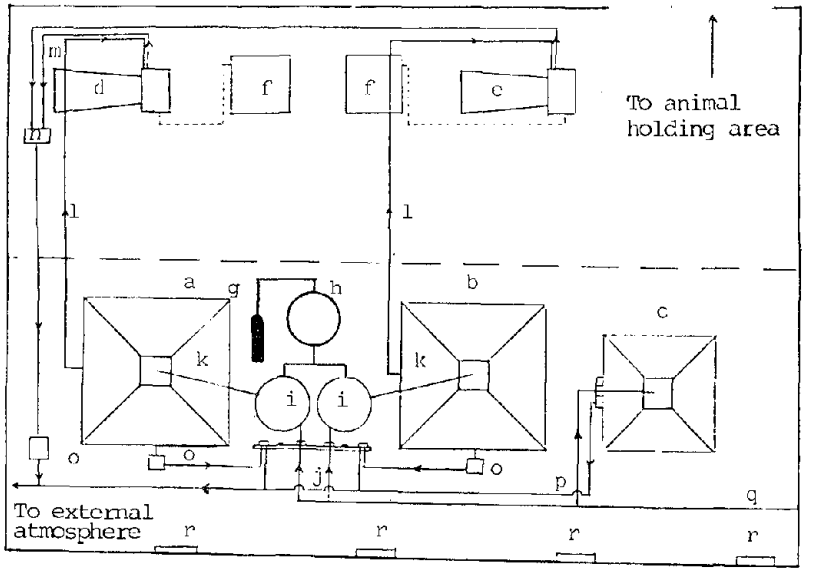
RESULTS

	Brood 1	Brood 2	Brood 3	All Broods
No. of F_1 vials	60			60
No. of sterile F_1 vials	14			14
No. of F_1 vials used in F_2	46			46
No. of F_2 vials set up	100			100
No. of F_2 vials scored	56			56
No. of F_2 vials containing lethals	19			19
Frequency of F_2 lethals	33.9%			33.9%
No. of F_3 vials set up				
No. of F_3 vials scored				
No. of F_3 vials containing lethals				
Frequency of F_3 lethals				

FIGURE 1a

Methyl Bromide

Schematic Lay-out of Exposure Area

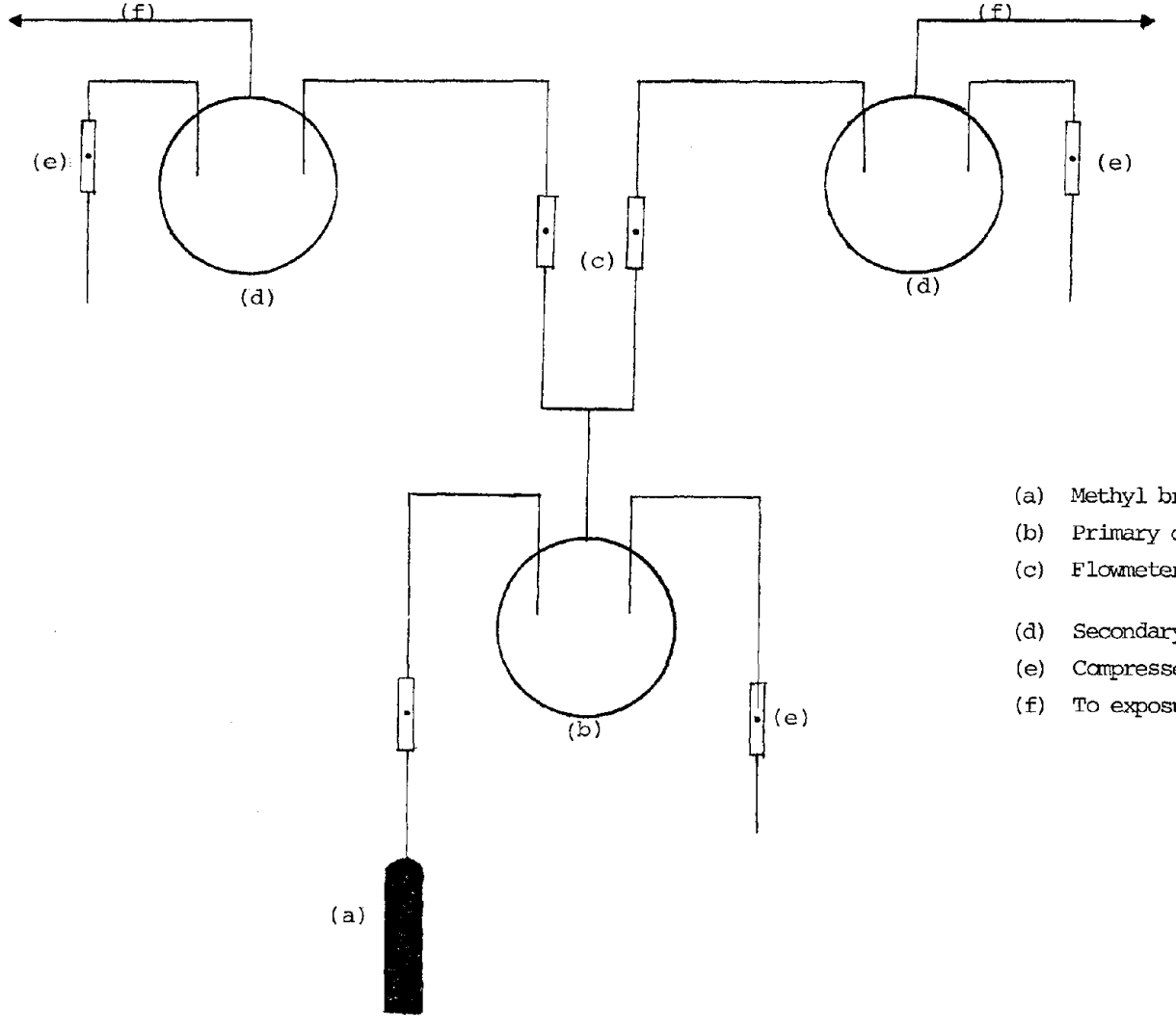


a High level exposure chamber
b Low level exposure chamber
c Air control exposure chamber
d Miran monitoring high level exposure chamber
e Miran monitoring low level exposure chamber
f Pen recorders
g Methyl bromide gas cylinder
h Primary dilution vessel
i Secondary dilution vessel
j Flow meter control panel for atmosphere generation
k Gas transfer line
l Sampling line
m Miran extract line
n Sampling flow rate control panel
o Scrubber
p Exposure chamber extract
q Compressed air line
r High efficiency extract

FIGURE 1b

Methyl Bromide

Schematid Lay-out of Vapour Generation Apparatus



(a) Methyl bromide gas cylinder

(b) Primary dilution vessel

(c) Flowmeters - controlled flow of diluted methyl bromide

(d) Secondary dilution vessel

(e) Compressed air

(f) To exposure chambers

FIGURE 2

Methyl Bromide
Typical Calibration Graph for Low Level
8 June 1979

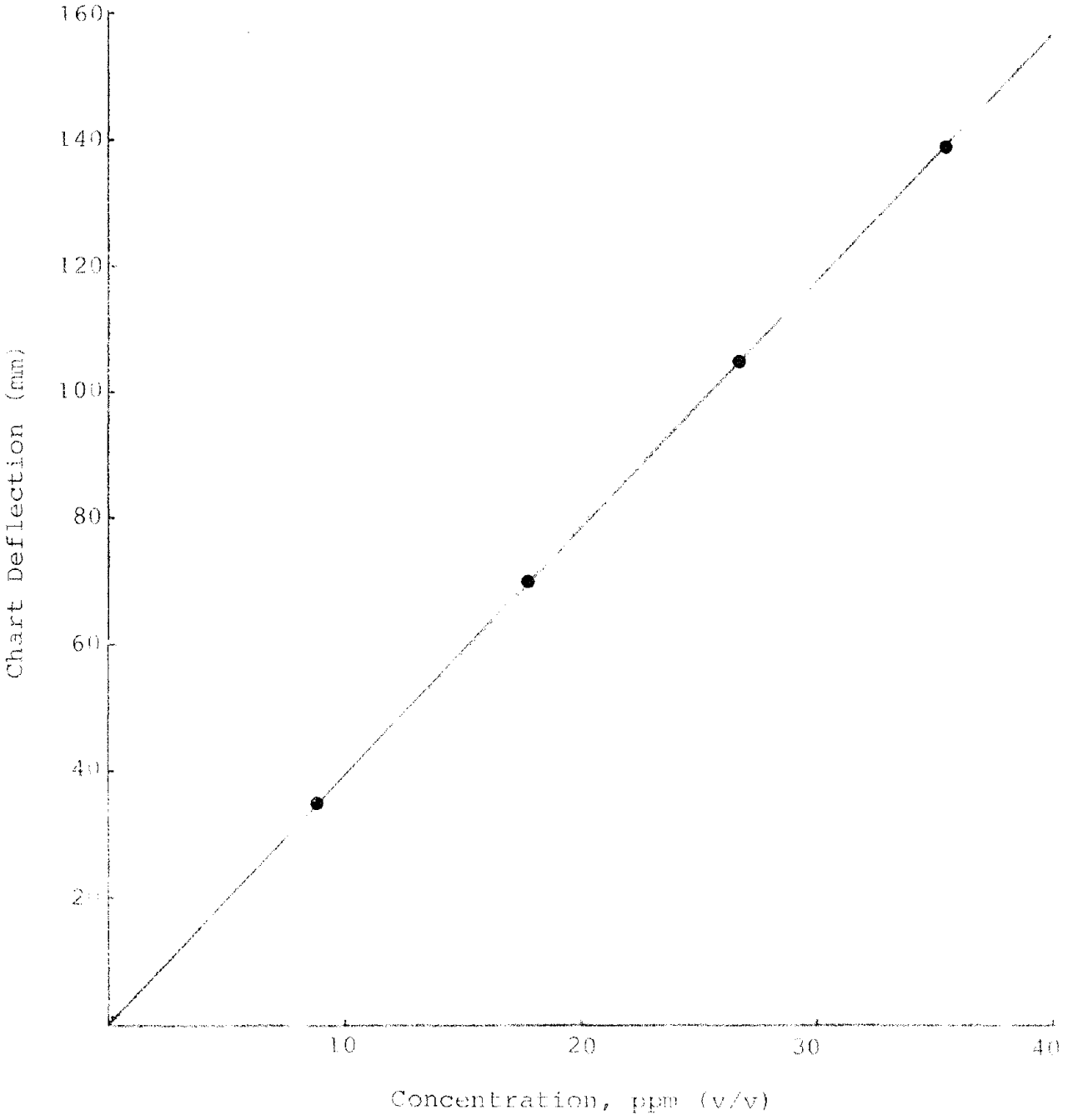
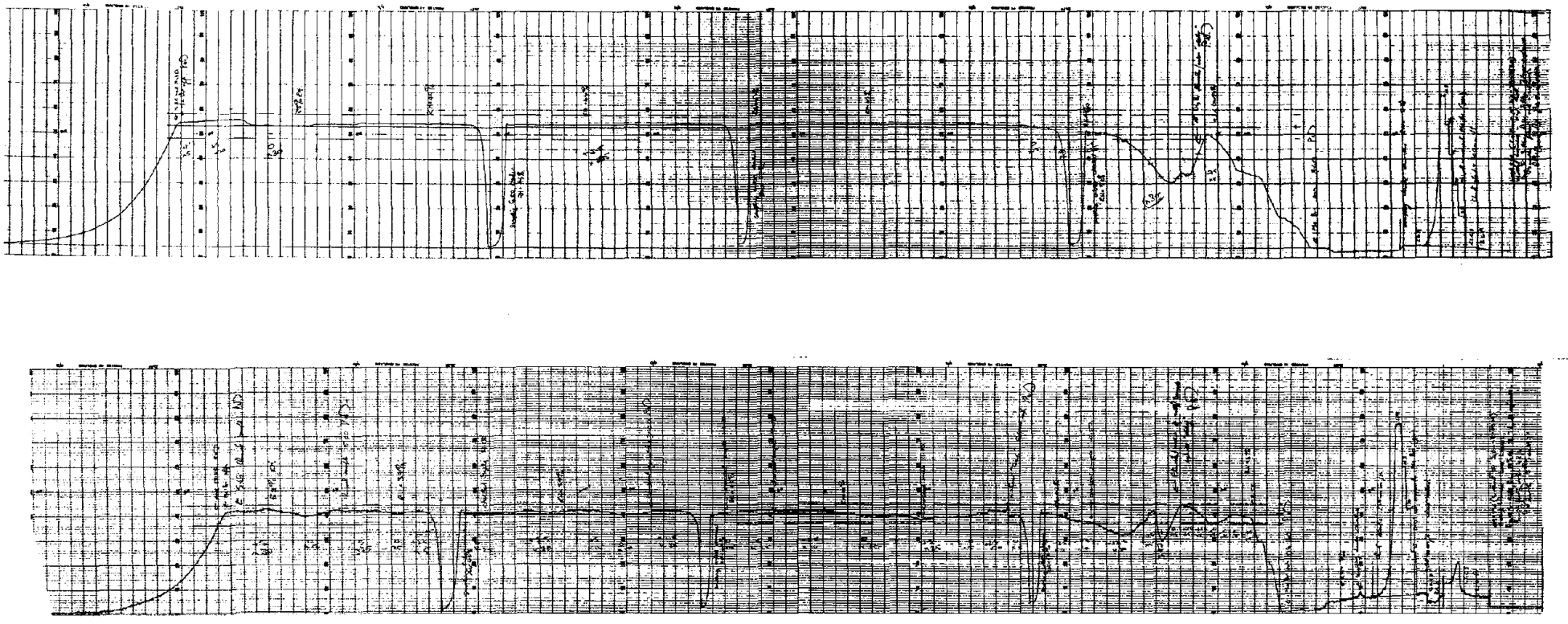


FIGURE 3

Methyl Bromide

Sample Record Chart of IR Absorption at 3.4 μm



APPENDIX DIET

Methyl Bromide
Diet Analysis

Spratt's Patent Ltd

Central House
Cambridge Road
Barking
Essex IG11 8NL

Telephone
01-594 7121
Telegrams
Spratt's Barking
Telex 897669

CERTIFICATE OF ANALYSIS

PRODUCT: LAD 1

BATCH NO: 027938

DATE OF MANUFACTURE: 2ND MAY, 1979.

