Report Number 35

TIER II MUTAGENIC SCREENING OF 13 NIOSH PRIORITY COMPOUNDS

INDIVIDUAL COMPOUND REPORT HEXACHLORO-1,3-BUTADIENE

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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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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

Hexachloro-1,3-butadiene was subjected to a tier II mutagenic test screening programme. The assays used were the following.

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

2. Dominant lethal test in male rats with exposure to atmospheres containing 10 ppm or 50 ppm hexachloro-1,3-butadiene for 7 h/day for 5 consecutive days. Analysis of test atmospheres was by continuous infra-red adsorption monitoring at a wavelength of 11.7 μm.

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

4. Cytogenetic test in male and female rat bone marrow cells using the same exposure conditions as in (2) or a single exposure of 7 h 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 25 ppm for 1 h.

The results obtained were as follows:

1. Hexachloro-1,3-butadiene at an atmospheric concentration of 50 ppm was severely toxic to mice (all died) and less toxic to rats. Even 10 ppm atmospheres affected body weight gain in mice and rats.

2. There was an erratic increase in the number of grain counts per nucleus at certain high concentrations of hexachloro-1,3-butadiene in the absence of S-9 mix. The compound was very toxic in the presence of S-9 mix. Difficulties were experienced in the interpretation of these results.

3. There were no effects attributable to hexachloro-1,3-butadiene in the dominant lethal test on pregnancy frequency, number of corpora lutea or implantations, or the frequency of early deaths.

   Corpora lutea counts were significantly lower than control values in females in Week 1 mated with male rats exposed to 10 ppm atmospheres. Total implantations were also significantly lower in females in Week 1 mated with male rats exposed to 50 ppm atmospheres.

4. No effect of hexachloro-1,3-butadiene was seen on the frequencies of cells with aberrations in rat bone marrow. A statistically significant response was observed, however, in female rats exposed to a 10 ppm atmosphere for 7 h, then killed 6 h later ($P<0.05$).

5. In the sperm abnormality test only mice exposed to 10 ppm hexachloro-1,3-butadiene were examined since all of the 50 ppm atmosphere mice died. There were no increases in abnormal sperm frequency in this dose group.

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

It was concluded that hexachloro-1,3-butadiene did not affect the incidence of mutagenic events in mammals and Drosophila as detectable in these in vivo experiments. Neither was there reproducible evidence for UDS induction in cultured, human fibroblasts.

INTRODUCTION

Hexachloro-1,3-butadiene (CAS No. 87-68-3) (perchlorobutadiene) is the fully chlorinated product of butadiene obtained in commercial quantities as a by-product in some chlorinated hydrocarbon processes (e.g. perchloroethylene production).

It has been used as a vineyard fumigant and has been suggested as an insecticide and herbicide (Laska et al, 1976). The largest use in the U.S. is for the recovery of "snift" or chlorine containing gas in chlorine plants. It is also used as an intermediate in lubricant production, as a solvent and in heat transfer and hydraulic fluids.

The high resistance of hexachloro-1,3-butadiene to chemical, biological and physical degradation makes it an environmentally stable chemical. It has been detected in the ppb range in water, soil, some aquatic organisms (Laska et al, 1976) and food samples (Yip, 1976) taken on the lower Mississippi river, Louisiana.

Properties

Structure:	$Cl_2C{=}CCl.CCl{=}CCl_2$
Mol. wt.	260.79
M.P.	-21°C
B.P.	215°C
Sp. grav.	1.6820 (25°C)
Ref. index	1.5542 (20°C)
Flash point	none
Solubility	insoluble in water, soluble in alcohol and ether.

Toxicology

The oral LD_{50} in rats is 90 mg/kg and in mice it is 110 mg/kg. Inhalation studies gave an LC_{50} in mice of 235 ppm over a 4 h period.

In a limited carcinogenicity study, no tumours were found in rats after 6 months administration of hexachloro-1,3-butadiene at levels of 2-7 mg/kg in the diet (Murzakaev, 1967). Dow Chemical Company has conducted a 2 year toxicity study with Sprague-Dawley rats given diets delivering up to 20 mg/kg/day. No toxicity was seen at 0.2 mg/kg/day, but 2.0 mg/kg/day induced kidney damage. Renal tubular epithelial hyperplasia was noted and there was an increase in urinary coproporphyrin. The highest dose (20 mg/kg/day) resulted in renal tubular adenomas and adenocarcinomas, some of which metastasised to the lung (Kociba et al, 1977).

Studies of the nephrotoxic effect indicate that within 4 h of 300 mg/kg, i.p. to rats there was decreased urine osmolality, glomerular filtration rate and drug excretion (Davis et al, 1980). At 24 h elevated blood urea nitrogen was found, but no signs of liver damage up to 48 h. Control rats excreted 40% of ^{14}C labelled dose (i.p.) in the faeces and 30% in urine whereas rats with hexachloro-1,3-butadiene induced nephrotoxicity excreted only 7% in faeces and 6% in urine. All the labelled biliary material and 87% of that in urine was water soluble. Since hepatic glutathione is depleted within 5 h of administration, it seems likely that glutathione conjugates are formed. Other chlorinated butadienes form epoxides when incubated with liver homogenates (Bartsch et al, 1979). While such a compound could explain the mutagenicity in Ames' test (Salmonella typhimurium TA 1535 and TA 100) of hexachlorobutadiene in the presence of liver homogenates (Simmon, 1977), an epoxide is not likely to be formed in the absence of the homogenate. Hence, the reported activity in the absence of the homogenate (Simmon, 1977) is probably through some other mechanism. It is, perhaps, as well to remember that epoxides are ofted used as stabilisers in commercially available halogenated hydrocarbons and that such an additive (e.g. butylene oxide) could be responsible for the bacterial mutagenicity.

In a reproductive study with Sprague-Dawley rats fed up to 20 mg/kg/day for 90 days before mating, then through mating, gestation and lactation, toxic signs were seen in the groups of adults given 2.0 or 20 mg/kg/day, but not when given 0.2 mg/kg/day (Schwetz et al, 1977). The toxic effects were, as expected, loss in body weight gain and changes in kidney structure. There were no effects upon pregnancy or neonatal survival and development. No toxic effects were observed in neonatal rats in groups given diets delivering 0.2 or 2.0 mg/kg/day.

It is against this background of animal data that genetic toxicity tests were initiated. The objective of this report is to describe the methods and results obtained in tests for mutagenicity using test systems more complex than the hitherto used bacterial tests. The exposure conditions used were:

Human fibroblasts:	up to 1,000 µg/ml for 3 h.
Mice and rats:	10 ppm and 50 ppm for 7 h/day for one or 5 days.
Drosophila:	25 ppm for 1 h.

MATERIALS AND METHODS

CHEMICALS

Test Substance

Three 1 kg cans of hexachloro-1,3-butadiene, Batch No. 15719 (stated purity 98%), were received from Aldrich Chemical Compnay Limited on 18 March 1980. 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	9 May 1980	10-11	10	220♂ 176♀	19-23 May 1980 26 May 1980	Single dose cytogenetic slides not suitable.
		8-10	None	800 x 10	None	DL matings.
Mouse	7 May 1980	10-12	12	44♂	19-23 May 1980	-
Rat	22 August 1980	10-11	10	130♂ 130♀	1 September 1980	Single dose cytogenetics only.

Pre-experiment Acceptance Tests

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

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

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

Animal Management

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

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

They were housed individually in cages in a room with a light intensity of approximately 200 lux, a 12 h light-dark cycle, approximately 10 air changes per hour, temperature maintained at ca 22°C with extreme limits of 19°C and 27°C, and relative humidity ca 50%, with extreme limits of 38% and 70%.

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

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

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

Diet

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

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

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

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

Allocation of Rats and Mice to Cages and Treatment Groups

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

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

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

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

Treatment groups were colour coded as follows:

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

Animal Identification

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

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

Animal Positioning in the Exposure Chambers

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

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

The treatment groups were constituted as follows:-

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

ATMOSPHERE GENERATION AND EXPOSURE

Exposure Chambers

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

Exposures to hexachloro-1,3-butadiene 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 10 air changes per hour.

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

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

The generating apparatus and exposure chambers (Figures 1a and 1b) were positioned behind a screen in a room with a high efficiency exhaust system designed to ensure a safe working environment for laboratory personnel. The monitoring equipment was located on the outside of the screen at the opposite end of the room. The laboratory atmosphere was continuously monitored for any traces of the test compound. Exposure personnel wore breathing apparatus until it was shown that the room environment was clear of any possible contamination by hexachloro-1,3-butadiene. 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.

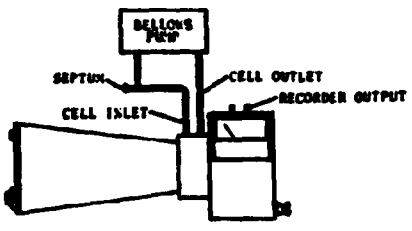
Calibration and Analytical Development

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 hexachloro-1,3-butadiene 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 hexachloro-1,3-butadiene were calibrated each day before vapour generation commenced.

The calibration was performed using a closed loop calibration system (see diagram below). Known volumes of hexachloro-1,3-butadiene 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 :	11.7 µm	11.7 µm
Pathlength :	21.75 m	21.75 m
Absorbence Range :	0.25 A	1 A
Slit Width :	1 mm	1 mm
Meter Response :	4	4
Recorder Voltage :	1 V	1 V
Chart Speed :	120 mm/h	120 mm/h

Calibration Data

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

Where:

C = Concentration (ppm)
V = Sample volume (µl)
ρ = Liquid density (g/cm³)
M = Molecular weight of test sample
$\frac{(RT)}{(P)}$ = Molar volume of gas (24.3 at 23°C)
5.64 = Volume of Miran sample chamber (l)

Example of the Calculation for V

Compound: Hexachloro-1,3-butadiene

C = 50 ppm
ρ = 1.665 g/cm³
M = 260.76

$$V = \frac{C \times M}{\rho} \times \frac{5.64}{10^3 \times 24.3\ \mu l}$$

$$= \frac{50 \times 260.76}{1.665} \times \frac{5.64}{10^3 \times 24.3\ \mu l}$$

$$= 1.82\ \mu l$$

Therefore, to construct a calibration curve to cover the 50 ppm range, 0.75 µl samples of hexachloro-1,3-butadiene 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 hexachloro-1,3-butadiene contained in a glass, gas washing or Drechsel bottle immersed in a temperature controlled water bath at 40°C. The nitrogen/hexachloro-1,3-butadiene vapour mixture so generated was ducted through 7/16" ID stainless steel piping to a glass mixing vessel and diluted with filtered, compressed air. The resulting mixture of hexachloro-1,3-butadiene/air was ducted through 7/8" stainless steel piping to the top of the exposure chamber.

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

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

Homogeneity Data

Before starting the animal exposures, chamber concentrations at both the high and low levels were determined by continuous monitoring for periods of 7 h. In addition, samples were measured from different areas (at least 9) of the animal holding zone to confirm uniformity of hexachloro-1,3-butadiene concentration.

Measurement of Chamber Concentrations

Atmospheric concentrations of hexachloro-1,3,-butadiene were monitored continuously during the 7 h exposure period from the breathing zone of the animals. A separate monitoring system was used for each concentration level. Stainless steel sampling lines, fitted with a particulate filter (Whatman Mini-Filter, Grade 80) and positioned on a central reference point in each exposure chamber were connected to the infra-red gas analysers. The sampling flow rate was approximately 5 l/min.

