Report Number 28

TIER II MUTAGENIC SCREENING OF 13 NIOSH PRIORITY COMPOUNDS

INDIVIDUAL COMPOUND REPORT
BUTYLENE OXIDE

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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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Principal Investigator

TABULATIONS

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

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

Example:

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

Abbreviations on Chromosomal Aberration Tables and Appendix Tables:

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

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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

Butylene oxide 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 exposure of 3 h duration and concentrations up to 7,300 mg/ml of culture medium.

2. Dominant lethal test in male rats with exposure to atmospheres containing 250 ppm or 1,000 ppm butylene oxide for 7 h per day for 5 consecutive days. Analysis of test atmospheres was by continuous infra-red absorption monitoring at a wavelength of 11.0 μm.

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

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

5. Sex-linked recessive lethal (SLRL) test in *Drosophila melanogaster* with exposure to atmospheres of 1,000 ppm

The results obtained were as follows:

1. There was no increase in UDS in cells treated with butylene oxide.

2. There were no effects attributable to butylene oxide in the dominant lethal test on pregnancy frequency, numbers of corpora lutea or implantations, or the frequency of early deaths.

3. Sperm abnormality frequency was not affected by treatment.

4. Sex-linked recessive lethal mutation frequency was not increased.

It was concluded that butylene oxide was devoid of genetic effects detectable in these experiments.

INTRODUCTION

1,2-Butylene oxide (1,2-epoxybutane; propyloxirane; 1-butene oxide; CAS No. 109-99-9) is a colourless liquid which is soluble in water and miscible with most organic solvents. It can be prepared by the epoxidation of 1-butene with peroxyacetic acid or catalytically with molecular oxygen when 1-butene is in the liquid phase. The main use of butylene oxide has been as a corrosion inhibitor or acid scavenger in chlorinated solvents such as trichloroethylene and methyl chloroform at levels of 3-8%. Inclusion of about 0.25-0.5% of butylene oxide during the preparation of vinyl chloride and co-polymers has been used to minimise both container corrosion and metal pick-up. Another use is in the co-polymerisation of butylene oxide with other alkylene oxides to yield polyethers and polyurethanes. A summary of its physical and chemical properties follows:

Formula	$CH_3CH_2CH\underset{O}{\diagdown\!\diagup}CH_2$
Mol. wt.	72.10
Sp. gr. ($20^{o}C$)	0.8312
B.P. (760 mm Hg)	$63^{o}C$
Refractive index	1.3855
Flash point	$5^{o}F$

Butylene oxide was found to be non-carcinogenic when applied as a 10% solution in acetone to the skin of ICR/Ha female Swiss mice (Van Duuren et al, 1967).

McCann et al, (1975) reported that it was weakly mutagenic for Salmonella typhimurium in their standard Ames' assay. It is possible that, in both the carcinogenicity test and the Ames' test mentioned the butylene oxide would evaporate quite rapidly. This effect would mean that neither the mice nor the bacteria were properly exposed. Inhalation studies are presently in progress with rats and mice in an NCI/NTP-sponsored study. Using an enclosed system, Cline et al, (1978) observed a large response from Escherichia coli WP2 uvrA and Salmonella typhimurium TA 100; small responses were also obtained from WP2 and TA 1535 strains of these bacteria. These results have been partially substantiated at Inveresk Research International. No mutagenic response was seen in the Salmonella strains in a pre-incubation test in which a rather low high dose (333 µg) was used, but large responses were obtained from strains TA 1535 and TA 100 in vapour assays (e.g. a x 10 background response from TA 1535 in an atmosphere of 0.02%).

MATERIALS AND METHODS

CHEMICALS

Test Substance

Five 250 g bottles and 2 x 250 g bottles, Batch No. 17047 (stated purity 99%) were received from Aldrich Chemical Company Limited on 3 October 1979 and 1 November 1979 respectively. The test material was a clear, colourless liquid and was retained in the dark under ambient conditions in the company dispensary until used. A sample has been retained for analysis, should this be necessary.

Positive Control 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	12 October 1979	11-12	10	220♂	24 October 1979	Single dose cytogenetics.
				176♀	29 October 1979 to 2 November 1979	Multiple dose cytogenetics.
Mouse	10 October 1979	11-13	5	44♂		
Rat	2 November 1979	8-10	None	80♀ x10	None	DL matings.

Pre-experiment Acceptance Tests

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

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

1. Ileal contents are incubated in selenite broth.

2. Lung, liver and kidney samples are incubated on blood agar plates.

3. Lung sample is plated on McConkey's medium.

4. Liver sample which was plated onto blood agar is then taken into a selenite tube.

5. All samples in selenite broth are incubated for 24 h, then plated on McConkey's medium for 24 h.

6. Smears are prepared and stained. Any Gram-negative bacteria are then put through Enterotubes for identification.

Animal Management

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

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

They were housed individually in cages in a room with a light intensity of approximately 200 lux, a 12 h light-dark cycle, approximately 10 air changes per hour, temperature maintained at ca 22°C with extreme limits of 23.5°C and 17°C, and relative humidity ca 50%, with extreme limits of 60% and 32% in the completed portions of the experiment.

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

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

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

Diet

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

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

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

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

Allocation of Rats and Mice to Cages and Treatment Groups

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

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

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

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

Treatment groups were colour coded as follows:

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

Animal Identification

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

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

Animal Positioning in the Exposure Chambers

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

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

The treatment groups were constituted as follows:-

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

ATMOSPHERE GENERATION AND EXPOSURE

Exposure Chambers

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

Exposures to butylene oxide 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 also was ventilated at the rate of 11 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 butylene oxide. 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.

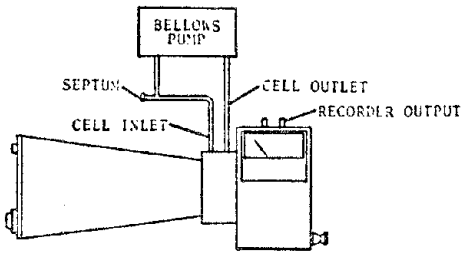
Calibration and Analytical Development

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 butylene oxide 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 butylene oxide were calibrated each day before vapour generation commenced.

The calibration was performed using a closed loop calibration system (see diagram below). Known volumes of butylene oxide 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 :	11.0 µm	11.0 µm
Pathlength :	15.75 m	6.75 m
Absorbence Range :	1.0 A	1.0 A
Slit Width :	0.5 mm	0.5 mm
Meter Response :	1	1
Recorder Voltage :	1.0 V	1.0 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: Butylene oxide

C = 1,000 ppm
ρ = 0.837 g/cm^3
M = 72.11

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

$$= \frac{1{,}000 \times 72.11}{0.837} \times \frac{5.64}{10^3 \times 24.06\ \mu l}$$

$$= 20.2\ \mu l$$

Therefore, to construct a calibration curve to cover the 1,000 ppm range, 5 µl samples of butylene oxide 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 butylene oxide contained in a glass gas washing, or Drechsel bottle immersed in a temperature controlled water bath at 28°C. The nitrogen/butylene oxide vapour mixture so generated was ducted through a vertical stainless steel piping approximately 1.5 m long to a glass mixing vessel and diluted with filtered, compressed air. The resulting mixture of butylene oxide/air was ducted through a 3/4" ID stainless steel tube to the apex 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 butylene oxide concentration.

Measurement of Chamber Concentrations

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

Photo-reduced traces showing exposure chamber concentrations 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 butylene oxide reservoir (a 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 butylene oxide 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) and 8-[^{3}H]-deoxyguanosine (26.4 Ci/mmol) were obtained from the Radiochemical Centre, Amersham, England.

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

Test Solutions

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

Cells

Unscheduled DNA synthesis, following treatment with test compound, was measured in human embryonic intestinal cells (Flow 11,000), 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 gentamicin (50 µg/ml). This medium was obtained from Flow Laboratories.

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

Animals

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

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

Preparation of the 9,000 g Supernatant Fluid from Livers

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

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

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

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

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

co-factor solution	9 parts
liver preparation	1 part

Preliminary Toxicity Test

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

The cells were harvested and suspended in growth medium as for sub-culture, sedimented by centrifugation at 200 g for 5 min and resuspended in fresh culture medium at a density of 5×10^4 cells/ml. 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 in dimethylsulphoxide, and 10 µl samples were added to duplicate cell suspensions to give final concentrations ranging from 20 to 1,226 µg/ml. To each control culture were 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 was used as follows:

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

No cells showed any damage in this test, so, in the main tests concentrations were increased to a maximum of 7,300 µg/ml. In the presence of S-9 mix, toxicity was extremely severe at concentrations of butylene oxide in excess of 525 µg/ml.

DNA Repair Assay (Method 1)

The cells were harvested, sedimented, suspended in fresh culture medium at a density of 5 x 10^4 cells/ml and 2 ml samples of this suspension were pipetted into 35 mm tissue culture Petri dishes containing 3 sterile coverslips (Lux Scientific Corporation, California, U.S.A.). These were then incubated at 37°C in a humid atmosphere of 5% CO_2 in air for 72 h. The medium from each of the dishes was then replaced with 2 ml of arginine-deficient DMEM supplemented with 5% heat inactivated foetal bovine serum and the plates incubated for 24 h. The medium was then replaced with a further 2 ml of arginine-deficient DMEM and the incubation continued for a further 48 h. At the end of this time the cultures were divided into 2 groups and 100 µl of S-9 mix added to one of them. Solutions of hydroxyurea (250 mM) in sterile distilled water and 6-[^{3}H]-thymidine (21 Ci/mmol) were added to each culture giving final concentrations of 2.5 mM and 10 µCi/ml respectively. Butylene oxide was dissolved in dimethylsulphoxide and dilutions were made from this to give the test solutions of concentrations as listed in Tables UDS-1 and 2. Triplicate wells, with and without S-9 mix, received 10 µl samples of test compound solution. 10 µl samples of dimethylsulphoxide were added to negative control cultures.

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

4-NQO	10 µg/ml
2-AAN	3 µg/ml or 16 µg/ml

After incubation for 3 h at 37°C in an atmosphere of 5% CO_2 in air the cultures were repeatedly rinsed in phosphate buffered saline (PBS) which removed loose cells and soluble [^{3}H]-thymidine they were then incubated for 10 min in sodium citrate (1%) and finally fixed in methanol:acetic acid (3:1) for 18 h. For ease of handling during processing for autoradiography the coverslips were air dried and attached, cells uppermost, to clean microscope slides with a drop of mountant DePeX. The cells were then processed for autoradiography and stained.

Autoradiography

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

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

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

Quantification of Repair Synthesis

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

DNA Repair Assay (Method 2)

Flow 11,000 cells were harvested, sedimented and suspended in fresh culture medium at a density of 5×10^4 cells/ml. 5 ml samples were dispensed into 60 mm tissue culture Petri dishes (Nunclon Delta) which were incubated in a humid atmosphere of 5% CO_2 in air at 37°C for 72 h. The medium from each of the Petri dishes was then replaced with 5 ml of arginine-deficient medium supplemented with 5% heat inactivated foetal bovine serum and the dishes incubated for 24 h. The medium was then replaced with a further 5 ml of arginine-deficient DMEM and the incubation continued for a further 48 h. The dishes were then randomly divided into 2 groups and 500 µl of S-9 mix were added to one group. Solutions of hydroxyurea and [^{3}H]-deoxyguanosine (26 Ci/mmol) were added to each dish giving final concentrations 2.5 mM and 10 µCi/ml respectively. Butylene oxide was dissolved in dimethylsulphoxide at a concentration of The positive control compounds, 4-nitroquinoline-N-oxide, for S-9 free cultures and 2-aminoanthracene for S-9 supplemented cultures, were dissolved in dimethylsulphoxide and

diluted 1:100 in the culture medium to give final concentrations of 1 µg/ml. 10 µl of butylene oxide in DMSO, at a concentration of 0.837 µg/ml, was added to the cultures, both in the presence and absence of S-9 mix, to give a final concentration of 8.37 µg/ml.

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

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

Incorporation of Label in the Presence of S-9 Mix

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

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

CYTOGENETIC ANALYSIS OF RAT BONE MARROW CELLS

Metaphase Cell Preparations

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

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

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

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

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

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

Slide Reading

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

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

The number of abnormalities was recorded on sheets of the design shown in Appendix Form-1. Abnormalities looked for were: gaps, breaks, fragments, dicentrics, translocations (within the limitations of the staining methods) 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 7 December 1979) by neck dislocation.

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

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

Assessment

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

The following types of sperm were not scored:

(1) separated tails and heads.

(2) clumps of sperm.

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

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

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

I Normal

II Abnormal

A. hook upturned or elongated.

B. banana-shaped head.

C. amorphous head.

D. abnormal tail (sharp, 180^{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, $In(1)\ SC^{S1L}\ SC^{8R} + S\ SC^{S1}\ SC^{8}\ w^{aB}$.

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

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

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

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

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

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

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 100.4-502.0 ppm (250 ppm target concentration) and 252.5-1,262.5 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 butylene oxide was occurring. The results are shown in Table AT-3, where it can be seen that the maximum deviations encountered were -2% at the 250 ppm target concentration and $\pm$ 2% 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)

10 October 1979 Delivery: From the 44 mice received, 4 were haphazardly selected for PEAT. None of the mice showed clinical signs of infection or disease and there were no significant observations at autopsy. There were no histological, microbiological or parasitological findings.

12 October 1979 Delivery: Ten male and 10 female rats were haphazardly selected for PEAT. There were no significant observations made on the female rats at any stages of examination. In the male rats, 2 showed minimal perivascular lymphocytes and 2 others showed some bronchial lymphocytic accumulation.

It was concluded that both the rats and the mice were suitable for the intended experiments.

Clinical Observations and Body Weights

There was little evidence of toxicity in either rats or mice exposed to 250 ppm butylene oxide atmospheres, but at 4 times that concentration there were marked effects, particularly in the male mice used in the sperm abnormality test (Tables BW-1 to 4). Rats and mice were subdued by the end of each exposure period and showed little response to auditory stimuli. In addition, mice showed ataxia. This symptom was particularly severe in mice 344♂ and 347♂ on the second day of exposure and both of these mice were dead by the following morning, so, they were sent for autopsy. They were replaced by 2 previously unexposed mice, Nos. 401♂ and 402♂ but 401♂ died within an hour of the end of exposure on its first day. On Day 5 of exposure, before exposure, 349♂ was tremulous, rigid and showed no righting reflex, so it was kept, but not exposed on this day. The day after the final exposure, mice 342♂, 343♂, 346♂ and 350♂ were found dead in their cages. These mice were sent for autopsy.

Body weights were decreased in both male and female rats exposed to 1,000 ppm butylene oxide. Reductions in body weights were also seen in rats dosed with ethyl methanesulphonate for 5 days.

Autopsy Findings

For mice 342♂ and 343♂ there were no signs of disease or injury. The only significant observation made on 344♂ was irregular areas of congestion in all lung lobes. In 347♂ the lungs were severely congested. Lung congestion was also noted in 350♂, but additionally the urinary bladder was distended and blood filled. The animal was in an advanced state of autolysis. Mouse 346♂ was also partially autolysed. In the distended urinary bladder of this mouse there was a 1 mm diameter, round, white, solid inclusion.

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 butylene oxide (Tables UDS-1 and 2). Nevertheless, there was ample indication that the substance was reaching the cells. In the first experiment in the presence of S-9 mix, cells exposed to butylene oxide in excess of 525 µg/ml were not usable since it appeared that no tritiated thymidine was incorporated, presumably because of toxicity. The tendency towards a reduction in grain counts with increasing concentrations of butylene oxide in the presence of S-9 mix did suggest that perhaps lower concentrations should be tested. The experiment was repeated in the presence of S-9 mix, using a high concentration of 84 µg/ml. There was no indication of UDS induction (Table UDS-2). Toxicity was much less severe in the absence of S-9 mix, however, and silver grains could be counted following exposure even to 3,650 µg/ml (Table UDS-1). The substances used (4-nitroquinoline-N-oxide and 2-aminoanthracene) in concurrent positive control groups evoked significant levels of unscheduled DNA synthesis from the cells. These positive control substances, however, are not appropriate for the demonstration of short patch repair when measured by Method 2.

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

CYTOGENETIC ANALYSIS OF RAT BONE MARROW CELLS

Data are presented in Tables CA-MD-M-1 to CA-F48-2 and Appendix Tables CA-MD-M to CA-F48. Data were analysed for statistical significance by the one-sided Student's *t* test of the Freeman-Tukey transformed data.

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 butylene oxide atmospheres.

On the other hand, treatment with 100 mg EMS/kg/day for 5 days induced large increases in the frequencies of cells with chromatid damage. In both male and female rats this damage included gaps as well as chromatid breaks with fragments.

In the single exposure test rats treated with ethyl methanesulphonate, there were significant increases in aberrant cell frequencies in males and females at the 6 h and 24 h sampling times and in the females at the 48 h sampling time ($P<0.05$ or less). There was no significant response in the 48 h sampling time males. These responses were significant if all aberrant cells were analysed or if cells containing only gaps were discarded. The damage was mainly to chromatids and there was a tendency for damage other than gaps to appear in the 24 h samples.

Butylene oxide exposure groups did not show any increase in the frequency of aberrant cells except in the 6 h sample time males exposed to 1,000 ppm butylene oxide ($P<0.05$). If cells only containing gaps were excluded from the analysis, then the statistical significance of the difference from the air control group was reduced.

This result with butylene oxide is probably insufficient evidence on which to base a conclusion for the clastogenic potential of this compound *in vivo*: the effect was small and was not observed in female rats.

DOMINANT LETHAL TEST

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

Pregnancy frequency was calculated in 2 ways: firstly, by considering as pregnant females with corpora lutea graviditatis (Tables DL-1) and secondly and more reliably, by considering as pregnant only females with implantations (Table DL-2). With neither method was there any effect upon pregnancy frequency due to butylene oxide treatment, but there were reductions in Weeks 2 and 3 in the positive control group.

Corpora lutea graviditatis counts (Table DL-3) were not reduced in either of the butylene oxide treated groups; these counts were greatly reduced, however, in Weeks 1-3 of the positive control group.

Implantations per pregnancy (Table DL-4) were unaffected by butylene oxide treatment except in Week 4, 1,000 ppm exposure group where there was a small, but significant reduction ($P<0.01$). There were substantial reductions in Weeks 1-4 of the positive control group. The apparent lack of statistical significance in Week 2 is a result of the low number of degrees of freedom, there being only 2 pregnancies in this week-group.

The frequencies of live implantations (Table DL-5) and live implantations + late deaths (Table DL-6) followed very closely the pattern of total implantations per pregnancy. However, there were no significant reductions at all in these parameters in butylene oxide treated groups compared with the air control group.

Analysis of the proportions of early deaths by various statistical methods (Tables DL-7 to 9) did not indicate any effects attributable to butylene oxide treatment. There were marked increases in early deaths, compared with the air control group, in Weeks 1-4 of the positive control group.

It appears that under these conditions of treatment, butylene oxide had no identifiable effect on male rats in this dominant lethal assay.

SPERM ABNORMALITY TEST

There were no increases in the frequencies of abnormal sperm in any of the categories examined (Table SA-1 and 2 and Appendix Table SA) following treatment with 250 ppm or 1,000 ppm butylene oxide for 7 h/day on 5 consecutive days. The positive control group showed significant increases in abnormalities overall ($P<0.01$), amorphous head ($P<0.01$) and folded tail ($P<0.05$).

SEX-LINKED RECESSIVE LETHAL TEST IN DROSOPHILA

There was no information on the toxicity of butylene oxide to flies, so, a preliminary study was made (Table RL-1). The study was made on 24 October 1979 using a dynamic atmosphere generated at the same time as the rodents were exposed for the other mutagenicity tests in this programme. Flies exposed to 250 ppm butylene oxide atmospheres for up to 7 h showed no signs of toxicity. In the 1,000 ppm atmosphere, there was some loss of activity after 4 h and after 7 h some flies appeared to be dead.

