Report Number 34

TIER II MUTAGENIC SCREENING OF 13 NIOSH PRIORITY COMPOUNDS

INDIVIDUAL COMPOUND REPORT BIS(2-METHOXYETHYL) ETHER

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JULY 7, 1981

Supported by

NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH
Division of Biomedical and Behavioural Science
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Contract No. 210-78-0026 (9)

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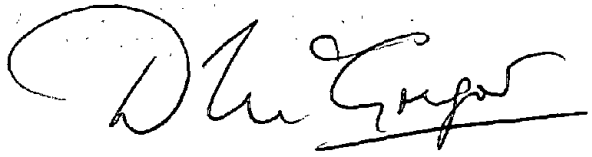
AUTHENTICATION

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



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Project No. 409959 Report No. 1190

TABULATIONS

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

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

Example:

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

Abbreviations on Chromosomal Aberration Tables and Appendix Tables:

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

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LOCATION OF EXPERIMENT

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

DISCLAIMER

"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

Bis(2-methoxyethyl) ether (diglyme) 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 19,000 µg/ml of culture medium.

2. Dominant lethal test in male rats with exposure to atmospheres containing 250 ppm or 1,000 ppm bis(2-methoxyethyl) ether for 7 h/day for 5 consecutive days. Analysis of test atmospheres was by continuous infrared absorption monitoring at a wavelength of 9.7 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 durations 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 250 ppm for 2.75 h.

The results obtained were as follows:

1. There was no increase in UDS in cells treated with bis(2-methoxyethyl) ether.

2. Clinical signs of toxicity were seen in both mice and rats exposed to 1,000 ppm bis(2-methoxyethyl) ether atmospheres. Effects were more severe in mice, 4 deaths occurring on day 4 of the 5 day exposure schedule.

3. Male rat fertility was drastically reduced following exposure to 1,000 ppm bis(2-methoxyethyl) ether in the dominant lethal test. Pregnancy frequency and implantations per pregnancy were particularly reduced in assessment Weeks 6 and 7 but these characters returned to normal values by Week 10. There was also some evidence that early death frequency had been increased by the treatment of the male rats by 1,000 ppm atmospheres.

4. Following multiple exposure of female rats to 1,000 ppm atmospheres, there was a significant reduction in total aberrant cell frequency ($P<0.05$). A decrease in total aberrant cell frequency was also seen in male rats 48 h after single exposures to either 250 ppm or 1,000 ppm atmospheres ($P<0.05$). The only significant increase was in male rats 6 h after a single exposure to a 250 ppm atmosphere ($P<0.05$).

5. Abnormal sperm frequency was increased, most of the effect being due to rises in sperm with amorphous heads.

6. SLRL frequency was increased, but in an inconsistent manner, in Drosophila. Although the results were not reproduced, the evidence did suggest that there may be some mutagenic potential in bis(2-methoxyethyl) ether.

It was concluded that bis(2-methyoxyethyl) ether may be a weak mutagen, but the adverse effect upon reproductive capacity is probably the more important aspect of its toxicology.

INTRODUCTION

Bis(2-methoxyethyl) ether (CAS No. 111-96-6) or diglyme is a solvent which is particularly useful in the reaction medium for Grignard and similar syntheses.

Properties

Formula	$(CH_3OCH_2CH_2)_2O$
Mol. wt.	134.18
Sp. gr.	0.937 (25°C)
M.P.	-64°C
B.P. (760 mm Hg)	161°C
Refractive index (20°C)	1.4073
Flash point (open cup)	70°C
Solubility	It is miscible with water, alcohol, ether and hydrocarbon solvents.

Toxicology

No published data have come to our attention relating to the pharmacology or toxicology of bis(2-methoxyethyl) ether. This compound has been subjected to Ames' test at Inveresk Research International Limited and the unpublished results from 2 experiments are as follows:

Mutagenicity Testing of Bis(2-methoxyethyl) ether in *Salmonella typhimurium* TA 1535, TA 1537, TA 1538, TA 98 and TA 100 and *Escherichia coli* WP2 uvrA (pKM101)

Test A

Substance	Quantity per plate	TA 1535		TA 1537		TA 1538		TA 98		TA 100	
		with S-9	without S-9	with S-9	without S-9	with S-9	without S-9	with S-9	without S-9	with S-9	without S-9
Phosphate Buffer	100 µl	11	6	10	9	24	10	21	32	94	72
2-Amino-anthracene	0.5 µg	31	9	22	13	150	11	147	30	169	71
Bis(2-methoxy-ethyl) ether	0.3 µl	6	5	6	6	27	10	22	25	90	88
	1.0 µl	10	8	3	8	19	7	24	38	90	73
	3.3 µl	10	7	8	13	22	12	25	28	91	63
	10.0 µl	8	10	5	6	28	10	25	30	102	80
	33.3 µl	9	8	7	10	37	9	32	21	125	82
	100.0 µl	7TL	5TL	4TL	VTL	14TL	VTL	9TL	1VTL	80TL	39TL

TL = thin lawn of microcolonies
VTL = very thin lawn of microcolonies

Mutagenicity Testing of Bis(2-methoxyethyl) ether in
Salmonella typhimurium TA 1535, TA 1537, TA 1538,
TA 98 and TA 100 and Escherichia coli WP2 uvrA (pKM101)

Test B

Substance	Quantity per plate	TA 1535		E. coli WP2 uvrA (pKM101)		TA 1538		TA 98		TA 100	
		with S-9	without S-9	with S-9	without S-9	with S-9	without S-9	with S-9	without S-9	with S-9	without S-9
Phosphate Buffer	100 µl			17		23		31		83	
2-Amino-anthracene	0.5 µg			25		132		175		160	
Bis(2-methoxy-ethyl) ether	20.0 µl			23		27		41		94	
	30.0 µl			23		32		44		111	
	40.0 µl			30		28		53		128	
	50.0 µl			30		28		47		137	
	60.0 µl			20		20		49		137	
	70.0 µl			NT		NT		NT		150	

NT = not tested

It was not entirely clear that bis(2-methoxyethyl) ether is a mutagen in this system, but ther were small increases in the numbers of mutants per plate at dose levels of 40 µl per plate (TA 98 and E. coli WP2 uvrA (pKM1010) with S-9) and 50 µl per plate (TA 100 with S-9).

Of greater importance, however, is the fact that bis(2-methoxyethyl) ether can be considered to be a condensation product of 2-methoxyethanol which has significant effects upon spermatogenesis (Nagano et al, 1979).

The work described in this report involves the testing of bis(2-methoxyethyl) ether in various systems for mutagenic potential in order that the risks associated with human exposure to the compound can be better evaluated. The exposure cpmdotopms ised were:

Human fibroblasts: up to 19,000 µg/ml for 3 h.

Mice and rats: 250 ppm and 1,000 ppm for 7 h/day for one or 5 days.

Drosophila: 250 ppm for 2.75 h.

MATERIALS AND METHODS

CHEMICALS

Test Substance

Five, 500 g bottles of bis(2-methoxyethyl) ether, Batch No. 21150, (stated purity 99%) were received from Aldrich Chemical Company Limited on 30 June 1980. The test material was a clear, colourless liquid and was retained in the dark under ambient conditions in the company dispensary until used. Whenever a bottle was opened and a sample withdrawn, the remainder was resealed under nitrogen. A sample has been retained for analysis, should this be necessary.

Positive Control Substance

Ethyl methanesulphonate (EMS) (stated purity 98%) was obtained from Koch-Light Laboratories, Colnbrook, Bucks and retained in a refrigerator in the company dispensary until used.

ANIMALS AND ANIMAL MANAGEMENT

Animals

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

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

These animals were obtained on the following dates.

Species	Date of Receipt	Age (Weeks)	Quarantine (Days)	Number (Sex)	Dates of Exposure	Comment
Rat	27 June 1980	10-11	10	220♂ 176♀	7-11 July 1980 14 July 1980	Multiple exposure. Single exposure.
	11 July 1980 etc	8-10	None	30♀ x 10	None	DL matings.
Mouse	2 July 1980	10-12	5	44♂	7-11 July 1980	

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 19°C and 25°C, and relative humidity ca 50%, with extreme limits of 46% and 75%.

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

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

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

Diet

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

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

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

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

Allocation of Rats and Mice to Cages and Treatment Groups

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

This complete process was repeated for the female rats and male B6C3F$_1$ mice. The mice were kept on a separate rack from the rats.

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

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

Treatment groups were colour coded as follows:

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

Animal Identification

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

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

Animal Positioning in the Exposure Chambers

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

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

The treatment groups were constituted as follows:-

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

ATMOSPHERE GENERATION AND EXPOSURE

Exposure Chambers

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

Exposures to bis(2-methoxyethyl) ether were carried out in 1.5 m^3 capacity chambers constructed of stainless steel and glass. The animals occupied a volume of 0.02 m^3 and were confined to a single tier of cages of 0.4 m^3 in volume (the breathing zone). The breathing zone was ventilated at the rate of 12 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 was ventilated at the rate of 10 air changes per hour.

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

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

The generating apparatus and exposure chambers (Figures 1a and 1b) were positioned behind a screen in a room with a high efficiency exhaust system designed to ensure a safe working environment for laboratory personnel. The monitoring equipment was located on the outside of the screen at the opposite end of the room. The laboratory atmosphere was continuously monitored for any traces of the test compound. Exposure personnel wore breathing apparatus until it was shown that the room environment was clear of any possible contamination by bis(2-methoxyethyl) ether. 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 (5 l/min) through stainless steel sample lines of 3/8" 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.

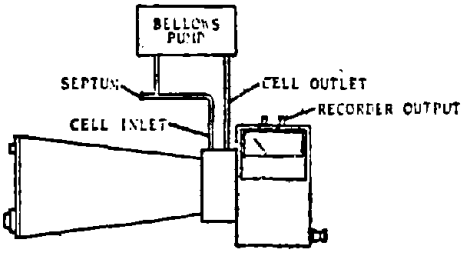
Calibration and Analytical Development

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 bis(2-methoxyethyl) ether 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 bis(2-methoxyethyl) ether were calibrated each day before vapour generation commenced.

The calibration was performed using a closed loop calibration system (see diagram below). Known volumes of bis(2-methoxyethyl) ether 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.



SCHEMATIC DIAGRAM OF CLOSED LOOP CALIBRATION SYSTEM

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

Analytical Conditions

Instrument Settings:

	Low Level	High Level
Wavelength :	9.7 μm	9.7 μm
Pathlength :	20.25 m	3.75 m
Absorbence Range :	1 A	1 A
Slit Width :	1 mm	1 mm
Meter Response :	4	1
Recorder Voltage :	1 V	1 V
Chart Speed :	120 mm/h	120 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 (μl)
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 (l)

Example of the Calculation for V

Compound:

C = 250 ppm
ρ = 0.937 g/cm^3
M = 134.18

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

$$= \frac{250 \times 134.18}{0.937} \times \frac{5.64}{10^3 \times 24.06\ \mu l}$$

$$= 8.4\ \mu l$$

Therefore, to construct a calibration curve to cover the 250 ppm range, 3.0 μl samples of bis(2-methoxyethyl) ether were injected into the analyser.

Atmosphere Generation

Schematic diagrams showing the vapour generating apparatus, exposure chambers and monitoring equipment is presented in Figures 1a and 1b. The test atmospheres were produced by bubbling dry, oxygen-free nitrogen (BOC Limited) through a liquid reservoir of bis(2-methoxyethyl) ether contained in a glass, gas washing or Drechsel bottle immersed in a temperature controlled water bath at 50°C. The nitrogen/bis(2-methoxyethyl) ether vapour mixture so generated was ducted through 7/16" ID stainless steel piping to a glass mixing vessel and diluted with filtered, compressed air. The resulting mixture of bis(2-methoxyethyl) ether/air was ducted through 7/8" stainless steel piping to the top of the exposure chamber.

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

The required atmospheric concentrations within the exposure chambers were maintained by finely regulating the flow of 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 bis(2-methoxyethyl) ether concentration.

Measurement of Chamber Concentrations

Atmospheric concentrations of bis(2-methoxyethyl) ether 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 5 l/min.

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

Test Compound Utilisation

At the beginning of each exposure day, the bis(2-methoxyethyl) ether reservoir (a gas washing or Drechsel bottle) was replenished with test compound. Utilisation of test material was calculated on a daily basis by weighing the bottle before vapour generation began and deducting the weight of the 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 bis(2-methoxyethyl) ether were arranged in a single tier inside the exposure chamber. Air control animals were stacked in 2 tiers.

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

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

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

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

Positive Control Groups in Animal Tests

Preparation of Dosing Solutions

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

Treatment of Rats and Mice with Ethyl methanesulphonate

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

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

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

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

UNSCHEDULED DNA SYNTHESIS ASSAY

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

Chemicals

The positive control 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) was 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 BDH Limited, Poole, Dorset, U.K.).

Cells

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

Culture Maintenance and Growth Media

Cells in 175 cm^2 Nunc flasks were routinely maintained at 37°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°C, 30 min) foetal calf serum, (10% v/v), gentamycin (50 µg/ml) and glutamine (2 mM). DMEM (10x concentrated) and antibiotics were obtained from Gibco Europe Limited, Paisley, Scotland, and serum from Flow Laboratories, Irvine, Scotland.

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

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

Animals

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

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

Preparation of the 9,000 g Supernatant Fluid from Livers

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

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

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

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

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

co-factor solution	9 parts
liver preparation	1 part

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. One 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 directly in culture medium to give concentrations of up to 9.50 mg/ml. To each control culture was added 10 µl of dimethylsulphoxide.

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 used was 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.

Even at 9.50 mg/ml there was less than 25% of the cells showing any damage. In the repair assay a high dose level of 19.0 mg/ml was selected.

DNA Repair Assay

The cells were harvested, sedimented, suspended in fresh culture medium at a density of 5 x 10^4 cells/ml and 2 ml samples of this suspension were pipetted into 35 mm tissue culture Petri dishes containing 3 sterile coverslips (Lux Scientific Corporation, California, U.S.A.). These were then incubated at 37°C in a humid atmosphere of 5% CO_2 in air for 72 h. The medium from each of the dishes was then replaced with 2 ml of arginine-deficient DMEM supplemented with 5% heat inactivated foetal bovine serum and the plates incubated for 24 h. The medium was then replaced with a further 1 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 200 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. Bis(2-methoxyethyl) ether was dissolved in culture medium at a concentration of 19.0 mg/ml and dilutions were made from this to give the lower concentration test solutions. Triplicate wells, with and without S-9 mix, received the test compound solution. 10 µl samples of dimethylsulphoxide were added to culture medium for the 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	1.25 µg/ml
2-AAN	5 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.

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 14 August 1980) 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^{o} angle or tight coiling only).

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

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

SEX-LINKED RECESSIVE LETHAL TEST IN DROSOPHILA MELANOGASTER

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

Strains

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

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

Medium

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

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

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

Exposures

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

*Nipogen: bacteriostatic agent (BDH Limited).

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

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

Toxicity Test

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

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

Recessive Lethal Test

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

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

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

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

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

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

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

STATISTICAL EVALUATION

Cytogenetics Tests

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

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

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

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

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

Dominant Lethal Assay

The variates analysed were:

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

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

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

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

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

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

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

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

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

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 2 moments of the distribution, consequently, likelihood methods cannot be applied. Instead, θ_i terms are estimated by weighted least squares, given the value of ϕ, by minimising.

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

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

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

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

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

Sperm Abnormalities Test

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

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

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

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

Sex-linked Recessive Lethal Test

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

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

F_2 Generation

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

F_3 Generation

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

Against this background, the criteria for result assessment were:

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

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

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

RESULTS

Instrument Calibration

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

Calibration ranges adopted were 90-450 ppm (250 ppm target concentration) and 300-1,500 ppm (1,000 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 bis(2-methoxyethyl) ether was occurring. The results are shown in Table AT-3, where it can be seen that the maximum deviations encountered was <1% at the 250 ppm target concentration and +1% at the 1,000 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 250 ppm and 1,000 ppm were obtained and recorded in Table AT-4.

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.

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)

27 June 1980 Delivery: Ten male and 10 female rats were haphazardly selected for PEAT. One male had red encrustations about one eye and another had a 3 mm bald patch. No other significant clinical observations were made. At

autopsy one male rat had grey foci on all lung lobes, but otherwise the animals were normal. The microbiological/ parasitological examination did not reveal any pathogens. Lung lesions suggestive of a viral infection were present in all animals and one female rat had hydronephrosis.

2 July 1980 Delivery: Four male mice were haphazardly selected for PEAT. There were no significant observations made at any of the examinations of these animals.

Clinical Observations and Body Weights

Male and female rats and male $B6C3F_1$ mice exposed to 1,000 ppm bis(2-methoxyethyl) ether were subdued and unresponsive to audio stimuli during the exposure periods. The mice also appeared hunched and were ataxic. Four mice were found dead on the morning of the fourth exposure day. Surviving mice were not subjected to further exposure to the 1,000 ppm atmosphere. Piloerection and red staining around the head were observed in EMS treated rats during the dosing period.

Male rats exposed to 1,000 ppm and male $B6C3F_1$ mice exposed to 250 and 1,000 ppm bis(2-methoxyethyl) ether showed a reduction in body weight over the multiple exposure period. Female rats were not affected. Dosing with EMS for 5 days caused a reduction in body weight for both rats and mice.

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 bis(2-methoxyethyl) ether (Table UDS-1). The highest concentration used was 19.0 mg/ml.

The positive control substances, 4-nitroquinoline-N-oxide and 2-aminoanthracene, evoked significant levels of UDS in the cells.

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 250 ppm or 1,000 ppm bis(2-methoxyethyl) ether atmospheres. There was a significant reduction in total aberrant cell frequency in female rats exposed to 1,000 ppm atmospheres ($P<0.05$). EMS treatment induced significant increases in both male and female rats ($P<0.05$), although this disappeared in male rats when cells only with gaps were excluded from the analysis.

In the single dose cytogenetic test a significant increase in the frequency of cells with any kind of aberration was seen only in male rats exposed to 250 ppm atmospheres and sampled after 6 h. Males in the 48 h sample showed significant decreases in aberrant cell frequency following exposure to both 250 ppm and 1,000 ppm atmospheres. EMS treatment induced significant increases in aberrant cell frequency at the 6 h ($P<0.001$), 24 h ($P<0.01$) and 48 h ($P<0.001$) sampling times. If cells only with gaps are excluded from the analysis, these increases remain significant. In female rats, increases occurred after 6 h ($P<0.05$), 24 h ($P<0.001$) and 48 h ($P<0.001$). However, if cells only with gaps are excluded from analysis then significance remains at 48 h, but not at 6 h or 24 h.

The significant increase in aberration frequency, described above, in male rats exposed to a 250 ppm atmosphere probably has no biological significance since there is no indication of a dose-related effect. However, decreases in aberration frequency do seem to be prevalent and should not be ignored. While these decreases also may have no biological significance, they may be related to the blood dysplasias known to be induced by glycol ethers (Carpenter *et al* 1956). With special reference to 2-butoxyethanol, these workers showed that the severe haemoglobinuria evident in many rats dying after oral dosing was produced by *in vivo* haemolysis, the kidney injury also induced not being responsible. Erythrocyte osmotic fragility studies suggested that the older or weaker erythrocytes were destroyed and promptly replaced from the bone marrow. The effect is even more pronounced with butoxyacetic acid, a metabolite of 2-butoxyethanol. However, there is less severe effect upon erythrocyte fragility by certain other glycol ethers, including 2-methoxyethanol. The condensation product of 2-methoxyethanol, bis(2-methoxyethyl) ether, was not studied. Should this compound have a similar effect upon rats and cause haemoglobinuria, then the eventual effect upon cell population

dynamics in bone marrow may be the basis for alterations in the proportion of cells carrying chromosomal aberrations. It should be pointed out, however, that in these experiments bis(2-methoxyethyl) ether did not induce haemoglobinuria.

DOMINANT LETHAL TEST

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

Pregnancy frequency was calculated in 2 ways: firstly by considering as pregnant females with corpora lutea graviditatis (Table DL-1) and secondly and more reliably, by considering as pregnant only females with implantations (Table DL-2). The results obtained by these methods were very similar. Pregnancy frequency was satisfactory in the filtered air control group and in the 250 ppm bis(2-methoxyethyl) ether atmosphere group in all assessment weeks. Large reductions in pregnancy frequency occurred in females mated with male rats exposed to 1,000 ppm atmospheres. These large reductions did not occur immediately after cessation of dosing, but in assessment Weeks 4-9, particularly in Weeks 5-7 when pregnancy frequencies were only about 10%. Recovery from the influence of the compound was complete in Week 10. In the EMS treated group there were large reductions in pregnancy frequency in Weeks 2 and 3.

Corpora lutea graviditatis numbers per pregnancy (Table DL-3) were reduced in the 1,000 ppm atmosphere group in Week 3 ($P<0.05$), but to a much greater extent in Weeks 6 and 7. These latter frequencies were not significant according to the method of statistical analysis used only because the pregnancy frequencies also were low, therefore the numbers of degrees of freedom were also low. No reductions in numbers of corpora lutea graviditatis per pregnancy occurred in the 250 ppm atmosphere group. In the EMS treated group the numbers of corpora lutea were reduced in Weeks 1-4.