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

Test Compound Utilisation

At the beginning of each exposure day, the hexachloro-1,3-butadiene reservoir (a gas washing or Drechsel bottle) was replenished with test compound. Utilisation of test material was calculated on a daily basis by weighing the bottle before vapour generation began and deducting the weight of the bottle and remaining test compound on completion of the exposure period.

Exposure Procedure

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

Each animal was removed from its housing cage, examined for any signs of ill health, the ear number checked, and then individually accommodated inside a stainless steel grid compartment. The animals were then transferred to the exposure room and placed inside the exposure chamber according to the daily exposure location chart.

Animals exposed to hexachloro-1,3-butadiene 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 BDH Limited, Poole, Dorset, U.K.).

Cells

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

Culture Maintenance and Growth Media

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

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

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

Animals

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

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

Preparation of the 9,000 g Supernatant Fluid from Livers

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

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

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

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

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

co-factor solution	9 parts
liver preparation	1 part

Preliminary Toxicity Test

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

The cells were harvested and suspended in growth medium as for sub-culture, sedimented by centrifugation at 200 g for 5 min and resuspended in fresh culture medium at a density of 5 x 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 each control culture was added 10 µl of dimethylsulphoxide.

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

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

Based on these results, 8 concentrations of hexachloro-1,3-butadiene were selected for use in the repair assay.

DNA Repair Assay

The cells were harvested, sedimented, suspended in fresh culture medium at a density of 5 x 10^5 cells/ml and 2 ml samples of this suspension were pipetted into 35 mm tissue culture Petri dishes containing 3 sterile coverslips (Lux Scientific Corporation, California, U.S.A.). These were then incubated at 37°C in a humid atmosphere of 5% CO_2 in air for 72 h. The medium from each of the dishes was then replaced with 2 ml of arginine-deficient DMEM supplemented with 5% heat inactivated foetal bovine serum and the plates incubated for 24 h. The medium was then replaced with a further 1 ml of arginine-deficient DMEM and the incubation continued for a further 48 h. At the end of this time the cultures were divided into 2 groups and 200 µl of S-9 mix added to one of them. Solutions of hydroxyurea (250 mM) in sterile distilled water and 6-[^{3}H]-thymidine (21 Ci/mmol) were added to each culture giving final concentrations of 2.5 mM and 10 µCi/ml respectively. Hexachloro-1,3-butadiene was dissolved in dimethylsulphoxide and dilutions were made from this to give the test solutions. 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	1.25 and 10 µg/ml
2-AAN	5 and 50 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 $\pm$ the standard deviation.

CYTOGENETIC ANALYSIS OF RAT BONE MARROW CELLS

Metaphase Cell Preparations

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

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

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

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

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

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

Slide Reading

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

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

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

DOMINANT LETHAL TESTING IN MALE RATS

Mating

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

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

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

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

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

Assessment

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

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

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

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

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

SPERM ABNORMALITIES TEST IN MICE

Preparation

Mice were killed 5 weeks from the last day of dosing (i.e., Friday 27 June 1980) by neck dislocation.

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

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

Assessment

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

The following types of sperm were not scored:

(1) separated tails and heads.

(2) clumps of sperm.

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

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

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

I Normal

II Abnormal

A. hook upturned or elongated.

B. banana-shaped head.

C. amorphous head.

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

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

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

SEX-LINKED RECESSIVE LETHAL TEST IN DROSOPHILA MELANOGASTER

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

Strains

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

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

Medium

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

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

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

Exposures

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

*Nipogen: bacteriostatic agent (BDH Limited).

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

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

Toxicity Test

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

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

Recessive Lethal Test

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

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

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

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

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

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

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

STATISTICAL EVALUATION

Cytogenetics Tests

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

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

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

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

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

Dominant Lethal Assay

The variates analysed were:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

These data were analysed using the GLIM programme package interactively. The value of ϕ was generally assumed to be independent of treatment effects, except for the positive control which was analysed using a separate ϕ estimate. The GLIM programme provided the estimates β_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 $\theta_i = \exp(\beta_i)/(1 + \exp(\beta_i))$.

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_1 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_2 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 4.1-20.7 ppm or, occasionally, 41.4 ppm (10 ppm target concentration) and 20.7-103.5 ppm (50 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 hexachloro-1,3-butadiene was occurring. The results are shown in Table AT-3, where it can be seen that the maximum deviations encountered was -3.2% at the 10 ppm target concentration and -2.7% at the 50 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 10, 25 and 50 ppm were obtained and recorded in Tables AT-4 and 5.

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)

7 May 1980 Delivery: Four male mice were selected haphazardly for PEAT. Clinical examination and autopsy did not reveal any signs of infection or disease. The microbiological/parasitological examination identified 2 organisms

in cultures from lungs of one mouse, but not in the other 3 mice. These organisms were a yeast and an α-haem Streptococcus sp. The possibility of contamination cannot be ruled out. At histopathology the lungs of this same mouse showed a very mild periarterial inflammatory reaction.

9 May 1980 Delivery: Ten male and 10 female rats were haphazardly selected for PEAT. There were no significant clinical observations, but at autopsy 3 animals had notable peculiarities: one male had a (right) globular shaped kidney; in one female liver and kidneys were pale; and in a second female lung lobes had numberous grey foci. Microbiological/parasitological examination was without significant observation except for one female from which α-haem Streptococcus and Carinae pyogenes were isolated. Histologically, one male and 2 female rats were totally free from lesions, but all others showed some mild lung pathology. This took the form of bronchial associated lymphoid tissue accumulation and interstitial pneumonitis, probably due to Sendai virus which is endemic in Charles River rats.

Clinical Observations and Body Weights

Rats exposed to 10 ppm atmospheres appeared to be normal, but mice were slightly subdued during dosing on Days 2-5. At the 50 ppm dose level, rats also were subdued and by Day 5 of dosing they were inactive and showing little response to audio stimuli. Rat 381♂ (dominant lethal test) was inactive, hunched and had laboured breathing on Day 5. By the following day it was dead. Mice were very severely affected by the 50 ppm atmosphere. On Day 1 they were all subdued and had their eyes closed. The following morning 7 out of 10 mice were dead. The remaining 3 mice were killed in extremis: one was ataxic, had eyes closed, tremors and laboured breathing; 2 were motionless in their cages and showed only slight respiratory movements. Rats exposed on a single occasion were subdued in their activity by this experience.

Body weights were severely affected by 5 days exposure to 50 ppm hexachloro-1,3-butadiene (Tables BW-1 to 4). There were substantial weight losses in both male and female rats. Even exposure to 10 ppm atmospheres induced some weight loss in male mice and female rats. Weight gain in the male rats was rather less than in the air control animals.

UNSCHEDULED DNA SYNTHESIS ASSAY

The initial toxicity test, conducted in the absence of S-9 mix, at least 75% of the cells exposed to 1.7 mg/ml solutions were dead or damaged whereas less than 25% were damaged by 0.17 mg/ml concentrations. Results of the tritiated thymidine incorporation assay are given in Tables UDS-1, 2 and 3.

In the first of these assays, all cells exposed to 31 µg/ml concentrations or more, in the presence of S-9 mix, were killed, whereas 1,000 µg/ml was tolerated if no S-9 mix was present. High mean values for the numbers of silver grains per nucleus were found in the absence of S-9 mix at hexachloro-1,3-butadiene concentrations of 125, 250 and 1,000 µg/ml. At 500 µg/ml, however, normal grain counts were encountered. At these high concentrations there were relatively few cells on the coverslips and the nuclei were small.

The assay was repeated, using a high concentration of 20 µg/ml. At this concentration, both in the presence and absence of S-9 mix, and at 10 µg/ml in the absence of S-9 mix there were very few cells available for gain counting, so, these results were not transferred to Table UDS-2. The inadequate data were:

S-9 Mix	Dose	No. of Cells	Mean Grain Count ± S.D.
-	20 µg	27	3.59 ± 2.08
-	10 µg	61	1.87 ± 1.55
+	20 µg	0	-

At lower concentrations there was no indications of UDS induction. Hence, the only high grain counts were in the first experiment in the absence of S-9 mix.

There were, then, some inadequacies in the data, but there was a suggestion, which required confirmation, that hexachloro-1,3-butadiene might induce UDS in these cells. The experiment was conducted a third time in the absence of S-9 mix (Table UDS-3). On this occasion, doses ranged from 8 µg/ml to 250 µg/ml. The highest dose level at which there was not unacceptably high toxicity was 63 µg/ml. There was no indication of UDS induction at this, or any lower, dose level. Neither is there a ready explanation for the difference in toxicity of the compound in the absence of S-9 mix, particularly in the first compared with the second and third experiments.

The tritiated deoxyguanosine incorporation assay was to be used to confirm the results of the first assay. Difficulties

were experienced, however, in this assay, particularly in the presence of S-9 mix. Essentially, the labelled deoxyguanosine was not adequately taken up by the cells, so, the radioactivity in the isolated RNA and DNA samples was too low for the results to be significant. The experiments performed on this portion of the assay are the subject of a separate report.

CYTOGENETIC ANALYSIS OF RAT BONE MARROW CELLS

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

In the multiple exposure cytogenetic test, there were no indications of chromosomal damage induction in either the male or the female rats exposed to 10 ppm or 50 ppm hexachloro-1,3-butadiene. EMS treatment only induced a significant increase in the frequency of cells with aberrations in male rats ($P<0.05$) after multiple exposure. This significance was reduced if cells containing only gaps were excluded from the analysis.

In the single exposure portion of the experiment there were no significant increases in aberrant cell frequency in male rats exposed to hexachloro-1,3-butadiene and sampled at any of the 3 standard times (i.e. 6 h, 24 h and 48 h after the end of exposure). In female rats the only significant increase was in the 6 h sample, ($P<0.05$), but even here there were only 7 aberrations and these occurred in the 10 ppm atmosphere group. In the 50 ppm atmosphere group there were only 2 aberrations. This lack of a dose related response reduces the importance of this result. EMS treatment increased frequency of cells with aberrations in male rats at the 6 h ($P<0.01$), 24 h ($P<0.01$) and 48 h ($P<0.05$) sampling times. If cells only with gaps are excluded, then there was still a significant increase at the 24 h sampling time ($P<0.001$). Female rats also showed increases at the 6 h ($P<0.001$), 24 h ($P<0.001$) and 48 h ($P<0.01$) sampling times. When cells only with gaps were excluded there were significant increases at the 6 h ($P<0.05$) and 24 h ($P<0.001$) sampling times.

It is concluded that hexachloro-1,3-butadiene had no effect upon the frequencies of aberrant cells.

DOMINANT LETHAL TEST

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

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

Corpora lutea graviditatis counts (Table DL-3) were not reduced in either of the hexachloro-1,3-butadiene groups; these counts were greatly reduced, however, in Weeks 2 and 3 ($P<0.001$), of the positive control group. The small significant reduction ($P<0.05$) in Week 1 of the 10 ppm group (13.0 ± 0.62) was a consequence of a much higher than normal Air Control group result (15.1 ± 0.64).

Implantations per pregnancy (Table DL-4) were greatly reduced in Weeks 1-4 of the positive control group. They were unaffected by hexachloro-1,3-butadiene treatment, however, apart from Week 1 of the 50 ppm group ($P<0.01$). Although statistically significant, it is not clear that the small reduction was induced by treatment with hexachloro-1,3-butadiene.

