Report Number 22

TIER II MUTAGENIC SCREENING OF 13 NIOSH PRIORITY COMPOUNDS

INDIVIDUAL COMPOUND REPORT 2-METHOXYETHANOL

Douglas B. McGregor

November 1, 1980

Supported by

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

Contract No. 210-78-0026

Inveresk Research International Limited
Musselburgh EH21 7UB, Scotland

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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."

D.B. McGregor, B.Sc., Ph.D.
Principal Investigator

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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CONTENTS (continued)

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

"The opinions, findings and conclusions expressed herein are not necessarily those of the National Institute for Occupational Safety and Health, nor does mention of company names or products constitute endorsement by the National Institute for Occupational Safety and Health." NIOSH Project Officer: Richard W. Niemeier.

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SUMMARY

2-Methoxyethanol (ethylene glycol monoethyl ether) 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 9.66 mg/ml of culture medium.

2. Dominant lethal test in male rats with exposure to atmospheres containing 25 ppm or 500 ppm 2-methoxyethanol for 7 h per day for 5 consecutive days. Analysis of test atmospheres was by continuous infrared absorption monitoring at a wavelength of 8.8 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 500 ppm for 0.25 h or 25 ppm for 1 h.

   Additional studies (not scheduled as part of this programme) were undertaken. These were SLRL studies in Drosophila with exposure by inhalation of static atmospheres and Ames test.

The results obtained were as follows:

1. There was no increase in UDS in cells treated with 2-methoxyethanol.

2. Male rat fertility was drastically reduced following exposure to 500 ppm 2-methoxyethanol in the dominant lethal test. Pregnancy frequency and implantations per pregnancy were particularly reduced in assessment Weeks 3-8, but these characters returned to normal values by Week 10. There was no convincing evidence that early death frequency had been increased by the treatment of the male rats.

3. Abnormal sperm frequency was slightly increased ($P<0.05$), most of the effect being due to rises in sperm with banana-shaped heads and amorphous heads ($P<0.01$).

4. The frequencies of chromosomal aberrations were not increased significantly or in a dose related manner in the rat bone marrow cells.

5. SLRL frequency was increased to small and variable extents in Drosophila. Experiments were not reliably reproducible, but the weight of evidence strongly suggested that a genetic effect was induced in the flies.

It was concluded that 2-methoxyethanol had no indisputable mutagenic effect, but there was good evidence for adverse effects upon certain stages of spermatogenesis. Consequently, careful re-evaluation of the toxicology of the compound is advisable.

INTRODUCTION

Properties

2-Methoxyethanol (ethylene glycol monomethyl ether) (CAS No. 109-86-4) has multiple usage in industry and occurs widely in the non-industrial environment. Synonyms for the compound are methyl cellosolve and Dowanol EM.

It is a volatile, colourless liquid with a mild ethereal odour and a bitter taste. A summary of its physical and chemical properties follows.

Formula	$HOCH_2CH_2OCH_3$
Mol. wt.	76.09
Sp. gr. ($20^{\circ}/4^{\circ}C$)	0.966
($25^{\circ}/25^{\circ}C$)	0.963
B.P. (760 mm Hg)	124.2
Vapour pressure, mm Hg ($25^{\circ}C$)	9.7
Refractive index ($25^{\circ}C$)	1.400
Flash point, $^{\circ}F$ (open cup)	120
Vapour density (air = 1)	2.6
Percent in satd. air ($25^{\circ}C$)	1.28

It is miscible with water, alcohol, ether, glycerol, acetone, dimethylformamide.

Synthesis can be from ethylene oxide and methanol or from ethylene glycol and diazomethane.

Human Exposure

2-Methoxyethanol is used in laboratories and in industrial plants which, in the U.S., are estimated to number over 10,000. In a survey conducted in 1972-74, but referring to 1970, an estimated 97,000 people were exposed to the chemical. People exposed more commonly work in the following industries:

Industry	Estimated Plants	Estimated People	Estimated Exposures
Furniture and Fixtures	177	2,152	26,863
Paper and Allied Products	244	7,179	7,345
Printing and Publishing	2,468	12,404	14,214

Industry	Estimated Plants	Estimated People	Estimated Exposures
Chemicals and Allied Products	879	22,507	23,745
Fabricated Metal Products	236	7,814	14,453
Real Estate	277	7,762	7,762

The occupations which lead people more commonly into contact with 2-methoxyethanol are:

Occupation	Estimated Plants	Estimated People	Estimated Exposures
Chemists	661	2,254	2,314
Chemical Technicians	450	2,167	3,010
Draftsmen	66	2,110	2,110
Managers and Superintendents	277	6,376	6,376
Bookbinders	1,046	3,989	3,989

The diverse uses of this chemical include (1) anti-icing additive in military jet aircraft, (2) paint stripping (along with dichloromethane, a known mutagen to bacteria even in the absence of stabilisers), (3) solvent for varnishes, wood stains and enamels, (4) solvent for low-viscosity cellulose acetate, natural resins, some synthetic resins and some alcohol-soluble dyes (e.g. inks). It can also be found in nail varnishes, nail varnish removers and, previously, in certain acne preparations. However, in 1979 the FDA recalled benzoyl peroxide-containing acne preparations in which 2-methoxyethanol was used as a solvent.

Toxicology

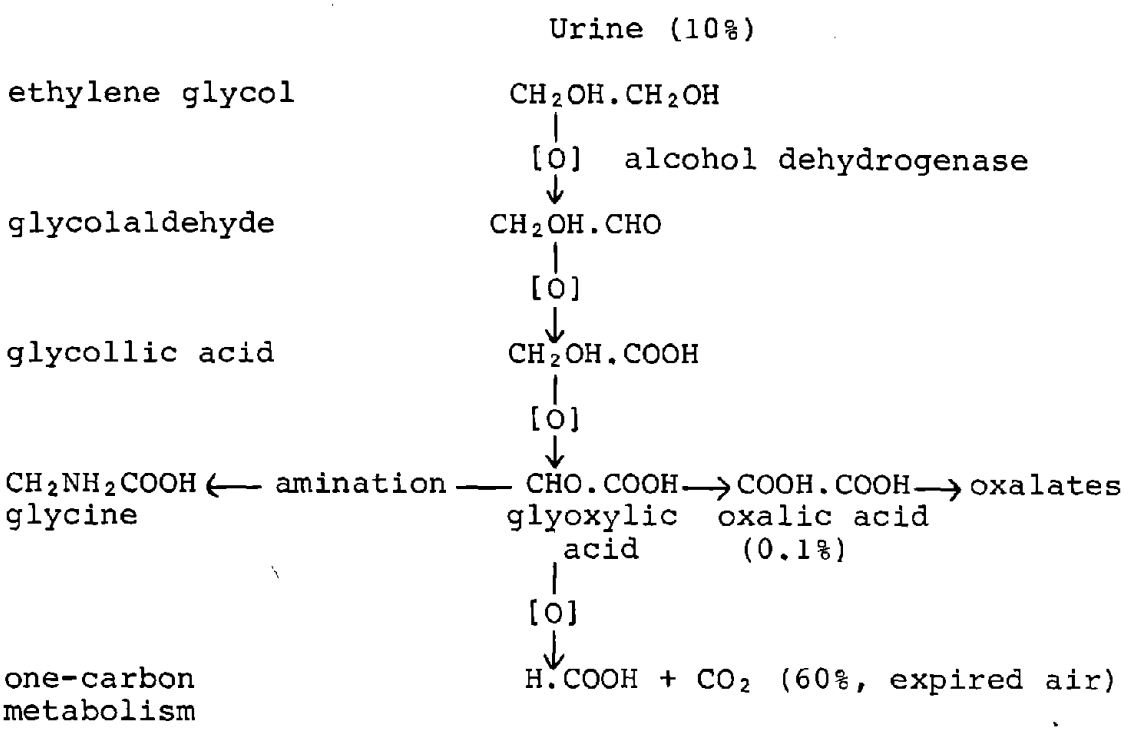
2-Methoxyethanol has low single-dose toxicity, the data from rats giving an i.p. LD_{50} (rat) of 2.46 g/kg and an i.v. LD_{50} (rat) of 2.14 g/kg. The inhalation LC_{50} (mouse) is 1,460 ppm. Comparative studies by Carpenter et al (1956) give single dose oral LD_{50} as 3.4 g/kg in rats, 0.95 g/kg in guinea pigs and 0.89 g/kg in rabbits.

With high dosage levels narcosis was observed, but at lower dosage levels deaths were delayed and accompanied by lung oedema, slight liver injury and marked kidney injury.

Studies with dogs and rabbits (Wiley et al, 1938) in which 2-methoxyethanol was given by injection revealed anuria, calcified casts in the urine, irritation of the bladder mucosa, oedema and haemorrhage in the lungs, hyperaemia of the liver and spleen, tubular degeneration of the cortico-medullary zone of the kidneys and testicular damage manifest as desquamation of the spermatic epithelium and the formation of variable numbers of spermatid giant cells. (The observation of testicular injury is particularly relevant to the interpretation of the results obtained in the genetic tests to be described in this report.) There was no increase in urinary oxalic acid in either dogs or rabbits and there was no increase in methanol or formic acid in rabbit urine. The absence of these substances suggests that 2-methoxyethanol is not metabolised, by demethylation, to ethylene glycol since, under similar conditions, ethylene glycol increased urinary oxalic acid 3-fold.

Exposure of rats to atmospheric 2-methoxyethanol at concentrations of 800 or 1,600 ppm induced systemic intoxication, with the most serious effects being upon the kidneys (Gross et al, 1938).

There seems to have been a single, fatal human exposure (Young and Woolner, 1946) in which approximately 200 ml mixed with rum was drunk. At autopsy there was haemorrhagic gastritis, marked degeneration of the renal tubules and fatty degeneration of the liver. Urine from this man contained no methanol, thereby supporting the results of the dog and rabbit studies (Wiley et al, 1938) in which it appeared that 2-methoxyethanol was not hydrolysed. Nitter-Hauge (1970), however, suggested that in man there is a common metabolic pathway resulting in excessive oxalate production from ethylene glycol and 2-methoxyethanol. Ethylene glycol biotransformation in rabbits is probably as follows (Gessner et al, 1961).



In cases of less severe exposure, by inhalation or by skin contact, CNS and haematological effects are most obvious. Macrocytic anaemia and an abnormal leucocyte profile are common findings in man, along with a high frequency of mental retardation, neurological symptoms, drowsiness and fatigue (Parsons and Parsons, 1938; Greenburg et al, 1938).

No reproduction studies appear to have been published, although the homologue 2-ethoxyethanol has been used in teratogenicity tests with mice, rats and rabbits (Stenger et al, 1971). Some foetal skeletal defects were found in rats given more than 100 µl/kg/day, but no other defects were described in the 3 species. The types of skeletal defects were not described.

Recent studies of 2-methoxyethanol and ethylene glycol (among other compounds) for their effects mainly upon mouse testes support the earlier work with dogs and rabbits described above (Nagano et al, 1979) and are of particular interest in the context of this test programme. Ethylene glycol, at oral doses up to 4,000 mg/kg for 5 weeks, had no significant effect upon testicular weight whereas 2-methoxyethanol at only 250 mg/kg did reduce significantly testicular weight.

Mean ± S.D. of Absolute and Relative Organ Weights of JCL-ICR Mice Treated with Ethylene Glycol or 2-Methoxyethanol for 5 Weeks

Compound	Daily Dose (mg/kg)	n	Testes		Vesicular and Coagulating Glands	
			Absolute (mg)	Relative (%)	Absolute (mg)	Relative (%)
Control		20	291 ± 25	0.76 ± 0.08	376 ± 59	0.99 ± 0.16
2-Methoxyethanol	2,000	1	54	0.15	258	0.71
	1,000	5	74 ± 8	0.10 ± 0.02	287 ± 25	0.75 ± 0.08
	500	5	82 ± 13	0.22 ± 0.03	323 ± 66	0.86 ± 0.17
	250	5	162 ± 37	0.42 ± 0.10	361 ± 26	0.94 ± 0.09
	125	5	300 ± 28	0.79 ± 0.10	373 ± 73	0.98 ± 0.06
	62.5	5	263 ± 57	0.68 ± 0.14	354 ± 73	0.95 ± 0.12
Ethylene glycol	4,000	5	274 ± 9	0.70 ± 0.03	392 ± 27	1.04 ± 0.05
	2,000	5	262 ± 36	0.69 ± 0.09	414 ± 48	1.08 ± 0.15
	1,000	5	285 ± 13	0.75 ± 0.02	346 ± 59	0.91 ± 0.14
	500	5	298 ± 18	0.78 ± 0.04	368 ± 35	0.97 ± 0.12

In the 250 mg/kg 2-methoxyethanol group, spermatozoa and spermatids were seen in small numbers only in some of the tubules and there was a clear reduction in spermatocytes. The degenerative changes seen were even more obvious in higher dose groups so that, in the 2,000 mg/kg group, only Sertoli's cells were observed in the tubules, no germ cells whatsoever being present.

At 1,000 mg/kg, 2-methoxyethanol induced significant reductions in the combined weights of the vesicular and coagulating glands.

Such testicular atrophy is rarely noticed in mice dosed with ethylene glycol.

The work which is to be described in this report involves the testing of 2-methoxyethanol for mutagenic potential in the following assays.

1. Unscheduled DNA synthesis (UDS) assay in human diploid fibroblasts with exposures up to 3 h duration and concentrations up to 9.664 mg/ml of culture medium.

2. Dominant lethal test in male rats with exposure to atmospheres containing 25 ppm or 500 ppm 2-methoxyethanol for 7 h per day for 5 consecutive days.

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 or 48 h.

5. Sex-linked recessive lethal (SLRL) test in Drosophila melanogaster with exposure to atmospheres of 500 ppm for 0.25 h or 25 ppm for 1 h.

   Additional studies (not part of this contract) have been undertaken. These are SLRL studies in Drosophila with exposure by inhalation of static atmospheres and Ames test.

REPETITION OF EXPOSURES

The initial exposure of rats was not judged to be satisfactory, by the Principal Investigator, therefore, repetition was necessary using fresh batches of animals. Dates of exposure were as follows:

Date	Test	Comment
4-8 December 1978	Dominant lethal	Stopped after 5 assessment weeks.
	Sperm abnormality	Slides prepared, not read.
	Multi-dose cytogenetics	Slides prepared, not read.
	Single dose cytogenetics	Slides prepared, not read.
17 January 1979	Single dose cytogenetics	Not read, slide quality substandard.
22-26 January 1979	Dominant lethal	Completed.
	Sperm abnormality	Completed.
	Multi-dose cytogenetics	Completed.
12 March 1979	Single dose cytogenetics	Completed.

Data evaluated from the 4-8 December 1978 exposures are restricted to assessment Weeks 1-5 of the dominant lethal test. These will be described since they do provide corroborative evidence for the test assessments following the 22-26 January 1979 exposures. Other animal data from the 4-8 December 1978 exposure will not be described.

MATERIALS AND METHODS

CHEMICALS

Test Substance

Three 2 kg cans of 2-methoxyethanol, Batch No. 10632, were received from Aldrich Chemical Company Limited on 20 November 1978. The test material was a clear, colourless liquid and was retained in the dark under ambient conditions in the company dispensary until used. A sample has been retained for analysis, should this be necessary.

Positive Control Substances

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 (UK) Limited, Manston, Kent.

$B6C3F_1$ hybrid mice were obtained from Charles River (USA).

These animals were obtained on the following dates.

Species	Date of Receipt	Age (Weeks)	Quarantine (Days)	Number (Sex)	Dates of Exposure	Comment
Rat	24 November 1978	11-12	10	220♂ 176♀	4-8 December 1978	D.L. continued 5 weeks. Rest abandoned.
Mouse	29 November 1978	11-13	5	44♂	4-8 December 1878	Abandoned.
Rat	7 December etc.	8-10	none	80♀ x10	none	D.L. matings.
Rat	10 January 1979	9-10	7 or 12	220♂ 176♀	17 January and 22-26 January 1979	Single dose cytogenetics slides
Mouse	17 January 1979	10-12	5	44♂	22-26 January 1979	not suitable.
Rat	26 January etc.	8-10	none	80♀ x10	none	D.L. matings.
Rat	6 March 1979	10	6	130♂ 130♀	12 March 1979	Single dose cytogenetics only.

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 16°C and 24°C, and relative humidity ca 50%, with extreme limits of 28% and 54% in the completed portions of the experiment.

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.

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 2-methoxyethanol 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 6 air changes per hour. 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 6 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 a 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 about 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 2-methoxyethanol. 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.

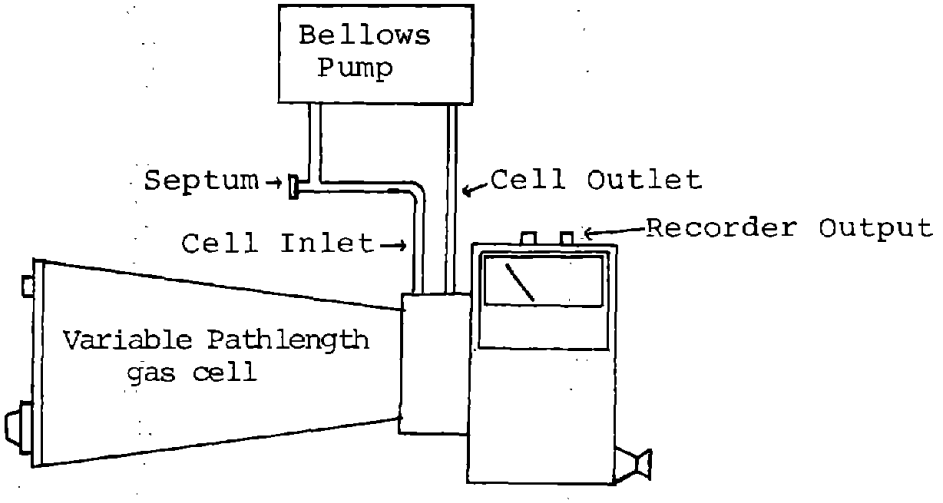
Calibration and Analytical Development

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 2-methoxyethanol 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 2-methoxyethanol were calibrated each day before vapour generation commenced.

The calibration was performed using a closed loop calibration system (see diagram below). Known volumes of 2-methoxyethanol were sequentially injected into the gas analyser via the closed loop calibration system through a rubber septum using a Hamilton glass micro syringe. After each injection the absorbence reading was allowed to stabilise as indicated on the chart recording.



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:	8.8 μm	8.8 μm
Pathlength:	8.25 m	0.75 m
Absorbence Range:	0.25 A	0.25 A
Slit Width:	0.5 mm	0.5 mm
Meter Response:	1	1
Recorder Voltage:	1 V	1 V
Chart Speed:	10 mm/h	10 mm/h

Calibration Data

$$C\ (ppm) = \frac{\rho V}{M} \times \frac{(RT)}{(P)} \frac{10^3}{5.64}$$

Where:

C = Concentration (ppm)
V = Sample volume in microlitres
ρ = Liquid density (g/cm^3)
M = Molecular weight of test sample
$\frac{(RT)}{(P)}$ = Molar volume of gas (24.06 at 20°C)
5.64 = Volume of Miran sample chamber in litres

Example of the Calculation for V

Compound: 2-methoxyethanol

C = 25 ppm
ρ = 0.965 g/cm^3
M = 76.1

$$V = \frac{C \times M}{\rho} \times \frac{5.64}{10^3 \times 24.06\ \mu l}$$

$$= \frac{25 \times 76.1}{0.965} \times \frac{5.64}{10^3 \times 24.06\ \mu l}$$

$$= 0.46\ \mu l$$

Therefore, to construct a calibration curve to cover the 25 ppm range, 0.2 μl samples of 2-methoxyethanol were injected into the analyser.

Atmosphere Generation

Schematic diagrams showing the vapour generating apparatus, exposure chambers and monitoring equipment are presented in Figures 1a, 1b. The test atmospheres were produced by bubbling dry, oxygen-free nitrogen (BOC Ltd.) through a liquid reservoir of 2-methoxyethanol contained in a glass gas washing, or Drechsel bottle immersed in a temperature controlled water bath at 37°C. The nitrogen/2-methoxyethanol vapour mixture so generated was ducted through a vertical glass column approximately 1.5 m long to a glass mixing vessel and diluted with filtered, compressed air. The resulting mixture of 2-methoxyethanol/air was ducted through a 5/16" ID nylon tube to the apex of the exposure chamber.

The atmospheres within 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 nitrogen 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 2-methoxyethanol concentration.

Measurement of Chamber Concentrations

Atmospheric concentrations of 2-methoxyethanol 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. The sampling flow rate was approximately 4 l/min.

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

Test Compound Utilisation

At the beginning of each exposure day, the 2-methoxyethanol reservoir (Drechsel bottle) was replenished with test compound. Utilisation of test material was calculated on a daily basis by weighing the Drechsel bottle before vapour generation began and deducting the weight of the Drechsel bottle and remaining test compound on completion of the exposure period.

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 2-methoxyethanol 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 cytogenetics 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	100 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 substances were 4-nitroquinoline-N-oxide, obtained from ICN K & K Laboratories, New York, U.S.A. and 2-aminoanthracene obtained from Aldrich Chemical Company, Gillingham, U.K.

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 Solutions

The test compound and positive controls were dissolved in dimethylsulphoxide ("AnalaR" grade from B.D.H. Limited, Poole, Dorset, U.K.).

Cells

Unscheduled DNA synthesis, following treatment with test compound, was measured in human embryonic intestinal cells (Flow 11,000 or Flow 2,002), passage 12-35 obtained from Flow Laboratories, Irvine, Scotland. These cell lines were chosen because of their higher permeability to some substrates than certain other human cell lines tested. Flow 2,002 line was used in Method 2 because the growth characteristics of Flow 11,000 had deteriorated to such an extent that they are no longer usable for these studies.

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 gentamicin (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

Preliminary Toxicity Test

This was done to establish the range of concentrations of test compound to be used in the DNA repair assay.

The cells were harvested and suspended in growth medium as for sub-culture, sedimented by centrifugation at 200 g for 5 min and resuspended in fresh culture medium at a density of 5×10^4 cells/ml. 1 ml samples of the suspension were pipetted into the wells of Linbro Multi-well plates (Flow Laboratories) which were incubated in a humid atmosphere of 5% CO_2 in air at 37°C for 72 h. The medium from each of the wells was then replaced with 1 ml of arginine-free-DMEM supplemented with 5% (v/v) heat inactivated foetal bovine serum and the plate incubated for a further 48 h.

The compound was dissolved in dimethylsulphoxide, at concentrations of 0.01, 0.1, 1.0, 10.0 and 100 mg/ml and 10 µl samples were added to duplicate cell suspensions to give final concentrations of 0.1, 1.0, 10, 100 and 1000 µg/ml. To each control culture were added 10 µl of dimethyl-sulphoxide.

After incubation for 3 h at 37°C in a humid atmosphere of 5% CO_2 in air the cultures were fixed with methanol, stained with Giemsa and examined for evidence of cellular damage. The grading was used as follows:

0 = no cells showing damage.
1 = under 25% of cells showing damage.
2 = 25-50% of cells showing damage.
3 = 50-75% of cells showing damage.
4 = 75-100% of cells showing damage.

In fact no toxicity was observed even at a concentration of 9.664 mg/ml which was selected as the highest in a series of 8 concentrations of 2-methoxyethanol in the repair assay.

DNA Repair Assay (Method 1)

The cells were harvested, sedimented, suspended in fresh culture medium at a density of 5×10^4 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. 2-Methoxyethanol was added directly to the cultures or diluted with dimethylsulphoxide to give final concentrations of 76, 151, 302, 604, 1,208, 2,416, 4,832, 9,664 µg/ml. Cultures with and without S-9 mix, received 10 µl samples of test compound solution. 10 µl samples of dimethylsulphoxide were added to negative control cultures.

The positive control compounds were 4-nitroquinoline-N-oxide (4-NQO) for S-9 free cultures and 2-aminoanthracene (2-AAN) for S-9 supplemented cultures. These were dissolved in dimethylsulphoxide in concentrations giving, on dilution 1:100 in the culture medium, the following levels:

4-NQO	8 and 10 µg/ml
2-AAN	6.26 µg/ml

After incubation for 3 h at 37°C in an atmosphere of 5% CO_2 in air 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.

DNA Repair Assay (Method 2)

Flow 2,002 cells were harvested, sedimented and suspended in fresh culture medium at a density of 5×10^4 cells/ml. 5 ml samples were dispensed into 60 mm tissue culture Petri dishes (Nunclon Delta) which were incubated in a humid atmosphere of 5% CO_2 in air at 37°C for 72 h. The medium from each of the Petri dishes was then replaced with 5 ml of arginine-deficient medium supplemented with 5% heat inactivated foetal bovine serum and the dishes incubated for 24 h. The medium was then replaced with a further 5 ml of arginine-deficient DMEM and the incubation continued for a further 48 h. The dishes were then randomly divided into 2 groups and 500 µl of S-9 mix were added to one group. Solutions of

hydroxyurea and [^{3}H]-deoxyguanosine (26 Ci/mmol) were added to each dish giving final concentrations 2.5 mM and 10 µCi/ml respectively. 50 µl of undiluted 2-methoxyethanol was added to the cultures, both in the presence and absence of S-9 mix, to give a final concentration of 9,664 µg/ml. The positive control compounds, 4-nitroquinoline-N-oxide, for S-9 free cultures, and 2-aminoanthracene, for S-9 supplemented cultures, were dissolved in dimethylsulphoxide and diluted 1:100 in the culture medium to give final concentrations of 1 µg/ml.

After a 4 h incubation at 37^{o}C in an atmosphere of 5% CO_2 in air the cultures were washed 3 times with phosphate buffered saline, harvested using a trypsin/EDTA/solution and suspended in 1 ml of saline-EDTA, pH 8.0. Cells were disrupted by 30 strokes of a glass pestle in a 1 ml capacity glass uniform homogeniser, 0.3 ml of 2 M-NaCl and 0.15 ml 10% (w/v) sodium lauryl sulphate added and the mixture incubated for 10 min at room temperature. The lysate was then vigorously shaken with 1.5 ml phenol-hydroxyquinoline (90 g phenol:10 ml water:0.1 g hydroxyquinoline) and centrifuged for 15 min at 3,000 r.p.m. in a MSE Chilspin bench centrifuge. The upper, aqueous phase was carefully removed and a 0.4 ml sample mixed with 4 ml caesium chloride (14.6 g caesium chloride in 10 ml 10 mM tris-HCl, 10 mM EDTA, pH 8.0). The solution was poured into polyallomer Beckman centrifuge tubes and overlaid with liquid paraffin. Tubes in a Beckman SW50.1 rotor were centrifuged at 35,000 r.p.m. in a Beckman LS-50 ultra centrifuge for 72 h to allow the gradient to form and DNA and RNA to band at their equilibrium densities. Gradients were fractioned by upward displacement with saturated caesium chloride using an ISCO density fractionator, (Model 640), 8 drop fractions being collected on 2.5 cm diameter GF/C filter discs (Whatman Limited). The filter discs were immersed for 10 min in 2 changes of ice-cold trichloroacetic acid (5% w/v) containing sodium pyrophosphate (20 mM), washed twice in ice-cold hydrochloric acid (0.5 M) and finally once in ethanol. After air drying, the discs were placed in scintillation fluid (PPO (0.32% w/v), and POPOP (0.032% w/v) in toluene) and analysed for radioactivity in a Beckman LA-100 liquid scintillation counter.

Gradient profiles of the DNA from cells incubated with the test compound were compared with the profiles of the DNA from cells incubated with 4-nitroquinoline-N-oxide, 2-aminoanthracene and dimethylsulphoxide.

Incorporation of Label in the Presence of S-9 Mix

Several experiments were carried out in the presence of S-9 mix in which it was found that no [^{3}H]-deoxyguanosine was incorporated by any of the cultures treated. In the belief that some component of the S-9 was perhaps metabolising the [^{3}H]-deoxyguanosine to a derivative which was not incorporated into nucleic acids, the experiment protocol was altered.

Cells which had been growing for 72 h in 5 ml of arginine-deficient medium were treated with hydroxyurea, S-9 mix and test compounds, 2-aminoanthracene or DMSO. After 3 h exposure at 37°C, this incubation medium was removed and the cells were washed twice with PBS. The cells were then covered with 5 ml of new arginine-deficient medium. [^{3}H]-deoxyguanosine was then added to 10 µCi/ml and the monolayers were incubated for a further 2½ h in the presence of the labelled precursor. The extraction procedure was the same as for those cells treated in the absence of S-9 mix.

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°C was added to the cells while they were agitated on a vortex mixer. Following incubation for 20 min in a 37°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°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), 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 26 January 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 Ltd.).

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

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

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

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 two 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.

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

The value of ϕ is estimated iteratively by equating the minimised value of $S(\phi)$ 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 tables. 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 each of the abnormal catergories 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.