By the following day, when survival was assessed, 78 (78%) of the flies originally exposed were still alive. Nine (9%) of the mortalities were suspected to be related to accidents in transfer between feeding vials and exposure chamber. Hatchability of eggs from females mated with exposed males was not adversely affected.

On the basis of these results, exposure conditions chosen for the main study on 29 October 1979 were:

1,000 ppm butylene oxide for 7 h.

Two breeding stocks (A and B) were exposed (Table RL-2) in the main test. After 5.5 h the flies appeared to have lost mobility and at 7 h about 30% of the exposed flies appeared to be dead. There was no evidence of induced sterility among the survivors.

A total of 3,524 F_2 generation vials were set up for analysis from butylene oxide exposed flies and 2,967 from air only exposed flies. In the air control group there were no F_2 lethals in Stock A and a single lethal (0.07%) in Stock B. Stock A butylene oxide exposed flies produced 2 lethals (0.12%), one in Brood 1 and one in Brood 2, while Stock B butylene oxide exposed flies produced a single lethal (0.07%).

One thousand, three hundred and forty eight F_3 generation vials were set up using Stock B flies exposed to butylene oxide and 600 F_3 generation vials were set up using air only exposed Stock B flies. In neither group were there any lethals.

From these results it would appear that butylene oxide did not induce any lethals in these flies.

Stock B flies were also exposed to ethyl methanesulphonate as a positive control. The exposure was for 5 h to 0.46% v/v ethyl methanesulphonate in sucrose. The frequency of lethals in the F_2 generation was 7.7%.

CONCLUSIONS

As toxic signs were seen in mammals and flies there is ample evidence that the butylene oxide was reaching the test animal. Had higher dose levels been used then the effect would probably have been an even higher mortality than was observed. Toxic concentrations also were achieved in the UDS assay.

In neither the UDS nor the *in vivo* assays, however, was there any evidence that butylene oxide is capable of inducing genetic damage.

REFERENCES

(1) Altham, P.M.E., Appl. Statistics, 27, (1978), 162.

(2) Cline, J.C., Thompson, C.Z. and McMahon, R.E., 9th Annual Meeting of Environmental Mutagen Society, San Francisco, March 9-13, (1978), 68.

(3) Van Duuren, B.L., Langseth, L., Goldschmidt, B.M. and Orris, L., J. Natl. Cancer Inst., 39, (1967), 1217.

(4) Kleinman, J.C., J. Amer. Statist. Assoc., 68, (1973), 46.

(5) Kupper, L.L. and Haseman, J.K., Biometrics, 34, (1978), 69.

(6) McCann, J., Chai, E., Yamasaki, E. and Ames, B.N., Proc. Natl. Acad. Sci., 12, (1975), 5135.

(7) Rinehart, R.R., Mutation Res., 7, (1969), 417.

(8) Rogers, A.W. in "Techniques of Autoradiography", Elsevier, Amsterdam, (1973).

(9) Spencer, W.P. and Stern, C., Genetics, 33, (1948), 43.

(10) Wedderburn, R.W.M., Biometrika, 61, (1974), 439.

(11) Williams, D.A., Biometrics, 31, (1975), 949.

(12) Würgler, F.E., Grat, U. and Berchtold, W., Arch. Genet., 48, (1975), 158.

(13) Würgler, F.E., Sobels, F.H. and Vogel, E. in Kilbey, B.J., Legator, M., Nichols, W. and Ramel, C., "Handbook of Mutagenicity Test Procedures", Elsevier, Amsterdam, (1977).

(14) Wyrobeck, A.J. and Bruce, W.R., Proc. Natl. Acad. Sci., U.S.A., 72, (1975), 4425.

TABLE AT-1

Butylene Oxide

Calibration Data for Low Level

Dose Level: 250 ppm v/v

Batch No.: 17047

Volume µl	Conc., ppm, (v/v)	Cumulative Chart Deflection, mm					
		24 October 1979	29 October 1979	30 October 1979	31 October 1979	1 November 1979	2 November 1979
0	0	0	0	0	0	0	0
2.0	100.4	29.0	29.0	29.0	29.0	28.0	29.0
4.0	200.8	57.0	58.0	57.0	56.0	57.0	57.0
6.0	301.2	85.0	85.0	84.0	84.0	84.0	84.0
8.0	401.6	110.0	111.0	110.0	110.0	109.0	109.0
10.0	502.0	136.0	136.0	136.0	135.0	134.5	134.5
Chart deflection (mm) for 250 ppm		72.0	72.0	72.0	72.0	72.0	72.0

Instrument Setting

Wavelength: 11.0 µm
Absorbence Range: 1.0 A
Slit Width: 0.5 mm
Meter Response: 1
Recorder Voltage: 1.0 V
Chart Speed: 120 mm/h

Calibration

Syringe: 10 µl Hamilton
Injection Volume: 2.0 µl
No. of Repeat Injections: 5

TABLE AT-2

Butylene Oxide
Calibration Data for High Level

Dose Level: 1,000 ppm v/v

Batch No.: 17047

Volume µl	Conc., ppm, (v/v)	Cumulative Chart Deflection, mm					
		24 October 1979	29 October 1979	30 October 1979	31 October 1979	1 November 1979	2 November 1979
0	0	0	0	0	0	0	0
5.0	252.5	33.0	33.0	33.0	33.0	32.5	32.5
10.0	505.0	64.0	65.0	64.0	65.0	64.5	63.5
15.0	757.5	94.0	94.0	95.0	94.0	94.0	93.5
20.0	1010.0	121.0	123.0	124.0	122.0	123.0	122.0
25.0	1262.5	148.0	149.0	150.0	148.0	149.0	148.5
Chart deflection (mm) for 1,000 ppm		120.0	122.0	122.0	122.0	122.0	122.0

Instrument Setting

Path Length: 6.75 m
Wavelength: 11.0 µm
Absorbence Range: 1.0 A
Slit Width: 0.5 mm
Meter Response: 1
Recorder Voltage: 1.0 V
Chart Speed: 120 mm/h

Calibration

Syringe: 10 µl Hamilton
Injection Volume: 5.0 µl
No. of Repeat Injections: 5

TABLE AT-3

Butylene Oxide

Chamber Atmosphere Homogeneity Data

Dose Level: 250 ppm and 1,000 ppm

Concentration / Sample Location	% Deviation from Reference Sampling Point	
	Low	High
Reference Point (R)	0	0
Right Centre (RC)	0	-1.0
Right Front (RF)	+1.0	-2.0
Centre Front (CF)	*NAD	-1.0
Left Front (LF)	+1.0	0
Left Centre (LC)	0	+2.0
Left Back (LB)	-2.0	+2.0
Centre Back (CB)	+1.0	+2.0
Right Back (RB)	0	+1.0

* No data available.

Top view of exposure chamber

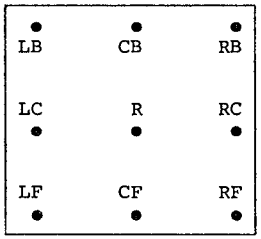


TABLE AT-4

Butylene Oxide
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)
	-10	-5	-2.5	-2	-1	0	+1	+2	+2.5	+5	+7.5	
Single	-	10	-	140	-	215	-	40	-	15	-	246.6
Multiple 1	10	25	-	220	-	120	-	15	-	30	-	247.1
Multiple 2	5	-	20	-	-	335	-	-	50	5	10	248.4
Multiple 3	10	30	85	-	-	235	-	-	35	25	-	248.6
Multiple 4	5	15	105	-	55	200	-	-	40	-	-	248.0
Multiple 5	-	10	5	-	35	220	15	-	35	-	-	249.5

TABLE AT-4 (continued)

Butylene Oxide
Target Concentration 1,000 ppm

Exposure Day	% Deviation from Target Concentration in Minutes												Time Averaged Concentration for 7 h (ppm)
	-12	-8	-5	-4	-3	-2	-1	0	+1	+2	+3	+4	
Single	-	-	5	-	-	-	40	300	50	-	-	20	1001.6
Multiple 1®	5	5	10	30	-	60	-	70	-	215	-	25	1003.3
Multiple 2	-	-	35	-	-	85	-	160	-	100	45	-	999.8
Multiple 3	-	5	-	10	-	15	120	190	80	-	-	-	996.4
Multiple 4	-	5	5	15	15	60	35	225	5	55	-	-	995.0
Multiple 5	-	5	-	-	-	5	5	60	85	150	10	60	1015.6

®Drosophila exposed for 7 h.

TABLE BW-1

Butylene Oxide
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	5 x 100 mg/kg EMS
Male	1	405.3 ± 33.5	397.1 ± 39.9	384.2 ± 27.3	387.8 ± 25.4
	2	394.8 ± 34.1	389.2 ± 39.7	361.8 ± 26.7	367.5 ± 24.5
	3	391.2 ± 32.4	383.2 ± 42.0	350.9 ± 26.6	343.6 ± 24.0
	4	391.4 ± 35.6	380.2 ± 44.7	347.6 ± 26.3	326.5 ± 25.6
	5	391.5 ± 37.5	382.8 ± 43.2	347.3 ± 25.4	312.2 ± 25.6
	Weight gain /loss	-13.8	-14.3	-36.9	-75.6
Female	1	228.9 ± 18.7	228.5 ± 17.0	235.0 ± 16.2	235.5 ± 24.1
	2	226.3 ± 16.8	225.0 ± 16.7	224.5 ± 15.8	221.9 ± 22.3
	3	227.3 ± 18.1	224.1 ± 17.1	218.7 ± 12.8	208.5 ± 19.4
	4	228.4 ± 19.5	224.7 ± 17.0	217.2 ± 10.9	200.5 ± 17.8
	5	230.3 ± 20.6	225.7 ± 15.8	218.6 ± 12.3	193.0 ± 17.3
	Weight gain /loss	1.4	-2.8	-16.4	-42.5

TABLE BW-2

Butylene Oxide
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	386.7 ± 20.3	405.6 ± 26.1	374.4 ± 27.0	375.2 ± 29.4
	24	384.6 ± 26.2	385.2 ± 19.3	382.3 ± 22.9	387.5 ± 24.1
	48	381.6 ± 23.9	370.3 ± 20.1	361.6 ± 23.7	385.5 ± 21.8
Female	6	243.0 ± 20.3	231.8 ± 22.0	217.1 ± 16.3	233.1 ± 16.9
	24	239.7 ± 14.2	226.1 ± 7.4	229.4 ± 20.2	226.9 ± 18.4
	48	234.4 ± 15.2	236.3 ± 24.2	235.8 ± 15.3	231.8 ± 13.3

TABLE BW-3

Butylene Oxide
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	400.6 ± 19.2	386.6 ± 14.3	384.7 ± 30.4	387.7 ± 28.0
2	397.0 ± 20.1	381.0 ± 16.4	367.8 ± 27.2	368.2 ± 23.2
3	392.7 ± 24.6	372.7 ± 18.2	359.0 ± 23.5	346.4 ± 21.9
4	393.5 ± 25.1	371.1 ± 14.2	354.9 ± 22.8	330.0 ± 20.7
5	396.9 ± 26.0	376.0 ± 14.8	354.7 ± 21.8	315.3 ± 19.9
Weight gain/loss	-3.7	-10.6	-30.0	-72.4

TABLE BW-4

Butylene Oxide
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 200 mg/kg EMS
1	24.8 ± 1.3	24.0 ± 0.8	23.8 ± 0.8	24.2 ± 0.9
2	24.7 ± 1.6	23.9 ± 0.7	22.7 ± 0.9	24.5 ± 0.8
3	25.1 ± 1.4	24.0 ± 0.7	(a) 23.2 ± 1.2	24.6 ± 0.8
4	24.8 ± 1.5	24.7 ± 0.8	22.9 ± 1.2	23.6 ± 0.5
5	25.2 ± 1.1	24.7 ± 0.7	22.9 ± 1.1	23.6 ± 0.8
Weight gain/loss	0.4	0.7	-	-0.6

(a) Two mice died and were replaced by 2 fresh mice.

TABLE UDS-1

Butylene Oxide
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	12.1 ± 7.6	3.7 ± 3.1
4-Nitroquinoline-N-oxide	-	10	-	75.2 ± 19.6
2-Aminoanthracene	3	-	65.0 ± 27.8	-
Butylene Oxide	66	57	11.3 ± 6.0	2.2 ± 1.7
	131	114	11.0 ± 6.6	4.1 ± 2.6
	263	228	9.7 ± 5.8	10.8 ± 4.3
	525	456	8.7 ± 5.2	13.7 ± 6.0
	-	913	-	7.8 ± 4.2
	-	1,825	-	8.8 ± 5.4
	-	3,650	-	3.8 ± 3.2
	-	7,300	-	T

T = Toxic, cells dead

TABLE UDS-2

Butylene Oxide
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	-	2.4 ± 2.2	-
4-Nitroquinoline-N-oxide	-	-	-	-
2-Aminoanthracene	16	-	53.1 ± 17.7	-
Butylene Oxide	11	-	4.2 ± 2.4	-
	21	-	5.4 ± 3.5	-
	42	-	3.0 ± 2.3	-
	84	-	3.9 ± 2.6	-

TABLE CA-MD-M-1

Butylene Oxide
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	450	7	1	1	1	-	-	-
250 ppm, 7 h/day	454	10	1	-	1	-	-	-
1,000 ppm, 7 h/day	450	13	-	-	-	-	-	-
EMS, 100 mg/kg/day	438	46	30	1	1	-	-	9 Exchanges 3 Multi Aberrations

TABLE CA-MD-M-2

Butylene Oxide
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	0.328	0.057		0.185	0.042	
250 ppm	0.309	0.054	-0.24	0.193	0.039	0.14
1,000 ppm	0.361	0.057	0.41	0.141	0.042	-0.75
EMS, 100 mg/kg	0.794	0.054	5.95***	0.560	0.039	6.54***

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

TABLE CA-MD-F-1

Butylene Oxide
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	417	20	2	-	-	-	-	1 Exchange
250 ppm, 7 h/day	500	7	-	-	4	-	-	-
1,000 ppm, 7 h/day	483	20	1	-	-	-	-	-
EMS, 100 mg/kg/day	500	55	28	1	-	-	-	8 Exchanges 5 Fragments 1 Chromosomal Fragment

TABLE CA-MD-F-1 (Supplementary)

Butylene Oxide
Cytogenetic Analysis of Rat Bone Marrow Cells
Supplementary Observations
Females

Multiple Dosing — Sampling Time: 6 h

Group	Animal No.	Observations
EMS, 100 mg/kg/day	315♀	Telomeric stickiness between two chromosomes

TABLE CA-MD-F-2

Butylene Oxide
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	0.447	0.061		0.227	0.038	
250 ppm	0.322	0.061	-1.46	0.141	0.038	-1.60
1,000 ppm	0.425	0.061	-0.26	0.164	0.038	-1.17
EMS, 100 mg/kg	0.794	0.061	4.03***	0.503	0.038	5.12***

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

TABLE CA-M6-1

Butylene Oxide
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	10	-	-	1	-	-	2 Chromatid Fragments
250 ppm, 7 h/day	450	4	-	-	-	-	-	2 Chromosomal Fragments 2 Chromatid Fragments
1,000 ppm, 7 h/day	400	15	1	2	-	-	-	2 Chromatid Fragments
EMS, 250 mg/kg/day	359	31	6	-	1	-	-	2 Chromatid Fragments

TABLE CA-M6-1 (Supplementary)

Butylene Oxide
Cytogenetic Analysis of Rat Bone Marrow Cells
Supplementary Observations
Males

Single Dosing — Sampling Time: 6 h

Group	Animal Number	Observation
Air Control, 7 h/day	3♂	1 Chromosome split at centromere
250 ppm, 7 h/day	32♂	2 Chromosomes split at centromere

TABLE CA-M6-2

Butylene Oxide
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	0.330	0.049		0.180	0.034	
250 ppm	0.228	0.052	-1.44	0.174	0.035	-0.13
1,000 ppm	0.482	0.055	2.06*	0.241	0.038	1.21
EMS, 250 mg/kg	0.624	0.055	4.00***	0.339	0.038	3.14**

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

*P<0.05

**P<0.01

***P<0.001

TABLE CA-M24-1

Butylene Oxide
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Males

Single Dosing

Sampling Time: 24 h

Treatment Group	Animals Observed	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	10	400	15	1	-	1	-	-	8 Chromatid Fragments
250 ppm, 7 h/day	10	300	7	2	-	1	-	-	1 Chromatid Fragment
1,000 ppm, 7 h/day	10	450	13	1	-	1	-	-	1 Chromatid Fragment
EMS, 250 mg/kg/day	10	364	30	24	1	-	2	-	7 Multi Aberrations 10 Exchanges 3 Chromosomal Fragments 11 Chromatid Fragments 1 Fragmented Chromosomal

TABLE CA-M24-2

Butylene Oxide
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	0.472	0.073		0.304	0.054	
250 ppm	0.371	0.084	-0.91	0.240	0.062	-0.78
1,000 ppm	0.362	0.069	-1.10	0.185	0.051	-1.62
EMS, 250 mg/kg	0.761	0.069	2.88**	0.632	0.051	4.45***

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

**P<0.01

***P<0.001

TABLE CA-M48-1

Butylene Oxide
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	6	1	-	-	-	-	-
250 ppm, 7 h/day	400	4	-	-	-	-	-	-
1,000 ppm, 7 h/day	350	8	-	-	1	-	-	1 Exchange
EMS, 250 mg/kg/day	500	3	-	-	-	-	-	1 Multi Aberration

TABLE CA-M48-2

Butylene Oxide
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	0.259	0.047		0.160	0.018	
250 ppm	0.214	0.053	-0.64	0.140	0.021	-0.72
1,000 ppm	0.303	0.056	0.59	0.169	0.022	0.30
EMS, 250 mg/kg	0.220	0.047	-0.58	0.160	0.018	0.00

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

TABLE CA-F6-1

Butylene Oxide
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	350	9	1	-	-	-	-	1 Chromatid Fragment
250 ppm, 7 h/day	450	11	1	1	-	-	-	2 Chromatid Fragments
1,000 ppm, 7 h/day	363	7	1	1	-	-	-	-
EMS, 250 mg/kg/day	385	26	5	-	-	1	-	2 Chromatid Fragments

TABLE CA-F6-2

Butylene Oxide
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	0.376	0.065		0.197	0.041	
250 ppm	0.341	0.057	-0.40	0.206	0.036	0.15
1,000 ppm	0.327	0.060	-0.55	0.207	0.038	0.16
EMS, 250 mg/kg	0.636	0.060	2.94**	0.358	0.038	2.85**

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

TABLE CA-F24-1

Butylene Oxide
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Females

Single Dosing

Sampling Time: 24 h

Treatment Group	Animals Observed	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	10	400	12	4	-	-	-	-	3 Chromatid Fragments
250 ppm, 7 h/day	10	350	15	-	1	-	-	-	8 Chromatid Fragments
1,000 ppm, 7 h/day	10	441	10	-	-	-	-	-	1 Chromatid Fragment
EMS, 250 mg/kg/day	10	228	23	6	-	1	-	-	12 Chromatid Fragments 3 Multi Aberrations 1 Exchange

TABLE CA-F24-2

Butylene Oxide
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	0.406	0.077		0.289	0.053	
250 ppm	0.505	0.082	0.88	0.269	0.057	-0.25
1,000 ppm	0.325	0.073	-0.77	0.164	0.050	-1.71
EMS, 250 mg/kg	0.767	0.089	3.07**	0.494	0.061	2.54*