The frequencies of live implantations (Table DL-5) and live implantations and late deaths (Table DL-6) followed the pattern of total implantations per pregnancy. In the 50 ppm atmosphere group there were no late deaths while in the 10 ppm atmosphere group there were only 2. These data compare with the air control group total of 7 over the 10 assessment weeks.

SPERM ABNORMALITY TEST

All mice exposed to 50 ppm hexachloro-1,3-butadiene died before sperm samples could be taken. In the group exposed to 10 ppm concentration atmospheres there were no significant increases in the frequencies of abnormal sperm in any of the categories examined (Table SA-1 and 2 and Appendix Table SA). EMS treatment increased the frequency of Category C, amorphous head.

SEX-LINKED RECESSIVE LETHAL TEST IN DROSOPHILA

There was no information available on the toxicity of hexachloro-1,3-butadiene to flies, so, some preliminary studies were made (Table RL-1). The first of these was on 13 May 1980 when flies were exposed to 50 ppm for 1 h, at which time no toxic effects were observed. The concentration was increased to 100 ppm and again, no toxic signs were seen after 40 min. At this time the concentration was increased to 120 ppm where it was maintained for 20 min before the flies showed any signs of toxicity. 1.5 h at this concentration induced obvious toxic effects, these being an inability to fly and even difficulty in walking without falling over. Exposure was terminated and all flies were dead the following morning.

On 14 May 1980 the concentration was maintained at 75 ppm. 100 min of exposure induced a general hyperactivity and an inability to walk in some flies. This effect was seen in all flies by 180 min, when exposure was terminated. Flies were alive at this stage, but they were all dead by the following morning, whether they had been exposed for 1, 2.5 or 3 h.

On 15 May 1980 the concentration was maintained at 25 ppm and flies were removed after 0.5, 1 and 2 h. Even after 2 h the activity of the flies appeared normal, but by the following morning all of these flies were dead (Table RL-1). Survival was normal, however, after exposure for 0.5 or 1 h and egg hatchability was not impaired.

Following these preliminary studies, the main study was initiated on 28 May 1980 using 25 ppm hexachloro-1,3-butadiene atmospheres for 1 h. No signs of toxicity were apparent under these experimental conditions.

Two breeding stocks (A and B) were used (Table RL-2), but no F_2 generation lethals were found in the air control groups in a total of 3,595 vials set up and 3,119 vials scored. Also, there were no F_3 generation lethals in a total of 500 vials set up, 486 scored.

Flies exposed to hexachloro-1,3-butadiene yielded a single lethal in the F_2 generation (Brood 1, Stock A), the frequency in that particular brood being 0.18%. A single lethal was also found in the F_3 generation (Brood 1, Stock B), the frequency being 0.20%. These frequencies are certainly not significant and it is concluded that hexachloro-1,3-butadiene did not induce a recessive lethal mutations in these Drosophila.

CONCLUSIONS

Toxicity was observed under the experimental conditions in the UDS assay, in the mice and in the rats used for the various tests. In the Drosophila sex-linked recessive lethal assay, the test conditions (25 ppm for 1 h) did not induce recognisable signs of toxicity, but preliminary studies had shown that 25 ppm for 2 h was lethal to the flies. Therefore, it is unlikely that the test environment could have been made much more severe than was actually used. It is concluded, then, that hexachloro-1,3-butadiene was tested to the practical limits of these assays, as described by the protocols used.

The UDS assay gave results which were difficult to interpret (this is presently being repeated). The other assays, however, gave no indication that hexachloro-1,3-butadiene might have any genotoxic potential.

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TABLE AT-1

Hexachloro-1,3-butadiene
Calibration Data for Low Level

Dose Level: 10 ppm v/v

Batch No.: 15719

Volume µl	Conc., ppm, (v/v)	Cumulative Chart Deflection, mm						
		19 May 1980	20 May 1980	21 May 1990	22 May 1980	23 May 1980	28 May* 1980	1 Sept.* 1980
0	0	0	0	0	0	0	0	0
0.15	4.14	30.0	30.0	31.0	25.5	32.0	-	34.5
0.30	8.28	57.0	60.0	60.5	50.0	54.0	27.5	61.5
0.45	12.42	81.5	86.5	86.5	76.0	78.5	-	92.0
0.60	16.56	102.0	110.0	110.0	98.0	100.0	52.5	119.5
0.75	20.70	122.0	133.0	132.0	118.0	122.0	-	146.5
0.90	24.84	-	-	-	-	-	75.0	-
1.20	33.12	-	-	-	-	-	93.0	-
1.50	41.40	-	-	-	-	-	110.5	-
Chart deflection (mm) for 10 ppm		67.0	68.0	68.0	63.0	66.0	74.0	75.0

*Drosophila main test, dose level = 25 ppm

*1 September 1980 - repeat acute cytogenetics (409959 MR)

Instrument Setting

Pathlength: 21.75 m
Wavelength: 11.7 µm
Absorbance Range: 0.25 A
Slit Width: 1 mm
Meter Response: 4
Recorder Voltage: 1 V
Chart Speed: 120 mm/h

Calibration

Syringe: 1 µl Hamilton
Injection Volume: 0.15 µl (0.30)
No. of Repeat Injections: 5

TABLE AT-2

Hexachloro-1,3-butadiene
Calibration Data for High Level

Dose Level: 50 ppm v/v

Batch No.: 15719

Volume µl	Conc., ppm, (v/v)	Cumulative Chart Deflection, mm					
		19 May 1980	20 May 1980	21 May 1980	22 May 1980	23 May 1980	1 Sept.* 1980
0	0	0	0	0	0	0	0
0.75	20.7	29.0	26.0	31.5	34.0	34.0	42.0
1.50	41.4	54.0	49.0	54.0	60.0	60.5	68.0
2.25	62.1	70.0	63.0	71.0	78.0	80.0	85.5
3.0	82.8	82.0	76.0	85.0	88.0	92.0	101.0
3.75	103.5	92.0	86.5	95.5	100.0	104.0	114.0
Chart deflection (mm) for 50 ppm		61.5	54.5	63.0	68.0	69.0	76.0

*1 September 1980 - repeat acute cytogenetics (409959 MR)

Instrument Setting

Pathlength: 21.75 m
Wavelength: 11.7 µm
Absorbance Range: 1 A
Slit Width: 1 mm
Meter Response: 4
Recorder Voltage: 1 V
Chart Speed: 120 mm/h

Calibration

Syringe: 1 µl Hamilton
Injection Volume: 0.75 µl
No. of Repeat Injections: 5

TABLE AT-3

Hexachloro-1,3-butadiene
Chamber Atmosphere Homogeneity Data

Dose Level: 10 ppm and 50 ppm

Sample Location	% Deviation from Reference Sampling Point	
	Low	High
Reference Point (R)	0	0
Right Centre (RC)	-3.2	-0.9
Right Front (RF)	-3.2	-0.9
Centre Front (CF)	-0.8	-1.8
Left Front (LF)	-3.2	-2.7
Left Centre (LC)	-1.6	0
Left Back (LB)	-1.6	0
Centre Back (CB)	-2.4	0
Right Back (RB)	0	-0.9

Top view of exposure chamber

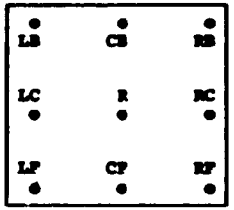


TABLE AT-4

Hexachloro-1,3-butadiene
Atmospheric Analysis by Infra-red Spectroscopy
Target Concentration 10 ppm

Exposure Day	% Deviation from Target Concentration in Minutes								Time Averaged Concentration for 7 h (ppm)
	-10.0	-7.5	-5.0	-2.5	0	+2.5	+5.0	+7.5	
Single	-	-	4	-	152	181	83	-	10.20
Multiple 1	-	-	-	125	35	105	130	25	10.19
Multiple 2	-	-	-	105	90	70	135	20	10.17
Multiple 3	-	-	-	10	155	255	-	-	10.15
Multiple 4	-	-	-	35	330	35	20	-	10.02
Multiple 5	10	15	55	115	155	70	-	-	9.86

TABLE AT-4 (continued)

Hexachloro-1,3-butadiene
Target Concentration 50 ppm

Exposure Day	% Deviation from Target Concentration in Minutes									Time Averaged Concentration for 7 h (ppm)
	-12.5	-10.0	-7.5	-5.0	-2.5	0	+2.5	+5.0	+7.5	
Single	-	-	1	56.5	107.5	10	15	140	90	49.94
Multiple 1	-	25	40	145	105	105	-	-	-	48.17
Multiple 2	-	15	-	-	5	90	170	140	-	51.15
Multiple 3	10	25	20	80	70	110	105	-	-	49.00
Multiple 4	-	-	-	20	65	90	65	155	25	51.03
Multiple 5	-	-	-	20	30	205	90	70	5	50.52

TABLE AT-5

Hexachloro-1,3-butadiene
Atmospheric Analysis by Infra-red Spectroscopy
Target Concentration 25 ppm

Exposure Day	% Deviation from Target Concentration in Minutes						Time Averaged Concentration for 1 h (ppm)
	-7.5	-5.0	-2.5	0	+2.5	+5.0	
Drosophila Main Test	5	20	5	15	5	10	24.64

TABLE BW-1

Hexachloro-1,3-butadiene
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)	10 ppm	50 ppm	250 mg/kg EMS
Male	1	389.4 ± 22.9	386.6 ± 22.4	390.9 ± 18.0	391.2 ± 20.9
	2	398.0 ± 21.7	391.2 ± 22.9	377.7 ± 19.9	370.5 ± 38.8
	3	400.5 ± 23.8	391.1 ± 23.1	370.1 ± 20.6	366.4 ± 17.1
	4	403.1 ± 24.9	394.0 ± 23.0	356.9 ± 14.7	357.1 ± 16.5
	5	405.7 ± 23.6	392.9 ± 25.9	351.9 ± 12.7	349.0 ± 16.9
	Weight gain /loss	16.3	6.3	-39.0	-42.2
Female	1	239.8 ± 17.2	231.8 ± 12.0	229.9 ± 9.1	236.5 ± 10.1
	2	241.9 ± 15.9	230.6 ± 12.5	221.1 ± 11.6	227.8 ± 9.9
	3	243.3 ± 13.8	230.0 ± 11.7	214.4 ± 12.3	218.8 ± 9.3
	4	243.2 ± 13.0	229.9 ± 11.9	213.9 ± 14.2	211.4 ± 9.4
	5	244.5 ± 13.3	228.1 ± 11.2	213.8 ± 14.5	206.8 ± 10.6
	Weight gain /loss	4.7	-3.7	-16.1	-29.7

TABLE BW-2 (Repeat)

Hexachloro-1,3-butadiene
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)	10 ppm	50 ppm	250 mg/kg IPMS
Male	6	367.0 ± 10.0	363.1 ± 17.7	356.0 ± 10.7	364.8 ± 12.2
	24	370.3 ± 16.5	352.8 ± 12.9	349.8 ± 16.2	371.1 ± 21.3
	48	356.5 ± 12.2	362.3 ± 12.4	359.5 ± 18.1	368.5 ± 12.5
Female	6	209.4 ± 20.1	224.8 ± 18.3	215.6 ± 16.4	216.4 ± 11.7
	24	215.6 ± 14.2	215.9 ± 12.1	215.6 ± 10.1	215.7 ± 20.5
	48	218.0 ± 13.1	218.4 ± 14.4	217.0 ± 18.5	221.1 ± 10.9