Calibration ranges adopted were 10.8-54.1 ppm (25 ppm target concentration) and 108.2-649.2 ppm (500 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 2-methoxyethanol was occurring. The results are shown in Table AT-3, where it can be seen that the maximum deviations encountered was -6% at the 25 ppm target concentration and -3% at the 500 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 25 ppm and 500 ppm were obtained and recorded in Tables AT-4 to 6.

Deviations from the target concentrations of more than $\pm$ 10% were limited to a few minutes, so, the exposures were considered to be acceptable and the remaining portions of the experiments allowed to proceed. (This was not the situation when animals were exposed on 4-8 December 1978. Data from this aborted experiment are given in Table AT-4.)

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)

24 November 1978 Delivery Ten male and 10 female rats were haphazardly selected for PEAT. There were no significant clinical observations and the microbiological/parasitological assays did not reveal any infections. At autopsy, 4 male rats presented notable features. R4♂, R7♂ and R8♂ had haemorrhagic foci on the kidneys. In addition, the lungs of R4♂ were slightly emphysemic. R10♂ kidneys were slightly mottled and there were haemorrhagic foci on the thymus. Histopathology showed lymphoid nodules in the lungs of all male rats and 7 of the 10 female rats examined. These were mild lesions and did not warrant rejection of the group.

28 November 1978 Delivery Four male mice were haphazardly selected for PEAT. The only notable observation was haemorrhagic patches on the lungs which, in the view of the pathologist, were probably induced at death.

10 January 1978 Delivery Ten male and 10 female rats were haphazardly selected for PEAT. There were no notable clinical observations and the microbiological/parasitological assays did not reveal infections. Autopsy findings of significance were restricted to 4 rats. 14B♂, 6B♂ and 10B♂ had raised patches on the kidneys. 3B♂ had enlarged Peyer's patches. Lymphocytic foci were present in the lungs of 4 females and 4 males; in the livers of 2 females; in the lungs and livers of one female and 2 males; in the lungs and kidneys of 2 females and one male; in the liver and kidneys of one female; and in the lungs, liver, kidney and heart of one male. No histopathology was evident in 2 males. All of these lesions were very mild and did not constitute a reason for rejection of the batch of rats.

18 January 1978 Delivery Four male mice were haphazardly selected for PEAT. There were no notable observations made clinically, at autopsy, histologically or during the microbiological/parasitological investigations.

7 March 1978 Delivery Ten male and 10 female rats were haphazardly selected for PEAT. There were no notable clinical or autopsy observations and the microbiological/parasitological assays did not reveal infections. Lymphocytic foci were present in the lungs of 5 female and 6 male rats; in the lungs and liver of one female and one male rat; in the lungs and thymus of one female rat; in the lungs, liver and thymus of one female rat; and in the lungs, liver and kidneys of one male rat. No histopathology was evident in one female and one male rat.

Clinical Observations and Body Weights

No clinical signs of toxicity to the rats or mice were observed during or after exposure to atmospheres containing either 25 ppm or 500 ppm 2-methoxyethanol. Body weight changes were slight during the 5 day exposure period (Tables BW-1, 3 and 4 and Appendix Tables BW-1, 3 and 4). In mice there was a reduction in mean body weight in the 500 ppm 2-methoxyethanol group of 4.4 g, but there was a similar reduction (of 4.2 g) in the air only control group. Female rats showed virtually no weight change over the 5 day exposure period, but male rats tended to show less weight gain in the 500 ppm 2-methoxyethanol exposure group than in the air only controls. This small difference in the male rats was evident in the cytogenetic and the dominant lethal test groups. Dosing with EMS for 5 days adversely affected body weights of male and female rats, but not of the male mice 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 indication of any increase in the number of silver grains per nucleus at any concentration of 2-methoxyethanol (Table UDS-1). On the contrary, at the highest concentration used (10 µl or 9.664 mg/ml) in the presence of S-9 mix there was a suggestion of repair inhibition. The substances used - 4-nitroquinoline-N-oxide and 2-aminoanthracene - in concurrent positive control groups evoked significant levels of unscheduled DNA synthesis from the cells. These positive control substances, however, are not appropriate for the demonstration of short patch repair when measured by Method 2.

The tritiated deoxyguanosine incorporation assay was used to confirm the results of the first assay. During the course of these experiments, the permeability of both cell lines to deoxyguanosine decreased dramatically, this reduction being aggravated by the addition of S-9 mix to the incubation medium. In consequence, the measured incorporation of radioactivity was insufficient to provide any reasonable analysis of data produced. (A more detailed account of these findings is to be reported separately.)

CYTOGENETIC ANALYSIS OF RAT BONE MARROW CELLS

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

In the multiple exposure cytogenetic test, there were no indications of induction of chromosomal damage in either male or female rats exposed to 25 ppm or 500 ppm 2-methoxyethanol atmospheres. Data were analysed for statistical significance by the permutation test as well as the one-sided Student's t test of the Freeman-Tukey transformed data. Chromatid breaks were rather common in the females exposed to the 25 ppm atmosphere ($0.025<P<0.050$), but this frequency was not continued in the 500 ppm atmosphere exposed group and the effect was not found in the males. The significant value (according to statistical analysis) was probably a chance occurrance.

The single exposure test male rats showed a small increase in the gap frequency at the 48 h sample time in the 500 ppm 2-methoxyethanol atmosphere exposed group, but not at the 6 h or 24 h sample times. The difference from the control group result was not significant.

In females exposed to 25 ppm or 500 ppm atmospheres small increases over the concurrent control levels in gap frequency were again observed at the 48 h sampling time. The increases were not exposure concentration related and were totally restricted to gap frequencies: break frequencies were not affected. The frequencies of cells with aberrations other than gaps were increased in the males at the 24 h and 48 h sample times in the 500 ppm exposure group, but the result pattern was not at all clear in the females. High values were found only in the 25 ppm exposure group at the 6 h and 24 h sample times. Furthermore, none of these increases was statistically significant according to the permutation test or the one-sided Student's t test.

DOMINANT LETHAL TEST

Experiment I Data Given in Tables DL-1 to DL-6 and Appendix Tables DL Curtailed

The first dominant lethal test was stopped since the atmospheric concentrations of 2-methoxyethanol were not within the required limits. Matings and assessments were allowed to continue for 5 weeks. Although these data were not analysed in the same detail as were those from the second, complete experiment they are reported here since they provide supporting evidence for the conclusions reached.

Pregnancy frequency and total implantations per female were normal in the high concentration exposed group in assessment Weeks 1 and 2, but these were greatly reduced in Weeks 3 and 4 while, in Week 5 there were no implantations recorded. There were no reductions in pregnancy frequency or total implantations per female in the low concentration exposed group. No clear indications were evident of biologically significant increases in early deaths. In the high concentration exposed group in Week 3 there were 5 early deaths in 33 total implantations from 5 implantation bearing pregnancies.

Experiment II Data Given in Tables DL-7 to DL-16, Figures 4 to 6 and Appendix Tables DL

Corpora lutea graviditatis counts (Table DL-7) were reduced only in the 500 ppm 2-methoxyethanol atmosphere exposed groups (particularly in assessment Weeks 3-7) and in the EMS dosed group, Weeks 1-4. In the high concentration 2-methoxyethanol exposed group there were no corpora lutea in Week 6.

Pregnancy frequency was calculated in 2 ways: firstly, by considering as pregnant females with corpora lutea graviditatis (Table DL-8) and secondly and more reliably, by considering as pregnant only females with implantations (Table DL-9, Figure 4). The results obtained by these methods were very similar. Pregnancy frequency was satisfactory in the filtered air control group and in the 25 ppm atmosphere exposed group in all assessment weeks, except for the air control group, Week 4. Satisfactory frequencies were also obtained in the 500 ppm atmosphere exposed group in Weeks 1-3 and 9-10 and in the EMS dosed group in Weeks 5-10.

Implantations per pregnancy (Table DL-10, Figure 5) were reduced in the 500 ppm atmosphere exposed group in assessment Weeks 3-8 and in the EMS dosed group in Weeks 1-4. 2-Methoxyethanol had a particularly marked effect in Weeks 5 and 6: there were no implantations at all in Week 6.

The frequencies of live implantations (Table DL-11) followed very closely the pattern of total implantations per pregnancy.

Similarly, if live implantations and late deaths are pooled (Table DL-12) the results are very similar to the total implantations per pregnancy results. No reductions were seen in the 25 ppm atmosphere exposed group, compared with the air control group, whereas there were substantial reductions in the 500 ppm atmosphere exposed group in assessment Weeks 3-8 and in the EMS dosed group in 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-13) did not indicate any increase in these frequencies in the 2-methoxyethanol treated groups. In the EMS dosed group, the only indications of increased numbers of affected pregnancies were in Weeks 6-8 in pregnancies with one or more (>0) early deaths.

Analysis of the proportions of early deaths, assuming that the untreated females contribute to the variation (Table DL-14, Figure 6), did not reveal significant increases in the 2-methoxyethanol treated groups. The single, high value seen in the 500 ppm atmosphere exposed group in Week 7 was probably an artefact of low pregnancy and implantation frequencies and the integral nature of the recorded data. It is not considered to be of biological importance.

Other elevated values observed were: 500 ppm group, Week 3; 500 ppm group, Week 8.

Analysis of early deaths frequency, following the Freeman-Tukey poisson transformation (Table DL-15), did not indicate that 2-methoxyethanol treatment affected the results, except possibly in the 500 ppm atmosphere group in Week 8. This significant difference could be due to the low air control value as much as an elevated treatment group value. EMS treatment, on the other hand, increased the frequency markedly in Week 2. Even this result must be treated with some reserve, however, since the total implantations per pregnancy averaged only 2.0 ± 1.0 in this group and week.

If the early deaths frequencies are analysed following the Freeman-Tukey binomial transformation (Table DL-16), the significant effect due to EMS in Week 2 is supported by a statistically significant result in Week 3. There were no effects seen due to 25 ppm 2-methoxyethanol atmosphere exposures, but statistically significant results were obtained in the 500 ppm atmosphere exposed group in Weeks 3, 4, 5, 7 and 8.

Hence, 3 different statistical analytical methods allow 3 different conclusions to be drawn. If there really is a dominant lethal effect due to 2-methoxyethanol, it occurs at exposure levels which also induce marked anti-fertility effects and the action is not directly upon mature sperm, but upon early stages of spermatogenesis.

SPERM ABNORMALITY TEST

The overall frequency of abnormal sperm was increased from 5.24% in the air exposed control group to 9.38% in the 500 ppm 2-methoxyethanol atmosphere exposed group (Table SA-1 and SA-2 and Appendix Table SA). This difference was significant ($P<0.05$). Most of the increase was due to abnormalities of the sperm head: Category B, the banana-shaped head where the hook had totally disappeared and Category C, the amorphous head. The frequencies of these characters increased from 0.64% and 2.02% respectively, in controls, to 1.30% and 5.11% respectively in the 500 ppm 2-methoxyethanol atmosphere exposed group. The difference in Category C was significant, with $P<0.01$.

SEX-LINKED RECESSIVE LETHAL TEST IN DROSOPHILA

There was no information on the toxicity of 2-methoxyethanol to flies, so a preliminary study was made (Table RL-1).

A dose ranging experiment was undertaken on 4 December 1978 in which flies were exposed to 25 ppm or 500 ppm for 1 h, 3 h or 7 h. All flies were dead before 3 h. The experiment was re-run on 6 December when flies were exposed for 15 min or 1 h to 500 ppm. In this second experiment, the flies withstood exposure to 2-methoxyethanol much better than in the first test. However, in case the flies in the main test should react as in the first dose ranging study, exposures in the SLRL test were restricted to 25 ppm for 1 h or 500 ppm for 15 min.

Two breeding stocks (A and B) were exposed (Table RL-2), but with neither stock was there any recessive lethals in the first brood, which covers Days 1-3 of the spermatogenesis cycle. The results of the second brood, covering Days 3-8 of spermatogenesis, and the third brood, covering Days 8-11, were conflicting for the 2 stocks of flies. Stock A gave 3 recessive lethals, all of them being in Brood 3, whereas Stock B gave 2 recessive lethals, both of them being in Brood 2. Thus, although the frequencies were relatively high (see p. 42), 2-methoxyethanol was not clearly responsible for these recessive lethal mutations. No recessive lethals were induced by 2-methoxyethanol in the F_3 generation. On the other hand, 0.4% EMS feeding for 5 h did induce a high frequency of lethals in the F_2 generation.

Outside the scope of the programme, the possible mutagenic activity of 2-methoxyethanol was checked. The concentrations of test compound were assumed from calculations in these tests which, unfortunately, gave equivocal results.

The first repeat test gave results which were clearly positive: 240 ppm for 7 h on 5 days gave 5 lethals out of 304 and 48 ppm for 7 h on 7 days gave 13 lethals out of 948 chromosomes.

In the second experiment, flies were exposed to a nominal 1,100 ppm 2-methoxyethanol for 2 h, then successive broods assessed. There was 40% survival in this experiment and the numbers of lethals scored were:

Brood 1	2/1075
Brood 2	2/1044
Brood 3	0/1049

A chronic exposure experiment also yielded negative results. Flies in this test were exposed to 36 ppm atmospheres for 7 h/day over 10 consecutive days. There were no lethals in 932 chromosomes tested, whereas in a concurrent negative control there were 2 lethals in 516 chromosomes tested. One difference between the first test, on one hand, and the second and third, on the other hand, was that the flies in the first test were sometimes returned to old medium bottles. Such bottles would contain ethanol, which might have some influence upon the metabolism of 2-methoxyethanol.

The fourth experiment also involved exposure to a static atmosphere of 2-methoxyethanol. The concentration was 120 ppm and exposure was for 7 h/day for 6 days. At the end of each exposure period, flies were returned to either clean or yeasty medium bottles. The results were as follows:

	120 ppm Clean Bottles	120 ppm Yeasty Bottles
No. of animals exposed	225	225
No. and % survival*	179(80%)	102(45%)
No. of lethals scored at F_2	3/551	6/525
% lethals scored at F_2	0.54%	1.14%
No. sterile at F_2	36	62

* Lower survival in the Yeasty Bottles group due to more flies sticking in the rather adhesive medium.

These results were judged to be positive and in agreement with the first of these extra experiments. There was little support, however, for an effect due to yeasty medium.

In subsequent tests exposure was via the food. 2 ml of 5% sucrose solution was placed in a dish and a filter paper wick placed in the dish. The test compound was given in the sucrose solution. (This technique is, apparently, more reproducible than using sintered glass discs since, with the latter method, there is no opportunity for flies to select preferred areas of dampness where they can feed. Consequently, the disc method may inhibit some flies from feeding as much as others, therefore, their intake of test compound will be less.)

In Experiment 5 (the first with the feeding technique) treatment was continuous for 3 days, the flies were mated for one day to allow matured sperm to be shed, then treated continuously for a further 3 days. The objective of this experiment was to examine the possibility of an interaction between 2-methoxyethanol and ethanol. The results were as follows:

	50 µl 2-ME	50 µl 2-ME +50 µl EtOH	25 µl 2-ME +25 µl EtOH
No. of males exposed	100	100	50
% survival after 3 and 7 days*	43%, 4%	77%, 34%	76%, 68%
No. of lethals scored at F_2	No progeny	4/345	Not examined
% lethals scored at F_2	-	1.16%	-

* Figures shown of 77% and 76% were mostly due to mechanical losses.

It appeared from this test that ethanol may protect flies from the toxic effects of 2-methoxyethanol. Also, the sex-linked recessive lethal results were clearly positive from the only group scored (50 µl 2-methoxyethanol + 50 µl ethanol in 2 ml 5% sucrose).

Using a similar treatment regime, the experimental design of Experiment 5 was extended in Experiment 6.

	40 µl 2-ME	30 µl 2-ME	20 µl 2-ME	60 µl 2-ME +40 µl EtOH	40 µl 2-ME +40 µl EtOH	20 µl 2-ME +40 µl EtOH
No. of males exposed	100	100	100	100	100	100
% survival after 7 days	12%	20%	40%	46%	50%	52%
No. of lethals scored at F_2	1/190	No progeny	2/514	0/495	No progeny	0/525
% lethals scored at F_2	0.53%	-	0.39%	0.00%	-	0.00%

These results provide supporting evidence for an effect of some kind by ethanol upon 2-methoxyethanol toxicity, but no support for either the mutagenicity of 2-methoxyethanol or the enhancing effect of ethanol.

DISCUSSION AND CONCLUSIONS

The data available show that careful evaluation of 2-methoxyethanol is necessary. The experiments reported here show that there is a profound effect upon male fertility, although recovery does occur, and this anti-fertility effect is paralleled by increased frequencies of aberrations in those sperm which do mature. It is not clear whether there is a true dominant lethal effect because proper assessment of any such effect - or lack of one - is complicated by the low fertility.

While no effect upon cell cycle and cell population dynamics was noted in the cytogenetics study, the possibility cannot be refuted: to investigate it, a different experimental design would have to be adopted. It could be concluded, however, that 2-methoxyethanol did not induce chromosomal aberrations in rat bone marrow in these experiments.

The sex-linked recessive lethal test was repeated several times in attempts to clarify a situation which repetition made no less obscure. The conclusions which can be drawn from this series of experiments must be guarded, but there does seem to be evidence for genotoxic activity of 2-methoxyethanol in *Drosophila* and a protecting effect of ethanol against the systemic toxicity of this ether. It was difficult to reproduce the mutagenic effects judged to be significant and the conditions for reliable demonstration of a mutagenic effect were not identified.

The UDS assays certainly did not demonstrate activity suggesting that 2-methoxyethanol could induce repair synthesis. On the contrary, in the absence of S-9 mix there was some inhibition of UDS. This could indicate either that 2-methoxyethanol or a metabolite is not reacting directly with DNA, or that metabolites generated *in vivo* differ qualitatively or quantitatively from those exerting the weak *in vitro* effect. The UDS assay result could be a consequence of a specific effect upon UDS enzymes or an unconnected, non-specific toxic effect upon the cultured cells.

To the knowledge of the investigators, the only other mutagenicity test conducted with 2-methoxyethanol was an Ames' test conducted at Inveresk Research International in 1977. The results obtained following pre-incubation for 30 min at 37°C and incubation of the plates for 2 days were (means of duplicate plates):

Test Material	Quantity per plate	TA 1535		TA 1538		TA 98		TA 100	
		S-9 + co-factors	S-9 only	S-9 + co-factors	S-9 only	S-9 + co-factors	S-9 only	S-9 + co-factors	S-9 only
Water	0.2 ml	15	20	18	25	27	24	131	118
2-Amino-anthracene	0.5 µg	86	43	1959	94	1359	122	1895	193
2-Methoxy-ethanol	333 µg	23	17	31	20	39	19	91	98
	1.0 µg	19	22	32	23	28	26	86	84
	3.3 mg	25	18	29	18	33	32	94	87
	10.0 mg	20	16	24	13	30	20	99	88
	33.3 mg	14	18	34	21	50	22	129	103

In view of the elevated mean obtained at 33.3 mg per plate with S. typhimurium TA 98 in presence of S-9 with added co-factors, this portion of the experiment was repeated, except that higher dose levels were used:

Test Material	Quantity per Plate	TA 98
		S-9 + co-factors
Water	0.2 ml	31
2-Amino-anthracene	0.5 µg	1720
2-Methoxy-ethanol	39 mg	27
	73 mg	36
	116 mg	31
	154 mg	29
	193 mg	33 (thin lawn-toxicity)

These results do not suggest that 2-methoxyethanol can induce point mutations in bacteria. Hence, there is no clear evidence for a mutagenic effect in any system.

More detailed studies of the effects upon testicular cells would be most useful in reaching an understanding of the significance of the dominant lethal test and sperm abnormality test results. Metabolic studies also are long overdue. With this information, the OSHA permissible exposure limit of 25 ppm can be reviewed and, if necessary, further restrictions placed upon the usage of 2-methoxyethanol.

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TABLE AT-1

2-Methoxyethanol
Calibration Data for Low Level

Dose Level: 25 ppm v/v

Batch No.: 10632

Volume µl	Conc., ppm, (v/v)	Cumulative Chart Deflection, mm						
		17 January 1979	22 January 1979	23 January 1979	24 January 1979	25 January 1979	26 January 1979	12 March 1979
0	0	0	0	0	0	0	0	0
0.2	10.8	28.0	30.0	27.5	29.0	29.0	28.5	23.5
0.4	21.6	56.0	58.0	56.0	57.5	57.5	57.5	47.0
0.6	32.4	84.0	86.0	84.0	85.0	84.0	85.0	68.0
0.8	43.2	111.0	112.5	111.0	112.5	111.0	111.5	91.0
1.0	54.1	137.5	138.5	137.5	138.5	137.0	137.5	114.0
Chart deflection (mm) for 25 ppm		64.0	65.0	64.0	65.0	64.0	64.0	53.0

Instrument Setting

Pathlength:	8.25 m
Wavelength:	8.8 µm
Absorbence Range:	0.25 A
Slit Width:	0.5 mm
Recorder Voltage:	1 V
Chart Speed:	120 mm/h

Calibration

Syringe:	1 µl Hamilton
Injection Volume:	0.2 µl
No. of Repeat Injections:	x 5

TABLE AT-2

2-Methoxyethanol
Calibration Data for High Level

Dose Level: 500 ppm v/v

Batch No.: 10632

Volume µl	Conc., ppm, (v/v)	Cumulative Chart Deflection, mm						
		17 January 1979	22 January 1979	23 January 1979	24 January 1979	25 January 1979	26 January 1979	12 March 1979
0	0	0	0	0	0	0	0	0
2	108.2	27.0	27.0	27.5	27.0	26.0	27.5	28.0
4	216.4	53.0	51.5	56.0	55.0	53.0	54.0	56.0
6	324.6	75.0	77.0	83.0	79.0	80.5	81.0	83.0
8	432.8	99.5	105.0	109.0	104.5	106.0	107.0	106.0
10	541.0	124.5	130.5	132.0	131.0	131.0	132.0	130.0
12	649.2	147.0	151.5	157.0	155.5	154.0	157.0	153.5
Chart deflection (mm) for 500 ppm		115.0	120.0	124.0	123.0	122.0	122.0	123.0

Instrument Setting

Pathlength: 0.75 m
Wavelength: 8.8 µm
Absorbence Range: 0.25 A
Slit Width: 0.5 mm
Recorder Voltage: 1 V
Chart Speed: 120 mm/h

Calibration

Syringe: 5 µl Hamilton
Injection Volume: 2.0 µl
No. of Repeat Injections: x 6

TABLE AT-3

2-Methoxyethanol

Chamber Atmosphere Homogeneity Data

Dose Level: 25 ppm and 500 ppm

Sample Location	% Deviation from Reference Point	
	Low	High
Reference Point (R)	0	0
Right Centre (RC)	-6	+1
Right Front (RF)	-6	-1
Centre Front (CF)	-6	-3
Left Front (LF)	-6	-1
Left Centre (LC)	+2	-1
Left Back (LB)	+2	+1
Centre Back (CB)	+2	+1
Right Back (RB)	+2	-1

Top view of exposure chamber

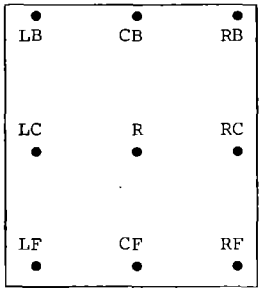


TABLE AT-4

2-Methoxyethanol
Concentrations in the Dominant Lethal Tests (curtailed)

Target Concentration 25 ppm

Exposure Day	% Deviation From Target Concentration in Minutes																							Time Averaged Concentration for 7 h (ppm)
	-32	-28	-20	-12	-10	-8	-6	-4	-2	0	2	4	6	8	10	12	16	20	28	30	48	54	68	116	
1	2	30	10	58	33	8	59	25	-	68	8	-	22	-	-	-	20	-	14	-	5	55	10	3	26.8
2	-	-	-	-	80	120	106	46	20	29	-	11	-	8	-	-	-	7	-	5	-	-	-	-	23.7
3	-	-	-	-	-	-	20	154	55	165	-	-	30	-	-	-	-	-	-	-	-	-	-	-	24.6
4	-	-	-	-	3	7	15	-	-	142	70	113	30	40	-	-	-	-	-	-	-	-	-	-	25.5
5	-	-	-	-	17	33	23	22	20	98	63	120	20	10	-	-	-	-	-	-	-	-	-	-	25.1

Target Concentration 500 ppm

Exposure Day	% Deviation From Target Concentration in Minutes													Time Averaged Concentration for 7 h (ppm)
	-10	-8	-6	-4	-2	0	2	4	6	8	10	12	14	
1	-	35	10	95	48	35	33	66	5	131	45	-	35	507*
2	-	-	-	-	10	120	60	145	30	15	40	-	-	516
3	-	-	6	-	25	70	119	98	95	7	-	-	-	514
4	-	10	15	15	125	235	25	-	-	-	-	-	-	495
5	-	-	28	72	107	109	38	66	-	-	-	-	-	496

* Concentration within exposure chamber at 170 ppm for 2¼ h before start of 7 h period.