	FOUND ANALYSIS
MOSITURE	9.6%
CRUDE FAT	4.0%
CRUDE PROTEIN	21.1%
ASH	5.7%
CALCIUM	1.08%
PHOSPHORUS	0.75%
NITRATE	$\leq$ 1.0 mg/kg
NITRITE	2.6 mg/kg
SELENIUM	0.26 mg/kg
LEAD	4.0 mg/kg
ARSENIC	$\leq$ 0.20 mg/kg
CADMIUM	$\leq$ 0.20 mg/kg
MERCURY	0.023mg/kg
AFLATOXINS	NONE DETECTED
TOTAL P.C.B	NONE DETECTED
TOTAL D.D.T.	0.018 mg/kg
DIELDRIN	NONE DETECTED
LINDANE	0.13 mg/kg
HEPTACHLOR	NONE DETECTED
MALATHION	0.44 mg/kg
TOTAL VIABLE ORGANISMS	1.0×10^3 /grm
E. COLI TYPE 1	NONE DETECTED
SALMONELLA SPECIES	NONE DETECTED
MOULDS.	NONE DETECTED

SIGNED .....

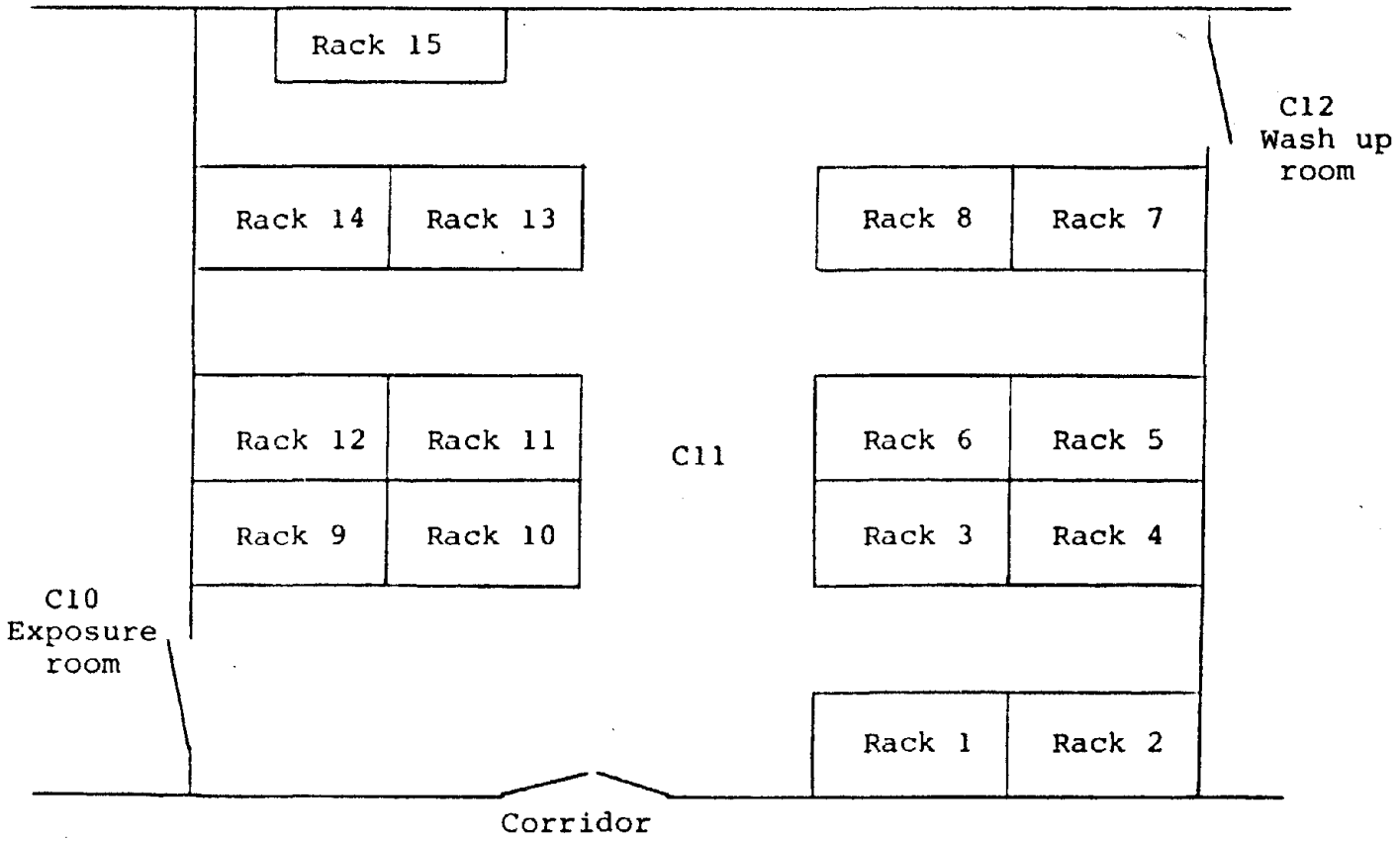
DATE .....

Registered in England Number 216746

APPENDIX Loc-1

Methyl Bromide

Animal Holding Room Plan



Rack 1, 2 - Dominant lethal ♂
Rack 3, 4, 5, 6 - Single dose cytogenetics ♂
Rack 7, 8 - Single dose + multi-dose cytogenetics ♂
Rack 9, 10, 11, 12 - Single dose cytogenetics ♀
Rack 13, 14 - Single dose + multi-dose cytogenetics ♀
Rack 15 - Sperm abnormality mice

APPENDIX Loc-2

Methyl Bromide

Examples of Animal Location During Exposure

Exposure Location Sheet

Project No: 409959 Test Concentration: 0

Test Compound: Air Control Tier No: 1

Exposure Chamber No: 1 Multi-dose Cytogenetic ♂ and ♀

Day of Study: 2

LEFT

Group Cage Treatment				
1	281	285	289	-
	282	286	290	-
	283	287	-	-
♀	284	288	-	-

FRONT REAR

Group Cage Treatment				
2	121	125	129	-
	122	126	130	-
	123	127	-	-
♂	124	128	-	-

RIGHT

SIGNED: _____ DATE: _____

APPENDIX Loc-2 (continued)

Methyl Bromide
Exposure Location Sheet

Project No: 409959

Test Compound: Air Control

Exposure Chamber No: 1

Day of Study: 2

Test Concentration: 0

Tier No: 2

Dominant Lethal of Sperm Ab. mice

LEFT

Group Cage Treatment				
3	361	365	369	-
	362	366	370	-
	363	367	-	-
	364	368	-	-

FRONT REAR

Group Cage Treatment				
4	321	325	329	-
	322	326	330	-
	323	327	-	-
	324	328	-	-

RIGHT

SIGNED: _____ DATE: _____

APPENDIX LOC-2 (continued)

Methyl Bromide
Exposure Location Sheet

Project No: 409959
Test Compound: Methyl Bromide
Exposure Chamber No: 2
Day of Study: 2

Test Concentration: Low
Tier No: 1

LEFT

Group Cage 4 Treatment: Sperm Ab.			
331	332	333	334
335	336	337	338
339	340	-	-
-	-	-	-

Group Cage 1 Treatment: Dom Lethal			
371	372	373	374
375	376	377	378
379	380	-	-
-	-	-	-

FRONT REAR

Group Cage 3 Treatment: Multi-dose Cyt ♀			
291	292	293	294
295	296	297	298
299	300	-	-
-	-	-	-

Group Cage 2 Treatment: Multi-dose Cyt ♂			
131	132	133	134
135	136	137	138
139	140	-	-
-	-	-	-

RIGHT

Signed: _____ Date: _____

APPENDIX Loc-2 (continued)

Methyl Bromide
Exposure Location Sheet

Project No: 409959
Test Compound: Methyl Bromide
Exposure Chamber No: 3
Day of Study: 2

Test Concentration: High
Tier No: 1

LEFT

Group Cage 4 Treatment: Sperm Ab.			
341	342	343	344
345	246	347	348
349	350	-	-
-	-	-	-

Group Cage 1 Treatment: Dom Lethal			
381	382	383	384
385	386	387	388
389	390	-	-
-	-	-	-

FRONT

REAR

Group Cage 3 Treatment: Multi-dose Cyt ♀			
301	302	303	304
305	306	307	308
309	310	-	-
-	-	-	-

Group Cage 2 Treatment: Multi-dose Cyt ♂			
141	142	143	144
145	146	147	148
149	150	-	-
-	-	-	-

RIGHT

Signed: _____ Date: _____

APPENDIX FORM-1

Methyl Bromide

CYTOGENETIC SCORE SHEET **NIOSH**

Contract No. 210-78-0026 **Substance:**
Assessor: **Decoded group:**
Signature: **Slide No.:** **Decoded animal No.:**
Date(s) Single/Multi-dose **Time(h):**
Slide quality:
Excellent _____ **Good** _____ **Acceptable** _____ **Poor** _____ **Uncountable** _____

Vernier	Chromatid			Chromosomal			Markers			Multi-Abn.	Misc. Abn.	Observation
	GAP	BwF	Bw/oF	GAP	BwF	Bw/oF	EXC.	DIC	RG			
1.												
2.												
3.												
4.												
5.												
6.												
7.												
8.												
9.												
10.												
11.												
12.												
13.												
14.												
15.												
16.												
17.												
18.												
19.												
20.												
21.												
22.												
23.												
24.												
25.												

APPENDIX FORM-2

Methyl Bromide

Assessors	Signature

Contract No. 210-78-0026

DOMINANT LETHAL ASSESSMENT

NIOSH

Dose Group:

Week No.	Male No.																				Total	Signature(s) and Date
	Female No.	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
1	Corpora lutea																					
	Total Implants																					
	Live Implants																					
	Early Deaths																					
	Late Deaths																					
2	Corpora lutea																					
	Total Implants																					
	Live Implants																					
	Early Deaths																					
	Late Deaths																					
3	Corpora lutea																					
	Total Implants																					
	Live Implants																					
	Early Deaths																					
	Late Deaths																					
4	Corpora lutea																					
	Total Implants																					
	Live Implants																					
	Early Deaths																					
	Late Deaths																					
5	Corpora lutea																					
	Total Implants																					
	Live Implants																					
	Early Deaths																					
	Late Deaths																					

APPENDIX FORM-3

Methyl Bromide

Contract No. 210-78-0026 NIOSH

SPERM ABNORMALITIES ASSESSMENT

Assessor: De-coder

Date(s) assessed: Date de-coded:

Slide No.	Normal	Abnormality					Total Abnormal	Total examined	De-coded Information	
		A	B	C	D	E			Animal No.	Group

APPENDIX TABLE BW-1

Methyl Bromide
Multiple Exposure Cytogenetics Test
Individual Body Weights (g)

Air Control (0 ppm)

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	121	394	394	393	397	395
	122	407	418	421	426	429
	123	385	387	393	391	395
	124	342	347	350	350	354
	125	371	372	381	385	386
	126	364	371	375	382	381
	127	371	375	376	382	387
	128	371	376	381	387	388
	129	356	356	360	365	365
	130	389	392	399	400	406
	Mean ± S.D.	375.0 ± 19.1	378.8 ± 20.2	382.9 ± 20.1	384.7 ± 20.1	388.6 ± 20.7

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	281	217	216	220	219	217
	282	224	221	229	229	232
	283	195	197	198	194	197
	284	247	242	243	243	242
	285	217	219	217	218	217
	286	230	235	239	238	238
	287	241	246	251	253	251
	288	218	219	225	224	225
	289	192	193	193	192	193
	290	214	219	219	218	222
	Mean ± S.D.	219.5 ± 17.5	220.7 ± 17.2	223.4 ± 18.5	222.8 ± 19.5	223.4 ± 18.6

APPENDIX TABLE BW-1 (continued)

Methyl Bromide

Multiple Dosing: 20 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	131	339	340	348	344	347
	132	379	376	381	381	384
	133	370	367	373	376	376
	134	385	384	395	393	393
	135	382	382	384	390	386
	136	367	368	376	382	380
	137	325	332	335	334	337
	138	351	350	357	362	368
	139	367	366	370	372	372
	140	380	379	380	394	391
	Mean ± S.D.	364.5 ± 20.0	364.4 ± 18.0	369.9 ± 18.2	372.8 ± 20.5	373.4 ± 18.5

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	291	211	215	217	221	220
	292	221	217	226	223	226
	293	238	240	245	247	246
	294	252	249	254	247	250
	295	205	203	203	204	208
	296	255	257	257	255	255
	297	205	205	207	203	205
	298	201	201	211	208	210
	299	236	231	235	235	234
	300	228	225	228	229	232
	Mean ± S.D.	225.2 ± 19.8	224.3 ± 19.7	228.3 ± 19.2	227.2 ± 18.8	228.6 ± 18.0

APPENDIX TABLE BW-1 (continued)

Methyl Bromide

Multiple Dosing: 70 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	141	351	354	350	351	354
	142	376	375	377	384	383
	143	396	403	406	412	412
	144	360	355	365	365	370
	145	386	384	388	390	391
	146	361	364	371	375	376
	147	384	383	390	389	397
	148	368	371	370	374	377
	149	355	353	354	360	362
	150	359	363	366	364	367
	Mean ± S.D.	369.6 ± 15.1	370.5 ± 16.1	373.7 ± 17.1	376.4 ± 17.9	378.9 ± 17.4

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	301	200	201	204	201	204
	302	233	231	236	238	237
	303	230	227	226	229	233
	304	176	176	178	178	178
	305	204	203	204	205	209
	306	200	199	201	203	203
	307	220	222	224	226	223
	308	179	176	178	175	179
	309	242	242	245	244	244
	310	244	248	248	246	248
	Mean ± S.D.	212.8 ± 24.7	212.5 ± 25.5	214.4 ± 25.4	214.5 ± 25.9	215.8 ± 25.3

APPENDIX TABLE BW-1 (continued)