TABLE BW-3

Hexachloro-1,3-butadiene
Dominant Lethal Assay
Group Mean Body Weights (g) for the Dosing Period of Male CD Rats

Day	Air Control (0 ppm)	10 ppm	50 ppm	5 x 100 mg/kg EMS
1	378.4 ± 17.9	387.4 ± 23.2	374.5 ± 14.5	400.9 ± 12.9
2	388.7 ± 20.5	391.4 ± 22.4	362.2 ± 16.1	390.0 ± 14.0
3	390.2 ± 21.9	391.3 ± 23.5	356.1 ± 14.7	375.9 ± 16.1
4	394.0 ± 22.3	393.7 ± 24.2	342.9 ± 13.2	362.5 ± 17.1
5	398.2 ± 22.7	395.9 ± 26.2	340.8 ± 15.7	353.6 ± 18.5
Weight gain/loss	19.8	8.5	-46.6	-47.3

TABLE BW-4

Hexachloro-1,3-butadiene
Sperm Abnormalities Test
Group Mean Body Weights (g) for the Dosing Period of Male B6C3F$_1$ Mice

Day	Air Control (0 ppm)	10 ppm	50 ppm	5 x 200 mg/kg EMS
1	26.3 ± 1.2	28.0 ± 1.1	26.4 ± 1.6	27.5 ± 1.6
2	26.7 ± 1.4	26.7 ± 1.3	†	27.1 ± 1.4
3	27.0 ± 1.3	28.3 ± 1.3	†	27.2 ± 1.5
4	27.1 ± 1.4	27.9 ± 1.4	†	27.3 ± 1.7
5	27.5 ± 1.4	27.8 ± 1.4	†	26.2 ± 1.8
Weight gain/loss	1.2	-0.2	†	-1.3

† = all animals dead

TABLE UDS-1

Hexachloro-1,3-butadiene
Unscheduled DNA Synthesis

Substance	Concentration (µg/ml)		Mean Number of Grains/Nucleus ± S.D.	
	With S-9	Without S-9	With S-9	Without S-9
Dimethylsulphoxide	10,000	10,000	3.6 ± 9.6	7.1 ± 4.3
4-Nitroquinoline-N-oxide	-	1.25	-	97.0 ± 31.1
2-Aminoanthracene	5	-	78.9 ± 46.2	-
Hexachloro-1,3-butadiene	8	8	4.8 ± 2.8	4.2 ± 2.8
	16	16	6.9 ± 4.7	5.0 ± 8.5
	31	31	T	6.7 ± 4.3
	63	63	T	6.7 ± 3.9
	125	125	T	19.0 ± 10.7
	250	250	T	24.6 ± 11.0
	500	500	T	5.7 ± 3.7
	1,000	1,000	T	15.6 ± 10.5

T = Toxic, all cells dead

TABLE UDS-2

Hexachloro-1,3-butadiene
Unscheduled DNA Synthesis

Substance	Concentration (pg/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	1.5 ± 2.5	3.5 ± 8.6
4-Nitroquinoline-N-oxide	-	10	-	48.9 ± 28.3
2-Aminoanthracene	50	-	18.1 ± 8.6	-
Hexachloro-1,3-butadiene	0.15	0.15	3.6 ± 11.8	3.5 ± 3.4
	0.30	0.30	2.4 ± 5.8	3.3 ± 2.3
	0.60	0.60	2.4 ± 4.0	2.2 ± 1.4
	1.25	1.25	2.4 ± 6.3	2.8 ± 2.0
	2.50	2.50	2.2 ± 2.0	5.0 ± 4.0
	5.00	5.00	3.0 ± 7.8	3.9 ± 2.5
	10.00	10.00	3.0 ± 5.9	*
	20.00	20.00	0	*

* = Insufficient cells present on the coverslips for reasonable estimation

TABLE UDS-3

Hexachloro-1-3-butadiene
Unscheduled DNA Synthesis

Substance	Concentration (µg/ml)	Mean number of Grains/Nucleus ± S.D.
	Without S-9	Without S-9
Dimethylsulphoxide	10,000	5.5 ± 4.2
4-Nitroquinoline-N-oxide	0.6	47.7 ± 13.8
Hexachloro-1-3-butadiene	8	7.1 ± 5.3
	16	6.1 ± 4.5
	31	9.2 ± 5.3
	63●	4.2 ± 6.0
	125	T
	250	T

T = Toxic, all cells dead
● = Toxic level with relatively few cells remaining in coverslips i.e. only 74 nuclei counted

TABLE CA-MD-M-1

Hexachloro-1,3-butadiene
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	400	2	1	-	-	-	-	1 Chromatid Fragment
10 ppm, 7 h/day	450	8	-	-	-	-	-	2 Chromatid Fragments
50 ppm, 7 h/day	500	5	2	-	-	-	-	1 Chromatid Fragment
EMS, 100 mg/kg/day	450	11	3	1	-	-	-	5 Chromatid Fragments 1 Chromosomal Fragment 2 Exchanges 2 Robertsonian Translocations 1 Pair of Minutes 1 Multi Aberration

TABLE CA-MD-M-2

Hexachloro-1,3-butadiene
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	0.240	0.056		0.190	0.041	
10 ppm	0.318	0.052	1.01	0.184	0.039	-0.10
50 ppm	0.281	0.050	0.55	0.200	0.037	0.18
EMS, 100 mg/kg	0.410	0.052	2.23*	0.304	0.039	2.02

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

*p<0.05

TABLE CA-MD-F-1

Hexachloro-1,3-butadiene
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Females

Multiple Dosing

Sampling Time: 6 h

Group	Number of Spreads Observed	Observed Aberrations						Miscellaneous
		Chromatid			Chromosome			
		Gap	B w F	B w/o F	Gap	B w F	B w/o F	
Air Control, 7 h/day	500	10	1	-	-	1	-	2 Chromatid Fragments
10 ppm, 7 h/day	500	8	-	-	-	-	-	1 Chromatid Fragment
50 ppm, 7 h/day	500	4	1	-	-	-	-	-
EMS, 100 mg/kg/day	464	14	3	2	1	-	-	1 Multi Aberration 2 Chromatid Fragments

TABLE CA-MD-F-2

Hexachloro-1,3-butadiene
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	0.322	0.044		0.200	0.033	
10 ppm	0.291	0.044	-0.51	0.160	0.033	-0.86
50 ppm	0.211	0.044	-1.80	0.160	0.033	-0.86
EMS, 100 mg/kg	0.423	0.044	1.64	0.270	0.033	1.51

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

TABLE CA-M6-1

Hexachloro-1,3-butadiene
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Males

Sampling Time: 6 h

Single Dosing

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	1	-	-	-	-	-	2 Chromatid Fragments
10 ppm, 7 h/day	486	-	-	-	-	1	-	1 Chromatid Fragment
50 ppm, 7 h/day	500	4	1	-	-	-	-	1 Chromatid Fragment
EMS, 250 mg/kg/day	450	11	4	-	-	-	-	1 Chromosomal Fragment

TABLE CA-M6-1 (Supplementary)

Hexachloro-1,3-butadiene
Cytogenetic Analaysis of Rat Bone Marrow Cells
Supplementary Observations
Males

Single Dosing **Sampling Time: 6 h**

Group	Animal No./Sex	Miscellaneous Observations
Air Control, 7 h/day	5♂	1 Chromosome split at centromere
50 ppm, 7 h/day	70♂	1 Chromosome split at centromere

TABLE CA-M6-2

Hexachloro-1,3-butadiene
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	0.191	0.040		0.180	0.031	
10 ppm	0.180	0.040	-0.19	0.180	0.031	-0.01
50 ppm	0.241	0.040	0.89	0.180	0.031	0.00
EMS, 250 mg/kg	0.392	0.042	3.47**	0.241	0.033	1.35

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

**p<0.01

TABLE CA-M24-1

Hexachloro-1,3-butadiene
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Males

Single Dosing

Sampling Time: 24 h

Group	Number of Spreads Observed	Observed Aberrations						Miscellaneous
		Chromatid			Chromosome			
		Gap	B w F	B w/o F	Gap	B w F	B w/o F	
Air Control, 7 h/day	500	2	1	-	-	-	-	-
10 ppm, 7 h/day	400	-	-	-	-	-	-	-
50 ppm, 7 h/day	500	3	-	-	-	-	-	-
EMS, 250 mg/kg/day	500	3	5	1	1	-	-	7 Chromatid Fragments 2 Exchanges 2 Multi Aberrations

TABLE CA-M24 and F24-1 (Supplementary)

Hexachloro-1,3-butadiene
Cytogenetic Analysis of Rat Bone Marrow Cells
Males and Females

Single Dosing — Sampling Time: 24 h

Group	Animal No./Sex	Miscellaneous Observations
Air Control, 7 h/day	13 ♂	2 Chromosomes split at centromere
10 ppm, 7 h/day	207 ♀	1 Chromosome split at centromere

TABLE CA-M24-2

Hexachloro-1,3-butadiene
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	0.200	0.035		0.160	0.026	
10 ppm	0.141	0.039	-1.13	0.141	0.029	-0.52
50 ppm	0.200	0.035	0.00	0.141	0.026	-0.55
EMS, 250 mg/kg	0.357	0.035	3.14**	0.330	0.026	4.70***

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

**p<0.01
***p<0.001

TABLE CA-M48-1

Hexachloro-1,3-butadiene
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	-	-	-	-	-	-	1 Chromatid Fragment
10 ppm, 7 h/day	500	2	1	-	-	-	-	-
50 ppm, 7 h/day	450	2	-	-	-	-	-	-
EMS, 250 mg/kg/day	500	10	6	-	-	-	-	1 Robertsonian Translocation 1 Exchange 3 Chromatid Fragments

TABLE CA-M48-1 (Supplementary)

Hexachloro-1,3-butadiene
Cytogenetic Analysis of Rat Bone Marrow Cells
Supplementary Observations
Males

Single Dosing — Sampling Time: 48 h

Group	Animal No./Sex	Miscellaneous Observations
10 ppm, 7 h/day	56♂	1 Chromosome split at centromere

TABLE CA-M48-2

Hexachloro-1,3-butadiene
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	0.160	0.039		0.160	0.029	
10 ppm	0.200	0.039	0.73	0.160	0.029	0.00
50 ppm	0.185	0.041	0.43	0.141	0.031	-0.48
EMS, 250 mg/kg	0.302	0.039	2.60*	0.227	0.029	1.62

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

*p<0.05

TABLE CA-F6-1

Hexachloro-1,3-butadiene
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	479	-	-	-	-	-	-	-
10 ppm, 7 h/day	500	5	-	-	-	-	-	2 Chromatid Fragments
50 ppm, 7 h/day	450	1	1	-	-	-	-	-
EMS, 250 mg/kg/day	350	5	4	-	1	-	-	-

TABLE CA-F6-2

Hexachloro-1,3-butadiene
Cytogenetic Analysis of Rat Bone Marrow Cells
Summary of Oberved 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	0.144	0.031		0.145	0.025	
10 ppm	0.260	0.031	2.60*	0.180	0.025	1.00
50 ppm	0.185	0.033	0.89	0.163	0.026	0.49
EMS, 250 mg/kg	0.371	0.037	4.67***	0.241	0.030	2.45*