TABLE AT-5

2-Methoxyethanol
Atmospheric Analysis
Target Concentration 25 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	+20	+27.5	
Multiple 1					60	200	40	30	50	25	15				24.7
Multiple 2	11		10		139	50	205	5							24.4
Multiple 3					10	85	110	160	30	5	5	5	5	5	25.4
Multiple 4		3		20	65	52	145	60	60	15					25.0
Multiple 5					75	80	170	30	65						24.9

Target Concentration 500 ppm

*Exposure Day	% Deviation from Target Concentration in Minutes													Time Averaged Concentration for 7 h (ppm)
	-10	-8	-6	-4	-2	0	+2	+4	+6	+8	+10	+12	+14	
Multiple 1	5	20	30	50	50	70	100	80	15					499.0
Multiple 2				5	25	145	115	40	55	35				511.1
Multiple 3			10	45	130	105	100	20	10					498.1
Multiple 4			10	45	85	55	90	85	25	25				505.5
Multiple 5			25	20		90	135	85	35	30				509.9

*Multiple exposure performed on 22-26 January 1979

TABLE AT-6

2-Methoxyethanol
Atmospheric Analysis
Target Concentration 25 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	+15	+20	+25	
Single	5	0	20	18	45	87	150	50	22	15	0	5	0	3	5	24.9

Target Concentration 500 ppm

*Exposure Day	% Deviation From Target Concentration in Minutes											Time Averaged Concentration for 7 h (ppm)
	-12	-10	-8	-6	-4	-2	0	+2	+4	+6	+8	+10	
Single	10	0	25	30	67	55	90	65	25	33	15	0	490

*Single exposure performed on 12 March 1979

TABLE BW-1

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

Sex	Exposure Day	Air Control (0 ppm)	25 ppm	500 ppm	5 x 100 mg/kg EMS
Male	1	301 ± 12	308 ± 14	298 ± 10	309 ± 13
	2	305 ± 14	316 ± 15	300 ± 9	306 ± 14
	3	311 ± 14	316 ± 15	294 ± 8	298 ± 14
	4	312 ± 18	317 ± 16	298 ± 10	285 ± 15
	5	313 ± 17	323 ± 16	301 ± 12	277 ± 17
	Weight gain/loss	12	15	3	-32
Female	1	215 ± 14	208 ± 16	217 ± 12	209 ± 12
	2	216 ± 15	207 ± 14	215 ± 12	206 ± 11
	3	217 ± 13	208 ± 15	211 ± 17	202 ± 11
	4	216 ± 15	212 ± 15	214 ± 18	199 ± 11
	5	216 ± 17	211 ± 16	218 ± 19	193 ± 11
	Weight gain/loss	1	3	1	-16

TABLE BW-2

2-Methoxyethanol
Single Exposure Cytogenetics Test
Group Mean Body Weights (g) for Male and Female CD Rats

Sex	Sampling Time (h post exposure)	Air Control (0 ppm)	25 ppm	500 ppm	EMS 250 mg/kg
Male	6	300 ± 12	294 ± 14	290 ± 20	292 ± 16
	24	291 ± 11	289 ± 16	297 ± 14	292 ± 19
	48	293 ± 12	298 ± 13	286 ± 24	287 ± 14
Female	6	208 ± 19	207 ± 14	201 ± 15	203 ± 18
	24	209 ± 17	204 ± 12	203 ± 23	206 ± 15
	48	217 ± 18	207 ± 18	218 ± 14	215 ± 14

TABLE BW-3

2-Methoxyethanol
Dominant Lethal Assay
Group Mean Body Weights (g) for the Dosing Period of Male CD Rats

Exposure Day	Air Control (0 ppm)	25 ppm	500 ppm	5 x 100 mg/kg EMS
1	326 ± 20	311 ± 24	308 ± 11	323 ± 14
2	332 ± 20	318 ± 22	308 ± 12	314 ± 12
3	336 ± 22	319 ± 23	304 ± 14	309 ± 10
4	337 ± 21	320 ± 22	303 ± 14	297 ± 9
5	338 ± 23	323 ± 25	308 ± 16	286 ± 9
Weight gain/loss	12	12	0	-37

TABLE BW-4

2-Methoxyethanol
Sperm Abnormalities Test
Group Mean Body Weights (g) for the Dosing Period of Male $B6C3F_1$ Mice

Exposure Day	Air Control (0 ppm)	25 ppm	500 ppm	5 x 100 mg/kg EMS
1	29.7 ± 1.2	25.9 ± 0.7	28.5 ± 1.0	27.1 ± 1.3
2	25.9 ± 1.0	25.8 ± 1.0	25.9 ± 1.3	26.7 ± 1.8
3	30.1 ± 1.0	25.4 ± 0.8	24.3 ± 1.3	26.3 ± 1.8
4	25.0 ± 1.2	25.1 ± 1.1	23.7 ± 0.9	27.4 ± 1.5
5	25.5 ± 1.1	25.4 ± 0.8	24.1 ± 1.2	27.2 ± 1.5
Weight gain/loss	-4.2	-0.5	-4.4	0.1

TABLE UDS-1

2-Methoxyethanol
Unscheduled DNA Synthesis

Substance	Concentration (µg/ml)		Mean Number of Grains/Nucleus ± S.D.	
	With S-9	Without S-9	With S-9	Without S-9
Dimethylsulphoxide	10,000	10,000	9.3 ± 4.2	13.1 ± 6.5
4-Nitroquinoline-N-oxide	-	8	-	>100
	-	10	-	>100
2-Aminoanthracene	6.25	-	79.3 ± 21.1	-
2-Methoxyethanol	76	76	14.0 ± 4.3	13.7 ± 7.1
	151	151	12.5 ± 3.2	13.7 ± 7.2
	302	302	11.9 ± 3.6	12.3 ± 5.7
	604	604	8.9 ± 3.4	12.5 ± 5.9
	1,208	1,208	8.8 ± 2.9	11.3 ± 4.8
	2,416	2,416	11.5 ± 5.4	11.8 ± 4.8
	4,832	4,832	10.4 ± 5.1	14.5 ± 6.2
	9,663	9,663	1.1 ± 1.0	13.1 ± 5.3

TABLE CA-MD-M-1

2-Methoxyethanol
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	430	37	2	-	-	-	-	1 Chromatid Fragment
25 ppm, 7 h/day	500	28	1	2	2	1	-	-
500 ppm, 7 h/day	500	45	-	2	3	1	-	-
EMS, 100 mg/kg/day	400	64	5	2	1	-	-	-

TABLE CA-MD-M-2

2-Methoxyethanol
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	0.663	0.049		0.258	0.047	
25 ppm	0.540	0.049	-1.78	0.220	0.047	-0.57
500 ppm	0.647	0.049	-0.23	0.200	0.047	-0.88
EMS, 100 mg/kg	0.827	0.055	2.44**	0.277	0.052	0.26

S.E. of mean = Standard error of Freeman-Tukey binomial transformation mean.
**P<0.025

TABLE CA-MD-F-1

2-Methoxyethanol
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	492	44	1	-	1	-	-	-
25 ppm, 7 h/day	312	35	4	2	1	-	-	-
500 ppm, 7 h/day	370	41	1	-	-	-	-	1 Chromatid Fragment
EMS, 100 mg/kg/day	412	83	7	1	6	1	-	1 Chromatid Fragment

TABLE CA-MD-F-2

2-Methoxyethanol
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	0.596	0.059		0.162	0.040	
25 ppm	0.765	0.071	1.83*	0.304	0.048	2.29**
500 ppm	0.689	0.066	1.04	0.201	0.045	0.65
EMS, 100 mg/kg	0.943	0.062	4.04***	0.333	0.042	2.95***

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

*p<0.050

**p<0.025

***p<0.005

TABLE CA-M6-1

2-Methoxyethanol
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	471	13	-	-	1	-	-	1 Chromatid Fragment
25 ppm, 7 h/day	500	12	1	1	2	-	-	3 Chromatid Fragments
500 ppm, 7 h/day	453	19	-	1	1	-	-	-
EMS, 250 mg/kg/day	500	43	4	2	2	2	-	1 Chromosomal Fragment 1 Chromatid Fragment

TABLE CA-M6-2

2-Methoxyethanol
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	0.349	0.063		0.168	0.037	
25 ppm	0.381	0.063	0.36	0.220	0.037	0.99
500 ppm	0.438	0.063	1.00	0.199	0.037	0.58
EMS, 250 mg/kg	0.626	0.063	3.13***	0.227	0.037	1.11

S.E. of mean = Standard error of Freeman-Tukey binomial transformation mean.
***p<0.005

TABLE CA-M24-1

2-Methoxyethanol
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	18	1	-	5	-	-	-
25 ppm, 7 h/day	500	28	1	-	2	-	-	1 Chromatid Fragment 1 Chromosomal Fragment
500 ppm, 7 h/day	500	19	4	-	7	-	-	-
EMS, 250 mg/kg/day	409	17	5	1	4	-	-	1 Chromosomal Fragment 1 Chromatid Fragment 2 Exchanges

TABLE CA-M24-2

2-Methoxyethanol
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	0.413	0.087		0.180	0.068	
25 ppm	0.508	0.087	0.77	0.191	0.068	0.11
500 ppm	0.451	0.087	0.31	0.220	0.068	0.42
EMS, 250 mg/kg	0.703	0.087	2.35**	0.381	0.068	2.08**

S.E. of mean = Standard error of Freeman-Tukey binomial transformation mean.
**p<0.025

TABLE CA-M48-1

2-Methoxyethanol
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	19	3	-	-	-	-	1 Chromatid Fragment
25 ppm, 7 h/day	500	20	2	-	-	-	-	1 Chromatid Fragment
500 ppm, 7 h/day	500	35	2	1	1	-	-	4 Chromatid Fragments
EMS, 250 mg/kg/day	350	21	1	-	-	-	1	-

TABLE CA-M48-2

2-Methoxyethanol
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	0.433	0.074		0.220	0.034	
25 ppm	0.438	0.074	0.05	0.191	0.034	-0.61
500 ppm	0.532	0.074	0.95	0.251	0.034	0.63
EMS, 250 mg/kg	0.482	0.088	0.43	0.197	0.041	-0.43

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

TABLE CA-F6-1

2-Methoxyethanol
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	500	26	1	-	-	-	-	3 Chromatid Fragments
25 ppm, 7 h/day	489	17	5	2	-	-	-	1 Chromatid Fragment
500 ppm, 7 h/day	500	25	1	-	-	-	-	-
EMS, 250 mg/kg/day	500	35	5	1	2	-	-	1 Chromatid Fragment

TABLE CA-F6-2

2-Methoxyethanol
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	0.488	0.075		0.211	0.044	
25 ppm	0.394	0.075	-0.89	0.227	0.044	0.26
500 ppm	0.445	0.075	-0.41	0.160	0.044	-0.81
EMS, 250 mg/kg	0.539	0.075	0.48	0.261	0.044	0.81

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

TABLE CA-F24-1

2-Methoxyethanol
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	453	30	2	1	3	1	-	-
25 ppm, 7 h/day	500	34	5	3	5	-	-	2 Chromatid Fragments 1 Translocation 1 Exchange
500 ppm, 7 h/day	500	37	2	1	5	1	-	1 Chromatid Fragment
EMS, 250 mg/kg/day	443	56	11	2	7	-	1	3 Chromosomal Fragments 1 Chromatid Fragment 1 Exchange

TABLE CA-F24-2

2-Methoxyethanol
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	0.540	0.065		0.259	0.046	
25 ppm	0.603	0.065	0.69	0.320	0.046	0.94
500 ppm	0.546	0.065	0.06	0.221	0.046	-0.57
EMS, 250 mg/kg	0.737	0.065	2.16**	0.354	0.046	1.46

S.E. of mean = Standard error of Freeman-Tukey binomial transformation mean.
**P<0.025

TABLE CA-F48-1

2-Methoxyethanol
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	500	19	1	-	-	-	-	-
25 ppm, 7 h/day	500	36	-	-	-	-	-	-
500 ppm, 7 h/day	500	32	-	-	-	-	-	-
EMS, 250 mg/kg/day	392	20	5	1	3	-	-	2 Chromatid Fragments

TABLE CA-F48-2

2-Methoxyethanol
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	0.375	0.068		0.171	0.027	
25 ppm	0.516	0.068	1.46	0.141	0.027	-0.81
500 ppm	0.481	0.068	1.10	0.141	0.027	-0.81
EMS, 250 mg/kg	0.490	0.068	1.19	0.295	0.027	3.29***

S.E. of mean = Standard error of Freeman-Tukey binomial transformation mean.
***P<0.005

TABLE DL-1

2-Methoxyethanol
Dominant Lethal Test in Rats
Pregnancy Frequency (Females with Implantations)
Curtailed Test

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)		25 ppm		500 ppm		5 x 100 mg/kg EMS	
1	17/20	85%	18/20	90%	14/20	70%	15/20	75%
2	16/20	80%	16/20	80%	18/20	90%	9/20	45%
3	19/20	95%	20/20	100%	5/20	25%	7/20	35%
4	19/20	95%	17/20	85%	2/20	10%	15/19	79%
5	18/20	90%	16/20	80%	0/20	0%	18/20	90%

TABLE DL-2

2-Methoxyethanol
Dominant Lethal Test in Rats
Total Implantations per Pregnancy
Curtailed Test

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	25 ppm	500 ppm	5 x 100 mg/kg EMS
1	12.2	13.6	13.7	9.9
2	12.5	12.9	11.2	1.6
3	13.7	12.4	6.6	1.3
4	14.2	14.8	12.0	7.9
5	13.9	13.3		12.6

TABLE DL-3

2-Methoxyethanol
Dominant Lethal Test in Rats
Live Implantations per Pregnancy
Curtailed Test

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	25 ppm	500 ppm	5 x 100 mg/kg EMS
1	11.9	13.2	12.7	3.1
2	12.0	12.1	10.9	0.0
3	13.1	12.0	5.4	0.0
4	13.3	14.3	11.5	5.9
5	13.9	13.2		12.6

TABLE DL-4

2-Methoxyethanol
Dominant Lethal Test in Rats
Live Implantations and Late Deaths per Pregnancy
Curtailed Test

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	25 ppm	500 ppm	5 x 100 mg/kg EMS
1	12.2	13.3	12.9	3.2
2	12.1	12.1	10.9	0.0
3	13.2	12.1	5.6	0.0
4	13.3	14.3	11.5	5.9
5	13.9	13.2		12.6

TABLE DL-5

2-Methoxyethanol
Dominant Lethal Test in Rats
Frequency of Pregnancies with One or More or Two or More Early Deaths
Curtailed Test

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)		25 ppm		500 ppm		5 x 100 mg/kg EMS	
	>0	>1	>0	>1	>0	>1	>0	>1
1	0/17	0/17	4/18	0/18	4/14	4/14	14/15	14/15
2	6/16	5/16	6/16	4/16	4/18	4/18	9/9	4/9
3	10/19	9/19	7/20	0/20	3/5	1/5	7/7	2/7
4	12/19	11/19	5/17	3/17	1/2	0/2	13/15	8/15
5	0/18	0/18	3/16	0/16	0/0	0/0	0/18	0/18

TABLE DL-6

2-Methoxyethanol
Dominant Lethal Test in Rats
Early Deaths as a Proportion of Total Implantations
Curtailed Test

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	25 ppm	500 ppm	5 x 100 mg/kg EMS
1	0.0%	1.6%	5.7%	67.6%
2	3.5%	6.3%	2.0%	100.0%
3	4.2%	2.8%	15.2%	100.0%
4	6.3%	3.2%	4.2%	25.2%
5	0.0%	1.4%	-	0.0%

TABLE DL-7

2-Methoxyethanol
Dominant Lethal Test in Rats
Total Number of Corpora Lutea per Pregnancy

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	25 ppm	500 ppm	5 x 100 mg/kg EMS
1	12.7 ± 0.39	12.1 ± 0.43	11.8 ± 0.36	8.6 ± 0.58
2	11.9 ± 0.49	12.6 ± 0.47	11.9 ± 0.48	3.5 ± 0.50
3	12.5 ± 0.75	13.1 ± 0.75	9.3 ± 0.87	3.0 ± 0.71
4	11.9 ± 0.87	12.3 ± 0.78	8.0 ± 1.28	9.0 ± 1.10
5	12.2 ± 0.29	12.4 ± 0.29	1.3 ± 0.74	11.2 ± 0.83
6	13.3 ± 0.42	13.6 ± 0.41		12.6 ± 0.62
7	11.8 ± 0.45	12.3 ± 0.49	8.5 ± 1.41	11.6 ± 0.49
8	13.0 ± 0.49	12.2 ± 0.50	10.9 ± 0.78	12.0 ± 0.60
9	12.1 ± 0.46	12.8 ± 0.47	12.1 ± 0.52	11.8 ± 0.43
10	12.5 ± 0.58	12.8 ± 0.58	10.3 ± 0.58	12.9 ± 0.69

TABLE DL-8

2-Methoxyethanol
Dominant Lethal Test in Rats
Pregnancy Frequency (Females with Corpora Lutea Graviditatis)

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	25 ppm	500 ppm	5 x 100 mg/kg EMS
1	85%	85%	100%	85%
2	85%	95%	90%	40%
3	100%	100%	85%	60%
4	75%	80%	60%	85%
5	95%	95%	40%	80%
6	100%	100%	50%	90%
7	100%	90%	20%	100%
8	100%	100%	67%	95%
9	95%	95%	80%	90%
10	100%	100%	95%	100%

TABLE DL-9

2-Methoxyethanol
Dominant Lethal Test in Rats
Pregnancy Frequency (Females with Implantations)

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)		25 ppm		500 ppm		5 x 100 mg/kg EMS	
1	17/20	85%	14/20	70%	20/20	100%	10/20	50%
2	17/20	85%	19/20	95%	18/20	90%	2/20	10%
3	20/20	100%	20/20	100%	15/20	75%	4/20	20%
4	13/20	65%	16/20	80%	6/20	30%	12/20	60%
5	19/20	95%	19/20	95%	3/20	15%	16/20	80%
6	19/20	95%	20/20	100%	0/20	0%	18/20	90%
7	20/20	100%	18/20	90%	2/20	10%	18/20	90%
8	20/20	100%	19/19	100%	8/18	44%	17/19	89%
9	19/20	95%	18/20	90%	15/20	75%	18/20	90%
10	19/19	100%	19/19	100%	19/20	95%	20/20	100%

TABLE DL-10

2-Methoxyethanol
Dominant Lethal Test in Rats
Total Implantations per Pregnancy

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	25 ppm	500 ppm	5 x 100 mg/kg EMS
1	12.8 ± 0.52	11.1 ± 0.57	11.5 ± 0.48	6.4 ± 0.78
2	11.6 ± 0.65	12.2 ± 0.62	11.1 ± 0.63	2.0 ± 1.00
3	12.5 ± 0.74	13.0 ± 0.74	8.3 ± 0.85	2.3 ± 0.48
4	11.6 ± 1.01	12.1 ± 0.91	6.0 ± 1.48	8.3 ± 1.29
5	11.7 ± 0.37	12.1 ± 0.37	1.0 ± 0.92	11.6 ± 0.88
6	13.3 ± 0.51	12.8 ± 0.50	0.0 ± 0.00	11.9 ± 0.95
7	12.1 ± 0.57	12.6 ± 0.60	7.0 ± 1.81	12.3 ± 0.67
8	13.3 ± 0.68	12.6 ± 0.69	8.9 ± 1.07	11.9 ± 0.95
9	12.8 ± 0.57	12.9 ± 0.59	12.1 ± 0.64	11.8 ± 0.54
10	12.4 ± 0.64	13.3 ± 0.64	10.2 ± 0.64	12.5 ± 0.72

TABLE DL-11

2-Methoxyethanol
Dominant Lethal Test in Rats
Live Implantations per Pregnancy

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	25 ppm	500 ppm	5 x 100 mg/kg EMS
1	11.9 ± 0.53	11.0 ± 0.59	11.1 ± 0.49	6.1 ± 0.89
2	11.1 ± 0.70	11.8 ± 0.66	10.8 ± 0.68	0.0 ± 0.00
3	12.0 ± 0.74	12.5 ± 0.74	7.8 ± 0.86	1.8 ± 0.63
4	11.6 ± 0.99	12.1 ± 0.89	6.0 ± 1.46	8.3 ± 1.29
5	11.0 ± 0.41	11.6 ± 0.41	1.0 ± 1.02	11.3 ± 0.87
6	12.7 ± 0.51	12.3 ± 0.50	0.0 ± 0.00	11.0 ± 0.86
7	11.5 ± 0.54	11.3 ± 0.57	6.5 ± 1.72	11.4 ± 0.62
8	12.9 ± 0.70	12.2 ± 0.72	8.0 ± 1.11	10.8 ± 0.84
9	11.7 ± 0.66	11.7 ± 0.67	10.9 ± 0.74	11.2 ± 0.54
10	12.0 ± 0.65	12.7 ± 0.65	10.0 ± 0.65	12.0 ± 0.74

TABLE DL-12

2-Methoxyethanol
Dominant Lethal Test in Rats
Live Implantations and Late Deaths per Pregnancy

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	25 ppm	500 ppm	5 x 100 mg/kg EMS
1	11.9 ± 0.53	11.0 ± 0.59	11.1 ± 0.49	6.1 ± 0.89
2	11.1 ± 0.70	11.8 ± 0.66	10.8 ± 0.68	0.0 ± 0.00
3	12.3 ± 0.74	12.6 ± 0.74	7.8 ± 0.86	1.8 ± 0.63
4	11.6 ± 0.99	12.1 ± 0.89	6.0 ± 1.46	8.3 ± 1.29
5	11.0 ± 0.39	11.6 ± 0.39	1.0 ± 0.99	11.3 ± 0.87
6	12.7 ± 0.52	12.3 ± 0.51	0.0 ± 0.00	11.1 ± 0.87
7	11.5 ± 0.54	11.3 ± 0.57	6.5 ± 1.71	11.4 ± 0.61
8	13.0 ± 0.69	12.2 ± 0.71	8.0 ± 1.10	10.8 ± 0.84
9	11.7 ± 0.65	11.8 ± 0.66	11.0 ± 0.73	11.2 ± 0.54
10	12.0 ± 0.65	12.7 ± 0.65	10.0 ± 0.65	12.0 ± 0.74

TABLE DL-13

2-Methoxyethanol

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)		25 ppm		500 ppm		5 x 100 mg/kg EMS	
	>0	>1	>0	>1	>0	>1	>0	>1
1	6/17	4/17	2/14	0/14	5/20	2/20	2/10	1/10
2	6/17	2/17	4/19	1/19	4/18	2/18	2/20	1/20
3	4/20	1/20	6/20	2/20	5/15	2/15	2/4	0/4
4	0/13	0/13	1/16	0/16	0/6	0/6	0/12	0/12
5	9/19	3/19	5/19	2/19	0/3	0/3	4/16	1/16
6	8/19	2/19	9/20	0/20	0/0	0/0	11/18	4/18
7	7/20	2/20	9/18	5/18	1/2	0/2	11/18	3/18
8	4/20	1/20	7/19	1/19	5/8	1/8	12/17	2/17
9	10/19	4/19	10/18	2/18	7/15	4/15	9/18	3/18
10	6/19	1/19	9/19	2/19	2/19	1/19	8/20	1/20

TABLE DL-14

2-Methoxyethanol
Dominant Lethal Test in Rats
Analysis of Proportions of Early Deaths Assuming Between
Female Variation (Logistic Scale)

Multiple Dosing

Assessment Week from Dosing	[1]Treatment Group	$\hat{\mu} \pm$ S.E. $\hat{\mu}$		Percentage Mean Probability of Early Deaths
		Total Implants a Linear Covariate	[2]Assuming Extra-Binomial Variation	
1	1	-2.950 ± 0.4260	-2.700 ± 0.3485	6.3%
	2	-4.479 ± 0.8874	-4.380 ± 0.8791	1.2%
	3	-3.461 ± 0.4708	-3.445 ± 0.4666	3.1%
	4	0.206 ± 0.1797		
2	1	-3.028 ± 0.5073	-2.985 ± 0.5035	4.8%
	2	-3.411 ± 0.6007	-3.470 ± 0.5928	3.0%
	3	-3.502 ± 0.6245	-3.299 ± 0.5757	3.6%
	4	-0.208 ± 0.1125		
3	1	-3.727 ± 0.5993	-3.856 ± 0.5966	2.1%
	2	-3.276 ± 0.5005	-3.467 ± 0.4929	3.0%
	3	-2.769 ± 0.5087	-2.533 ± 0.4458	7.4%
	4	-0.149 ± 0.0880		
4	1	-17.76 ± 104.7	-13.24 ± 37.08	0.0%
	2	-15.74 ± 51.11	-5.263 ± 1.003	0.5%
	3	-15.03 ± 65.13	-12.44 ± 50.72	0.0%
	4	1.814 ± 7.265		

1. 1 = Air Control; 2 = 2-methoxyethanol, 25 ppm; 3 = 2-methoxyethanol, 500 ppm; 4 = ethyl methanesulphonate, 100 mg/kg, orally.

2. Pure binomial variation assumed in Weeks 4 and 6; This assumption invalid in all other weeks.

TABLE DL-14 (continued)

2-Methoxyethanol

Multiple Dosing

Assessment Week from Dosing	[1]Treatment Group	$\hat{\mu} \pm$ S.E. $\hat{\mu}$		Percentage Mean Probability of Early Deaths
		Total Implants a Linear Covariate	[2]Assuming Extra-Binomial Variation	
5	1	-2.768 ± 0.3897	-2.688 ± 0.3441	6.4%
	2	-3.345 ± 0.4975	-3.238 ± 0.4374	3.8%
	3	-7.642 ± 25.45	-8.566 ± 25.38	0.0%
	4	0.091 ± 0.1888		
6	1	-3.010 ± 1.072	-3.091 ± 0.3082	4.4%
	2	-3.395 ± 1.246	-3.308 ± 0.3393	3.5%
	3	[3]_	[3]_	[3]_
	4	-0.021 ± 0.3691		
7	1	-3.027 ± 0.5083	-2.997 ± 0.5057	4.8%
	2	-2.501 ± 0.4476	-2.358 ± 0.4031	8.6%
	3	-1.424 ± 1.120	-1.486 ± 1.107	18.5%
	4	0.133 ± 0.1308		
8	1	-3.660 ± 0.5686	-3.795 ± 0.5673	2.2%
	2	-3.323 ± 0.4826	-3.369 ± 0.4820	3.3%
	3	-2.327 ± 0.5842	-1.962 ± 0.4725	12.3%
	4	-0.167 ± 0.0856		

1. 1 = Air Control; 2 = 2-methoxyethanol, 25 ppm; 3 = 2-methoxyethanol, 500 ppm; 4 = ethyl methanesulphonate, 100 mg/kg, orally.
2. Pure binomial variation assumed in Weeks 4 and 6; This assumption invalid in all other weeks.
3. Zero information.

TABLE DL-14 (continued)

2-Methoxyethanol

Multiple Dosing

Assessment Week from Dosing	[1] Treatment Group	$\hat{\mu} \pm$ S.E. $\hat{\mu}$		Percentage Mean Probability of Early Deaths
		Total Implants a Linear Covariate	Assuming Extra-Binomial Variation	
9	1	-2.494 ± 0.4398	-2.459 ± 0.4334	7.9%
	2	-2.435 ± 0.4389	-2.400 ± 0.4332	8.3%
	3	-2.365 ± 0.4760	-2.371 ± 0.4751	8.5%
	4	0.074 ± 0.1173		
10	1	-3.433 ± 0.4118	-3.482 ± 0.4087	3.0%
	2	-2.956 ± 0.3647	-3.085 ± 0.3302	4.4%
	3	-4.192 ± 0.6215	-4.134 ± 0.6067	1.6%
	4	-0.092 ± 0.1201		

1. 1 = Air Control; 2 = 2-methoxyethanol, 25 ppm; 3 = 2-methoxyethanol, 500 ppm; 4 = ethyl methanesulphonate, 100 mg/kg, orally.

TABLE DL-15

2-Methoxyethanol
Dominant Lethal Test in Rats
Early Death Frequency, Freeman-Tukey Poisson Transformation

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	25 ppm	500 ppm	5 x 100 mg/kg EMS
1	1.80 ± 0.217	1.20 ± 0.239	1.43 ± 0.200	1.36 ± 0.244
2	1.62 ± 0.208	1.39 ± 0.197	1.40 ± 0.202	3.07 ± 0.659
3	1.32 ± 0.179	1.50 ± 0.179	1.61 ± 0.207	1.71 ± 0.408
4	1.00 ± 0.067	1.09 ± 0.061	1.00 ± 0.099	1.00 ± 0.000
5	1.85 ± 0.217	1.51 ± 0.217	1.00 ± 0.545	1.40 ± 0.183
6	1.70 ± 0.187	1.64 ± 0.182	0.00 ± 0.000	2.03 ± 0.210
7	1.65 ± 0.272	2.11 ± 0.286	1.71 ± 0.859	2.05 ± 0.228
8	1.35 ± 0.180	1.56 ± 0.184	2.05 ± 0.284	2.20 ± 0.250
9	2.04 ± 0.294	2.04 ± 0.302	2.02 ± 0.331	1.83 ± 0.210
10	1.49 ± 0.167	1.75 ± 0.167	1.19 ± 0.167	1.63 ± 0.188

TABLE DL-16

2-Methoxyethanol

Dominant Lethal Test in Rats

Early Death Frequency, Freeman-Tukey Binomial Transformation

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	25 ppm	500 ppm	5 x 100 mg/kg EMS
1	0.495 ± 0.0613	0.360 ± 0.0676	0.417 ± 0.0565	0.653 ± 0.2080
2	0.476 ± 0.0715	0.401 ± 0.0676	0.443 ± 0.0694	2.468 ± 0.1342
3	0.371 ± 0.0792	0.408 ± 0.0792	0.706 ± 0.0915	1.195 ± 0.4181
4	0.287 ± 0.0360	0.318 ± 0.0324	0.546 ± 0.0529	0.407 ± 0.0576
5	0.539 ± 0.0627	0.424 ± 0.0627	0.785 ± 0.1578	0.427 ± 0.0565
6	0.469 ± 0.0518	0.445 ± 0.0505	0.000 ± 0.0000	0.586 ± 0.0517
7	0.464 ± 0.0891	0.580 ± 0.0940	1.302 ± 0.2819	0.573 ± 0.0591
8	0.365 ± 0.0817	0.431 ± 0.0838	0.926 ± 0.1291	0.634 ± 0.0606
9	0.568 ± 0.0860	0.576 ± 0.0884	0.584 ± 0.0968	0.530 ± 0.0638
10	0.417 ± 0.0516	0.475 ± 0.0516	0.406 ± 0.0516	0.491 ± 0.0755

TABLE SA-1

2-Methoxyethanol
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	4264	9	29	91	94	13	236	0.20	0.64	2.02	2.09	0.29	5.24
25 ppm, 7 h/day	8683	10	31	116	119	41	317	0.11	0.34	1.29	1.32	0.46	3.52
500 ppm, 7 h/day	9062	10	130	511	250	37	938	0.10	1.30	5.11	2.50	0.37	9.38
EMS, 100 mg/kg/day	8655	14	27	116	155	33	345	0.16	0.30	1.29	1.72	0.37	3.83

* 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)

TABLE SA-2

2-Methoxyethanol
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	8.41 ± 1.736	16.17 ± 2.758	28.01 ± 4.206	26.14 ± 4.366	10.24 ± 2.844	43.98 ± 5.631
25 ppm, 7 h/day	7.12 ± 1.294	11.48 ± 2.056	22.62 ± 3.135	22.23 ± 3.254	12.74 ± 2.120	37.29 ± 4.197
500 ppm, 7 h/day	6.13 ± 1.227	21.77 ± 1.950	43.85** ± 2.974	30.77 ± 3.087	10.83 ± 2.011	60.50* ± 3.982
EMS, 100 mg/kg/day	8.26 ± 1.294	11.27 ± 2.056	27.01 ± 3.135	25.14 ± 3.254	11.64 ± 2.120	41.54 ± 4.197

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)

* = $P<0.05$

** = $P<0.01$

TABLE RL-1

2-Methoxyethanol

Drosophila Dose Ranging Experiment

Day		Air Control (0 ppm)		25 ppm		500 ppm	
		0 h	0 h	1 h	3 h	0.25 h	1 h
0	No. of males exposed	-	-	100	100	*50	*50
1	No. and % survival	-	-	82 (82%)	23 (23%)	45 (97%)	40 (88%)
2	No. of eggs laid by 10♀	110	95	112	105	178	195
3	No. and % hatched	109 (99%)	77 (81%)	109 (97%)	97 (92%)	164 (92%)	185 (95%)

*Fewer flies used as stocks of suitably aged flies depleted by earlier exposure to 25 ppm or 500 ppm 2-methoxyethanol for times which were lethal.