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

TABLE CA-F48-1

Butylene Oxide
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Females

Single Dosing — Sampling Time: 48 h

Group	Number of Spreads Observed	Observed Aberrations						Miscellaneous
		Chromatid			Chromosome			
		Gap	B w F	B w/o F	Gap	B w F	B w/o F	
Air Control, 7 h/day	500	7	-	2	-	-	-	1 Multi Aberration
250 ppm, 7 h/day	450	8	1	1	-	-	-	-
1,000 ppm, 7 h/day	500	8	1	7	1	-	-	1 Chromatid Fragment
EMS, 250 mg/kg/day	450	19	2	6	1	-	-	6 Chromatid Fragments 1 Chromosomal Fragment 1 Translocation

TABLE CA-F48-2

Butylene Oxide
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	0.289	0.060		0.191	0.048	
250 ppm	0.307	0.063	0.20	0.185	0.050	-0.09
1,000 ppm	0.314	0.060	0.29	0.225	0.048	0.51
EMS, 250 mg/kg	0.489	0.063	2.31*	0.337	0.050	2.11*

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

TABLE DL-1

Butylene Oxide
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	80%	100%	90%	90%
2	95%	95%	95%	75%
3	100%	95%	100%	30%
4	90%	100%	100%	90%
5	100%	100%	95%	100%
6	95%	100%	85%	95%
7	95%	100%	100%	100%
8	95%	100%	95%	95%
9	100%	100%	95%	90%
10	100%	100%	94%	100%

TABLE DL-2

Butylene Oxide
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	14/19	74%	17/20	85%	18/20	90%	14/20	70%
2	18/20	90%	19/20	95%	19/20	95%	2/20	10%
3	20/20	100%	18/20	90%	20/20	100%	3/20	15%
4	18/20	90%	19/19	100%	17/19	89%	17/20	85%
5	20/20	100%	19/20	95%	17/20	85%	19/20	95%
6	19/20	95%	19/20	95%	17/20	85%	18/19	95%
7	19/20	95%	19/20	95%	20/20	100%	20/20	100%
8	19/20	95%	20/20	100%	18/20	90%	19/20	95%
9	20/20	100%	20/20	100%	19/20	95%	17/20	85%
10	14/14	100%	18/18	100%	16/18	89%	14/14	100%

TABLE DL-3

Butylene Oxide
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] 13.2 ± 0.56	13.3 ± 0.51	14.1 ± 0.49	8.57 ± 0.80***
2	12.9 ± 0.57	13.2 ± 0.56	13.8 ± 0.56	5.00 ± 3.00
3	12.9 ± 0.51	13.9 ± 0.54	14.3 ± 0.51	1.33 ± 0.88**
4	13.9 ± 0.66	14.9 ± 0.65	12.6 ± 0.68	12.1 ± 0.70
5	13.6 ± 0.54	13.4 ± 0.55	11.8 ± 0.58*	11.6 ± 0.85
6	14.0 ± 0.53	13.1 ± 0.53	14.0 ± 0.56	13.7 ± 0.59
7	15.7 ± 0.51	14.6 ± 0.51	14.9 ± 0.49	13.9 ± 0.43*
8	13.8 ± 0.46	14.0 ± 0.45	13.6 ± 0.47	12.8 ± 0.35
9	14.3 ± 0.48	14.7 ± 0.48	14.7 ± 0.49	13.7 ± 0.64
10	13.4 ± 0.63	13.4 ± 0.56	13.7 ± 0.59	12.0 ± 1.05

1 = Mean ± standard error of mean

*P<0.05
**P<0.01
***P<0.001

TABLE DL-4

Butylene Oxide
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] 12.9 ± 0.49	13.0 ± 0.45	13.3 ± 0.43	5.9 ± 0.80***
2	12.6 ± 0.76	13.1 ± 0.74	13.4 ± 0.74	2.5 ± 0.50
3	13.3 ± 0.46	12.9 ± 0.49	13.9 ± 0.46	1.0 ± 0.00**
4	14.0 ± 0.48	13.4 ± 0.47	12.0 ± 0.50**	7.6 ± 1.05***
5	12.4 ± 0.56	12.5 ± 0.57	12.1 ± 0.61	9.7 ± 1.00*
6	13.8 ± 0.47	13.4 ± 0.47	13.9 ± 0.50	13.9 ± 0.60
7	14.5 ± 0.39	14.5 ± 0.39	15.3 ± 0.38	13.8 ± 0.63
8	12.8 ± 0.56	12.8 ± 0.54	13.4 ± 0.57	12.5 ± 0.39
9	13.1 ± 0.58	14.4 ± 0.58	14.4 ± 0.59	13.4 ± 0.66
10	12.4 ± 0.85	12.6 ± 0.75	12.2 ± 0.80	11.9 ± 1.06

1 = Mean ± standard error of mean

*P<0.05
**P<0.01
***P<0.001

TABLE DL-5

Butylene Oxide
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] 12.8 ± 0.51	12.7 ± 0.46	12.6 ± 0.45	0.7 ± 0.50***
2	11.7 ± 0.90	12.0 ± 0.88	11.7 ± 0.88	0.0 ± 0.00*
3	12.4 ± 0.53	12.5 ± 0.56	12.9 ± 0.53	0.0 ± 0.00**
4	12.6 ± 0.60	12.6 ± 0.58	11.2 ± 0.62	3.9 ± 1.00***
5	11.9 ± 0.64	11.8 ± 0.66	11.1 ± 0.69	8.8 ± 0.92*
6	13.2 ± 0.47	12.6 ± 0.47	13.4 ± 0.50	13.4 ± 0.51
7	14.1 ± 0.44	13.9 ± 0.44	14.7 ± 0.43	13.4 ± 0.66
8	11.9 ± 0.54	12.5 ± 0.53	12.7 ± 0.56	11.9 ± 0.38
9	11.8 ± 0.60	13.4 ± 0.60	13.4 ± 0.62	12.6 ± 0.72
10	12.0 ± 0.84	12.1 ± 0.74	11.4 ± 0.78	10.8 ± 1.07

1 = Mean ± standard error of mean

*P<0.05
**P<0.01
***P<0.001

TABLE DL-6

Butylene Oxide
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] 12.8 ± 0.50	12.7 ± 0.45	13.0 ± 0.44	0.7 ± 0.50***
2	11.8 ± 0.93	12.1 ± 0.90	11.9 ± 0.90	0.0 ± 0.00*
3	12.5 ± 0.51	12.6 ± 0.54	13.0 ± 0.51	0.0 ± 0.00**
4	12.8 ± 0.56	12.7 ± 0.54	11.5 ± 0.57	4.3 ± 0.98***
5	11.9 ± 0.63	12.1 ± 0.64	11.2 ± 0.68	8.9 ± 0.94*
6	13.3 ± 0.47	12.6 ± 0.47	13.7 ± 0.50	13.4 ± 0.52
7	14.2 ± 0.44	14.0 ± 0.44	14.8 ± 0.43	13.5 ± 0.66
8	12.0 ± 0.52	12.5 ± 0.51	12.9 ± 0.54	12.0 ± 0.38
9	12.0 ± 0.61	13.5 ± 0.61	13.5 ± 0.63	12.7 ± 0.70
10	12.1 ± 0.84	12.1 ± 0.74	11.6 ± 0.78	10.8 ± 1.07

1 = Mean ± standard error of mean

*P<0.05
**P<0.01
***P<0.001

TABLE DL-7

Butylene Oxide
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	2/14	0/14	6/17	0/17	5/18	0/18	13/14	12/14
2	9/18	2/18	12/19	5/19	9/19	6/19	2/2	2/2
3	9/20	5/20	6/18	0/18	12/20	5/20	3/3	0/3
4	6/18	4/18	7/19	2/19	6/17	2/17	16/17	14/17
5	6/20	2/20	5/19	2/19	10/17	3/17	9/19	3/19
6	8/19	2/19	11/19	3/19	4/17	0/17	8/18	1/18
7	6/19	0/19	9/19	2/19	8/20	2/20	5/20	1/20
8	9/19	5/19	7/20	0/20	8/18	2/18	6/19	3/19
9	12/20	3/20	13/20	5/20	11/19	5/19	9/17	3/17
10	4/14	1/14	6/18	2/18	9/16	1/16	8/14	5/14

TABLE DL-8

Butylene Oxide
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] 1.202 ± 0.1691	1.499 ± 0.1535	1.393 ± 0.1491	4.479 ± 0.4280***
2	1.849 ± 0.2957	2.143 ± 0.2878	2.222 ± 0.2878	3.439 ± 0.2929
3	1.878 ± 0.2103	1.471 ± 0.2217	2.086 ± 0.2103	2.414 ± 0.0000
4	1.962 ± 0.2870	1.701 ± 0.2794	1.585 ± 0.2953	3.749 ± 0.2700***
5	1.527 ± 0.2038	1.480 ± 0.2091	2.025 ± 0.2211	1.874 ± 0.2421
6	1.673 ± 0.1799	1.934 ± 0.1799	1.333 ± 0.1902	1.669 ± 0.1856
7	1.447 ± 0.1880	1.747 ± 0.1800	1.639 ± 0.1755	1.390 ± 0.1586
8	1.951 ± 0.2067	1.495 ± 0.2014	1.710 ± 0.2123	1.620 ± 0.2319
9	2.133 ± 0.2300	2.102 ± 0.2300	2.042 ± 0.2360	1.878 ± 0.2162
10	1.456 ± 0.2134	1.553 ± 0.1882	1.841 ± 0.1996	2.153 ± 0.2977

1 = Mean ± standard error of mean

***P<0.001

TABLE DL-9

Butylene Oxide
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] 0.327 ± 0.0464	0.411 ± 0.0421	0.375 ± 0.0409	2.411 ± 0.1683***
2	0.606 ± 0.1361	0.653 ± 0.1325	0.661 ± 0.1325	2.555 ± 0.0472*
3	0.510 ± 0.0596	0.407 ± 0.0629	0.563 ± 0.0596	2.334 ± 0.0000**
4	0.540 ± 0.0858	0.456 ± 0.0835	0.455 ± 0.0883	1.600 ± 0.1417***
5	0.451 ± 0.0700	0.419 ± 0.0718	0.609 ± 0.0759	0.666 ± 0.1134
6	0.447 ± 0.0481	0.524 ± 0.0481	0.346 ± 0.0509	0.434 ± 0.0444
7	0.376 ± 0.0486	0.459 ± 0.0486	0.413 ± 0.0474	0.375 ± 0.0457
8	0.539 ± 0.0535	0.407 ± 0.0522	0.456 ± 0.0550	0.448 ± 0.0625
9	0.595 ± 0.0654	0.552 ± 0.0654	0.533 ± 0.0671	0.521 ± 0.0661
10	0.433 ± 0.0616	0.438 ± 0.0543	0.525 ± 0.0576	0.726 ± 0.1514

1 = Mean ± standard error of mean

*P<0.05
**P<0.01
***P<0.001

TABLE SA-1

Butylene Oxide
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	9690	10	18	157	38	87	310	0.10	0.18	1.57	0.38	0.87	3.10
250 ppm, 7 h/day	9725	10	13	144	38	70	275	0.10	0.13	1.44	0.38	0.70	2.75
1,000 ppm, 7 h/day	4587	1	8	60	23	31	123	0.02	0.17	1.27	0.49	0.66	2.61
EMS, 200 mg/kg/day	9488	17	16	266	82	131	512	0.17	0.16	2.66	0.82	1.31	5.12

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

TABLE SA-2

Butylene Oxide
Sperm Abnormality Test in Mice
Means of Freeman-Tukey Binomial Transformation
± Standard Error

Multiple Dosing

Dose Group	Abnormality Category					
	A	B	C	D	E	Total
Air Control, 7 h/day	6.72 ± 1.064	9.13 ± 1.083	15.18 ± 1.670	12.71 ± 1.499	18.59 ± 1.605	35.31 ± 2.231
250 ppm, 7 h/day	6.72 ± 1.064	7.21 ± 1.083	24.31 ± 1.670	12.08 ± 1.499	16.93 ± 1.605	33.20 ± 2.231
1,000 ppm, 7 h/day	4.43 ± 1.682	7.76 ± 1.713	20.81 ± 2.641	14.66 ± 2.369	17.10 ± 2.538	31.76 ± 3.528
EMS, 200 mg/kg/day	8.51 ± 1.064	8.45 ± 1.083	32.36** ± 1.670	17.82* ± 1.499	22.71 ± 1.605	44.94** ± 2.231

A = Hook up-turned or hook elongated

B = Banana-shaped head

C = Amorphous head

D = Folded tail

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

*$P<0.05$

**$P<0.01$

TABLE RL-1

Butylene Oxide

Drosophila Dose Ranging Experiment

Day		250 ppm			1,000 ppm			Control
		1 h	3 h	7 h	1 h	3 h	7 h	
0	No. of males exposed	100	100	100	100	100	100	
1	No. and % survival	100/100%	88/88%	94/94%	*39/39%	97/97	78/78	
2	No. of eggs laid by 55♀	120	112	114	115	95	95	118
3	No. and % hatched	102/85%	90/80%	112/98%	108/94%	71/75%	81/85%	115/97%

* Some flies lost from the exposure chamber.

TABLE RL-2

Butylene Oxide

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	30.10.79	2.11.79	7.11.79
F_2 set up	13.11.79	15.11.79	21.11.79
F_2 scored	26.11.79	3.12.79	6.12.79
F_2 repeats scored			
F_3 set up			
F_3 scored			
F_3 repeats scored			

RESULTS

	Brood 1	Brood 2	Brood 3	All Broods
No. of F_1 vials	107	107	106	320
No. of sterile F_1 vials	21	12	25	58
No. of F_1 vials used in F_2	83	95	81	259
No. of F_2 vials set up	603	600	594	1197
No. of F_2 vials scored	575	484	559	1618
No. of F_2 vials containing lethals	0	0	0	0
Frequency of F_2 lethals	0	0	0	0
No. of F_3 vials set up				
No. of F_3 vials scored				
No. of F_3 vials containing lethals				
Frequency of F_3 lethals				

TABLE RL-2 (continued)

Butylene Oxide

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	30.10.79	2.11.79	7.11.79
F_2 set up	13.11.79	15.11.79	20.11.79
F_2 scored	30.11.79	3.12.79	7.12.79
F_2 repeats scored	17.12.79		
F_3 set up			7.12.79
F_3 scored			18.12.79
F_3 repeats scored			

RESULTS

	Brood 1	Brood 2	Brood 3	All Broods
No. of F_1 vials	108	108	106	322
No. of sterile F_1 vials	28	8	18	54
No. of F_1 vials used in F_2	80	100	88	268
No. of F_2 vials set up	571	600	599	1770
No. of F_2 vials scored	480	461	488	1429
No. of F_2 vials containing lethals	1	0	0	1
Frequency of F_2 lethals	0.20%	0	0	0.069%
No. of F_3 vials set up			600	600
No. of F_3 vials scored			572	572
No. of F_3 vials containing lethals			0	0
Frequency of F_3 lethals			0	0

TABLE RL-2 (continued)

Butylene Oxide

Drosophila SLRL Procedure and Results

Compound: Butylene Oxide **Concentration:** 1,000 ppm **Stock:** A
Length of Exposure: 7 h **Test exposure given:** 22.10.79

	Brood 1	Brood 2	Brood 3
F_1 set up	30.10.79	2.11.79	7.11.79
F_2 set up	14.11.79	16.11.79	22.11.79
F_2 scored	29.11.79	3.12.79	5.12.79
F_2 repeats scored	17.12.79		
F_3 set up			
F_3 scored			
F_3 repeats scored			

RESULTS

	Brood 1	Brood 2	Brood 3	All Broods
No. of F_1 vials	86	86	84	256
No. of sterile F_1 vials	16	10	17	43
No. of F_1 vials used in F_2	70	76	67	213
No. of F_2 vials set up	622	601	602	1825
No. of F_2 vials scored	562	540	554	1656
No. of F_2 vials containing lethals	1	1	0	2
Frequency of F_2 lethals	0.17%	0.18%	0	0.12%
No. of F_3 vials set up				
No. of F_3 vials scored				
No. of F_3 vials containing lethals				
Frequency of F_3 lethals				

TABLE RL-2 (continued)

Butylene Oxide

Drosophila SLRL Procedure and Results

Compound: Butylene Oxide **Concentration:** 1,000 ppm **Stock:** B

Length of Exposure: 7 h **Test exposure given:** 29.10.79

	Brood 1	Brood 2	Brood 3
F_1 set up	30. 10.79	2.11.79	7.11.79
F_2 set up	14.11.79	16.11.79	21.11.79
F_2 scored	29.11.79	1.12.79	4.12.79
F_2 repeats scored	17.2.79		
F_3 set up	29.11.79	1.12.79	4.12.79
F_3 scored	10.12.79	14.12.79	17.12.79
F_3 repeats scored			

RESULTS

	Brood 1	Brood 2	Brood 3	All Broods
No. of F_1 vials	75	74	71	220
No. of sterile F_1 vials	21	8	10	39
No. of F_1 vials used in F_2	54	66	61	181
No. of F_2 vials set up	500	590	609	1699
No. of F_2 vials scored	473	492	546	1511
No. of F_2 vials containing lethals	1	0	0	1
Frequency of F_2 lethals	0.21%	0	0	0.066%
No. of F_3 vials set up	452	496	400	1348
No. of F_3 vials scored	406	465	381	1252
No. of F_3 vials containing lethals	0	0	0	0
Frequency of F_3 lethals	0	0	0	0

TABLE RL-2 (continued)

Butylene Oxide

Drosophila SLRL Procedure and Results

Compound: Ethyl methanesulphonate Concentration: 0.46%v/v Stock: B

Length of Exposure: 5 h Test exposure given: 29.10.79

	Brood 1	Brood 2	Brood 3
F_1 set up	30.10.79		
F_2 set up	18.11.79		
F_2 scored	5.12.79		
F_2 repeats scored	17.12.79		
F_3 set up			
F_3 scored			
F_3 repeats scored			

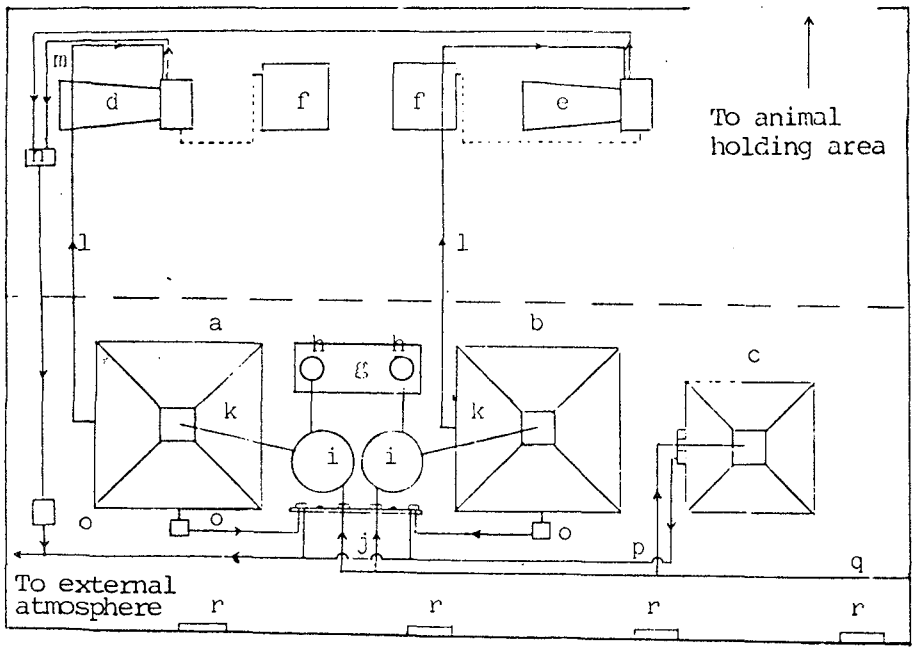
RESULTS

	Brood 1	Brood 2	Brood 3	All Broods
No. of F_1 vials	50			50
No. of sterile F_1 vials	18			18
No. of F_1 vials used in F_2	31			31
No. of F_2 vials set up	301			301
No. of F_2 vials scored	270			270
No. of F_2 vials containing lethals	21			21
Frequency of F_2 lethals	7.7%			7.7%
No. of F_3 vials set up				
No. of F_3 vials scored				
No. of F_3 vials containing lethals				
Frequency of F_3 lethals				