Total implantations per pregnancy (Table DL-4) were reduced in the 1,000 ppm atmosphere group in Week 4 ($P<0.05$), slightly in Week 5, but they were greatly reduced in Weeks 6 and 7 ($P<0.001$). No reductions occurred in the 250 ppm atmosphere group. EMS treatment induced reductions in implantation frequencies in Weeks 1-4.

The numbers of live implantations per pregnancy (Table DL-5) were reduced in the 1,000 ppm atmosphere group in Weeks 4-7, but particularly in Weeks 6 and 7 ($P<0.001$). There were no reductions in the 250 ppm atmosphere group. EMS treatment induced reductions in live implantations per pregnancy in Weeks 1-5.

Live implantations and late deaths per pregnancy (Table DL-6) followed a pattern very similar to the live implantations per pregnancy pattern described above. In the 1,000 ppm atmosphere group there were significant reductions from the concurrent air control values in Weeks 4-7, but there were no

reductions in the 250 ppm atmosphere group. EMS treatment induced reductions in Weeks 1-5. No implantations survived in Week 2 of this group.

A review of the data showing pregnancies with either (1) one or more early deaths or (2) two or more early deaths (Table DL-7) showed a possible increase in Week 5 of the 1,000 ppm atmosphere group, but in no assessment week of the 250 ppm atmosphere group. There were increases in these proportions in Weeks 1-4 of the EMS treated group.

Analysis of the frequency of early deaths per pregnancy, following the Freeman-Tukey poisson transformation (Table DL-8) indicated a significant increase in Week 5 of the 1,000 ppm atmosphere, but not in any week of the 250 ppm atmosphere group. In the EMS treated group there were increases in Weeks 1-4. A similar analysis, but following the Freeman-Tukey binomial transformation gave significant increases in the frequency of early deaths per pregnancy in the 1,000 ppm atmosphere group in Weeks 5 ($P<0.01$) and 6 ($P<0.001$). In Week 7 of the 250 ppm atmosphere group there was a significant decrease in this frequency. EMS treatment gave increases in Weeks 1-4 which were statistically significant in Weeks 1 ($P<0.001$) and 4 ($P<0.001$).

SPERM ABNORMALITY TEST

No increases in the frequencies of abnormal sperm were noted following exposure to 250 ppm bis(2-methoxyethyl) ether, but large increases were seen in mice exposed to 1,000 ppm atmospheres. All the categories of abnormalities were involved to some extent, but the greatest response was in the incidence of amorphous heads, which increased from 2.18% in the air control to 20.87% in the 1,000 ppm atmosphere group.

It was concluded that bis(2-methoxyethyl) ether does increase the frequency of abnormal sperm.

Some increases in sperm abnormality frequencies were also seen in the 200 mg EMS/kg treated group. While some increases were observed in categories C, D and E, the largest increase was seen in D, the folded tail category.

SEX-LINKED RECESSIVE LETHAL TEST IN DROSOPHILA

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

A dose ranging experiment was conducted on 1 July 1980 in which flies were exposed to 1,000 ppm atmospheres. Within 10 min the Drosophila were no longer able to fly. Exposure was stopped, but even after 2 h in air there were very few fully active individuals.

Exposure was re-started with fresh Drosophila. The concentration of bis(2-methoxyethyl) ether used was 100 ppm. There were apparently no adverse effects upon the flies by this concentration within 2.5 h.

On 2 July 1980, Drosophila were exposed to 250 ppm atmospheres for 2 h, 2.8 h or 4 h. In 1.25 h the flies were at the bottom of the exposure chamber, but still active. The first batch of flies was removed from the exposure chamber following 2 h exposure and these regained normal activity in 2 h. The second batch of flies was not able to fly 1 h after the end of exposure and the third batch of flies was unable to move, apart from occassional twitching. The following day, 94% of flies were still alive after the 2 h exposure, 68% after the 2.8 h exposure and 0% after the 4 h exposure. Hatchability of eggs from the 2 h and 2.8 h exposure flies was not impaired.

On the basis of these results, 2 breeding stocks (A and B) were exposed on 15 July 1980 to a 250 ppm bis(2-methoxyethyl) ether atmosphere for 2.75 h (Table RL-2).

In air control groups, there were 3 F_2 generation lethals in Stock A and 2 in Stock B. The 3 Stock A lethals all occurred in Brood 1 (0.52%), which covers Days 1-3 of the spermatogenesis cycle, while in Stock B one lethal was in Brood 1 (0.18%) and one was in Brood 2 (0.18%), which covers Days 3-8 of the spermatogenesis cycle.

In the bis(2-methoxyethyl) ether exposed flies, there were 6 lethals in Stock A and one in Stock B. The F_2 generation lethals in Stock A were: one in Brood 1 (0.18%), one in Brood 2 (0.19%) and 4 in Brood 3 (0.81%) which covers Days 8-11 of spermatogenesis. The single Stock B lethal occurred in Brood 3 (0.18%). The Stock A, Brood 3 result was certainly high, but it was not repeated in Stock B flies. It is difficult to interpret this result because the concurrent air control (Stock A, Brood 1) also showed an unusually high frequency of recessive lethals. In an attempt

to put these results in perspective, however, it may be useful to consider historical air control data in these stocks of flies.

The Stocks A and B were established in 1979 by splitting a randomly bred colony of flies. Breeding continued to be random and the 2 stocks of flies had environmental conditions which were essentially identical. The only reason for establishing these stocks in the first place was to allow parallel tests to be duplicated. The F_2 generation frequencies of SLRL in air control group flies over the period 1979-1980 were:

Stock A: 9/12253 (0.07%)
Stock B: 7/9930 (0.07%)

Many fewer F_3 generation flies have been examined and the corresponding data are:

Stock A: 0/1445 (0.00%)
Stock B: 2/2215 (0.09%)

The air control groups sizes varied in these different experiments but were generally around 500 flies and in these groups, which totalled 52, more than one SLRL occurred in only 3 groups. These were:

Stock A, F_2, Brood 1: 3/571 = 0.53% (this test)
Stock B, F_2, Brood 1: 3/527 = 0.57%
Stock B, F_3, Brood 3: 2/576 = 0.35%

Therefore, while it cannot be confidently concluded that bis(2-methoxyethyl) ether exposure was responsible for the high SLRL frequency in Stock B, Brood 3 it is also rather improbable that another "spontaneous" high frequency should have occurred in the same experiment as an uncommonly high air control group value.

In the F_3 generation there were no SLRL in the air control group in which 500 vials were set up and 490 were scored. Flies exposed to 250 ppm bis(2-methoxyethyl) ether, however, produced 6 lethals in Brood 1 out of 498 vials set up and 484 scored (1.24%). No lethals were detected in Broods 2 or 3. It is very unlikely that this high frequency was a result of treatment since 4/6 lethals were derived from one male and 2/6 were derived from another. Mapping experiments showed that the lethals from the former of these 2 males all map in the same part of the chromosome, indicating that these 4 lethals were not independent events.

DISCUSSION AND CONCLUSIONS

The use of the UDS assay and cytogenetic analysis of rat bone marrow cells failed to demonstrate any genotoxic effects of bis(2-methoxyethyl) ether. Nevertheless, careful evaluation of this compound as a hazard to man is necessary. In the dominant lethal assay a significant antifertility effect was demonstrated and there also seemed to be an increase in the frequencies of early deaths in assessment Weeks 5 and 7 in the 1,000 ppm atmosphere group. These increases were statistically significant, but the number of implantations per pregnancy were very low. It has been shown that, in untreated CD-1 mice the proportion of early deaths increases in pregnancies with less than about 4 implantations, when compared with pregnancies of more usual size (Anderson et al, 1980).

Similar studies have not been conducted in CD rats, but it is possible that the effects observed in this experiment are due to a similar phenomenon. No effects at all were seen in the 250 ppm atmosphere group in the dominant lethal study. Similar antifertility effects were seen in a study with 2-methosyethanol at this laboratory (NIOSH Contract No. 210-86-0026, Report No. 22).

The examination of mouse sperm for abnormalities also revealed that bis(2-methoxyethyl) ether was having a deleterious effect. There was again a similarity with the effects of 2-methoxyethanol in that the frequency of Category C, amorphous heads, was most clearly increased.

The SLRL test in Drosophila yielded results that were suggestive of a genetic effect, although they were not entirely clear and remain unconfirmed. The problems experienced in the interpretation of these results were similar to those reported with 2-methoxyethanol (NIOSH Contract No. 210-86-0026, Report No. 22). It should be mentioned that in a series of 13 compounds tested in this way under the same contract only 2-methoxyethanol and bis(2-methoxyethyl) ether presented these interpretative problems.

Tests with S. typhimurium (see p. 5 and 6) suggested that, at best, bis(2-methoxyethyl) ether is a very weak bacterial mutagen. It appears, therefore, that the reproductive effects of the compound are more important that the mutagenicity. The antifertility effects should be examined in more detail and, perhaps, studies undertaken to establish whether there is any correlation between antifertility and the incidence of abnormal sperm.

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TABLE AT-1

Bis(2-methoxyethyl) ether
Calibration Data for Low Level

Dose Level: 250 ppm v/v

Batch No.: 21150

Volume µl	Conc., ppm, (v/v)	Cumulative Chart Deflection, mm						
		7 July 1980	8 July 1980	9 July 1980	10 July 1980	11 July 1980	14 July 1980	15 July 1980
0	0	0	0	0	0	0	0	0
3.0	90	34.0	32.0	38.5	30.0	38.0	39.0	37.0
6.0	180	73.0	64.0	75.0	68.5	66.0	76.0	75.0
9.0	270	102.0	95.0	103.0	101.5	102.5	111.0	108.0
12.0	360	135.5	120.0	127.0	136.5	140.5	141.0	138.0
15.0	450	157.0	156.0	153.0	161.0	171.5	169.0	167.0
Chart deflection (mm) for 250 ppm		97.0	88.0	97.0	94.0	97.0	102.0	100.0

Instrument Setting

Pathlength: 20.25 m
Wavelength: 9.7 µm
Absorbence Range: 1
Slit Width: 1 mm
Meter Response: 4
Recorder Voltage: 1 V
Chart Speed: 120 mm/h

Calibration

Syringe: 10 µl Hamilton
Injection Volume: 3.0 µl
No. of Repeat Injections: 5

TABLE AT-2

Bis(2-methoxyethyl) ether
Calibration Data for High Level

Dose Level: 1,000 ppm v/v

Batch No.: 21150

Volume µl	Conc., ppm,(v/v)	Cumulative Chart Deflection, mm					
		7 July 1980	8 July 1980	9 July 1980	10 July 1980	11 July 1980	14 July 1980
0	0	0	0	0	0	0	0
10.0	300	40.0	32.0	37.0	34.0	36.0	32.0
20.0	600	82.0	67.5	74.0	69.0	70.0	61.5
30.0	900	116.5	104.0	110.0	106.5	105.0	97.0
40.0	1200	148.0	137.0	143.5	140.5	138.5	131.0
50.0	1500	177.0	165.0	172.0	168.0	170.5	160.0
Chart deflection (mm) 1000 ppm		127.0	113.0	121.0	116.0	115.0	107.0

Instrument Setting

Pathlength: 3.75 m
Wavelength: 9.7 µm
Absorbence Range: 1
Slit Width: 1 mm
Meter Response: 1
Recorder Voltage: 1 V
Chart Speed: 120 mm/h

Calibration

Syringe: 10 µl Hamilton
Injection Volume: 10.0 µl
No. of Repeat Injections: 5

TABLE AT-3

Bis(2-methoxyethyl) ether
Chamber Atmosphere Homogeneity Data

Dose Level: 250 ppm and 1,000 ppm

Sample Location	% Deviation from Reference Sampling Point	
	Low	High
Reference Point (R)	0	0
Right Centre (RC)	0	-0.9
Right Front (RF)	0	+0.8
Centre Front (CF)	0	+1.0
Left Front (LF)	0	0
Left Centre (LC)	0	+0.8
Left Back (LB)	0	+1.0
Centre Back (CB)	0	+0.8
Right Back (RB)	0	+0.8

Top view of exposure chamber

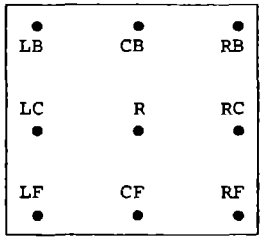


TABLE AT-4

Bis(2-methoxyethyl) ether
Atmospheric Analysis by Infra-red Spectroscopy
Target Concentration 250 ppm

Exposure Day	% Deviation from Target Concentration in Minutes												Time Averaged Concentration for 7 h (ppm)
	-15	-7.5	-5.0	-2.5	0	+2.5	+5.0	+7.5	+10.0	+12.5	+15.0	+20.0	
Single	10	10	67	70	90	68	40	-	10	25	20	10	253.3
Multiple 1	-	-	10	60	135	200	15	-	-	-	-	-	252.2
Multiple 2	-	-	-	5	220	110	85	-	-	-	-	-	254.1
Multiple 3	-	5	55	65	220	60	15	-	-	-	-	-	248.5
Multiple 4	-	-	-	15	320	30	45	10	-	-	-	-	252.0
Multiple 5	-	10	20	155	165	70	-	-	-	-	-	-	247.7

TABLE AT-4 (continued)

Bis(2-methoxyethyl) ether
Target Concentration 1,000 ppm

Exposure Day	% Deviation from Target Concentration in Minutes														Time Averaged Concentration for 7 h (ppm)
	-17.5	-15	-12.5	-10	-7.5	-5.0	-2.5	0	+2.5	+5.0	+7.5	+10.0	+12.5	+15.0	
Single	15	27.5	-	-	45	30	40	92.5	15	30	5	55	50	15	1008.6
Multiple 1	-	-	35	20	5	20	10	75	195	35	25	-	-	-	1001.2
Multiple 2	-	-	-	-	-	10	30	50	305	25	-	-	-	-	1018.2
Multiple 3	-	-	-	-	-	10	50	280	70	10	-	-	-	-	1001.2
Multiple 4	-	-	-	-	-	5	10	155	250	-	-	-	-	-	1013.7
Multiple 5	-	-	-	-	-	5	-	290	125	-	-	-	-	-	1006.8

TABLE AT-4 (continued)

Bis(2-methoxyethyl) ether
Target Concentration 250 ppm

Exposure Day	% Deviation from Target Concentration in Minutes								Time Averaged Concentration (165 min)
	-5.0	-2.5	0	+2.5	+5.0	+7.5	+10	+12.5	
Drosophila Main Test	17	5	78	15	20	20	0	10	254.8

TABLE BW-1

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

Sex	Day	Air Control (0 ppm)	250 ppm	1,000 ppm	EMS 5 x 100 mg/kg
Male	1	389.9 ± 16.2	391.9 ± 15.4	389.0 ± 16.7	398.8 ± 17.2
	2	393.0 ± 19.3	394.6 ± 15.7	384.3 ± 15.8	388.6 ± 16.0
	3	398.3 ± 21.6	399.8 ± 15.9	383.8 ± 16.0	377.0 ± 15.0
	4	398.2 ± 22.9	403.0 ± 15.2	383.1 ± 17.7	363.1 ± 12.5
	5	403.1 ± 24.5	404.8 ± 17.0	385.7 ± 18.4	361.7 ± 13.6
	Weight gain/ loss	13.2	12.9	-3.3	-37.1
Female	1	234.7 ± 24.3	239.1 ± 17.1	244.3 ± 9.7	244.6 ± 24.4
	2	235.8 ± 20.6	234.8 ± 15.5	239.2 ± 11.0	234.8 ± 22.4
	3	237.2 ± 19.5	240.2 ± 13.8	241.2 ± 12.7	224.1 ± 21.0
	4	237.5 ± 20.0	238.4 ± 15.7	241.7 ± 10.4	217.7 ± 22.6
	5	239.7 ± 19.2	240.5 ± 15.1	250.7 ± 10.2	217.3 ± 24.5
	Weight gain/ loss	5.0	1.4	6.4	-27.3

TABLE BW-2

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

Sex	Sampling Time (Hours Post Exposure)	Air Control (0 ppm)	250 ppm	1,000 ppm	250 mg/kg EMS
Male	6	433.3 ± 22.4	424.3 ± 14.9	446.9 ± 22.9	435.9 ± 27.8
	24	422.0 ± 18.1	422.2 ± 25.2	432.8 ± 27.6	433.8 ± 23.4
	48	411.0 ± 29.2	415.5 ± 19.3	421.1 ± 13.9	432.2 ± 14.0
Female	6	257.3 ± 20.5	255.8 ± 25.1	273.2 ± 18.1	247.0 ± 16.6
	24	255.1 ± 30.1	260.3 ± 22.0	247.1 ± 13.9	253.4 ± 18.6
	48	266.0 ± 27.8	254.6 ± 19.2	250.0 ± 23.3	263.2 ± 18.0

TABLE BW-3

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

Day	Air Control (0 ppm)	250 ppm	1,000 ppm	5 x 100 mg/kg EMS
1	385.3 ± 16.1	380.5 ± 21.7	387.2 ± 15.1	384.7 ± 16.2
2	387.2 ± 16.9	381.8 ± 20.3	385.1 ± 13.4	376.7 ± 17.3
3	389.8 ± 18.5	386.0 ± 20.9	387.4 ± 14.0	363.9 ± 15.9
4	389.8 ± 20.7	388.5 ± 21.6	383.0 ± 14.6	349.8 ± 17.2
5	392.7 ± 19.9	391.2 ± 22.4	385.4 ± 14.6	348.5 ± 17.0
Weight gain/loss	7.4	10.7	-1.8	-36.2

TABLE BW-4

Bis(2-methoxyethyl) ether
Sperm Abnormalities Test
Group Mean Body Weights (g) for the Dosing Period of Male B6C3F$_1$ Mice

Day	Air Control (0 ppm)	250 ppm	1,000 ppm	5 x 100 mg/kg EMS
1	23.8 ± 0.9	23.7 ± 1.3	23.3 ± 1.1	24.8 ± 0.9
2	24.1 ± 0.6	23.9 ± 1.2	22.2 ± 0.8	23.9 ± 1.0
3	24.3 ± 0.7	24.5 ± 1.4	21.4 ± 1.3	23.8 ± 1.1
4	24.5 ± 0.8	23.8 ± 1.1	*	23.7 ± 0.8
5	25.0 ± 0.8	23.1 ± 1.2	*	22.7 ± 0.7
Weight gain/loss	1.2	-0.6	-	-2.1

* - 3 mortalities, surviving animals not exposed.