TABLE RL-2

2-Methoxyethanol

Drosophila SLRL Procedure and Results

Compound: 2-Methoxyethanol **Concentration:** 25 ppm **Stock:** A
Length of Exposure: 1 h **Test exposure given:** 11.12.78

	Brood 1	Brood 2	Brood 3
F_1 set up	12.12.78	15.12.78	20.12.78
F_2 set up	27.12.78	29.12.78	4. 1.79
F_2 scored	8. 1.79	11. 1.79	15. 1.79
F_2 repeats scored	19. 1.79	-	-
F_3 set up	-	-	15. 1.79
F_3 scored	-	-	30. 1.79
F_3 repeats scored	-	-	-

RESULTS

	Brood 1	Brood 2	Brood 3	All Broods
No. of F_1 vials	96	52	50	198
No. of sterile F_1 vials	67	26	43	136
No. of F_1 vials used in F_2	29	27	7	63
No. of F_2 vials set up	307	379	133	819
No. of F_2 vials scored	284	365	132	781
No. of F_2 vials containing lethals	-	-	2	2
Frequency of F_2 lethals	-	-	1.5%	0.24%
No. of F_3 vials set up	-	-	12	12
No. of F_3 vials scored	-	-	11	11
No. of F_3 vials containing lethals	-	-	-	-
Frequency of F_3 lethals	-	-	-	-

Total No. sterile vials = 186

TABLE RL-2 (continued)

2-Methoxyethanol
Drosophila SLRL Procedure and Results

Compound: 2-Methoxyethanol **Concentration:** 25 ppm **Stock:** B
Length of Exposure: 1 h **Test exposure given:** 11.12.78

	Brood 1	Brood 2	Brood 3
F_1 set up	12.12.78	15.12.78	20.12.78
F_2 set up	27.12.78	29.12.78	4. 1.79
F_2 scored	8. 1.79	11. 1.79	15. 1.79
F_2 repeats scored	18. 1.79	-	30. 1.79
F_3 set up	8. 1.79	11. 1.79	15. 1.79
F_3 scored	19. 1.79	22. 1.79	30. 1.79
F_3 repeats scored	-	-	8. 2.79

RESULTS

	Brood 1	Brood 2	Brood 3	All Broods
No. of F_1 vials	95	89	47	231
No. of sterile F_1 vials	15	42	36	93
No. of F_1 vials used in F_2	78+	58	19	155
No. of F_2 vials set up	524	492	221	1237
No. of F_2 vials scored	512	478	203	1193
No. of F_2 vials containing lethals	1	4	0	5
Frequency of F_2 lethals	0.19%	0.81%	-	0.40%
No. of F_3 vials set up	403	406	190	999
No. of F_3 vials scored	378	390	177	945
No. of F_3 vials containing lethals	1	-	-	1
Frequency of F_3 lethals	0.24%	-	-	0.10%

Total No. sterile vials = 200

TABLE RL-2 (continued)

2-Methoxyethanol

Drosophila SLRL Procedure and Results

Compound: 2-Methoxyethanol **Concentration:** 500 ppm **Stock:** A
Length of Exposure: 15 min **Test exposure given:** 12.12.78

	Brood 1	Brood 2	Brood 3
F_1 set up	12.12.78	15.12.78	20.12.78
F_2 set up	27.12.78	29.12.78	4. 1.79
F_2 scored	9. 1.79	12. 1.79	15. 1.79
F_2 repeats scored	20. 1.79	22. 1.79	29. 1.79
F_3 set up	-	-	15. 1.79
F_3 scored	-	-	29. 1.79
F_3 repeats scored	-	-	-

RESULTS

	Brood 1	Brood 2	Brood 3	All Broods
No. of F_1 vials	100	97	56	253
No. of sterile F_1 vials	40	23	27	90
No. of F_1 vials used in F_2	60	64	30	154
No. of F_2 vials set up	428	502	420	1350
No. of F_2 vials scored	421	481	386	1288
No. of F_2 vials containing lethals	-	-	3	3
Frequency of F_2 lethals	-	-	0.71%	0.22%
No. of F_3 vials set up	-	-	322	322
No. of F_3 vials scored	-	-	307	307
No. of F_3 vials containing lethals	-	-	-	-
Frequency of F_3 lethals	-	-	-	-

Total No. sterile vials = 163

TABLE RL-2 (continued)

2-Methoxyethanol

Drosophila SLRL Procedure and Results

Compound: 2-Methoxyethanol **Concentration:** 500 ppm **Stock:** B
Length of Exposure: 15 min **Test exposure given:** 12.12.78

	Brood 1	Brood 2	Brood 3
F_1 set up	12.12.78	15.12.78	20.12.78
F_2 set up	27.12.78	29.12.78	4. 1.79
F_2 scored	9. 1.79	12. 1.79	15. 1.79
F_2 repeats scored	-	-	27. 1.79
F_3 set up	9. 1.79	12. 1.79	15. 1.79
F_3 scored	19. 1.79	23. 1.79	29. 1.79
F_3 repeats scored	-	-	8. 2.79

RESULTS

	Brood 1	Brood 2	Brood 3	All Broods
No. of F_1 vials	100	93	46	239
No. of sterile F_1 vials	22	64	32	118
No. of F_1 vials used in F_2	73	36	19	128
No. of F_2 vials set up	409	477	77	963
No. of F_2 vials scored	364	457	71	892
No. of F_2 vials containing lethals	-	2	-	2
Frequency of F_2 lethals	-	0.42%	-	0.20%
No. of F_3 vials set up	372	259	67	698
No. of F_3 vials scored	360	250	61	671
No. of F_3 vials containing lethals	-	-	-	-
Frequency of F_3 lethals	-	-	-	-

Total No. sterile vials = 215

TABLE RL-2 (continued)

2-Methoxyethanol

Drosophila SLRL Procedure and Results

Compound: Ethyl methanesulphonate **Concentration:** 0.4% v/v **Stock:** A
Length of Exposure: 5 h **Test exposure given:** 12.12.78

	Brood 1	Brood 2	Brood 3
F_1 set up	13.12.78	-	-
F_2 set up	27.12.78	-	-
F_2 scored	10. 1.79	-	-
F_2 repeats scored	23. 1.79	-	-
F_3 set up	-	-	-
F_3 scored	-	-	-
F_3 repeats scored	-	-	-

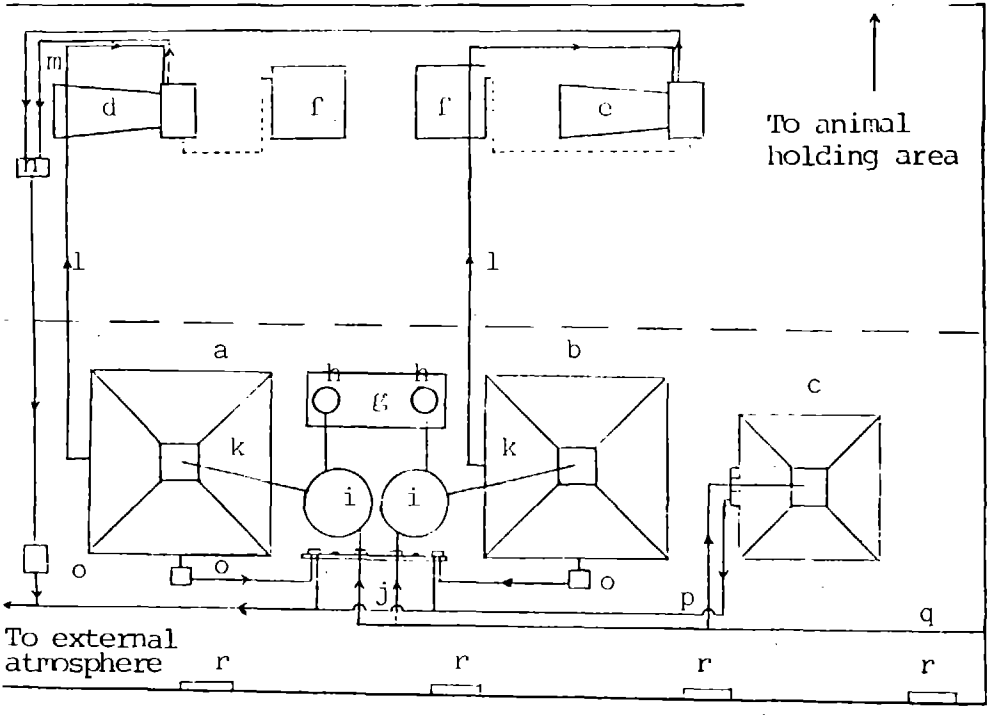
RESULTS

	Brood 1	Brood 2	Brood 3	All Broods
No. of F_1 vials	50	-	-	50
No. of sterile F_1 vials	0	-	-	0
No. of F_1 vials used in F_2	50	-	-	50
No. of F_2 vials set up	107	-	-	107
No. of F_2 vials scored	95	-	-	95
No. of F_2 vials containing lethals	18	-	-	18
Frequency of F_2 lethals	18.91%	-	-	18.9%
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

2-Methoxyethanol

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 Temperature controlled water baths
h Drechsel bottle
i Mixing vessel for dilution of test compound
j Flow meter control panel for atmosphere generation
k Vapour 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

2-Methoxyethanol

Schematic Lay-out of Vapour Generation Apparatus

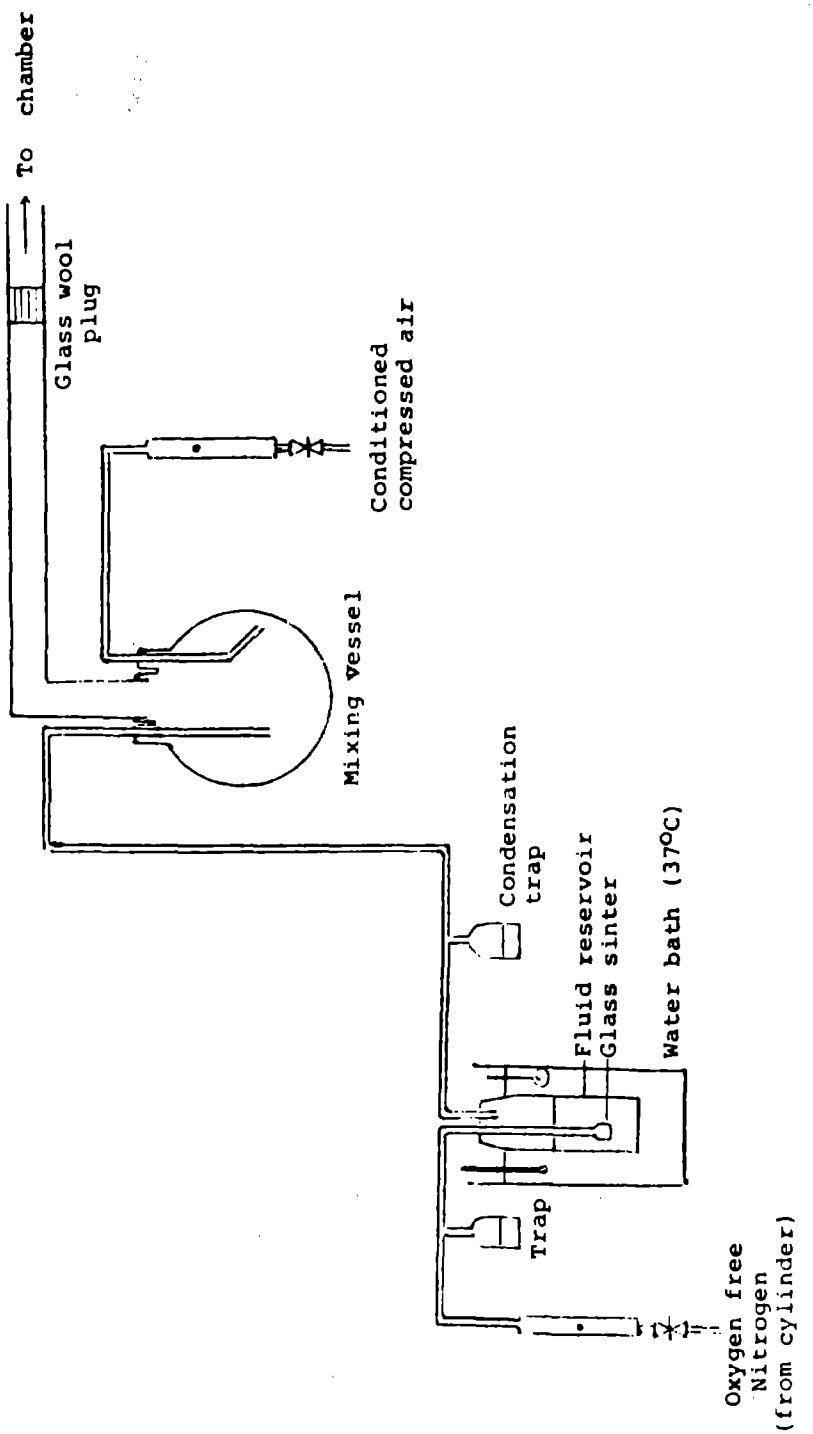


FIGURE 2

2-Methoxyethanol
Calibration Graph for Low Level
25 January 1979

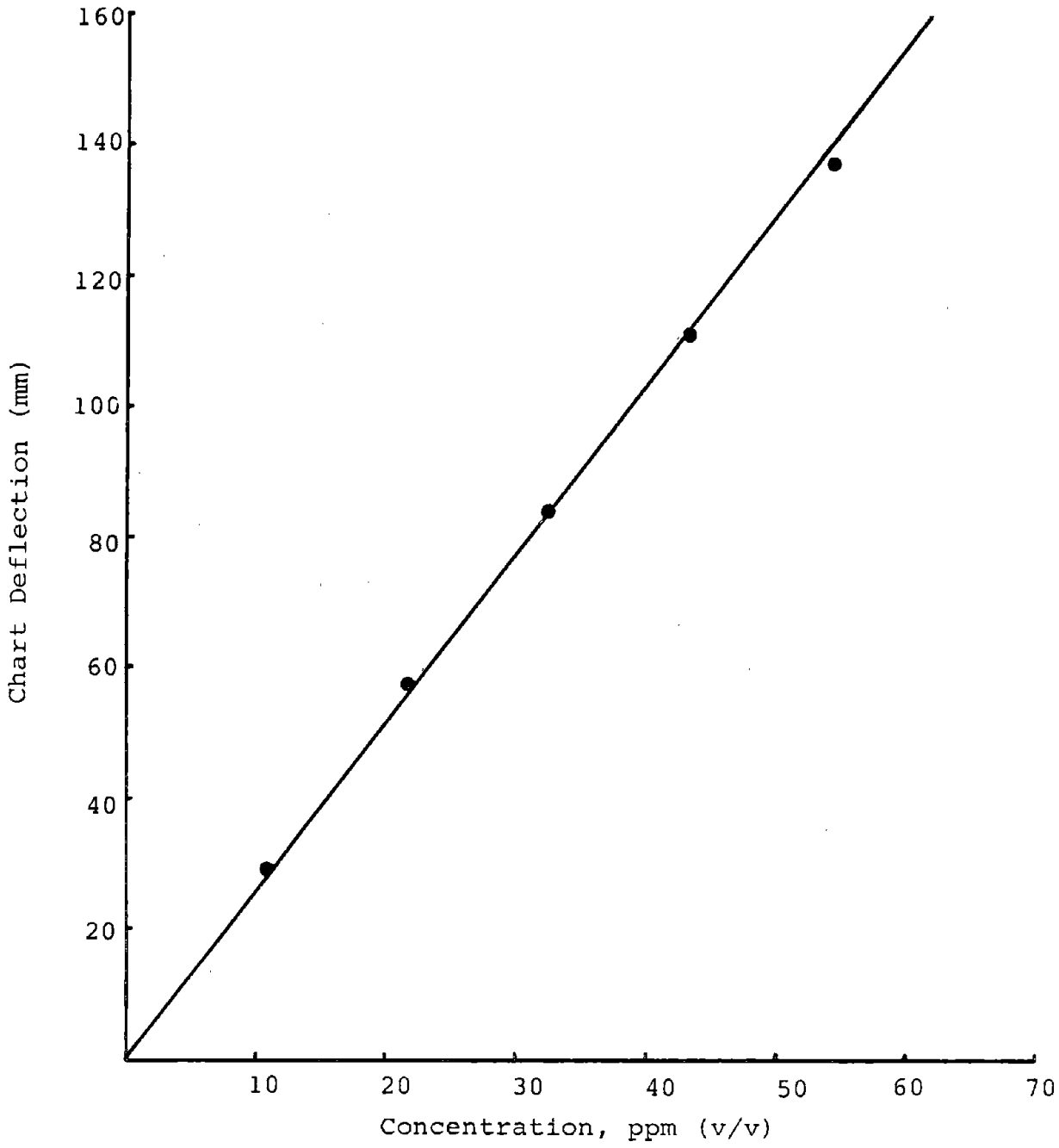


FIGURE 3

2-Methoxyethanol

Sample Record Chart of IR Absorption at 8.8 µm

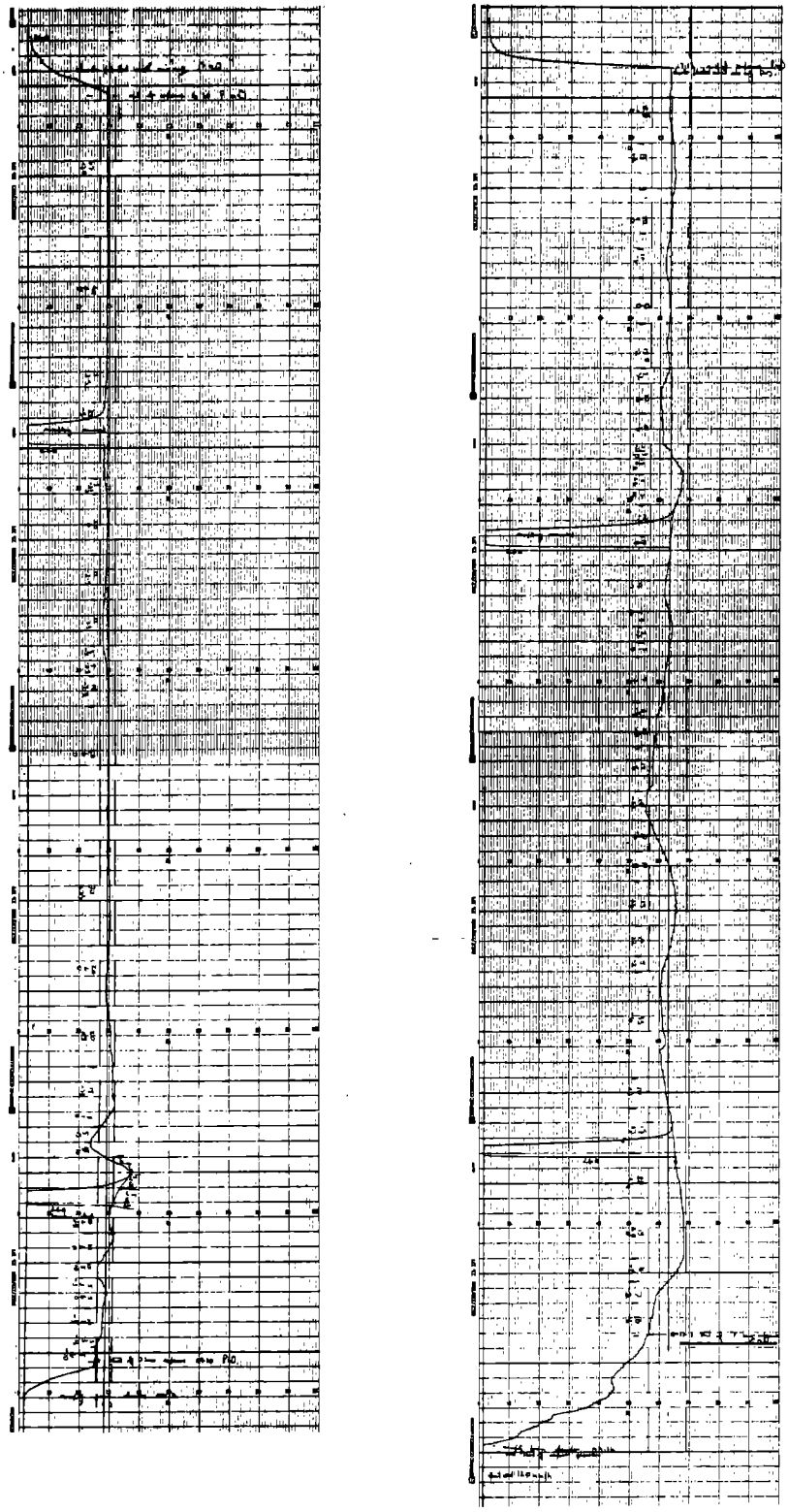


FIGURE 4

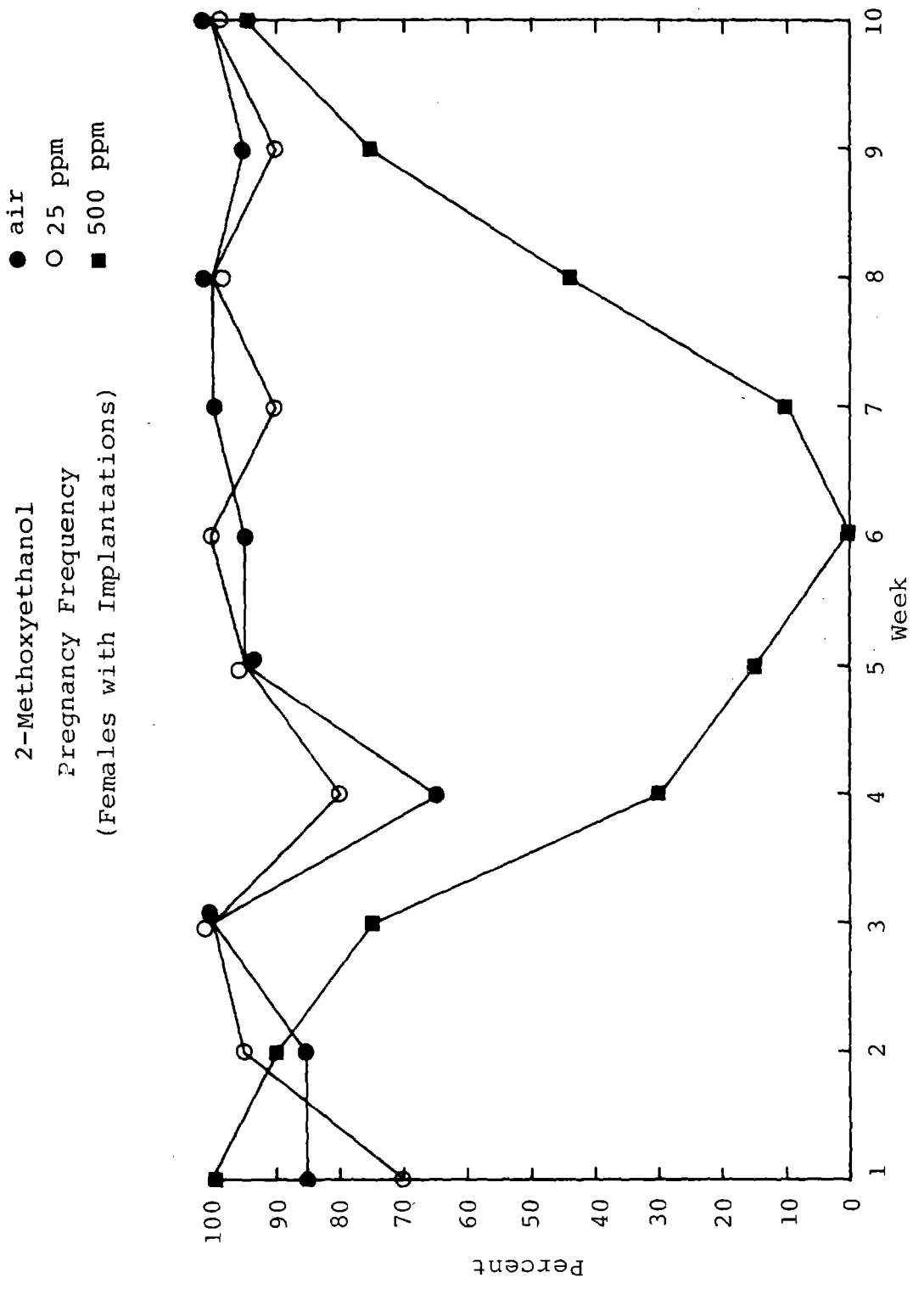

2-Methoxyethanol
Pregnancy Frequency
(Females with Implantations)
● air
○ 25 ppm
■ 500 ppm
Percent
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
Week

FIGURE 5

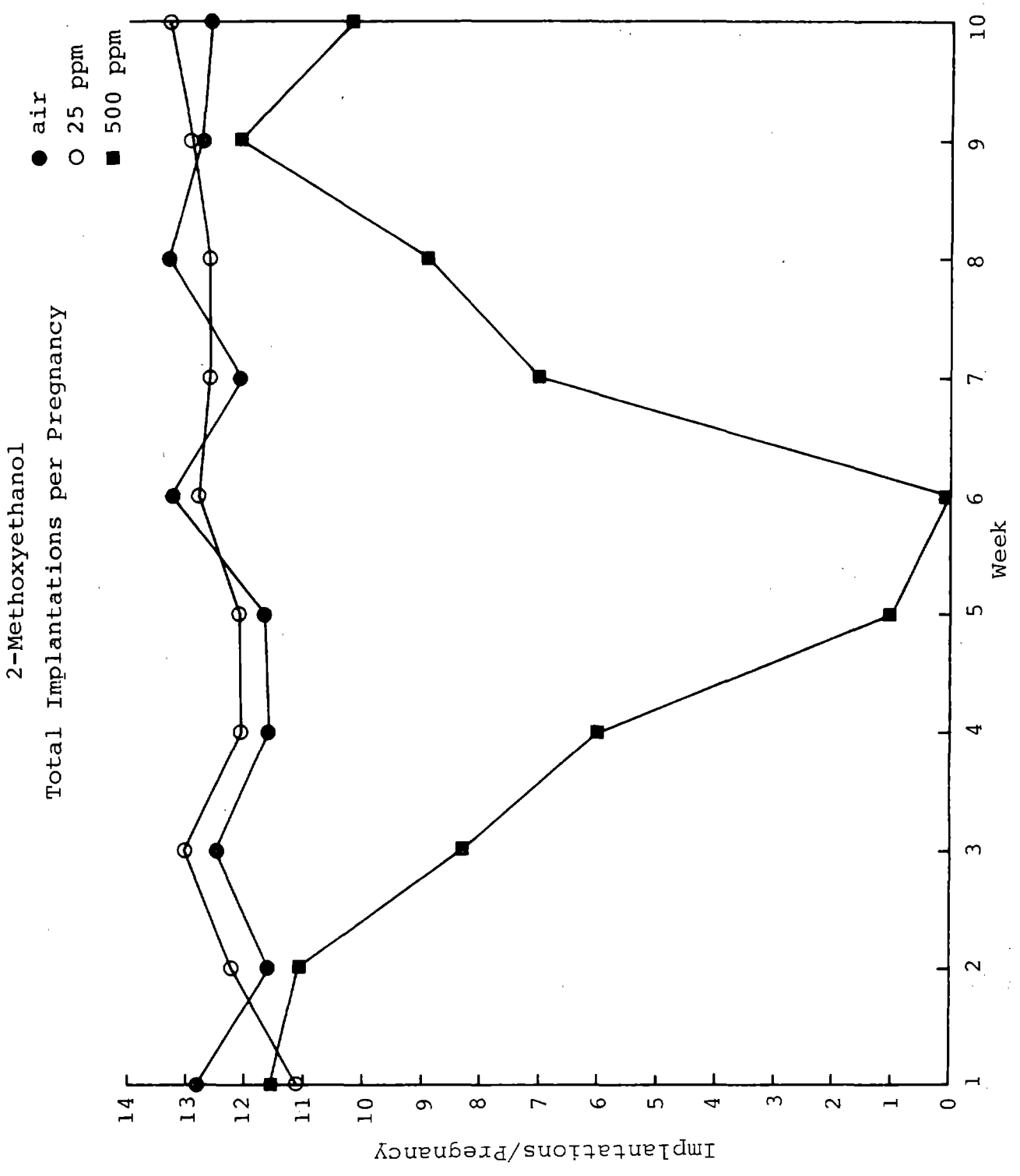
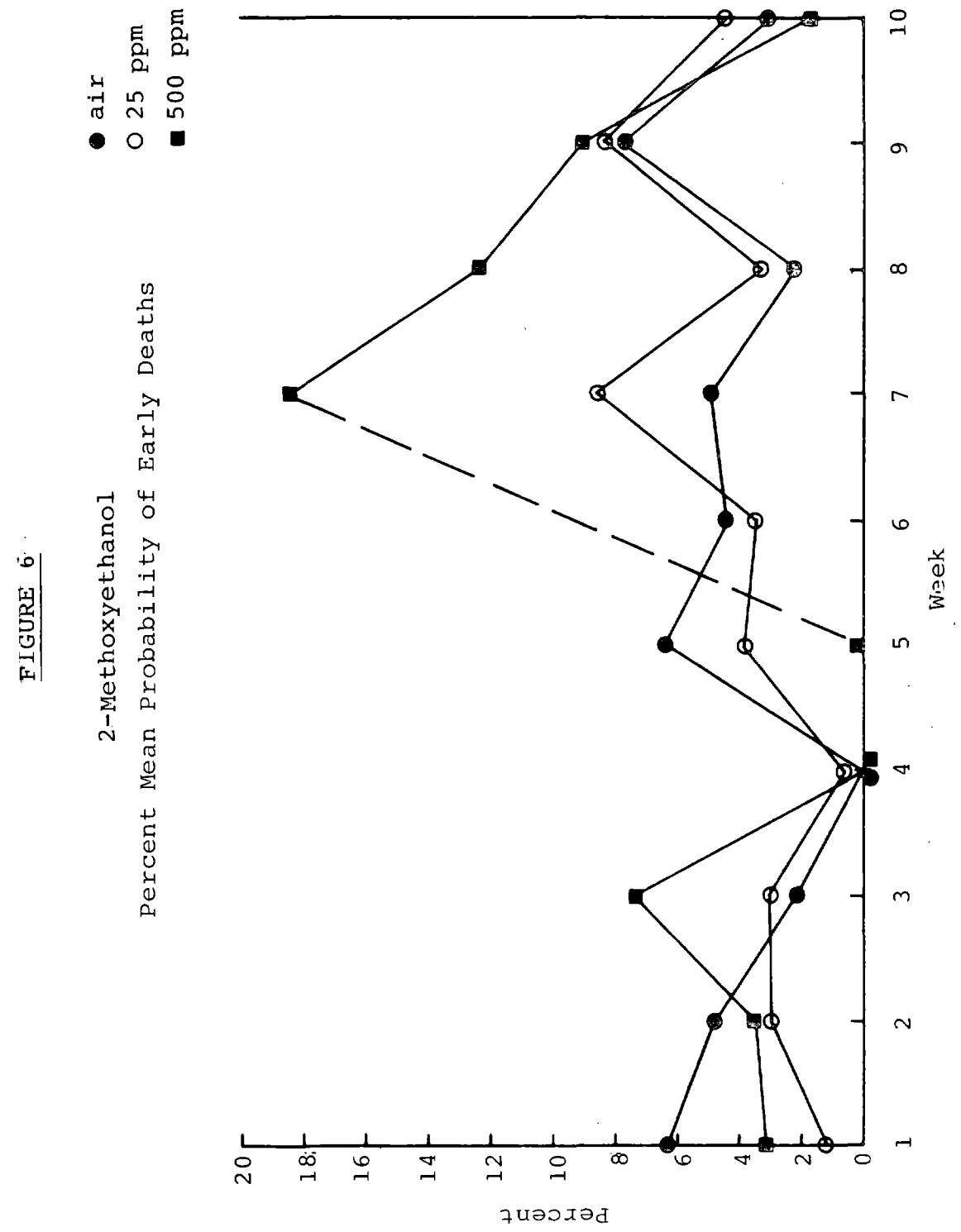