FIGURE 1a

Butylene Oxide

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

Butylene Oxide

Schematic Lay-out of Apparatus

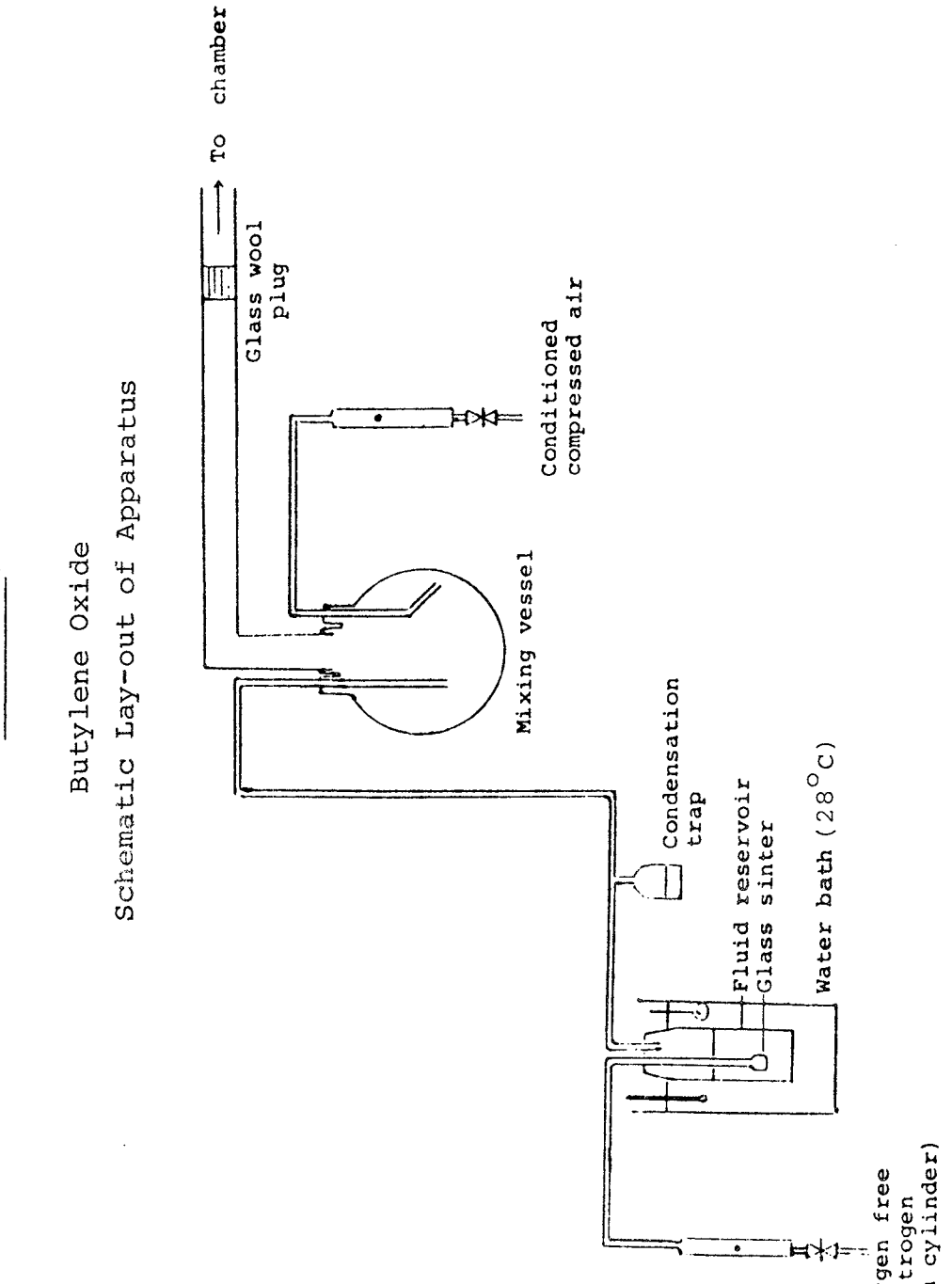


FIGURE 2

Butylene Oxide
Typical Calibration Graph for High Level
2 November 1979

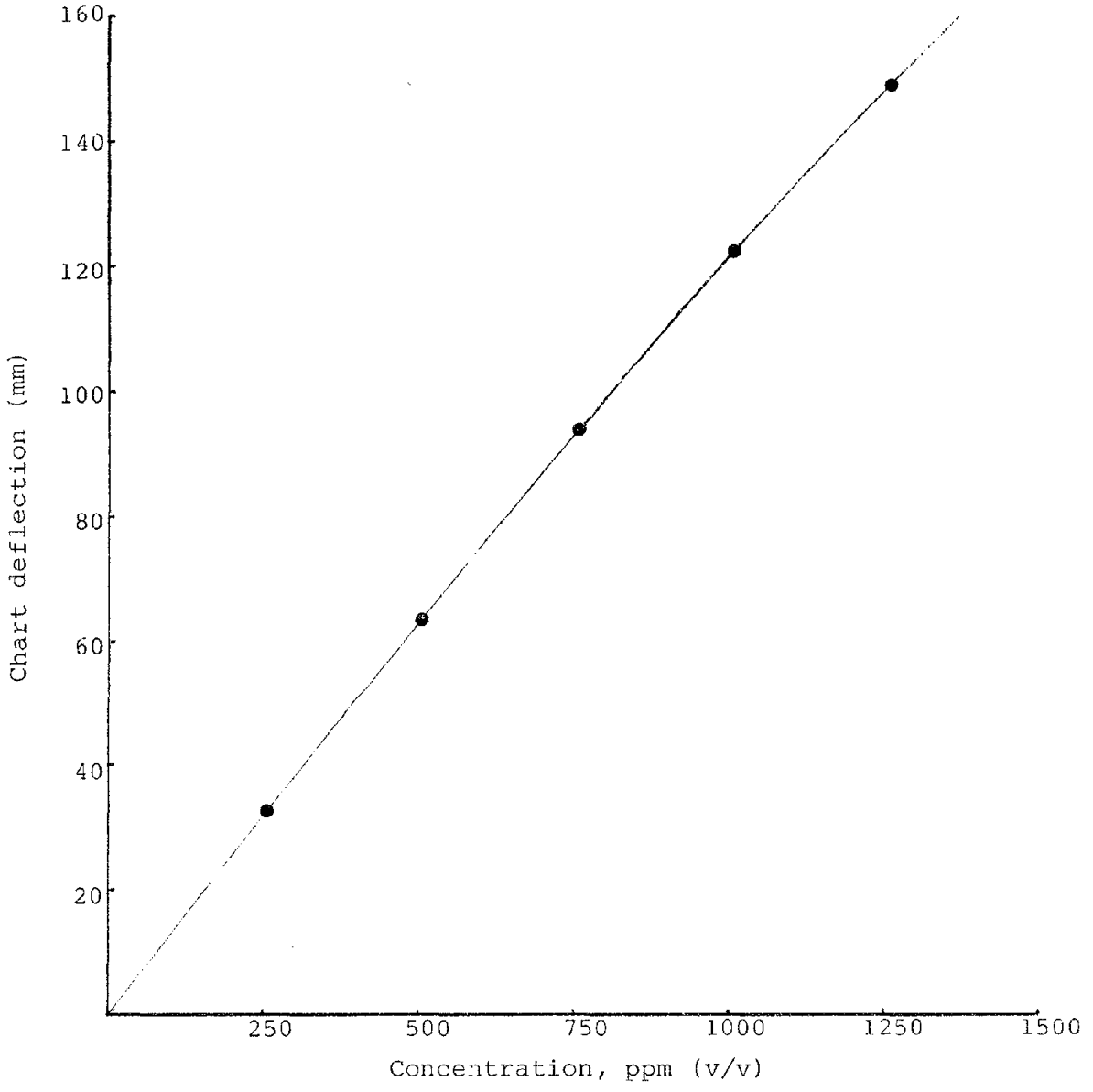
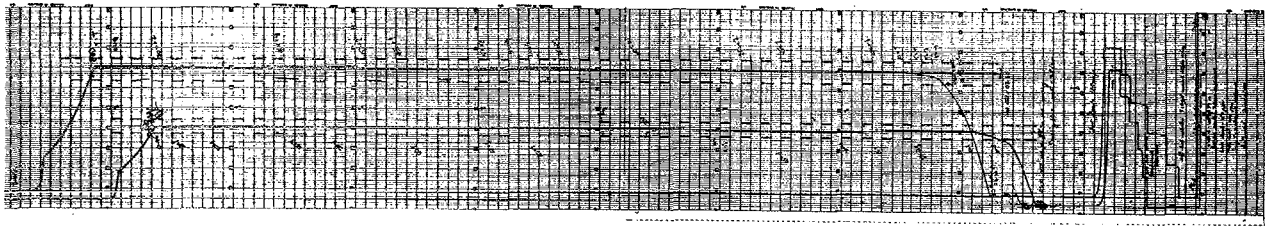


FIGURE 3

Butylene Oxide
Sample Record Chart for IR Absorption at 11.0 μm



APPENDIX DIET

Butylene Oxide
Diet Analysis

Spratt's Patent Ltd

Central House
Cambridge Road
Barking
Essex IG11 8NL

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

CERTIFICATE OF ANALYSIS

PRODUCT: LAD 1

BATCH NO: 247956

DATE OF MANUFACTURE: 24TH OCTOBER 1979

	FOUND ANALYSIS
MOISTURE.	9.3 %
CRUDE FAT	3.9 %
CRUDE PROTEIN	20.8 %
ASH	5.5 %
CALCIUM	0.96 %
PHOSPHORUS	0.85 %
NITRATE	< 1.0 mg/kg
NITRITE	1.0 mg/kg
SELENIUM	0.28 mg/kg
LEAD	1.5 mg/kg
ARSENIC	< 0.20 mg/kg
CADMIUM	0.23 mg/kg
MERCURY	0.047 mg/kg
AFLATOXINS	NONE DETECTED
TOTAL P.C.B	NONE DETECTED
TOTAL D.D.T.	0.051 mg/kg
DIELDRIN	NONE DETECTED
LINDANE	0.008 mg/kg
HEPTACHLOR	0.009 mg/kg
MALATHION	0.16 mg/kg
TOTAL VIABLE ORGANISMS	4.0×10^3/GRM
E. COLI TYPE 1	NONE DETECTED
SALMONELLA SPECIES	NONE DETECTED
MOULDS.	200/GRM

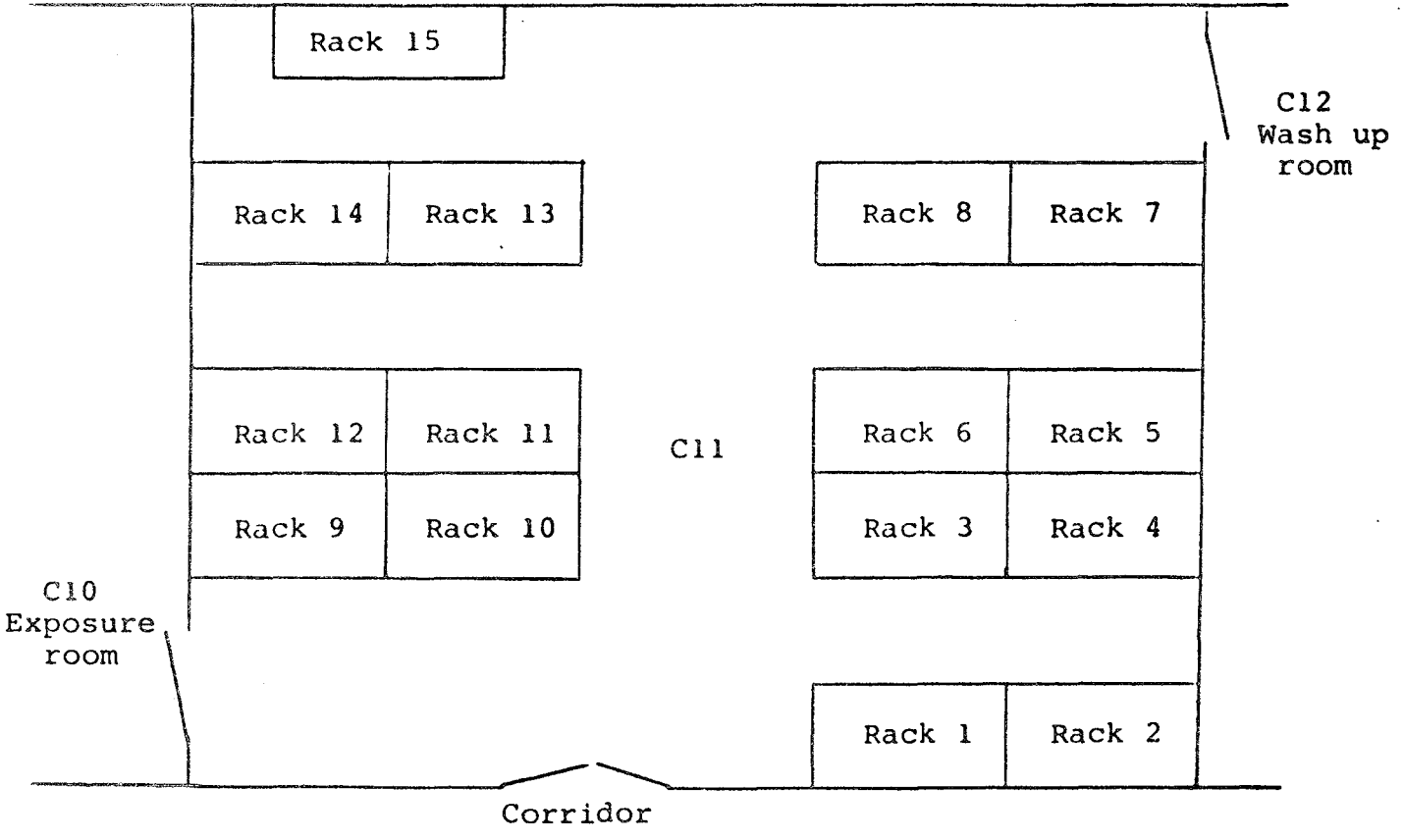
SIGNED .....

DATE .....

APPENDIX Loc-1

Butylene Oxide

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

Butylene Oxide

Examples of Animal Location During Exposure
Exposure Location Sheet

Project No: 409959 Test Concentration: 0

Test Compound: Air Control Tier No: 1

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

Day of Study: 2

LEFT

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

FRONT REAR

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

RIGHT

SIGNED: _____ DATE: _____

APPENDIX Loc-2 (continued)

Butylene Oxide

Exposure Location Sheet

Project No: 409952

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)

Butylene Oxide

Exposure Location Sheet

Project No: 409959

Test Compound: Butylene Oxide

Exposure Chamber No: 2

Day of Study: 2

Test Concentration: Low

Tier No: 1

LEFT

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

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

FRONT

REAR

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

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

RIGHT

Signed: _____ Date: _____

APPENDIX Loc-2 (continued)

Butylene Oxide

Exposure Location Sheet

Project No: 409959

Test Compound: Butylene Oxide

Exposure Chamber No: 3

Day of Study: 2

Test Concentration: High

Tier No: 1

LEFT

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

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

FRONT REAR

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

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

RIGHT

Signed: _____ Date: _____

APPENDIX FORM-1

Butylene Oxide

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.	Obser-vation
	GAP	BwF	Bw/oF	GAP	BwF	Bw/oF	EXC.	DIC	RG			
1.												
2.												
3.												
4.												
5.												
6.												
7.												
8.												
9.												
10.												
11.												
12.												
13.												
14.												
15.												
16.												
17.												
18.												
19.												
20.												
21.												
22.												
23.												
24.												
25.												

APPENDIX FORM-2

Butylene Oxide

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

Butylene Oxide

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

Butylene Oxide
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	473	453	442	444	457
	122	384	381	380	385	385
	123	370	359	357	363	372
	124	384	366	373	380	380
	125	427	413	394	382	379
	126	420	435	434	441	433
	127	440	424	425	430	428
	128	395	388	391	391	394
	129	374	357	352	346	344
	130	386	372	364	352	343
	Mean ± S.D.	405.3 ± 33.5	394.8 ± 34.1	391.2 ± 32.4	391.4 ± 35.6	391.5 ± 37.5

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	281	207	204	207	208	213
	282	217	219	217	221	225
	283	246	238	230	236	239
	284	220	221	224	224	224
	285	225	216	211	205	201
	286	259	251	254	260	264
	287	239	238	245	243	242
	288	227	228	232	231	231
	289	248	248	250	252	257
	290	201	202	203	204	207
	Mean ± S.D.	228.9 ± 18.7	226.3 ± 16.8	227.3 ± 18.1	228.4 ± 19.5	230.3 ± 20.6

APPENDIX TABLE BW-1 (continued)

Butylene Oxide

Multiple Dosing: 250 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	131	424	424	433	420	420
	132	353	350	348	343	361
	133	386	370	355	346	348
	134	405	390	380	379	383
	135	349	345	336	334	340
	136	372	365	365	365	373
	137	468	447	431	440	438
	138	380	369	369	373	371
	139	382	375	357	342	333
	140	452	457	458	460	461
	Mean ± S.D.	397.1 ± 39.9	389.2 ± 39.7	383.2 ± 42.0	380.2 ± 44.7	382.8 ± 43.2

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	291	212	211	213	215	212
	292	227	228	223	224	225
	293	230	224	215	215	219
	294	265	264	266	264	263
	295	241	238	237	240	237
	296	219	217	219	215	218
	297	211	210	209	208	210
	298	243	230	229	231	232
	299	214	209	209	210	214
	300	223	219	221	225	227
	Mean ± S.D.	228.5 ± 17.0	225.0 ± 16.7	224.1 ± 17.1	224.7 ± 17.0	225.7 ± 15.8

APPENDIX TABLE BW-1 (continued)

Butylene Oxide

Multiple Dosing: 1,000 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	141	390	367	357	358	359
	142	338	320	302	300	305
	143	361	335	333	337	344
	144	387	362	342	330	326
	145	355	336	327	319	315
	146	388	368	358	358	363
	147	385	361	350	348	342
	148	394	368	367	364	365
	149	431	410	394	384	373
	150	413	391	379	378	381
	Mean ± S.D.	384.2 ± 27.3	361.8 ± 26.7	350.9 ± 26.6	347.6 ± 26.3	347.3 ± 25.4

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	301	205	196	195	198	198
	302	237	224	217	215	219
	303	227	214	211	210	208
	304	239	225	221	218	218
	305	220	210	210	213	215
	306	236	228	218	215	212
	307	246	244	235	236	242
	308	227	218	212	211	216
	309	261	244	235	227	230
	310	252	242	233	229	228
	Mean ± S.D.	235.0 ± 16.2	224.5 ± 15.8	218.7 ± 12.8	217.2 ± 10.9	218.6 ± 12.3

APPENDIX TABLE BW-1 (continued)

Butylene Oxide

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	151	380	361	338	320	307
	152	396	379	355	333	320
	153	417	385	358	341	322
	154	429	414	391	382	370
	155	385	367	347	331	318
	156	375	356	329	307	290
	157	386	368	345	327	311
	158	400	375	346	328	312
	159	337	320	297	282	271
	160	373	350	330	314	301
	Mean	387.8	367.5	343.6	326.5	312.2
	± S.D.	± 25.4	± 24.5	± 24.0	± 25.6	± 25.6