TABLE UDS-1

Bis(2-methoxyethyl) ether
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	3.6 ± 9.6	7.1 ± 4.3
4-Nitroquinoline-N-oxide	-	1.25	-	97.0 ± 31.1
2-Aminoanthracene	5	-	78.9 ± 46.2	-
Bis(2-methoxyethyl) ether	148	148	2.2 ± 4.3	4.5 ± 4.5
	297	297	2.4 ± 2.5	2.5 ± 3.5
	594	594	3.4 ± 7.8	4.7 ± 3.6
	1,188	1,188	3.1 ± 7.4	3.6 ± 7.7
	2,375	2,375	7.0 ± 11.2	2.6 ± 2.6
	4,750	4,750	4.2 ± 7.7	4.8 ± 7.7
	9,500	9,500	4.1 ± 5.4	4.0 ± 3.6
	19,000	19,000	4.6 ± 6.8	5.4 ± 11.5

TABLE CA-MD-M-1

Bis(2-methoxyethyl) ether
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	310	6	-	-	-	-	-	1 Chromatid Fragment
250 ppm, 7 h/day	309	13	3	-	-	-	-	3 Chromatid Fragments
1,000 ppm, 7 h/day	445	10	-	-	1	-	-	-
EMS, 100 mg/kg/day	344	20	5	3	2	-	-	2 Chromatid Fragments

TABLE CA-MD-M-2

Bis(2-methoxyethyl) ether
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	0.326	0.0600		0.183	0.0418	
250 ppm	0.465	0.0600	1.634	0.280	0.0418	1.646
1,000 ppm	0.342	0.0530	0.192	0.141	0.0369	-0.742
EMS, 100 mg/kg	0.533	0.0600	2.433*	0.269	0.0418	1.463

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

TABLE CA-MD-F-1

Bis(2-methoxyethyl) ether
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	301	17	2	1	1	-	-	1 Exchange 1 Robertsonian Translocation
250 ppm, 7 h/day	337	15	3	-	-	-	-	1 Chromatid Fragment 2 Chromatid Fragments
1,000 ppm, 7 h/day	278	2	-	-	1	-	-	1 Pair of Minutes 3 Chromatid Fragments
EMS, 100 mg/kg/day	376	34	15	3	1	-	-	7 Chromatid Fragments 1 Exchange 1 Pair of Minutes

TABLE CA-MD-F-1 (Supplementary)

Bis(2-methoxyethyl) ether
Cytogenetic Analysis of Rat Bone Marrow Cells
Supplementary Observations
Females

Multiple Dosing

Sampling Time: 6 h

Group	Animal Number	Miscellaneous Observations
Air Control, 7 h/day	285 287	1 Chromosome split at centromere Some Chromosomes split at centromere
250, ppm, 7 h/day	291 292 299	Some Chromosomes split at centromere 2 Chromosomes split at centromere Some Chromosomes split at centromere
1,000 ppm, 7 h/day	307 310	1 Chromosome split at centromere 4 Chromosomes split at centromere
EMS, 100 mg/kg/day	316	1 Chromosome split at centromere

TABLE CA-MD-F-2

Bis(2-methoxyethyl) ether
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	0.566	0.0613		0.293	0.0624	
250 ppm	0.547	0.0574	-0.231	0.357	0.0584	0.741
1,000 ppm	0.341	0.0613	-2.595*	0.230	0.0624	-0.713
EMS, 100 mg/kg	0.749	0.0513	2.285*	0.489	0.0522	2.408*

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

TABLE CA-M6-1

Bis(2-methoxyethyl) ether
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	400	10	-	-	-	-	-	1 Chromatid Framgment
250 ppm, 7 h/day	300	16	3	-	-	-	-	-
1,000 ppm, 7 h/day	450	12	1	-	-	-	-	2 Chromatid Fragments 2 Pairs of Minutes
EMS, 250 mg/kg/day	256	26	5	-	3	-	-	3 Chromatid Fragments

TABLE CA-M6-2

Bis(2-methoxyethyl) ether
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	0.335	0.0605		0.165	0.0468	
250 ppm	0.531	0.0699	2.126*	0.224	0.0541	0.824
1,000 ppm	0.352	0.0570	0.209	0.196	0.0441	0.482
EMS, 250 mg/kg	0.697	0.0647	4.091***	0.443	0.0500	4.057***

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

*P<0.05

***P<0.001

TABLE CA-M24-1

Bis(2-methoxyethyl) ether
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Males

Single Dosing

Sampling Time: 6 h

Group	Number of Spreads Observed	Observed Aberrations						Miscellaneous
		Chromatid			Chromosome			
		Gap	B w F	B w/o F	Gap	B w F	B w/o F	
Air Control, 7 h/day	500	3	-	-	-	-	-	1 Miscellaneous Aberration
250 ppm, 7 h/day	450	4	-	-	2	1	-	2 Miscellaneous Aberrations
1,000 ppm, 7 h/day	500	5	-	-	-	-	-	-
EMS, 250 mg/kg/day	500	31	5	1	1	-	-	1 Miscellaneous Aberration 4 Multi Aberrations 2 Exchanges 1 Chromatid Fragment 1 Robertsonian Translocation

TABLE CA-M24-2

Bis(2-methoxyethyl) ether
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	0.211	0.0478		0.180	0.0350	
250 ppm	0.286	0.0503	1.086	0.196	0.0369	0.315
1,000 ppm	0.207	0.0478	-0.061	0.141	0.0350	-0.805
EMS, 250 mg/kg	0.526	0.0478	4.670***	0.287	0.0350	2.159*

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

*P<0.05

***P<0.001

TABLE CA-M48-1

Bis(2-methoxyethyl) ether
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	4	6	1	1	1	-	3 Chromatid Fragments 1 Chromosomal Fragment
250 ppm, 7 h/day	450	-	-	-	-	-	-	1 Chromatid Fragment
1,000 ppm, 7 h/day	450	-	-	-	-	-	-	2 Chromatid Fragments
EMS, 250 mg/kg/day	500	20	20	2	-	-	-	1 Chromosomal Fragment 5 Chromatid Fragments 5 Exchanges 1 Multi Aberration

TABLE CA-M48-2

Bis(2-methoxyethyl) ether
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	0.308	0.0450		0.250	0.0423	
250 ppm	0.163	0.0474	-2.231*	0.163	0.0446	-1.414
1,000 ppm	0.163	0.0474	-2.231*	0.163	0.0446	-1.414
EMS, 250 mg/kg	0.509	0.0450	3.150**	0.369	0.0423	1.987

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

*P<0.05

**P<0.01

TABLE CA-F6-1

Bis(2-methoxyethyl) ether
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	351	11	1	-	-	-	-	-
250 ppm, 7 h/day	173	12	1	-	-	-	-	-
1,000 ppm, 7 h/day	393	25	1	-	-	-	-	1 Chromatid Fragment
EMS, 250 mg/kg/day	400	45	9	-	2	-	-	3 Chromatid Fragments

TABLE CA-F6-2

Bis(2-methoxyethyl) ether
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	0.402	0.0678		0.181	0.0373	
250 ppm	0.546	0.0959	1.226	0.202	0.0527	0.331
1,000 ppm	0.504	0.0678	1.057	0.192	0.0373	0.207
EMS, 250 mg/kg	0.714	0.0678	3.252**	0.353	0.0373	3.262**

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

TABLE CA-F24-1

Bis(2-methoxyethyl) ether
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	409	2	-	-	-	-	-	-
250 ppm, 7 h/day	450	3	-	-	-	-	-	1 Chromatid Fragment
1,000 ppm, 7 h/day	500	1	-	-	-	-	-	2 Miscellaneous Aberrations
EMS, 250 mg/kg/day	500	16	1	-	2	-	-	1 Multi Aberration 1 Chromatid Fragment

TABLE CA-F24-2

Bis(2-methoxyethyl) ether
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	0.205	0.0515		0.161	0.0304	
250 ppm	0.206	0.0515	0.012	0.163	0.0304	0.047
1,000 ppm	0.191	0.0489	-0.199	0.171	0.0289	0.244
EMS, 250 mg/kg	0.381	0.0489	2.478*	0.179	0.0289	0.446

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

TABLE CA-F48-1

Bis(2-methoxyethyl) ether
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	443	3	-	-	-	-	-	-
250 ppm, 7 h/day	450	2	2	-	-	-	-	1 Pair of Minutes 2 Chromatid Fragments
1,000 ppm, 7 h/day	420	2	-	-	-	-	-	-
EMS, 250 mg/kg/day	450	20	20	4	-	-	-	9 Chromatid Fragments 2 Chromosomal Fragments 10 Exchanges 1 Pair of Minutes 3 Multi Aberrations

TABLE CA-F48-2

Bis(2-methoxyethyl) ether
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	0.198	0.0587		0.142	0.0438	
250 ppm	0.272	0.0587	0.899	0.228	0.0438	1.392
1,000 ppm	0.194	0.0587	-0.048	0.149	0.0438	0.123
EMS, 250 mg/kg	0.561	0.0587	4.374***	0.478	0.0438	5.437***

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

TABLE CA-M48 and F48 (Supplementary)

Bis(2-methoxyethyl) ether
Cytogenetic Analysis of Rat Bone Marrow Cells
Supplementary Observations
Males and Females

Single Dosing

Sampling Time: 48 h

Group	Animal No./Sex	Miscellaneous Aberrations
250 ppm, 7 h/day	53♂	1 Chromosome split at centromere
1,000 ppm, 7 h/day	241♀	1 Chromosome split at centromere

TABLE DL-1

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

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	250 ppm	1,000 ppm	5 x 100 mg/kg EMS
1	90%	100%	80%	75%
2	95%	95%	95%	60%
3	100%	95%	80%	40%
4	100%	95%	65%	90%
5	95%	95%	16%	90%
6	100%	100%	60%	95%
7	100%	100%	30%	90%
8	95%	100%	65%	95%
9	100%	95%	60%	100%
10	100%	95%	85%	100%

TABLE DL-2

Bis(2-methoxyethyl) ether
Dominant Lethal Test in Rats
Pregnancy Frequency (Females with Implantations)

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)		250 ppm		1,000 ppm		5 x 100 mg/kg EMS	
1	18/20	90%	19/20	95%	15/20	75%	15/20	75%
2	19/20	95%	18/20	90%	19/20	95%	1/20	5%
3	18/20	90%	18/19	95%	16/20	80%	3/20	15%
4	19/20	95%	19/20	95%	10/20	50%	17/20	85%
5	19/20	95%	18/20	90%	2/19	11%	18/20	90%
6	20/20	100%	20/20	100%	2/20	10%	18/19	95%
7	19/20	95%	20/20	100%	2/20	10%	18/20	90%
8	19/20	95%	20/20	100%	8/20	40%	19/20	95%
9	19/20	95%	19/20	95%	10/20	50%	20/20	100%
10	18/20	90%	19/20	95%	17/20	85%	20/20	100%

TABLE DL-3

Bis(2-methoxyethyl) ether
Dominant Lethal Test in Rats
Total Number of Corpora Lutea per Pregnancy

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	250 ppm	1,000 ppm	5 x 100 mg/kg EMS
1	[1] 12.7 ± 0.54	12.5 ± 0.53	12.6 ± 0.59	10.8 ± 0.76
2	13.3. ± 0.50	13.2 ± 0.52	13.3 ± 0.50	5.0 ± 0.00
3	14.8 ± 0.79	13.8 ± 0.79	12.4 ± 0.84*	1.0 ± 0.58**
4	12.2 ± 0.54	12.5 ± 0.54	11.1 ± 0.74	8.6 ± 0.97**
5	13.8 ± 0.55	12.6 ± 0.57	12.5 ± 1.71	12.6 ± 0.77
6	13.4 ± 0.55	12.3 ± 0.55	7.0 ± 1.75**	13.0 ± 0.54
7	12.2 ± 0.34	11.8 ± 0.33	2.5 ± 1.04***	11.4 ± 0.84
8	11.8 ± 0.62	11.9 ± 0.60	11.9 ± 0.96	11.8 ± 0.50
9	13.7 ± 0.57	13.2 ± 0.57	12.0 ± 0.79	13.6 ± 0.55
10	12.9 ± 0.51	12.3 ± 0.50	12.6 ± 0.52	13.6 ± 0.63

1 = Mean ± standard error of mean

*P<0.05

**P<0.01

***P<0.001

TABLE DL-4

Bis(2-methoxyethyl) ether
Dominant Lethal Test in Rats
Total Implantations per Pregnancy

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	250 ppm	1,000 ppm	5 x 100 mg/kg EMS
1	[1] 13.0 ± 0.63	12.0 ± 0.61	13.4 ± 0.69	7.8 ± 0.86***
2	13.6 ± 0.54	12.4 ± 0.55	13.7 ± 0.54	1.0 ± 0.00
3	13.8 ± 0.78	12.5 ± 0.78	12.4 ± 0.82	1.0 ± 0.00**
4	12.4 ± 0.60	12.3 ± 0.60	9.9 ± 0.82*	8.2 ± 1.06**
5	13.5 ± 0.53	13.1 ± 0.55	11.0 ± 1.64	11.9 ± 0.74
6	13.0 ± 0.47	13.6 ± 0.47	2.5 ± 1.49***	12.5 ± 0.60
7	12.0 ± 0.31	11.7 ± 0.31	2.0 ± 0.97***	11.4 ± 0.72
8	12.1 ± 0.68	12.3 ± 0.67	11.8 ± 1.06	12.4 ± 0.50
9	11.6 ± 0.63	12.4 ± 0.63	10.4 ± 0.87	12.8 ± 0.64
10	12.3 ± 0.50	12.1 ± 0.49	11.5 ± 0.52	12.5 ± 0.82

1 = Mean ± standard error of mean

*P<0.05

**P<0.01

***P<0.001

TABLE DL-5

Bis(2-methoxyethyl) ether
Dominant Lethal Test in Rats
Live Implantations per Pregnancy

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	250 ppm	1,000 ppm	5 x 100 mg/kg EMS
1	[1] 12.4 ± 0.70	11.6 ± 0.68	12.4 ± 0.77	1.2 ± 0.63***
2	13.1 ± 0.55	11.9 ± 0.57	13.1 ± 0.55	0.0 ± 0.00
3	13.3 ± 0.78	11.6 ± 0.78	11.6 ± 0.82	0.3 ± 0.33**
4	11.9 ± 0.64	11.8 ± 0.64	9.5 ± 0.88*	4.6 ± 0.68***
5	13.3 ± 0.54	12.4 ± 0.55	9.5 ± 1.65*	11.3 ± 0.71*
6	12.3 ± 0.46	13.3 ± 0.46	1.5 ± 1.45***	12.0 ± 0.62
7	11.2 ± 0.36	11.3 ± 0.35	2.0 ± 1.10***	10.9 ± 0.74
8	11.5 ± 0.72	11.6 ± 0.70	11.0 ± 1.11	11.9 ± 0.53
9	11.1 ± 0.62	12.0 ± 0.62	10.0 ± 0.86	11.9 ± 0.59
10	12.3 ± 0.50	12.1 ± 0.49	11.5 ± 0.52	12.5 ± 0.82

1 = Mean ± standard error of mean
*P<0.05
**P<0.01
***P<0.001

TABLE DL-6

Bis(2-methoxyethyl) ether
Dominant Lethal Test in Rats
Live Implantations and Late Deaths per Pregnancy

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	250 ppm	1,000 ppm	5 x 100 mg/kg EMS
1	[1] 12.5 ± 0.69	11.6 ± 0.67	12.4 ± 0.75	1.2 ± 0.63***
2	13.2 ± 0.56	12.1 ± 0.57	13.1 ± 0.56	0.0 ± 0.00
3	13.3 ± 0.78	11.6 ± 0.78	11.6 ± 0.82	0.3 ± 0.33**
4	11.9 ± 0.64	11.8 ± 0.64	9.5 ± 0.88*	4.7 ± 0.71***
5	13.3 ± 0.54	12.4 ± 0.55	9.5 ± 1.65*	11.3 ± 0.71*
6	12.3 ± 0.46	13.3 ± 0.46	1.5 ± 1.47***	12.0 ± 0.62
7	11.3 ± 0.33	11.5 ± 0.32	2.0 ± 1.01***	10.9 ± 0.74
8	11.5 ± 0.72	11.6 ± 0.70	11.0 ± 1.11	11.9 ± 0.52
9	11.1 ± 0.62	12.0 ± 0.62	10.0 ± 0.85	12.0 ± 0.60
10	12.3 ± 0.48	12.2 ± 0.47	11.6 ± 0.50	12.6 ± 0.77

1 = Mean ± standard error of mean
*P<0.05
**P<0.01
***P<0.001

TABLE DL-7

Bis(2-methoxyethyl) ether
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)		250 ppm		1,000 ppm		5 x 100 mg/kg EMS	
	>0	>1	>0	>1	>0	>1	>0	>1
1	7/18	2/18	7/19	0/19	8/15	3/15	14/15	14/15
2	6/19	2/19	5/18	1/18	10/19	3/19	1/1	0/1
3	8/18	1/18	9/18	4/18	4/16	2/16	2/3	0/3
4	7/19	3/19	7/19	2/19	3/10	1/10	16/17	13/17
5	4/19	0/19	8/18	3/18	2/2	1/2	7/18	2/18
6	10/20	4/20	7/20	0/20	1/2	1/2	7/18	2/18
7	9/19	3/19	4/20	0/20	0/2	0/2	7/18	2/18
8	8/19	2/19	9/20	4/20	4/8	2/8	7/19	2/19
9	6/19	2/19	8/19	1/19	3/10	1/10	12/20	3/20
10	4/18	1/18	9/19	5/19	7/17	3/17	7/20	2/20

TABLE DL-8

Bis(2-methoxyethyl) ether
Dominant Lethal Test in Rats
Early Death Frequency, Freeman-Tukey Poisson Transformation

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	250 ppm	1,000 ppm	5 x 100 mg/kg EMS
1	[1] 1.631 ± 0.2091	1.521 ± 0.2036	2.003 ± 0.2291	5.077 ± 0.4245***
2	1.524 ± 0.1864	1.431 ± 0.1915	1.860 ± 0.1864	2.414 ± 0.0000
3	1.669 ± 0.2616	1.955 ± 0.2616	1.634 ± 0.2775	1.943 ± 0.4714
4	1.637 ± 0.1955	1.598 ± 0.1955	1.497 ± 0.2694	3.816 ± 0.2985***
5	1.298 ± 0.1879	1.816 ± 0.1930	2.780 ± 0.5791*	1.696 ± 0.2301
6	1.854 ± 0.1869	1.495 ± 0.1869	2.073 ± 0.5912	1.631 ± 0.1984
7	1.785 ± 0.1685	1.283 ± 0.1642*	1.000 ± 0.5193	1.631 ± 0.1984
8	1.673 ± 0.2065	1.783 ± 0.2013	1.890 ± 0.3183	1.598 ± 0.1905
9	1.554 ± 0.1914	1.634 ± 0.1914	1.497 ± 0.2639	1.988 ± 0.1998
10	1.355 ± 0.2185	1.955 ± 0.2127	1.712 ± 0.2248	1.568 ± 0.1832

1 = Mean ± standard error of mean
*P<0.05
***P<0.001

TABLE DL-9

Bis(2-methoxyethyl) ether
Dominant Lethal Test in Rats
Early Death Frequency, Freeman-Tukey Binomial Transformation

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	250 ppm	1,000 ppm	5 x 100 mg/kg EMS
1	[1] 0.457 ± 0.0643	0.449 ± 0.0626	0.559 ± 0.0705	2.386 ± 0.1646***
2	0.415 ± 0.0520	0.401 ± 0.0535	0.494 ± 0.0520	2.334 ± 0.0000
3	0.443 ± 0.0738	0.572 ± 0.0738	0.478 ± 0.0782	1.818 ± 0.5163
4	0.465 ± 0.0636	0.454 ± 0.0636	0.507 ± 0.0877	1.431 ± 0.0825***
5	0.351 ± 0.0514	0.496 ± 0.0528	0.837 ± 0.1585**	0.487 ± 0.0632
6	0.512 ± 0.0698	0.394 ± 0.0698	1.516 ± 0.2208***	0.461 ± 0.0559
7	0.513 ± 0.0487	0.363 ± 0.0474*	0.654 ± 0.1500	0.497 ± 0.0623
8	0.472 ± 0.0705	0.499 ± 0.0687	0.704 ± 0.1086	0.452 ± 0.0548
9	0.449 ± 0.0590	0.454 ± 0.0590	0.503 ± 0.0813	0.547 ± 0.0518
10	0.380 ± 0.0599	0.532 ± 0.0584	0.478 ± 0.0617	0.463 ± 0.0713

1 = Mean ± standard error of mean

*P<0.05

**P<0.01

***P<0.001

TABLE SA-1

Bis(2-methoxyethyl) ether
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	9486	31	28	218	77	160	514	0.31	0.28	2.18	0.77	1.60	5.14
250 ppm, 7 h/day	8576	18	25	190	46	145	424	0.20	0.28	2.11	0.51	1.61	4.71
1,000 ppm, 7 h/day	4736	118	121	1461	239	325	2264	1.68	1.73	20.87	3.41	4.64	32.3
EMS, 200 mg/kg/day	8271	22	25	338	145	199	729	0.24	0.28	3.76	1.60	2.21	8.10

* 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 RL-1

Bis(2-methoxyethyl) ether
Drosophila Dose Ranging Experiment

Day		250 ppm						Date & Initial
		2 h		2 h 50 min		4 h		
0	No. of males exposed	50		50		50		2.7.80 KT
1	No. & % survival	47	94	34	68	0	0	3.7.80 KT
2	No. of eggs laid by 4 females	262		284		0		4.7.80 KT
3	No. & % hatched	231	88	273	96	0	0	5.7.80 KT

Comments: Control:- eggs laid = 140 = 95%
eggs hatched = 133

Time chosen for test exposure: 2 3/4 h

TABLE RL-2

Bis(2-methoxyethyl) ether
Drosophila SLRL Procedure and Results

Compound: Air Concentration: - Stock: A
Length of Exposure: - Test exposure given: -

	Brood 1	Brood 2	Brood 3
F_1 set up	16.7.80	19.7.80	24.7.80
F_2 set up	29.7.80	31.7.80	6.8.80
F_2 scored	12.8.80	13.8.80	20.8.80
F_2 repeats scored	26.8.80	29.8.80	-
F_3 set up	-	-	20.8.80
F_3 scored	-	-	2.9.80
F_3 repeats scored	-	-	-

RESULTS

	Brood 1	Brood 2	Brood 3	All Broods
No. of F_1 vials	100	100	88	288
No. of sterile F_1 vials	19	34	28	81
No. of F_1 vials used in F_2	81	66	60	207
No. of F_2 vials set up	609	600	600	1809
No. of F_2 vials scored	571	527	575	1673
No. of F_2 vials containing lethals	3	0	0	3
Frequency of F_2 lethals	0.52	0	0	0.18
No. of F_3 vials set up	0	0	500	500
No. of F_3 vials scored	0	0	490	490
No. of F_3 vials containing lethals	0	0	0	0
Frequency of F_3 lethals	0	0	0	0

TABLE RL-2 (continued)

Bis(2-methoxyethyl) ether
Drosophila SLRL Procedure and Results

Compound: Air **Concentration:** - **Stock:** B
Length of Exposure: - **Test exposure given:** -

	Brood 1	Brood 2	Brood 3
F_1 set up	16.7.80	19.7.80	24.7.80
F_2 set up	29.7.80	31.7.80	8.8.80
F_2 scored	11.8.80	15.8.80	21.8.80
F_2 repeats scored	26.8.80	-	-
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	97	91	70	258
No. of sterile F_1 vials	25	20	27	72
No. of F_1 vials used in F_2	72	71	43	186
No. of F_2 vials set up	600	599	556	1755
No. of F_2 vials scored	560	544	534	1638
No. of F_2 vials containing lethals	1	1	0	2
Frequency of F_2 lethals	0.18	0.18	0	0.12
No. of F_3 vials set up	0	0	0	0
No. of F_3 vials scored	0	0	0	0
No. of F_3 vials containing lethals	0	0	0	0
Frequency of F_3 lethals	0	0	0	0

TABLE RL-2 (continued)

Bis(2-methoxyethyl) ether

Drosophila SLRL Procedure and Results

Compound: Bis(2-methoxy-ethyl) ether **Concentration:** 250 ppm **Stock:** A
Length of Exposure: 2 3/4 h **Test exposure given:** 15.7.80

	Brood 1	Brood 2	Brood 3
F_1 set up	16.7.80	19.7.80	24.7.80
F_2 set up	28.7.80	1.8.80	5.8.80
F_2 scored	11.8.80	14.8.80	18.8.80
F_2 repeats scored	25.8.80	-	-
F_3 set up	-	14.8.80	18.8.80
F_3 scored	-	28.8.80	2.9.80
F_3 repeats scored	-	-	-

RESULTS

	Brood 1	Brood 2	Brood 3	All Broods
No. of F_1 vials	72	69	62	203
No. of sterile F_1 vials	14	13	15	42
No. of F_1 vials used in F_2	58	56	47	161
No. of F_2 vials set up	602	599	600	1801
No. of F_2 vials scored	539	520	490	1548
No. of F_2 vials containing lethals	1	1	4	6
Frequency of F_2 lethals	0.18	0.19	0.81	0.38
No. of F_3 vials set up	0	496	400	896
No. of F_3 vials scored	0	471	391	862
No. of F_3 vials containing lethals	0	0	0	0
Frequency of F_3 lethals	0	0	0	0

Comments: Had already scored A Brood 1 before remembering that F_3 was required from it, F_3 was therefore taken from B Brood $1F_2$.