2-Methoxyethanol
Total Implantations per Pregnancy
● air
○ 25 ppm
■ 500 ppm
Implantations/Pregnancy
Week
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14

FIGURE 6


2-Methoxyethanol
Percent Mean Probability of Early Deaths
● air
○ 25 ppm
■ 500 ppm
Percent
Week
0
2
4
6
8
10
12
14
16
18
20
1
2
3
4
5
6
7
8
9
10

APPENDIX DIET

2-Methoxyethanol
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: SPRATT'S LABORATORY DIET 1

Batch No: 237825

Date of Manufacture: 23rd October 1978

	Found Analysis
Moisture	6.4%
Crude Fat	3.2%
Crude Protein	20.8%
Ash	5.6%
Calcium	1.0%
Phosphorus	0.78%
Nitrate	< 1.0 mg/kg
Nitrite	2.78 mg/kg
Selenium	0.32 mg/kg
Lead	< 1.0 mg/kg
Arsenic	< 0.2 mg/kg
Cadmium	< 0.2 mg/kg
Mercury	0.045mg/kg
Aflatoxins	None detected
Total P.C.B.	None detected
Total D.D.T.	0.085mg/kg
Dieldrin	0.003mg/kg
Lindane	0.003mg/kg
Heptachlor	None detected
Malathion	0.034mg/kg
Total Viable Organisms	5.0×10^3/gm
E. Coli Type 1	None detected
Salmonella Species	None detected
Moulds	75/gm

Signed .....

Date 25th January 1979

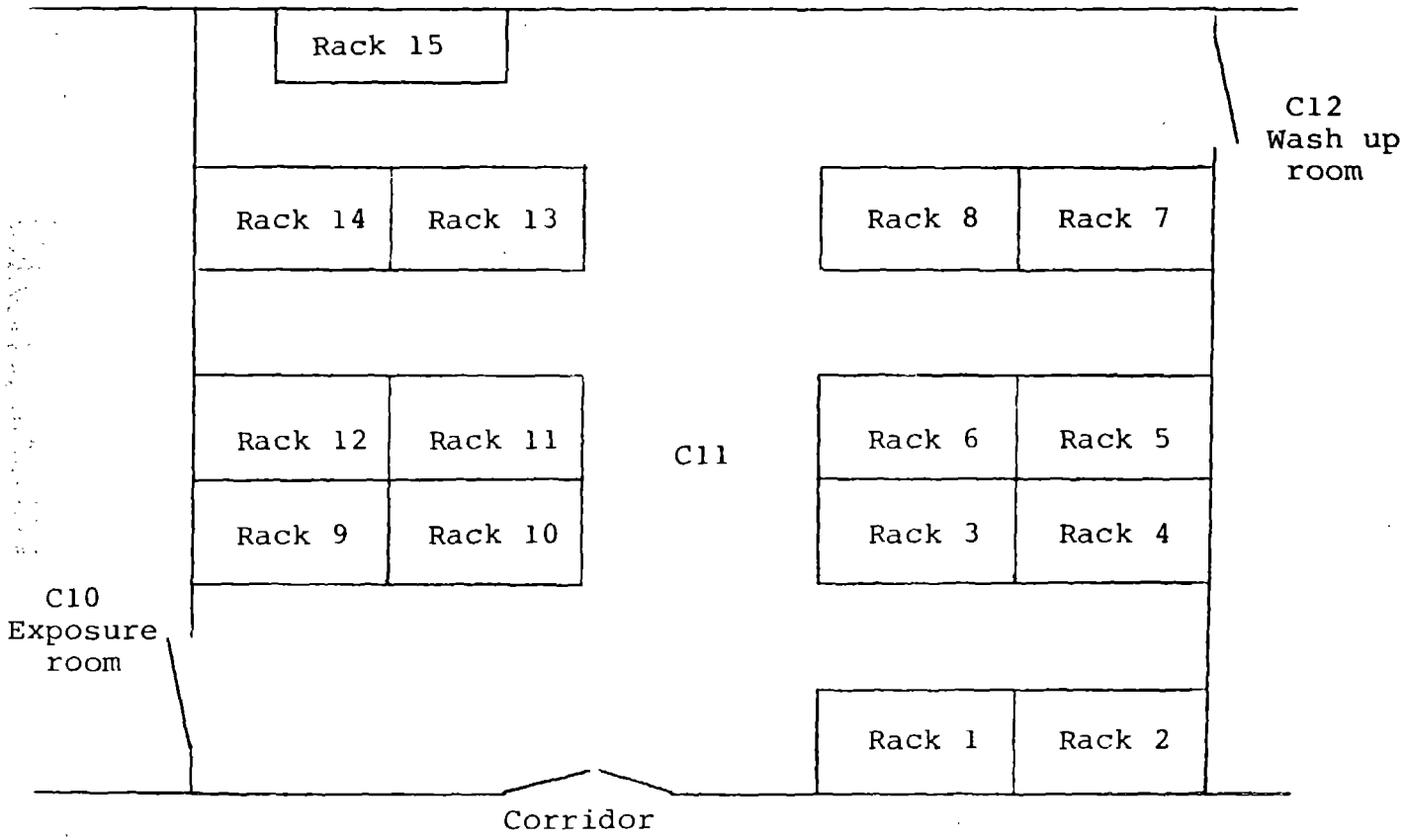
Registered in England Number 536740
Registered Office Old Change House,
4-8 Cannon Street, London EC4M 6XB

A member of the Spillers Group

APPENDIX Loc-1

2-Methoxyethanol

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

2-Methoxyethanol
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 Sub Acute Cytogen ♂ 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)

2-Methoxyethanol
Exposure Location Sheet

Project No: 409951

Test Compound: Air Control

Exposure Chamber No: 1

Day of Study: 2

Test Concentration: 0

Tier No: 2

Dominant Lethal ♂
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)

2-Methoxyethanol
Exposure Location Sheet

Project No: 409959

Test Compound: 2-Methoxyethanol

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: Sub Acute Cyt ♀			
291	292	293	294
295	296	297	298
299	300	-	-
-	-	-	-

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

RIGHT

Signed: _____ Date: _____

APPENDIX Loc-2 (continued)

2-Methoxyethanol
Exposure Location Sheet

Project No: 409959

Test Compound: 2-Methoxyethanol

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: Sub Acute Cyt ♀			
301	302	303	304
305	306	307	308
309	310	-	-
-	-	-	-

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

RIGHT

Signed: _____ Date: _____

APPENDIX FORM-1

2-Methoxyethanol

CYTOGENETIC SCORE SHEET **NIOSH**

Contract No. 210-78-0026 **Substance:**

Assessor: **Decoded group:** **Decoded animal No.:**

Signature: **Slide No.:**

Date(s) **Acute/Sub-acute** **Time(h):**

Slide quality:

Excellent _____ **Good** _____ **Acceptable** _____ **Poor** _____ **Uncountable** _____

Vernier	Chromatid			Chromosomal			Markers			Multi-Abn.	Misc. Abn.	Obser-vation
	GAP	BmF	Bw/oF	GAP	BmF	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

2-Methoxyethanol

Assessors	Signature

Contract No. 210-78-0026

NIOSH

DOMINANT LETHAL ASSESSMENT

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

2-Methoxyethanol

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

2-Methoxyethanol
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	314	316	323	325	304
	122	290	292	294	280	297
	123	287	291	266	303	304
	124	297	301	305	308	306
	125	314	315	317	307	320
	126	314	311	319	325	321
	127	300	300	306	308	314
	128	289	285	294	298	293
	129	286	313	317	321	319
	130	314	329	337	344	352
	Mean ± S.D.	301 ± 12	305 ± 14	311 ± 14	312 ± 18	313 ± 17

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	281	219	221	222	216	204
	282	200	204	206	206	192
	283	222	226	230	230	227
	284	198	196	201	197	203
	285	235	236	239	239	240
	286	217	217	217	215	217
	287	197	197	201	201	203
	288	220	222	223	227	228
	289	234	233	228	233	240
	290	206	203	205	200	204
	Mean ± S.D.	215 ± 14	216 ± 15	217 ± 13	216 ± 15	216 ± 17

APPENDIX TABLE BW-1 (continued)

2-Methoxyethanol

Multiple Dosing: 25 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	131	307	315	317	300	320
	132	289	300	301	302	305
	133	322	327	325	326	330
	134	310	318	317	320	324
	135	305	312	312	318	324
	136	303	311	316	319	326
	137	286	288	285	288	288
	138	323	332	330	333	339
	139	331	340	339	341	345
	140	308	314	315	320	324
	Mean ± S.D.	308 ± 14	316 ± 15	316 ± 15	317 ± 16	323 ± 16

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	291	186	187	184	189	189
	292	237	228	236	238	242
	293	211	216	213	217	215
	294	211	209	211	214	210
	295	202	201	201	204	204
	296	182	183	186	190	187
	297	216	216	215	221	220
	298	208	204	204	208	208
	299	210	205	210	215	215
	300	219	219	219	221	221
	Mean ± S.D.	208 ± 16	207 ± 14	208 ± 15	212 ± 15	211 ± 16

APPENDIX TABLE BW-1 (continued)

2-Methoxyethanol

Multiple Dosing: 500 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	141	287	290	285	291	289
	142	286	286	284	293	296
	143	300	297	292	297	300
	144	300	298	292	297	302
	145	310	305	303	306	314
	146	295	295	286	280	278
	147	309	310	307	307	312
	148	300	300	290	296	297
	149	285	300	294	302	306
	150	311	314	304	314	315
	Mean ± S.D.	298 ± 10	300 ± 9	294 ± 8	298 ± 10	301 ± 12

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	301	230	230	233	237	244
	302	226	229	230	232	241
	303	195	197	195	196	201
	304	227	221	221	229	230
	305	210	208	208	204	209
	306	212	204	210	213	218
	307	204	204	204	207	216
	308	231	225	177	178	181
	309	223	223	225	227	227
	310	210	210	210	212	215
	Mean ± S.D.	217 ± 12	215 ± 12	211 ± 17	214 ± 18	218 ± 19

APPENDIX TABLE BW-1 (continued)

2-Methoxyethanol

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	151	295	293	292	284	281
	152	295	295	288	278	270
	153	292	285	270	256	243
	154	313	307	301	295	290
	155	300	293	290	275	266
	156	307	310	308	296	290
	157	325	327	317	301	287
	158	329	326	318	307	304
	159	318	310	298	283	273
	160	317	309	294	274	266
	Mean ± S.D.	309 ± 13	306 ± 14	298 ± 14	285 ± 15	277 ± 17

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	311	206	200	191	184	178
	312	209	203	199	198	189
	313	221	217	212	208	202
	314	190	190	190	191	183
	315	192	192	183	181	176
	316	222	217	214	210	205
	317	208	214	208	203	198
	318	205	200	201	198	192
	319	224	219	213	208	200
	320	216	209	210	208	205
	Mean ± S.D.	209 ± 12	206 ± 11	202 ± 11	199 ± 11	193 ± 11

APPENDIX TABLE BW-2

2-Methoxyethanol
Single Exposure Cytogenetics Test
Individual Body Weights (g)

Air Control (0 ppm)

Sex	Animal Number	6 h Acute Weight	Animal Number	24 h Acute Weight	Animal Number	48 h Acute Weight
Male	1	304	11	308	21	284
	2	314	12	290	22	292
	3	292	13	293	23	295
	4	305	14	277	24	284
	5	297	15	282	25	293
	6	316	16	295	26	301
	7	300	17	298	27	321
	8	274	18	280	28	277
	9	304	19	280	29	285
	10	296	20	308	30	300
	Mean	300		291		293
	± S.D.	± 12		± 11		± 12

Sex	Animal Number	6 h Acute Weight	Animal Number	24 h Acute Weight	Animal Number	48 h Acute Weight
Female	161	225	171	218	181	226
	162	202	172	235	182	200
	163	201	173	191	183	228
	164	221	174	198	184	242
	165	189	175	203	185	211
	166	222	176	209	186	202
	167	186	177	241	187	209
	168	244	178	200	188	207
	169	197	179	203	189	221
	170	193	180	193	190	219
	Mean	208		209		217
	± S.D.	± 19		± 17		± 13

APPENDIX TABLE BW-2 (continued)

2-Methoxyethanol

Single Dosing: 25 ppm

Sex	Animal Number	6 h Acute Weight	Animal Number	24 h Acute Weight	Animal Number	48 h Acute Weight
Male	31	268	41	301	51	310
	32	287	42	269	52	285
	33	310	43	295	53	306
	34	302	44	293	54	303
	35	320	45	283	55	301
	36	289	46	303	56	312
	37	288	47	293	57	281
	38	291	48	312	58	292
	39	297	49	282	59	309
	40	292	50	201	60	278
	Mean	294		289		298
	± S.D.	± 14		± 16		± 13

Sex	Animal Number	6 h Acute Weight	Animal Number	24 h Acute Weight	Animal Number	48 h Acute Weight
Female	191	191	201	193	211	242
	192	229	202	193	212	203
	193	230	203	186	213	221
	194	203	204	218	214	199
	195	195	205	215	215	191
	196	216	206	212	216	180
	197	207	207	215	217	220
	198	201	208	195	218	204
	199	202	209	195	219	196
	200	192	210	214	220	215
	Mean	207		204		207
	± S.D.	± 14		± 12		± 18

APPENDIX TABLE BW-2 (continued)

2-Methoxyethanol

Single Dosing: 500 ppm

Sex	Animal Number	6 h Acute Weight	Animal Number	24 h Acute Weight	Animal Number	48 h Acute Weight
Male	61	337	71	298	81	263
	62	287	72	292	82	308
	63	265	73	315	83	286
	64	291	74	312	84	296
	65	286	75	283	85	338
	66	280	76	301	86	257
	67	277	77	286	87	290
	68	276	78	275	88	281
	69	304	79	295	89	263
	70	300	80	315	90	281
	Mean	290		297		286
	± S.D.	± 20		± 14		± 24

Sex	Animal Number	6 h Acute Weight	Animal Number	24 h Acute Weight	Animal Number	48 h Acute Weight
Female	221	187	231	235	241	205
	222	186	232	179	242	208
	223	205	233	207	243	221
	224	208	234	188	244	239
	225	193	235	221	245	232
	226	190	236	231	246	227
	227	220	237	185	247	208
	228	213	238	185	248	227
	229	224	239	222	249	219
	230	183	240	174	250	194
	Mean	201		203		218
	± S.D.	± 15		± 23		± 14

APPENDIX TABLE BW-2 (continued)

2-Methoxyethanol

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sex	Animal Number	6 h Acute Weight	Animal Number	24 h Acute Weight	Animal Number	48 h Acute Weight
Male	91	320	101	320	111	266
	92	262	102	275	112	315
	93	278	103	311	113	270
	94	294	104	275	114	287
	95	308	105	303	115	299
	96	283	106	295	116	290
	97	292	107	284	117	288
	98	301	108	260	118	280
	99	292	109	289	119	283
	100	289	110	307	120	288
	Mean	292		292		287
	± S.D.	± 16		± 19		± 14

Sex	Animal Number	6 h Acute Weight	Animal Number	24 h Acute Weight	Animal Number	48 h Acute Weight
Female	251	205	261	200	271	207
	252	221	262	196	272	207
	253	175	263	202	273	217
	254	206	264	187	274	226
	255	189	265	218	275	188
	256	232	266	197	276	224
	257	201	267	217	277	226
	258	225	268	218	278	233
	259	188	269	190	279	200
	260	190	270	235	280	221
	Mean	203		206		215
	± S.D.	± 18		± 15		± 14

APPENDIX TABLE BW-3

2-Methoxyethanol
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	324	339	341	343	352
	362	302	306	311	314	321
	363	306	308	316	312	318
	364	355	357	368	365	369
	365	306	310	306	312	316
	366	308	317	324	320	302
	367	342	352	355	358	366
	368	347	351	357	355	338
	369	342	349	352	354	359
	370	330	335	332	341	341
	Mean ± S.D.	326.0 ± 20	332 ± 20	336 ± 22	337 ± 21	338 ± 23

APPENDIX TABLE BW-3 (continued)

2-Methoxyethanol

Multiple Dosing: 25 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	371	320	327	327	334	334
	372	327	335	342	345	347
	373	305	315	317	322	326
	274	267	281	286	291	297
	375	280	287	282	289	296
	376	322	331	333	311	298
	377	317	322	322	326	330
	378	304	306	301	302	310
	379	353	353	356	355	370
	380	311	319	320	324	329
	Mean ± S.D.	311 ± 24	318 ± 22	319 ± 23	320 ± 22	324 ± 24

APPENDIX TABLE BW-3 (continued)

2-Methoxyethanol

Multiple Dosing: 500 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	381	317	322	324	317	324
	382	327	326	321	324	331
	383	290	288	282	280	284
	384	304	301	297	293	295
	385	305	302	300	300	305
	386	315	315	316	320	325
	387	305	307	301	300	308
	388	294	294	287	286	288
	389	309	303	303	301	305
	390	315	318	311	305	313
	Mean ±S.D.	308 ± 11	308 ± 12	304 ± 14	303 ± 14	308 ± 16

APPENDIX TABLE BW-3 (continued)

2-Methoxyethanol

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	391	306	302	298	288	279
	392	315	305	298	287	271
	393	308	307	304	297	291
	394	313	305	300	286	275
	395	321	306	310	298	288
	396	330	322	312	297	284
	397	328	320	314	297	289
	398	317	309	302	296	287
	399	346	337	326	317	305
	400	344	330	322	305	286
	Mean	323	314	309	297	286
	± S.D.	± 14	± 12	± 10	± 9	± 9

APPENDIX TABLE BW-4

2-Methoxyethanol
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	30	27	30	25	26
	322	29	25	30	24	25
	323	28	26	30	25	25
	324	31	28	32	28	28
	325	28	25	28	24	24
	326	30	25	30	24	25
	327	30	26	30	25	26
	328	31	26	30	25	25
	329	29	25	30	24	25
	330	31	26	31	26	26
	Mean ± S.D.	29.7 ± 1.2	25.9 ± 1.0	30.1 ± 1.0	25.0 ± 1.2	25.5 ± 1.1

APPENDIX TABLE BW-4 (continued)

2-Methoxyethanol

Multiple Dosing: 25 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	331	27	28	27	27	27
	332	26	25	25	24	25
	333	25	26	25	25	25
	334	27	27	26	25	25
	335	26	26	26	26	26
	336	25	25	25	25	25
	337	26	25	25	25	26
	338	26	26	26	26	26
	339	25	25	24	23	24
	340	26	25	25	25	25
	Mean ± S.D.	25.9 ± 0.7	25.8 ± 1.0	25.4 ± 0.8	25.1 ± 1.1	25.4 ± 0.8

APPENDIX TABLE BW-4 (continued)

2-Methoxyethanol

Multiple Dosing: 500 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	341	27	24	22	22	23
	342	30	28	26	25	26
	343	28	25	24	24	24
	344	29	27	25	24	25
	345	29	25	24	23	23
	346	30	27	26	25	26
	347	28	25	23	23	23
	348	28	25	24	23	24
	349	28	27	25	24	24
	350	28	26	24	24	23
	Mean ± S.D.	28.5 ± 1.0	25.9 ± 1.3	24.3 ± 1.3	23.7 ± 0.9	24.1 ± 1.2

APPENDIX TABLE BW-4 (continued)

2-Methoxyethanol

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	351	28	28	28	29	26
	352	26	26	26	26	27
	353	28	28	27	28	29
	354	29	30	29	30	30
	355	25	25	25	27	27
	356	28	27	27	28	28
	357	26	24	24	26	26
	358	26	25	23	25	25
	359	27	27	27	28	28
	360	28	27	27	27	26
	Mean ± S.D.	27.1 ± 1.3	26.7 ± 1.8	26.3 ± 1.8	27.4 ± 1.5	27.2 ± 1.5

APPENDIX TABLE CA-MD-M

2-Methoxyethanol
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	108/4	50	25	23	1							58.0 x 107.2
					1							72.0 x 102.0
	108/5		25	24	1							46.4 x 105.0
124	20/3	50	21	20	1							41.1 x 106.8
	20/4		14	13	1							37.8 x 105.3
	20/5		10	9	1							45.6 x 103.8
	20/2		5	5								
125	100/6	50	25	25								
	100/7		25	23	1							32.1 x 113.3
					1							61.9 x 111.5
123	26/3	50	25	21	1							39.5 x 112.7
					1							56.4 x 110.6
					1							36.4 x 108.6
					1							47.7 x 106.8
	26/4		25	22	1							34.5 x 112.0
					1							56.9 x 111.6
					1							35.3 x 109.0
127	158/4	50	25	22	1							38.9 x 108.7
					1							45.6 x 104.9
					1							36.0 x 104.3
	158/5		25	23	1							59.3 x 110.7
					1							42.1 x 109.1
129	140/4	50	25	22	1							51.1 x 112.5
					1							49.9 x 110.7
						1						31.7 x 106.3

APPENDIX TABLE CA-MD-M (continued)

2-Methoxyethanol

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
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
129	140/5		25	23	1							48.2 x 106.1
					1							47.2 x 103.7
130	106/3	50	25	23	1							62.0 x 107.8
					1							39.2 x 102.3
	106/4		22	21	1							62.2 x 103.0
	106/5		3	3								
128	157/1	19	8	6	2							43.8 x 107.8
					1						1 Chromatid Fragment	41.6 x 107.8
	157/2		8	7	1							59.1 x 97.0
	157/3		3	3								
	157/4-5		0	0								
126	56/6	11	5	4	1							59.0 x 107.7
	56/7		6	5		1						63.7 x 111.5
	56/1-5		0	0								
122	41/4	50	25	22	1							57.0 x 108.2
					1							48.6 x 102.0
					1							34.6 x 101.5
	41/5		17	16	1							53.4 x 104.8
	41/3		8	7	1							56.5 x 105.8

APPENDIX TABLE CA-MD-M (continued)

2-Methoxyethanol

Males

Multiple Dosing: 25 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		
132	50/1	50	25	23	1							51.0 x 106.8
					1							33.2 x 99.7
	50/3		14	13	1							37.8 x 99.0
	50/7		11	11								
137	47/3	50	25	24	1							34.6 x 107.1
	47/5		25	25								
133	66/6	50	25	22	1							47.1 x 106.3
					1							45.2 x 105.9
					1							58.0 x 104.9
	66/7		25	23					1			43.4 x 109.2
					1							62.4 x 109.0
135	5/5	50	25	22	1							55.7 x 110.5
					1							38.0 x 109.4
					1							57.3 x 108.3
	5/4		25	22	1							44.7 x 110.5
					1							40.2 x 108.7
					1							66.4 x 106.4
134	52/5	50	25	23	1							67.5 x 112.0
						1						32.6 x 111.0
	52/1		25	24	1							36.2 x 110.5
131	67/4	50	25	25								
	67/1		25	24	1							41.9 x 98.0

APPENDIX TABLE CA-MD-M (continued)

2-Methoxyethanol
Males

Multiple Dosing: 25 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	119/2	50	22	21	1							53.2 x 99.9
	119/3		25	23	1							52.6 x 104.3
							1					45.4 x 103.8
	119/4		3	3								
138	9/5	50	9	9								
			7	5	1							58.5 x 107.5
					1							43.8 x 100.4
	9/3		21	19	1							55.9 x 106.1
					1							60.5 x 94.7
	9/2		13	12	1							39.3 x 108.9
139	137/3	50	25	24				1				35.3 x 109.5
	137/4		25	25								
136	59/6	50	25	21	1							32.4 x 110.7
					1							60.3 x 110.1
								1				55.2 x 109.5
							1					41.1 x 108.3
	59/7		25	23	1							56.7 x 110.8
					1							35.9 x 110.5

APPENDIX TABLE CA-MD-M (continued)

2-Methoxyethanol

Males

Multiple Dosing: 500 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		
145	38/2	50	25	24	1							29.5 x 104.7
	38/4		25	25								
141	121/3	50	25	23	1							71.2 x 113.2
					1							57.4 x 112.0
	121/5		25	24	1							62.7 x 108.8
142	138/3	50	25	23	1							42.8 x 111.0
									1			56.7 x 107.4
	138/4		25	24	1							55.5 x 110.7
150	4/1	50	25	24	1		1					53.9 x 111.4
	4/3		25	23	1							51.4 x 111.9
					1							60.0 x 108.9
143	97/1	50	25	22	1							43.2 x 105.7
					1							55.2 x 104.4
					1							59.4 x 103.5
	97/2		25	22	1							46.0 x 107.4
					1							55.3 x 103.0
					1							58.6 x 103.2
144	39/6	50	25	21	1							35.3 x 109.4
					1							30.3 x 108.7
					1							63.2 x 107.9
					1							32.8 x 106.9
	39/7		25	23	1							40.0 x 111.8
					1							60.2 x 109.8

APPENDIX TABLE CA-MD-M (continued)