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

*p<0.05
***p<0.001

TABLE CA-F24-1

Hexachloro-1,3-butadiene
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Females

Single Dosing

Sampling Time: 24 h

Group	Number of Spreads Observed	Observed Aberrations						Miscellaneous
		Chromatid			Chromosome			
		Gap	B w F	B w/o F	Gap	B w F	B w/o F	
Air Control, 7 h/day	500	1	-	-	-	-	-	-
10 ppm, 7 h/day	500	1	1	-	-	-	-	1 Chromatid Fragment 1 Exchange 1 Pair of Minutes
50 ppm, 7 h/day	500	1	-	-	-	-	-	-
EMS, 250 mg/kg/day	500	15	12	-	3	-	-	3 Chromatid Fragments 4 Exchanges 5 Multi Aberrations

TABLE CA-F24-2

Hexachloro-1,3-butadiene
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	0.160	0.032		0.141	0.027	
10 ppm	0.199	0.032	0.86	0.191	0.027	1.32
50 ppm	0.160	0.032	0.00	0.141	0.027	-0.00
EMS, 250 mg/kg	0.532	0.032	8.27***	0.415	0.027	7.19***

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

***p<0.001

TABLE CA-F48-1

Hexachloro-1,3-butadiene
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	400	-	1	-	-	-	-	1 Chromatid Fragment
10 ppm, 7 h/day	350	1	-	-	-	-	-	-
50 ppm 7 h/day	400	2	-	-	-	-	-	-
EMS, 250 mg/kg/day	300	7	5	-	1	-	-	2 Multi Aberrations 2 Exchanges 4 Chromatid Fragments

TABLE CA-F48-2

Hexachloro-1,3-butadiene
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	0.190	0.048		0.190	0.041	
10 ppm	0.169	0.051	-0.31	0.141	0.044	-0.82
50 ppm	0.190	0.048	0.00	0.141	0.041	0.85
EMS, 250 mg/kg	0.385	0.043	3.04**	0.302	0.037	2.01

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

**p<0.01

TABLE DL-1

Hexachloro-1,3-butadiene
Dominant Lethal Test in Rats
Pregnancy Frequency (Females with Corpora Lutea Graviditatis)

Assessment Week from Dosing	Air Control (0 ppm)	10 ppm	50 ppm	5 x 100 mg/kg EMS
1	90%	95%	84%	85%
2	90%	100%	84%	75%
3	95%	95%	84%	45%
4	100%	95%	95%	95%
5	90%	100%	89%	94%
6	95%	90%	89%	100%
7	95%	100%	84%	100%
8	100%	100%	89%	90%
9	100%	100%	95%	100%
10	100%	90%	89%	95%

TABLE DL-2

Hexachloro-1,3-butadiene
Dominant Lethal Test in Rats
Pregnancy Frequency (Females with Implantations)

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)		10 ppm		50 ppm		5 x 100 mg/kg EMS	
1	18/20	90%	19/20	95%	16/19	84%	13/20	65%
2	17/20	85%	19/19	100%	16/18	89%	8/20	40%
3	19/20	95%	19/20	95%	16/18	89%	6/20	30%
4	19/20	95%	19/20	95%	17/18	94%	18/20	90%
5	17/20	85%	20/20	100%	17/18	94%	16/18	89%
6	19/20	95%	18/20	90%	17/18	94%	19/20	95%
7	19/20	95%	19/20	95%	16/18	89%	18/20	90%
8	20/20	100%	20/20	100%	17/18	94%	18/20	90%
9	20/20	100%	19/20	95%	18/18	100%	19/20	95%
10	19/20	95%	18/20	90%	17/18	94%	18/19	95%

TABLE DL-3

Hexachloro-1,3-butadiene
Dominant Lethal Test in Rats
Total Number of Corpora Lutea per Pregnancy

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	10 ppm	50 ppm	5 x 100 mg/kg EMS
1	[1] 15.1 ± 0.64	13.0 ± 0.62*	13.4 ± 0.67	12.4 ± 1.76
2	13.7 ± 0.52	12.8 ± 0.49	12.4 ± 0.53	4.3 ± 0.53***
3	13.3 ± 0.56	13.1 ± 0.56	12.4 ± 0.61	2.2 ± 0.87***
4	13.4 ± 0.49	12.2 ± 0.49	13.3 ± 0.52	10.6 ± 0.90*
5	12.9 ± 0.51	13.0 ± 0.47	13.1 ± 0.51	13.1 ± 0.44
6	13.3 ± 0.58	13.7 ± 0.59	12.4 ± 0.61	13.2 ± 0.70
7	12.4 ± 0.53	13.6 ± 0.53	12.9 ± 0.57	13.3 ± 0.54
8	13.3 ± 0.45	12.9 ± 0.45	12.7 ± 0.48	12.4 ± 0.41
9	12.4 ± 0.54	12.8 ± 0.56	13.1 ± 0.57	13.4 ± 0.35
10	13.5 ± 0.57	13.8 ± 0.58	13.2 ± 0.60	13.9 ± 0.72

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

TABLE DL-4

Hexachloro-1,3-butadiene
Dominant Lethal Test in Rats
Total Implantations per Pregnancy

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	10 ppm	50 ppm	5 x 100 mg/kg EMS
1	[1] 13.7 ± 0.36	13.1 ± 0.35	12.2 ± 0.38**	9.3 ± 1.07**
2	13.2 ± 0.57	12.5 ± 0.54	11.6 ± 0.59	1.3 ± 0.16***
3	13.2 ± 0.59	13.5 ± 0.59	12.5 ± 0.64	1.3 ± 0.33***
4	13.1 ± 0.54	12.5 ± 0.54	13.5 ± 0.58	9.5 ± 1.06**
5	13.1 ± 0.59	12.8 ± 0.55	13.2 ± 0.59	12.9 ± 0.54
6	12.8 ± 0.65	13.7 ± 0.67	11.7 ± 0.69	12.7 ± 0.50
7	11.8 ± 0.56	13.0 ± 0.56	12.3 ± 0.61	13.1 ± 0.46
8	12.5 ± 0.49	12.1 ± 0.49	13.2 ± 0.53	11.2 ± 0.66
9	12.2 ± 0.41	12.2 ± 0.42	12.4 ± 0.43	12.6 ± 0.48
10	13.6 ± 0.54	14.3 ± 0.56	13.2 ± 0.57	13.5 ± 0.82

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

TABLE DL-5

Hexachloro-1,3-butadiene
Dominant Lethal Test in Rats
Live Implantations per Pregnancy

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	10 ppm	50 ppm	5 x 100 mg/kg EMS
1	[1] 13.2 ± 0.57	12.8 ± 0.56	10.9 ± 0.61**	4.2 ± 1.03***
2	12.5 ± 0.61	12.1 ± 0.58	11.1 ± 0.63	0.0 ± 0.00***
3	12.5 ± 0.60	12.6 ± 0.60	12.0 ± 0.65	0.0 ± 0.00***
4	12.5 ± 0.57	11.9 ± 0.57	12.7 ± 0.60	6.1 ± 1.11***
5	12.4 ± 0.56	12.3 ± 0.51	12.7 ± 0.56	12.0 ± 0.59
6	12.3 ± 0.63	13.3 ± 0.65	11.2 ± 0.67	12.3 ± 0.55
7	11.4 ± 0.59	12.4 ± 0.59	11.7 ± 0.64	12.4 ± 0.51
8	12.1 ± 0.51	11.5 ± 0.51	12.8 ± 0.55	10.6 ± 0.63
9	11.5 ± 0.41	11.8 ± 0.42	11.8 ± 0.43	11.7 ± 0.49
10	12.7 ± 0.61	13.4 ± 0.63	12.6 ± 0.64	12.9 ± 0.79

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

TABLE DL-6

Hexachloro-1,3-butadiene
Dominant Lethal Test in Rats
Live Implantations and Late Deaths per Pregnancy

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	10 ppm	50 ppm	5 x 100 mg/kg EMS
1	[1] 13.2 ± 0.57	12.8 ± 0.56	10.9 ± 0.61**	4.3 ± 1.01***
2	12.5 ± 0.61	12.1 ± 0.58	11.1 ± 0.63	0.0 ± 0.00***
3	12.5 ± 0.60	12.6 ± 0.60	12.0 ± 0.65	0.0 ± 0.00***
4	12.5 ± 0.57	11.9 ± 0.57	12.7 ± 0.60	6.1 ± 1.12***
5	12.5 ± 0.55	12.4 ± 0.51	12.7 ± 0.55	12.1 ± 0.59
6	12.4 ± 0.64	13.3 ± 0.66	11.2 ± 0.68	12.3 ± 0.55
7	11.4 ± 0.59	12.4 ± 0.59	11.7 ± 0.64	12.6 ± 0.54
8	12.1 ± 0.51	11.5 ± 0.51	12.8 ± 0.55	10.6 ± 0.63
9	11.6 ± 0.41	11.8 ± 0.42	11.8 ± 0.43	11.8 ± 0.49
10	12.8 ± 0.61	13.4 ± 0.62	12.6 ± 0.64	12.9 ± 0.79

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

TABLE DL-7

Hexachloro-1,3-butadiene
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)		10 ppm		50 ppm		5 x 100 mg/kg EMS	
	>0	>1	>0	>1	>0	>1	>0	>1
1	6/18	2/18	5/19	1/19	6/16	3/16	12/13	11/13
2	8/17	2/17	6/19	1/19	7/16	1/16	8/8	2/8
3	9/19	3/19	11/19	3/19	7/16	1/16	6/6	1/6
4	8/19	3/19	8/19	3/19	9/17	1/17	18/18	15/18
5	6/17	4/17	6/20	2/20	5/17	3/17	8/16	4/16
6	7/19	1/19	5/18	1/18	5/17	1/17	5/19	2/19
7	8/19	1/19	8/19	3/19	7/16	3/16	8/18	1/18
8	7/20	2/20	6/20	3/20	6/17	0/17	9/18	2/18
9	9/20	2/20	5/19	1/19	10/18	1/18	14/19	2/19
10	9/19	4/19	10/18	4/18	8/17	1/17	9/18	2/18

TABLE DL-8

Hexachloro-1,3-butadiene
Dominant Lethal Test in Rats
Early Death Frequency, Freeman-Tukey Poisson Transformation

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	10 ppm	50 ppm	5 x 100 mg/kg EMS
1	[1] 1.553 ± 0.2599	1.411 ± 0.2529	1.963 ± 0.2756	4.453 ± 0.4126***
2	1.786 ± 0.2065	1.516 ± 0.1953	1.664 ± 0.2128	2.597 ± 0.1198*
3	1.816 ± 0.2137	2.023 ± 0.2137	1.664 ± 0.2328	2.634 ± 0.2196*
4	1.711 ± 0.2245	1.711 ± 0.2245	1.929 ± 0.2373	3.820 ± 0.2278***
5	1.706 ± 0.2254	1.497 ± 0.2078	1.580 ± 0.2254	1.927 ± 0.2545
6	1.560 ± 0.1860	1.466 ± 0.1911	1.493 ± 0.1967	1.449 ± 0.1824
7	1.634 ± 0.1977	1.711 ± 0.1979	1.756 ± 0.2154	1.669 ± 0.1856
8	1.568 ± 0.1907	1.589 ± 0.1907	1.499 ± 0.2068	1.788 ± 0.1982
9	1.739 ± 0.1857	1.442 ± 0.1905	1.826 ± 0.1957	2.119 ± 0.1659
10	1.881 ± 0.2216	1.981 ± 0.2277	1.743 ± 0.2343	1.788 ± 0.1982