TABLE RL-2 (continued)

Bis(2-methoxyethyl) ether
Drosophila SLRL Procedure and Results

Compound: Bis(2-methoxy-ethyl) ether **Concentration:** 250 ppm **Stock:** B
Length of Exposure: 2 3/4 h **Test exposure given:** 15.7.80

	Brood 1	Brood 2	Brood 3
F_1 set up	16.7.80	19.7.80	24.7.80
F_2 set up	28.7.80	31.7.80	5.8.80
F_2 scored	12.8.80	14.8.80	18.8.80
F_2 repeats scored	-	-	-
F_3 set up	12.8.80	-	-
F_3 scored	-	-	-
F_3 repeats scored	-	-	-

RESULTS

	Brood 1	Brood 2	Brood 3	All Broods
No. of F_1 vials	83	77	70	230
No. of sterile F_1 vials	9	17	11	37
No. of F_1 vials used in F_2	74	60	59	193
No. of F_2 vials set up	616	600	598	1814
No. of F_2 vials scored	556	504	543	1603
No. of F_2 vials containing lethals	0	0	1	1
Frequency of F_2 lethals	0	0	0.18	0.06
No. of F_3 vials set up	498	0	0	0
No. of F_3 vials scored	484	0	0	484
No. of F_3 vials containing lethals	6	0	0	6
Frequency of F_3 lethals	1.24	0	0	1.24

Comments: Six F_3 lethals all derived from the progeny of only 2 males. Four from male number 86 all map in same place.

TABLE RL-2 (continued)

Bis(2-methoxyethyl) ether

Drosophila SLRL Procedure and Results

Compound: Ethyl methanesulphonate **Concentration:** 0.4% v/v **Stock:** A

Length of Exposure: 5 h **Test exposure given:** 16.7.80

	Brood 1	Brood 2	Brood 3
F_1 set up	17.7.80	-	-
F_2 set up	1.8.80	-	-
F_2 scored	13.8.80	-	-
F_2 repeats scored	26.8.80	-	-
F_3 set up	-	-	-
F_3 scored	-	-	-
F_3 repeats scored	-	-	-

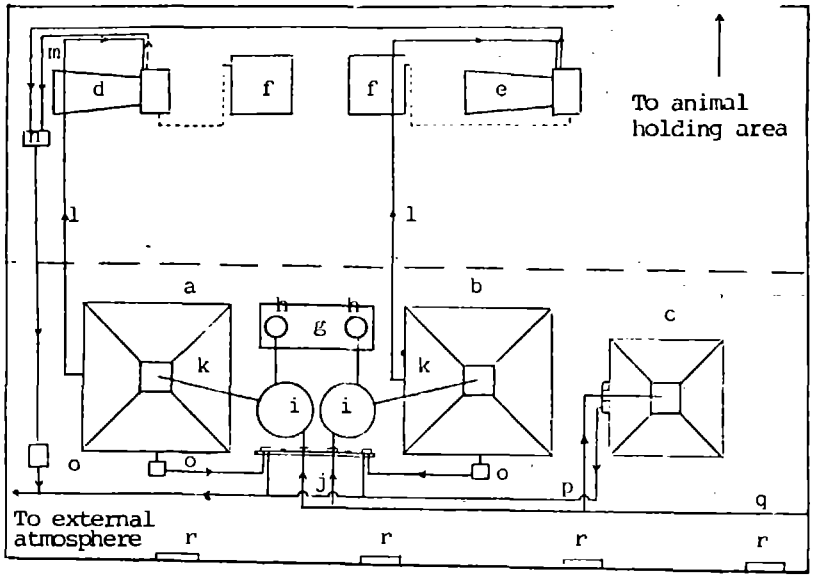
RESULTS

	Brood 1	Brood 2	Brood 3	All Broods
No. of F_1 vials	67	-	-	-
No. of sterile F_1 vials	2	-	-	-
No. of F_1 vials used in F_2	65	-	-	-
No. of F_2 vials set up	195	-	-	-
No. of F_2 vials scored	179	-	-	-
No. of F_2 vials containing lethals	36	-	-	-
Frequency of F_2 lethals	20.1%	-	-	-
No. of F_3 vials set up	0	-	-	-
No. of F_3 vials scored	0	-	-	-
No. of F_3 vials containing lethals	0	-	-	-
Frequency of F_3 lethals	0	-	-	-

FIGURE 1a

Bis(2-methoxyethyl) ether

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

Bis(2-methoxyethyl) ether

Schematic Lay-out of Vapour Generation Apparatus

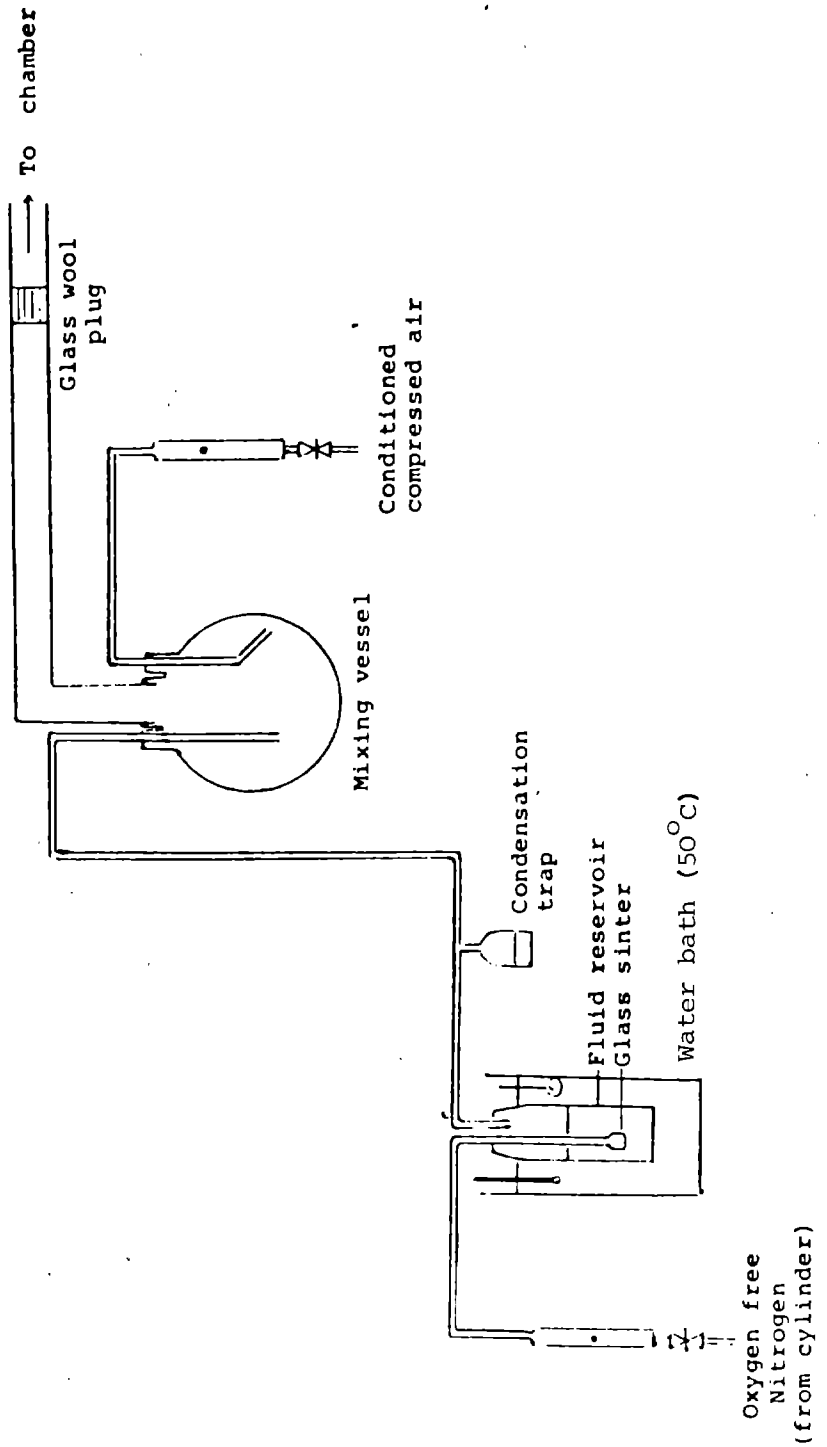


FIGURE 2

Bis(2-methoxyethyl) ether
Typical Calibration Graph for Low Level
9 July 1980

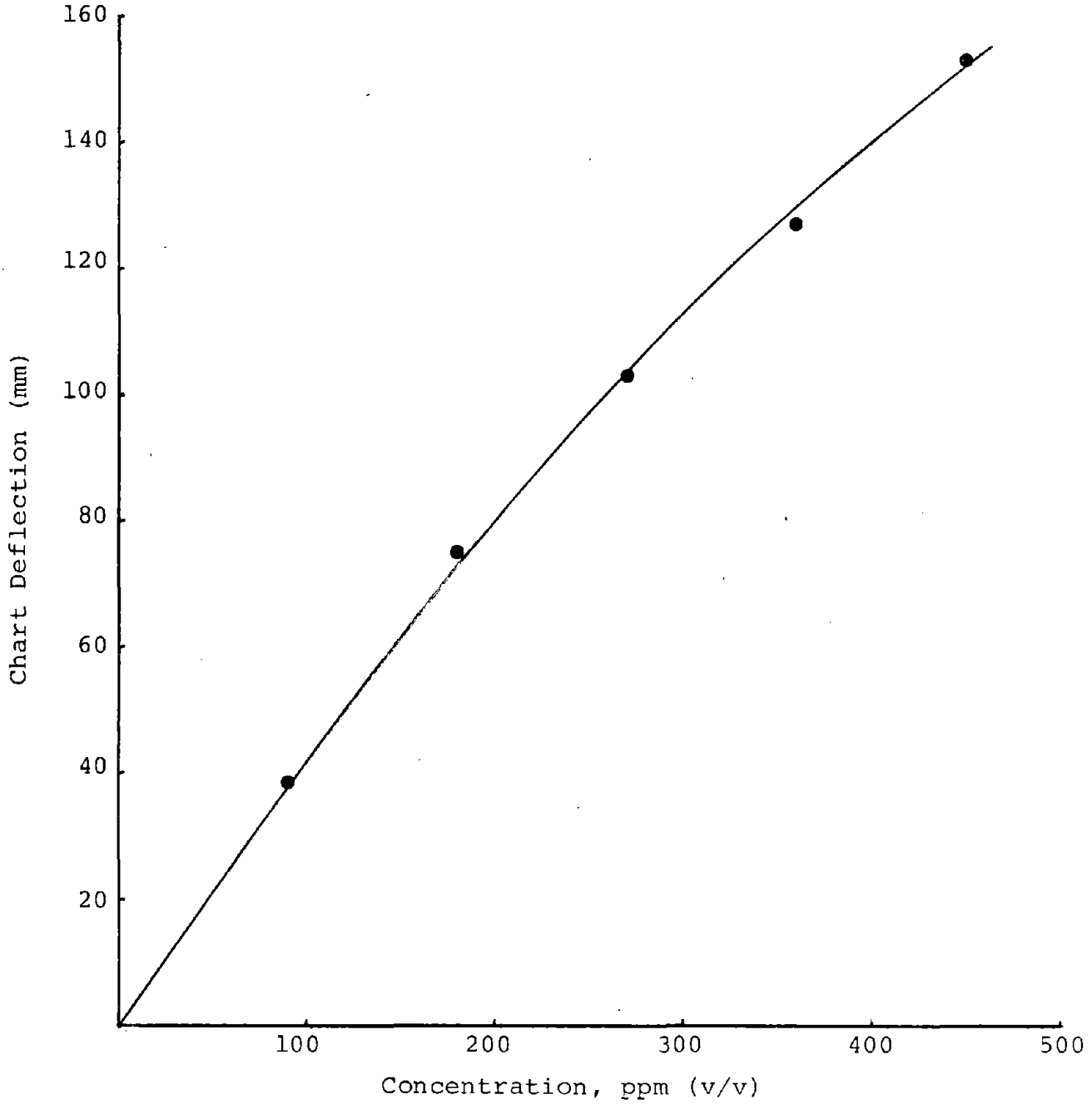
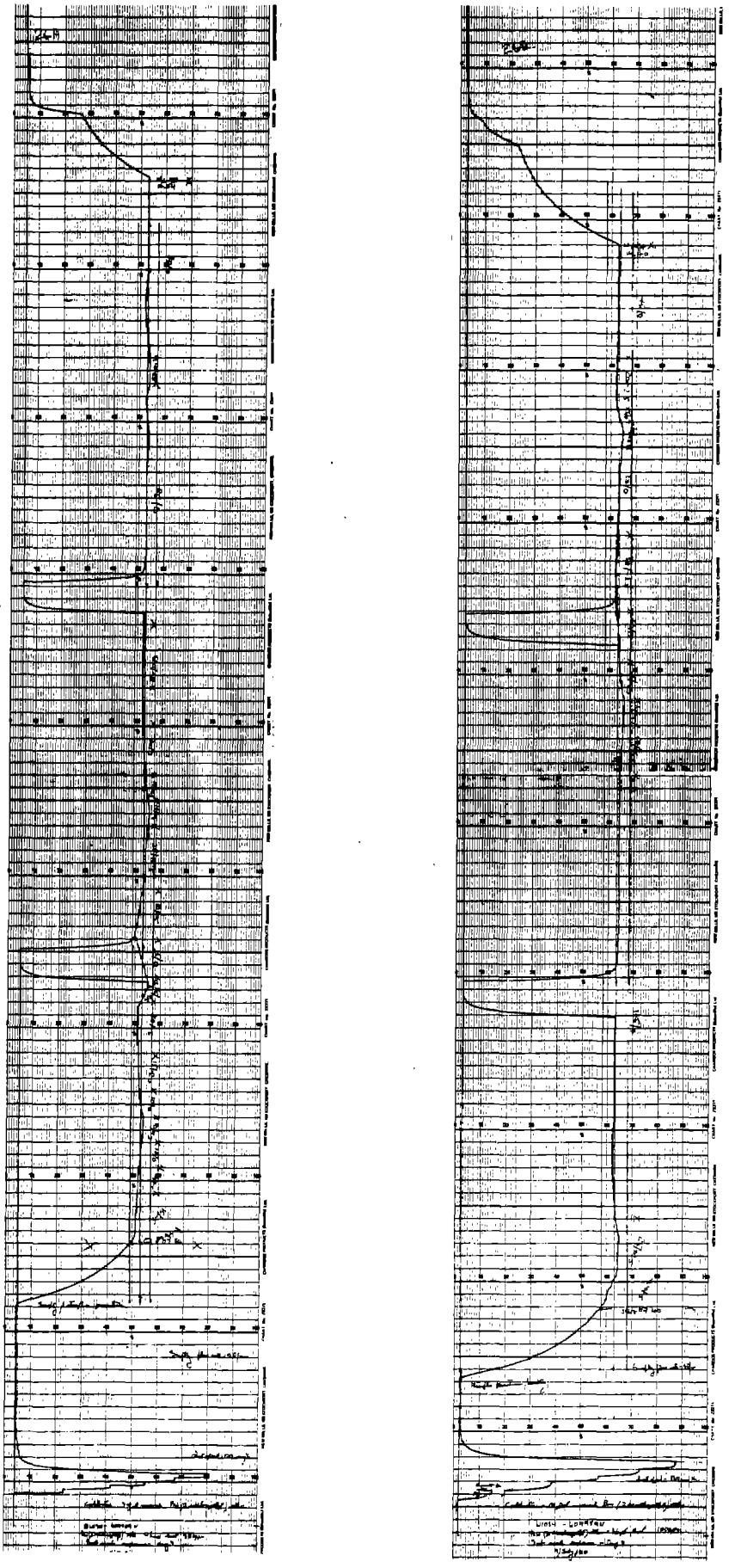


FIGURE 3

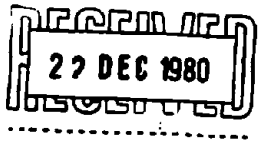
Bis(2-methoxyethyl) ether

Sample Record Chart of IR Absorption at 9.7 µm



APPENDIX DIET

Bis(2-methoxyethyl) ether
Diet Analysis



Spratt's Patent Ltd

Central House
Cambridge Road
Barking
Essex IG11 8NL

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01-594 7121
Telegrams
Spratt's Barking
Telex 897669

CERTIFICATE OF ANALYSIS

Product: LAD 1

Batch No: 098072

Date of Manufacture: 9th July 1980P

	Found Analysis
Moisture	8.4 %
Crude Fat	3.3 %
Crude Protein	20.9 %
Ash	5.3 %
Calcium	0.97 %
Phosphorus	0.82 %
Nitrate	11.0 mg/kg
Nitrite	2.0 mg/kg
Selenium	0.19 mg/kg
Lead	1.5 mg/kg
Arsenic	0.50 mg/kg
Cadmium	0.27 mg/kg
Mercury	0.02 mg/kg
Aflatoxins	NONE DETECTED
Total P.C.B.	NONE DETECTED
Total D.D.T.	0.040 mg/kg
Dieldrin	0.005 mg/kg
Lindane	0.010 mg/kg
Heptachlor	0.003 mg/kg
Malathion	NONE DETECTED
Total Viable Organisms	5.6×10^3/grm
E. Coli Type 1	NONE DETECTED
Salmonella Species	NONE DETECTED
Moulds	NONE DETECTED

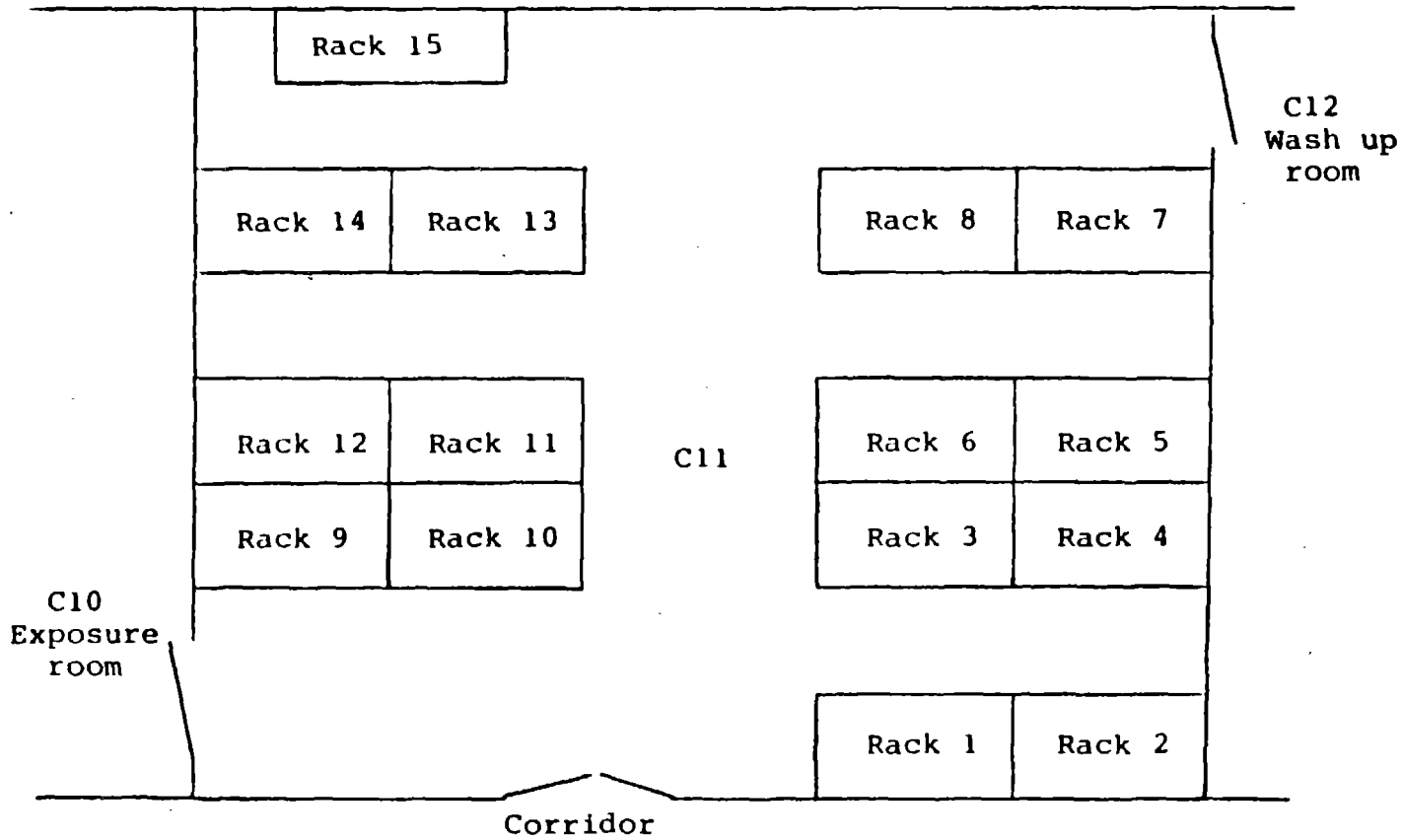
Signed .....