Methyl Bromide

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	151	357	335	235	323	312
	152	397	390	374	358	340
	153	368	367	352	337	331
	154	318	317	310	300	295
	155	350	350	344	340	335
	156	367	360	351	341	332
	157	355	355	341	330	317
	158	352	347	335	321	312
	159	390	386	371	345	329
	160	365	365	359	347	333
	Mean	361.9	358.8	347.2	334.2	323.6
	± S.D.	± 21.9	± 20.7	± 18.8	± 16.4	± 14.0

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	311	200	200	190	183	177
	312	239	233	224	215	207
	313	222	223	215	208	204
	314	231	234	229	227	227
	315	200	195	190	184	180
	316	206	205	195	191	185
	317	219	219	210	205	200
	318	218	224	224	218	204
	319	201	200	191	182	177
	320	241	245	242	239	235
	Mean	217.7	217.8	211.0	205.2	199.6
	± S.D.	± 15.8	± 17.1	± 17.8	± 19.9	± 20.3

APPENDIX TABLE BW-2

Methyl Bromide
Single Exposure Cytogenetics Test
Individual Body Weights (g)

Air Control (0 ppm)

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Male	1	434	11	390	21	416
	2	406	12	416	22	395
	3	422	13	395	23	371
	4	354	14	409	24	408
	5	433	15	460	25	393
	6	423	16	398	26	364
	7	399	17	386	27	380
	8	406	18	382	28	422
	9	400	19	406	29	360
	10	380	20	406	30	423
	Mean ± S.D.	405.7 ± 24.8		404.8 ± 22.2		393.2 ± 23.8

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Female	161	245	171	233	181	247
	162	214	172	227	182	225
	163	227	173	221	183	216
	164	267	174	237	184	244
	165	244	175	242	185	247
	166	274	176	261	186	261
	167	237	177	257	187	264
	168	220	178	256	188	226
	169	256	179	232	189	242
	170	227	180	255	190	262
	Mean ± S.D.	241.1 ± 20.0		242.8 ± 15.1		243.4 ± 16.7

APPENDIX TABLE BW-2 (continued)

Methyl Bromide

Single Dosing: 20 ppm

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Male	31	416	41	404	51	405
	32	368	42	422	52	384
	33	427	43	446	53	396
	34	410	44	395	54	395
	35	383	45	409	55	430
	36	404	46	403	56	369
	37	369	47	408	57	395
	38	357	48	395	58	401
	39	384	49	436	59	401
	40	401	50	384	60	389
	Mean ± S.D.	391.9 ± 23.2		410.2 ± 19.2		396.2 ± 15.7

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Female	191	202	201	252	211	263
	192	231	202	211	212	225
	193	236	203	219	213	249
	194	256	204	235	214	214
	195	270	205	261	215	220
	196	237	206	247	216	249
	197	256	207	239	217	223
	198	216	208	231	218	225
	199	234	209	224	219	213
	200	230	210	232	220	273
	Mean ± S.D.	236.8 ± 19.9		235.1 ± 15.3		235.4 ± 21.4

APPENDIX TABLE BW-2 (continued)

Methyl Bromide

Single Dosing: 70 ppm

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Male	61	458	71	376	81	385
	62	351	72	380	82	400
	63	393	73	396	83	413
	64	402	74	358	84	368
	65	410	75	373	85	370
	66	341	76	413	86	445
	67	368	77	397	87	387
	68	425	78	406	88	405
	69	384	79	377	89	386
	70	407	80	396	90	388
	Mean ± S.D.	393.9 ± 34.9		387.2 ± 17.0		394.7 ± 22.6

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Female	221	222	231	264	241	252
	222	220	232	264	242	230
	223	214	233	260	243	237
	224	238	234	254	244	228
	225	228	235	267	245	251
	226	252	236	221	246	261
	227	245	237	227	247	277
	228	260	238	308	248	225
	229	225	239	243	249	250
	230	246	240	268	250	200
	Mean ± S.D.	235.0 ± 15.4		257.6 ± 24.3		241.1 ± 21.7

APPENDIX TABLE BW-2 (continued)

Methyl Bromide

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Male	91	395	101	381	111	407
	92	448	102	413	112	450
	93	359	103	426	113	434
	94	428	104	433	114	399
	95	401	105	415	115	386
	96	454	106	387	116	405
	97	400	107	406	117	430
	98	390	108	438	118	411
	99	415	109	444	119	416
	100	372	110	417	120	426
	Mean ± S.D.	406.2 ± 30.6		416.0 ± 20.6		416.4 ± 18.8

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Female	251	246	261	217	271	216
	252	226	262	242	272	250
	253	230	263	237	273	270
	254	211	264	253	274	237
	255	230	265	258	275	226
	256	242	266	237	276	227
	257	238	267	251	277	231
	258	251	268	229	278	251
	259	229	269	257	279	222
	260	226	270	236	280	180
	Mean ± S.D.	232.9 ± 11.6		241.7 ± 13.2		231.0 ± 24.2

APPENDIX TABLE BW-3

Methyl Bromide
Dominant Lethal Assay
Individual Body Weights (g)

Multiple Dosing: Air Control (0 ppm)

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	361	382	387	389	392	394
	362	359	376	374	380	383
	363	351	353	350	354	357
	364	355	359	359	364	370
	365	365	366	371	365	372
	366	357	366	369	368	377
	367	416	425	432	435	444
	368	352	360	364	361	363
	369	367	362	373	370	376
	370	365	373	383	385	385
	Mean ± S.D.	366.9 ± 19.5	372.7 ± 20.9	376.5 ± 22.4	377.4 ± 23.3	382.1 ± 24.2

APPENDIX TABLE BW-3 (continued)

Methyl Bromide

Multiple Dosing: 20 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	371	406	411	421	412	416
	372	343	346	352	351	357
	373	375	381	387	384	386
	374	344	340	348	346	349
	375	383	383	393	390	392
	376	388	390	391	393	396
	377	353	360	358	357	369
	378	352	354	363	365	366
	379	360	355	367	363	365
	380	368	377	386	385	392
	Mean	367.2	369.7	376.6	374.6	378.8
	± S.D.	± 20.7	± 22.3	± 22.8	± 21.3	± 20.8

APPENDIX TABLE BW-3 (continued)

Methyl Bromide

Multiple Dosing: 70 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	381	366	370	381	376	382
	382	362	364	371	371	376
	383	357	367	378	370	378
	384	380	380	393	391	394
	385	385	400	408	403	403
	386	360	365	394	372	367
	387	357	365	367	362	367
	388	415	424	427	430	431
	389	392	392	400	397	397
	390	352	355	357	353	356
	Mean ± S.D.	372.6 ± 20.0	378.2 ± 21.2	387.6 ± 21.0	382.5 ± 22.8	385.1 ± 21.8

APPENDIX TABLE BW-3 (continued)

Methyl Bromide

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	391	356	352	338	324	319
	392	381	386	377	366	359
	393	384	375	360	343	330
	394	368	360	349	333	328
	395	406	400	380	365	353
	396	348	344	335	325	317
	397	402	397	382	372	365
	398	350	354	349	340	331
	399	363	367	352	338	331
	400	392	387	381	368	357
	Mean	375.0	372.2	360.3	347.4	339.0
	± S.D.	± 21.1	± 19.8	± 18.3	± 18.6	± 17.7

APPENDIX TABLE BW-4

Methyl Bromide
Sperm Abnormality Test
Individual Body Weights (g)

Multiple Dosing: Air Control (0 ppm)

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	321	18	19	20	21	21
	322	21	21	22	21	21
	323	19	19	20	20	20
	324	20	20	20	20	20
	325	22	22	22	22	23
	326	20	21	21	21	21
	327	20	20	20	21	20
	328	22	22	22	22	23
	329	21	22	22	22	21
	330	21	21	22	23	22
	Mean ± S.D.	20.4 ± 1.3	20.7 ± 1.2	21.1 ± 1.0	21.3 ± 0.9	21.2 ± 1.1

APPENDIX TABLE BW-4 (continued)

Methyl Bromide

Multiple Dosing: 20 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	331	22	22	23	24	24
	332	17	18	18	18	18
	333	18	18	18	19	20
	334	19	18	18	19	19
	335	22	22	22	22	22
	336	21	22	22	22	22
	337	21	21	21	22	22
	338	19	19	20	20	21
	339	21	22	22	23	23
	340	20	21	22	22	22
	Mean ± S.D.	20.0 ± 1.7	20.3 ± 1.8	20.6 ± 2.0	21.1 ± 2.0	21.3 ± 1.8

APPENDIX TABLE BW-4 (continued)

Methyl Bromide

Multiple Dosing: 70 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	341	19	19	20	20	20
	342	19	20	20	20	20
	343	20	21	22	22	22
	344	19	19	19	19	18
	345	20	21	21	21	21
	346	19	19	20	19	20
	347	21	22	22	22	23
	348	18	18	18	18	18
	349	21	21	22	21	22
	350	21	21	22	22	20
	Mean	19.7	20.1	20.6	20.4	20.4
	± S.D.	± 1.1	± 1.3	± 1.4	± 1.4	± 1.6

APPENDIX TABLE BW-4 (continued)

Methyl Bromide

Multiple Dosing: Ethyl methanesulphonate, 200 mg/kg

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	401	26	27	25	28	26
	402	30	30	29	31	30
	403	29	29	28	30	29
	404	30	29	30	30	29
	405	27	27	27	29	27
	406	30	30	30	30	27
	407	28	27	25	26	25
	408	27	26	26	28	26
	409	31	31	32	32	30
	410	30	30	30	31	29
	Mean ± S.D.	28.8 ± 1.7	28.6 ± 1.7	28.2 ± 2.4	29.5 ± 1.8	27.8 ± 1.8

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	351	19	18	18	20	19
	354	19	17	17	18	19
	359	22	22	21	21	22
	360	21	19	19	19	21
	Mean ± S.D.	20.3 ± 1.5	19.0 ± 2.2	18.8 ± 1.7	19.5 ± 1.3	20.3 ± 1.5

Nos. 351-360 wrongly dosed with 400 mg/kg/day ethyl methanesulphonate on Day 1 of dosing, surviving animals continued on test and were dosed with 200 mg/kg/day ethyl methanesulphonate for the remaining 4 dosing days.

Nos. 401-410 are intended to replace the original group (351-360).

APPENDIX TABLE CA-MD-M

Methyl Bromide
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Males

Multiple Dosing: Air Control (0 ppm) Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
121	40/1	50	25	24	2							36.0 x 105.6
	40/2		25	24	2							28.7 x 103.6
126	129/1	50	25	25								
	129/2		25	25								
127	155/1	50	25	25								
	155/2		25	25								
128	44/2	50	25	25								
	44/3		25	25								
124	102/1	50	25	25								
	102/2		25	24	1							43.1 x 102.7
122	4/1	50	25	25								
	4/2		25	25								
125	10/1	50	25	24	1							39.3 x 111.7
	10/2		25	25								
129	137/1	50	25	25								
	137/2		25	25								
130	152/1	50	25	25								
	152/2		25	25								
123	9/1	50	25	25								
	9/2		25	25								

APPENDIX TABLE CA-MD-M (continued)

Methyl Bromide
Males

Multiple Dosing: 20 ppm

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
140	53/1	50	25	25								
	53/2		25	24	1							29.2 x 110.1
135	141/2	50	25	25								
	141/3		25	24	1							39.4 x 107.2
139	154/1	50	25	25								
	154/2		25	25								
136	125/1	50	25	25								
	125/2		25	25								
137	83/1	50	25	25								
	83/2		25	25								
134	118/1	50	25	25								
	118/2		25	25								
132	19/3	50	25	25								
	19/1		25	25								
138	100/1	50	25	25								
	100/2		25	24	1							22.9 x 111.3
131	77/1	50	25	24		1						38.3 x 105.4
	77/2		25	25								
133	26/1	50	25	24	1							43.1 x 107.3
	26/2		25	24	1							47.5 x 100.6

APPENDIX TABLE CA-MD-M (continued)

Methyl Bromide
Males

Multiple Dosing: .70 ppm

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
142	110/2	50	25	24				1				28.8 x 105.5
	110/3		25	25								-
150	37/1	50	25	25								-
	37/2		25	24	1							26.9 x 106.7
143	52/1	50	25	24	2							41.3 x 105.8
	52/2		25	25								-
145	115/1	50	25	23		1						27.8 x 105.3
						1						27.3 x 105.0
	115/2		25	24		1						38.0 x 111.8
146	25/1	50	25	25								-
	25/2		25	25								-
148	3/2	50	25	25								-
	3/3		25	25								-
149	143/1	50	25	25								-
	143/2		25	24	1							45.7 x 102.7
144	93/1	50	25	24	1							65.3 x 88.0
	93/2		25	24	1							30.4 x 111.4
141	56/1	50	25	21	1							36.9 x 111.9
						1						38.3 x 111.4
					1	1						47.4 x 110.0
					1							48.1 x 108.8
	56/2		25	24				1				31.8 x 105.9
147	21/1	50	25	24	1							36.9 x 109.5
	21/2		25	25								-

APPENDIX TABLE CA-MD-M (continued)

Methyl Bromide

Males

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg — Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
156	51/1	50	25	24	1							32.1 x 108.5
	51/2		25	25								
153	86/1	50	25	25								
	86/2		25	24							1 Chromatid Fragment	45.7 x 103.9
157	139/1	50	25	25								
	139/2		25	25								
154	58/1	50	25	24		1						40.6 x 107.0
	58/2		25	22		1						44.7 x 102.0
						3		1				30.5 x 101.0
						1					1 Chromatid Fragment	66.9 x 109.3
151	136/1	50	25	23				1				35.5 x 109.8
								1				38.9 x 108.6
	136/2		25	23	1							42.3 x 103.0
					1							29.9 x 101.3
155	32/1	50	25	24	1							42.6 x 109.5
	32/2		25	25								
159	92/1	50	25	24	1							28.7 x 104.5
	92/2		25	25								
160	78/1	50	25	25								
	78/2		25	24		3						39.4 x 108.8

APPENDIX TABLE CA-MD-M (continued)

Methyl Bromide
Males

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
158	123/1	50	25	22	1							38.8 x 111.4
					1							31.4 x 110.0
											1 Chromatid Fragment	33.4 x 107.6
	123/2		25	25								
152	138/1	50	25	25								
	138/2		25	24	1							25.5 x 106.1