2-Methoxyethanol
Males

Multiple Dosing: 500 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		
148	48/6	50	25	22	1							59.8 x 110.5
					1							31.5 x 110.0
					1							39.0 x 109.0
	48/7		25	22	1							24.4 x 108.5
					1							30.8 x 107.8
					1							54.2 x 107.5
149	11/3	50	25	21	1							38.8 x 112.4
					1							35.1 x 112.0
					1							58.9 x 111.8
					1							59.4 x 111.1
	11/4		25	21	1							43.0 x 111.8
					1			1				44.0 x 110.8
					1							45.1 x 108.6
					1							41.1 x 107.9
147	64/1	50	25	21	1							40.8 x 112.3
					1							47.5 x 112.3
							1					46.3 x 110.2
								1				37.0 x 109.4
	64/2		25	22	1							37.3 x 112.5
					1							58.0 x 111.9
								1				62.6 x 111.4

APPENDIX TABLE CA-MD-M (continued)

2-Methoxyethanol
Males

Multiple Dosing: 500 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		
146	23/5	50	25	22	1							60.8 x 110.6
					1							58.0 x 107.5
					1							54.5 x 106.4
	23/4		20	18	1							45.0 x 103.7
					1							45.1 x 103.2
	23/2		5	4	1							60.6 x 104.6

APPENDIX TABLE CA-MD-M (continued)

2-Methoxyethanol

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
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
152	116/4	50	25	23	1							57.9 x 105.6
						1						34.0 x 105.7
	116/5		25	22		1		1				70.2 x 106.8
							1					60.5 x 107.1
					1							61.7 x 103.2
158	61/3	50	25	21	1							62.4 x 111.0
					1							41.7 x 109.5
					1							63.6 x 109.1
					1							63.8 x 106.4
	61/5		25	18	1							64.6 x 110.9
					1							39.8 x 109.7
					2							62.0 x 109.6
					1							70.3 x 109.7
					2							69.4 x 109.0
					1							63.5 x 109.0
					1							58.0 x 108.9
160	8/1	50	25	19	1							38.9 x 112.6
					1							56.8 x 111.7
					1							39.2 x 111.8
					1							43.9 x 111.1
					1							45.9 x 110.8
					1							60.0 x 110.7

APPENDIX TABLE CA-MD-M (continued)

2-Methoxyethanol

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
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
160	8/2		25	20	1							36.8 x 110.3
					1							61.1 x 107.8
					1							58.0 x 107.8
					1							60.5 x 105.5
					1	1						69.2 x 104.4
153	55/4	50	25	22	1							54.0 x 108.8
					2							41.5 x 107.9
					2							35.6 x 107.0
	55/5		25	22			1					44.6 x 105.6
					1							47.4 x 104.2
					1							50.4 x 104.1
159	117/2	50	25	21	1							40.2 x 111.3
					1							60.1 x 108.4
					1							32.4 x 106.9
					1							47.6 x 105.9
	117/1		25	22	2							34.9 x 109.2
					1							67.3 x 103.1
					1							34.8 x 101.9
155	76/1	50	5	4	1							34.7 x 106.2
	76/2		6	4	1							67.0 x 102.5
					1							70.5 x 99.3
	76/3		7	5	1							63.4 x 114.2
					1							57.4 x 101.0

APPENDIX TABLE CA-MD-M (continued)

2-Methoxyethanol

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
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
155	76/4		17	14	2							37.3 x 111.9
					1							35.0 x 109.9
					1							56.7 x 105.9
	76/5		15	13	1							39.7 x 109.4
					1							54.4 x 103.4
156	115/4	50	25	21	1							49.4 x 110.5
					1							58.8 x 107.3
					1							52.5 x 105.9
					1	1						52.3 x 105.3
	115/2		25	21		1						60.3 x 108.7
					1							53.1 x 106.4
					1							48.4 x 106.5
					1							55.6 x 103.7
151	142/2	50	23	22	1							57.1 x 103.0
	142/3		25	22	1							60.6 x 109.5
					1							63.7 x 107.5
					1							64.2 x 105.8
	142/4		2	1	1							38.7 x 102.0
154	74/1-7	0	0	0								

APPENDIX TABLE CA-MD-F

2-Methoxyethanol
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
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
286	216/3	50	16	15	1							55.5 x 101.4
	216/4		13	11	1							63.1 x 102.6
					1							45.0 x 100.2
	216/5		21	17	1							58.8 x 108.5
					1							54.4 x 107.0
					1							54.9 x 105.6
					1							49.5 x 102.0
287	318/4	50	19	18	1							40.3 x 106.3
	318/3		21	20	1							40.6 x 99.3
	318/1		10	10								
283	186/6	50	25	22	2							57.9 x 111.3
					1							60.7 x 107.6
					1							44.0 x 105.3
	186/7		25	24	2							50.1 x 105.2
289	300/2	50	25	24	1							59.4 x 104.9
	300/1		25	23	1							48.2 x 104.9
					1							48.9 x 104.1
282	201/6	50	25	23	1							67.8 x 111.9
					1							35.5 x 106.7
	201/7		25	24	1							64.5 x 110.7

APPENDIX TABLE CA-MD-F (continued)

2-Methoxyethanol
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
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
288	317/6	50	25	18	1							34.6 x 111.3
					1							63.0 x 110.8
					1							35.3 x 110.0
					1							53.8 x 109.8
					1							36.5 x 109.4
								1				33.1 x 109.4
					1							34.9 x 109.0
	317/7		25	21	1							53.5 x 111.9
					2							33.5 x 108.5
					1							57.2 x 107.9
					1							39.6 x 106.4
290	266/1	50	11	10	1							40.6 x 98.2
	266/2		2	1	1							36.4 x 109.9
	266/3		19	16	1							62.4 x 106.9
					1							36.9 x 104.2
					1							44.6 x 104.4
	266/4		18	15	1							45.7 x 109.5
					1							37.1 x 108.1
					1							55.1 x 105.1
281	268/2	50	25	24	1							60.5 x 106.9
	268/3		16	16								
	268/1		9	9								

APPENDIX TABLE CA-MD-F (continued)

2-Methoxyethanol

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		
285	260/1	50	25	25								
	260/3		6	6								
	260/4		19	16	1							48.6 x 103.4
					1							55.3 x 101.3
						1						61.8 x 101.4
284	180/1	42	10	10								
	180/2		5	5								
	180/3		2	2								
	180/4		11	11								
	180/5		14	13	1							39.2 x 102.6

APPENDIX TABLE CA-MD-F (continued)

2-Methoxyethanol

Females

Multiple Dosing: 25 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		
299	297/1-5	0	0									
300	279/1	50	25	22	1							56.0 x 113.4
					1							59.3 x 107.2
					1							51.4 x 106.4
	279/7		25	21				1				59.5 x 109.6
						1						40.4 x 109.1
					1							38.2 x 105.6
					1							45.1 x 104.2
296	219/5	50	13	12		1						53.7 x 104.2
	219/6		22	17	1							45.0 x 113.6
					1							57.8 x 110.9
						1						56.8 x 110.3
					1							66.2 x 107.2
					1							40.6 x 95.2
	219/7		13	12	1							45.1 x 100.0
	219/4		2	1	1							47.9 x 107.5
291	227/1-7	0	0	0								
297	207/6	50	25	23	1							30.2 x 105.1
					1							38.6 x 105.2
	207/7		25	24	1							30.8 x 106.8
293	226/1-7	0	0	0								
295	165/1	50	25	21	1							61.7 x 107.7
					2							59.1 x 107.7
					1							33.4 x 107.1
					1							32.9 x 106.0

APPENDIX TABLE CA-MD-F (continued)

2-Methoxyethanol
Females

Multiple Dosing: 25 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	165/2		16	15	1							61.0 x 104.1
	165/3		9	7	1							41.1 x 108.9
					1							52.3 x 108.0
292	210/7	12	12	10	2							47.5 x 107.1
					1							60.0 x 106.0
	210/1-6		0	0								
298	169/4	50	25	20	1							51.4 x 109.3
							1					57.1 x 108.2
					1							58.9 x 107.4
					1							42.1 x 105.3
					1							53.7 x 104.2
	169/1		25	22			1					57.4 x 106.3
					1							66.2 x 104.8
					1							57.3 x 104.8
294	212/3	50	25	23	1							42.9 x 106.4
					1							49.6 x 101.6
	212/4		13	11		1						36.8 x 102.8
					1							51.5 x 99.2
	212/2		12	11	1							54.5 x 99.7

APPENDIX TABLE CA-MD-F (continued)

2-Methoxyethanol
Females

Multiple Dosing: 500 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		
302	298/3	50	25	25								
	298/4		25	21	1							47.1 x 107.9
					1							39.2 x 105.6
					1							44.5 x 104.4
					1							35.9 x 104.6
305	198/1	38	7	7								
	198/2		10	9		1						62.5 x 101.6
	198/3		8	7	1							41.7 x 103.7
	198/4-5		0	0								
	198/6		10	10								
	198/7		3	2	1							59.8 x 110.3
308	208/1	50	25	20	1							56.5 x 110.8
					1							44.1 x 108.0
					1						1 Chromatid Fragment	35.0 x 106.4
					1							54.5 x 105.8
					1							51.1 x 104.8
	208/2		25	21	1							44.3 x 108.9
					1							61.2 x 108.1
					1							56.4 x 107.9
					1							65.3 x 107.7
307	224/2	50	25	21	1							39.7 x 108.0
					1							29.2 x 106.3
					1							56.9 x 105.6
					2							54.2 x 105.0

APPENDIX TABLE CA-MD-F (continued)

2-Methoxyethanol

Females

Multiple Dosing: 500 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		
307	224/3		25	21	1							52.0 x 110.0
					1							48.3 x 107.0
					1							47.4 x 105.8
					1							50.5 x 105.4
301	281/1	50	25	22	1							58.0 x 111.0
					1							57.8 x 109.2
					1							51.3 x 107.3
	281/2		25	22	1							39.8 x 109.3
					1							60.5 x 107.4
					1							54.0 x 107.7
310	164/1-5	0	0	0								
303	257/1	50	25	24	1							56.7 x 101.5
	257/2		8	8								
	257/3		17	16	1							52.0 x 105.5
306	183/7	50	25	22	1							52.3 x 105.3
					1							54.1 x 103.5
					1							52.9 x 100.5
	183/6		25	22	1							62.9 x 108.4
					1							57.5 x 107.5
					1							38.6 x 104.1
304	199/5	32	14	13	1							40.6 x 101.2
	199/3		1	0	1							34.4 x 108.2
	199/1		17	16	1							30.3 x 108.0
	199/2,4, 6 & 7		0	0								

APPENDIX TABLE CA-MD-F (continued)

2-Methoxyethanol

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		
319	277/2	50	25	19	1							34.6 x 113.6
					1							36.0 x 112.1
						1						35.7 x 112.1
					1							35.2 x 110.3
					2							32.6 x 109.9
					1							31.9 x 109.1
	277/3		25	22	1							38.4 x 108.3
					2							65.5 x 108.3
					1							61.3 x 105.9
318	221/6	50	25	20	1							39.4 x 112.0
					1							39.8 x 111.6
					2							44.2 x 110.0
								1				62.4 x 107.6
					1							41.8 x 107.3
	221/7		25	18	1							42.4 x 113.9
					1							37.8 x 112.5
					1							57.6 x 108.0
					1							61.8 x 107.5
					1	1						53.4 x 107.2
					1							30.2 x 106.7
					1							54.6 x 106.7
314	234/3	50	25	22	1							41.5 x 110.2
					1							64.2 x 109.7
					1							39.6 x 109.7

APPENDIX TABLE CA-MD-F (continued)

2-Methoxyethanol

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		
314	234/1		25	21	1							55.0 x 113.1
					1							54.4 x 112.8
					1							53.2 x 110.8
					1							33.9 x 103.0
315	236/1	50	25	20	1							46.9 x 108.5
					1		1					45.2 x 108.2
					1	1						42.1 x 106.8
											1 Chromatid Fragment	45.4 x 105.5
								1				57.9 x 104.9
	236/2		21	16	1							36.5 x 111.4
					1							34.8 x 110.8
					2							37.8 x 110.2
					1							35.6 x 108.1
					1							58.5 x 104.7
	236/4		4	3	1							35.5 x 103.6
313	215/4	50	25	20	1							62.0 x 103.1
					1							57.6 x 110.0
					1							60.3 x 107.9
					1							68.2 x 107.4
					1							62.4 x 106.1

APPENDIX TABLE CA-MD-F (continued)

2-Methoxyethanol

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		
313	215/5		25	19		1		2				61.9 x 111.0
					1							36.6 x 109.8
					1							38.5 x 110.0
					1							64.0 x 109.8
					1							72.1 x 109.3
					1							71.1 x 109.6
311	302/3	50	25	14	1				1			58.2 x 114.4
					1							38.7 x 108.5
					1							69.9 x 112.3
					2							37.5 x 110.0
								1				41.3 x 109.8
					1							41.9 x 109.2
					2							35.2 x 108.9
						1						56.9 x 106.9
					1							59.8 x 105.5
					2							58.2 x 104.8
					1	1						34.3 x 104.0
	302/4		25	16	1							50.9 x 111.2
					1							51.0 x 111.2
					1							33.1 x 110.2
					2							47.1 x 108.2
					1							33.1 x 107.3
					1							36.5 x 105.8
					1							52.9 x 105.5
					1							48.1 x 101.4
					2							32.4 x 99.8

APPENDIX TABLE CA-MD-F (continued)

2-Methoxyethanol

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		
320	168/1-7	0	0	0								
317	258/4	50	25	21	1							46.8 x 110.9
					1							46.5 x 110.8
					1							45.0 x 109.5
					1							65.1 x 108.8
	258/5		25	23	1							55.6 x 111.2
					1							40.5 x 109.7
316	275/2	50	25	24				1				49.9 x 106.9
	275/3		25	22	1							58.3 x 107.0
					1							43.3 x 106.1
					1							59.0 x 105.2
312	276/3	12	7	5	1							42.1 x 105.8
					1							41.7 x 103.8
	276/4		3	2		1						57.1 x 103.7
	276/5		2	2								
	276/1,2, 6 & 7		0	0								

APPENDIX TABLE CA-M6

2-Methoxyethanol
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		
3	43/1	50	25	22	1							37.5 x 101.9
					1							60.0 x 102.2
					1							61.4 x 102.3
	43/2		25	24	1							40.5 x 106.3
9	115/1	50	25	25								
	115/2		25	25								
7	105/2	50	25	25								
	105/3		25	25								
2	80/1	50	25	25								
	80/4		25	25								
4	153/1	50	25	23	1							42.9 x 105.2
					1							36.0 x 102.7
	153/2		18	17	1							61.9 x 98.2
	153/3		7	7								

APPENDIX TABLE CA-M6 (continued)

2-Methoxyethanol
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
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
10	135/1	50	25	24	1							46.3 x 109.1
	135/2		25	25								
1	136/1	50	25	22	1							42.2 x 112.7
					1							55.5 x 109.8
								1				57.9 x 109.8
	136/3		25	24	1							41.5 x 108.2
5	148/4	50	25	24	1							47.7 x 100.3
	148/5		25	24							1 Chromatid Fragment	36.6 x 100.8
6	75/1	50	25	24	1							52.5 x 106.1
	75/2		25	25								
8	156/1	21	13	13								
	156/2		2	2								
	156/3		6	6								
	156/4		0	0								
	156/5		0	0								

APPENDIX TABLE CA-M6 (continued)

2-Methoxyethanol

Males

Single Dosing: 25 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		
31	93/5	50	25	25								
	93/4		25	24	1							64.3 x 109.4
35	10/1	50	25	24							1 Chromatid Fragment	62.9 x 108.0
	10/2		25	24				1				45.1 x 110.6
36	34/1	50	25	25								
	34/3		25	25								
34	58/1	50	11	11								
	58/2		16	14	1							57.6 x 100.6
					1							40.6 x 103.6
	58/3		17	15			1				1 Chromatid Fragment	42.8 x 105.7
					1							33.5 x 97.5
	58/4		6	6								
39	50/1	50	25	21	1							64.6 x 109.3
						1						29.6 x 107.9
					1							31.4 x 105.8
					1							37.9 x 102.8
	50/3		25	25								

APPENDIX TABLE CA-M6 (continued)

2-Methoxyethanol
Males

Single Dosing: 25 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		
38	74/1	50	25	22							1 Chromatid Fragment	65.3 x 103.9
					1							47.1 x 100.5
					1							32.7 x 97.0
	74/2		25	25								
32	67/1	50	25	25								
	67/2		25	25								
40	158/2	50	25	24	1							37.7 x 110.3
	158/3		25	25								
33	60/2	50	25	24	1							37.3 x 110.9
	60/3		25	23	1							67.2 x 111.6
								1				62.6 x 109.0
37	71/3	50	25	25								
	71/4		25	25								

APPENDIX TABLE CA-M6 (continued)

2-Methoxyethanol

Males

Single Dosing: 500 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		
66	98/1	50	25	24				1				45.5 x 107.2
	98/3		25	22	1							41.9 x 112.9
					1							53.2 x 112.9
					1							50.0 x 112.1
65	64/4	50	25	25								
	64/5		25	24	1							59.4 x 114.0
70	62/1	50	25	25								
	62/2		25	25								
69	137/1	50	11	11								
	137/2		13	13								
	137/3		9	9								
	137/4		9	9								
	137/5		8	8								
67	53/1	3	0	0								
	53/2		2	2								
	53/3		1	1								
	53/4		0	0								
	53/5		0	0								

APPENDIX TABLE CA-M6 (continued)

2-Methoxyethanol
Males

Single Dosing: 500 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		
63	139/1	50	25	24	1							63.3 x 109.9
	139/2		25	19	1							70.8 x 104.1
							1					67.2 x 98.4
					1							65.7 x 109.0
					1							64.8 x 111.7
					1							63.2 x 109.5
					1							54.4 x 104.6
62	72/1	50	25	23	1							58.3 x 105.0
					1							38.7 x 103.4
	72/2		25	25								
64	40/1	50	25	22	1							35.5 x 108.9
					1							65.5 x 107.1
					1							37.2 x 106.6
	40/3		25	25								
68	96/1	50	25	22	1							46.3 x 112.6
					1							58.2 x 109.9
					1							30.5 x 109.0
	96/2		25	25								
61	83/4	50	25	24	1							72.2 x 112.0
	83/2		25	25								

APPENDIX TABLE CA-M6 (continued)

2-Methoxyethanol

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
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
95	28/3	50	25	22	1							64.5 x 107.7
					1							31.6 x 107.7
								1				30.3 x 107.6
	28/1		25	23	2							65.7 x 112.4
					1							69.5 x 110.4
97	132/1	50	25	23	2							54.9 x 106.9
					1							62.8 x 107.2
	132/3		25	20	1							65.3 x 109.5
					1							43.8 x 109.4
					1							35.2 x 109.7
					1							32.7 x 108.9
					1							65.4 x 108.9
94	52/1	50	25	25								
	52/2		25	25								
92	99/2	50	25	22	1							38.4 x 105.1
					1		1					64.0 x 103.1
								1				38.7 x 101.6
	99/3		25	23		1						64.5 x 105.9
					1							69.2 x 101.1

APPENDIX TABLE CA-M6 (continued)

2-Methoxyethanol

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
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
100	94/1	50	25	19	1							61.8 x 110.1
					1							64.2 x 110.3
									2			33.8 x 108.0
						1					1 Chromatid Fragment	41.4 x 107.7
					2							69.5 x 105.5
					1							69.6 x 102.9
	94/2		25	22		1						45.6 x 107.7
							1					38.2 x 107.0
					1							40.5 x 104.9
93	76/1	50	25	24	1							59.5 x 106.5
	76/2		25	22	1	1						33.6 x 112.4
					1							67.2 x 110.0
					1							68.1 x 108.7
91	9/1	50	25	24	1							28.2 x 110.1
	9/2		25	24	1							55.9 x 111.9
96	146/1	50	25	22	1							34.8 x 108.6
					1							34.9 x 108.0
											1 Chromosomal Fragment	43.7 x 105.0

APPENDIX TABLE CA-M6 (continued)

2-Methoxyethanol

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
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
96	146/5		25	21	1							64.0 x 111.0
					1							27.6 x 104.7
					1							55.9 x 104.7
					1							45.2 x 102.9
98	22/5	50	25	24	1							41.7 x 112.3
	22/4		25	22	1							37.3 x 113.4
					1							64.1 x 111.0
					1							64.7 x 109.7
99	147/1	50	25	22	1							62.6 x 102.9
					1							42.8 x 100.9
					1							45.5 x 100.9
	147/2		14	13	1							37.4 x 95.9
	147/3		7	6	1							60.0 x 98.3
	147/4		4	4								

APPENDIX TABLE CA-M24

2-Methoxyethanol
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
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
18	16/3	50	25	25								
	16/1		25	25								
12	12/4	50	25	23	1	1						64.2 x 109.8
					1							37.3 x 109.8
	12/5		25	24	1							64.3 x 109.0
11	103/1	50	25	25								
	103/2		25	25								
13	89/1	50	25	24	1							52.3 x 114.6
	89/2		25	21	1							57.9 x 113.6
								1				38.0 x 111.7
								1				45.5 x 112.2
					1							29.2 x 109.2
15	116/2	50	25	24	1							59.5 x 112.9
	116/4		25	23	1							73.6 x 106.4
					1							25.0 x 105.9

APPENDIX TABLE CA-M24 (continued)

2-Methoxyethanol

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
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
20	6/5	50	25	22	1							28.2 x 110.9
					1							31.4 x 111.4
					1							58.3 x 111.4
	6/4		25	23				1				66.0 x 107.7
					1							60.9 x 107.5
17	140/1	50	25	24	1							55.8 x 110.6
	140/3		25	25								
14	41/5	50	25	22	1			1				56.8 x 111.2
					1							53.2 x 111.2
					1							44.9 x 110.5
	41/4		25	25								
19	81/2	50	25	25								
	81/3		25	25								
16	8/1	50	25	25								
	8/2		25	23	1							73.2 x 105.0
								1				24.9 x 102.9

APPENDIX TABLE CA-M24 (continued)

2-Methoxyethanol

Males

Single Dosing: 25 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		
44	118/5	50	25	25								
	118/1		25	25								
48	114/3	50	25	24	1							63.0 x 110.9
	114/2		25	23	1							54.5 x 106.5
								1				35.9 x 106.5
42	133/1	50	25	24	1							60.4 x 114.0
	133/2		25	23	1							51.7 x 113.7
					1							50.7 x 113.7
49	14/2	50	25	24	1							63.9 x 113.5
	14/4		25	25								
47	134/2	50	25	24	1							58.3 x 111.0
	134/4		25	24				1				30.2 x 110.6
46	27/1	50	25	24	1							51.8 x 111.6
	27/2		25	23	1							70.2 x 105.9
					1							37.9 x 103.6
43	160/1	50	24	20	1							37.1 x 105.0
					1							41.0 x 105.0
					2							67.0 x 102.9
					1							42.9 x 101.4

APPENDIX TABLE CA-M24 (continued)

2-Methoxyethanol

Males

Single Dosing: 25 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		
43	160/2		23	22	1							59.1 x 99.8
	160/3		3	3								
41	55/1	50	25	20	1							114.1 x 25.1
											1 Chromosomal Fragment	97.5 x 25.8
					1							101.8 x 24.0
					1							106.6 x 21.0
					1							81.1 x 14.0
41	55/2		25	24	1							120.1 x 7.9
45	79/1	50	25	24	1							102.2 x 16.2
	79/2		25	24	1							77.5 x 24.1
50	152/1	50	24	21	1						1 Chromatid Fragment	111.2 x 17.9
						1						88.2 x 5.2
					1							71.0 x 3.9
	152/3		25	22	1							87.2 x 13.4
					1							82.8 x 10.1
					1							71.9 x 8.0
	152/4		1	1								

APPENDIX TABLE CA-M24 (continued)

2-Methoxyethanol

Males

Single Dosing: 500 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		
80	178/4	50	25	25								
	178/3		25	25								
73	87/3	50	25	25								
	87/4		25	24	1							50.2 x 113.8
74	122/2	50	25	22	1							60.0 x 113.0
						1						57.2 x 113.0
								1				30.0 x 112.5
	122/3		25	23				1				70.9 x 112.8
					1							56.3 x 109.9
77	90/4	50	25	25								
	90/3		25	23	2							25.5 x 112.5
								1				55.0 x 111.0
76	97/1	50	25	23		1						54.0 x 110.0
					1							41.4 x 107.3
	97/2		7	7								
	97/3		18	16	1							45.1 x 112.7
					1							54.8 x 111.0
72	86/3	50	25	20	1	1		1				57.6 x 107.4
					1							61.3 x 106.5
					1							39.7 x 104.0
					1							55.0 x 102.8
					1							30.5 x 101.6

APPENDIX TABLE CA-M24 (continued)

2-Methoxyethanol

Males

Single Dosing: 500 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	86/5		25	20	1							41.7 x 107.8
								1				38.5 x 104.6
					1							39.3 x 103.2
					1							58.4 x 101.1
								1				58.5 x 101.1
75	30/1	50	25	25								
	30/3		25	23	1							69.5 x 109.0
								1				38.1 x 109.6
78	107/5	50	25	25								
	107/2		25	25								
71	111/1	50	25	25								
	111/2		25	23	1							54.0 x 111.2
					1							51.8 x 110.0
79	130/1	50	25	25								
	130/2	25	24	23		1						38.0 x 107.6

APPENDIX TABLE CA-M24 (continued)

2-Methoxyethanol

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	4/5	50	25	22	1							58.8 x 104.6
					1							53.8 x 104.6
								1				52.5 x 104.6
	4/4		25	25								
103	37/5	50	25	24	1							75.7 x 109.0
	37/4		25	24	1							37.3 x 110.0
104	119/5	50	25	23	1							37.5 x 105.0
								1				32.1 x 103.5
	119/1		25	24	1							33.8 x 107.8
105	126/1	1	1	1								
	126/2-5		0	0								
102	26/1	50	25	24	1							53.8 x 108.0
	26/4		25	23	1							57.4 x 110.7
					1							42.8 x 109.6
108	129/2	50	25	22	1							67.2 x 110.1
								1				58.5 x 110.0
					1							57.9 x 110.3

APPENDIX TABLE CA-M24 (continued)

2-Methoxyethanol

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	129/3		25	23	1							64.4 x 110.0
					1							59.4 x 109.9
109	143/3	50	25	24	1							42.0 x 109.1
	143/4		25	24							1 Chromatid Fragment	33.1 x 109.9
110	7/2	50	25	23				1				54.5 x 109.6
						2						58.2 x 109.1
	7/5		25	23		1						61.7 x 110.6
						1						52.4 x 107.9
101	108/3	50	25	25								
	108/5		25	20							1 Exchange	32.1 x 109.9
											1 Exchange	33.1 x 109.9
					1							44.0 x 109.8
							1					29.5 x 108.8
											1 Chromosomal Fragment	28.0 x 108.1
107	33/1	8	2	2								
	33/3		2	2								
	33/5		4	2	1							71.1 x 104.0
					1	1						51.1 x 92.0
	33/2,4		0	0								

APPENDIX TABLE CA-M48

2-Methoxyethanol
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		
25	85/1	50	25	24	1							54.9 x 98.3
	85/2		25	25								
27	154/1	50	25	25								
	154/2		25	24		1						64.1 x 108.9
28	48/5	50	25	24	1							40.9 x 109.9
	48/2		25	24							1 Chromatid Fragment	65.2 x 112.2
26	92/5	50	25	24	1							61.8 x 113.3
	92/3		25	25								
23	66/4	50	25	24	1							69.2 x 114.2
	66/1		25	25								
22	42/3	50	25	25								
	42/4		25	25								

APPENDIX TABLE CA-M48 (continued)

2-Methoxyethanol

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		
21	35/2	50	25	24	1							72.3 x 112.9
	35/3		25	21	1							63.0 x 111.1
					2							48.0 x 110.2
					1							48.0 x 110.4
					1							47.5 x 110.3
30	124/4	50	25	23		1						62.6 x 109.8
					1							38.1 x 110.0
	124/2		25	23	1							62.0 x 111.6
					1							59.4 x 110.9
24	54/2	50	25	24	1							60.8 x 111.2
	54/3		25	24	1							67.1 x 110.5
29	29/3	50	25	22	1							58.3 x 109.6
					1							61.9 x 110.0
					1							67.2 x 109.9
	29/2		25	23		1						66.1 x 103.0
					1							38.3 x 111.6

APPENDIX TABLE CA-M48 (continued)

2-Methoxyethanol
Males

Single Dosing: 25 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		
52	110/2	50	25	25								
	110/3		25	24	1							67.0 x 109.5
57	5/1	50	25	23		1						62.0 x 109.1
					1							60.5 x 109.1
	5/2		25	24	1							25.5 x 104.7
51	36/1	50	25	24	1							36.9 x 106.8
	36/2		25	24	1							67.9 x 109.3
56	128/4	50	25	22	1							71.4 x 113.6
					1							69.9 x 113.4
					1							45.2 x 112.5
	128/3		25	23	1							70.5 x 110.8
					1							67.1 x 110.9
58	131/5	50	25	24	1							43.8 x 112.8
	131/4		25	23	1							67.6 x 114.2
					1							64.2 x 114.0