Date 17/12/80

APPENDIX Loc-1

Bis(2-methoxyethyl) ether

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

Bis(2-methoxyethyl) ether
Examples of Animal Location During Exposure
Exposure Location Sheet

Project No: 409959

Test Compound: Air Control

Exposure Chamber No: 1

Day of Study: 2

Test Concentration: 0

Tier No: 1

Multi-dose Cytogenetic ♂ and ♀

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)

Bis(2-methoxyethyl) ether
Exposure Location Sheet

Project No: 409959

Test Compound: Air Control

Exposure Chamber No: 1

Day of Study: 2

Test Concentration: 0

Tier No: 2

Dominant Lethal ♂
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)

Bis(2-methoxyethyl) ether
Exposure Location Sheet

Project No: 409959 Test Concentration: Low

Test Compound: Bis(2-methoxyethyl) ether Tier No: 1

Exposure Chamber No: 2

Day of Study: 2

LEFT

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

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

FRONT REAR

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

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

RIGHT

Signed: _____ Date: _____

APPENDIX Loc-2 (continued)

Bis(2-methoxyethyl) ether
Exposure Location Sheet

Project No: 409959 Test Concentration: High

Test Compound: Bis(2-methoxyethyl) ether Tier No: 1

Exposure Chamber No: 3

Day of Study: 2

LEFT

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

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

FRONT REAR

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

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

RIGHT

Signed: _____ Date: _____

APPENDIX FORM-1

Bis(2-methoxyethyl) ether

CYTOGENETIC SCORE SHEET NIOSH

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

Vernier	Chromatid			Chromosomal			Markers			Multi-Abn.	Misc. Abn.	Observation
	GAP	Bm/F	Bm/oF	GAP	Bm/F	Bm/oF	EXC.	DIC	RG			
1.												
2.												
3.												
4.												
5.												
6.												
7.												
8.												
9.												
10.												
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20.												
21.												
22.												
23.												
24.												
25.												

APPENDIX FORM-2

Bis(2-methoxyethyl) ether

Assessors	Signature

Contract No. 210-78-0026

DOMINANT LETHAL ASSESSMENT

NIOSH

Dose Group:

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

APPENDIX FORM-3

Bis(2-methoxyethyl) ether

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

Bis(2-methoxyethyl) ether
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	374	367	367	362	369
	122	389	391	405	395	402
	123	425	426	442	444	450
	124	395	396	399	405	406
	125	385	389	391	393	400
	126	371	376	385	384	385
	127	379	382	385	390	390
	128	387	391	393	393	397
	129	408	426	426	427	440
	130	386	386	390	389	392
	Mean ± S.D.	389.9 ± 16.2	393.0 ± 19.3	398.3 ± 21.6	398.2 ± 22.9	403.1 ± 24.5

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	281	240	238	239	240	241
	282	236	233	229	231	236
	283	264	266	261	262	265
	284	250	251	248	250	251
	285	225	221	227	225	223
	286	226	220	229	230	234
	287	270	269	274	274	274
	288	185	209	208	207	212
	289	234	234	237	236	238
	290	217	217	220	220	223
	Mean ± S.D.	234.7 ± 24.3	235.8 ± 20.6	237.2 ± 19.5	237.5 ± 20.0	239.7 ± 19.2

APPENDIX TABLE BW-1 (continued)

Bis(2-methoxyethyl) ether

Multiple Dosing: 250 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	131	393	389	400	403	405
	132	390	395	397	401	404
	133	392	391	398	405	404
	134	402	402	407	408	407
	135	403	403	410	407	415
	136	388	387	393	396	397
	137	410	418	419	422	425
	138	356	361	366	370	370
	139	381	389	388	394	391
	140	404	411	420	424	430
	Mean ± S.D.	391.9 ± 15.4	394.6 ± 15.7	399.8 ± 15.9	403.0 ± 15.2	404.8 ± 17.0

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	291	232	228	230	228	230
	292	228	227	230	227	227
	293	279	269	269	273	275
	294	246	238	250	249	248
	295	235	229	234	231	235
	296	242	246	253	250	249
	297	236	226	238	233	235
	298	240	238	238	236	238
	299	211	210	221	217	222
	300	242	237	239	240	246
	Mean ± S.D.	239.1 ± 17.1	234.8 ± 15.5	240.2 ± 13.8	238.4 ± 15.7	240.5 ± 15.1

APPENDIX TABLE BW-1 (continued)

Bis(2-methoxyethyl) ether

Multiple Dosing: 1,000 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	141	401	396	387	393	396
	142	386	380	377	379	383
	143	400	390	393	394	400
	144	387	385	385	383	392
	145	382	381	387	387	387
	146	406	396	393	395	397
	147	400	399	403	395	395
	148	399	397	394	393	394
	149	380	372	374	375	375
	150	349	347	345	337	338
	Mean ± S.D.	389.0 ± 16.7	384.3 ± 15.8	383.5 ± 16.0	383.1 ± 17.7	385.7 ± 18.4

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	301	248	240	241	241	252
	302	254	246	253	250	263
	303	252	255	255	253	262
	304	246	236	245	244	252
	305	226	219	221	223	235
	306	250	247	252	255	261
	307	234	228	224	228	237
	308	256	251	254	247	252
	309	237	232	232	237	240
	310	240	238	235	239	253
	Mean ± S.D.	244.3 ± 9.7	239.2 ± 11.0	241.2 ± 12.7	241.7 ± 10.4	250.7 ± 10.2

APPENDIX TABLE BW-1 (continued)

Bis(2-methoxyethyl) ether

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	151	383	368	357	345	349
	152	418	405	386	372	366
	153	395	389	379	366	366
	154	412	404	398	382	382
	155	407	394	380	365	351
	156	415	403	390	376	384
	157	383	372	366	350	352
	158	410	398	386	365	365
	159	400	392	378	364	358
	160	365	361	350	346	344
	Mean	398.8	388.6	377.0	363.1	361.7
	± S.D.	± 17.2	± 16.0	± 15.0	± 12.5	± 13.6

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	311	241	229	217	213	208
	312	261	250	236	230	231
	313	285	272	261	258	258
	314	228	226	216	210	209
	315	253	242	238	234	241
	316	271	257	243	238	232
	317	249	239	223	211	224
	318	203	195	189	181	178
	319	234	228	217	208	204
	320	221	210	201	194	188
	Mean	244.6	234.8	224.1	217.7	217.3
	± S.D.	± 24.4	± 22.4	± 21.0	± 22.6	± 24.5

APPENDIX TABLE BW-2

Bis(2-methoxyethyl) ether
Single Exposure Cytogenetics Test
Individual Body Weights (g)

Air Control (0 ppm)

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Male	1	407	11	420	21	402
	2	423	12	401	22	441
	3	437	13	420	23	386
	4	458	14	417	24	361
	5	449	15	450	25	392
	6	413	16	435	26	420
	7	406	17	428	27	422
	8	448	18	421	28	394
	9	470	19	440	29	434
	10	422	20	388	30	458
	Mean ± S.D.	433.3 ± 22.4		422.0 ± 18.1		411.0 ± 29.2

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Female	161	247	171	259	181	250
	162	285	172	277	182	275
	163	265	173	243	183	248
	164	241	174	285	184	261
	165	248	175	258	185	316
	166	267	176	247	186	265
	167	243	177	202	187	274
	168	221	178	307	188	242
	169	276	179	250	189	304
	170	280	180	223	190	225
	Mean ± S.D.	257.3 ± 20.5		255.1 ± 30.1		266.0 ± 27.8

APPENDIX TABLE BW-2 (continued)

Bis(2-methoxyethyl) ether

Single Dosing: 250 ppm

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Male	31	414	41	425	51	435
	32	427	42	421	52	395
	33	428	43	410	53	403
	34	425	44	410	54	430
	35	420	45	450	55	439
	36	457	46	385	56	405
	37	407	47	405	57	379
	38	413	48	405	58	420
	39	415	49	470	59	423
	40	437	50	441	60	426
	Mean ± S.D.	424.3 ± 14.9		422.2 ± 25.2		415.5 ± 19.3

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Female	191	237	201	275	211	254
	192	282	202	277	212	232
	193	227	203	274	213	250
	194	238	204	257	214	270
	195	235	205	242	215	285
	196	245	206	262	216	266
	197	262	207	238	217	240
	198	251	208	252	218	230
	199	275	209	300	219	277
	200	306	210	226	220	242
	Mean ± S.D.	255.8 ± 25.1		260.3 ± 22.0		254.6 ± 19.2

APPENDIX TABLE BW-2 (continued)

Bis(2-methoxyethyl) ether

Single Dosing: 1,000 ppm

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Male	61	487	71	425	81	403
	62	433	72	474	82	421
	63	463	73	385	83	441
	64	435	74	430	84	415
	65	412	75	407	85	397
	66	419	76	459	86	427
	67	450	77	456	87	436
	68	445	78	417	88	418
	69	465	79	420	89	432
	70	460	80	455	90	421
	Mean ± S.D.	446.9 ± 22.9		432.8 ± 27.6		421.1 ± 13.9

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Female	221	278	231	235	241	247
	222	291	232	240	242	261
	223	277	233	251	243	225
	224	306	234	241	244	286
	225	268	235	250	245	229
	226	260	236	276	246	240
	227	267	237	242	247	225
	228	285	238	264	248	285
	229	255	239	229	249	236
	230	245	240	243	250	266
	Mean ± S.D.	273.2 ± 18.1		247.1 ± 13.9		250.0 ± 23.3

APPENDIX TABLE BW-2 (continued)

Bis(2-methoxyethyl) ether

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sex	Animal Number	6 h Sample	Animal Number	24 h Sample	Animal Number	48 h Sample
		Weight		Weight		Weight
Male	91	446	101	408	111	450
	92	415	102	447	112	441
	93	474	103	446	113	442
	94	453	104	407	114	434
	95	402	105	461	115	408
	96	401	106	446	116	437
	97	439	107	459	117	438
	98	405	108	450	118	409
	99	461	109	404	119	438
	100	463	110	410	120	425
	Mean ± S.D.	435.9 ± 27.8		433.8 ± 23.4		432.2 ± 14.0

Sex	Animal Number	6 h Sample	Animal Number	24 h Sample	Animal Number	48 h Sample
		Weight		Weight		Weight
Female	251	252	261	254	271	250
	252	264	262	239	272	256
	253	250	263	254	273	307
	254	217	264	280	274	259
	255	252	265	265	275	265
	256	252	266	268	276	263
	257	254	267	240	277	275
	258	268	268	267	278	250
	259	221	269	215	279	265
	260	240	270	252	280	242
	Mean ± S.D.	247.0 ± 16.6		253.4 ± 18.6		263.2 ± 18.0

APPENDIX TABLE BW-3

Bis(2-methoxyethyl) ether
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	400	403	402	407	406
	362	405	415	422	423	425
	363	368	368	369	364	369
	364	413	411	416	416	422
	365	366	375	373	367	374
	366	372	373	377	377	376
	367	385	385	389	393	393
	368	380	375	378	380	385
	369	388	390	395	397	399
	370	376	377	377	374	378
	Mean ± S.D.	385.3 ± 16.1	387.2 ± 16.9	389.8 ± 18.5	389.8 ± 20.7	392.7 ± 19.9

APPENDIX TABLE BW-3 (continued)

Bis(2-methoxyethyl) ether

Multiple Dosing: 250 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	371	373	376	377	378	380
	372	399	405	406	410	418
	373	365	365	369	373	371
	374	358	363	371	370	375
	375	395	398	405	410	411
	376	405	400	407	406	409
	377	399	391	398	397	398
	378	350	354	356	355	361
	379	403	406	409	416	420
	380	358	360	362	370	369
	Mean ± S.D.	380.5 ± 21.7	381.8 ± 20.3	386.0 ± 20.9	388.5 ± 21.6	391.2 ± 22.4

APPENDIX TABLE BW-3 (continued)

Bis(2-methoxyethyl) ether

Multiple Dosing: 1,000 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	381	407	408	402	399	403
	382	370	369	374	367	363
	383	382	380	375	374	374
	384	394	391	395	392	389
	385	393	390	395	391	396
	386	396	386	396	391	399
	387	411	403	410	404	401
	388	373	377	380	375	380
	389	373	380	380	378	383
	390	373	367	367	359	366
	Mean	387.2	385.1	387.4	383.0	385.4
	± S.D.	± 15.1	± 13.4	± 14.0	± 14.6	± 14.6

APPENDIX TABLE BW-3 (continued)

Bis(2-methoxyethyl) ether

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	391	392	382	367	348	345
	392	365	356	344	330	330
	393	357	342	332	318	320
	394	390	382	368	353	351
	395	397	391	374	361	358
	396	397	392	374	367	364
	397	377	374	363	348	348
	398	409	398	388	377	380
	399	373	368	359	342	338
	400	390	382	370	354	351
	Mean ± S.D.	384.7 ± 16.2	376.7 ± 17.3	363.9 ± 15.9	349.8 ± 17.2	348.5 ± 17.0

APPENDIX TABLE BW-4

Bis(2-methoxyethyl) ether
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	24	24	24	24	24
	322	24	25	25	24	25
	323	24	24	24	24	25
	324	23	24	24	25	24
	325	25	24	24	24	25
	326	22	23	23	23	24
	327	24	24	25	25	26
	328	25	24	25	25	26
	329	24	25	25	26	26
	330	23	24	24	25	25
	Mean	23.8	24.1	24.3	24.5	25.0
	± S.D.	± 0.9	± 0.6	± 0.7	± 0.8	± 0.8

APPENDIX TABLE BW-4 (continued)

Bis(2-methoxyethyl) ether

Multiple Dosing: 250 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	331	23	25	26	24	23
	332	23	24	25	24	24
	333	24	24	25	24	24
	334	25	25	26	25	25
	335	25	24	25	24	23
	336	23	24	24	24	22
	337	22	21	21	21	21
	338	26	25	25	25	24
	339	23	23	24	23	22
	340	23	24	24	24	23
	Mean ± S.D.	23.7 ± 1.3	23.9 ± 1.2	24.5 ± 1.4	23.8 ± 1.1	23.1 ± 1.2

APPENDIX TABLE BW-4 (continued)

Bis(2-methoxyethyl) ether

Multiple Dosing: 1,000 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	341	25	23	23	-	-
	342	24	21	20	+	-
	343	23	21	20	+	-
	344	25	22	22	-	-
	345	23	23	23	-	-
	346	22	22	23	-	-
	347	23	22	20	+	-
	348	23	23	21	-	-
	349	23	23	21	-	-
	350	22	22	21	-	-
	Mean ± S.D.	23.3 ± 1.1	22.2 ± 0.8	21.4 ± 1.3	-	-

+ = Day 4: No. 342 and 347 found dead, 343 killed in extremis, remaining animals not exposed.

APPENDIX TABLE BW-4 (continued)

Bis(2-methoxyethyl) ether

Multiple Dosing: Ethyl methanesulphonate, 200 mg/kg

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	351	26	25	25	24	23
	352	24	23	22	23	22
	353	25	25	25	23	23
	354	24	23	23	23	23
	355	24	23	23	24	23
	356	24	23	23	24	22
	357	26	25	24	25	23
	358	26	25	25	25	24
	359	24	23	23	23	22
	360	25	24	25	23	22
	Mean ± S.D.	24.8 ± 0.9	23.9 ± 1.0	23.8 ± 1.1	23.7 ± 0.8	22.7 ± 0.7

APPENDIX TABLE CA-MD-M

Bis(2-methoxyethyl) ether
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
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
124	124/4	50	21	19	1							29.9 x 111.0
					1							29.0 x 106.0
	124/3		25	25								
	124/5		4	4								
122	150/5	50	25	23	1							36.5 x 110.6
											1 Chromatid Fragment	67.5 x 104.3
	150/3		11	11								
	150/2		14	14								
129	122/1-5	0	0	0								
126	128/1	40	5	5								
	128/2		6	6								
	128/3		12	12								
	128/4		9	9								
	128/5		8	8								
121	36/1-5	0	0	0								
130	11/1	50	21	20	1							43.2 x 96.2
	11/4		25	25								
	11/5		4	4								
127	22/1-5	0	0	0								
123	127/1	50	9	9								
	127/2		11	10	1							32.4 x 107.1
	127/3		19	19								
	127/4		11	11								

APPENDIX TABLE CA-MD-M (continued)

Bis(2-methyoxyethyl) ether

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		
125	18/2	50	25	24	1							36.0 x 109.8
	18/3		25	25								
128	9/1	20	6	6								
	9/2		4	4								
	9/3		5	5								
	9/4		1	1								
	9/5		4	4								

APPENDIX TABLE CA-MD-M (continued)

Bis(2-methoxyethyl) ether

Males

Multiple Dosing: 250 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		
137	131/2	49	21	21								
	131/3		18	18								
	131/5		10	9		1						58.1 x 110.2
135	90/5	50	25	25								
	90/3		25	24	1							58.9 x 112.9
140	38/1	50	11	10	1							59.9 x 96.2
	38/2		13	13								
	38/3		10	10								
	38/4		8	8								
	38/5		8	8								
138	135/1-5	0	0	0								
134	134/1	50	16	15	1							31.0 x 113.1
	134/2		20	19	1							40.0 x 114.0
	134/4		14	13	1							39.0 x 100.0
131	27/5	10	8	8								
	27/4		2	2								
	27/1-3		0	0								
132	86/1-5	0	0	0								
133	132/1-5	0	0	0								

APPENDIX TABLE CA-MD-M (continued)

Bis(2-methoxyethyl) ether

Males

Multiple Dosing: 250 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		
136	24/1	50	17	12	1							32.2 x 113.5
						1						29.5 x 110.8
					1							34.4 x 110.9
					1							27.0 x 102.8
					1							31.5 x 96.0
	24/3		9	9								
	24/2		20	19							2 Chromatid Fragments	65.1 x 106.3
	24/4		4	4								
139	72/4	50	14	11	1							63.5 x 109.2
					1	1						63.2 x 107.0
											1 Chromatid Fragment	29.9 x 94.8
	72/3		15	15								
	72/2		14	12	1							26.3 x 105.1
					1							53.1 x 103.0
	72/1		7	7								

APPENDIX TABLE CA-MD-M (continued)

Bis(2-methoxyethyl) ether

Males

Multiple Dosing: 1,000 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	57/5	50	25	25								
	57/4		25	25								
144	116/1-5	0	0	0								
141	64/2	50	25	25								
	64/3		25	24	1							62.9 x 105.8
145	145/1	50	25	24	1							22.0 x 106.0
	145/5		25	25								
147	34/1	50	25	25								
	34/3		25	25								
149	120/5	50	6	5	1							56.0 x 108.7
	120/1		14	14								
	120/2		20	19	1							61.4 x 102.2
	120/3		10	10								
146	93/3	50	25	25								
	93/2		25	22	1							38.0 x 106.5
					1							68.9 x 106.1
					1							61.9 x 98.9
142	37/5	45	16	16								
	37/3		6	6								
	37/4		3	3								
	37/1		12	11				1				53.5 x 101.2
	37/2		8	8								

APPENDIX TABLE CA-MD-M (continued)

Bis(2-methoxyethyl) ether

Males

Multiple Dosing: 1,000 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		
143	111/5	50	21	21								
	111/4		23	23								
	111/3		6	5	1							65.9 x 112.1
150	89/1	50	10	10								
	89/2		14	14								
	89/3		9	8	1							26.9 x 112.0
	89/4		12	11	1							46.1 x 109.9
	89/5		5	5								