APPENDIX TABLE CA-MD-F

Methyl Bromide
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Females

Multiple Dosing: Air Control (0 ppm) Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
282	164/1	50	25	24				1				32.4 x 108.2
	164/2		25	25								
283	169/1	50	25	25								
	169/2		25	25								
290	312/1	50	25	24	1							31.9 x 114.3
	312/2		25	24		1						23.1 x 109.6
285	170/1	50	25	24	1							67.1 x 98.6
	170/2		25	25								
289	297/1	50	25	23	3							34.7 x 109.9
					1	1						58.3 x 105.3
	297/2		25	25								
281	200/2	50	25	25								
	200/3		25	25								
286	289/1	50	25	25								
	289/2		25	25								
288	204/1	50	25	25								
	204/2		25	25								
287	315/1	50	25	23				1				45.6 x 103.9
					1							37.4 x 103.7
	315/2		25	25								
284	262/1	50	25	25								
	262		25	25								

APPENDIX TABLE CA-MD-F (continued)

Methyl Bromide

Females

Multiple Dosing: 20 ppm

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
295	301/1	50	25	24	1							35.4 x 109.8
	301/2		25	25								
299	314/1	50	25	25								
	314/2		25	25								
298	260/1	50	25	25								
	260/2		25	25								
300	213/2	50	25	25								
	213/3		25	25								
296	285/1	50	25	24		1						43.2 x 110.5
	285/2		25	25								
294	278/1	50	25	25								
	278/2		25	25								
297	243/1	50	25	25								
	243/2		25	24	2							43.0 x 105.9
293	186/1	50	25	24	1							32.3 x 108.0
	186/2		25	25								
292	179/1	50	25	24	1							42.2 x 103.7
	179/2		25	25								
291	237/1	50	25	25								
	237/2		25	24	1							49.8 x 103.2

APPENDIX TABLE CA-MD-F (continued)

Methyl Bromide
Females

Multiple Dosing: 70 ppm

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
302	270/1	50	25	25								
	270/2		25	25								
307	181/1	50	25	25								
	181/2		25	25								
301	216/2	50	25	25								
	216/3		25	25								
303	212/1	50	25	25								
	212/2		25	25								
304	253/2	50	25	23	1						1 Chromatid Fragment	34.3 x 110.0 48.9 x 108.2
	253/3		25	24			1	1				29.7 x 111.7
308	163/1	50	25	25								
	163/2		25	25								
310	197/1	50	25	25								
	197/2		25	25								
309	303/1	50	25	25								
	303/3		25	25								
305	275/1	50	25	25								
	275/2		25	25								
306	185/2	50	25	25								
	185/3		25	25								

APPENDIX TABLE CA-MD-F (continued)

Methyl Bromide

Females

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
313	246/1	50	25	23		1						33.6 x 105.7
						1						34.9 x 101.7
	246/3		25	25								
315	192/2	50	25	25								
	192/3		25	25								
316	211/1	50	25	24	1							59.7 x 110.0
	211/2		25	23	1							30.3 x 113.2
					2							59.8 x 104.8
319	252/1	50	25	25								
	252/2		25	25								
320	238/1	50	25	25								
	238/3		25	24							1 Chromatid Fragment	32.8 x 109.9
314	218/1	50	25	25								
	218/2		25	25								
317	299/1	50	25	23		1						41.8 x 104.6
					1			1				37.5 x 103.6
	299/2		25	22	1							46.6 x 109.4
							1	1			1 Chromatid Fragment	46.0 x 107.8
					1							29.5 x 107.5
311	296/1	50	25	25								
	296/2		25	25								

APPENDIX TABLE CA-MD-F (continued)

Methyl Bromide
Females

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg — Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
318	283/1	50	25	25								
	283/2		25	24		1						36.0 x 109.0
312	298/2	50	25	25								
	298/3		25	23	1							49.2 x 113.9
						1						54.1 x 110.2

APPENDIX TABLE CA-M6

Methyl Bromide
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Males

Single Dosing: Air Control (0 ppm) Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
7	140/2	50	25	24				1				14.9 x 96.0
	140/1		25	25								
8	16/1	50	25	24	1							7.4 x 114.2
	16/3		25	24	1							7.7 x 102.0
1	68/4	50	25	24	1							10.1 x 100.0
	68/5		25	25								
3	41/4	50	25	25								
	41/3		25	24				1				11.6 x 94.2
2	150/4	50	25	24	1							12.9 x 96.4
	150/1		25	25								
6	128/5	50	25	25								
	128/3		25	25								
5	87/1	50	25	24	1							5.3 x 98.7
	87/2		25	25								
9	2/4	50	25	22	1							13.5 x 91.2
					1	1						12.8 x 88.4
								1				9.1 x 102.0
	2/3		25	22	1							14.9 x 94.5
					1							15.0 x 91.5
					1			1				11.9 x 92.8

APPENDIX TABLE CA-M6 (continued)

Methyl Bromide
Males

Single Dosing: Air Control (0 ppm) Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
10	62/3	50	25	24					1			14.1 x 82.9
	62/4		25	25								
4	114/1	50	25	24	1							11.3 x 95.5
	114/4		25	22	1							13.4 x 92.8
					1							13.1 x 115.5
					1							12.8 x 96.2

APPENDIX TABLE CA-M6 (continued)

Methyl Bromide

Males

Single Dosing: 20 ppm

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
34	55/5	50	25	25								
	55/4		25	24	1							8.2 x 114.9
36	126/1	50	25	25								
	126/2		25	25								
31	124/2	50	25	25								
	124/3		25	25								
32	135/4	50	25	25								
	135/2		25	25								
37	22/5	50	25	25								
	22/4		25	24	1							14.0 x 90.1
33	46/5	50	25	23	1							14.8 x 100.2
					1							13.5 x 89.6
	46/4		25	23	1			1				13.4 x 96.0
					1							11.3 x 102.2
35	109/2	50	25	25								
	109/4		25	25								
38	151/2	0	0	0								
	151/4		0	0								
	151/1		0	0								
	151/3		0	0								
	151/5		0	0								

APPENDIX TABLE CA-M6 (continued)

Methyl Bromide

Males

Single Dosing: 20 ppm

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
40	60/3	50	22	22								
	60/2		14	13				1				33.0 x 85.3
			14	14								
39	42/4	50	25	25								
	42/3		25	25								

APPENDIX TABLE CA-M6 (continued)

Methyl Bromide

Males

Single Dosing: 70 ppm

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
70	11/4	50	25	25								
	11/3		25	25								
68	31/5	50	25	24	1							11.8 x 115.9
	31/4		25	24	1							13.5 x 95.8
61	34/3	50	25	25								
	34/4		25	25								
63	147/1	50	25	23	1							9.9 x 101.8
					1							9.3 x 98.8
	147/4		25	25								
67	73/5	50	25	25								
	73/1		25	23	1							10.0 x 106.4
								1				10.0 x 112.5
66	79/4	50	25	24	1							15.0 x 94.0
	79/2		25	24	1							7.9 x 97.1
65	160/4	50	25	25								
	160/1		25	25								
64	145/4	50	25	24	1							4.8 x 108.3
	145/3		25	25								
62	122/2	50	25	25								
	122/1		25	25								
69	127/4	50	25	25								
	127/5		25	24	1							15.1 x 105.8

APPENDIX TABLE CA-M6 (continued)

Methyl Bromide

Males

Single Dosing: Ethyl methanesulphonate, 250 mg/kg — Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
91	81/3	50	25	23		1						12.5 x 95.0
					1							8.2 x 87.8
	81/5		25	25								
99	144/3	50	25	21	1							12.0 x 92.9
					1							9.9 x 98.1
					1							9.3 x 97.4
						1						8.1 x 88.0
	144/1		25	25								
97	48/1	50	25	24	1							8.9 x 96.4
	48/4		25	25								
100	80/4	50	25	23	1							10.9 x 86.9
					1							9.4 x 88.0
	80/1		25	21	1							11.9 x 89.8
					1							9.0 x 84.0
					1							9.1 x 84.6
					1							9.8 x 88.8
92	5/1	50	25	20	1							13.8 x 107.9
					3							13.8 x 100.0
					2							13.5 x 102.8
					1							13.2 x 89.8
					1							12.8 x 105.9
	5/2		25	23	1							13.9 x 97.5
					1							12.8 x 99.4
98	148/1	50	25	24		1						7.9 x 88.3
	148/3		25	23		1						9.9 x 86.9
					1							8.7 x 89.4

APPENDIX TABLE CA-M6 (continued)

Methyl Bromide
Males

Single Dosing: Ethyl methanesulphonate 250 mg/kg

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
93	97/2	50	25	25								
	97/1		25	22	1							12.9 x 92.5
					1							10.8 x 88.0
					1							6.3 x 98.1
96	14/3	50	25	22	1							12.9 x 88.1
					1							12.4 x 89.2
						1						12.5 x 97.3
	14/2		25	22		1						14.6 x 91.9
					1							13.8 x 89.0
						1						13.3 x 90.0
95	35/5	50	25	24	1							13.8 x 100.8
	35/1		25	24	1							13.3 x 117.0
94	88/5	50	25	24	1							11.5 x 86.0
	88/1		25	24	2							12.9 x 88.4

APPENDIX TABLE CA-M24

Methyl Bromide
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Males

Single Dosing: Air Control (0 ppm) Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
14	64/4	50	25	25								
	64/1		25	25								
17	45/1	50	25	25								
	45/3		25	25								
20	103/1	50	25	24	1							36.5 x 92.5
	103/2		25	25								
18	157/3	50	25	25								
	157/5		25	25								
13	76/1	50	25	24	1							32.4 x 95.3
	76/2		25	23	1							35.1 x 73.0
12	47/1	50	25	25			1					34.7 x 74.1
	47/2		25	25								
16	82/3	50	25	25								
	82/5		25	25								
19	74/1	50	25	25								
	74/5		25	25								
11	146/2	50	25	24							1 Chromatid Fragment	16.9 x 116.5
	146/4		25	25								
15	153/2	50	25	25								
	153/1		25	25								

APPENDIX TABLE CA-M24 (continued)

Methyl Bromide

Males

Single Dosing: 20 ppm

Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
41	57/1	50	25	25								
	57/3		25	25								
42	133/1	50	25	25								
	133/3		25	24	1							33.5 x 72.3
46	71/3	50	25	25								
	71/5		25	25								
50	98/2	50	25	23	1							31.1 x 73.8
					2							31.2 x 91.6
	98/4		25	25								
48	134/3	50	25	25								
	134/4		25	25								
45	84/2	50	25	24	1							35.4 x 94.7
	84/3		25	25								
49	85/2	50	25	25								
	85/5		25	25								
44	39/3	50	25	25								
	39/2		25	24		1						33.4 x 97.5
47	70/1	50	25	25								
	70/4		25	24							1 Dicentric	36.8 x 115.0
43	12/3	50	25	25								
	12/2		25	24		1						37.6 x 105.3

APPENDIX TABLE CA-M24 (continued)

Methyl Bromide

Males

Single Dosing: 70 ppm

Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
72	59/2	50	25	25								
	59/3		25	25								
78	121/1	50	25	25								
	121/4		25	25								
77	13/1	50	25	25								
	13/2		25	25								
73	50/4	50	25	24		1						34.4 x 71.8
	50/5		25	25								
80	96/3	50	25	24	1							31.1 x 70.1
	96/5		25	25								
74	27/2	50	25	24	1							33.1 x 74.3
	27/5		25	25								
76	18/3	50	25	25								
	18/2		25	25								
75	15/1	50	25	25								
	15/4		25	25								
71	38/3	50	25	25								
	38/5		25	25								
79	61/3	50	25	25								
	61/5		25	25								

APPENDIX TABLE CA-M24 (continued)

Methyl Bromide
Males

Single Dosing: Ethyl methanesulphonate, 250 mg/kg — Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
101	111/2	50	25	21	1							33.9 x 97.8
						1						36.9 x 96.1
						1						37.0 x 87.8
					2							37.5 x 70.2
	111/1		25	21	1	2						31.4 x 63.8
					1							31.2 x 98.9
					1							32.8 x 73.4
					1							33.7 x 71.1
103	65/3	50	25	22	3	1			1			35.2 x 74.6
					1							37.3 x 68.8
					1	2						37.5 x 75.5
	65/4		25	19	1		1					29.2 x 68.2
					1		1					33.7 x 89.9
					1							35.4 x 90.9
					2		1					35.7 x 76.4
					1							37.9 x 75.6
					1							38.0 x 91.4
105	24/2	50	25	21		1						30.3 x 76.9
							1					32.5 x 99.5
									1			32.5 x 93.2
					1	2						32.4 x 77.4

APPENDIX TABLE CA-M24 (continued)

Methyl Bromide

Males

Single Dosing: Ethyl methanesulphonate, 250 mg/kg — Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
105	24/5		25	17		1						33.5 x 70.3
									1			33.1 x 75.0
					1							33.0 x 78.7
						1						35.2 x 86.3
					2							35.4 x 77.0
					1							35.4 x 72.3
					1	1						37.7 x 73.1
					1	2	1					37.3 x 95.0
107	89/1	50	25	18							1 Multiple Aberration	31.6 x 72.3
											1 Multiple Aberration	32.1 x 75.9
						2						32.2 x 77.6
						3						31.8 x 81.1
					1	2						32.6 x 103.2
					1							35.0 x 92.3
					2	2						37.9 x 82.5
	89/5		25	18		1						32.8 x 67.4
						1						33.4 x 100.5
						3						36.8 x 78.5
						1						37.1 x 77.8
					1							37.1 x 68.9
					2	4						40.0 x 76.9
					1	2						40.3 x 80.9

APPENDIX TABLE CA-M24 (continued)

Methyl Bromide
Males

Single Dosing: Ethyl methanesulphonate, 250 mg/kg — Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
109	149/2	50	25	11	1							33.5 x 68.4
					2	3						33.1 x 75.0
					1	2						33.2 x 76.7
											1 Multiple Aberration	32.8 x 79.7
						5						33.4 x 91.2
						1						33.1 x 91.6
					1							33.0 x 92.7
						1						32.9 x 93.5
						4						33.3 x 94.0
									1			33.8 x 98.2
											1 Multiple Aberration	34.0 x 101.6
						1						35.5 x 98.4
											1 Multiple Aberration	35.8 x 95.6
						1						35.7 x 94.6