APPENDIX TABLE CA-M48 (continued)

2-Methoxyethanol
Males

Single Dosing: 25 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		
55	70/4	50	25	24	1							43.1 x 108.6
	70/1		25	24	1							56.0 x 112.1
59	39/2	50	25	23							1 Chromatid Fragment	58.4 x 111.6
					1							58.4 x 111.5
	39/5		25	23	1							58.3 x 111.9
					1	1						56.8 x 112.2
60	102/3	50	25	24	1							63.9 x 111.4
	102/4		25	25								
54	142/4	50	25	24	1							67.5 x 108.0
	142/5		25	25								
53	106/4	50	25	25								
	106/5		25	25								

APPENDIX TABLE CA-M48 (continued)

2-Methoxyethanol

Males

Single Dosing: 500 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		
85	18/1	50	25	23	1							68.0 x 111.4
					1							46.5 x 110.4
	18/2		25	21	1							60.0 x 110.7
					1							37.0 x 107.8
											1 Chromatid Fragment	38.7 x 107.9
					1							40.2 x 107.0
86	127/2	50	25	20		1						62.5 x 101.4
								1				62.3 x 101.5
					1							48.4 x 108.8
					1							41.4 x 108.7
					1							33.1 x 100.2
	127/3		25	21	1							68.0 x 111.3
					1							42.2 x 110.3
					1							44.0 x 108.2
					1							45.4 x 108.3
84	19/1	50	25	23	1							34.4 x 111.0
					1							39.7 x 108.3
	19/2		25	19	1							71.6 x 109.8
					1							69.9 x 110.0
					1							48.0 x 109.8
					1							45.4 x 109.9
					2							42.0 x 110.1
					1							40.5 x 110.1

APPENDIX TABLE CA-M48 (continued)

2-Methoxyethanol
Males

Single Dosing: 500 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		
83	77/1	50	25	25								
	77/2		25	25								
87	23/1	50	25	25								
	23/2		25	25								
81	88/2	50	25	23	1							63.5 x 111.9
							1					62.2 x 112.1
	88/4		25	23	1							45.5 x 109.0
					2							41.0 x 109.2
89	21/1	50	25	22	1							39.7 x 111.3
					1							70.3 x 105.7
					1							66.4 x 105.2
	21/4		25	24	1							42.3 x 110.8
90	145/1	50	25	22	1							71.7 x 109.4
					1							68.8 x 109.3
											2 Chromatid Framgents	62.5 x 109.2
	145/2		25	21	1							68.3 x 110.5
					1							68.0 x 110.3
											1 Chromatid Fragment	67.8 x 110.5
					1							67.6 x 110.7
82	113/3	50	25	25								
	113/4		25	23		1						52.1 x 107.7
					1							49.1 x 109.1
88	95/3	50	25	25								
	95/2		25	25								

APPENDIX TABLE CA-M48 (continued)

2-Methoxyethanol

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		
117	2/1-5	0	0	0								
114	1/1	50	25	24		1						65.5 x 110.5
	1/2		25	23	1							58.9 x 109.8
					1							53.3 x 109.5
119	31/2	50	25	23	1							62.3 x 108.7
					1							59.4 x 107.8
	31/3		25	23	1							56.4 x 107.4
					1							62.7 x 106.4
120	109/1	50	25	21	1							68.9 x 109.9
					1							61.9 x 109.8
					1							61.5 x 109.7
					1							58.9 x 110.0

APPENDIX TABLE CA-M48 (continued)

2-Methoxyethanol

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		
120	109/2		25	18	1							73.5 x 111.2
					1							72.9 x 111.4
					1							71.3 x 111.4
					1							70.0 x 111.6
					1							69.8 x 111.5
										1		69.5 x 111.2
					1							67.8 x 110.8
112	38/1	50	25	25								
	38/2		25	25								
116	45/3	50	22	22								
	45/5		25	25								
	45/1		3	3								
115	123/1-5	0	0	0								
111	141/1	50	25	23	1							70.0 x 111.2
					1							42.5 x 111.3
	141/4		25	24	1							68.5 x 109.9
113	61/4	50	25	24	1							54.9 x 107.9
	61/5		25	24	1							60.7 x 113.8
118	84/1-5	0	0	0								

APPENDIX TABLE CA-F6

2-Methoxyethanol
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
		Fer Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
167	265/3	50	25	23	1							38.6 x 110.9
					1							31.2 x 108.3
	265/2		25	25								
165	308/1	50	25	24	1							39.4 x 109.0
	308/3		25	24	1							65.8 x 110.3
166	235/1	50	11	10							1 Chromatid Fragment	49.1 x 105.2
	235/2		11	11								
	235/3		14	13	1							27.2 x 101.2
	235/4		14	13	1							54.8 x 97.0
168	316/2	50	25	25								
	316/3		25	25								
163	203/1	50	25	23	1							31.3 x 109.3
					1							54.6 x 108.0
	203/3		25	25								
161	296/2	50	25	22	2							34.9 x 109.0
					1							58.1 x 108.4
					1							32.8 x 107.4

APPENDIX TABLE CA-F6 (continued)

2-Methoxyethanol

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		
161	296/1		25	22	1							42.8 x 107.2
					1							31.2 x 107.0
					1							62.9 x 105.9
169	275/1	50	25	25								
	275/4		25	23	1							63.5 x 112.8
					1							34.3 x 111.9
162	240/1	50	25	24	1							66.3 x 104.4
	240/2		25	24	1							41.1 x 111.5
164	313/1	50	23	20	2							52.7 x 101.7
					1							52.3 x 99.5
											1 Chromatid Fragment	52.4 x 96.9
	313/2		21	19	1							32.8 x 106.2
					1							32.4 x 97.4
	313/3		6	5	1							31.9 x 106.8
170	295/1	50	25	23		1						36.6 x 109.0
											1 Chromatid Fragment	51.5 x 101.5
	295/2		25	24	1							49.9 x 104.0

APPENDIX TABLE CA-F6 (continued)

2-Methoxyethanol
Females

Single Dosing: 25 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		
194	218/2	50	14	14								
	218/2		8	5	1							35.5 x 109.1
					1							35.7 x 109.3
					2							44.5 x 108.2
	218/3		10	9	1							60.6 x 95.9
	218/4		9	9								
	218/5		9	8	1							67.6 x 95.1
196	194/1	50	25	25								
	194/2		25	25								
191	253/1	50	25	25								
	253/2		25	25								
199	210/1	50	25	25								
	210/4		25	25								
198	234/1	50	25	20			1					72.4 x 100.9
					1							72.2 x 99.9
						1						64.6 x 108.1
					1	1						64.5 x 108.7
					1	1						61.9 x 94.5

APPENDIX TABLE CA-F6 (continued)

2-Methoxyethanol
Females

Single Dosing: 25 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		
198	234/2		25	21	1		1					71.5 x 105.9
						1						67.7 x 112.2
						1						67.8 x 107.7
					1							65.4 x 110.1
200	318/1	50	25	24	1							65.9 x 103.8
	318/4		25	25								
197	231/1	39	6	5	1							57.9 x 102.4
	231/3		16	16								
	231/4		6	6								
	231/2		9	9								
	231/5		2	2								
193	220/2	50	25	24	1							36.3 x 105.9
	220/3		25	24							1 Chromatid Fragment	30.0 x 102.5
192	227/5	50	25	24	1							35.6 x 107.4
	227/4		25	25								
195	170/2	50	25	25								
	170/4		22	20	1							29.3 x 102.4
					1							38.7 x 100.9
	170/5		3	3								

APPENDIX TABLE CA-F6 (continued)

2-Methoxyethanol
Females

Single Dosing: 500 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		
221	243/2	50	25	22	1							35.5 x 110.5
					2							35.0 x 110.3
					1							34.9 x 110.3
	243/3		25	25								
229	297/2	50	25	22	1							58.4 x 108.9
					1							58.3 x 107.8
					1							57.1 x 107.8
	297/2		25	21	1							37.8 x 108.9
					1							43.0 x 108.6
					1							35.2 x 107.8
					1							42.6 x 107.7
224	200/2	50	25	25								
	200/1		25	25								
225	224/1	50	25	25								
	224/2		25	24	1							65.2 x 108.0
223	299/1	50	25	25								
	299/2		25	25								

APPENDIX TABLE CA-F6 (continued)

2-Methoxyethanol
Females

Single Dosing: 500 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	258/1	50	12	11	1							30.7 x 100.9
	258/2		18	18								
	258/3		19	18	1							59.9 x 103.1
	258/4		1	1								
222	232/1	50	25	23	1							72.8 x 111.2
						1						62.0 x 110.9
	232/2		25	24	1							66.7 x 113.0
228	256/1	50	25	25								
	256/2		25	24	1							58.9 x 108.8
227	213/1	50	25	22	1							70.6 x 113.4
					1							64.6 x 113.0
					1							72.4 x 112.5
	213/2		25	25								
230	222/1	50	25	23	1							67.0 x 107.8
					1							68.2 x 107.7
	222/2		25	22	1							49.7 x 102.0
					1							69.5 x 108.6
					1							32.3 x 104.4

APPENDIX TABLE CA-F6 (continued)

2-Methoxyethanol

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	306/1	50	25	25								
	306/2		25	25								
258	182/1	50	25	22	1							63.2 x 108.9
					1							63.5 x 108.4
											1 Chromatid Fragment	29.1 x 106.8
	182/2		25	25								
260	254/3	50	25	25								
	254/2		25	24	1							37.1 x 107.1
252	259/1	50	25	25								
	259/2		25	24	1							44.1 x 106.5
259	207/1	50	21	16	1							68.8 x 96.8
					1							64.0 x 110.8
							1					35.7 x 104.1
								1				33.8 x 96.9
					1							30.7 x 102.5
	307/2		25	19	2							53.4 x 113.6
					1							58.5 x 112.7
					2							37.0 x 108.9
					2							62.3 x 107.0
					1							65.3 x 106.4
					1							40.5 x 106.1
	307/3		4	4								
253	236/1	50	25	21	1							38.7 x 111.9
					1							44.5 x 111.0
						1						45.0 x 108.5
					1							72.4 x 106.4

APPENDIX TABLE CA-F6 (continued)

2-Methoxyethanol
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
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
253	236/2		25	21	1							40.5 x 112.5
					1							64.0 x 110.3
					1							61.3 x 108.0
					1							62.5 x 105.0
255	188/1	50	25	25								
	188/2		25	25								
257	292/2	50	25	25								
	292/3		25	25	1							29.2 x 108.2
					1							26.8 x 107.5
251	169/1	50	25	19		1						37.5 x 109.2
					1							61.6 x 108.8
					1							32.5 x 108.0
						1						60.5 x 108.2
					1							40.4 x 107.7
					1							66.4 x 103.2
	169/2		25	25								
254	212/5	50	11	8				1				61.8 x 112.0
					1							30.5 x 107.6
					1							59.9 x 102.9
	212/4		25	21		1						64.5 x 104.0
					1							32.1 x 101.4
					1							48.1 x 94.1
					1							46.3 x 94.2
	212/3		14	12		1						35.5 x 108.4
					1							56.0 x 104.6

APPENDIX TABLE CA-F24

2-Methoxyethanol
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
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
180	166/1	50	25	25								
	166/3		25	23	1							67.9 x 110.0
					1							71.6 x 110.0
171	263/4	50	7	6	1							42.2 x 112.8
	263/5		2	2								
	263/3		20	18				1				62.2 x 111.0
								1				63.4 x 103.4
	263/2		21	14	1							58.2 x 115.0
					2							29.5 x 110.1
					1							27.6 x 110.0
					1							25.7 x 108.3
					1				1			33.2 x 95.9
					1							42.7 x 95.8
					2							54.1 x 94.1
173	249/1	50	25	25								
			25	23	1							69.5 x 110.5
					1							66.4 x 109.7
176	168/2	50	25	22				1				60.7 x 109.7
					1							49.7 x 110.1
					1							43.1 x 108.7

APPENDIX TABLE CA-F24 (continued)

2-Methoxyethanol

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
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
176	168/4		25	24	1							45.2 x 106.6
174	201/3	3	2	2								
	201/2		1	1								
	201/4,5,1		0	0								
178	176/1	50	25	25								
	176/3		25	24	1							66.9 x 108.2
179	241/3	50	25	23	1							36.6 x 106.6
					1	1						51.0 x 95.1
	241/4		25	24	1							24.5 x 97.8
177	300/1	50	25	24	1							71.1 x 99.6
	300/2		16	16								
	300/3		9	8	1							34.9 x 108.5
172	172/2	50	14	14								
	172/3		24	21	1							109.1 x 17.1
					2		1					89.5 x 14.1
					1							113.5 x 11.8
	172/4		12	12								
175	276/1	50	25	23	1							114.1 x 20.2
					1							80.4 x 16.0
	276/2		25	23	1							120.0 x 17.8
						1						94.8 x 12.2

APPENDIX TABLE CA-F24 (continued)

2-Methoxyethanol

Females

Single Dosing: 25 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		
210	309/3	50	25	21	1							48.0 x 111.0
						1						34.1 x 108.2
							1					64.8 x 107.2
							1					47.2 x 103.9
	309/4		25	23	1							32.7 x 112.0
								1				51.6 x 102.8
203	320/3	50	25	23	1							65.7 x 112.5
					1							37.6 x 112.5
	320/2		25	23		1		1				67.5 x 103.1
					1			1				29.2 x 107.4
201	215/5	50	25	22				1				29.1 x 113.5
						1						31.7 x 112.1
					1							42.5 x 111.1
	215/2		25	24	1							32.6 x 108.0
202	293/4	50	25	24	1							42.3 x 111.4
	293/2		25	24							1 Chromatid Fragment	45.6 x 114.0
209	217/3	50	25	22	1							40.4 x 110.0
											1 Chromatid Fragment	35.0 x 108.5
					1							48.0 x 106.0
	217/4		25	24	1							43.4 x 107.2
205	317/1	50	25	23	1			1				89.0 x 20.0
					1							118.2 x 13.0

APPENDIX TABLE CA-F24 (continued)

2-Methoxyethanol
Females

Single Dosing: 25 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		
205	317/2		25	22	1							107.0 x 18.6
					1							102.0 x 16.0
							1					113.0 x 13.8
206	281/4	50	25	19	1							87.9 x 22.8
					1							103.8 x 20.5
					1							80.8 x 19.8
					3	2					1 Translocation	101.8 x 15.9
					1							114.2 x 15.0
					2							85.9 x 11.0
	281/2		25	22							1 Exchange	108.0 x 17.0
					1							122.8 x 16.4
					1							118.9 x 12.8
204	192/2	50	25	24	1							82.0 x 23.0
	192/3		25	24	1							119.0 x 17.9
207	185/2	50	25	24	1							82.2 x 26.6
	185/3		19	18	1							70.0 x 24.9
	185/4		6	6								
208	310/2	50	25	21	1							119.0 x 18.6
					1							119.0 x 16.1
					1							118.2 x 14.5
					1							85.2 x 21.8
	310/3		25	25								

APPENDIX TABLE CA-F24 (continued)

2-Methoxyethanol
Females

Single Dosing: 500 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		
237	209/4	50	25	24	1							25.0 x 110.7
	209/3		25	25								
232	184/1	50	25	25								
	184/2		25	23	1							63.0 x 110.5
					1							40.4 x 111.9
235	251/4	50	25	21	1							31.7 x 112.2
					1							33.3 x 111.9
					1							36.6 x 111.9
					2							39.6 x 111.7
	251/1		25	22	2							35.9 x 110.2
					1							29.6 x 110.3
								2				27.2 x 108.5
238	315/1	50	25	22				1				64.9 x 111.8
					1							32.0 x 109.8
					1							33.5 x 111.9
	315/2		25	25								
239	242/3	50	25	23		1						65.0 x 109.6
						1						35.8 x 110.0
	242/1		25	24	1							42.3 x 110.0
233	225/1	50	11	11								
	225/2		17	17								

APPENDIX TABLE CA-F24 (continued)

2-Methoxyethanol

Females

Single Dosing: 500 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		
233	225/3		19	19								
	225/4		3	3								
236	277/1	50	25	22	1				1			78.9 x 20.2
					1							121.2 x 19.9
					1							108.9 x 14.9
236	277/2		25	21	2							91.9 x 15.8
					1							78.8 x 11.8
											1 Chromatid Fragment	100.8 x 11.1
					1							104.1 x 10.1
234	311/1	50	21	20	2							85.1 x 5.9
	311/2		25	24	1							85.0 x 23.4
	311/3		4	4								
240	163/1	50	25	23	1							84.9 x 24.0
					1							82.1 x 22.9
	163/2		25	22	3							48.6 x 24.8
					1							85.9 x 20.1
							1					108.0 x 16.8
231	304/2	50	25	22	1			1				112.5 x 14.1
					1							82.9 x 12.9
					1							86.1 x 5.6
	304/5		25	21	2							75.5 x 19.0
					1							92.1 x 14.9
								1				80.2 x 12.2
					1							110.0 x 11.0

APPENDIX TABLE CA-F24 (continued)

2-Methoxyethanol

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		
270	211/2	50	21	16						1		54.2 x 112.9
											2 Chromosomal Fragments	59.7 x 110.0
											1 Chromatid Fragment	70.1 x 107.9
					2							50.2 x 104.0
					1							56.7 x 97.4
	211/4		24	21	1							37.6 x 110.4
					2							62.5 x 104.1
								1				62.9 x 102.7
	211/5		5	4				1				37.7 x 107.0
263	216/4	50	5	4	1							29.0 x 107.9
	216/5		22	15	1							50.4 x 111.9
					1							49.1 x 104.0
					1	1						64.6 x 101.8
					1		1					26.2 x 90.4
					1							61.7 x 90.4
					2							32.2 x 90.4
						1						26.2 x 90.3
	216/3		23	19		2						70.0 x 111.0
					1							56.7 x 103.0
						1						38.5 x 101.5
					1							39.6 x 100.6
269	204/1	49	12	11		1						57.2 x 103.5
	204/2		8	6			1	1				27.5 x 106.5
						1						38.6 x 104.6

APPENDIX TABLE CA-F24 (continued)

2-Methoxyethanol
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	204/3		9	6	1							58.3 x 105.7
								1				58.3 x 106.5
					1							33.2 x 100.4
	204/4		10	9	1							33.9 x 102.3
	204/5		10	9	1							26.9 x 102.9
264	177/1	9	4	4								
	177/2		3	3								
	177/4		2	2								
	177/3,5		0	0								
261	173/1	35	9	9								
	173/2		7	7								
	173/3		5	5								
	173/4		7	7								
	173/5		7	6	1							62.1 x 104.9
267	319/2	50	25	17	1							33.8 x 110.2
					1							73.1 x 108.2
					1			1				73.2 x 108.0
					1							73.7 x 108.0
					1							60.5 x 107.6
					1	1						34.9 x 106.4
					1							51.3 x 106.2
						1						57.6 x 104.0

APPENDIX TABLE CA-F24 (continued)

2-Methoxyethanol
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		
267	319/4		25	19				1				48.1 x 109.5
					1							42.7 x 109.3
					1							31.6 x 107.9
					1							62.2 x 107.9
					1							42.4 x 101.7
					1							44.9 x 106.9
266	298/5	50	25	22	2							63.1 x 113.1
					1							30.7 x 111.7
					1							29.4 x 111.6
	298/3		25	24	1							57.6 x 111.5
262	206/1	50	15	14	1							55.0 x 93.9
	206/2		22	19	1							43.1 x 112.1
					2						1 Exchange	62.5 x 111.0
					1							64.0 x 93.0
	206/3		12	11	1							47.5 x 94.2
	206/4		1	1								
268	260/1	50	25	23	1							71.5 x 109.2
								1				75.1 x 105.6
	260/2		25	24	1							44.2 x 113.4
265	233/1	50	8	7	1							100.6 x 10.9
	233/2		9	8	1							82.4 x 6.8
	233/3		10	7	1							80.1 x 18.1
					3							110.8 x 16.6
											1 Chromosomal Fragment	107.0 x 17.1
	233/4		7	5	1							85.0 x 4.9
					1							86.8 x 4.0
265	233/5		16	13	1							72.2 x 19.0
					1							92.1 x 11.9
						2						117.0 x 11.2

APPENDIX TABLE CA-F48

2-Methoxyethanol
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		
188	312/2	50	25	19	1						1 Chromatid Fragment	104.5 x 3.1
					1							103.9 x 3.1
					2							118.6 x 6.1
					1							131.8 x 8.0
					1							108.7 x 11.4
					1							121.9 x 13.8
	312/3		25	23		1						124.5 x 5.0
					1							99.5 x 5.8
181	285/1	50	25	25								
	285/2		25	25								
183	239/1	50	25	25								
	239/2		25	25								
186	274/2	50	25	24	1							70.8 x 109.2
	274/5		25	25								

APPENDIX TABLE CA-F48 (continued)

2-Methoxyethanol
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
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
182	278/5	50	25	24	1							41.2 x 111.9
	278/2		25	25								
185	294/1	50	25	22	1							59.0 x 109.5
					1							55.0 x 110.2
					1							33.0 x 109.9
	294/4		25	24	1							59.6 x 109.6
190	246/1	50	25	24	1							63.5 x 112.2
	246/2		25	25								
187	174/3	50	25	25								
	174/1		25	25								
184	187/4	50	25	23	1							57.2 x 110.2
					1							56.0 x 110.2
	187/2		25	24	1							35.1 x 109.9
189	271/1	50	25	24	1							57.3 x 108.9
	271/2		25	25								

APPENDIX TABLE CA-F48 (continued)

2-Methoxyethanol

Females

Single Dosing: 25 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		
211	247/1	50	25	25								
	247/2		25	25								
220	186/1	50	25	23	1							65.0 x 113.0
					1							55.6 x 110.1
	186/2		25	21	1							59.9 x 111.7
					2							46.1 x 111.7
					1							44.5 x 111.9
					1							36.9 x 109.1
217	240/1	50	25	24	1							30.2 x 108.0
	240/2		25	20	1							35.3 x 107.1
					1							43.0 x 106.5
					1							57.8 x 105.3
					1							55.5 x 103.9
					1							45.1 x 104.0
216	267/1	50	25	23	1							36.1 x 106.3
					1							35.9 x 106.2
	267/2		25	24	1							67.2 x 108.6
214	257/1	50	25	22	1							30.0 x 101.9
					1							38.5 x 101.9
					1							27.0 x 94.6

APPENDIX TABLE CA-F48 (continued)

2-Methoxyethanol
Female

Single Dosing: 25 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		
214	257/3		25	22	2							64.6 x 108.5
					1							63.0 x 108.6
					1							51.6 x 107.6
212	282/2	50	25	25								
	282/3		25	24	1							45.5 x 105.2
213	190/3	50	25	23	1							38.9 x 109.9
					1							34.7 x 106.6
	190/5		25	25								
218	238/1	50	25	24	1							60.3 x 109.6
	238/5		25	25								
215	250/5	50	25	24	1							66.0 x 113.0
	250/2		25	25								
219	268/5	50	25	20	1							65.0 x 110.1
					1							60.8 x 110.0
					1							40.8 x 108.9
					1							38.0 x 108.6
					1							39.1 x 108.8
	268/4		25	22	1							66.0 x 110.6
					1							38.5 x 108.4
					1							38.9 x 104.0

APPENDIX TABLE CA-F48 (continued)

2-Methoxyethanol

Females

Single Dosing: 500 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		
249	195/1	50	25	22	1							37.5 x 110.1
					1							34.0 x 111.1
					1							28.8 x 110.6
	195/2		25	25								
248	167/1	50	25	24	1							57.4 x 109.7
	167/2		25	23	2							57.6 x 113.0
					1							66.5 x 108.5
247	303/1	50	25	24	1							30.9 x 108.0
	303/2		25	25								
245	193/4	50	25	25								
	193/1		25	23	1							70.0 x 106.2
					1							54.5 x 109.0
243	286/5	50	25	25								
	286/3		25	25								
250	202/4	50	25	23	1							70.2 x 114.0
					1							54.0 x 108.3

APPENDIX TABLE CA-F48 (continued)

2-Methoxyethanol
Females

Single Dosing: 500 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		
250	202/5		25	22	1							63.8 x 112.3
					3							66.5 x 110.2
					3							72.6 x 107.6
244	164/5	50	25	23	1							53.0 x 110.7
					1							47.0 x 110.0
	164/1		25	25								
241	197/5	50	25	23	1							67.7 x 110.9
					1							63.0 x 110.4
	197/4		25	25								
242	279/1	50	25	25								
	279/2		25	21	1							68.1 x 112.3
					1							67.9 x 112.4
					1							62.2 x 112.4
					1							43.2 x 110.0
246	289/2	50	25	22	1							63.4 x 111.3
					1							48.6 x 110.4
					1							46.0 x 110.5
	289/4		25	23	1							61.5 x 114.0
					1							59.3 x 114.1

APPENDIX TABLE CA-F48 (continued)

2-Methoxyethanol

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		
278	284/1	32	0	0								
	284/2		3	3								
	284/3		13	13								
	284/4		12	12								
	284/5		4	4								
272	214/1	50	25	21	1							54.3 x 109.7
								1				35.5 x 110.2
					1							34.5 x 109.5
						1	1					29.1 x 108.4
	214/2		25	21	1							64.9 x 112.2
					1							64.0 x 112.6
					1							46.4 x 112.6
					1							42.3 x 109.9
271	226/1	50	25	22				1				54.2 x 109.8
					1							48.2 x 110.1
					1							43.9 x 110.2
	226/2		25	24	1							69.3 x 112.2
276	208/1	50	25	24	1							62.0 x 112.1
	208/2		25	23	1							60.8 x 110.3
											1 Chromatid Fragment	58.8 x 109.8

APPENDIX TABLE CA-F48 (continued)

2-Methoxyethanol

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	189/1	7	1	1								
	189/3		6	6								
	189/2,4 5		0	0								
273	245/2	50	25	22	1							42.2 x 110.2
					1							36.7 x 109.0
											1 Chromatid Fragment	46.1 x 109.5
	245/3		25	20	1							70.1 x 107.7
								1				61.8 x 108.0
					1	2						60.7 x 108.1
					1							57.4 x 107.6
					1							55.0 x 107.7
280	270/1	3	3	3								
	270/2-5		0	0								
279	196/2	50	25	25								
	196/5		20	20								
	196/1		5	5								
275	314/4	50	25	23	1							44.3 x 108.6
					1							62.5 x 108.1
	314/5		25	25								
274	252/1	50	25	24	1							51.8 x 114.5
	252/3		25	24		2						68.7 x 111.0

APPENDIX TABLE DL CURTAILED

2-Methoxyethanol
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	4	9	14	16	14	12	12	13	14	15	13	14	0	0	10	10	11	13	12	14	220
	Total Implants	0	7	11	15	14	12	12	13	13	15	13	14	0	0	10	10	11	13	12	13	208
	Live Implants	0	7	11	15	14	12	12	12	13	15	12	14	0	0	10	10	10	12	12	12	203
	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	1	0	0	1	0	0	0	0	0	1	1	0	1	5
2	Corpora lutea	14	12	12	11	0	13	0	12	13	13	14	12	0	13	13	10	0	13	10	16	201
	Total Implants	14	12	13	10	0	13	0	12	12	13	14	12	0	13	13	10	0	13	10	16	200
	Live Implants	13	11	13	10	0	11	0	12	11	12	14	12	0	12	13	10	0	12	10	16	192
	Early Deaths	1	1	0	0	0	2	0	0	1	1	0	0	0	1	0	0	0	0	0	0	7
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
3	Corpora lutea	14	15	12	9	12	15	17	17	8	11	15	15	13	13	11	11	12	*-	16	0	250
	Total Implants	15	15	12	12	12	16	16	15	14	12	15	14	13	14	12	11	13	*-	16	0	261
	Live Implants	15	14	12	12	10	15	15	15	14	12	14	13	12	13	11	10	13	*-	16	0	248
	Early Deaths	0	1	0	0	2	0	1	0	0	0	1	1	1	1	1	1	0	*-	0	0	11
	Late Deaths	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	*-	0	0	2
4	Corpora lutea	11	10	12	16	13	14	14	0	11	10	20	14	12	13	12	11	14	12	16	13	248
	Total Implants	10	13	15	18	14	16	16	0	13	14	19	17	12	14	13	11	14	13	14	13	269
	Live Implants	9	12	15	17	13	11	15	0	13	13	18	16	12	14	13	9	13	12	14	13	252
	Early Deaths	1	1	0	1	1	5	1	0	0	1	1	1	0	0	0	2	1	1	0	0	17
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	Corpora lutea	13	17	14	13	16	14	16	13	0	18	10	16	0	15	13	17	13	12	12	16	258
	Total Implants	13	17	13	13	14	14	14	13	0	18	10	16	0	13	13	17	13	12	12	16	251
	Live Implants	13	17	13	13	14	14	14	13	0	18	10	16	0	13	13	17	13	12	12	16	251
	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