APPENDIX TABLE CA-MD-M (continued)

Bis(2-methoxyethyl) ether

Males

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
157	46/1	50	13	11	1							32.0 x 114.0
					1		1					27.3 x 104.6
	46/2		19	18	1						1 Chromatid Fragment	69.1 x 104.5
	46/5		6	5				1				36.2 x 107.0
	46/4		12	12								
151	6/3	44	9	8	1							61.5 x 107.0
	6/2		15	15								
	6/4		5	4	1							59.1 x 102.0
	6/1		6	6								
	6/5		9	7	1							40.0 x 100.1
					1							32.0 x 94.9
160	48/1-5	0	0	0								
158	114/1-5	0	0	0								
154	146/1-5	0	0	0								
152	158/1	50	23	22	1							32.8 x 105.1
	158/2		23	23								
	158/3		4	4								
156	79/1	50	18	16	1							30.0 x 111.1
					1							39.8 x 106.9
	79/2		19	17		2	1					61.8 x 109.0
					1							70.1 x 107.1
	79/3		13	12	2							36.1 x 110.2

APPENDIX TABLE CA-MD-M (continued)

Bis(2-methoxyethyl) ether

Males

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
153	67/1	50	25	23	1							34.6 x 110.0
					1			1				29.9 x 102.2
	67/3		12	12								
	67/5		13	10	1							53.0 x 105.0
					1							44.0 x 99.5
					1							71.4 x 97.0
155	13/1	50	18	18								
	13/3		16	16								
	13/4		16	16								
159	52/1	50	10	8							1 Chromatid Fragment	56.9 x 109.2
					1							52.0 x 104.0
	52/2		12	10		1						50.0 x 101.9
					1							26.0 x 99.8
	52/3		11	11								
	52/4		9	9								
	52/5		8	7		2	1					67.0 x 102.8

APPENDIX TABLE CA-MD-F

Bis(2-methoxyethyl) ether
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		
289	282/1-5	0	0	0								
281	196/1-5	0	0	0								
285	178/1	50	25	21	1							25.1 x 110.5
											1 Exchange	54.4 x 109.0
					1							44.3 x 102.6
					1							46.4 x 101.0
	178/2		10	9	1							71.5 x 97.2
	178/3		15	14	1							62.7 x 110.8
283	287/1	50	24	23				1				66.0 x 95.0
	287/2		25	23	1							66.7 x 109.6
					3							35.1 x 108.6
	287/3		1	1								
287	182/1	50	25	24		1						63.8 x 106.0
	182/2		25	23	1							27.2 x 106.1
					1							53.2 x 104.6
288	169/1	50	25	25								
	169/2		21	21								
	169/3		4	3	1							42.5 x 103.8
284	284/1	33	10	8	1							26.5 x 104.6
					1							53.1 x 93.3
	284/2		4	4								
	284/3		5	4	1							57.8 x 95.2
	284/4		8	7			1					68.1 x 110.2
	284/5		6	6								

APPENDIX TABLE CA-MD-F (continued)

Bis(2-methoxyethyl) ether

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		
286	288/1	50	15	15								
	288/2		12	11		1						27.3 x 94.6
	288/3		17	15							1 Robertsonian Translocation	32.6 x 113.2
					1							62.8 x 99.9
	288/4		6	6								
282	310/1-5	0	0	0								
290	171/1	18	7	7								
	171/2		6	5	1							58.3 x 107.7
	171/3		5	5								
	171/4,5		0	0								

APPENDIX TABLE CA-MD-F (continued)

Bis(2-methoxyethyl) ether

Females

Multiple Dosing: 250 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		
294	294/1-5	1	0	0								
	294/2		1	1								
296	184/1	50	25	22		1						62.3 x 102.2
											1 Chromatid Fragment	22.3 x 98.4
					1							55.6 x 95.6
	184/2		16	14	1							37.6 x 109.2
					1							32.8 x 104.4
	184/3		9	8	1							32.3 x 100.2
293	292/1	50	16	16								
	292/2		25	24	1							36.1 x 105.6
	292/3		9	8	1							47.6 x 99.1
292	246/1	50	25	25								
	246/2		9	9								
	246/4		16	16								
	246/3		0	0								
298	295/1-5	0	0	0								
300	198/2	50	24	21	1							29.7 x 105.9
					1							26.1 x 105.2
					1							28.1 x 103.6
	198/3		8	8								
	198/1		18	17	1							62.0 x 108.6
299	232/5	50	25	24		1						56.0 x 101.6
	232/4		9	8	1							25.2 x 97.6
	232/1		16	16								

APPENDIX TABLE CA-MD-F (continued)

Bis(2-methoxyethyl) ether

Females

Multiple Dosing: 250 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		
291	187/1	50	25	21	1							20.5 x 110.2
					1							29.6 x 110.4
						1						35.8 x 110.1
					1							33.2 x 109.2
	187/2		25	25								
297	291/1	36	7	7								
	291/3		10	10								
	291/4		17	14							1 Chromosomal Fragment	20.5 x 109.3
					1							62.2 x 106.0
											1 Chromosomal Fragment	59.0 x 103.4
	291/5		2	2								
	291/2		0	0								
295	250/1-5	0	0	0								

APPENDIX TABLE CA-MD-F (continued)

Bis(2-methoxyethyl) ether

Females

Multiple Dosing: 1,000 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		
310	249/1	50	25	24							1 Pair of Minutes	60.0 x 102.9
	249/2		25	23							1 Chromatid Fragment	62.9 x 99.7
											2 Chromatid Fragments	57.5 x 97.7
303	271/1-5	0	0	0								
308	217/1	50	14	14								
	217/2		9	9								
	217/3		16	15				1				36.5 x 104.1
	217/4		11	11								
309	280/1-5	0	0	0								
307	194/1	50	25	25								
	194/2		25	25								
302	197/1	50	25	24	1							32.5 x 110.2
	197/2		25	25								
306	253/3	14	7	7								
	253/1		3	2	1							63.6 x 107.8
	253/2		1	1								
	253/4		1	1								
	253/5		2	2								
304	276/1-5	0	0	0								
305	305/1	50	21	21								
	305/2		20	20								
	305/3		9	9								

APPENDIX TABLE CA-MD-F (continued)

Bis(2-methoxyethyl) ether
Females

Multiple Dosing: 1,000 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		
301	224/5	14	5	5								
	224/2		1	1								
	224/1		4	4								
	224/4		4	4								
	224/3		0	0								

APPENDIX TABLE CA-MD-F (continued)

Bis(2-methoxyethyl) ether

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		
314	306/1	50	9	9								
	306/2		25	22	1							23.2 x 112.1
											2 Chromatid Fragments	28.1 x 109.0
											1 Exchange	42.2 x 101.1
	306/3		16	15		1						42.0 x 104.9
312	318/1	50	16	15	1							58.9 x 102.4
	318/2		21		2							32.1 x 95.4
											1 Chromatid Fragment	31.5 x 98.5
	318/3		13	12	1							32.0 x 95.3
316	239/2	50	25	21	1							45.1 x 107.0
					2							44.6 x 107.1
					1							22.1 x 106.9
					1							69.5 x 104.9
	239/3		25	20	1							30.8 x 113.0
											1 Chromatid Fragment	28.6 x 111.4
					1							24.6 x 110.7
								1				32.5 x 109.8
						1						56.7 x 107.2
319	212/1	50	23	17	1							62.2 x 105.4
						1						59.6 x 105.4
						1						42.8 x 105.6
					1							30.6 x 102.8
					1							68.4 x 101.1
					2							70.5 x 101.0

APPENDIX TABLE CA-MD-F (continued)

Bis(2-methoxyethyl) ether

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	212/2		21	19	1							48.3 x 99.4
					1							65.9 x 99.3
	212/3		6	5		1						34.7 x 109.9
317	206/1	50	20	17	1							57.3 x 112.2
						1						33.8 x 105.0
											1 Chromatid Fragment	60.6 x 99.7
	206/2		23	18	1							60.4 x 105.9
					1							28.0 x 104.3
					1						1 Chromatid Fragment	60.8 x 104.4
					1	1						36.5 x 104.4
						1						61.5 x 101.8
	206/3		7	7								
313	227/1	50	25	22	1							62.2 x 108.4
					1							60.0 x 108.0
							2					67.3 x 106.9
	227/2		25	22	1	1						61.5 x 108.4
											1 Pair of Minutes	52.2 x 107.7
						1						47.4 x 106.0
311	166/3	50	25	20	2	1	1					59.6 x 109.5
						2						38.3 x 108.3
						1					1 Chromatid Fragment	40.6 x 106.6
						1						50.7 x 105.9
					1							38.0 x 103.7

APPENDIX TABLE CA-MD-F (continued)

Bis(2-methoxyethyl) ether

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		
311	166/4		23	22	1							41.5 x 97.0
	166/1		2	2								
315	173/1	11	3	2	1							67.0 x 104.7
	173/2		4	4								
	173/3		2	2								
	173/4		1	1								
	173/5		1	1								
320	208/2	2	1	1								
	208/4		1	1								
	208/1, 3,5		0	0								
318	274/1	13	1	1								
	274/2		4	3	1							27.2 x 107.1
	274/3		3	2	1							53.8 x 104.5
	274/4		2	2								
	274/5		3	3								

APPENDIX TABLE CA-M6

Bis(2-methoxyethyl) ether

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		
1	45/2	50	25	22	1							61.0 x 111.8
					1							68.3 x 110.3
					1							70.3 x 108.7
	45/1		3	3								
	45/3		22	22								
9	123/3	50	25	25								
	123/1		25	25								
3	133/3	50	25	25								
	133/4		25	25								
7	143/3	50	25	25								
	143/2		25	25								
5	53/1-5	0	0	0								
4	56/1	50	25	24	1							63.9 x 109.2
	56/2		25	22	1							32.6 x 107.5
					1							32.7 x 105.9
											1 Chromatid Fragment	43.2 x 107.6
8	35/2	50	24	23	1							32.7 x 105.9
	35/1		25	25								
	35/3		1	1								
6	10/2	50	25	25								
	10/3		25	24	1							37.6 x 108.0
2	3/2	50	25	24	1							55.5 x 112.4
	3/4		25	24	1							26.7 x 109.3
10	85/1-5	0	0	0								

APPENDIX TABLE CA-M6 (continued)

Bis(2-methoxyethyl) ether
Males

Single Dosing: 250 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		
33	75/1	50	25	24		1						23.0 x 97.9
	75/2		25	23	1							51.3 x 105.0
						1						42.4 x 102.3
34	97/1-5	0	0	0								
35	91/4	50	25	22	1							61.2 x 113.6
					1							36.9 x 107.1
					1							36.8 x 106.1
	91/5		25	23	1							27.0 x 105.9
					1							67.7 x 103.1
31	76/2	50	25	25								
	76/4		25	24	1							32.9 x 109.8
32	130/1	50	25	24	1							36.7 x 101.9
	130/2		25	24	1							65.2 x 105.2
37	54/2	50	25	23	1							59.6 x 110.6
					1							28.2 x 109.3
	54/3		25	23	1							50.4 x 103.8
						1						32.6 x 101.8
40	32/2	50	25	23	1							38.0 x 109.4
					1							51.4 x 108.7
	32/3		25	23	1							30.1 x 108.9
					1							41.0 x 108.3
39	155/1-5	0	0	0								
38	40/1-5	0	0	0								
36	92/1-5	0	0	0								

APPENDIX TABLE CA-M6 (continued)

Bis(2-methoxyethyl) ether
Males

Single Dosing: 1,000 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		
67	62/2	50	25	25								
	62/3		25	25								
63	69/3	50	25	25								
	69/4		25	24	1							42.3 x 112.8
66	47/1	50	25	24	1							40.3 x 107.9
	47/2		25	21							1 Chromatid Fragment	31.0 x 110.6
						1					2 Pairs of Minutes	62.9 x 109.4
					1							60.3 x 107.9
					1							55.3 x 108.3
70	70/2	50	25	24	1							28.6 x 105.0
	70/3		25	25								
61	140/1	50	25	24	1							54.8 x 106.7
	140/2		25	23	1							46.0 x 106.9
					2							34.0 x 105.0
68	51/3	50	25	25								
	51/4		25	24	1							61.6 x 105.2
62	19/1-5	0	0	0								
64	80/2	50	25	25								
	80/3		25	25								
65	83/2	50	25	25								
	83/3		25	25								
69	82/2	50	25	23	1							58.8 x 112.2
					1							50.4 x 104.8
	82/4		25	24							1 Chromatid Fragment	56.8 x 101.2

APPENDIX TABLE CA-M6 (continued)

Bis(2-methoxyethyl) ether

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	115/1	5	3	3								
	115/2		1	1								
	115/3		1	1								
	115/4		0	0								
	115/5		0	0								
95	29/3	1	1	1								
	29/1,2, 4,5		0	0								
92	30/2	50	18	17	1							37.8 x 104.4
	30/1		25	20	1							39.4 x 110.4
											1 Chromatid Fragment	56.1 x 106.1
					1							33.3 x 103.9
						1						34.2 x 102.8
											1 Chromatid Fragment	38.7 x 98.4
	30/4		7	7								
91	8/3	50	25	19	1							47.9 x 110.5
					1							48.4 x 109.6
					1							41.9 x 108.1
					1							42.3 x 108.0
					1							33.6 x 106.6
											1 Chromatid Fragment	53.3 x 111.6
	8/1		25	21	1							66.6 x 106.3
					1							53.4 x 106.5
								1				43.0 x 105.5
						1						60.0 x 105.5

APPENDIX TABLE CA-M6 (continued)

Bis(2-methoxyethyl) ether

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		
94	94/1-5	0	0	0								
93	129/1-5	0	0	0								
100	151/3	50	25	23	1							35.2 x 109.3
					1							54.4 x 103.9
	151/2		25	23	1							38.4 x 107.5
					1							32.6 x 105.1
99	12/3	50	25	22	1							64.3 x 106.5
						1						23.0 x 106.1
					1							53.8 x 105.6
	12/1		25	22				1				63.1 x 110.0
					1							69.2 x 105.0
						1						38.6 x 104.3
98	144/1	50	25	22	1	1						53.2 x 108.8
					1							59.2 x 108.8
					2							68.1 x 106.6
	144/2		25	23	3			1				59.0 x 107.7
					2							46.9 x 107.0
97	42/1-5	0	0	0								

APPENDIX TABLE CA-M24

Bis(2-methoxyethyl) ether
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Males

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
19	44/3	50	25	25								
	44/4		25	25								
11	113/3	50	25	25								
	113/2		25	24							1 Miscellaneous Aberration	55.1 x 108.2
16	126/2	50	25	25								
	126/3		25	23	1							66.9 x 110.0
					1							58.2 x 101.8
20	153/3	50	25	25								
	153/5		25	25								
18	106/4	50	25	25								
	106/5		25	25								
17	43/5	50	25	25								
	43/4		25	25								
14	33/1	50	25	25								
	33/2		25	25								
15	125/2	50	25	25								
	125/1		25	25								
12	103/1	50	25	25								
	103/2	50	25	25								
13	63/1	50	25	24	1							58.3 x 111.9
	63/4		25	25								

APPENDIX TABLE CA-M24 (continued)

Bis(2-methoxyethyl) ether

Males

Single Dosing: 250 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		
47	73/5	50	25	25								
	73/3		25	24				1	1			30.5 x 110.3
41	157/3	50	25	25								
	157/1		25	25								
49	4/2	50	25	25								
	4/3		25	25								
43	26/5	50	25	25								
	26/2		25	23	1							63.0 x 109.8
					1							56.6 x 107.3
50	60/2	50	25	25								
	60/3		25	25								
44	160/5	50	25	25								
	160/3		25	23							1 Miscellaneous Aberration	54.0 x 108.7
											1 Miscellaneous Aberration	37.0 x 105.6
46	119/1	50	25	25								
	119/3		25	25								
42	1/1-5	0	0	0								
45	25/2	50	25	24	1							54.8 x 111.6
	25/3		25	24	1							42.7 x 110.9
48	55/2	50	25	25								
	55/3		25	24				1				27.3 x 111.7

APPENDIX TABLE CA-M24 (continued)

Bis(2-methoxyethyl) ether
Males

Single Dosing: 1,000 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	49/2	50	25	25								
	49/1		25	25								
71	121/1	50	25	25								
	121/5		25	25								
78	17/5	50	25	25								
	17/3		25	25								
72	78/1	50	25	25								
	78/2		25	25								
76	99/1	50	25	25								
	99/2		25	25								
79	88/3	50	25	24	1							33.0 x 107.0
	88/2		25	22	1							30.3 x 110.0
					1							30.8 x 106.0
					1							55.0 x 104.0
74	16/1	50	25	25								
	16/4		25	25								
77	154/2	50	25	25								
	154/3		25	25								
73	118/5	50	25	25								
	118/2		25	25								
75	39/1	50	25	24	1							35.2 x 113.3
	39/4		25	25								

APPENDIX TABLE CA-M24 (continued)

Bis(2-methoxyethyl) ether

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		
107	87/5	50	25	25								
	87/3		25	24	1							67.0 x 106.0
104	59/3	50	25	23	1							43.3 x 106.0
					1							61.6 x 106.0
	59/1		25	24	1							59.1 x 106.2
103	50/3	50	25	23	1							28.8 x 107.9
					1							36.8 x 107.6
	50/2		25	24	1							28.6 x 106.7
108	41/3	50	25	24	1							54.2 x 109.0
	41/2		25	21	1							70.0 x 109.0
					1							68.3 x 107.3
					2							63.3 x 106.7
					1							60.7 x 106.7
105	61/1	50	25	25								
	61/2		25	24	1							51.8 x 108.0
110	138/2	50	25	22	2							30.0 x 109.5
					2							64.6 x 108.7
					1							59.2 x 106.0
	138/5		25	21	1	1						45.6 x 109.7
											1 Miscellaneous Aberration	55.9 x 110.0
					1							59.0 x 105.6
					1							48.9 x 104.9

APPENDIX TABLE CA-M24 (continued)

Bis(2-methoxyethyl) ether

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		
106	149/1	50	25	24				1				43.7 x 112.1
	149/4		25	22	1							36.3 x 114.4
											1 Multi Aberration	35.2 x 113.4
											1 Multi Aberration	60.2 x 112.6
109	141/1	50	25	22							1 Multi Aberration	63.1 x 114.4
					1							60.3 x 114.7
					1							55.8 x 114.0
	141/2		25	21							1 Exchange	69.0 x 114.3
					2	3	1				1 Exchange	68.2 x 113.5
					3							68.1 x 113.4
					1	1					1 Chromatid Fragment	61.7 x 114.0
101	21/4	50	25	24							1 Robertsonian Translocation	34.3 x 113.9
	21/3		25	25								
102	152/1	50	25	24							1 Multi Aberration	51.6 x 113.6
	152/3		25	25								

APPENDIX TABLE CA-M48

Bis(2-methoxyethyl) ether
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		
30	7/1	50	25	24		2						40.3 x 105.3
	7/2		25	24							1 Chromatid Fragment	38.1 x 110.3
23	2/1	50	0	0								
	2/2		25	25								
	2/3		25	24							2 Chromatid Fragment	29.1 x 107.8
27	74/1	50	25	25								
	74/2		25	25								
24	96/2	50	25	23				1				60.4 x 110.9
					1							25.7 x 108.3
	96/3		25	24	1							26.5 x 109.1
26	77/4	50	10	10								
	77/2		20	18	1		1					29.0 x 102.7
						1						38.4 x 98.9
	77/1		8	7		2			1		1 Chromosomal Fragment	59.3 x 108.1
	77/5		12	12								
29	109/1	50	25	25								
	109/4		25	25								
21	101/1	50	25	25								
	101/3		25	25								
22	5/4	50	25	25								
	5/5		25	24	1							28.0 x 109.3
28	156/5	50	25	24		1						57.6 x 103.5
	156/1		18	18								
	156/2		7	7								

APPENDIX TABLE CA-M48 (continued)

Bis(2-methoxyethyl) ether
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	100/1	50	25	25								
	100/2		25	25								

APPENDIX TABLE CA-M48 (continued)

Bis(2-methoxyethyl) ether

Males

Single Dosing: 250 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		
51	81/1	50	25	25								
	81/2		25	25								
57	102/1	50	25	25								
	102/3		25	25								
53	65/1	50	25	25								
	65/2		25	25								
56	31/4	50	25	25								
	31/2		25	25								
60	48/1	50	25	25								
	48/2		25	25								
58	112/1	50	25	25								
	112/3		25	25								
59	98/2	50	25	25								
	98/3		25	25								
54	71/1	50	25	25								
	71/3		25	24							1 Chromatid Fragment	66.1 x 105.0
55	110/1-5	0	0	0								
52	66/1	50	25	25								
	66/2		22	22								
	66/3		3	3								

APPENDIX TABLE CA-M48 (continued)