APPENDIX TABLE CA-M24 (continued)

Methyl Bromide

Males

Single Dosing: Ethyl methanesulphonate, 250 mg/kg — Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
109	149/3		25	12		2						33.8 x 70.0
					1	1						33.9 x 70.9
						2						33.9 x 72.4
						3	1					34.3 x 72.7
					1							33.9 x 82.8
						4						34.1 x 94.0
							1					34.4 x 97.2
						2						38.3 x 95.5
					1	1						38.5 x 94.8
					1							38.7 x 90.4
						1						38.4 x 69.1
						1						38.2 x 68.4
						1						40.6 x 69.8
108	95/2	50	25	20		3						32.7 x 93.5
						4						34.8 x 96.4
						1						34.6 x 86.1
						4						34.8 x 78.0
						5						37.4 x 88.5

APPENDIX TABLE CA-M24 (continued)

Methyl Bromide

Males

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
108	95/3		25	15	1							31.4 x 68.9
						2						31.9 x 69.5
						1						32.2 x 72.2
						1						33.0 x 95.5
						7						35.3 x 103.6
					1							35.2 x 103.3
						1						34.7 x 102.4
					1							35.4 x 94.4
					1							35.0 x 81.8
					1							34.8 x 75.5
110	91/4	50	25	20		4						9.8 x 115.7
						3						17.3 x 115.1
					1							32.5 x 115.4
						2						37.1 x 115.9
					1							34.1 x 113.5
	91/1		25	19		1						14.6 x 118.1
						1						16.3 x 117.8
						4						32.9 x 117.8
					1	1						35.9 x 118.1
						1						37.8 x 117.2
						4						34.6 x 117.2

APPENDIX TABLE CA-M24 (continued)

Methyl Bromide

Males

Single Dosing: Ethyl methanesulphonate, 250 mg/kg — Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
106	36/4	50	25	15		1						15.6 x 117.6
						1						16.1 x 117.7
											1 Chromatid Fragment	21.7 x 117.6
					1	5						35.6 x 117.3
						1						36.1 x 117.2
						1						45.0 x 116.3
					3	6						33.4 x 114.3
					1							33.2 x 114.3
											1 Multiple Aberration	11.5 x 114.5
					1	2						16.2 x 113.5
	36/2		25	15	1							13.7 x 117.2
											1 Chromatid Fragment	16.5 x 117.6
											1 Multiple Aberration	18.7 x 118.1
					1	2						20.7 x 118.2
					1							21.8 x 118.0
						1						44.5 x 118.4
						2						41.9 x 115.3
						6						38.7 x 115.7
											1 Multiple Aberration	36.6 x 115.5
											1 Chromatid Fragment	21.1 x 115.8

APPENDIX TABLE CA-M24 (continued)

Methyl Bromide

Males

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
102	69/2	50	25	21	1	2						21.7 x 115.8
						1						21.7 x 115.9
					1							35.6 x 115.9
						3						42.2 x 114.6
	69/1		25	19	2	3						18.0 x 117.8
					1	1						22.8 x 118.2
						2						37.9 x 117.3
					2							43.4 x 114.8
						1						42.3 x 115.6
											1 Chromatid Fragment	41.1 x 115.6
104	130/2	50	25	15							1 Chromatid Fragment	14.3 x 116.9
					1	5						16.9 x 117.5
					1	1						18.5 x 117.8
					1							39.4 x 117.6
					1							40.0 x 117.7
											1 Multiple Aberration	43.4 x 117.8
					2	2						42.2 x 115.2
					1	1						39.6 x 115.4
											1 Multiple Aberration	39.4 x 115.3
						6						20.9 x 115.7

APPENDIX TABLE CA-M24 (continued)

Methyl Bromide

Males

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
104	130/5		25	18		2						16.8 x 117.3
						6						17.0 x 117.3
											1 Multiple Aberration	17.5 x 117.6
					2	6						38.5 x 117.4
						1						39.3 x 117.5
					1							40.8 x 114.9
						9						14.3 x 115.8

APPENDIX TABLE CA-M48

Methyl Bromide
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Males

Single Dosing: Air Control (0 ppm) Sampling Time: 48 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
27	20/1	50	25	25								
	20/5		25	25								
25	119/1	50	25	25								
	119/2		25	25								
29	132/1	50	25	24		1						36.4 x 97.8
	132/2		25	25								
28	105/1	50	25	25								
	105/3		25	25								
24	72/1	50	25	25								
	72/2		25	24	1							37.1 x 70.3
22	106/3	50	25	23	1							11.4 x 83.3
					1							9.0 x 83.4
	106/5		25	23	1							16.1 x 112.7
						1						6.5 x 109.6
23	7/3	50	25	25								
	7/5		25	24	1							17.1 x 70.3
21	8/3	50	25	24	1							15.2 x 90.6
	8/2		25	25								
26	6/2	50	25	25								
	6/4		25	24	1							9.2 x 99.4
30	29/4	50	25	25								
	29/5		25	25								

APPENDIX TABLE CA-M48 (continued)

Methyl Bromide
Males

Single Dosing: 20 ppm

Sampling Time: 48 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
57	112/2	50	25	24		1						21.9 x 103.3
	112/3		25	25								
56	94/2	50	25	25								
	94/4		25	25								
51	30/3	50	25	25								
	30/5		25	24		1						34.7 x 96.7
59	23/1	50	25	24		1						28.9 x 93.8
	23/2		25	25								
53	156/2	50	25	25								
	156/4		25	25								
52	117/1	50	25	24	1							4.1 x 67.5
	117/5		25	25								
58	66/1	50	25	25								
	66/5		25	25								
55	104/1	50	25	25								
	104/2		25	24	1							10.6 x 90.2
54	17/1-5	0	0	0								
60	131/3	50	25	25								
	131/5		25	25								

APPENDIX TABLE CA-M48 (continued)

Methyl Bromide
Males

Single Dosing: 70 ppm

Sampling Time: 48 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
83	1/1	50	25	25								
	1/2		25	24	1							39.4 x 100.3
89	120/2	50	25	25								
	120/3		25	24		1						25.7 x 102.0
87	43/1	50	25	24	1	1						40.8 x 100.8
	43/2		25	25								
82	142/4	50	25	25								
	142/3		25	23	1							24.0 x 93.7
					1							26.7 x 89.9
86	63/1	50	25	24	1							53.6 x 91.0
	63/2		25	24	1							32.8 x 97.7
85	67/1	50	25	25								
	67/4		25	24		1						85.1 x 6.0
90	158/1	50	25	24	1							10.6 x 97.6
	158/2		25	24	1							12.7 x 97.5
84	113/1-5	0	0	0								
81	90/5	50	25	25								
	90/1		25	25								
88	107/1	50	25	25								
	107/2		25	25								

APPENDIX TABLE CA-M48 (continued)

Methyl Bromide

Males

Single Dosing: Ethyl methanesulphonate, 250 mg/kg — Sampling Time: 48 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
116	75/1	50	25	21		1						41.7 x 109.4
						1						30.8 x 110.3
							1					30.1 x 110.3
					1							29.4 x 110.4
	75/2		25	22	1							35.6 x 109.6
					1							29.3 x 107.5
					1							35.5 x 106.5
114	99/3	50	25	25								
	99/5		25	24	1							20.2 x 99.5
119	33/2	50	25	23	2							8.0 x 100.5
								1				18.4 x 101.1
	33/3		25	24	2	1	1					13.5 x 98.0
112	49/1	50	25	25								
	49/2		25	24	1							16.3 x 73.1
117	101/1	50	25	25								
	101/5		25	25								
111	159/1	50	25	25								
	159/2		25	25								
120	28/4	50	25	25								
	28/3		25	25								
118	108/1	50	25	24		1						6.9 x 99.5
	108/4		25	23	1							8.7 x 93.7
					1							10.4 x 93.8

APPENDIX TABLE CA-M48 (continued)

Methyl Bromide

Males

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sampling Time: 48 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
115	54/3	50	25	24		1						9.1 x 92.7
	54/2		25	19	1							18.7 x 89.1
											1 Multi Aberration	16.7 x 88.3
					1							13.7 x 88.2
											1 Multi Aberration	13.2 x 89.2
					2							12.3 x 88.7
					1	2						11.3 x 88.1
								1				11.5 x 87.8
113	116/3	50	25	24			1					8.5 x 111.0
	116/2		25	24	1							2.8 x 111.8

APPENDIX TABLE CA-F6

Methyl Bromide
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Females

Single Dosing: Air Control (0 ppm) *Sampling Time:* 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
164	274/1	50	25	25								
	274/3		25	23				1				13.0 x 118.1
								1				12.3 x 115.2
170	222/2	50	25	24		1						12.8 x 86.2
	222/1		25	23	1							12.3 x 95.9
					1							10.0 x 96.2
161	228/4	16	1	1								
	228/3		2	2								
	228/2		4	4								
	228/1		2	2								
	228/5		7	6	1							7.1 x 100.6
167	300/3	50	25	24	1							8.0 x 114.0
	300/1		25	24	1	1						10.8 x 112.1
169	162/1	50	25	25								10.3 x 94.5
	162/2		25	24	1							
165	247/5	34	5	5								
	247/1		4	4								
	247/2		13	12				1				10.0 x 93.0
	247/3		8	7							1 Exchange	11.8 x 119.1
	247/4		4	2	1							0.4 x 87.6
								1				33.2 x 98.4
163	201/4	50	25	23							1 Pair Chromsome Minutes	13.5 x 93.5
					1							12.3 x 87.9
	201/3		25	23	2							12.9 x 89.0
					1							12.8 x 97.8

APPENDIX TABLE CA-F6 (continued)

Methyl Bromide
Females

Single Dosing: Air Control (0 ppm) Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
168	176/5	50	25	22	1							15.0 x 110.0
					1							14.8 x 106.1
					1							15.2 x 99.2
	176/4		25	23	1							14.1 x 110.2
					1							13.3 x 86.2
166	288/5	50	25	22	1							15.1 x 95.1
					1							14.9 x 87.8
					1							14.3 x 101.3
	288/1		25	24	1							13.1 x 93.2
162	310/5	50	25	24	2							10.8 x 93.7
	310/2		25	24	1							9.4 x 89.2

APPENDIX TABLE CA-F6 (continued)

Methyl Bromide

Females

Single Dosing: 20 ppm

Sampling Time 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
195	269/3	50	25	23	1							12.4 x 98.6
						1						12.9 x 90.2
	269/5		25	23	1							12.6 x 101.8
					1							12.2 x 94.1
192	295/1	50	25	24		1						8.8 x 94.0
	295/3		25	25								
193	206/3	50	25	25								
	206/2		25	25								
191	284/2	50	25	25								
	284/4		25	25								
198	311/1	50	25	24	1							13.8 x 94.7
	311/3		25	25								
194	215/5	24	16	15	1							11.9 x 117.3
	215/3		3	3								
	215/4		4	4								
	215/1		0	0								
	215/2		1	1								
197	182/3	50	25	25								
	182/2		25	23	1							13.2 x 91.0
					1							12.0 x 97.8

APPENDIX TABLE CA-F6 (continued)

Methyl Bromide

Females

Single Dosing: 20 ppm

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
199	202/3	50	21	21								
	202/1		25	25								
	202/4		4	4								
196	286/3	50	25	22				1				11.6 x 112.9
					1							11.3 x 94.3
											1 Pair Chromosome Minutes	8.0 x 96.4
	286/2		25	24				1				8.6 x 87.4
200	220/4	50	25	25								
	220/2		25	25								

APPENDIX TABLE CA-F6 (continued)

Methyl Bromide
Females

Single Dosing: 70 ppm

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
227	233/3	50	25	25								
	233/4		25	24	1							12.1 x 97.9
221	194/4	50	25	23	1							10.6 x 95.0
								1				10.0 x 103.1
	194/2		25	24	1							14.0 x 94.0
229	287/2	50	17	16								30.5 x 92.1
	287/5		25	24	1							6.3 x 114.9
	287/4		8	8								
230	171/1	50	25	23	1							14.9 x 91.1
					1							12.2 x 94.2
	171/3		25	24	1							10.3 x 90.2
223	307/3	7	2	2								
	307/4		2	2								
	307/1		1	1								
	307/2		2	2								
	307/5		0	0								
228	191/5	50	25	24	1							13.7 x 101.0
	191/3		25	25								
225	320/2	50	25	25								
	320/5		25	25								
224	305/4	50	25	24	1							10.9 x 68.9
	305/1		25	24	1							8.7 x 83.1

APPENDIX TABLE CA-F6 (continued)

Methyl Bromide

Females

Single Dosing: 70 ppm

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
226	239/3	36	6	5				1				12.9 x 95.0
	239/1		23	23								
	239/5		0	0								
	239/4		0	0								
	239/2		7	7								
222	282/2	50	22	21	1							9.9 x 99.8
	282/3		25	24	1							11.8 x 90.0
	282/1		3	3								

APPENDIX TABLE CA-F6 (continued)

Methyl Bromide

Females

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
255	195/3	8	5	5								
	195/4		0	0								
	195/1		1	1								
	195/5		0	0								
	195/2		2	2								
259	304/3	50	25	23				1				7.8 x 95.0
					1							1.9 x 101.9
	304/2		25	24	1							4.5 x 103.0
260	240/1	0	0	0								
	250/2		0	0								
	240/3		0	0								
	240/4		0	0								
	240/5		0	0								
258	308/1	0	0	0								
	308/2		0	0								
	308/3		0	0								
	308/4		0	0								
	308/5		0	0								
251	241/1	50	25	24	1							10.9 x 94.9
	241/2		25	22							1 Exchange	13.2 x 95.3
					1			1				10.8 x 93.1
					1							10.8 x 91.5

APPENDIX TABLE CA-F6 (continued)