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

TABLE DL-9

Hexachloro-1,3-butadiene
Dominant Lethal Test in Rats
Early Death Frequency, Freeman-Tukey Binomial Transformation

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	10 ppm	50 ppm	5 x 100 mg/kg EMS
1	[1] 0.416 ± 0.0960	0.387 ± 0.0934	0.614 ± 0.1019	1.762 ± 0.2058***
2	0.448 ± 0.0640	0.444 ± 0.0605	0.486 ± 0.0660	2.377 ± 0.029***
3	0.486 ± 0.0613	0.561 ± 0.0613	0.462 ± 0.0668	2.379 ± 0.0447***
4	0.464 ± 0.0624	0.488 ± 0.0624	0.525 ± 0.0659	1.488 ± 0.1426***
5	0.453 ± 0.0590	0.412 ± 0.0544	0.432 ± 0.0590	0.537 ± 0.0745
6	0.429 ± 0.0518	0.390 ± 0.0532	0.448 ± 0.0547	0.405 ± 0.0541
7	0.492 ± 0.0638	0.469 ± 0.0638	0.497 ± 0.0695	0.463 ± 0.0570
8	0.445 ± 0.0544	0.454 ± 0.0544	0.407 ± 0.0590	0.528 ± 0.0550
9	0.490 ± 0.0516	0.402 ± 0.0529	0.513 ± 0.0544	0.592 ± 0.0496
10	0.503 ± 0.0701	0.526 ± 0.0721	0.506 ± 0.0741	0.495 ± 0.0534

1 = Mean ± standard error of mean
***p<0.001

TABLE SA-2

Hexachloro-1,3-butadiene
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	3.84 ± 0.779	8.49 ± 0.908	23.37 ± 2.420	18.65 ± 2.420	20.33 ± 2.317	37.73 ± 3.312
10 ppm, 7 h/day	6.39* ± 0.821	7.8 ± 0.957	26.71 ± 2.551	19.93 ± 2.810	25.21 ± 2.442	41.92 ± 3.492
50 ppm, 7 h/day	†-	-	-	-	-	-
EMS, 200 mg/kg/day	4.87 ± 0.779	8.77 ± 0.908	31.47* ± 2.420	20.85 ± 2.665	24.14 ± 2.317	46.06 ± 3.312

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)

† = All animals dead as a result of dosing

*P<0.05

TABLE EL-1

Hexachloro-1,3-butadiene
Drosophila Dose Ranging Experiment

Day		25 ppm						Date & Initial
		½ h		1 h		2 h		
0	No. of males exposed	100		100		100		15.5.80 KT
1	No. & % survival	100	100	98	98	0	0	16.5.80 KT
2	No. of eggs laid by 10 females	265		316		0		17.5.80 KT
3	No. & % hatched	247	93%	281	88.9	0	0	18.5.80 KT

Comments: Control:- eggs laid = 302
eggs hatched = 282 = 93%

Time chosen for test exposure: 1 h
First exposure done at 75 ppm, all flies killed.

TABLE EL-2 (continued)

Hexachloro-1,3-butadiene
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	29.5.80	1.6.80	6.6.80
F_2 set up	12.6.80	17.6.80	20.6.80
F_2 scored	24.6.80	30.6.80	4.7.80
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	100	97	88	285
No. of sterile F_1 vials	12	17	16	45
No. of F_1 vials used in F_2	88	80	72	240
No. of F_2 vials set up	600	608	592	1800
No. of F_2 vials scored	542	509	545	1596
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 BL-2 (continued)

Hexachloro-1,3-butadiene
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	29.5.80	1.6.80	6.6.80
F_2 set up	12.6.80	17.6.80	20.6.80
F_2 scored	24.6.80	2.7.80	3.7.80
F_2 repeats scored	-	-	-
F_3 set up	-	-	3.7.80
F_3 scored	-	-	15.7.80
F_3 repeats scored	-	-	-

RESULTS

	Brood 1	Brood 2	Brood 3	All Broods
No. of F_1 vials	100	100	91	291
No. of sterile F_1 vials	22	28	30	80
No. of F_1 vials used in F_2	78	72	61	211
No. of F_2 vials set up	600	595	600	1795
No. of F_2 vials scored	526	458	539	1523
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	-	-	500	500
No. of F_3 vials scored	-	-	486	486
No. of F_3 vials containing lethals	-	-	0	0
Frequency of F_3 lethals	-	-	0	0

TABLE XL-2

Hexachloro-1,3-butadiene

Drosophila SLRL Procedure and Results

Compound: Hexachloro-1,3-butadiene Concentration: 25 ppm Stock: A
Length of Exposure: 1 h Test exposure given: 28.5.80

	Brood 1	Brood 2	Brood 3
F_1 set up	29.5.80	1.6.80	6.6.80
F_2 set up	11.6.80	13.6.80	18.6.80
F_2 scored	23.6.80	26.6.80	4.7.80
F_2 repeats scored	-	10.7.80	-
F_3 set up	-	-	-
F_3 scored	-	-	-
F_3 repeats scored	-	-	-

RESULTS

	Brood 1	Brood 2	Brood 3	All Broods
No. of F_1 vials	101	98	78	277
No. of sterile F_1 vials	12	19	12	43
No. of F_1 vials used in F_2	89	79	66	234
No. of F_2 vials set up	600	593	600	1793
No. of F_2 vials scored	534	538	573	1645
No. of F_2 vials containing lethals	1	0	0	1
Frequency of F_2 lethals	0.188	0	0	0.061
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)

Hexachloro-1,3-butadiene

Drosophila SLRL Procedure and Results

Compound: Hexachloro-1,3-butadiene Concentration: 25 ppm Stock: B

Length of Exposure: 1 h Test exposure given: 28.5.80

	Brood 1	Brood 2	Brood 3
F_1 set up	29.5.80	1.6.80	6.6.80
F_2 set up	11.6.80	13.6.80	19.6.80
F_2 scored	24.6.80	27.6.80	1.7.80
F_2 repeats scored	-	-	-
F_3 set up	24.6.80	27.6.80	1.7.80
F_3 scored	7.7.80	10.7.80	14.7.80
F_3 repeats scored	-	-	-

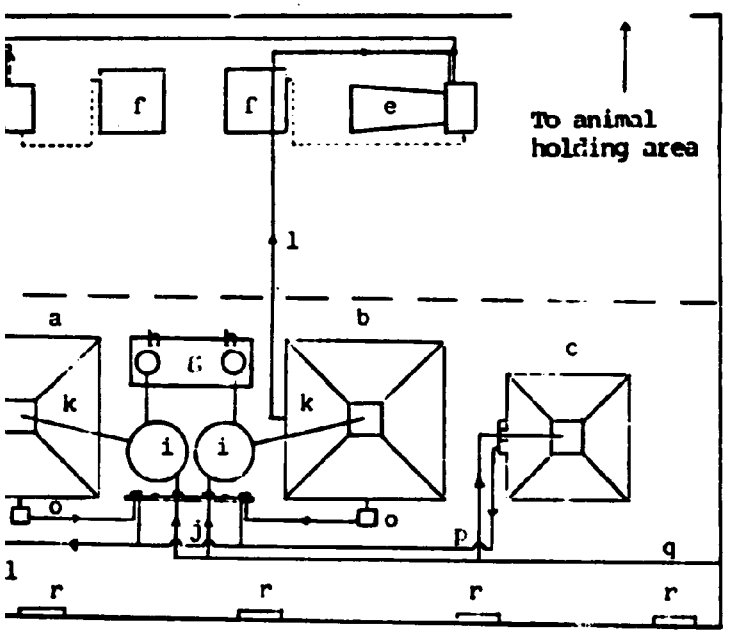
RESULTS

	Brood 1	Brood 2	Brood 3	All Broods
No. of F_1 vials	98	97	93	288
No. of sterile F_1 vials	15	19	26	60
No. of F_1 vials used in F_2	83	78	68	229
No. of F_2 vials set up	601	601	609	1811
No. of F_2 vials scored	517	525	574	1616
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	500	500	400	1400
No. of F_3 vials scored	479	481	378	1338
No. of F_3 vials containing lethals	1	0	0	1
Frequency of F_3 lethals	0.201	0	0	0.07%