Bis(2-methoxyethyl) ether

Males

Single Dosing: 1,000 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		
81	105/1-5	0	0	0								
82	159/2	50	25	25								
	159/5		25	25								
88	108/3	50	25	25								
	108/4		25	25								
89	104/1	50	25	25								
	104/5		25	25								
83	117/2	50	25	25								
	117/3		25	25								
87	84/2	50	25	25								
	84/3		25	25								
90	58/5	50	25	25								
	58/1		25	25								
85	68/1	50	25	25								
	68/2		25	25								
86	23/1	50	25	25								
	23/4		25	25								
84	20/2	50	25	24							2 Chromatid Fragment	61.7 x 107.7
	20/3		25	25								

APPENDIX TABLE CA-M48 (continued)

Bis(2-methoxyethyl) ether

Males

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
117	95/1	50	25	23	1	1	2				1 Chromosomal Fragment	30.2 x 111.9
					1							31.4 x 108.2
	95/2		25	21							1 Exchange	64.1 x 112.9
											1 Chromatid Fragment	59.7 x 113.5
					1							33.3 x 112.0
					1							27.9 x 111.3
119	14/1	50	25	24	1						1 Exchange	68.1 x 113.0
	14/3		25	25								
111	147/1	50	25	24	1							30.6 x 105.7
	147/2		25	24	1							51.6 x 105.5
114	107/1	50	25	25								
	107/3		25	24	1							41.6 x 108.0
113	28/1	50	25	23		1						37.7 x 105.8
					1							25.2 x 104.7
	28/4		25	24	1							62.4 x 110.5
116	136/1	50	25	24							1 Chromatid Fragment	58.2 x 109.7
	136/5		25	24		1						33.0 x 103.7
112	137/1	50	25	21		2						55.0 x 113.3
					1	2						60.1 x 109.0
						1						56.4 x 105.6
											2 Exchange 1 Chromatid Fragment	51.9 x 106.1
	137/2		25	22	1							26.2 x 107.2
					1							52.2 x 106.2
					1							35.0 x 102.7

APPENDIX TABLE CA-M48 (continued)

Bis(2-methoxyethyl) ether

Males

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
115	142/3	50	25	21		2						68.2 x 106.0
					1	3					1 Exchange	44.5 x 107.8
					1	1					1 Chromatid Fragment	42.5 x 106.8
						2					1 Chromatid Fragment	37.5 x 107.0
	142/4		25	23							1 Multi Aberration	40.7 x 106.5
					1							39.7 x 106.5
120	15/2	50	25	25								
	15/4		25	25								
118	139/2	50	25	23		4						67.4 x 104.8
					1							44.2 x 104.1
	139/5		25	23	1							35.3 x 105.7
					1							30.5 x 105.9

APPENDIX TABLE CA-F6

Bis(2-methoxyethyl) ether
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
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
161	205/1	33	10	10								
	205/2		7	7								
	205/3		4	4								
	205/3		4	4								
	205/4		5	4	1							46.3 x 98.0
	205/5		7	7								
163	293/4	18	11	11								
	293/2		6	5	1							41.0 x 98.5
	293/3		1	1								
	293/1,5		0	0								
170	245/1	50	25	22	1							28.7 x 104.7
					1							36.6 x 100.5
					2							64.0 x 98.5
	254/2		25	25								
165	213/2	50	20	20								
	213/1		25	24	1							66.2 x 106.5
	213/4		5	5								
168	195/1-5	0	0	0								
167	303/1-5	0	0	0								
164	216/2	50	25	25								
	216/1		25	23	1							34.2 x 111.6
					1							64.0 x 110.2
166	170/3	50	25	25								
	170/2		25	25								

APPENDIX TABLE CA-F6 (continued)

Bis(2-methoxyethyl) ether
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		
169	283/1	50	25	25								
	283/3		25	23		1						49.3 x 111.0
					1							61.1 x 108.8
162	163/3	50	25	25								
	163/2		25	24	1							55.3 x 107.6

APPENDIX TABLE CA-F6 (continued)

Bis(2-methoxyethyl) ether

Females

Single Dosing: 250 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		
197	214/2	35	13	12	1							66.0 x 104.7
	214/1		14	14								
	214/3		3	3								
	214/5		5	4	2							25.5 x 106.5
	214/4		0	0								
193	235/3	50	23	23								
	235/2		23	20	1							53.7 x 105.8
					1							70.3 x 99.2
					1							67.7 x 94.7
	235/1		4	3	1							62.2 x 112.1
192	290/1	38	5	5								
	290/2		18	16	1							63.1 x 106.6
					1							62.3 x 104.2
	290/4		2	1	1							25.6 x 106.4
	290/1		13	13								
	290/5		0	0								
196	252/1-5	0	0	0								
194	257/1-5	0	0	0								
191	236/1-5	0	0	0								
198	200/1-5	0	0	0								
199	315/1-5	0	0	0								
200	192/1-5	0	0	0								
195	251/1		25	23	1							64.3 x 110.4
					1	1						55.7 x 103.8

APPENDIX TABLE CA-F6 (continued)

Bis(2-methoxyethyl) ether
Females

Single Dosing: 1,000 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		
227	221/1-5	0	0	0								
225	243/1-5	0	0	0								
230	230/2	43	10	9	1							31.9 x 103.1
	230/2		15	13	2							34.2 x 106.8
					1							25.1 x 102.0
	230/4		5	5								
	230/1		6	5	1							64.2 x 102.9
	230/5		7	7								
224	240/3	50	21	20	1							64.1 x 104.8
	240/3		25	19	1							31.2 x 110.7
					1							53.9 x 108.8
					1							51.9 x 108.9
					1							32.5 x 107.4
					1							29.8 x 106.9
					1							27.6 x 106.8
	240/1		4	4								
222	179/2	50	25	22	1							61.7 x 111.0
					1							61.9 x 111.0
					1							58.7 x 109.4
	179/4		25	25								
223	229/3	50	25	25								
	229/2		25	25								

APPENDIX TABLE CA-F6 (continued)

Bis(2-methoxyethyl) ether

Females

Single Dosing: 1,000 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		
226	207/3	50	25	24	1							29.6 x 107.9
	207/1		25	21	1							58.0 x 107.3
					1							58.7 x 106.4
					1							58.7 x 103.6
					1							68.2 x 103.4
228	211/3	50	25	24	1							23.6 x 101.3
	211/4		25	24		1						29.8 x 105.6
229	242/1	50	18	16	1							60.4 x 98.7
											1 Chromatid Fragment	65.7 x 102.8
	242/2		25	22	1							36.0 x 106.5
					1							32.6 x 102.2
					1							48.4 x 102.4
	242/3		7	7								
221	300/2	50	25	25								
	300/4		25	25								

APPENDIX TABLE CA-F6 (continued)

Bis(2-methoxyethyl) ether

Females

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

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		
254	254/1-5	0	0	0								
256	275/1-5	0	0	0								
252	190/2	50	25	22		1						32.9 x 108.5
					2							40.5 x 104.4
					1							42.7 x 101.5
	190/3		25	22							1 Chromatid Fragment	46.9 x 108.5
					1							65.4 x 107.4
					1							71.0 x 104.4
255	189/3	50	25	23	1							47.9 x 112.2
						1						64.4 x 109.0
	189/2		25	20	1							48.9 x 109.1
					1							62.3 x 109.1
					1							22.3 x 108.8
						1						59.3 x 107.1
						1						60.5 x 105.2
251	168/2	50	25	22	1							41.3 x 109.3
					1							43.2 x 107.9
					2							39.1 x 103.3
	168/3		25	22	1							29.0 x 105.7
						1						42.7 x 105.2
					1							60.6 x 102.9
253	289/2	50	25	25								
	289/4		25	24	1							51.8 x 96.1

APPENDIX TABLE CA-F6 (continued)

Bis(2-methoxyethyl) ether

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		
258	304/2	50	25	19	1	1						32.7 x 110.1
								1				41.3 x 107.4
					1							62.2 x 106.7
					1							38.2 x 104.1
					2			1				63.2 x 104.5
					1							43.2 x 102.8
	304/1		25	18	1							55.0 x 107.8
					1							61.5 x 104.2
					1							35.0 x 102.8
					1							29.5 x 102.3
					2							25.5 x 102.4
						1						29.2 x 101.3
					1							30.0 x 101.4
257	202/2	50	25	22	1							24.8 x 108.4
					1							69.9 x 106.3
						1						40.8 x 102.9
	202/3		25	21	2							35.8 x 110.7
					1							42.8 x 107.3
					1							59.1 x 107.1
					1	1						34.8 x 104.2
259	172/3	50	25	23	1							61.9 x 112.0
					1							62.6 x 103.9
	172/2		25	24	1							39.1 x 106.5

APPENDIX TABLE CA-F6 (continued)

Bis(2-methoxyethyl) ether
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		
260	311/2	50	25	22	1							28.4 x 109.0
					2							26.4 x 107.6
					1							55.3 x 104.0
	311/1		25	22							2 Chromatid Fragments	40.1 x 107.2
					1							38.9 x 106.6
					1							56.6 x 104.9

APPENDIX TABLE CA-F24

Bis(2-methoxyethyl) ether
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Females

Single Dosing: Air Control (0 ppm)

Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
178	266/1	50	25	25								
	266/3		25	25								
175	285/4	9	2	2								
	285/5		1	1								
	285/1		2	2								
	285/2		3	3								
	285/3		1	1								
174	193/2	50	25	25								
	193/5		25	25								
172	263/5	50	25	25								
	263/3		25	25								
176	286/3	50	25	25								
	286/1		25	24	1							60.0 x 107.0
173	223/5	50	25	25								
	223/4		25	25								
179	204/3	50	25	25								
	204/4		25	25								
171	273/1-5	0	0	0								
180	313/3	50	25	25								
	313/4		25	24	1							25.0 x 111.5
177	203/2	50	25	25								
	203/4		25	25								

APPENDIX TABLE CA-F24 (continued)

Bis(2-methoxyethyl) ether
Females

Single Dosing: 250 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		
202	161/2	50	25	25								
	161/4		25	24	1							33.5 x 105.0
207	233/2	50	25	25								
	233/1		25	25								
203	186/1	50	25	25								
	186/2		25	25								
204	320/5	50	25	25								
	320/4		25	25								
206	279/1-5	0	0	0								
208	215/1	50	25	25								
	215/3		25	25								
201	317/4	50	25	25								
	317/2		25	25								
209	164/1	50	25	24							1 Chromatid Fragment	61.1 x 112.2
	164/2		25	23	1							41.0 x 112.6
					1							40.4 x 112.7
205	185/4	50	25	25								
	185/2		25	25								
210	220/3	50	25	25								
	220/2		25	25								

APPENDIX TABLE CA-F24 (continued)

Bis(2-methoxyethyl) ether

Females

Single Dosing: 1,000 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		
239	249/1	50	25	25								
	249/4		25	25								
237	314/1	50	25	25								
	314/3		25	25								
240	209/2	50	25	25								
	209/5		25	23							1 Miscellaneous Aberration	50.2 x 106.1
											1 Miscellaneous Aberration	45.2 x 104.0
236	259/2	50	25	25								
	259/3		25	25								
232	238/2	50	25	25								
	238/5		25	25								
233	278/1	50	25	24	1							54.0 x 107.6
	278/4		25	25								
231	281/1	50	25	25								
	281/5		25	25								
235	199/5	50	25	25								
	199/1		25	25								
238	177/3	50	25	25								
	177/4		25	25								
234	176/1	50	25	25								
	176/2		25	25								

APPENDIX TABLE CA-F24 (continued)

Bis(2-methoxyethyl) ether

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	247/5	50	25	22	1							10.3 x 110.0
					1							59.6 x 109.0
					1							47.8 x 106.7
	247/3		25	24	1							56.3 x 107.0
263	210/3	50	25	25								
	210/2		25	25								
262	312/4	50	25	25								
	312/1		25	24	1							52.6 x 106.0
265	221/3	50	25	25								
	221/1		25	25								
269	301/5	50	25	22	1							65.0 x 109.0
					1							39.4 x 107.8
					2							44.8 x 103.3
	301/1		25	24	1							31.9 x 108.1
261	181/3	50	25	25								
	181/1		25	25								
270	298/2	50	25	25								
	298/3		25	25								
266	309/5	50	25	25								
	309/1		25	22	1							63.4 x 105.8
					1							56.0 x 100.3
								1				66.5 x 99.4
268	201/4	50	25	25								
	201/5		25	23				1				28.9 x 106.5
					1							27.8 x 106.7

APPENDIX TABLE CA-F24 (continued)

Bis(2-methoxyethyl) ether

Females

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
264	219/1	50	25	21	1							61.3 x 111.9
					1							63.4 x 110.2
					1							62.1 x 110.6
											1 Multi Aberration	55.6 x 110.9
	219/4		25	23							1 Chromatid Fragment	65.2 x 113.6
						1						62.1 x 112.2

APPENDIX TABLE CA-F48

Bis(2-methoxyethyl) ether
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		
187	234/2	50	25	25								
	234/4		25	24	1							64.5 x 105.9
188	316/1	50	25	25								
	316/2		19	19								
	316/3		6	6								
185	260/1	50	25	25								
	260/2		25	25								
186	237/1	50	25	25								
	237/3		25	25								
182	165/4	50	25	24	1							56.9 x 99.2
	165/5		25	24	1							70.0 x 104.2
181	261/1	50	25	25								
	261/2		25	25								
189	269/1	50	25	25								
	269/2		25	25								
183	162/2	50	25	25								
	162/3		25	25								
184	256/1-5	0	0	0								
190	167/2	43	20	20								
	167/3		11	11								
	167/4		0	0								
	167/5		7	7								
	167/1		5	5								

APPENDIX TABLE CA-F48 (continued)

Bis(2-methoxyethyl) ether

Females

Single Dosing: 250 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		
216	191/1	50	25	23							1 Pair of Minutes	45.5 x 114.3
											1 Chromatid Fragment	30.9 x 113.9
	191/2		25	24							1 Chromatid Fragment	63.2 x 112.1
218	272/1	50	25	24		1						30.5 x 109.7
	272/2		25	25								
213	225/1	50	25	24	1							59.1 x 111.0
	225/2		25	25								
215	270/2	50	25	25								
	270/4		25	25								
219	258/4	50	25	25								
	258/5		25	24		1						39.0 x 102.4
212	226/3	50	25	25								
	226/5		25	25								
220	308/1	50	25	25								
	308/2		25	25								
214	231/2	50	25	25								
	231/4		25	25								
217	262/1	50	25	25								
	262/2		14	13	1							32.2 x 93.1
	262/3		11	11								
211	241/1-5	0	0	0								

APPENDIX TABLE CA-F48 (continued)

Bis(2-methoxyethyl) ether
Females

Single Dosing: 1,000 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	264/1	50	25	25								
	264/3		25	25								
247	244/2	20	4	4								
	244/5		16	16								
	244/1-3-4		0	0								
245	228/1-5	0	0	0								
246	183/2	50	25	25								
	183/3		25	25								
242	319/1	50	25	25								
	319/3		25	25								
244	180/2	50	25	25								
	180/3		25	25								
250	218/1	50	25	24	1							65.0 x 106.5
	218/2		22	22								
	218/3		3	3								
248	268/1	50	25	25								
	268/2		25	25								
243	277/3	50	25	25								
	277/5		25	24	1							62.1 x 113.2
241	265/3	50	25	25								
	265/4		20	20								
	265/5		5	5								

APPENDIX TABLE CA-F48 (continued)

Bis(2-methoxyethyl) ether

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	229/1	50	25	25								
	229/3		25	25								
280	175/1	50	25	23	1							40.6 x 104.0
											1 Chromatid Fragment	42.5 x 99.3
	175/3		25	24		1						28.9 x 110.4
277	255/1-5	0	0	0								
273	188/2	50	25	14	1							68.0 x 111.8
							1					66.8 x 111.8
						1						63.4 x 112.8
					1							33.5 x 109.6
											1 Multi Aberration	38.9 x 109.8
					1							57.2 x 108.5
											1 Chromosomal Fragment	62.6 x 109.0
					1							63.2 x 108.8
					1							64.0 x 109.0
					1	1						67.5 x 108.9
						1					1 Chromatid Fragment	68.6 x 107.4
	188/4		25	22							1 Exchange	62.2 x 108.6
					1							29.2 x 103.6
					1						1 Chromosomal Fragment	65.8 x 104.1
279	174/2	50	25	24	1	2					3 Chromatid Fragment	50.0 x 108.2
	174/5		25	25								

APPENDIX TABLE CA-F48 (continued)

Bis(2-methoxyethyl) ether

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
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
276	296/2	50	25	24	1							64.4 x 112.2
	296/3		25	20	1							58.0 x 107.3
					1							55.0 x 107.9
						1						50.3 x 107.6
											1 Exchange	41.5 x 105.6
					1	1					1 Exchange	41.1 x 105.8
275	302/1	50	25	21							1 Multi Aberration	67.6 x 110.9
					1	1						66.4 x 110.7
						3					1 Exchange	61.2 x 110.3
						1					1 Exchange	54.8 x 110.2
	302/2		25	23							2 Exchange 2 Chromatid Fragments	63.9 x 106.3
						1	1					63.0 x 106.2
271	307/1	50	25	24			1					67.1 x 103.3
	307/3		25	25								
272	297/3	50	25	25								
	297/4		25	23	2	1					1 Exchange	58.3 x 113.2
					1							61.0 x 108.6
274	267/1	50	25	22		1	1				1 Exchange	57.3 x 104.5
					1	2					1 Exchange 1 Pair of minutes 2 Chromatid Fragments	53.4 x 104.3
						2						52.2 x 104.8
	267/3		25	23	1							36.3 x 103.9
											1 Multi Aberration	34.5 x 105.4

APPENDIX TABLE DL

Bis(2-methoxyethyl) ether
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	16	0	12	16	14	9	14	13	9	12	13	0	17	10	10	13	10	11	14	16	229
	Total Implants	16	0	12	13	14	11	15	13	10	15	13	0	15	9	12	10	12	11	16	17	234
	Live Implants	16	0	11	12	14	10	15	13	8	15	11	0	15	7	12	10	12	11	16	16	24
	Early Deaths	0	0	1	1	0	1	0	0	2	0	2	0	0	1	0	0	0	0	0	1	9
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
2	Corpora lutea	12	0	15	13	13	12	14	15	11	14	12	12	12	14	13	11	13	16	16	14	252
	Total Implants	16	0	15	12	14	11	15	9	13	15	14	12	13	15	14	13	14	13	16	15	259
	Live Implants	15	0	13	12	14	10	15	7	13	15	13	10	13	15	14	13	14	13	16	14	249
	Early Deaths	1	0	1	0	0	1	0	2	0	0	0	2	0	0	0	0	0	0	0	1	8
	Late Deaths	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	2
3	Corpora lutea	21	16	11	20	1	15	13	14	14	21	1	16	8	12	15	14	16	12	12	16	263
	Total Implants	12	14	11	15	0	14	12	12	15	15	0	16	12	13	14	14	16	14	14	15	248
	Live Implants	12	14	10	14	0	13	11	12	15	15	0	16	12	13	14	13	15	14	13	13	239
	Early Deaths	0	0	1	1	0	1	1	0	0	0	0	0	0	0	0	1	1	0	1	2	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	8	11	12	14	12	9	12	13	10	15	12	12	12	3	18	12	12	15	11	11	234
	Total Implants	8	14	11	15	16	8	15	15	10	12	12	13	13	0	13	11	13	15	11	11	236
	Live Implants	8	13	11	15	14	7	15	15	8	12	12	13	12	0	12	11	13	15	9	11	226
	Early Deaths	0	1	0	0	2	1	0	0	2	0	0	0	1	0	1	0	0	0	2	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

APPENDIX TABLE DL (continued)

Bis(2-methoxyethyl) ether

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	
5	Corpora lutea	14	17	16	10	12	18	14	11	12	9	15	17	14	12	0	13	13	16	11	18	262
	Total Implants	11	16	17	9	13	15	11	11	14	11	14	16	15	12	0	13	13	13	16	16	256
	Live Implants	10	16	17	9	12	15	11	11	13	11	14	16	15	12	0	12	13	13	16	16	252
	Early Deaths	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	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
6	Corpora lutea	13	16	12	15	13	14	17	16	13	10	9	16	15	12	8	14	11	15	10	18	267
	Total Implants	6	17	13	15	13	14	16	13	13	12	9	15	15	13	9	15	12	14	11	15	260
	Live Implants	6	16	12	14	13	12	14	13	13	11	8	15	15	13	7	14	12	12	11	14	245
	Early Deaths	0	1	1	1	0	2	2	0	0	1	1	0	0	0	2	1	0	2	0	0	14
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
7	Corpora lutea	11	13	13	12	10	11	2	12	10	13	13	14	13	12	10	13	11	13	14	13	233
	Total Implants	12	13	13	13	9	11	0	13	10	14	12	10	12	12	11	13	11	14	13	11	227
	Live Implants	12	13	13	11	8	10	0	13	9	14	12	9	12	11	11	12	9	12	13	9	213
	Early Deaths	0	0	0	2	1	1	0	0	0	0	0	1	0	1	0	1	2	2	0	1	12
	Late Deaths	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	2
8	Corpora lutea	15	10	15	11	9	13	17	14	10	8	9	12	0	10	13	12	11	13	13	10	225
	Total Implants	13	10	16	12	9	12	14	14	11	12	10	10	0	13	12	13	11	12	13	12	229
	Live Implants	13	10	14	12	9	10	14	14	11	12	9	10	0	12	12	12	10	11	13	11	219
	Early Deaths	0	0	2	0	0	2	0	0	0	0	1	0	0	1	0	1	1	1	0	1	10
	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)