*Missing value: ambiguous record of result

APPENDIX TABLE DL CURTAILED (continued)

2-Methoxyethanol

Multiple Dosing: 25 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	12	16	0	10	13	15	13	16	14	13	3	10	16	12	13	14	15	12	14	12	243
	Total Implants	12	16	0	10	13	15	13	16	15	13	0	10	16	14	13	14	15	13	14	12	244
	Live Implants	12	15	0	10	12	15	12	16	15	12	0	10	16	14	13	13	15	13	14	11	238
	Early Deaths	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	1	4
	Late Deaths	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2
2	Corpora lutea	8	17	18	13	14	17	0	14	0	12	0	13	0	13	15	16	13	13	12	12	220
	Total Implants	8	13	15	13	16	17	0	14	0	12	0	13	0	13	15	16	13	5	12	12	207
	Live Implants	8	13	15	13	16	15	0	14	0	11	0	13	0	13	10	16	12	3	10	12	194
	Early Deaths	0	0	0	0	0	2	0	0	0	1	0	0	0	0	5	0	1	2	2	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	7	14	12	11	14	15	15	16	*-	11	13	12	13	12	14	13	13	14	15	15	261
	Total Implants	6	12	11	9	14	17	12	16	*-	9	13	9	12	8	14	13	14	16	15	16	248
	Live Implants	6	12	10	8	14	17	12	15	*-	9	12	9	11	8	14	12	14	16	15	15	240
	Early Deaths	0	0	1	1	0	0	0	0	*-	0	1	0	1	0	0	1	0	0	0	1	7
	Late Deaths	0	0	0	0	0	0	0	1	*-	0	0	0	0	0	0	0	0	0	0	0	1
4	Corpora lutea	13	4	16	14	16	5	14	14	15	10	15	2	12	16	15	15	13	14	14	13	237
	Total Implants	14	0	16	13	17	0	16	15	15	15	13	0	15	14	14	15	16	14	14	15	251
	Live Implants	14	0	13	13	17	0	15	15	15	15	13	0	14	14	14	14	16	12	14	15	243
	Early Deaths	0	0	3	0	0	0	1	0	0	0	0	0	1	0	0	1	0	2	0	0	8
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	Corpora lutea	14	16	15	14	14	14	16	0	0	15	15	13	0	14	0	12	14	16	15	16	233
	Total Implants	14	14	15	14	14	14	16	0	0	15	14	13	0	13	0	12	14	15	14	16	212
	Live Implants	14	14	15	14	14	14	15	0	0	14	13	13	0	13	0	12	14	15	14	16	211
	Early Deaths	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	3
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

*Missing value: ambiguous record of result

APPENDIX TABLE DL CURTAILED (continued)

2-Methoxyethanol

Multiple Dosing: 500 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	
1	Corpora lutea	4	12	14	13	13	9	15	16	10	13	6	12	10	15	15	14	15	12	15	16	249
	Total Implants	0	10	14	13	13	0	15	16	0	13	0	0	0	14	14	12	15	12	15	16	192
	Live Implants	0	9	14	8	13	0	15	14	0	13	0	0	0	12	14	12	13	12	13	16	178
	Early Deaths	0	0	0	5	0	0	0	2	0	0	0	0	0	2	0	0	2	0	0	0	11
	Late Deaths	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	3
2	Corpora lutea	15	10	12	14	12	11	14	13	13	9	0	0	12	13	10	8	12	14	15	10	217
	Total Implants	15	9	12	14	12	11	14	13	13	1	0	0	12	13	11	8	4	14	15	10	201
	Live Implants	15	9	12	14	11	11	13	13	13	1	0	0	12	13	10	8	4	13	15	10	197
	Early Deaths	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	1	0	0	4
	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	5	0	4	5	2	0	14	10	0	2	4	6	3	0	3	0	0	0	4	10	72
	Total Implants	0	0	0	0	0	0	12	11	0	0	1	1	0	0	0	0	0	0	0	8	33
	Live Implants	0	0	0	0	0	0	12	8	0	0	0	0	0	0	0	0	0	0	0	7	27
	Early Deaths	0	0	0	0	0	0	0	3	0	0	1	0	0	0	0	0	0	0	0	1	5
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
4	Corpora lutea	3	0	1	0	0	0	10	0	10	0	0	0	3	0	0	0	0	1	0	0	28
	Total Implants	0	0	0	0	0	0	11	0	13	0	0	0	0	0	0	0	0	0	0	0	24
	Live Implants	0	0	0	0	0	0	10	0	13	0	0	0	0	0	0	0	0	0	0	0	23
	Early Deaths	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	Corpora lutea	3	0	3	0	0	4	1	5	0	3	3	2	0	5	0	0	0	0	0	0	29
	Total Implants	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Live Implants	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	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

APPENDIX TABLE DL CURTAILED (continued)

2-Methoxyethanol

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	12	12	14	11	7	9	0	13	2	14	11	11	14	11	13	14	0	11	3	10	192
	Total Implants	12	9	14	11	0	9	0	7	0	14	11	2	14	1	13	12	0	9	0	10	148
	Live Implants	2	0	12	7	0	4	0	2	0	4	2	0	4	1	3	2	0	3	0	1	47
	Early Deaths	10	9	2	4	0	5	0	5	0	10	9	2	9	0	10	10	0	6	0	9	100
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
2	Corpora lutea	0	10	12	10	11	5	14	15	0	10	13	8	11	13	0	15	13	10	8	12	190
	Total Implants	0	2	1	1	0	2	0	0	0	1	0	3	0	0	0	1	0	0	2	1	14
	Live Implants	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Early Deaths	0	2	1	1	0	2	0	0	0	1	0	3	0	0	0	1	0	0	2	1	14
	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	5	12	4	7	0	16	8	0	3	4	8	7	0	11	8	0	6	1	4	4	108
	Total Implants	0	0	0	1	0	0	1	0	0	2	1	0	0	1	1	0	2	0	0	0	9
	Live Implants	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Early Deaths	0	0	0	1	0	0	1	0	0	2	1	0	0	1	1	0	2	0	0	0	9
	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	5	6	9	13	*-	4	5	4	0	0	14	14	12	0	13	7	0	7	12	6	131
	Total Implants	3	2	12	14	*-	4	5	2	0	1	16	13	14	0	14	7	0	9	3	0	119
	Live Implants	1	0	11	8	*-	1	1	1	0	0	15	11	11	0	14	6	0	6	3	0	89
	Early Deaths	2	2	1	6	*-	3	4	1	0	1	1	2	3	0	0	1	0	3	0	0	30
	Late Deaths	0	0	0	0	*-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	Corpora lutea	9	17	0	15	8	14	15	11	12	18	15	15	11	15	12	0	16	14	18	13	248
	Total Implants	7	17	0	15	5	14	15	11	12	17	13	15	11	14	5	0	16	15	14	11	227
	Live Implants	7	17	0	15	5	14	15	11	12	17	13	15	11	14	5	0	16	15	14	11	227
	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

*Missing value: ambiguous record of result

APPENDIX TABLE DL

2-Methoxyethanol

Dominant Lethal Assessment

Multiple Dosing: Air Control (0 ppm)

Week No.	Male No.	361		362		263		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	13	13	13	14	0	0	0	14	11	10	14	14	13	11	12	12	13	13	11	14	215
	Total Implants	11	13	13	14	0	0	0	14	11	10	14	14	13	11	13	13	13	15	11	14	217
	Live Implants	11	12	12	13	0	0	0	14	11	10	11	14	13	9	13	13	11	10	11	14	202
	Early Deaths	0	1	0	1	0	0	0	0	0	0	3	0	0	2	0	0	2	5	0	0	14
	Late Deaths	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
2	Corpora lutea	13	14	14	10	0	0	10	13	10	10	13	13	0	11	11	10	13	12	12	13	202
	Total Implants	13	14	13	10	0	0	10	13	9	9	13	13	0	11	12	10	13	12	12	10	197
	Live Implants	12	13	13	10	0	0	7	13	9	7	12	13	0	11	12	9	13	12	12	10	188
	Early Deaths	1	1	0	0	0	0	3	0	0	2	1	0	0	0	0	1	0	0	0	0	9
	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	13	11	12	12	10	12	11	11	14	13	13	10	11	16	13	12	14	14	17	10	249
	Total Implants	13	11	12	12	9	12	11	11	14	13	13	10	11	16	15	12	15	14	16	10	250
	Live Implants	13	11	10	11	8	12	11	10	14	13	12	9	11	16	15	10	13	14	16	10	239
	Early Deaths	0	0	2	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	5
	Late Deaths	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	2	2	0	0	0	6
4	Corpora lutea	13	13	11	0	0	0	10	12	10	15	11	11	2	13	0	0	12	11	13	2	159
	Total Implants	13	13	11	0	0	0	10	12	11	15	10	11	0	12	0	0	12	8	13	0	151
	Live Implants	13	13	11	0	0	0	10	12	11	15	10	11	0	12	0	0	12	8	13	0	151
	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
5	Corpora lutea	11	14	12	12	14	0	11	11	13	13	13	12	13	11	10	13	11	14	14	10	232
	Total Implants	11	12	12	10	14	0	13	11	13	13	14	12	13	11	10	13	11	8	13	9	223
	Live Implants	11	12	12	6	11	0	13	10	13	12	13	12	13	11	8	13	10	7	12	9	208
	Early Deaths	0	0	0	3	3	0	0	1	0	1	1	0	0	0	2	0	1	1	1	0	14
	Late Deaths	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1

APPENDIX TABLE DL (continued)

2-Methoxyethanol

Multiple Dosing: Air Control (0 ppm)

Week No.	Male No.	361		362		363		364		365		366		367		368		389		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	15	13	15	11	10	12	1	11	14	16	13	17	11	14	12	18	15	11	11	253
	Total Implants	13	15	13	15	12	9	12	0	10	14	15	13	17	12	13	12	18	13	17	10	253
	Live Implants	13	14	13	12	12	8	11	0	10	14	14	13	17	12	13	11	18	12	17	8	242
	Early Deaths	0	1	0	3	0	1	1	0	0	0	1	0	0	0	0	1	0	1	0	2	11
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	Corpora lutea	15	12	12	10	14	13	10	13	11	12	9	10	12	13	12	12	12	11	12	11	236
	Total Implants	15	12	12	11	11	11	11	14	11	12	13	11	13	10	14	15	12	11	12	11	242
	Live Implants	14	12	12	11	10	11	11	9	10	12	13	11	13	9	13	15	10	10	12	11	229
	Early Deaths	1	0	0	0	1	0	0	5	1	0	0	0	0	0	1	0	2	1	0	0	12
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
8	Corpora lutea	15	12	12	13	13	14	13	15	12	15	12	13	12	13	11	13	11	13	14	13	259
	Total Implants	15	12	13	16	13	14	9	15	13	17	10	13	12	15	10	16	10	16	13	13	265
	Live Implants	15	11	13	13	13	14	9	15	13	16	8	13	12	15	10	16	10	16	13	13	258
	Early Deaths	0	1	0	3	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	6
	Late Deaths	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1
9	Corpora lutea	11	14	13	11	12	0	11	14	14	14	10	8	13	8	14	10	13	14	12	13	229
	Total Implants	11	15	15	14	14	0	11	14	14	14	6	15	12	9	15	10	12	14	15	13	243
	Live Implants	10	15	15	14	12	0	11	13	12	12	6	15	11	8	7	10	12	13	15	12	223
	Early Deaths	1	0	0	0	2	0	0	1	2	2	0	0	1	1	8	0	0	1	0	1	20
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	Corpora lutea	14	12	*-	11	11	12	12	13	11	13	11	12	14	12	12	12	16	14	15	11	238
	Total Implants	14	12	*-	10	11	12	13	14	11	13	8	11	13	13	13	12	16	14	13	12	235
	Live Implants	14	12	*-	9	10	12	13	14	11	13	8	11	13	13	13	11	15	14	11	11	228
	Early Deaths	0	0	*-	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	2	1	7
	Late Deaths	0	0	*-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

*Missing value: ambiguous record of result

APPENDIX TABLE DL (continued)

2-Methoxyethanol

Multiple Dosing: 25 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	15	11	15	11	2	14	2	0	12	11	0	10	2	9	0	13	12	12	14	10	175
	Total Implants	15	12	13	11	0	13	0	0	11	6	0	3	0	10	0	13	13	12	14	10	156
	Live Implants	15	12	12	11	0	12	0	0	11	6	0	3	0	10	0	13	13	12	14	10	154
	Early Deaths	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	Corpora lutea	14	12	15	13	10	13	0	8	12	10	15	11	15	11	13	15	13	12	16	12	240
	Total Implants	14	12	14	13	10	13	0	3	12	10	13	11	14	11	13	16	13	12	16	12	232
	Live Implants	14	12	14	13	10	9	0	3	11	10	13	10	14	11	13	16	12	12	16	12	225
	Early Deaths	0	0	0	0	0	4	0	0	1	0	0	1	0	0	0	0	1	0	0	0	7
	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	14	14	12	13	14	11	18	9	15	13	13	12	15	13	11	14	13	10	12	16	262
	Total Implants	14	15	12	13	14	12	18	9	15	12	13	12	15	13	12	14	9	10	11	16	259
	Live Implants	14	14	12	13	12	12	17	9	15	12	11	11	15	12	12	14	9	10	11	15	250
	Early Deaths	0	1	0	0	2	0	0	0	0	0	2	1	0	1	0	0	0	0	0	1	8
	Late Deaths	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
4	Corpora lutea	0	12	11	13	0	16	18	14	11	10	0	13	12	8	0	14	4	14	12	15	197
	Total Implants	0	12	12	13	0	15	18	14	11	11	0	13	11	7	0	14	2	14	12	15	194
	Live Implants	0	12	12	13	0	15	17	14	11	11	0	13	11	7	0	14	2	14	12	15	193
	Early Deaths	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	Corpora lutea	12	12	13	13	14	0	11	10	13	14	11	12	13	12	14	12	13	11	14	12	236
	Total Implants	12	12	13	11	14	0	11	7	13	14	11	12	13	11	14	13	13	11	12	12	229
	Live Implants	12	12	13	10	11	0	11	7	12	11	11	12	13	11	14	13	12	11	12	12	220
	Early Deaths	0	0	0	1	3	0	0	0	1	3	0	0	0	0	0	0	1	0	0	0	9
	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)

2-Methoxyethanol

Multiple Dosing: 25 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	14	13	12	14	15	16	10	14	13	14	14	14	14	12	14	13	13	14	14	14	271
	Total Implants	14	13	12	11	13	16	10	15	12	15	15	12	14	8	15	11	11	12	13	13	255
	Live Implants	13	12	12	11	13	14	10	14	12	14	15	11	13	8	15	11	11	12	12	12	245
	Early Deaths	1	1	0	0	0	1	0	1	0	1	0	1	1	0	0	0	0	0	1	1	9
	Late Deaths	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
7	Corpora lutea	11	13	15	12	12	11	14	11	0	13	12	0	15	14	12	7	12	12	15	10	221
	Total Implants	13	12	17	10	18	12	11	13	0	12	15	0	16	14	12	6	12	11	11	11	226
	Live Implants	13	12	16	10	9	12	11	12	0	10	12	0	15	12	12	6	11	11	10	9	203
	Early Deaths	0	0	1	0	9	0	0	1	0	2	3	0	1	2	0	0	0	0	1	2	22
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
8	Corpora lutea	12	11	12	11	15	10	11	12	*-	13	13	13	13	13	12	14	9	11	13	13	231
	Total Implants	15	11	12	11	15	11	11	14	*-	14	13	13	16	12	12	12	9	13	13	13	240
	Live Implants	15	11	11	10	15	11	11	14	*-	13	11	13	15	10	12	11	9	13	13	13	231
	Early Deaths	0	0	1	1	0	0	0	0	*-	1	2	0	1	1	0	1	0	0	0	0	8
	Late Deaths	0	0	0	0	0	0	0	0	*-	0	0	0	0	1	0	0	0	0	0	0	1
9	Corpora lutea	12	12	10	1	10	15	14	14	13	13	16	10	12	13	12	0	12	14	13	15	231
	Total Implants	12	14	11	0	13	10	15	14	13	13	14	10	10	13	12	0	12	14	14	18	232
	Live Implants	12	14	10	0	4	9	15	13	13	11	13	9	8	11	12	0	12	13	13	18	210
	Early Deaths	0	0	1	0	9	1	0	1	0	1	1	1	0	2	0	0	0	1	1	0	19
	Late Deaths	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	0	0	0	0	0	3
10	Corpora lutea	12	12	12	10	15	13	16	15	14	13	14	*-	10	12	15	12	11	10	15	13	244
	Total Implants	12	15	13	11	15	13	15	16	14	13	13	*-	12	12	16	13	9	12	15	13	252
	Live Implants	12	13	12	10	13	13	14	16	13	12	13	*-	12	11	16	13	8	12	15	13	241
	Early Deaths	0	2	1	1	2	0	1	0	1	1	0	*-	0	1	0	0	1	0	0	0	11
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	*-	0	0	0	0	0	0	0	0	0

*Missing value: ambiguous record of result

APPENDIX TABLE DL (continued)

2-Methoxyethanol

Multiple Dosing: 500 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	
1	Corpora lutea	12	13	11	10	9	13	15	10	10	12	14	11	14	11	13	11	13	13	11	10	236
	Total Implants	10	13	10	9	9	13	15	10	11	11	14	11	14	11	11	11	13	12	11	10	229
	Live Implants	10	13	10	9	8	11	15	10	11	10	14	11	14	9	11	11	13	12	10	10	222
	Early Deaths	0	0	0	0	1	2	0	0	0	1	0	0	0	2	0	0	0	0	1	0	7
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	Corpora lutea	9	14	15	13	0	10	11	13	0	12	10	12	14	15	13	13	12	12	11	5	214
	Total Implants	9	14	15	12	0	10	11	15	0	12	9	12	13	15	12	13	12	4	8	4	200
	Live Implants	9	14	15	12	0	10	11	15	0	11	9	12	11	15	12	12	12	4	8	2	194
	Early Deaths	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	1	0	0	0	2	6
	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	14	2	3	2	2	0	13	12	12	15	14	12	16	11	2	10	0	0	0	4	144
	Total Implants	14	0	0	1	2	0	9	12	13	15	14	11	8	11	1	10	0	3	0	1	125
	Live Implants	13	0	0	1	0	0	9	12	13	15	11	10	8	11	1	10	0	2	0	1	117
	Early Deaths	1	0	0	0	2	0	0	0	0	0	3	1	0	0	0	0	0	1	0	0	8
	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	3	2	0	2	4	2	0	13	2	0	3	8	15	6	0	0	2	0	0	0	62
	Total Implants	0	1	0	0	1	0	0	13	0	0	0	1	14	6	0	0	0	0	0	0	36
	Live Implants	0	1	0	0	1	0	0	13	0	0	0	1	14	6	0	0	0	0	0	0	36
	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
5	Corpora lutea	0	2	1	0	0	0	0	0	0	1	0	0	0	0	0	2	4	2	3	0	15
	Total Implants	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	3
	Live Implants	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	3
	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

APPENDIX TABLE DL (continued)

2-Methoxyethanol

Multiple Dosing: 500 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	2	0	0	2	0	2	1	0	0	0	0	1	0	4	4	3	0	2	0	4	25
	Total Implants	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Live Implants	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	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
7	Corpora lutea	0	0	2	0	4	0	0	0	13	0	0	0	7	0	0	0	0	0	0	0	26
	Total Implants	0	0	0	0	1	0	0	0	13	0	0	0	0	0	0	0	0	0	0	0	14
	Live Implants	0	0	0	0	0	0	0	0	13	0	0	0	0	0	0	0	0	0	0	0	13
	Early Deaths	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	Corpora lutea	4	0	0	9	5	2	14	10	13	14	2	0	*-	*-	15	16	0	1	0	0	105
	Total Implants	1	0	0	6	3	0	16	4	0	14	0	0	*-	*-	13	14	0	0	0	0	71
	Live Implants	0	0	0	6	3	0	16	1	0	13	0	0	*-	*-	12	13	0	0	0	0	64
	Early Deaths	1	0	0	0	0	0	0	3	0	1	0	0	*-	*-	1	1	0	0	0	0	7
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	*-	*-	0	0	0	0	0	0	0
9	Corpora lutea	0	0	12	10	6	0	13	15	15	13	0	13	15	10	11	12	12	12	2	12	183
	Total Implants	0	0	12	11	5	0	12	14	15	14	0	14	14	7	12	17	12	11	0	11	181
	Live Implants	0	0	12	11	5	0	12	14	14	12	0	14	14	5	11	11	9	10	0	10	164
	Early Deaths	0	0	0	0	0	0	0	0	1	2	0	0	0	2	1	6	3	1	0	0	16
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
10	Corpora lutea	1	0	7	15	4	9	13	12	11	11	10	10	17	14	11	12	8	11	10	10	196
	Total Implants	1	0	7	14	1	8	13	11	10	10	12	12	16	13	15	12	6	9	11	12	193
	Live Implants	1	0	7	14	1	8	13	11	10	10	12	12	16	13	15	12	6	8	9	12	190
	Early Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	3
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

*Missing value: ambiguous record of result

APPENDIX TABLE DL (continued)

2-Methoxyethanol

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	0	2	9	2	1	11	0	2	10	2	5	10	0	10	5	7	8	7	9	102
	Total Implants	0	0	0	9	0	0	9	0	0	8	0	0	8	0	7	1	6	6	4	6	64
	Live Implants	0	0	0	9	0	0	9	0	0	8	0	0	8	0	7	0	6	6	4	4	61
	Early Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2	3
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	Corpora lutea	2	0	0	0	0	2	0	1	0	2	0	0	0	0	3	4	1	0	1	0	16
	Total Implants	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	1	0	0	0	0	4
	Live Implants	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Early Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	1	0	0	0	0	4
	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	2	6	0	0	0	3	1	4	1	0	0	2	0	1	0	4	0	1	2	2	29
	Total Implants	0	0	0	0	0	3	0	2	0	0	0	0	0	1	0	3	0	0	0	0	9
	Live Implants	0	0	0	0	0	2	0	2	0	0	0	0	0	0	0	3	0	0	0	0	7
	Early Deaths	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	2
	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	5	0	12	3	11	12	0	2	6	12	10	12	0	7	4	12	2	10	1	4	125
	Total Implants	1	0	12	0	10	13	0	0	0	11	10	11	0	7	0	12	0	10	1	2	100
	Live Implants	1	0	12	0	10	13	0	0	0	11	10	11	0	7	0	12	0	10	1	2	100
	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
5	Corpora lutea	0	0	12	13	11	13	14	11	6	10	13	13	14	1	12	12	13	0	11	0	179
	Total Implants	0	0	13	13	13	14	14	11	6	10	13	14	14	1	11	14	13	0	11	0	185
	Live Implants	0	0	13	13	13	14	12	11	6	9	13	13	14	1	10	14	13	0	11	0	180
	Early Deaths	0	0	0	0	0	0	2	0	0	1	0	1	0	0	1	0	0	0	0	0	5
	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)

2-Methoxyethanol

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	6	0	14	13	10	13	12	12	10	14	13	10	14	14	0	12	12	14	18	16	227
	Total Implants	2	0	12	12	10	12	12	12	14	13	11	3	13	12	0	13	15	12	18	18	214
	Live Implants	2	0	12	10	9	12	12	10	12	12	10	3	13	12	0	12	13	11	17	16	198
	Early Deaths	0	0	0	2	1	0	0	2	1	1	1	0	0	0	0	1	2	1	1	2	15
	Late Deaths	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
7	Corpora lutea	12	7	10	14	12	11	13	12	8	11	3	9	0	15	12	14	13	11	12	13	212
	Total Implants	9	4	12	13	12	11	15	12	10	16	0	12	0	17	13	14	13	13	12	14	222
	Live Implants	8	4	11	12	11	11	11	11	10	14	0	12	0	16	11	14	12	13	11	14	206
	Early Deaths	1	0	1	1	1	0	4	1	0	2	0	0	0	1	2	0	1	0	1	0	16
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	Corpora lutea	0	1	14	14	11	11	13	16	12	9	12	9	11	8	13	12	*-	13	9	17	205
	Total Implants	0	0	13	14	11	2	14	16	13	9	12	11	13	8	12	14	*-	17	6	17	202
	Live Implants	0	0	12	13	10	2	14	15	12	8	11	11	13	7	11	12	*-	11	6	16	184
	Early Deaths	0	0	1	1	1	0	0	1	1	1	1	0	0	1	1	2	*-	6	0	1	18
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	*-	0	0	0	0
9	Corpora lutea	0	12	8	12	13	13	12	16	10	11	13	12	8	0	12	12	11	12	12	13	210
	Total Implants	0	11	7	11	12	13	12	16	8	9	12	12	11	0	14	12	11	14	13	15	213
	Live Implants	0	11	6	11	12	12	11	14	6	9	12	11	10	0	14	12	11	13	13	13	201
	Early Deaths	0	0	1	0	0	1	1	2	2	0	0	1	1	0	0	0	0	1	0	2	12
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	Corpora lutea	13	12	12	19	16	15	13	16	17	11	12	13	4	14	11	12	14	12	10	11	256
	Total Implants	14	12	13	16	16	14	15	15	16	10	12	13	2	14	9	10	13	12	11	12	249
	Live Implants	11	12	12	16	16	13	14	14	16	10	12	12	1	14	9	9	13	12	11	12	239
	Early Deaths	3	0	1	0	0	1	1	1	0	0	0	1	1	0	0	1	0	0	0	0	10
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

*Missing value: ambiguous record of result

APPENDIX TABLE SA

2-Methoxyethanol

Sperm Abnormality Assessment

Multiple Dosing: Air Control (0 ppm)
Low, 25 ppm
High, 500 ppm
Positive, Ethyl methanesulphonate, 100 mg/kg

Slide No.	Normal	Abnormality					Total Abnormal	Total Examined	De-coded Information	
		A	B	C	D	E			Animal No.	Group
328	893	4	13	31	58	1	107	1000	321	Air
358	962	0	2	25	9	2	38	1000	322	Air
338	-	-	-	-	-	-	*	*	323	Air
339	-	-	-	-	-	-	*	*	324	Air
322	962	4	5	18	8	3	38	1000	325	Air
329	-	-	-	-	-	-	*	*	326	Air
336	-	-	-	-	-	-	*	*	327	Air
324	-	-	-	-	-	-	*	*	328	Air
333	486	0	3	7	4	0	14	500	329	Air
325	961	1	6	10	15	7	39	1000	330	Air
346	941	2	1	19	28	9	59	1000	331	Low
343	955	0	4	9	23	9	45	1000	332	Low
360	962	2	0	20	9	7	38	1000	333	Low
344	969	0	5	9	15	2	31	1000	334	Low
347	-	-	-	-	-	-	*	*	335	Low
357	970	1	1	19	9	0	30	1000	336	Low
350	967	1	9	13	7	3	33	1000	337	Low
334	954	3	4	12	19	8	46	1000	338	Low
342	976	0	1	12	8	3	24	1000	339	Low
327	989	1	6	3	1	0	11	1000	340	Low

* Sample of poor quality - slide not readable

APPENDIX TABLE SA (continued)

2-Methoxyethanol

Multiple Dosing: Air Control (0 ppm)
Low, 25 ppm
High, 500 ppm
Positive, Ethyl methanesulphonate, 100 mg/kg

Slide No.	Normal	Abnormality					Total Abnormal	Total Examined	De-coded Information	
		A	B	C	D	E			Animal No.	Group
356	875	0	23	62	39	1	125	1000	341	High
335	919	0	18	29	34	0	81	1000	342	High
345	873	2	8	103	10	4	127	1000	343	High
332	945	2	1	33	16	3	55	1000	344	High
340	854	0	16	83	27	20	146	1000	345	High
326	945	0	11	32	11	1	55	1000	346	High
337	960	0	1	17	22	0	40	1000	347	High
351	925	0	17	36	18	4	75	1000	348	High
348	959	1	9	19	11	1	41	1000	349	High
341	801	5	26	97	62	3	193	1000	350	High
359	965	2	1	22	6	4	35	1000	351	+
349	941	3	6	36	9	5	59	1000	352	+
353	928	2	2	27	36	5	72	1000	353	+
354	980	0	2	9	9	0	20	1000	354	+
323	969	1	8	12	10	0	31	1000	355	+
321	-	-	-	-	-	-	*	*	356	+
331	981	1	3	11	3	1	19	1000	357	+
330	949	0	2	111	31	4	51	1000	358	+
352	952	1	1	16	25	5	48	1000	359	+
355	970	4	2	19	26	9	60	1000	360	+

* Sample of poor quality - slide not readable