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	311	219	209	196	187	178
	312	211	195	182	177	169
	313	229	215	201	194	188
	314	227	210	200	193	186
	315	228	217	205	199	191
	316	263	240	228	220	214
	317	282	264	241	230	219
	318	204	197	189	183	178
	319	240	225	211	200	192
	320	252	247	232	222	215
	Mean	235.5	221.9	208.5	200.5	193.0
	± S.D.	± 24.1	± 22.3	± 19.4	± 17.8	± 17.3

APPENDIX TABLE BW-2

Butylene Oxide
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	368	11	352	21	392
	2	370	12	358	22	369
	3	415	13	370	23	345
	4	400	14	407	24	390
	5	421	15	365	25	378
	6	377	16	366	26	352
	7	375	17	412	27	421
	8	383	18	396	28	284
	9	396	19	390	29	373
	10	362	20	430	30	412
	Mean ± S.D.	386.2 ± 20.3		384.6 ± 26.2		381.6 ± 23.9

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Female	161	227	171	264	181	234
	162	222	172	222	182	212
	163	233	173	251	183	211
	164	259	174	227	184	234
	165	220	175	235	185	228
	166	235	176	232	186	259
	167	248	177	228	187	232
	168	251	178	254	188	239
	169	248	179	252	189	248
	170	287	180	232	190	247
	Mean ± S.D.	243.0 ± 20.3		239.7 ± 14.2		234.4 ± 15.2

APPENDIX TABLE BW-2 (continued)

Butylene Oxide

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	440	41	370	51	376
	32	413	42	378	52	398
	33	406	43	410	53	388
	34	358	44	394	54	352
	35	432	45	357	55	391
	36	373	46	409	56	349
	37	396	47	374	57	368
	38	406	48	412	58	354
	39	433	49	380	59	342
	40	399	50	370	60	385
	Mean ± S.D.	405.6 ± 26.1		385.2 ± 19.3		370.3 ± 20.1

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Female	191	201	201	229	211	225
	192	265	202	214	212	241
	193	226	203	226	213	215
	194	231	204	221	214	254
	195	224	205	238	215	222
	196	232	206	223	216	226
	197	247	207	235	217	202
	198	267	208	220	218	231
	199	211	209	231	219	267
	200	214	210	224	220	280
	Mean ± S.D.	231.8 ± 22.0		226.1 ± 7.4		236.3 ± 24.2

APPENDIX TABLE BW-2 (continued)

Butylene Oxide

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	394	71	340	81	337
	62	344	72	396	82	352
	63	379	73	403	83	341
	64	400	74	379	84	374
	65	332	75	380	85	343
	66	395	76	399	86	371
	67	352	77	418	87	361
	68	409	78	360	88	355
	69	352	79	366	89	419
	70	387	80	382	90	363
	Mean ± S.D.	374.4 ± 27.0		382.3 ± 22.9		361.6 ± 23.7

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Female	221	216	231	227	241	258
	222	193	232	219	242	217
	223	215	233	225	243	235
	224	211	234	234	244	239
	225	204	235	209	245	223
	226	240	236	218	246	211
	227	221	237	207	247	241
	228	203	238	226	248	244
	229	246	239	264	249	255
	230	222	240	265	250	235
	Mean ± S.D.	217.1 ± 16.3		229.4 ± 20.2		235.8 ± 15.3

APPENDIX TABLE BW-2 (continued)

Butylene Oxide

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Male	91	410	101	371	111	395
	92	389	102	413	112	431
	93	355	103	359	113	383
	94	373	104	428	114	356
	95	375	105	420	115	381
	96	392	106	375	116	381
	97	339	107	367	117	372
	98	427	108	381	118	393
	99	346	109	375	119	360
	100	346	110	386†	120	403
	Mean ± S.D.	375.2 ± 29.4		387.5 ± 24.1		385.5 ± 21.8

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Female	251	216	261	202	271	235
	252	243	262	230	272	235
	253	205	263	215	273	209
	254	217	264	232	274	211
	255	253	265	200	275	238
	256	225	266	243	276	226
	257	252	267	250	277	248
	258	250	268	244	278	236
	259	238	269	226	279	248
	260	232	270	228	280	232
	Mean ± S.D.	233.1 ± 16.9		226.9 ± 18.4		231.8 ± 13.3

APPENDIX TABLE BW-3

Butylene Oxide
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	426	410	386	380	379
	362	377	365	351	351	359
	363	396	390	395	388	389
	364	413	397	386	383	379
	365	395	395	397	399	403
	366	406	413	417	429	431
	367	388	397	400	403	410
	368	435	435	431	431	437
	369	390	397	407	403	412
	370	380	371	357	368	370
	Mean	400.6	397.0	392.7	393.5	396.9
	± S.D.	± 19.2	± 20.1	± 24.6	± 25.1	± 26.0

APPENDIX TABLE BW-3 (continued)

Butylene Oxide

Multiple Dosing: 250 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	371	375	373	371	377	378
	372	390	375	362	359	364
	373	412	409	401	383	389
	374	386	394	391	386	391
	375	391	374	360	360	370
	376	407	397	383	379	387
	377	379	384	381	378	378
	378	385	382	378	379	386
	379	366	350	357	340	342
	380	375	372	363	370	375
	Mean ± S.D.	386.6 ± 14.3	381.0 ± 16.4	372.7 ± 18.2	371.1 ± 14.2	376.0 ± 14.8

APPENDIX TABLE BW-3 (continued)

Butylene Oxide

Multiple Dosing: 1,000 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	381	338	331	331	330	334
	382	364	347	342	337	335
	383	366	345	328	322	327
	384	395	375	374	379	379
	385	382	362	356	360	361
	386	425	410	399	393	393
	387	367	357	349	343	338
	388	418	398	381	369	360
	389	365	351	350	347	349
	390	427	402	380	369	371
	Mean ± S.D.	384.7 ± 30.4	367.8 ± 27.2	359.0 ± 23.5	354.9 ± 22.8	354.7 ± 21.8

APPENDIX TABLE BW-3 (continued)

Butylene Oxide

Multiple Dosing; Ethyl methanesulphonate, 100 mg/kg

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	391	376	357	331	315	301
	392	420	394	371	351	336
	393	405	378	352	337	319
	394	411	388	363	344	326
	395	406	380	356	337	320
	396	376	371	362	348	338
	397	405	380	356	340	326
	398	375	360	338	323	311
	399	323	312	295	281	270
	400	380	362	340	324	306
	Mean ± S.D.	387.7 ± 28.0	368.2 ± 23.2	346.4 ± 21.9	330.0 ± 20.7	315.3 ± 19.9

APPENDIX TABLE BW-4

Butylene Oxide
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	25	24	25	25	25
	322	27	28	28	28	28
	323	23	23	23	23	24
	324	26	26	26	26	26
	325	25	24	24	24	24
	326	26	26	25	25	25
	327	24	24	25	25	25
	328	23	23	24	23	25
	329	24	24	25	24	25
	330	25	25	26	25	25
	Mean ± S.D.	24.8 ± 1.3	24.7 ± 1.6	25.1 ± 1.4	24.8 ± 1.5	25.2 ± 1.1

APPENDIX TABLE BW-4 (continued)

Butylene Oxide

Multiple Dosing: 250 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	331	24	24	24	24	25
	332	23	24	24	25	25
	333	25	25	25	26	25
	334	24	24	24	25	25
	335	23	24	23	24	24
	336	25	25	25	26	26
	337	23	23	24	25	25
	338	24	23	23	24	24
	339	24	23	24	24	24
	340	25	24	24	24	24
	Mean ± S.D.	24.0 ± 0.8	23.9 ± 0.7	24.0 ± 0.7	24.7 ± 0.8	24.7 ± 0.7

APPENDIX TABLE BW-4 (continued)

Butylene Oxide

Multiple Dosing: 1,000 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	341	25	24	24	24	24
	342	24	23	23	24	23
	343	23	22	23	22	22
	344	24	22	†	†	†
	345	24	24	24	24	24
	346	23	22	22	22	22
	347	23	22	†	†	†
	348	25	24	25	24	24
	349	23	22	22	22	not exposed
	350	24	22	22	21	21
	401 (a)	-	-	22	†	†
	402 (a)	-	-	25	23	23
	Mean	23.8	22.7	23.2	22.9	22.9
	± S.D.	± 0.8	± 0.9	± 1.2	± 1.2	± 1.1

(a) 401 and 402 replaced 344 and 347 which died following 2 days exposure.

† = Animal dead.

APPENDIX TABLE BW-4 (continued)

Butylene Oxide

Multiple Dosing: Ethyl methanesulphonate, 200 mg/kg

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	351	23	23	23	24	22
	352	23	24	24	23	23
	353	25	25	26	24	25
	354	26	25	25	24	24
	355	24	24	25	24	24
	356	24	24	25	23	23
	357	24	26	24	23	23
	358	24	25	25	24	24
	359	25	25	25	24	24
	360	24	24	24	23	24
	Mean ± S.D.	24.2 ± 0.9	24.5 ± 0.8	24.6 ± 0.8	23.6 ± 0.5	23.6 ± 0.8

APPENDIX TABLE CA-MD-M

Butylene Oxide
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Males

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
123	145/5	50	13	12				1				2.9 x 110.6
	145/4		25	24	1							1.8 x 118.4
	145/1		12	11	1							8.9 x 89.2
124	12/4	50	25	24		1						5.1 x 121.9
	12/5		25	25								
126	69/5	50	25	25								
	69/3		25	23	1							3.8 x 94.5
					1							0.8 x 89.1
125	158/5	50	25	25								
	158/3		25	24			1					7.8 x 104.9
130	32/2	50	25	24	1							6.0 x 130.1
	32/3		25	25								
127	120/1	50	25	24	1							7.0 x 85.3
	120/5		25	25								
129	111/2	50	25	25								
	111/5		25	25								
121	22/5	50	25	25								
	22/4		25	25								
122	103/2	50	25	25								
	103/4		25	24	1							6.2 x 129.9

APPENDIX TABLE CA-MD-M (continued)

Butylene Oxide
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		
139	54/2	50	25	25								
	54/5		25	25								
135	26/1	50	14	13		1						28.9 x 124.5
	26/4		5	5								
	26/2		4	4								
	26/3		25	21	1							9.0 x 101.0
					1							7.7 x 109.4
					1							11.9 x 112.4
					1							10.8 x 119.0
	26/5		2	1	1							9.2 x 124.0
137	154/3	4	1	1								
	154/5		1	1								
	154/4		1	1								
	154/1		1	1								
	154/2		0	0								
136	84/2	50	25	25								
	84/3		25	25								
131	41/2	50	25	24				1				10.9 x 92.2
	41/1		25	23	1							14.2 x 97.9
					1							5.6 x 102.4
133	70/1	50	25	25								
	70/5		25	25								

APPENDIX TABLE CA-MD-M (continued)

Butylene Oxide
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		
138	14/3	50	25	23	1							3.8 x 128.8
					1							2.0 x 125.6
	14/3		25	24	1							8.2 x 119.0
132	44/3	50	18	18								
	44/4		20	20								
	44/1		12	12								
134	157/3	50	25	25								
	157/4		25	25								
140	5/4	50	25	25								
	5/1		25	25								

APPENDIX TABLE CA-MD-M (continued)

Butylene Oxide
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		
149	107/4	50	25	23	1							7.5 x 95.1
					1							2.0 x 94.6
	107/3		25	25								
148	126/5	50	25	24	1							6.3 x 124.4
	126/3		25	25								
145	124/2	50	25	24	1							4.5 x 103.5
	124/4		25	25								
141	50/5	50	25	23	1							13.7 x 116.1
					1							12.0 x 123.1
	50/1		25	24	1							6.1 x 99.1
146	33/3	50	25	25								
	33/5		25	25								
147	149/4	50	25	24	1							9.1 x 117.1
	149/3		25	25								
144	128/3	50	25	25								
	128/4		25	25								
143	135/5	50	15	14	1							9.3 x 128.1
	135/1		24	24								
	135/3		11	10	1							7.0 x 127.2
150	66/5	50	25	25								
	66/2		25	22	1							11.1 x 100.0
					1							9.1 x 98.0
					1							9.2 x 96.5

APPENDIX TABLE CA-MD-M (continued)

Butylene Oxide
Males

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
153	62/3	50	25	19	1							9.1 x 99.2
					1							0.0 x 90.0
					1							34.9 x 90.9
					3	1						30.0 x 96.1
					1							12.2 x 123.0
											1 Exchange	11.0 x 115.0
	62/4		25	21	1							6.2 x 116.3
											1 Multi Aberration	6.0 x 115.9
					2							3.0 x 100.2
					1							2.9 x 97.5
154	110/1-2	1	0	0								
	110/3		1	1								
	110/4-5		0	0								
159	67/3	50	25	20	1							10.3 x 100.1
					2							8.8 x 104.0
						1						7.0 x 100.5
						1						6.6 x 107.0
					1							6.0 x 105.2

APPENDIX TABLE CA-MD-M (continued)

Butylene Oxide
Males

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
159	67/4		25	17		1						7.9 x 84.9
											1 Multi Aberration	7.5 x 88.2
					1							7.2 x 96.0
					4						1 Exchange	6.7 x 101.0
											1 Multi Aberration	6.9 x 100.1
											1 Exchange	6.8 x 98.3
					1	2						7.2 x 95.9
					1							6.6 x 93.5
151	61/3	50	25	22	1							7.2 x 97.0
					1							6.6 x 93.2
					1	2						6.2 x 91.0
	61/1		25	24		1						5.0 x 97.0
152	108/2	50	25	23	1							9.5 x 114.0
					1							32.9 x 116.6
	108/3		25	23	1							13.0 x 104.2
					1							7.0 x 108.9
156	34/3	50	25	24		1						3.5 x 93.7
	34/2		25	21	1							6.1 x 95.0
						1						4.5 x 101.5
					1							4.1 x 101.2
						1						4.5 x 92.1

APPENDIX TABLE CA-MD-M (continued)

Butylene Oxide
Males

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
158	122/4	50	25	19		1						7.8 x 105.1
						1						4.0 x 108.0
					1							3.9 x 101.0
					1							1.2 x 108.5
						1						0.8 x 109.0
					1							33.8 x 107.1
	122/5		25	22	1							32.3 x 109.9
						2						30.5 x 93.1
						1						28.9 x 96.9
157	115/2	37	4	4								
	115/1		14	14								
	115/3		9	8							1 Exchange	6.6 x 114.5
	115/5		3	1	1							4.2 x 95.2
					1							2.8 x 88.1
	115/4		7	4		1						9.8 x 96.0
					1							31.8 x 91.8
						3	1				1 Exchange	34.9 x 122.1
155	55/2	50	25	19	1							5.0 x 127.1
					1							3.3 x 114.9
					1							3.7 x 115.4
						1						3.4 x 125.1
					1							3.4 x 126.0
					1							3.3 x 127.8

APPENDIX TABLE CA-MD-M (continued)

Butylene Oxide
Males

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
155	55/3		25	23		3		1			1 Exchange	10.9 x 125.1
					1							11.0 x 126.1
160	141/3	50	25	21		1						6.4 x 116.1
						1					1 Exchange	4.7 x 122.0
											1 Exchange	2.9 x 125.2
					1							0.4 x 117.1
	141/3		25	24	1	2					1 Exchange	9.8 x 116.2

APPENDIX TABLE CA-MD-F

Butylene Oxide
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Females

Multiple Dosing: Air Control (0 ppm)

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
289	271/4	50	25	22	1							3.5 x 100.5
					1							8.9 x 86.8
					1							3.8 x 97.0
	271/5		10	8	2							1.7 x 74.5
					1	1						34.2 x 80.1
	271/2		15	12	1							11.3 x 70.5
											1 Exchange	5.9 x 75.3
					1							3.8 x 75.0
286	229/2	5	2	2								
	229/5		2	2								
	229/1		0	0								
	229/3		0	0								
	229/4		1	1								
288	310/4	50	21	20	1							1.8 x 101.3
	310/5		25	24	2	1						4.2 x 117.1
	310/1		4	4								
285	318/3	50	9	9								
	318/2		5	5								
	318/1		13	13								
	318/4		23	23								
290	192/4	50	25	24	1							9.9 x 92.1
	192/5		25	25								

APPENDIX TABLE CA-MD-F (continued)

Butylene Oxide
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		
281	182/5	50	2	2								
	182/2		25	25								
	182/3		2	2								
	182/4		6	6								
	182/1		10	9	1							10.8 x 116.8
287	280/3	50	25	25								
	280/5		25	24	1							4.1 x 118.4
283	305/5	50	25	24	1							9.9 x 116.0
	305/4		25	25								
284	172/1	25	7	7								
	172/2		5	3	1							32.4 x 125.2
					1							32.2 x 125.1
	172/3		2	2	1							32.2 x 125.1
	172/4		3	3								
	172/5		8	8								
282	263/2	37	6	6								
	263/1		3	3								
	263/3		10	10								
	263/4		9	9								
	263/5		9	7	1							8.7 x 125.6
					1							31.8 x 116.9

APPENDIX TABLE CA-MD-F (continued)

Butylene Oxide
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
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
293	230/2	50	25	25								
	230/5		25	25								
295	186/4	50	25	25								
	186/2		25	25								
298	174/4	50	25	24	1							4.2 x 103.1
	174/2		25	25								
299	214/2	50	25	24				1				11.5 x 93.9
	214/3		25	24	1							11.0 x 101.3
291	201/3	50	25	25								
	201/5		25	25								
297	314/4	50	25	24	1							7.7 x 89.0
	314/3		25	25								
294	317/2	50	25	24	1							4.8 x 131.3
	317/3		25	24				1				34.0 x 120.4
292	204/5	50	25	24	1							9.9 x 108.1
	204/1		25	24	1							8.2 x 116.9
300	165/3	50	25	24				1				11.1 x 98.9
	165/2		25	25								
296	244/2	50	25	24	1							1.8 x 89.0
	244/1		25	24				1				4.7 x 99.1

APPENDIX TABLE CA-MD-F (continued)

Butylene Oxide
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		
303	295/2	50	25	23	1							0.0 x 91.0
					1							31.9 x 86.5
	295/1		25	23	1							11.2 x 90.4
					1							9.2 x 89.2
307	309/5	50	13	13								
	309/1		21	20	1							3.9 x 101.8
	309/4		6	6								
	309/2		4	4								
	309/3		6	5	1							3.4 x 92.1
308	286/5	50	25	24	1							13.0 x 97.3
	286/1		25	23		1						2.8 x 88.9
					1							34.2 x 91.3
304	288/3	50	25	23	1							10.0 x 92.0
					1							9.2 x 98.1
	288/2		25	24	1							9.5 x 98.4
309	267/5	50	25	24	1							6.0 x 95.3
	267/4		25	24	1							2.2 x 128.8
310	226/1	50	25	24	1							8.6 x 120.8
	226/5		25	25								
306	193/3	50	25	24	1							11.7 x 95.1
	193/2		25	23	1							13.0 x 93.4
					1							13.1 x 94.1

APPENDIX TABLE CA-MD-F (continued)

Butylene Oxide
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		
305	284/4	50	25	25								
	284/2		25	25								
302	265/1	33	8	7	2							7.0 x 98.2
	265/2		10	10								
	265/3		3	3								
	265/4		3	3								
	265/5		9	8	1							33.7 x 102.4
301	210/4	50	25	25								
	210/2		25	25								

APPENDIX TABLE CA-MD-F (continued)