FIGURE 1a

Hexachloro-1,3-butadiene

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

Hexachloro-1,3-butadiene
Schematic Lay-out of Apparatus

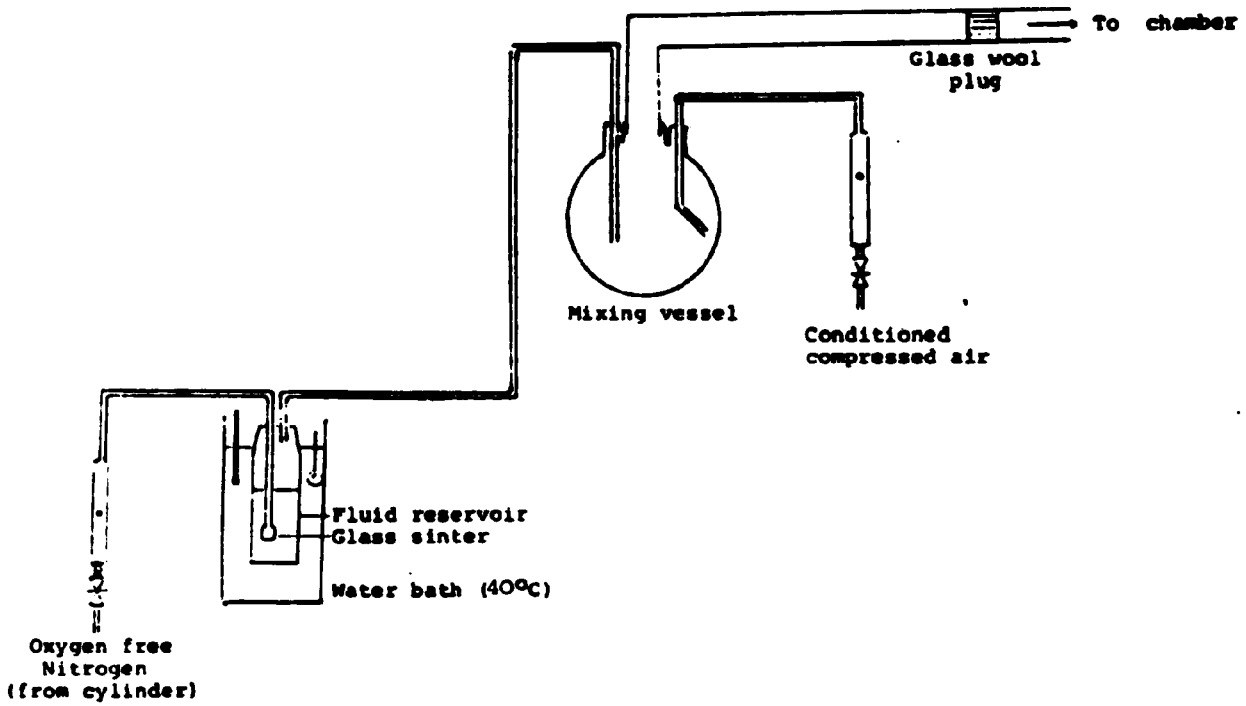


FIGURE 2

Hexachloro-1,3-butadiene
Typical Calibration Graph for High Level
21 May 1980

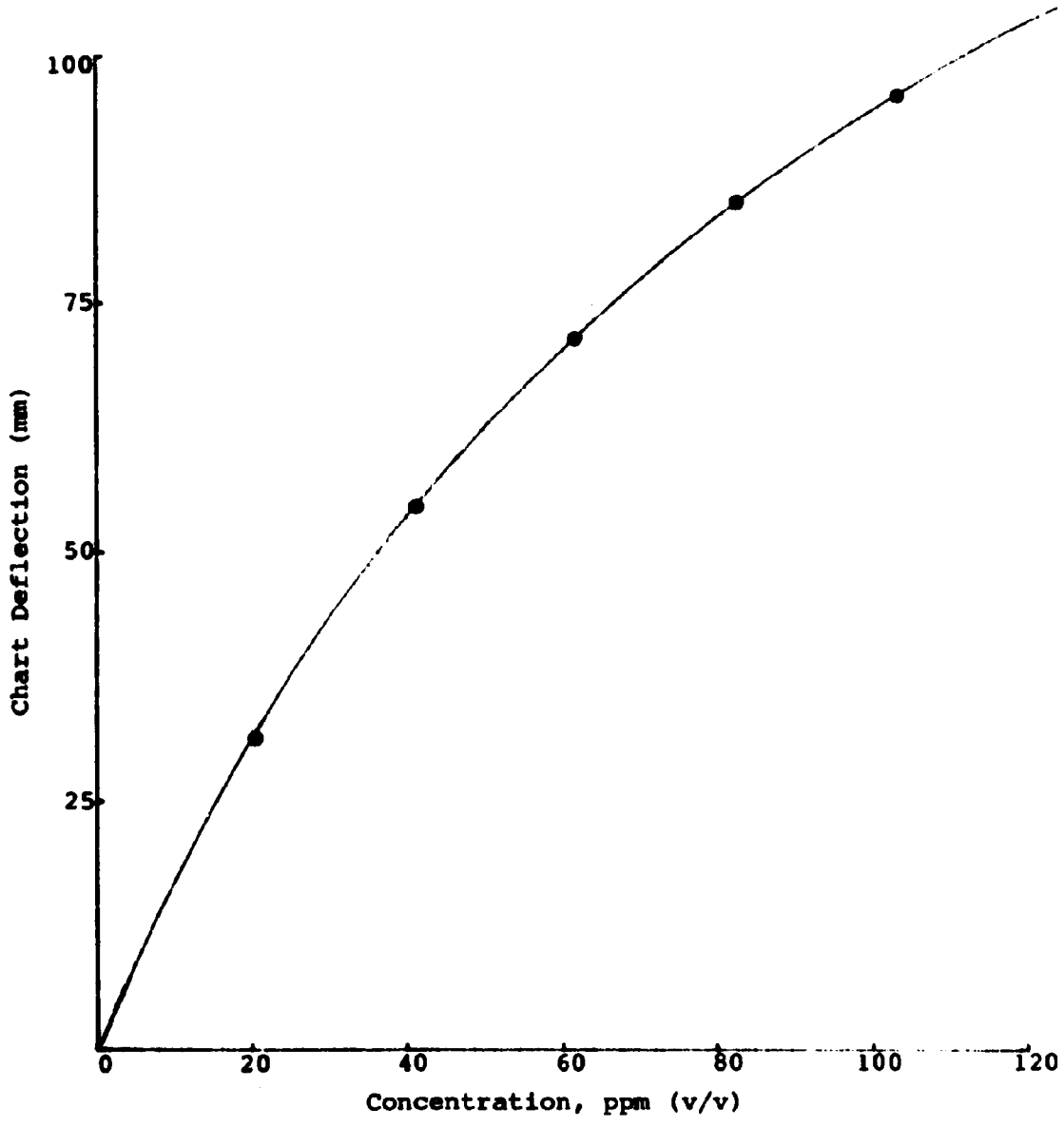


FIGURE 3

Hexachloro-1,3-butadiene
Sample Record Chart of IR Absorption at 8.3 μm

APPENDIX DIET

Hexachloro-1,3-butadiene
Diet Analysis

Spratt's Patent Ltd

Central House
Cambridge Road
Barking
Essex IG11 8NR

Telephone
01-594 7121
Telegrams
Spratt's Barking
Telex 896283

CERTIFICATE OF ANALYSIS

Product: LAD 2

Batch No: 078068

Date of Manufacture: 7th May 1980

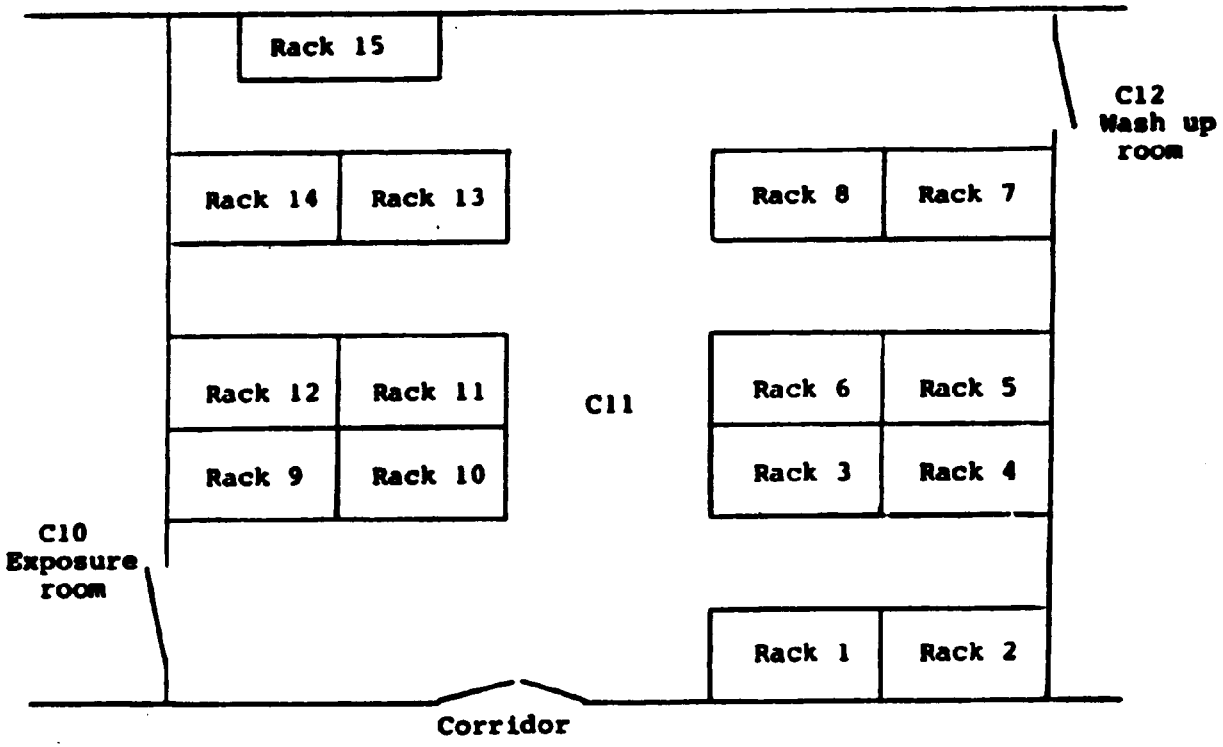
	Found Analysis
Moisture	8.1 %
Crude Fat	3.2 %
Crude Protein	20.9 %
Ash	5.5 %
Calcium	1.10 %
Phosphorus	0.78 %
Nitrate	16.0 mg/kg
Nitrite	< 1.0 mg/kg
Selenium	0.41 mg/kg
Lead	< 1.0 mg/kg
Arsenic	< 0.2 mg/kg
Cadmium	< 0.2 mg/kg
Mercury	0.02 mg/kg
Aflatoxins	NONE DETECTED
Total P.C.B.	NONE DETECTED
Total D.D.T.	0.003 mg/kg
Dieldrin	NONE DETECTED
Lindane	0.010 mg/kg
Heptachlor	0.004 mg/kg
Malathion	0.008 mg/kg
Total Viable Organisms	< 1.0×10^3/grm
E. Coli Type 1	NONE DETECTED
Salmonella Species	NONE DETECTED
Moulds	50/grm

Signed .....

Date .....

APPENDIX Loc-1

Hexachloro-1,3-butadiene
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

Hexachloro-1,3-butadiene
Examples of Animal Location During Exposure
Exposure Location Sheet

Project No: 409959
Test Compound: Air Control
Exposure Chamber No: 1
Day of Study: 2

Test Concentration: 0
Tier No: 1
Multi-dose Cytogenetic ♂ and ♀

LEFT

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

FRONT REAR

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

RIGHT

SIGNED: _____ DATE: _____

APPENDIX Lee-2 (continued)

Hexachloro-1,3-butadiene
Exposure Location Sheet

Project No: 409959
Test Compound: Air Control
Exposure Chamber No: 1
Day of Study: 2

Test Concentration: 0
Tier No: 2
Dominant I-thal ♂
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 IOC-2 (continued)

Hexachloro-1,3-butadiene
Exposure Location Sheet

Project No: 409959
Test Compound: Hexachloro-1,3-butadiene
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	-	-
-	-	-	-

RIGHT

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	-	-
-	-	-	-

FRONT

Signed: _____ Date: _____

APPENDIX Ies-2 (continued)

Hexachloro-1,3-butadiene
Exposure Location Sheet

Project No: 409939 Test Concentration: High

Test Compound: Hexachloro-1,3-butadiene Tier No: 1

Exposure Chamber No: 3

Day of Study: 2

LEFT

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

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

FRONT REAR

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

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

RIGHT

Signed: _____ Date: _____

APPENDIX FORM-1

Hexachloro-1,3-butadiene

[illegible] [illegible]

Contract No. 210-78-0036 Substance:

Assessor: Decoded group:

Signature: Slide No.: Decoded animal No.:

Date(s) Single/Multi-dose Time(h):

Slide quality:

Excellent ____ Good ____ Acceptable ____ Poor ____ Uncountable ____

Number	Chromatid			Chromosomal			Markers			Multi-Abn.	Misc. Abn.	Observation
	GAP	BrdU	Br+U	GAP	BrdU	Br+U	SAC.	DSC	TG			
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

Hexachloro-1,3-butadiene

Contract No. 210-78-0026

Dose Group:

REPRODUCTIVE LETHAL ASSESSMENT

Assessor	Signature

STUDY

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

Hexachloro-1,3-butadiene

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 BM-1

Hexachloro-1,3-butadiene
Multiple Exposure Cytogenetics Test
Individual Body Weights (g)

Air Control (0 ppm)

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	121M	390	401	406	405	409
	122M	345	358	356	360	361
	123M	398	405	401	406	400
	124M	368	376	378	384	386
	125M	397	404	405	400	403
	126M	427	437	443	449	446
	127M	383	388	399	400	400
	128M	398	405	409	415	420
	129M	377	390	386	383	400
	130M	411	416	422	429	432
	Mean ± S.D.	389.4 ± 22.9	398.0 ± 21.7	400.5 ± 23.8	403.1 ± 24.9	405.7 ± 23.6