Methyl Bromide

Females

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
256	174/1	50	25	24	1	1						12.9 x 97.2
	174/4		25	22	1							14.0 x 99.2
					1							14.1 x 97.5
					1							13.8 x 97.2
252	165/3	50	25	23	1							12.3 x 91.0
					1							11.7 x 90.5
	165/4		25	23	1							11.9 x 106.8
					1							10.2 x 95.4
257	208/3	50	25	23	1							10.2 x 102.8
					1							9.8 x 98.8
	208/1		25	23	1							10.8 x 110.6
					1							10.0 x 109.2
253	257/5	50	15	14	1							13.1 x 92.4
	257/1		4	3				1				34.9 x 124.0
	257/2		14	11	1			3				10.5 x 94.4
					1							7.6 x 95.2
								1				0.6 x 86.6
	257/3		9	8		1						10.1 x 86.0
	257/4		8	8								

APPENDIX TABLE CA-F6 (continued)

Methyl Bromide

Females

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
254	248/1	50	25	21	1							11.2 x 117.9
								1				10.5 x 99.3
					1							10.5 x 91.7
					1							10.5 x 89.1
	248/2		25	22	1							10.9 x 96.1
					1							10.8 x 118.9
					1							10.1 x 95.2

APPENDIX TABLE CA-F24

Methyl Bromide
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Females

Single Dosing: Air Control (0 ppm) Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
178	317/1	50	25	25								
	317/2		25	25								
171	306/2	50	25	25								
	306/5		25	25								
172	207/1	50	25	25								
	207/2		25	25								
179	234/5	50	25	25								
	234/4		25	24		1						31.3 x 73.8
174	224/2	50	19	18	1	1						35.1 x 99.8
	224/4		25	24	1							36.8 x 77.7
	224/5		6	5		1						34.4 x 83.7
175	313/1	50	25	25								
	313/4		25	25								
180	263/2	50	25	24	1							32.7 x 90.0
	263/3		25	25								
176	242/3	50	25	25								
	242/1		25	25								
173	236/1	50	25	23	1							14.0 x 116.3
					1							15.0 x 116.5
	236/2		25	25								
177	205/3	50	25	24	1							17.8 x 115.4
	205/5		25	23	1							20.2 x 118.2
					1							26.3 x 113.5

APPENDIX TABLE CA-F24 (continued)

Methyl Bromide
Females

Single Dosing: 70 ppm

Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
235	175/3	50	25	25								
	175/5		25	24			1					30.4 x 71.2
238	281/2	50	25	24								30.4 x 70.4
	281/3		25	25								
237	173/2	50	25	24	1							34.6 x 75.7
	173/4		25	25								
234	187/4	50	25	25								
	187/5		25	24	2							37.4 x 99.6
240	256/3	50	25	25								
	256/4		25	23		1						33.7 x 75.1
236	178/3	50	25	25					1			36.2 x 102.3
	178/5		25	25								
239	221/2	50	25	25								
	221/4		25	25								
233	210/4	50	25	25								
	210/1		25	25								
232	219/2	50	25	25								
	219/1		25	24							1 Chromatid Fragment	20.0 x 117.5
231	198/4	50	25	25								
	198/2		25	24	1							41.5 x 114.6

APPENDIX TABLE CA-F24 (continued)

Methyl Bromide

Females

Single Dosing: Ethyl methanesulphonate, 250 mg/kg — Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
263	225/4	50	25	13	1							32.8 x 69.8
											1 Multiple Aberration	32.7 x 73.8
					1							32.5 x 77.0
											1 Multiple Aberration	32.4 x 77.1
					1	1						33.0 x 79.5
					1	1						33.1 x 79.7
					1	1			2			33.1 x 79.9
						3						32.7 x 81.0
					1	1						33.1 x 94.0
											1 Multiple Aberration	33.5 x 95.5
					1	1						35.7 x 93.7
						1						34.6 x 68.2
	225/5		25	12		1						35.7 x 71.1
											1 Multiple Aberration	35.5 x 71.2
						1						35.7 x 77.1
						2		1	1			35.8 x 80.4
						3						35.0 x 98.7
						1						35.1 x 102.6
						1						36.8 x 100.1
					1							36.7 x 99.2
					1							37.2 x 97.5
											1 Multiple Aberration	36.6 x 80.0
											1 Multiple Aberration	36.3 x 78.5
											1 Multiple Aberration	38.9 x 82.5
						2						38.7 x 99.0

APPENDIX TABLE CA-F24 (continued)

Methyl Bromide
Females

Single Dosing: Ethyl methanesulphonate, 250 mg/kg — Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
261	271/2	50	25	15		1						34.5 x 72.3
					1							34.6 x 74.9
					1	2						34.1 x 77.6
						1						33.9 x 80.0
					1							36.6 x 95.3
					1	1						36.6 x 72.5
					1	2						36.4 x 70.2
					2							38.6 x 70.6
											1 Multiple Aberration	40.7 x 96.7
											1 Multiple Aberration	40.6 x 95.3
	271/3		25	16		1						35.8 x 74.4
						1						36.1 x 95.5
						1						35.3 x 97.5
						2						35.5 x 98.8
											1 Multiple Aberration	37.7 x 101.7
						3						37.5 x 98.7
					1	3						37.7 x 95.1
						1						37.9 x 74.3
						5						38.0 x 71.4

APPENDIX TABLE CA-F24 (continued)

Methyl Bromide

Females

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
268	255/2	50	25	19		1						35.2 x 70.8
						2						35.0 x 99.2
					2							35.2 x 102.0
					1	1			2			37.2 x 88.0
											1 Multiple Aberration	37.3 x 87.5
					1	2			3			39.1 x 71.3
	255/3		25	14		3						33.2 x 72.6
						2						32.8 x 73.4
					1							33.0 x 93.6
					1	1						33.2 x 100.9
						1						34.9 x 95.2
					1							34.9 x 94.2
						1			1			34.7 x 79.0
											1 Multiple Aberration	34.7 x 78.3
					1	1						34.5 x 74.8
											1 Multiple Aberration	37.3 x 73.5
											1 Multiple Aberration	37.3 x 76.3

APPENDIX TABLE CA-F24 (continued)

Methyl Bromide

Females

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
269	309/1	50	25	18	1	2						37.0 x 78.7
						1						39.9 x 74.8
						1						40.1 x 74.0
									1			42.0 x 77.3
					1	2						42.3 x 99.3
						1						42.2 x 100.2
					2							42.2 x 107.2
	309/5		25	18		2						30.0 x 74.1
						1						32.5 x 70.5
											1 Multiple Aberration	34.7 x 76.7
						1						34.5 x 92.8
					1							36.7 x 87.2
						3			1			37.0 x 78.4
					1							37.6 x 67.3

APPENDIX TABLE CA-F24 (continued)

Methyl Bromide

Females

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
267	249/1	50	25	20		2						33.2 x 76.5
						2						33.4 x 91.4
						5						34.9 x 97.3
						2			1			35.0 x 96.7
						2						34.9 x 82.1
	249/3		25	18	1							32.7 x 103.4
						4			1			34.8 x 98.8
						1						34.9 x 78.9
						2						34.8 x 73.5
						1						35.7 x 77.8
						1						36.2 x 101.9
						1						38.3 x 104.1
262	229/1	50	25	17		1						33.3 x 81.1
					1		1					33.4 x 82.3
											1 Multiple Aberration	33.4 x 101.4
						4						33.1 x 102.1
						5						35.5 x 99.1
											1 Multiple Aberration	35.4 x 80.8
						1						36.2 x 72.3
					3	3						36.4 x 74.8

APPENDIX TABLE CA-F24 (continued)

Methyl Bromide

Females

Single Dosing: Ethyl methanesulphonate, 250 mg/kg — Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
262	229/4		25	16	1							31.4 x 71.4
											1 Multiple Aberration	31.4 x 76.9
						1						32.5 x 95.5
						1						33.7 x 93.6
											1 Multiple Aberration	33.1 x 81.8
						1						33.0 x 79.8
						2			1			33.2 x 74.4
					1	2						35.7 x 71.3
					1	1						34.8 x 92.7
266	196/3	50	25	19		2						11.6 x 115.5
					1							20.6 x 115.4
					1							22.7 x 115.5
						4						23.4 x 115.8
						5			1			28.8 x 115.7
						1						33.1 x 116.3
	196/1		25	18							1 Chromosomal Fragment	16.2 x 116.2
											1 Multiple Aberration	16.5 x 116.3
						1						17.3 x 116.4
											1 Chromosomal Fragment	20.1 x 116.2
						1	1					24.3 x 116.5
						2						36.5 x 116.4
					1							40.1 x 116.1

APPENDIX TABLE CA-F24 (continued)

Methyl Bromide
Females

Single Dosing: Ethyl methanesulphonate, 250 mg/kg — Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
270	251/3	50	25	17		7						14.3 x 114.8
					1							14.8 x 114.7
					2	6						16.5 x 115.1
					2							37.4 x 115.3
						1						39.6 x 116.0
						1						43.2 x 117.0
					1							40.4 x 114.3
											1 Multiple Aberration	16.9 x 114.9
	251/5		25	16	1	5						16.7 x 118.0
											1 Multiple Aberration	17.6 x 118.2
					1	1						20.6 x 118.3
											1 Multiple Aberration	25.1 x 117.8
											1 Multiple Aberration	46.5 x 118.1
					1							46.3 x 116.2
						1						45.3 x 116.3
											1 Multiple Aberration	43.6 x 116.4
					1	4						36.4 x 117.1

APPENDIX TABLE CA-F24 (continued)

Methyl Bromide
Females

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
265	184/1	50	25	16	1							8.0 x 116.7
					1							8.6 x 116.8
						1		1			2 Chromatid Fragments	8.8 x 116.8
					1							22.7 x 117.3
					1							28.0 x 117.3
					1	2					1 Chromatid Fragment	29.4 x 116.8
						7						30.3 x 117.1
					1							35.0 x 117.1
						1						30.0 x 114.7
											1 Multiple Aberration	27.4 x 114.1
	184/4		25	16	1	4						8.5 x 116.7
					1							12.3 x 117.1
					1	2						16.9 x 118.0
					1	2					1 Ring Chromosomal	19.2 x 117.8
					1	3						45.9 x 115.0
											1 Chromatid Fragment	43.4 x 115.2
					1	2						42.5 x 115.0
											1 Multiple Aberration	40.5 x 114.7
					1						1 Chromatid Fragment	39.2 x 114.5

APPENDIX TABLE CA-F24 (continued)

Methyl Bromide

Females

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
264	290/2	50	25	15	2	1	1					18.6 x 117.0
											1 Chromatid Fragment	19.1 x 117.3
					1	2						20.4 x 117.0
					1	3						20.9 x 117.3
					2	10						21.2 x 117.3
						2						22.0 x 117.6
					1	3						22.2 x 117.6
					1	1						24.0 x 114.0
											1 Multiple Aberration	24.0 x 114.7
						3						25.1 x 115.2
	290/1		25	11		5						13.5 x 115.9
											1 Multiple Aberration	14.1 x 115.7
					2							15.0 x 115.6
					2	5						15.6 x 116.0
					2							15.6 x 116.0
					1							16.4 x 116.3
					1	5		1				17.3 x 117.2
					1	3						17.5 x 117.6
						3						18.6 x 117.0
											1 Multiple Aberration	20.6 x 114.8
					1	1						20.6 x 115.2
											1 Multiple Aberration	21.8 x 115.2
					1							22.0 x 115.6
					1							23.4 x 116.4

APPENDIX TABLE CA-F48

Methyl Bromide
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Females

Single Dosing: Air Control (0 ppm) Sampling Time: 48 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
182	266/1-5	0	0	0								
186	166/1	50	25	25					1			36.4 x 103.0
	166/2		25	25								
183	167/1	50	25	25								
	167/3		25	25								
189	292/1	50	25	24	1							15.3 x 93.7
	292/2		25	25								
185	279/1	50	25	25								
	279/2		25	25								
187	180/2	50	25	25								
	180/3		25	24	1							13.1 x 68.2
190	189/4	50	25	25								
	189/1		25	25								
181	168/4	50	25	25								
	168/1		25	24	1							10.3 x 98.9
184	232/2	50	25	25								
	232/4		25	25								
188	265/2	50	25	25								
	265/3		25	23	1							11.7 x 106.1
					1							15.5 x 115.7

APPENDIX TABLE CA-F48 (continued)

Methyl Bromide

Females

Single Dosing: 20 ppm

Sampling Time: 48 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
217	272/1	50	25	25								
	272/3		25	25								
216	254/1	50	25	25								
	254/2		25	25								
220	291/1	50	25	25								
	291/2		25	25								
218	226/1	50	25	25								
	226/2		25	25								
214	177/2	50	25	25								
	177/4		25	25								
213	316/3	50	25	25								
	316/4		25	24	1							14.4 x 98.1
215	264/1	50	25	25								
	264/2		25	25								
212	277/1	50	25	23	2							12.8 x 102.4
					1							13.8 x 112.0
	277/5		25	24	1							13.4 x 101.6
219	183/4	50	25	22	1							5.5 x 114.6
					1							5.7 x 114.9
					1							5.8 x 114.9
	183/5		25	25								
211	190/2	50	25	25								
	190/3		25	25								

APPENDIX TABLE CA-F48 (continued)

Methyl Bromide

Females

Single Dosing: 70 ppm

Sampling Time: 48 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
242	302/1	50	25	25								
	302/2		25	25								
249	280/1	50	25	25								
	280/2		25	25								
241	250/2	50	25	24	1							34.9 x 100.7
	250/5		25	24		1						32.5 x 111.5
243	161/2	50	25	25								
	161/3		25	24		1						94.2 x 21.4
247	203/1	50	25	23			1					21.3 x 98.0
						1						18.5 x 97.6
	203/2		25	22	1							31.4 x 99.7
					1	1						34.1 x 99.9
					1							34.5 x 96.0
245	227/4	50	25	24	1							29.5 x 89.5
	227/5		25	24	1							19.0 x 99.0
244	273/3	50	25	24	1							27.4 x 94.1
	273/2		25	25								
248	267/3	50	25	23	1							13.6 x 63.5
					1							15.0 x 63.9
	267/2		25	25								
246	223/2	50	25	25								
	223/3		25	23	1							17.0 x 99.9
					2							12.3 x 100.1
250	318/1	50	25	25								
	318/2		25	25								

APPENDIX TABLE CA-F48 (continued)