Butylene Oxide
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		
316	194/4	50	25	22		1						7.8 x 91.1
						1						3.9 x 99.8
					1							3.0 x 87.1
	194/5		25	20	1							9.4 x 97.6
					1							8.3 x 97.2
					1							5.3 x 93.0
					1							3.8 x 98.1
											1 Exchange	2.4 x 97.5
319	227/2	50	25	19	2							9.8 x 98.8
					3							6.3 x 94.0
					1							5.2 x 103.6
					1							4.3 x 97.5
											1 Exchange	2.9 x 106.1
					1							34.9 x 106.2
	227/4		25	18	1							11.3 x 97.2
											1 Exchange	9.2 x 94.0
					1							6.0 x 99.0
						1						6.2 x 102.6
							1					5.0 x 93.0
						1						3.9 x 106.2
						1					1 Exchange	3.9 x 103.0

APPENDIX TABLE CA-MD-F (continued)

Butylene Oxide
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		
317	275/2	50	25	21		1						13.1 x 94.1
					1							11.8 x 98.7
						1						9.9 x 100.9
					1							7.1 x 100.6
	275/3		25	20	1							12.0 x 93.9
						1						10.9 x 94.0
											1 Fragment	10.8 x 93.0
						2						10.0 x 98.2
					1							10.2 x 119.7
314	270/2	50	25	23		1						7.1 x 89.1
					1							7.2 x 101.5
	270/5		25	24		1					1 Chromosomal Fragment	12.5 x 107.5
320	301/5	50	25	21	3	1					1 Exchange	6.0 x 92.5
					1							5.8 x 93.0
					2							5.9 x 100.2
					1							0.1 x 94.0
	301/4		25	17		1						13.5 x 91.3
											1 Fragment	12.2 x 97.1
					3							10.9 x 87.1
					1							10.0 x 89.0
					1							9.0 x 100.1
					1							7.0 x 99.1
						1						6.9 x 91.1
						2						6.3 x 86.4

APPENDIX TABLE CA-MD-F (continued)

Butylene Oxide
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		
315	215/3	50	25	19	1							7.9 x 96.1
					1							6.2 x 90.3
						1						2.8 x 91.3
					1							0.0 x 92.8
					1							0.2 x 98.9
					1							33.9 x 104.0
	215/4		25	17		1						10.5 x 95.0
						1						10.1 x 103.6
						1						9.8 x 102.9
											2 Exchange	9.2 x 101.0
					1							7.6 x 98.0
					1							5.6 x 90.2
											1 Fragment	5.1 x 99.4
					1							1.8 x 95.8
318	282/1	50	25	22	1							7.0 x 89.0
					1							6.6 x 91.9
					1							6.1 x 91.0
	282/5		25	22	2							7.2 x 93.7
						2					1 Fragment	5.7 x 103.6
						3					1 Exchange 1 Fragment	2.0 x 102.0

APPENDIX TABLE CA-MD-F (continued)

Butylene Oxide
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	221/4	50	25	24		1						8.2 x 96.5
	221/3		25	24	1							7.5 x 98.8
312	268/4	50	25	23	1							6.3 x 100.6
					1							2.8 x 103.1
	268/5		25	24	1							9.0 x 121.9
313	222/5	60	25	21	1							9.1 x 121.2
					1							9.2 x 123.1
					1							8.9 x 128.8
					1							7.9 x 129.0
	222/4		25	23		1						9.0 x 119.0
					1							8.1 x 130.1

APPENDIX TABLE CA-M6

Butylene Oxide
Cytogenic 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
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
2	58/2	50	24	21	1							56.0 x 100.2
											1 Chromatid Fragment	54.8 x 98.7
					1							61.3 x 98.8
	58/3		25	25								
	58/5		1	1								
3	63/1	50	17	17								
	63/2		7	7								
	63/3		7	7								
	63/4		10	10								
	63/5		6	6								
	63/6		3	3								
10	113/3	50	25	25								
	113/5		25	25								
7	71/3	50	25	24							1 Chromatid Fragment	53.8 x 111.0
	71/5		25	25								
9	109/1	50	20	19	1							53.1 x 103.3
	109/2		21	21								
	109/3		9	9								
4	152/1	50	25	24	1							49.5 x 104.1
	152/4		25	24	1							43.5 x 111.1

APPENDIX TABLE CA-M6 (continued)

Butylene Oxide
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		
8	78/4	50	25	24	1			1				65.9 x 114.8
	78/5		25	24	1							59.5 x 107.8
6	143/5	50	25	24	1							64.9 x 110.0
	143/4		25	25								
5	104/2	50	25	23	1							63.2 x 113.1
					1							54.1 x 106.0
	104/7		25	25								
1	100/1	50	25	25								
	100/2		25	25								

APPENDIX TABLE CA-M6 (continued)

Butylene Oxide
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		
34	147/3	50	25	25								
	147/1		18	18								
	147/2		7	7								
38	7/1	50	18	17	1						1 Chromatid Fragment	57.1 x 113.0
	7/4		17	16	1							24.0 x 111.0
	7/3		11	11								
	7/2		4	3							1 Chromosomal Fragment 2 Chromatid Fragments	25.0 x 108.8
36	131/3	50	25	25								
	131/4		25	24	1							66.0 x 106.6
32	51/1	50	21	21								
	51/2		11	11								
	51/3		15	15								
	51/5		3	3								
35	4/2	50	25	25								
	4/4		25	25								
37	99/1	50	25	25								
	99/5		18	18								
	99/2		7	7								
39	93/1-7	0	0	0								

APPENDIX TABLE CA-M6 (continued)

Butylene Oxide
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		
31	85/5	50	25	25								
	85/4		25	25								
33	160/1	50	25	25								
	160/2		25	24	1							66.0 x 107.2
40	118/1	50	25	25								
	118/3		25	25								

APPENDIX TABLE CA-M6 (continued)

Butylene Oxide
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
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
65	30/1-7	0	0	0								
64	6/1	50	25	23			1					37.0 x 110.9
					1							35.4 x 110.0
	6/5		25	24			1					45.3 x 109.3
69	75/1	50	23	21	1							45.7 x 111.9
					1							63.0 x 107.8
	75/2		16	15	1							59.9 x 114.0
	75/3		11	11								
62	123/1-7	0	0	0								
70	138/6	50	25	25								
	138/2		25	24	1							33.7 x 110.0
68	148/3	50	25	24	1							34.3 x 109.4
	148/4		25	23	1							32.0 x 107.8
					1							52.0 x 102.0
61	3/7	50	25	24	1							48.1 x 104.2
	3/1		21	21								
	3/5		4	4								
63	133/1	50	22	20	1							42.1 x 109.6
											1 Chromatid Fragment	40.8 x 106.0
	133/2		20	19							1 Chromatid Fragment	33.0 x 108.2
	133/3		8	8								

APPENDIX TABLE CA-M6 (continued)

Butylene Oxide
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
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
66	106/3	50	25	23	1							68.3 x 108.2
					1							48.7 x 103.1
	106/2		25	24	1							47.0 x 105.8
67	121/3	50	25	24	1							34.1 x 103.0
	121/5		25	2		1						62.8 x 109.0
					1							59.3 x 99.0

APPENDIX TABLE CA-M6 (continued)

Butylene Oxide
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		
92	59/1	50	25	22	1							28.5 x 108.2
					1							40.1 x 101.9
					1							66.0 x 100.2
	59/4		25	22		1						43.0 x 109.2
					1							63.5 x 105.9
					1							38.5 x 103.0
96	56/3	50	25	25								
	56/4		25	22	1							59.2 x 113.0
					1	1						54.0 x 111.0
					1							38.0 x 108.1
91	94/1-7	0	0	0								
100	136/1	50	25	25								
	136/3		25	22	1							36.2 x 111.2
											1 Chromatid Fragment	41.8 x 110.1
						1						32.4 x 104.9
95	42/3	50	25	23	1							65.3 x 106.2
					1							39.0 x 101.1
	42/1		25	23	1							67.5 x 110.0
					1							33.1 x 106.7
93	16/1-7	0	0	0								
97	31/6	50	14	13	1							41.9 x 105.2
	31/7		18	17							1 Chromatid Fragment	62.0 x 107.0
	31/5		18	17	1							70.1 x 109.0

APPENDIX TABLE CA-M6 (continued)

Butylene Oxide
Males

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
99	18/2	50	25	23		1						34.2 x 112.0
					1							44.1 x 105.0
	18/3		9	7				1				28.0 x 112.0
					1							52.2 x 106.9
	18/1		16	15	1							59.0 x 111.0
94	19/2	50	25	16	1							45.9 x 114.2
					1							39.7 x 113.9
					1							37.1 x 113.2
					1	1						33.0 x 113.2
					1							36.8 x 111.4
						1						37.0 x 111.1
					1							41.0 x 111.0
					1							47.0 x 110.1
					1							45.3 x 107.5
	19/4		25	21	1							27.0 x 110.8
					1							34.9 x 111.0
					2							56.0 x 110.0
					1							64.9 x 110.0
98	13/1	9	3	3								
	13/2		0	0								
	13/3		4	4								
	13/4		2	2								
	13/5-7		0	0								

APPENDIX TABLE CA-M24

Butylene Oxide
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		
12	1/3	50	20	17	1							54.8 x 110.0
						1					1 Chromatid Fragment	40.2 x 99.6
					1						1 Chromatid Fragment	34.8 x 93.6
	1/2		23	22	1							40.5 x 99.1
	1/1		7	6	1							70.7 / 112.8
16	134/1	50	25	24	1							49.2 x 109.1
	134/2		19	18	1							50.7 x 102.2
	134/3		6	6								
19	64/2	50	13	11	1							67.3 x 108.5
											1 Chromatid Fragment	39.5 x 102.1
	64/3		22	20	1							63.9 x 109.2
											1 Chromatid Fragment	69.1 x 107.9
	64/4		14	14								
	64/5		1	1								
14	74/3	50	25	24							1 Chromatid Fragment	47.6 x 104.0
	74/4		25	24	1							36.8 x 104.9

APPENDIX TABLE CA-M24 (continued)

Butylene Oxide
Males

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
15	82/1-5	0	0	0								
11	92/3	50	25	23	1							33.7 x 107.0
								1				66.2 x 106.7
	92/2		16	15							1 Chromatid Fragment	67.3 x 109.4
	92/4		9	7	1							51.2 x 112.2
											1 Chromatid Fragment	67.1 x 105.0
13	88/1-5	0	0	0								
18	27/3	50	15	15								
	27/1		19	19								
	27/5		16	16								
20	40/3	50	21	18							1 Chromatid Fragment	40.9 x 105.3
					1							46.0 x 103.9
					1							39.6 x 98.1
	40/2		15	14	1							56.3 x 98.4
	40/1		14	13	1							66.4 x 97.7
17	119/1	50	25	25								
	119/4		19	19								
	119/3		4	4								
	119/5		2	2								

APPENDIX TABLE CA-M24 (continued)

Butylene Oxide
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		
50	140/1-5	0	0	0								
49	38/1-5	0	0	0								
46	8/1-5	0	0	0								
45	53/2	50	20	18	1							31.8 x 100.6
					2							62.4 x 98.8
	53/3		25	25								
	53/1		5	4		1						59.5 x 111.1
48	117/1	50	25	25								
	117/3		25	25								
41	129/3	50	25	25								
	129/5		25	25								
43	89/2	50	18	17							1 Chromatid Fragment	61.9 x 103.2
	89/3		25	24	1							41.4 x 108.0
	89/4		7	7								
47	11/1	50	23	22	1							25.0 x 100.2
	11/2		20	19				1				26.4 x 102.1
	11/3		7	7								
44	15/1	50	25	23	1							54.1 x 105.4
						1						56.5 x 104.0
	15/2		25	24	1							61.2 x 106.9
42	98/1-5	0	0	0								

APPENDIX TABLE CA-M24 (continued)

Butylene Oxide
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		
73	142/3	50	25	24	1							44.7 x 103.9
	142/2		25	24	1						1 Chromosomal Fragment	40.2 x 104.9
80	57/4	50	25	25								
	57/5		25	25								
74	79/2	50	22	21		1						64.8 x 106.7
	79		21	18	1							38.0 x 111.0
					1							30.0 x 101.3
					1							68.9 x 97.5
	79/4		7	6	1							37.4 x 108.9
75	101/1-5	0	0	0								
71	46/1	50	25	25								
	46/4		25	25								
78	90/1	50	25	25								
	90/4		25	24	1							42.3 x 108.3
76	23/3	50	25	25								
	23/4		25	25								
79	45/2	50	25	24	1							27.6 x 108.2
	45/3		25	25								
72	17/2	50	11	11								
	17/4		21	19	1							22.8 x 106.1
					1							65.1 x 106.1
	17/3		18	16	1							31.7 x 106.5
								1				31.9 x 105.1

APPENDIX TABLE CA-M24 (continued)

Butylene Oxide
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		
77	139/1	50	11	10	1							58.4 x 107.2
	139/2		19	18	1							31.5 x 101.5
	139/3		20	20								

APPENDIX TABLE CA-M24 (continued)

Butylene Oxide
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	39/1	24	14	8							1 Chromatid Fragment	58.8 x 108.6
					2	1					1 Chromatid Fragment	68.5 x 105.2
					1							40.6 x 105.2
											1 Multi Aberration	32.5 x 105.4
					1							56.6 x 104.7
					1							57.8 x 101.0
	39/3		10	10								
	39/2		0	0								
	39/4-5		0	0								
105	9/2	50	25	24	1							67.8 x 108.8
	9/3		25	20							1 Multi Aberration	29.0 x 112.3
						1						66.4 x 111.4
						1						31.0 x 108.2
					1						1 Chromatid Fragment	66.0 x 106.8
											1 Chromatid Fragment	64.4 x 106.6
103	68/2	39	10	9	1						1 Chromatid Fragment	30.5 x 101.6
	68/5		4	3	1							30.2 x 103.8
	68/3		11	11								
	68/1		0	0								
	68/4		14	11	1							53.7 x 108.0
					1							33.8 x 104.0
						1						58.8 x 93.7
107	77/2	1	1	1								
	77/1		0	0								
	77/3-5		0	0								

APPENDIX TABLE CA-M24 (continued)

Butylene Oxide
Males

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
102	72/2	50	17	17								
	72/5		25	24	1							59.5 x 107.9
	72/4		8	8								
108	127/3	50	8	6	1							72.3 x 105.1
					1	1					1 Chromosomal Fragment	
											1 Exchange	44.9 x 103.8
	127/4		25	25								
	127/1		17	14	1	1						36.7 x 109.0
					1	1					1 Exchange	
											1 Chromosomal Fragment	40.2 x 107.9
											1 Multi Aberration	39.8 x 107.5
110	24/1	50	25	18		2					1 Exchange	
											1 Chromosomal Fragment	37.5 x 108.0
											2 Chromatid Fragments	35.5 x 108.3
					4							36.4 x 106.8
					1							52.4 x 106.8
					1	1						59.0 x 106.6
											1 Exchange	38.4 x 103.4
											1 Multi Aberration	
					1	1					1 Exchange	
											1 Chromatid Fragment	30.1 x 101.8
	24/2		25	20		2						36.5 x 107.2
											1 Multi Aberration	70.1 x 105.0
						2	1				2 Exchanges	63.7 x 99.7
					1							63.1 x 99.3
					2	4					2 Chromatid Fragments	65.5 x 99.2
109	93/1-5	0	0	0								

APPENDIX TABLE CA-M24 (continued)

Butylene Oxide
Males

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
104	36/2	50	25	24		1						58.0 x 103.0
	36/3		21	16							2 Exchanges	65.6 x 107.7
											1 Exchange	63.4 x 107.9
											1 Chromatid Fragment	38.3 x 107.5
					1	1						33.4 x 107.7
											1 Fragmented Chromosomal	35.5 x 98.9
	36/4		4	4								
101	96/1	50	25	23	1	2			2			33.1 x 110.0
											1 Multi Aberration	60.1 x 100.6
	96/2		19	15		1						61.8 x 106.0
					1							59.8 x 105.0
					1							33.2 x 100.6
											1 Multi Aberration	59.7 x 94.6
	96/3		6	6								

APPENDIX TABLE CA-M48

Butylene Oxide
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		
24	114/1	50	25	25								
	114/3		25	25								
22	10/2	50	25	25								
	10/5		25	25								
26	102/2	50	25	25								
	102/3		25	25								
29	146/2	50	25	24	1							105.5 x 42.1
	146/5		25	23	1							72.8 x 42.8
					1							80.8 x 37.2
25	125/1	50	25	25								
	125/3		25	24		1						99.9 x 35.2
23	73/3	50	25	25								
	73/2		25	25								
30	95/3	50	25	25								
	95/4		25	24	1							105.5 x 41.8
21	76/2	50	25	25								
	76/4		25	24	1							79.0 x 37.2
28	116/1	50	25	25								
	116/3		25	24	1							87.1 x 40.0
27	81/3	50	25	25								
	81/4		25	25								

APPENDIX TABLE CA-M48 (continued)

Butylene Oxide
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
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
55	35/3	50	25	25								
	35/4		25	24	1							33.2 x 88.1
58	132/3	50	25	25								
	132/1		25	22	1							97.1 x 44.0
					1							104.0 x 34.2
					1							106.0 x 32.4
54	49/2	50	25	25								
	49/4		25	25								
60	65/1-5	0	0	0								
57	153/1	50	25	25								
	153/2		25	25								
56	137/2	50	25	25								
	137/5		25	25								
52	159/1-5	0	0	0								
59	52/1	50	25	25								
	52/2		25	25								
51	155/2	50	25	25								
	155/3		25	25								
53	130/1	50	25	25								
	130/2		25	25								

APPENDIX TABLE CA-M48 (continued)

Butylene Oxide
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
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
84	43/4	50	25	24	1							55.5 x 89.5
	43/5		20	19							1 Exchange	27.1 x 98.9
	43/3		5	5								
83	37/1-5	0	0	0								
90	28/2	50	25	25								
	28/5		25	25								
87	112/1-5	0	0	0								
86	91/1	50	25	25								
	91/3		25	23	1							99.0 x 42.0
					1							96.9 x 42.2
82	151/1	50	25	25								
	151/2		25	25								
88	87/1	50	25	25								
	87/3		25	25								
85	156/1	50	25	22	1							26.9 x 90.8
					1							34.0 x 90.6
					1			1				58.2 x 90.8
	156/3		25	23	1							35.0 x 87.0
					1							52.0 x 86.2
89	25/5	50	25	25								
	25/3		25	25								
81	48/1-5	0	0	0								

APPENDIX TABLE CA-M48 (continued)

Butylene Oxide

Males

Single Dosing: Ethyl methylsulphonate, 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		
111	20/1	50	25	25								
	20/2		25	25								
118	80/2	50	25	25								
	80/4		25	25								
120	97/1	50	25	24	1							101.8 x 43.1
	97/2		25	25								
119	60/2	50	25	25								
	60/3		25	25								
116	21/1	50	25	25								
	21/2		25	24							1 Multi Aberration	104.2 x 42.1
113	47/1	50	25	25								
	47/2		25	25								
114	86/2	50	25	24	1							77.7 x 40.6
	86/3		25	25								
117	2/1	50	25	25								
	2/2		25	25								
115	29/3	50	25	25								
	29/4		25	25								95.8 x 41.2
112	144/2	50	25	24	1							
	144/1		25	25								

APPENDIX TABLE CA-F6

Butylene Oxide
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Females

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
163	223/1	50	25	23	1							31.0 x 111.8
					1							60.0 x 105.9
	223/5		25	23	1							41.3 x 109.1
					1							65.5 x 99.9
169	269/3	50	25	24		1						59.7 x 112.0
	269/5		25	25								
170	273/4	50	25	25								
	273/5		25	24	1							35.0 x 110.0
161	260/1-7	0	0	0								
165	264/2	50	25	25								
	264/4		25	24							1 Chromatid Fragment	61.5 x 107.6
162	218/1-7	0	0	0								
164	312/5	50	25	24	1							40.0 x 108.2
	312/3		18	18								
	312/2		7	7								
166	303/2	50	25	23	1							28.1 x 111.0
					1							65.0 x 109.0
	303/3		25	24	1							61.8 x 110.2
167	231/6	50	25	25								
	231/1		21	21								
	231/2		4	4								