Bis(2-methoxyethyl) ether

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	
9	Corpora lutea	14	14	11	18	12	13	12	14	15	17	2	16	11	11	16	11	10	14	13	18	262
	Total Implants	9	12	11	13	12	13	11	12	14	16	0	12	10	10	12	10	6	14	12	11	220
	Live Implants	9	12	10	12	10	13	10	12	10	16	0	12	10	10	12	9	6	14	12	11	210
	Early Deaths	0	0	1	1	2	0	1	0	3	0	0	0	0	0	0	1	0	0	0	0	9
	Late Deaths	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
10	Corpora lutea	13	12	2	10	4	12	12	17	12	8	13	12	14	14	10	15	11	13	17	17	238
	Total Implants	14	12	0	11	0	12	13	13	14	12	13	12	13	14	5	14	10	14	15	16	227
	Live Implants	14	12	0	11	0	12	13	13	14	10	13	12	13	14	5	13	9	13	15	16	222
	Early Deaths	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	1	1	1	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
Σ	Corpora lutea	137	109	119	139	100	126	127	139	116	127	99	133	116	110	113	128	118	138	131	146	2465
	Total Implants	117	108	119	128	100	121	122	125	124	134	97	116	118	111	102	126	118	134	137	139	2396
	Live Implants	115	106	111	122	94	112	118	123	114	131	92	113	117	107	99	119	113	128	134	131	2299
	Early Deaths	2	2	7	6	6	9	4	2	8	3	4	3	1	3	3	7	5	6	3	6	90
	Late Deaths	0	0	1	0	0	0	0	0	2	0	1	0	0	1	0	0	0	0	0	2	7

APPENDIX TABLE DL (continued)

Bis(2-methoxyethyl) ether

Multiple Dosing: 250 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	12	2	13	4	12	13	13	15	14	15	11	12	13	12	13	13	16	12	13	240
	Total Implants	14	11	0	13	1	13	15	15	14	13	10	11	13	7	11	12	13	16	13	13	228
	Live Implants	14	11	0	13	1	12	14	14	14	13	9	11	13	7	10	12	12	16	12	13	221
	Early Deaths	0	0	0	0	0	1	1	1	0	0	1	0	0	0	1	0	1	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	12	13	12	16	15	0	3	18	14	8	15	13	13	11	18	14	12	14	9	11	241
	Total Implants	6	11	13	13	16	0	0	14	16	8	14	12	13	13	17	14	14	13	6	10	223
	Live Implants	6	11	12	11	16	0	0	14	15	8	13	12	13	12	17	14	14	11	6	10	215
	Early Deaths	0	0	1	0	0	0	0	0	1	0	1	0	0	1	0	0	0	2	0	0	6
	Late Deaths	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
3	Corpora lutea	14	15	12	11	14	18	*-	18	5	11	14	12	13	14	13	21	14	17	0	13	249
	Total Implants	13	15	7	13	10	17	*-	12	1	16	13	11	14	15	16	12	13	14	0	13	225
	Live Implants	13	15	7	13	5	17	*-	12	1	14	11	11	13	13	16	11	12	13	0	12	209
	Early Deaths	0	0	0	0	5	0	*-	0	0	2	2	0	1	2	0	1	1	1	0	1	16
	Late Deaths	0	0	0	0	0	0	*-	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	Corpora lutea	12	9	12	12	11	14	13	0	14	12	15	11	12	12	17	12	13	10	13	13	227
	Total Implants	13	10	13	13	12	13	14	0	14	10	15	11	15	12	13	3	15	11	14	13	234
	Live Implants	13	10	13	12	12	11	13	0	14	10	15	9	14	12	13	3	15	10	14	12	225
	Early Deaths	0	0	0	1	0	2	1	0	0	0	0	2	1	0	0	0	0	1	0	1	9
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

*- = Ambiguous Record of Result

APPENDIX TABLE DL (continued)

Bis(2-methoxyethyl) ether

Multiple Dosing: 250 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	
5	Corpora lutea	0	13	13	13	4	11	14	12	11	9	16	12	14	14	13	14	8	15	10	14	230
	Total Implants	0	10	12	9	0	10	17	15	12	10	16	16	15	14	12	15	12	15	14	12	236
	Live Implants	0	10	11	8	0	9	16	12	11	10	13	16	15	14	12	15	12	13	14	12	223
	Early Deaths	0	0	1	1	0	1	1	3	1	0	3	0	0	0	0	0	0	2	0	0	13
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	Corpora lutea	11	15	15	13	12	13	13	15	11	12	13	10	11	11	12	12	10	12	13	11	276
	Total Implants	11	12	15	13	12	15	12	17	14	13	13	15	14	14	15	13	14	12	14	14	272
	Live Implants	11	12	15	13	12	14	12	16	13	13	13	14	14	13	14	12	14	12	14	14	265
	Early Deaths	0	0	0	0	0	1	0	1	1	0	0	1	0	1	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	0	0
7	Corpora lutea	16	14	9	11	13	10	11	10	12	13	10	11	11	12	12	12	12	12	13	11	232
	Total Implants	15	11	11	11	10	12	11	10	12	13	13	10	11	11	14	12	11	12	13	11	234
	Live Implants	14	11	11	11	10	11	10	9	10	13	13	10	11	11	14	11	11	12	12	11	226
	Early Deaths	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	4
	Late Deaths	0	0	0	0	0	0	1	1	2	0	0	0	0	0	0	0	0	0	0	0	4
8	Corpora lutea	13	14	14	12	12	13	16	10	11	12	11	9	12	12	12	9	11	11	12	12	238
	Total Implants	12	15	14	11	11	15	13	14	10	9	11	10	12	12	13	12	12	12	13	14	241
	Live Implants	12	15	12	11	11	15	12	12	9	9	11	9	11	12	11	12	12	12	12	12	228
	Early Deaths	0	0	2	0	0	0	1	2	1	0	0	1	1	0	2	0	0	0	1	2	13
	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)

Bis(2-methoxyethyl) ether

Multiple Dosing: 250 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	
9	Corpora lutea	12	12	15	14	13	14	13	0	13	12	13	13	10	13	15	15	12	12	12	18	251
	Total Implants	10	12	16	14	10	12	15	0	13	12	12	13	10	12	15	15	11	12	7	15	236
	Live Implants	9	12	16	14	10	12	15	0	12	12	11	12	10	12	13	14	10	12	7	14	227
	Early Deaths	1	0	0	0	0	0	0	0	1	0	1	1	0	0	2	1	1	0	0	1	9
	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	12	0	10	16	13	14	16	10	10	16	10	11	12	11	15	12	13	11	13	9	234
	Total Implants	13	0	13	16	12	15	15	11	10	15	12	12	14	11	15	13	13	13	14	11	248
	Live Implants	13	0	12	13	12	15	14	11	9	15	12	11	11	11	14	13	13	10	12	9	230
	Early Deaths	0	0	1	3	0	0	1	0	0	0	0	1	3	0	1	0	0	3	2	2	17
	Late Deaths	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
Σ	Corpora lutea	114	117	114	131	111	119	112	106	114	118	134	117	121	125	140	136	121	129	117	132	2428
	Total Implants	107	107	114	126	94	122	112	108	116	119	129	121	131	121	141	121	128	130	108	126	2381
	Live Implants	105	107	109	119	89	116	106	100	108	117	121	115	125	117	134	117	125	121	103	119	2275
	Early Deaths	2	0	5	5	5	6	5	7	5	2	8	6	6	4	7	4	3	9	5	7	101
	Late Deaths	0	0	0	2	0	0	1	1	3	0	0	0	0	0	0	0	0	0	0	0	7

APPENDIX TABLE DL (continued)

Bis(2-methoxyethyl) ether

Multiple Dosing: 1,000 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	12	10	12	10	15	14	0	15	13	15	14	4	12	0	0	10	13	12	0	193
	Total Implants	14	16	13	14	13	15	14	0	11	10	15	16	0	12	0	0	10	14	14	0	201
	Live Implants	13	16	12	14	13	14	11	0	6	9	15	16	0	12	0	0	9	12	14	0	186
	Early Deaths	1	0	1	0	0	1	2	0	5	1	0	0	0	0	0	0	1	2	0	0	14
	Late Deaths	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
2	Corpora lutea	8	15	11	13	14	11	14	12	18	11	13	12	14	13	14	14	15	14	0	17	253
	Total Implants	9	16	14	12	15	12	15	12	13	12	14	14	14	12	15	14	16	15	0	17	261
	Live Implants	9	16	14	12	13	11	14	11	11	12	12	13	13	12	14	14	16	15	0	16	248
	Early Deaths	0	0	0	0	2	1	1	1	2	0	2	1	1	0	1	0	0	0	0	1	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	16	10	0	0	0	14	14	14	11	17	5	13	9	14	13	14	16	8	0	11	199
	Total Implants	8	9	0	0	0	16	18	14	11	17	1	11	9	15	14	14	14	13	0	14	198
	Live Implants	7	9	0	0	0	16	16	14	11	8	1	11	9	14	14	14	14	13	0	14	185
	Early Deaths	1	0	0	0	0	0	2	0	0	9	0	0	0	1	0	0	0	0	0	0	13
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	Corpora lutea	4	0	3	0	1	0	14	0	16	12	0	6	12	10	13	0	9	13	0	6	119
	Total Implants	0	0	0	0	0	0	13	0	13	12	0	6	10	7	11	0	11	11	0	5	99
	Live Implants	0	0	0	0	0	0	12	0	13	12	0	4	10	7	11	0	11	11	0	4	95
	Early Deaths	0	0	0	0	0	0	1	0	0	0	0	2	0	0	0	0	0	0	0	1	4
	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)

Bis(2-methoxyethyl) ether

Multiple Dosing: 1,000 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	
5	Corpora lutea	0	0	0	0	0	0	0	14	*-	0	0	0	0	0	0	11	1	0	0	0	26
	Total Implants	0	0	0	0	0	0	0	12	*-	0	0	0	0	0	0	10	0	0	0	0	22
	Live Implants	0	0	0	0	0	0	0	11	*-	0	0	0	0	0	0	8	0	0	0	0	19
	Early Deaths	0	0	0	0	0	0	0	1	*-	0	0	0	0	0	0	2	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
6	Corpora lutea	2	2	1	0	3	1	3	0	0	12	0	1	1	0	4	0	3	0	0	1	34
	Total Implants	0	2	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	5
	Live Implants	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	3
	Early Deaths	0	2	0	0	0	0	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
7	Corpora lutea	0	0	1	2	1	0	0	0	1	4	0	0	0	0	0	0	3	0	0	0	12
	Total Implants	0	0	0	0	0	0	0	0	1	3	0	0	0	0	0	0	0	0	0	0	4
	Live Implants	0	0	0	0	0	0	0	0	1	3	0	0	0	0	0	0	0	0	0	0	4
	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
8	Corpora lutea	10	0	1	1	2	0	3	0	2	17	10	11	0	0	14	17	6	0	0	14	108
	Total Implants	8	0	0	0	0	0	0	0	2	17	4	13	0	0	16	14	0	0	0	20	94
	Live Implants	6	0	0	0	0	0	0	0	1	17	3	13	0	0	16	12	0	0	0	20	88
	Early Deaths	2	0	0	0	0	0	0	0	1	0	1	0	0	0	0	2	0	0	0	0	6
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

* = Ambiguous record of result

APPENDIX TABLE DL (continued)

Bis(2-methoxyethyl) ether

Multiple Dosing: 1,000 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	
9	Corpora lutea	8	16	9	0	2	10	0	0	14	12	6	13	0	0	0	12	0	0	18	12	132
	Total Implants	3	14	8	0	0	0	0	0	14	12	3	13	0	0	0	13	0	0	13	11	104
	Live Implants	2	13	8	0	0	0	0	0	12	12	3	13	0	0	0	13	0	0	13	11	100
	Early Deaths	1	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0	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
10	Corpora lutea	14	13	13	12	14	11	0	0	11	15	12	14	0	14	9	13	12	10	14	13	214
	Total Implants	14	13	13	13	7	12	0	0	11	15	11	10	0	14	10	14	12	10	14	14	207
	Live Implants	13	13	12	13	6	12	0	0	11	14	11	8	0	14	10	13	12	10	12	12	196
	Early Deaths	1	0	1	0	0	0	0	0	0	1	0	2	0	0	0	1	0	0	2	2	10
	Late Deaths	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Σ	Corpora lutea	74	68	49	40	47	62	62	40	88	113	61	84	40	63	67	81	75	58	44	74	1290
	Total Implants	56	70	48	39	35	55	60	38	76	101	48	83	33	60	66	79	63	63	41	81	1195
	Live Implants	50	67	46	39	32	53	53	36	66	90	45	78	32	59	65	74	62	61	39	77	1124
	Early Deaths	6	3	2	0	2	2	6	2	10	11	3	5	1	1	1	5	1	2	2	4	69
	Late Deaths	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2

* = result omitted in error

APPENDIX TABLE DL (continued)

Bis(2-methoxyethyl) ether

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	11	15	5	7	10	12	12	12	0	0	8	0	15	13	9	0	14	11	8	0	162
	Total Implants	11	3	2	7	10	10	3	10	0	0	9	0	3	9	9	0	12	9	10	0	117
	Live Implants	0	0	0	1	0	1	0	2	0	0	0	0	0	0	1	0	4	9	0	0	18
	Early Deaths	11	3	2	6	10	9	3	8	0	0	9	0	3	9	8	0	8	0	10	0	99
	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	2	1	0	4	0	3	1	0	5	7	6	4	4	5	0	0	44
	Total Implants	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
	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	1	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
3	Corpora lutea	3	0	0	0	0	0	0	2	2	1	0	0	1	2	2	0	3	0	0	0	16
	Total Implants	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	3
	Live Implants	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
	Early Deaths	0	0	0	0	0	0	0	0	0	0	0	1	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	13	10	13	3	12	0	11	3	10	3	5	4	6	13	11	4	13	11	4	0	149
	Total Implants	13	11	9	0	14	0	12	7	8	4	1	5	4	12	9	3	14	12	2	0	140
	Live Implants	9	5	6	0	7	0	6	1	5	3	1	2	3	7	6	1	9	7	1	0	79
	Early Deaths	3	6	3	0	7	0	6	6	3	1	0	3	1	5	3	2	5	5	1	0	60
	Late Deaths	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1

APPENDIX TABLE DL (continued)

Bis(2-methoxyethyl) ether

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	
5	Corpora lutea	12	23	13	11	12	14	0	6	0	11	13	13	12	15	13	13	12	12	12	9	226
	Total Implants	13	17	11	7	11	10	0	11	0	12	13	14	10	16	12	16	14	6	7	14	214
	Live Implants	10	17	11	7	11	10	0	10	0	12	12	13	10	13	12	16	14	6	6	13	203
	Early Deaths	3	0	0	0	0	0	0	1	0	0	1	1	0	3	0	0	0	0	1	1	11
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	Corpora lutea	16	15	11	12	15	*	10	13	18	12	12	0	11	12	11	12	12	17	14	11	234
	Total Implants	4	13	13	13	12	*	11	13	14	12	10	0	15	13	12	12	13	15	15	15	225
	Live Implants	4	12	11	13	12	*	11	12	14	11	9	0	15	11	12	12	12	15	15	15	216
	Early Deaths	0	1	2	0	0	*	0	1	0	1	1	0	0	2	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
7	Corpora lutea	11	11	12	15	14	0	9	13	17	10	1	9	14	13	13	0	13	13	10	7	205
	Total Implants	12	13	12	14	12	0	11	14	12	11	1	9	13	11	14	0	13	13	12	8	205
	Live Implants	10	12	12	14	11	0	11	14	11	10	1	7	13	11	14	0	13	12	12	8	196
	Early Deaths	2	1	0	0	1	0	0	0	1	1	0	2	0	0	0	0	0	1	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
8	Corpora lutea	16	13	8	12	14	11	10	13	10	13	10	11	14	10	11	16	12	12	0	9	215
	Total Implants	15	12	9	7	15	12	10	13	14	13	13	12	16	11	14	12	12	11	0	14	235
	Live Implants	15	12	8	7	13	12	9	11	14	13	13	12	16	11	14	11	12	10	0	13	226
	Early Deaths	0	0	1	0	2	0	1	2	0	0	0	0	0	0	0	1	0	1	0	1	9
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

* = No result recorded

APPENDIX TABLE DL (continued)

Bis(2-methoxyethyl) ether

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	
9	Corpora lutea	16	10	16	17	13	14	12	13	9	13	13	15	16	14	9	13	16	15	11	17	272
	Total Implants	14	10	13	14	12	14	11	12	6	12	14	14	16	13	12	14	16	8	11	19	255
	Live Implants	13	9	12	14	12	14	11	11	6	11	14	14	13	11	11	11	15	8	10	18	238
	Early Deaths	1	1	1	0	0	0	0	1	0	1	0	0	3	2	1	2	1	0	1	1	16
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
10	Corpora lutea	16	13	14	13	13	14	15	10	19	12	15	15	13	15	5	14	11	15	16	13	281
	Total Implants	12	8	13	14	13	13	15	13	10	15	15	15	16	16	3	13	12	16	15	14	261
	Live Implants	12	4	13	14	12	13	15	12	10	15	15	13	16	16	2	13	11	16	15	13	250
	Early Deaths	0	2	0	0	1	0	0	1	0	0	0	2	0	0	1	0	1	0	0	1	9
	Late Deaths	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Σ	Corpora lutea	116	110	92	90	105	66	79	89	85	78	78	67	107	114	90	76	110	111	75	66	1804
	Total Implants	94	87	82	76	99	59	73	93	64	79	76	70	94	102	85	70	106	91	72	84	1656
	Live Implants	73	71	73	70	78	50	63	73	60	75	65	61	87	80	72	64	90	83	59	80	1427
	Early Deaths	20	14	9	6	21	9	10	20	4	4	11	9	7	22	13	5	16	8	13	4	225
	Late Deaths	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	4

* = result omitted in error

APPENDIX TABLE SA

Bis(2-methoxyethyl) ether
Sperm Abnormality Assessment

Multiple Dosing: Air Control (0 ppm)
Low, 250 ppm
High, 1,000 ppm
Positive, Ethyl methanesulphonate, 200 mg/kg

Slide No.	Normal	Abnormality					Total Abnormal	Total Examined	De-coded Information	
		A	B	C	D	E			Animal No.	Group
334	919	11	3	43	5	19	81	1000	321	Air
346	949	3	2	23	8	15	51	1000	322	Air
356	931	2	3	34	5	25	69	1000	323	Air
352	972	1	2	10	4	11	28	1000	324	Air
323	945	5	3	27	8	12	55	1000	325	Air
348	962	1	2	18	5	12	38	1000	326	Air
342	966	1	2	12	6	13	34	1000	327	Air
360	952	1	4	15	11	17	48	1000	328	Air
347	940	5	4	24	5	22	60	1000	329	Air
351	950	1	3	12	20	14	50	1000	330	Air
330	972	2	2	17	0	0	28	1000	331	Low
327	950	2	1	24	3	20	50	1000	332	Low
329	967	2	5	17	1	11	33	1000	333	Low
324	963	0	3	18	2	14	37	1000	334	Low
331	948	3	1	24	2	22	52	1000	335	Low
*							*	*	336	Low
357	936	5	2	26	8	23	64	1000	337	Low
353	940	1	6	28	14	18	67	1000	338	Low
359	951	2	3	19	9	16	49	1000	339	Low
324	996	1	2	23	7	21	54	1000	340	Low

* missing value

APPENDIS TABLE SA (continued)

Bis(2-methoxyethyl) ether

Multiple Dosing: Air Control (0 ppm)
Low, 250 ppm
High, 1,000 ppm
Positive, Ethyl methanesulphonate, 200 mg/kg

Slide No.	Normal	Abnormality					Total Abnormal	Total Examined	De-coded Information	
		A	B	C	D	E			Animal No.	Group
344	746	6	19	175	13	41	254	1000	341	High
328							DEAD ON DOSING		342	High
325							DEAD ON DOSING		343	High
355	710	20	17	188	20	45	290	1000	344	High
337	786	12	10	120	24	48	214	1000	345	High
333	667	15	34	226	21	37	333	1000	346	High
321							DEAD ON DOSING		347	High
326	816	0	3	53	100	28	184	1000	348	High
336	512	34	15	347	27	65	488	1000	349	High
335	509	31	23	352	34	51	491	1000	350	High
341	955	0	0	19	6	20	45	1000	351	+
358	922	2	6	49	6	15	78	1000	352	+
339	919	2	3	34	16	26	81	1000	353	+
350	899	4	5	57	11	24	101	1000	354	+
*							*	*	355	+
340	916	1	2	39	10	32	84	1000	356	+
354	888	3	3	45	36	25	112	1000	357	+
338	940	4	0	20	23	13	60	1000	358	+
343	927	1	1	35	17	19	73	1000	359	+
349	915	5	5	40	10	25	85	1000	360	+

* missing value