Methyl Bromide

Females

Single Dosing: Ethyl methanesulphonate, 250 mg/kg — Sampling Time: 48 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
276	235/1	50	25	25								
	235/4		25	24	1							39.1 x 95.0
272	209/3	50	25	23	1							32.8 x 114.7
						1						29.8 x 113.8
	209/2		25	23		1						29.2 x 99.7
					1							32.8 x 95.1
280	188/5	50	25	24	1							39.4 x 99.9
	188/4		25	25								
273	276/3	50	25	25								
	276/2		25	25								
271	319/1	50	25	25								
	319/3		25	24		1						24.5 x 102.7
278	268/1	50	25	25								
	268/2		25	22		1						34.5 x 112.0
					1							32.9 x 111.6
					1							30.1 x 109.1
279	193/1	50	25	23		1						38.8 x 107.6
					1							34.1 x 105.5
	193/3		25	25								
275	214/2	50	25	23		1						42.5 x 106.6
						1						41.9 x 106.5
	214/3		25	24			1					44.0 x 105.3

APPENDIX TABLE CA-F48 (continued)

Methyl Bromide

Females

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sampling Time: 48 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
277	261/2	50	25	24	1							13.4 x 94.8
	261/4		25	24			1					16.9 x 97.4
274	259/3	50	25	25								
	259/4		25	24	1							18.9 x 108.1

APPENDIX TABLE DL

Methyl Bromide
Dominant Lethal Assessment

Multiple Dosing: Air Control (0 ppm)

Week No.	Male No.	361		362		363		364		365		366		367		368		369		370		Total
	Female	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
1	Corpora lutea	15	0	15	13	13	13	13	12	11	11	14	11	0	0	15	7	0	0	15	11	187
	Total Implants	15	0	15	0	8	11	13	12	12	12	14	11	0	0	18	10	0	0	4	11	172
	Live Implants	15	0	15	4	4	11	13	11	11	10	14	11	0	0	18	9	0	0	2	11	159
	Early Deaths	0	0	0	2	3	0	0	1	1	2	0	0	0	0	0	1	0	0	0	0	10
	Late Deaths	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	3
2	Corpora lutea	12	23	15	13	13	15	14	20	0	11	11	11	18	14	15	13	16	20	0	13	267
	Total Implants	13	14	15	14	13	15	12	14	0	11	12	13	16	12	14	13	17	15	0	13	246
	Live Implants	7	13	14	13	13	10	11	12	0	8	12	13	15	12	12	13	15	15	0	11	219
	Early Deaths	2	0	1	1	0	5	0	1	0	3	0	0	1	0	2	0	2	0	0	2	20
	Late Deaths	4	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	7
3	Corpora lutea	2	11	13	11	14	14	13	14	11	16	9	14	12	13	13	12	15	10	13	13	243
	Total Implants	0	16	14	13	14	11	13	15	11	16	11	17	12	13	13	11	13	10	15	13	251
	Live Implants	0	15	13	10	13	3	12	15	11	16	7	15	12	13	13	10	13	10	14	11	226
	Early Deaths	0	1	1	0	1	2	1	0	0	0	0	2	0	0	0	1	0	0	1	2	12
	Late Deaths	0	0	0	3	0	6	0	0	0	0	4	0	0	0	0	0	0	0	0	0	13
4	Corpora lutea	9	12	11	13	6	17	12	8	15	15	0	12	12	11	0	12	12	11	0	17	205
	Total Implants	12	14	12	12	5	19	12	14	16	18	0	12	12	12	0	12	14	11	0	17	224
	Live Implants	12	14	11	12	2	14	12	0	15	17	0	12	12	12	0	10	9	11	0	12	187
	Early Deaths	0	0	1	0	2	5	0	10	1	1	0	0	0	0	0	2	5	0	0	5	32
	Late Deaths	0	0	0	0	1	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	5
5	Corpora lutea	11	10	10	12	0	13	16	15	12	12	14	14	14	14	12	14	13	3	11	13	233
	Total Implants	12	11	11	12	0	13	10	15	12	12	16	13	13	14	12	14	14	3	13	12	232
	Live Implants	11	9	9	8	0	12	9	13	11	11	15	12	13	14	0	14	14	0	13	12	20
	Early Deaths	0	0	2	0	0	1	0	2	0	0	0	0	0	0	0	0	0	1	0	0	6
	Late Deaths	1	2	0	4	0	0	1	0	1	1	1	1	0	0	12	0	0	2	0	0	26

APPENDIX TABLE DL (continued)

Methyl Bromide

Multiple Dosing: Air Control (0 ppm)

Week No.	Male No.	361		362		363		364		365		366		367		368		369		370		Total
	Female	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
6	Corpora lutea	13	14	14	8	14	15	12	12	2	11	13	13	14	13	12	15	2	15	19	15	246
	Total Implants	14	12	13	7	15	15	13	12	0	11	10	12	15	11	13	15	0	12	21	14	235
	Live Implants	9	11	13	6	15	8	12	12	0	10	10	12	12	11	13	15	0	3	16	14	202
	Early Deaths	2	0	0	1	0	3	1	0	0	1	0	0	1	0	0	0	0	4	2	0	15
	Late Deaths	3	1	0	0	0	4	0	0	0	0	0	0	2	0	0	0	0	5	3	0	18
7	Corpora lutea	16	15	3	15	0	17	14	20	19	13	14	6	11	15	12	14	14	10	13	13	251
	Total Implants	14	6	0	15	0	12	13	15	14	12	13	8	13	12	12	16	15	10	15	15	230
	Live Implants	13	5	0	14	0	12	6	15	14	12	13	8	13	12	11	16	14	10	15	15	218
	Early Deaths	0	0	0	1	0	0	2	0	0	0	0	0	0	0	1	0	1	0	0	0	5
	Late Deaths	1	1	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	7
8	Corpora lutea	16	12	14	14	0	14	14	15	17	7	14	14	16	9	12	11	14	11	18	8	250
	Total Implants	14	14	12	11	0	14	12	10	16	11	14	14	17	0	14	11	10	12	17	2	225
	Live Implants	11	14	12	9	0	12	12	10	16	10	13	13	14	0	14	11	10	11	15	0	207
	Early Deaths	3	0	0	1	0	0	0	0	0	1	0	1	2	0	0	0	0	0	2	2	12
	Late Deaths	0	0	0	1	0	2	0	0	0	0	1	0	1	0	0	0	0	1	0	0	6
9	Corpora lutea	13	12	?	16	12	14	13	13	14	14	15	16	11	10	12	15	16	13	12	12	253
	Total Implants	9	11	5	16	13	14	12	12	14	13	16	17	11	11	16	15	14	14	13	11	257
	Live Implants	9	11	0	16	12	14	12	11	14	8	16	15	2	11	13	14	14	14	13	9	228
	Early Deaths	0	0	1	0	1	0	0	1	0	0	0	1	6	0	2	0	0	0	0	1	13
	Late Deaths	0	0	4	0	0	0	0	10	0	5	0	1	3	0	1	1	0	0	0	1	12
10	Corpora lutea	14	14	13	14	12	13	10	17	0	16	14	18	14	12	14	13	12	11	14	14	265
	Total Implants	13	13	12	14	13	13	16	12	0	17	10	13	13	12	14	12	12	12	13	13	247
	Live Implants	13	11	11	14	7	2	15	11	0	17	8	13	11	4	14	11	8	11	10	13	204
	Early Deaths	0	2	1	0	5	4	1	1	0	0	1	0	2	7	0	1	4	1	2	0	32
	Late Deaths	0	0	0	0	1	7	0	0	0	0	1	0	0	1	0	0	0	0	1	0	11

APPENDIX TABLE DL (continued)

Methyl Bromide

Multiple Dosing: 20 ppm

Week No.	Male No.	371		372		373		374		375		376		377		378		379		380		Total
	Female	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
1	Corpora lutea	14	15	12	9	14	16	13	0	12	0	0	0	0	0	13	16	14	6	0	11	159
	Total Implants	15	6	10	8	14	14	17	0	12	0	0	0	0	0	2	9	14	4	0	11	136
	Live Implants	14	5	8	3	14	14	14	0	9	0	0	0	0	0	0	7	14	4	0	11	117
	Early Deaths	1	1	2	5	0	0	3	0	3	0	0	0	0	0	1	2	0	0	0	0	18
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
2	Corpora lutea	12	12	11	13	13	15	15	13	15	14	0	14	-*	13	0	16	0	14	14	16	220
	Total Implants	8	13	12	13	13	15	12	14	2	14	0	11	-*	14	0	16	0	16	6	11	190*
	Live Implants	7	12	12	13	11	15	12	14	2	10	0	11	-*	13	0	16	0	16	6	11	181
	Early Deaths	1	1	0	0	2	0	0	0	0	1	0	0	-*	1	0	0	0	0	0	0	6
	Late Deaths	0	0	0	0	0	0	0	0	0	3	0	0	-*	0	0	0	0	0	0	0	3
3	Corpora lutea	14	5	15	13	16	12	11	7	10	13	13	16	15	15	0	12	0	12	16	0	215
	Total Implants	12	5	15	13	14	11	12	0	13	14	13	15	14	15	0	12	0	12	14	0	204
	Live Implants	12	5	15	13	14	1	11	0	13	14	12	15	14	15	0	11	0	11	14	0	190
	Early Deaths	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	2
	Late Deaths	0	0	0	0	0	10	1	0	0	0	0	0	0	0	0	1	0	0	0	0	12
4	Corpora lutea	15	17	18	15	13	12	12	12	5	12	13	12	13	14	12	13	11	15	13	13	260
	Total Implants	16	15	14	13	14	12	12	14	0	13	14	12	13	12	11	14	11	14	14	8	246
	Live Implants	14	13	13	13	13	11	12	14	0	-*	13	12	13	12	11	12	8	10	6	8	213
	Early Deaths	2	2	1	0	1	1	0	0	0	1	1	0	0	0	0	2	2	1	2	0	16
	Late Deaths	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	1	3	6	-*	17
5	Corpora lutea	12	13	13	13	12	14	9	12	13	10	14	15	11	13	0	11	15	11	11	13	235
	Total Implants	12	12	13	11	12	14	10	13	15	9	16	14	11	14	0	11	10	14	11	10	232
	Live Implants	11	12	12	11	12	14	10	12	14	7	15	13	11	13	0	11	8	13	11	9	219
	Early Deaths	0	0	1	0	0	0	0	1	1	2	1	1	0	1	0	0	2	1	0	0	11
	Late Deaths	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2

APPENDIX TABLE DL (continued)

Methyl Bromide

Multiple Dosing: 20 ppm

Week No.	Male No.	371		372		373		374		375		376		377		378		379		380		Total
	Female	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
6	Corpora lutea	13	16	14	11	15	9	12	11	14	16	10	11	13	14	16	13	15	0	12	11	246
	Total Implants	16	15	13	11	14	11	12	14	12	16	11	11	13	14	16	14	15	0	13	11	252
	Live Implants	13	12	12	10	13	7	8	13	12	15	10	8	11	10	16	3	13	0	13	8	207
	Early Deaths	1	3	1	1	1	3	1	0	0	1	1	3	1	4	0	5	2	0	0	3	31
	Late Deaths	2	0	0	0	0	1	3	1	0	0	0	0	1	0	0	6	0	0	0	0	14
7	Corpora lutea	12	11	10	11	13	14	11	13	3	10	12	11	*	12	12	11	0	10	8	13	197
	Total Implants	14	11	10	14	13	14	14	12	0	12	12	16	*	13	11	10	0	10	10	11	207
	Live Implants	13	10	10	14	11	13	13	11	0	9	12	13	*	11	11	10	0	10	9	11	191
	Early Deaths	1	1	0	0	0	0	1	1	0	2	0	3	*	2	0	0	0	0	0	0	11
	Late Deaths	0	0	0	0	2	1	0	0	0	1	0	0	*	0	0	0	0	0	1	0	5
8	Corpora lutea	17	19	12	14	14	12	0	16	13	4	10	14	11	15	12	12	10	15	13	14	247
	Total Implants	17	19	12	19	13	13	12	12	14	0	11	13	12	16	12	13	10	13	14	14	259
	Live Implants	16	19	12	14	13	12	0	0	14	0	11	12	12	14	12	12	0	12	13	14	212
	Early Deaths	0	0	0	3	0	0	10	0	0	0	0	0	0	0	0	0	1	1	1	0	16
	Late Deaths	1	0	0	2	0	1	2	12	0	0	0	1	0	2	0	1	9	0	0	0	31
9	Corpora lutea	12	15	11	10	13	11	*	0	14	10	10	13	11	11	19	13	10	10	13	14	220
	Total Implants	12	13	11	12	14	12	*	0	13	10	10	13	11	11	15	14	10	13	13	13	220
	Live Implants	11	12	11	11	13	0	*	0	12	9	7	11	11	6	15	0	9	11	13	13	175
	Early Deaths	1	0	0	1	1	9	*	0	0	1	0	1	0	0	0	0	1	1	0	0	16
	Late Deaths	1	0	0	0	0	3	*	0	1	0	3	1	0	5	0	14	0	1	0	0	29
10	Corpora lutea	13	11	15	14	13	12	11	15	13	0	11	13	9	12	11	13	16	12	14	16	244
	Total Implants	13	11	14	11	14	11	13	15	14	0	12	11	12	11	12	15	15	9	18	15	246
	Live Implants	13	0	13	0	13	11	12	14	14	0	12	2	6	11	2	12	15	9	18	13	190
	Early Deaths	0	1	0	9	1	0	1	1	0	0	0	6	1	0	0	3	0	0	0	2	25
	Late Deaths	0	10	1	2	0	0	0	0	0	0	0	3	5	0	10	0	0	0	0	0	31

* Missing value : inconsistent result record

APPENDIX TABLE DL (continued)