APPENDIX TABLE CA-F6 (continued)

Butylene Oxide
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		
198	167/1	50	25	25								
	167/5		25	25								
197	259/2	50	25	23	1							34.8 x 113.2
					1							51.0 x 109.7
	259/3		25	25								
200	278/1	50	25	23	1							33.0 x 107.8
											1 Chromatid Fragment	52.7 x 105.0
	278/6		23	22	1							59.2 x 105.0
	278/2		2	2								
196	291/7	50	25	25								
	291/6		25	24	1							51.0 x 104.0
199	253/1	50	25	24	1							60.2 x 103.3
	253/2		25	23	1							36.0 x 109.9
					1							56.8 x 102.3
194	307/1	50	12	12								
	307/2		20	20								
	307/3		10	10								
	307/6		8	8								
193	320/1-7	0	0	0								
195	164/4	50	25	25								
	164/3		25	25								

APPENDIX TABLE CA-F6 (continued)

Butylene Oxide
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
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
191	245/2	50	15	14	1						1 Chromatid Fragment	55.0 x 100.1
	245/3		13	10		1						32.3 x 112.7
					1							64.1 x 108.0
					1							68.0 x 103.9
	245/4		14	14								
	245/5		8	7			1					59.4 x 101.9
192	211/3	50	25	25								
	211/2		25	25								

APPENDIX TABLE CA-F6 (continued)

Butylene Oxide
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		
226	266/6	50	25	23	1							64.0 x 113.6
					1							52.2 x 105.1
	266/7		25	23	1							36.4 x 108.8
						1						47.0 x 107.1
225	190/1	50	25	25								
	190/3		25	25								
224	166/6	50	25	24	1							56.2 x 107.9
	166/4		25	25								
223	293/1	13	11	11								
	293/2		2	2								
	293/3-7		0	0								
228	308/2	50	25	24	1							60.9 x 112.0
	308/4		18	17			1					58.0 x 101.0
	308/5		7	7								
227	281/1-7	0	0	0								
222	283/2	50	25	24	1							71.9 x 103.2
	283/4		25	25								
230	298/1	50	12	12								
	298/2		10	10								
	298/3		20	20								
	298/4		1	1								
	298/5		7	7								

APPENDIX TABLE CA-F6 (continued)

Butylene Oxide
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		
221	163/3	50	20	19	1							62.0 x 104.0
	163/2		22	22								
	163/4		8	8								

APPENDIX TABLE CA-F6 (continued)

Butylene Oxide
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	173/4	50	13	13								
	173/3		14	13	1							34.6 x 108.9
	173/1		12	11		1						42.0 x 104.0
	173/2		11	11								
255	202/1	50	17	15	1							64.0 x 107.8
					1							55.9 x 104.0
	202/2		25	22	1							45.0 x 105.9
						1						57.0 x 103.6
											1 Chromatid Fragment	31.0 x 102.3
	202/3		8	8								
253	176/1	35	12	10	1							36.1 x 104.1
					1							57.7 x 94.6
	176/2		12	10	1							61.9 x 108.8
					2							27.8 x 108.3
	176/3		6	5	1							59.1 x 100.8
	176/4		5	4	1							59.8 x 99.0
	176/5-7		0	0								
260	296/2	50	25	23	1							39.2 x 110.9
					1							57.0 x 104.9
	296/4		25	22	1							63.2 x 109.0
					1							57.0 x 107.2
						1						29.0 x 102.3
251	254/1-7	0	0	0								

APPENDIX TABLE CA-F6 (continued)

Butylene Oxide
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		
259	178/5	50	20	17	1							61.4 x 109.9
					1							31.1 x 105.0
					1							66.1 x 101.9
	178/2		25	22	1							60.0 x 109.2
									1			32.9 x 108.0
					1							37.9 x 105.0
	178/3		5	4	1							28.1 x 103.0
252	219/1	50	19	19								
	219/3		25	23	1							65.0 x 112.8
					1							26.5 x 102.0
	219/7		6	6								
254	179/2	50	21	21								
	179/3		10	10								
	179/4		19	17	1							27.0 x 106.0
											1 Chromatid Fragment	27.1 x 106.0
257	191/1-7	0	0	0								
256	216/4	50	18	16	1							38.1 x 113.2
						1						69.0 x 98.2
	216/3		19	18	1	1						32.1 x 111.9
	216/2		13	13								

APPENDIX TABLE CA-F24

Butylene Oxide
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		
176	294/5	50	25	22	1							66.6 x 105.2
					1						1 Chromatid Fragment	44.2 x 103.7
					1							70.3 x 102.8
	294/3		16	16								
	294/2		9	9								
177	279/1	50	25	25								
	279/2		25	25								
179	224/2	50	25	25								
	224/3		25	24		1						58.8 x 101.7
173	248/2	50	25	22		1						58.3 x 110.6
											1 Chromatid Fragment	39.0 x 110.3
					1						1 Chromatid Fragment	40.4 x 105.5
	248/4		20	16	1							38.4 x 106.0
					1							39.0 x 105.7
					1							37.2 x 100.5
					1							65.4 x 100.4
	248/3		5	5								
180	200/1	50	21	21								
	200/2		22	19		1						30.9 x 109.8
					1							33.0 x 107.2
					1							29.8 x 103.3
	200/3		7	7								
178	187/1-5	0	0	0								

APPENDIX TABLE CA-F24 (continued)

Butylene Oxide
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		
172	161/1	50	25	23		1						33.8 x 110.4
					1							54.0 x 108.0
	161/3		25	25								
171	252/1	50	25	24	1							33.9 x 112.1
	252/3		25	25								
174	234/1-5	0	0	0								
175	242/3	50	25	25								
	242/4		25	25								

APPENDIX TABLE CA-F24 (continued)

Butylene Oxide
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		
203	249/1	50	25	23							1 Chromatid Fragment	61.3 x 106.5
							1				3 Chromatid Fragments	60.5 x 102.0
	249/2		25	25								
208	277/2	50	25	22	1							30.0 x 107.4
					1							43.5 x 107.2
					1							62.4 x 107.1
	277/3		25	24							1 Chromatid Fragment	38.6 x 112.2
202	258/1	50	9	8	1							39.5 x 103.4
	258/2		7	6	1							50.8 x 106.2
	258/3		10	8	1							46.5 x 108.9
					1							61.9 x 105.7
	258/4		12	11							2 Chromatid Fragments	28.1 x 106.0
	258/5		12	11	1							62.9 x 97.6
209	198/1	50	13	13								
	198/2		9	9								
	198/3		24	23	1							68.1 x 107.3
	198/4		4	4								
204	175/1-5	0	0	0								
210	300/2	50	13	13								
	300/4		16	15	1							33.7 x 102.9
	300/5		21	20	1							56.4 x 106.0
201	289/2	50	25	23	1							37.9 x 111.3
					1							70.5 x 109.3
	289/4		25	24	1							70.9 x 104.5

APPENDIX TABLE CA-F24 (continued)

Butylene Oxide
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		
207	171/1-5	0	0	0								
206	168/2	50	25	25								
	168/2		15	15								
	168/4		10	8	1							65.0 x 112.3
											1 Chromatid Fragment	63.9 x 108.0
205	213/1-5	0	0	0								

APPENDIX TABLE CA-F24 (continued)

Butylene Oxide
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		
237	299/2	50	25									
	299/3		25									
236	283/5	50	25	23	1							29.4 x 102.3
					1							54.1 x 111.1
	283/2		25	23	1							40.3 x 107.3
					1							32.3 x 104.8
238	250/1-5	0	0	0								
233	302/1	50	25	25								
	302/3		25	21	1							66.9 x 106.7
								1				62.3 x 107.7
					1							72.0 x 105.5
											1 Chromatid Fragment	70.2 x 105.9
232	177/2	50	11	11								
	177/3		21	21								
	177/4		13	13								
	177/5		5	5								
231	206/1	50	25	25								
	206/2		25	23	1							69.1 x 110.1
					1							36.4 x 106.7
234	239/2	50	25	24	1							28.6 x 107.8
	239/3		25	25								
239	205/2	50	25	25								
	205/3		25	25								
240	217/3	50	25	25								
	217/4		25	25								

APPENDIX TABLE CA-F24 (continued)

Butylene Oxide
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		
235	261/3	41	6	6								
	261/5		11	11								
	261/2		14	14								
	261/4		6	5	1							39.5 x 114.2
	261/1		4	4								

APPENDIX TABLE CA-F24 (continued)

Butylene Oxide
Females

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
265	169/1-5	0	0	0								
266	199/2	50	25	16	1							59.6 x 110.7
					1						1 Exchange	
											2 Chromatid Fragments	58.0 x 110.0
					1							48.0 x 108.6
						1						34.7 x 107.3
											2 Chromatid Fragments	33.3 x 107.4
								1				72.6 x 105.7
					1	1						32.3 x 104.4
											1 Chromatid Fragment	70.1 x 105.2
											1 Chromatid Fragment	44.6 x 103.7
	199/3		25	19	1							42.6 x 110.3
					1							33.0 x 108.9
					1							38.0 x 109.2
						1					2 Chromatid Fragments	43.3 x 105.6
					1							66.0 x 103.5
						1						48.7 x 103.9
267	237/4	50	24	17	1							36.2 x 112.8
											1 Chromatid Fragment	28.4 x 110.5
					1							65.0 x 108.9
											1 Multi Aberration	27.3 x 106.0
						1						61.8 x 106.0
					1							61.6 x 104.5
					1							37.8 x 104.6

APPENDIX TABLE CA-F24 (continued)

Butylene Oxide
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	237/1		13	10							1 Chromatid Fragment	40.3 x 110.2
					2	1					1 Chromatid Fragment	61.0 x 97.2
					1							63.8 x 94.1
	237/3		13	10	1							33.0 x 111.0
					1							59.9 x 106.0
					1							58.0 x 105.2
262	232/1	18	6	6								
	232/2		9	7							1 Multi Aberration	57.8 x 111.1
					1							27.3 x 102.9
	232/3		3	3								
	232/4		0	0								
	232/5		0	0								
268	287/2	10	2	2								
	287/4		7	6	2							38.3 x 101.2
	287/5		1	1								
	287/3		0	0								
	287/1		0	0								
264	196/1-5	0	0	0								
261	256/1-5	0	0	0								
263	228/3	50	22	21	1							37.3 x 103.4
	228/5		15	15								
	228/2		13	13								

APPENDIX TABLE CA-F24 (continued)

Butylene Oxide
Females

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
269	243/3	50	25	25								
	243/1		25	22							1 Multi Aberration	66.0 x 109.5
					1							54.5 x 108.5
											1 Chromatid Fragment	59.3 x 106.9
270	184/1-5	0	0	0								

APPENDIX TABLE CA-F48

Butylene Oxide
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
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
186	226/4	50	25	25								
	226/5		25	24	1							31.0 x 90.2
188	276/1	50	25	25								
	276/3		25	25								
185	285/1	50	25	24							1 Multi Aberration	
	285/3		25	25								
190	255/1	50	25	25								
	255/2		25	25								
182	170/1	50	25	25								
	170/4		25	24	1							100.0 x 41.5
184	274/2	50	25	25								
	274/4		25	25								
187	214/1	50	25	24	1							69.0 x 37.3
	214/2		25	25								
183	233/3	50	25	24			1					69.0 x 40.8
	233/2		25	23			1					89.8 x 40.6
					1							80.0 x 40.1
181	236/2	50	25	25								
	236/3		25	25								
189	306/2	50	25	23	1							94.6 x 42.1
					2							78.5 x 39.8
	306/4		25	25								

APPENDIX TABLE CA-F48 (continued)

Butylene Oxide
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		
220	225/1	50	25	24	1							66.8 x 97.0
	225/2		25	24	1							69.0 x 39.7
218	292/4	50	25	24			1					94.9 x 45.1
	292/5		25	24	1							68.1 x 34.2
213	290/5	50	25	23	1							100.0 x 43.2
					1							97.2 x 43.5
	290/1		25	24	1							96.8 x 38.9
214	209/2	50	25	24		1						74.0 x 33.2
	209/3		25	24	1							99.1 x 30.9
216	297/3	50	25	25								
	297/4		25	25								
217	313/4	50	25	24	1							55.9 x 95.0
	313/3		25	25								
215	195/3	50	25	25								
	195/5		25	25								
211	315/1	50	25	25								
	315/5		25	25								
212	319/1-5	0	0	0								
219	212/2	50	25	25								
	212/3		25	25								

APPENDIX TABLE CA-F48 (continued)

Butylene Oxide
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		
242	311/3	50	25	25								
	311/4		15	15								
	311/1		10	10								
246	251/3	50	25	25								
	251/4		25	25								
249	185/1	50	25	25								
	185/4		25	25								
241	208/1	50	25	25								
	208/2		25	25								
245	316/1	50	25	25								
	316/3		25	23	1							46.5 x 90.0
					1							33.0 x 93.8
244	203/3	50	25	24	1							60.5 x 87.0
	203/4		25	25								
248	247/3	50	25	24				1				65.0 x 39.0
	247/4		25	25								
247	272/3	50	25	21			1					54.6 x 91.8
											1 Chromatid Fragment	60.0 x 93.9
							1					30.8 x 94.0
							1					30.0 x 94.1
	272/1		25	22			1					43.9 x 89.6
							2					36.0 x 92.9
							1					65.2 x 96.2

APPENDIX TABLE CA-F48 (continued)

Butylene Oxide
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		
243	197/2	50	25	23	1							40.0 x 89.8
					2							28.4 x 92.5
	197/3		25	24	2	1						34.5 x 88.7
250	188/1	50	25	25								
	188/2		25	25								

APPENDIX TABLE CA-F48 (continued)

Butylene Oxide
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		
279	220/2	50	25	23							1 Chromatid Fragment	73.0 x 42.0
											3 Chromatid Fragments	
											1 Chromosomal Fragment	70.0 x 38.9
	220/4		25	22	1							76.7 x 38.2
							1					102.8 x 34.3
											1 Translocation	80.5 x 34.1
277	162/4	50	25	22	1						1 Chromatid Fragment	35.6 x 86.5
					1							42.0 x 90.2
						1						25.0 x 93.6
	162/5		25	22	2							60.0 x 91.0
											1 Chromatid Fragment	32.5 x 97.3
					1							40.0 x 99.0
271	180/2	50	25	25								
	180/3		25	24	1							92.3 x 43.0
276	181/3	50	25	24	1							76.9 x 44.1
	181/2		25	25								
278	240/3	50	25	23	1							53.6 x 88.0
					1		1					28.0 x 94.5
	240/4		25	21	1							65.5 x 90.2
					1							37.9 x 90.5
							1					32.1 x 93.2
					1							38.2 x 92.8
274	246/1	50	25	24	1		1					106.2 x 31.4
	246/3		25	25								

APPENDIX TABLE CA-F48 (continued)

Butylene Oxide
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		
272	304/1	50	25	23				1				39.9 x 89.1
					2							40.8 x 90.2
	304/2		25	22	1							34.0 x 89.0
					1	1						44.9 x 89.5
					1		-					51.0 x 93.7
275	189/1-5	0	0	0								
280	257/1	50	25	25								
	257/3		25	25								
273	207/2	50	25	25								
	207/3		25	23			1					97.8 x 41.0
							1					100.1 x 37.1

APPENDIX TABLE DL

Butylene Oxide
Dominant Lethal Assesment

Multiple Dosing: Air Control (0 ppm)

Week No.	Male No. / Female	361		362		363		364		365		366		367		368		369		370		Total
		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
1	Corpora lutea	15	11	0	12	14	15	15	10	0	11	0	2	0	13	10	14	16	13	3	16	190
	Total Implants	15	13	0	12	12	16	13	10	0	13	0	0	0	14	9	12	14	12	*	16	181
	Live Implants	15	13	0	11	12	16	13	10	0	13	0	0	0	14	9	12	14	12	*	15	179
	Early Deaths	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	*	1	2
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	*	0	0
2	Corpora lutea	11	13	12	12	18	1	9	12	16	12	13	12	13	13	0	15	15	14	11	12	234
	Total Implants	2	14	12	16	17	0	15	11	13	12	13	12	15	11	0	17	13	14	5	14	226
	Live Implants	0	13	12	15	16	0	15	10	13	11	12	12	14	11	0	13	11	14	4	14	210
	Early Deaths	2	1	0	0	1	0	0	1	0	1	1	0	0	0	0	4	1	0	1	0	13
	Late Deaths	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	3
3	Corpora lutea	10	13	14	9	13	16	16	14	14	14	15	10	9	12	11	14	16	12	12	14	258
	Total Implants	16	15	15	9	13	16	10	13	13	16	15	12	10	12	9	14	16	13	13	15	265
	Live Implants	13	14	14	9	13	13	10	13	13	15	15	11	8	10	9	13	16	11	13	15	248
	Early Deaths	3	1	1	0	0	3	0	0	0	1	0	1	2	2	0	0	0	2	0	0	16
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
4	Corpora lutea	17	15	13	17	13	12	0	17	12	0	15	13	12	13	14	16	16	14	14	8	251
	Total Implants	15	14	14	12	15	12	0	19	13	0	14	13	12	13	13	17	16	15	15	10	252
	Live Implants	15	13	14	12	11	12	0	12	13	0	14	13	12	13	13	14	16	15	14	0	226
	Early Deaths	0	1	0	0	4	0	0	5	0	0	0	0	0	0	0	3	0	0	1	8	22
	Late Deaths	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	2	4
5	Corpora lutea	12	14	19	15	13	15	15	15	15	14	13	12	10	13	14	10	14	16	13	11	273
	Total Implants	12	12	13	13	14	13	13	13	15	14	13	12	4	11	14	10	13	15	12	11	247
	Live Implants	12	12	13	13	14	13	13	13	15	14	13	12	3	8	13	9	11	14	12	10	237
	Early Deaths	0	0	0	0	0	0	0	0	0	0	0	0	1	3	1	1	2	0	0	1	9
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1

* 370 Unassessable brown mass

APPENDIX TABLE DL (continued)

Butylene Oxide

Multiple Dosing: Air Control (0 ppm)

Week No.	Male No.	361		362		363		364		365		366		367		368		369		370		Total
	Female	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
6	Corpora lutea	13	13	14	11	13	0	13	15	12	15	14	13	16	17	22	13	14	12	12	14	256
	Total Implants	14	12	13	12	13	0	12	13	13	16	14	14	14	16	13	16	14	13	17	14	263
	Live Implants	14	12	11	12	10	0	11	12	13	16	14	13	14	16	11	15	13	13	17	13	250
	Early Deaths	0	0	2	0	2	0	1	1	0	0	0	1	0	0	1	0	1	0	0	1	10
	Late Deaths	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	3
7	Corpora lutea	18	14	19	15	15	16	15	19	21	12	13	16	14	17	15	14	14	15	0	16	308
	Total Implants	15	16	15	13	12	14	16	15	18	12	12	16	14	15	15	14	14	15	0	14	275
	Live Implants	15	16	15	13	11	14	15	15	18	12	11	15	14	15	14	14	13	14	0	14	268
	Early Deaths	0	0	0	0	1	0	1	0	0	0	1	0	0	0	1	0	1	1	0	0	6
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
8	Corpora lutea	9	16	11	14	12	13	13	16	0	12	17	14	11	12	16	17	15	13	16	15	252
	Total Implants	10	16	3	15	12	14	13	16	0	11	13	14	10	13	15	16	14	10	15	14	237
	Live Implants	10	16	3	13	12	10	13	14	0	10	11	13	9	13	15	15	11	10	15	13	216
	Early Deaths	0	0	0	2	0	4	0	2	0	0	2	1	1	0	0	1	3	0	0	1	17
	Late Deaths	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
9	Corpora lutea	14	12	17	14	16	12	13	11	14	18	14	14	16	16	16	15	13	13	12	15	285
	Total Implants	5	12	15	16	4	10	13	11	11	17	16	18	16	14	16	15	12	13	14	14	262
	Live Implants	5	11	14	16	4	10	11	4	10	16	16	15	13	13	15	12	12	13	13	13	238
	Early Deaths	0	1	1	0	0	0	1	7	1	0	0	3	3	1	1	1	0	0	1	1	22
	Late Deaths	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	2	0	0	0	0	4
10	Corpora lutea	13	12	12	9	17	15	11	17	18	12	16	11	*	*	*	*	12	12	*	*	187
	Total Implants	12	11	13	1	17	15	11	15	18	13	12	12					12	12			174
	Live Implants	12	11	12	1	16	15	10	15	18	12	12	12					10	12			168
	Early Deaths	0	0	1	0	1	0	1	0	0	0	0	0					2	0			5
	Late Deaths	0	0	0	0	0	0	0	0	0	1	0	0					0	0			1