Methyl Bromide

Multiple Dosing: 70 ppm

Week No.	Male No. / Female	381		382		383		384		385		386		387		388		389		390		Total
		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
1	Corpora lutea	15	14	0	12	12	14	0	12	19	0	16	13	13	0	14	16	0	12	12	16	210
	Total Implants	13	12	0	13	11	8	0	8	9	0	15	15	13	0	14	12	0	14	11	22	190
	Live Implants	12	11	0	13	8	2	0	7	8	0	15	15	13	0	14	11	0	14	10	21	174
	Early Deaths	1	1	0	0	2	0	0	1	1	0	0	0	0	0	0	1	0	0	1	1	9
	Late Deaths	0	0	0	0	1	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7
2	Corpora lutea	20	19	0	17	13	14	14	14	17	14	10	0	15	13	14	14	18	12	12	13	263
	Total Implants	16	18	0	17	12	13	17	13	15	14	11	0	16	12	15	14	15	8	12	13	251
	Live Implants	11	17	0	14	12	12	14	13	9	14	11	0	15	12	14	13	14	8	12	13	228
	Early Deaths	5	1	0	3	0	1	3	0	5	0	0	0	1	0	1	1	1	0	0	0	22
	Late Deaths	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
3	Corpora lutea	12	2	12	12	0	17	13	15	6	12	11	11	15	12	10	13	14	0	12	15	214
	Total Implants	12	0	12	12	0	16	13	13	0	13	13	12	15	11	13	15	12	0	12	15	209
	Live Implants	12	0	11	12	0	0	13	13	0	13	12	12	14	8	12	15	12	0	11	14	172
	Early Deaths	0	0	1	0	0	2	0	0	0	0	1	0	1	3	1	0	0	0	1	1	23
	Late Deaths	0	0	0	0	0	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14
4	Corpora lutea	12	11	11	13	13	15	12	12	13	7	11	11	14	15	12	13	10	15	14	13	247
	Total Implants	12	12	11	13	13	14	13	13	12	6	12	13	9	15	12	13	10	13	6	13	235
	Live Implants	11	0	10	12	7	12	13	13	12	0	11	3	4	13	9	10	8	11*	5	13	177
	Early Deaths	1	8	1	1	2	0	0	0	0	4	0	2	4	1	0	3	0	1	0	0	28
	Late Deaths	0	4	0	0	4	2	0	0	0	2	1	8	1	1	3	0	2	1	1	0	30
5	Corpora lutea	4	14	13	9	13	12	10	12	0	16	14	11	12	13	8	15	0	12	15	15	220
	Total Implants	0	14	14	10	13	13	12	12	0	19	13	11	14	13	3	15	0	11	13	16	216
	Live Implants	0	13	13	10	12	9	12	9	0	18	13	11	14	11	3	15	0	11	13	14	201
	Early Deaths	0	1	1	0	1	1	0	0	0	1	0	0	0	1	0	0	0	0	0	2	8
	Late Deaths	0	0	0	0	0	3	0	3	0	0	0	0	0	1	0	0	0	0	0	0	7

APPENDIX TABLE DL (continued)

Methyl Bromide

Multiple Dosing: 70 ppm

Week No.	Male No.	381		382		383		384		385		386		387		388		389		390		Total
	Female	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
6	Corpora lutea	0	17	14	13	14	13	13	16	0	13	12	12	16	16	0	9	10	10	7	12	217
	Total Implants	0	16	13	13	15	13	12	11	0	12	11	14	12	14	0	12	10	12	0	13	203
	Live Implants	0	11	0	13	15	13	12	11	0	12	11	11	11	14	0	12	10	11	0	11	178
	Early Deaths	0	3	8	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	2	16
	Late Deaths	0	2	5	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	9
7	Corpora lutea	17	13	13	13	12	11	12	11	14	13	14	15	12	13	15	14	10	3	13	0	238
	Total Implants	17	14	14	13	12	12	12	11	15	16	15	6	12	13	10	14	11	3	13	0	233
	Live Implants	15	12	6	13	9	11	11	11	14	15	15	4	12	10	9	13	11	3	8	0	202
	Early Deaths	2	1	2	0	3	1	0	0	1	1	0	0	0	0	1	1	0	0	0	0	13
	Late Deaths	0	1	6	0	0	0	1	0	0	0	0	2	0	3	0	0	0	0	5	0	18
8	Corpora lutea	0	13	14	14	11	16	12	14	14	14	0	16	11	15	8	11	0	13	15	13	224
	Total Implants	0	14	15	16	11	15	13	11	14	13	0	16	12	14	8	13	0	13	15	9	222
	Live Implants	0	10	15	6	9	15	12	11	13	8	0	16	11	14	7	9	0	13	13	8	190
	Early Deaths	0	3	0	9	1	0	1	0	1	3	0	0	1	0	0	0	0	0	2	1	22
	Late Deaths	0	1	0	1	1	0	0	0	0	2	0	0	0	0	1	4	0	0	0	0	10
9	Corpora lutea	11	13	11	12	13	12	9	12	15	14	12	12	15	13	16	13	13	12	14	14	256
	Total Implants	12	0	12	13	13	12	10	11	13	4	12	12	13	13	17	13	13	12	14	12	231
	Live Implants	12	0	11	13	7	10	5	3	11	0	12	12	13	12	16	12	12	11	12	2	186
	Early Deaths	0	0	1	0	0	1	2	5	2	2	0	0	0	1	1	1	1	1	2	7	27
	Late Deaths	0	0	0	0	6	1	3	3	0	2	0	0	0	0	0	0	0	0	0	3	18
10	Corpora lutea	11	8	16	11	13	15	15	8	0	13	17	11	12	12	12	11	13	12	17	14	241
	Total Implants	11	7	14	11	15	15	15	0	0	14	12	3	12	14	12	10	13	12	14	13	217
	Live Implants	9	7	14	11	12	15	14	0	0	14	10	3	12	14	12	10	13	5	14	11	200
	Early Deaths	2	0	0	0	3	0	1	0	0	0	2	0	0	0	0	0	0	7	0	2	17
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

APPENDIX TABLE DL (continued)

Methyl Bromide

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Week No.	Male No.	391		392		393		394		395		396		397		398		399		400		Total
	Female	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
1	Corpora lutea	2	8	13	0	2	15	0	4	10	2	5	2	11	0	2	15	1	8	12	7	119
	Total Implants	3	5	8	0	0	15	0	5	4	1	0	1	13	0	0	15	1	13	8	0	92
	Live Implants	3	1	8	0	0	8	0	2	0	0	0	1	9	0	0	5	1	6	8	0	52
	Early Deaths	0	4	0	0	0	7	0	3	4	1	0	0	4	0	0	8	0	7	0	0	38
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	2
2	Corpora lutea	3	5	2	0	19	13	1	0	0	13	10	9	4	7	9	12	0	0	11	17	135
	Total Implants	1	0	0	0	0	1	0	0	0	13	0	0	3	0	0	4	0	0	0	0	22
	Live Implants	1	0	0	0	0	1	0	0	0	0	0	0	3	0	0	4	0	0	0	0	9
	Early Deaths	0	0	0	0	0	0	0	0	0	13	0	0	0	0	0	0	0	0	0	0	13
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	Corpora lutea	0	2	0	0	1	0	4	10	2	10	3	9	0	4	0	0	2	0	5	0	52
	Total Implants	0	0	0	0	1	0	0	2	0	1	3	0	0	2	0	0	0	0	0	0	9
	Live Implants	0	0	0	0	1	0	0	2	0	1	3	0	0	2	0	0	0	0	0	0	9
	Early Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	Corpora lutea	11	10	2	14	0	12	17	12	7	11	15	12	13	5	0	10	9	11	9	15	195
	Total Implants	12	5	0	13	0	7	17	12	7	9	12	10	14	5	0	11	11	9	9	14	177
	Live Implants	8	1	0	7	0	0	8	8	2	6	9	5	11	5	0	9	9	2	3	1	94
	Early Deaths	2	1	0	3	0	3	4	2	3	0	3	2	1	0	0	0	0	3	1	1	29
	Late Deaths	2	3	0	3	0	4	5	2	2	3	0	3	2	0	0	2	2	4	5	12	54
5	Corpora lutea	0	15	15	9	11	12	12	14	5	12	10	15	12	12	13	12	13	10	15	9	226
	Total Implants	0	8	14	9	11	12	12	13	*	11	3	15	15	12	10	12	12	11	16	13	209
	Live Implants	0	6	12	3	9	11	11	12		7	3	14	13	12	9	0	11	10	13	10	166
	Early Deaths	0	2	1	1	1	1	1	1		0	0	1	1	0	1	0	1	0	3	2	17
	Late Deaths	0	0	1	5	1	0	0	0		4	0	0	1	0	0	12	0	1	0	1	26

* Unscorable

APPENDIX TABLE DL (continued)

Methyl Bromide

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Week No.	Male No.	391		392		393		394		395		396		397		398		399		400		Total
	Female	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
6	Corpora lutea	12	11	12	15	15	13	0	13	13	9	13	11	2	13	11	11	15	14	11	13	227
	Total Implants	12	12	9	15	14	13	0	13	12	12	15	11	0	13	11	11	14	15	9	13	224
	Live Implants	11	11	7	12	13	10	0	13	12	12	15	11	0	12	11	10	12	14	8	12	206
	Early Deaths	0	1	2	3	1	2	0	0	0	0	0	0	0	1	0	0	2	1	1	1	15
	Late Deaths	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	3
7	Corpora lutea	13	14	13	11	9	14	12	10	14	9	12	14	14	14	16	12	23	15	11	16	266
	Total Implants	13	14	14	11	9	14	12	10	15	6	11	14	16	14	15	4	15	16	12	13	248
	Live Implants	12	11	13	8	8	14	10	10	14	5	11	14	13	10	15	3	12	14	5	13	215
	Early Deaths	1	1	1	1	1	0	2	0	1	1	0	0	2	2	0	1	3	1	0	0	18
	Late Deaths	0	2	0	2	0	0	0	0	0	0	0	0	1	2	0	0	0	1	7	0	15
8	Corpora lutea	14	13	12	14	15	14	15	10	15	15	14	12	12	12	0	15	13	11	13	15	254
	Total Implants	14	13	11	12	15	15	15	10	13	17	12	12	12	13	0	15	13	11	15	16	254
	Live Implants	12	7	11	12	14	14	8	0	11	17	12	11	12	13	0	11	13	10	14	9	211
	Early Deaths	2	5	0	0	1	1	0	4	0	0	0	1	0	0	0	2	0	1	1	4	22
	Late Deaths	0	1	0	0	0	0	7	6	2	0	0	0	0	0	0	2	0	0	0	3	21
9	Corpora lutea	11	12	13	10	12	18	7	12	14	12	12	15	16	10	10	12	9	7	11	12	235
	Total Implants	12	14	13	11	13	13	7	13	15	9	15	16	8	13	12	13	11	2	11	12	233
	Live Implants	12	14	12	10	13	7	5	8	15	4	13	13	4	9	12	13	4	0	11	12	191
	Early Deaths	0	0	0	1	0	6	2	3	0	4	2	3	4	3	0	0	7	2	0	0	37
	Late Deaths	0	0	1	0	0	0	0	2	0	1	0	0	0	1	0	0	0	0	0	0	5
10	Corpora lutea	14	16	16	13	14	13	0	12	12	15	11	13	11	13	2	12	15	13	16	14	243
	Total Implants	14	16	15	4	13	13	0	12	12	15	12	13	5	13	1	12	13	12	16	16	227
	Live Implants	13	16	15	0	13	10	0	10	12	11	12	6	4	13	1	12	13	11	15	13	200
	Early Deaths	1	0	0	2	0	3	0	2	0	4	0	7	0	0	0	0	0	1	1	3	24
	Late Deaths	0	0	0	2	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	3

APPENDIX TABLE SA

Methyl Bromide

Sperm Abnormality Assessment

Multiple Dosing: Air Control (0 ppm)
Low, 20 ppm
High, 70 ppm
Positive, Ethyl methanesulphonate, 200 mg/kg

Slide No.	Normal	Abnormality					Total Abnormal	Total Examined	De-coded Information	
		A	B	C	D	E			Animal No.	Group
332	970	2	2	15	5	6	30	1000	321	Air
337	975	0	2	8	5	10	25	1000	322	Air
325	947	6	5	20	3	19	53	1000	323	Air
329	973	1	2	10	6	8	27	1000	324	Air
326	965	2	1	14	6	12	35	1000	325	Air
331	979	2	2	12	2	3	21	1000	326	Air
341	971	0	2	11	5	11	29	1000	327	Air
323								0**	328	Air
333	961	0	7	12	12	8	39	1000	329	Air
340	972	1	1	13	3	10	28	1000	330	Air
334								0**	331	Low
336	975	5	3	8	6	3	25	1000	332	Low
359	977	0	1	14	3	5	23	1000	333	Low
354	953	7	3	13	7	13	43	1000	334	Low
344	978	1	1	10	3	7	22	1000	335	Low
335	978	2	3	12	2	3	22	1000	336	Low
352	970	3	3	11	3	10	30	1000	337	Low
348	964	3	1	15	6	11	36	1000	338	Low
351	970	1	5	14	4	6	30	1000	339	Low
343	967	2	1	10	8	12	33	1000	340	Low

**No sperm observed on slides

APPENDIX TABLE SA (continued)

Methyl Bromide

Multiple Dosing: Air Control (0 ppm)
Low, 20 ppm
High, 70 ppm
Positive, Ethyl methanesulphonate, 200 mg/kg

Slide No.	Normal	Abnormality					Total Abnormal	Total Examined	De-coded Information	
		A	B	C	D	E			Animal No.	Group
324								0**	341	High
357	979	1	3	5	1	11	21	1000	342	High
356	970	2	5	10	5	8	30	1000	343	High
353	974	1	2	13	2	8	26	1000	344	High
346	984	2	4	6	0	4	16	1000	345	High
358	990	0	2	5	3	1	11	1000	346	High
355	973	2	2	10	8	5	27	1000	347	High
339	975	2	2	13	1	7	25	1000	348	High
360	969	1	2	10	5	14	32	1000	349	High
								*	350	High
1	939	2	1	29	11	18	61	1000	1	+
2	915	1	2	37	21	24	85	1000	2	+
3	921	1	2	43	18	15	79	1000	3	+
4	933	4	2	32	10	19	67	1000	4	+
5	933	2	3	38	10	14	67	1000	5	+
6	799	14	8	120	28	31	201	1000	6	+
7	923	4	4	41	19	9	77	1000	7	+
8	852	9	3	88	27	21	148	1000	8	+
9	935	5	1	38	7	14	65	1000	9	+
10	914	6	4	43	15	18	86	1000	10	+

*Animal dead

**No sperm observed on slides