* 367, 368, 370 not assessed because identity uncertain

APPENDIX TABLE DL (continued)

Butylene Oxide

Multiple Dosing: 250 ppm

Week No.	Male No. / Female	371		372		373		374		375		376		377		378		379		380		Total
		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
1	Corpora lutea	11	12	13	2	15	12	17	16	2	15	12	13	12	14	1	12	14	12	13	13	231
	Total Implants	11	12	14	0	12	13	17	14	0	13	13	14	9	15	0	13	13	12	14	12	221
	Live Implants	11	12	14	0	12	12	16	14	0	13	13	13	8	15	0	12	13	12	14	11	215
	Early Deaths	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	0	1	6
	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	14	15	10	11	19	11	11	15	13	15	14	16	0	13	14	18	5	13	12	251
	Total Implants	13	17	14	12	14	13	12	10	16	13	15	15	18	0	15	15	12	4	9	12	249
	Live Implants	13	16	13	12	12	13	12	8	15	12	14	13	16	0	13	14	11	0	9	12	228
	Early Deaths	0	1	1	0	2	0	0	2	0	1	1	2	1	0	2	1	1	4	0	0	19
	Late Deaths	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	2
3	Corpora lutea	0	15	4	12	12	17	15	11	13	17	14	15	9	15	16	12	18	13	13	14	256
	Total Implants	0	13	0	11	13	17	12	11	13	15	12	15	14	13	13	12	8	13	13	14	232
	Live Implants	0	13	0	10	13	17	11	10	13	15	12	14	13	12	13	12	8	12	13	14	225
	Early Deaths	0	0	0	1	0	0	1	1	0	0	0	0	1	1	0	0	0	1	0	0	6
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
4	Corpora lutea	10	16	12	13	13	18	23	16	14	15	12	11	21	17	19	15	12	9	14	12	292
	Total Implants	10	13	13	14	12	17	18	14	14	15	14	10	15	15	12	13	11	*	14	11	255
	Live Implants	10	13	13	13	11	17	12	12	14	15	13	9	14	15	12	13	11	*	12	11	240
	Early Deaths	0	0	0	1	1	0	6	1	0	0	1	1	0	0	0	0	0	*	0	0	13
	Late Deaths	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	*	0	0	2
5	Corpora lutea	12	10	13	10	9	12	15	3	15	14	17	12	13	15	15	12	11	20	15	14	257
	Total Implants	12	10	13	10	10	13	14	0	14	15	15	12	12	13	6	15	12	13	15	13	237
	Live Implants	12	10	13	10	10	12	13	0	8	14	14	12	12	13	5	15	10	13	15	13	224
	Early Deaths	0	0	0	0	0	0	0	0	3	1	1	0	0	0	1	0	2	0	0	0	8
	Late Deaths	0	0	0	0	0	1	1	0	3	0	0	0	0	0	0	0	0	0	0	0	5

* 379 Unassessable brown mass

APPENDIX TABLE DL (continued)

Butylene Oxide

Multiple Dosing: 250 ppm

Week No.	Male No. / Female	371		372		373		374		375		376		377		378		379		380		Total
		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
6	Corpora lutea	14	20	12	14	1	15	12	12	11	15	13	13	8	12	13	11	15	12	14	13	250
	Total Implants	13	14	13	16	0	15	8	15	13	15	11	13	14	16	13	11	15	15	13	11	254
	Live Implants	13	13	12	16	0	13	7	13	11	15	10	12	13	15	13	11	14	14	13	11	239
	Early Deaths	0	1	1	0	0	2	1	2	2	0	1	0	1	1	0	0	1	1	0	0	14
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
7	Corpora lutea	15	14	14	15	13	13	12	16	16	16	17	20	2	17	14	12	13	13	15	12	279
	Total Implants	14	16	13	14	12	11	14	17	17	14	19	16	0	15	14	12	14	15	16	13	276
	Live Implants	13	16	12	14	11	11	12	17	16	14	19	16	0	14	14	10	13	15	15	12	264
	Early Deaths	1	0	1	0	1	0	2	0	1	0	0	0	0	1	0	2	1	0	0	1	11
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
8	Corpora lutea	19	15	17	14	13	15	13	12	18	15	12	13	13	10	13	15	14	12	15	12	280
	Total Implants	12	14	14	14	13	13	13	12	17	13	13	13	13	10	12	15	16	6	11	12	256
	Live Implants	12	14	13	14	13	13	12	12	16	13	13	13	13	10	11	14	15	6	11	11	249
	Early Deaths	0	0	1	0	0	0	1	0	1	0	0	0	0	0	1	1	1	0	0	1	7
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	Corpora lutea	14	13	13	16	15	17	15	13	17	14	12	17	15	14	14	12	17	14	17	15	294
	Total Implants	14	14	13	17	15	15	12	15	17	15	14	16	15	14	11	13	17	14	15	12	288
	Live Implants	14	13	13	14	14	15	11	14	17	14	13	16	13	13	9	11	15	14	15	10	268
	Early Deaths	0	1	0	2	1	0	1	1	0	1	1	0	2	1	2	2	2	0	0	1	18
	Late Deaths	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2
10	Corpora lutea	14	14	12	13	14	13	12	14	16	14	12	12	*	*	12	13	12	18	13	14	242
	Total Implants	16	13	12	9	14	14	13	14	15	13	11	14			13	13	2	15	13	12	218
	Live Implants	15	13	11	9	13	14	12	14	15	13	11	14			11	13	2	13	13	12	8
	Early Deaths	1	0	1	0	1	0	1	0	0	0	0	0			2	0	0	2	0	0	8
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0			0	0	0	0	0	0	0

* 377 not assessed because identity uncertain

APPENDIX TABLE DL (continued)

Butylene Oxide

Multiple Dosing: 1,000 ppm

Week No.	Male No. / Female	381		382		383		384		385		386		387		388		389		390		Total
		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
1	Corpora lutea	11	0	13	16	15	15	12	14	14	14	12	17	13	15	12	0	17	19	9	15	253
	Total Implants	11	0	13	15	16	15	12	14	14	14	11	11	15	14	13	0	14	14	9	14	239
	Live Implants	11	0	13	14	16	15	12	13	9	14	10	11	14	12	13	0	13	14	9	14	227
	Early Deaths	0	0	0	1	0	0	0	1	0	0	1	0	0	1	0	0	1	0	0	0	5
	Late Deaths	0	0	0	0	0	0	0	0	5	0	0	0	1	1	0	0	0	0	0	0	7
2	Corpora lutea	14	13	16	13	18	13	17	11	16	13	15	14	11	12	11	0	13	14	14	14	262
	Total Implants	16	13	8	13	11	14	17	11	19	14	16	15	13	12	10	0	12	11	14	16	255
	Live Implants	12	13	4	12	10	13	17	11	15	12	16	15	13	0	10	0	11	11	14	14	223
	Early Deaths	4	0	4	1	1	1	0	0	2	2	0	0	0	12	0	0	0	0	0	2	29
	Late Deaths	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	1	0	0	0	3
3	Corpora lutea	13	12	14	17	14	14	15	9	16	14	16	16	19	12	15	12	12	15	13	18	286
	Total Implants	13	14	15	15	14	14	15	10	16	14	16	16	11	13	16	12	12	12	13	17	278
	Live Implants	13	13	15	15	13	13	14	9	14	13	15	16	5	13	14	10	10	12	13	17	257
	Early Deaths	0	1	0	0	1	1	1	1	2	1	1	0	4	0	2	2	2	0	0	0	19
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	2
4	Corpora lutea	18	17	10	4	12	10	5	14	10	12	14	12	10	9	13	4	12	14	13	14	227
	Total Implants	9	13	12	0	13	9	0	15	10	13	14	14	10	10	11	*	12	13	12	14	204
	Live Implants	6	13	10	0	11	9	0	12	10	13	13	14	10	9	11		12	13	11	14	191
	Early Deaths	2	0	1	0	1	0	0	2	0	0	0	0	0	1	0		0	0	1	0	8
	Late Deaths	1	0	1	0	1	0	0	1	0	0	1	0	0	0	0		0	0	0	0	5
5	Corpora lutea	0	12	13	12	14	14	5	8	10	14	2	14	2	10	13	13	13	11	12	13	205
	Total Implants	0	12	14	12	14	14	4	11	10	14	0	13	0	9	13	12	18	11	11	13	205
	Live Implants	0	11	13	12	14	14	2	11	10	12	0	12	0	8	12	11	18	11	10	7	188
	Early Deaths	0	1	1	0	0	0	2	0	0	2	0	1	0	1	1	1	0	0	1	4	15
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2

* 388 Unassessable brown mass

APPENDIX TABLE DL (continued)

Butylene Oxide

Multiple Dosing: 1,000 ppm

Week No.	Male No. / Female	381		382		383		384		385		386		387		388		389		390		Total
		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
6	Corpora lutea	13	14	16	13	0	15	16	17	13	14	19	11	15	12	0	13	0	12	11	14	238
	Total Implants	15	14	16	14	0	15	14	18	11	14	12	10	17	14	0	18	0	9	13	12	236
	Live Implants	14	14	14	14	0	14	14	17	10	14	12	10	17	14	0	15	0	9	13	12	227
	Early Deaths	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	4
	Late Deaths	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	2	0	0	0	0	5
7	Corpora lutea	14	16	18	13	15	12	14	12	15	14	16	15	17	18	15	18	18	13	15	10	298
	Total Implants	15	14	18	13	16	13	16	12	17	15	16	15	17	15	15	15	17	16	14	17	306
	Live Implants	15	14	18	11	15	13	15	12	17	13	16	15	16	15	15	13	16	16	14	15	294
	Early Deaths	0	0	0	2	1	0	1	0	0	1	0	0	1	0	0	2	1	0	0	1	10
	Late Deaths	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	2
8	Corpora lutea	15	15	15	2	12	12	13	14	13	15	13	15	0	13	14	15	11	14	14	11	246
	Total Implants	14	12	17	0	13	10	13	14	12	15	14	14	0	14	14	15	11	14	14	12	242
	Live Implants	13	12	17	0	12	8	12	13	12	13	14	14	0	14	13	14	11	11	14	12	229
	Early Deaths	1	0	0	0	1	0	1	1	0	2	0	0	0	0	1	1	0	2	0	0	10
	Late Deaths	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	3
9	Corpora lutea	16	13	11	0	14	15	12	14	17	23	18	13	15	12	14	13	17	15	13	14	279
	Total Implants	16	13	11	0	14	16	13	14	17	18	17	15	15	11	14	12	14	14	14	16	274
	Live Implants	14	13	11	0	14	16	13	12	16	17	15	13	15	10	13	9	13	13	14	14	255
	Early Deaths	0	0	0	0	0	0	0	2	1	1	2	2	0	1	1	3	1	1	0	2	17
	Late Deaths	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
10	Corpora lutea	13	12	11	12	1	12	18	17	14	16	*	*	0	14	19	10	13	11	16	11	220
	Total Implants	12	12	6	11	0	12	13	12	13	18			0	14	15	9	12	12	13	11	195
	Live Implants	11	11	5	10	0	11	13	12	13	16			0	13	14	8	12	12	12	10	183
	Early Deaths	1	1	1	1	0	1	0	0	0	2			0	0	1	0	0	0	1	1	10
	Late Deaths	0	0	0	0	0	0	0	0	0	0			0	1	0	1	0	0	0	0	2

* 386 not assessed because identity uncertain

APPENDIX TABLE DL (continued)

Butylene Oxide

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Week No.	Male No. / Female	391		392		393		394		395		396		397		398		399		400		Total
		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
1	Corpora lutea	9	5	0	10	2	6	11	6	3	9	10	16	5	4	7	7	5	8	11	0	134
	Total Implants	7	2	0	8	0	4	9	2	0	10	5	10	1	0	6	6	0	4	8	0	82
	Live Implants	7	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	10
	Early Deaths	0	2	0	7	0	4	9	2	0	10	4	9	1	0	6	6	0	4	8	0	72
	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	1	0	0	3	2	2	1	1	0	0	11	6	2	6	2	3	1	0	3	8	52
	Total Implants	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	2	5
	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	3	0	0	0	0	0	0	2	5
	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	1	0	1	0	0	0	0	1	3	0	3	1	0	0	0	0	0	0	0	0	10
	Total Implants	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0	0	0	3
	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	1	0	0	1	0	0	0	0	1	0	0	0	0	3
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	Corpora lutea	10	*	0	8	14	2	10	11	10	13	17	16	13	15	13	7	12	0	10	14	195
	Total Implants	8	2	0	8	8	0	8	3	3	12	11	17	13	6	8	1	12	0	5	5	130
	Live Implants	3	1	0	1	4	0	3	0	0	6	4	14	11	2	6	0	9	0	3	0	67
	Early Deaths	4	1	0	7	4	0	5	3	2	5	7	3	2	4	2	1	3	0	0	4	57
	Late Deaths	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	2	1	6
5	Corpora lutea	7	10	12	2	14	13	12	13	8	17	17	12	12	10	10	11	11	18	2	11	222
	Total Implants	4	11	14	1	13	12	12	10	7	15	12	12	11	12	2	12	6	15	0	4	185
	Live Implants	4	10	12	0	9	11	9	10	7	13	12	12	11	12	2	11	5	14	0	4	168
	Early Deaths	0	0	2	1	4	1	3	0	0	1	0	0	0	0	0	1	1	1	0	0	15
	Late Deaths	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2

* Corpora lutea counts missing

APPENDIX TABLE DL (continued)

Butylene Oxide

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	
8	Corpora lutea	11	11	0	17	12	14	11	16	16	13	12	15	17	9	16	14	11	11	14	17	257
	Total Implants	11	13	0	16	12	15	10	16	15	14	13	14	19	11	19	14	13	15	12	12	264
	Live Implants	11	11	0	16	12	14	10	15	14	13	13	14	17	10	18	13	13	14	12	12	252
	Early Deaths	0	1	0	0	0	1	0	1	0	1	0	0	2	1	1	1	0	1	0	0	10
	Late Deaths	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
7	Corpora lutea	12	13	17	12	13	15	12	15	15	14	14	11	13	13	16	15	14	10	16	17	283
	Total Implants	13	13	18	11	14	14	9	15	16	10	14	7	14	13	16	15	14	16	16	18	276
	Live Implants	13	13	17	10	14	14	9	14	16	9	14	7	13	10	16	15	14	15	16	18	267
	Early Deaths	0	0	1	1	0	0	0	0	0	1	0	0	1	2	0	0	0	0	0	0	6
	Late Deaths	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	3
8	Corpora lutea	14	12	13	11	11	12	14	12	12	14	0	15	13	12	16	15	13	10	13	12	244
	Total Implants	14	13	11	10	12	10	12	12	13	14	0	15	11	13	16	12	14	10	14	12	238
	Live Implants	14	12	11	10	11	10	12	11	11	10	0	15	10	13	14	12	14	10	14	12	226
	Early Deaths	0	1	0	0	0	0	0	1	2	4	0	0	1	0	2	0	0	0	0	0	11
	Late Deaths	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
9	Corpora lutea	15	12	0	13	18	13	14	15	10	13	1	15	17	16	0	13	7	12	16	13	233
	Total Implants	13	14	0	14	17	13	14	14	10	15	0	16	15	16	0	14	5	12	13	12	227
	Live Implants	12	14	0	14	17	13	12	12	9	14	0	16	14	15	0	13	4	12	13	10	214
	Early Deaths	1	0	0	0	0	0	2	2	0	1	0	0	1	1	0	1	1	0	0	2	12
	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	15	12	10	9	13	1	14	10	12	15	*	*	*	*	*	*	12	16	17	12	168
	Total Implants	14	12	10	7	14	1	14	10	14	15							12	15	16	12	166
	Live Implants	11	12	10	5	12	0	14	10	14	13							11	12	16	11	151
	Early Deaths	3	0	0	2	2	1	0	0	0	2							1	3	0	1	15
	Late Deaths	0	0	0	0	0	0	0	0	0	0							0	0	0	0	0

396, 397, 398, not assessed because identity uncertain.

APPENDIX TABLE SA

Butylene Oxide

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	954	3	3	21	6	13	46	1000	321	Air
333	978	0	2	10	7	3	22	1000	322	Air
351	981	1	0	7	1	10	19	1000	323	Air
324	956	0	3	21	2	18	44	1000	324	Air
325	973	0	1	14	3	9	27	1000	325	Air
357	960	1	2	24	2	11	40	1000	326	Air
336	979	2	1	13	2	3	21	1000	327	Air
349	970	1	3	15	4	7	30	1000	328	Air
338	969	2	1	14	5	9	31	1000	329	Air
321	970	0	2	18	6	4	30	1000	330	Air
344	986	0	0	12	0	2	14	1000	331	Low
337	977	1	0	13	5	4	23	1000	332	Low
340	978	1	3	11	1	6	22	1000	333	Low
360	979	0	1	10	3	7	21	1000	334	Low
355	976	0	2	14	1	7	24	1000	335	Low
335	970	1	1	19	4	5	30	1000	336	Low
359	966	2	1	13	6	12	34	1000	337	Low
356	977	0	0	14	2	7	23	1000	338	Low
347	957	2	0	17	11	13	43	1000	339	Low
353	959	3	5	21	5	7	41	1000	340	Low

APPENDIX TABLE SA (continued)

Butylene Oxide

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
358	974	0	1	17	4	4	26	1000	341	High
341							Animal dead*		342	High
327							Animal dead*		343	High
352							Animal dead*		344	High
354	984	1	1	4	6	4	16	1000	345	High
345							Animal dead*		346	High
346							Animal dead		347	High
342	690	0	2	5	2	11	20	710	348	High
329	971	0	0	16	7	6	29	1000	349**	High
339							Animal dead*		350	High
350	965	1	1	14	8	11	35	1000	351	+
323	898	2	1	55	23	21	102	1000	352	+
331	937	2	3	39	5	14	63	1000	353	+
322	939	6	3	29	8	15	61	1000	354	+
328	952	0	1	25	5	17	48	1000	355	+
348	972	0	0	16	7	5	28	1000	356	+
330	937	2	3	29	14	15	63	1000	357	+
332	960	1	2	24	6	7	40	1000	358	+
343	977	1	0	16	2	4	23	1000	359	+
326	951	2	2	19	4	22	49	1000	360	+

* Animal Nos. 344, 347 - dead on Day 2
342, 343, 346, 350 - dead after 5 day dosing regime

** Animal No. 349 - not exposed on Day 5

Additional High Dose Group Mice

Animal No. 402 exposure Day 3, 4, 5 - A B C D E
0 4 18 4 6 = 32/1000

Animal No. 401 exposure Day 3 - dead on Day 4