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	281M	233	233	236	236	237
	282M	230	238	240	233	241
	283M	236	236	237	238	240
	284M	218	227	227	231	228
	285M	240	243	246	246	248
	286M	264	260	266	264	262
	287M	267	267	261	261	265
	288M	219	220	225	226	227
	289M	235	236	239	242	240
	290M	256	251	256	255	257
	Mean ± S.D.	239.8 ± 17.2	241.9 ± 15.9	243.3 ± 13.8	243.2 ± 13.0	244.5 ± 13.3

APPENDIX TABLE BN-1 (continued)

Hexachloro-1,3-butadiene

Multiple Dosing: 10 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	131M	392	392	394	390	398
	132M	397	400	402	400	404
	133M	380	388	386	395	394
	134M	390	398	396	405	398
	135M	396	403	401	406	406
	136M	371	375	379	385	377
	137M	373	376	375	378	378
	138M	342	345	343	346	340
	139M	397	403	403	399	392
	140M	428	432	432	436	442
	Mean ± S.D.	386.6 ± 22.4	391.2 ± 22.9	391.1 ± 23.1	394.0 ± 23.0	392.9 ± 25.9

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	291M	233	228	226	224	224
	292M	255	253	251	250	250
	293M	231	236	234	230	225
	294M	229	232	228	230	230
	295M	223	217	219	217	218
	296M	239	238	238	238	234
	297M	237	237	237	239	236
	298M	239	236	236	239	234
	299M	221	219	221	221	220
	300M	211	210	210	211	210
	Mean ± S.D.	231.8 ± 12.0	230.6 ± 12.5	230.0 ± 11.7	229.9 ± 11.9	228.1 ± 11.2

APPENDIX TABLE BM-1 (continued)

Hexachloro-1,3-butadiene

Multiple Dosing: 50 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	141M	398	387	385	363	363
	142M	392	386	381	364	362
	143M	388	369	370	359	349
	144M	392	374	355	358	346
	145M	375	364	361	353	353
	146M	383	374	371	346	348
	147M	364	353	345	336	332
	148M	382	355	340	340	334
	149M	430	418	407	388	371
	150M	405	397	386	362	361
	Mean ± S.D.	390.9 ± 18.0	377.7 ± 19.9	370.1 ± 20.6	356.9 ± 14.7	351.9 ± 12.7

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	301M	226	220	212	214	213
	302M	247	238	231	235	235
	303M	218	208	206	205	203
	304M	225	215	215	212	214
	305M	235	225	224	226	221
	306M	240	237	223	221	221
	307M	236	232	225	225	226
	308M	224	205	118	184	182
	309M	224	215	210	205	207
	310M	224	216	210	212	216
	Mean ± S.D.	229.9 ± 9.1	221.1 ± 11.6	214.4 ± 12.3	213.9 ± 14.2	213.8 ± 14.5

APPENDIX TABLE HH-1 (continued)

Hexachloro-1,3-butadiene

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	151M	411	401	379	367	353
	152M	413	404	384	374	369
	153M	381	373	365	353	343
	154M	373	362	346	340	329
	155M	375	360	348	337	332
	156M	398	379	361	343	337
	157M	378	372	372	371	365
	158M	392	386	370	362	359
	159M	363	350	344	341	330
	160M	428	418	395	383	373
	Mean ± S.D.	391.2 ± 20.9	370.5 ± 38.8	366.4 ± 17.1	357.1 ± 16.5	349.0 ± 16.9

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	311M	232	222	212	201	193
	312M	231	219	216	217	212
	313M	245	237	231	225	221
	314M	240	228	214	205	197
	315M	256	247	232	221	216
	316M	245	235	232	221	220
	317M	230	218	210	201	196
	318M	229	228	219	215	212
	319M	221	215	208	202	198
	320M	236	229	215	206	203
	Mean ± S.D.	236.5 ± 10.1	227.8 ± 9.9	218.8 ± 9.3	211.4 ± 9.4	206.8 ± 10.6

APPENDIX TABLE BN-2

Hexachloro-1,3-butadiene
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	375	11	379	21	348
	2	356	12	407	22	346
	3	369	13	365	23	357
	4	364	14	372	24	347
	5	349	15	379	25	378
	6	381	16	350	26	348
	7	368	17	366	27	378
	8	370	18	374	28	354
	9	360	19	360	29	360
	10	378	20	351	30	349
	Mean ± S.D.	367.0 ± 10.0		370.3 ± 16.5		356.5 ± 12.2

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Female	161	190	171	218	181	219
	162	195	172	233	182	235
	163	207	173	235	183	203
	164	203	174	204	184	230
	165	245	175	206	185	212
	166	233	176	203	186	195
	167	232	177	212	187	229
	168	200	178	210	188	230
	169	203	179	236	189	217
	170	186	180	199	190	210
	Mean ± S.D.	209.4 ± 20.1		215.6 ± 14.2		218.0 ± 13.1

APPENDIX TABLE BW-2 (continued)

Hexachloro-1,3-butadiene

Single Dosing: 10 ppm

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Male	31	345	41	351	51	390
	32	343	42	348	52	360
	33	352	43	356	53	361
	34	365	44	343	54	374
	35	363	45	384	55	365
	36	368	46	347	56	351
	37	381	47	361	57	363
	38	363	48	456	58	359
	39	401	49	340	59	354
	40	350	50	342	60	346
	Mean ± S.D.	363.1 ± 17.7		352.8 ± 12.9		362.3 ± 12.4

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Female	191	239	201	237	211	218
	192	227	202	205	212	244
	193	211	203	213	213	214
	194	265	204	212	214	203
	195	200	205	208	215	206
	196	209	206	218	216	226
	197	230	207	204	217	198
	198	225	208	238	218	232
	199	227	209	210	1219(b)	214
	1200(a)	215	210	214	220	229
	Mean ± S.D.	224.8 ± 18.3		215.9 ± 12.1		218.4 ± 14.4

(a) 1200 replaced No. 200 - animal littered down

(b) 1219 replaced No. 219 - animal appeared emaciated

APPENDIX TABLE BH-2 (continued)

Hexachloro-1,3-butadiene

Single Dosing: 50 ppm

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Male	61	352	71	357	81	367
	62	349	72	379	82	337
	63	353	73	354	83	357
	64	359	74	348	84	360
	65	365	75	336	85	344
	66	360	76	322	86	346
	67	356	77	354	87	347
	68	363	78	340	88	400
	69	371	79	341	89	368
	70	332	80	367	90	369
	Mean ± S.D.	356.0 ± 10.7		349.8 ± 16.2		359.5 ± 18.1

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Female	1221(c)	234	231	214	241	219
	222	210	232	218	242	216
	223	205	233	207	243	205
	224	237	234	207	244	241
	225	202	235	227	1245(d)	208
	226	189	236	219	246	217
	227	212	237	197	247	190
	228	237	238	215	248	222
	229	223	239	231	249	252
	230	207	240	221	250	200
	Mean ± S.D.	215.6 ± 16.4		215.6 ± 10.1		217.0 ± 16.5

(c) 1221 replaced No. 221 - animal found dead

(d) 1245 replaced No. 245 - animal appeared emaciated

APPENDIX TABLE BM-2 (continued)

Hexachloro-1,3-butadiene

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	376	101	369	111	364
	92	346	102	365	112	375
	93	353	103	395	113	365
	94	368	104	328	114	394
	95	373	105	403	115	361
	96	370	106	381	116	363
	97	380	107	350	117	349
	98	346	108	369	118	371
	99	367	109	379	119	381
	100	369	110	372	120	362
	Mean ± S.D.	364.8 ± 12.2		371.1 ± 21.3		368.5 ± 12.5

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Female	251	217	261	204	271	227
	252	208	262	206	272	225
	253	222	263	260	273	222
	1254(a)	216	264	216	274	214
	255	213	265	190	275	212
	256	242	266	207	276	197
	257	220	267	214	277	225
	258	211	268	240	278	235
	259	196	269	200	279	230
	260	219	270	220	280	224
	Mean ± S.D.	216.4 ± 11.7		215.7 ± 20.5		221.1 ± 10.9

(a) 1254 replaced No. 254 - animal thought to be pregnant

APPENDIX TABLE BW-3

Hexachloro-1,3-butadiene
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	361M	368	375	379	380	386
	362M	377	382	385	391	393
	363M	373	387	381	387	395
	364M	400	418	423	426	431
	365M	365	372	376	377	377
	366M	393	400	400	410	415
	367M	385	394	394	400	402
	368M	357	367	363	368	373
	369M	357	367	371	370	374
	370M	409	425	430	431	436
	Mean ± S.D.	378.4 ± 17.9	388.7 ± 20.5	390.2 ± 21.9	394.0 ± 22.3	398.2 ± 22.7

APPENDIX TABLE BM-3 (continued)

Hexachloro-1,3-butadiene

Multiple Dosing: 10 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	371M	380	382	382	385	384
	372M	367	371	371	377	378
	373M	399	411	412	417	421
	374M	420	424	420	427	429
	375M	357	360	360	362	361
	376M	356	362	360	360	362
	377M	416	418	420	419	429
	378M	378	379	380	379	380
	379M	398	401	403	406	406
	380M	403	401	405	405	407
	Mean ± S.D.	387.4 ± 23.2	391.4 ± 22.4	391.3 ± 23.5	393.7 ± 24.2	395.9 ± 26.2

APPENDIX TABLE BM-3 (continued)

Hexachloro-1,3-butadiene

Multiple Dosing: 50 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	381M	375	363	358	338	328
	382M	384	379	368	351	353
	383M	357	350	348	336	333
	384M	364	344	340	333	330
	385M	383	376	372	350	357
	386M	398	382	366	350	350
	387M	368	354	351	339	336
	388M	366	353	352	345	339
	389M	393	381	376	368	367
	390M	357	340	330	319	315
	Mean ± S.D.	374.5 ± 14.5	362.2 ± 16.1	356.1 ± 14.7	342.9 ± 13.2	340.8 ± 15.7

APPENDIX TABLE BW-3 (continued)

Hexachloro-1,3-butadiene

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	391M	388	374	368	358	350
	392M	390	385	369	353	349
	393M	410	400	393	378	370
	394M	414	396	380	362	352
	395M	416	404	390	375	365
	396M	399	377	365	346	335
	397M	417	414	401	388	381
	398M	380	370	345	331	319
	399M	398	386	374	358	345
	400M	397	394	374	376	370
	Mean ± S.D.	400.9 ± 12.9	390.0 ± 14.0	375.9 ± 16.1	362.5 ± 17.1	353.6 ± 18.5

APPENDIX TABLE BW-4

Hexachloro-1,3-butadiene
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	321M	28	28	29	29	29
	322M	26	27	27	27	28
	323M	25	25	26	25	25
	324M	26	26	26	27	27
	325M	25	25	26	26	26
	326M	25	25	25	26	27
	327M	27	27	27	27	28
	328M	27	28	28	27	28
	329M	26	27	27	27	27
	330M	28	29	29	30	30
	Mean ± S.D.	26.3 ± 1.2	26.7 ± 1.4	27.0 ± 1.3	27.1 ± 1.4	27.5 ± 1.4

APPENDIX TABLE BW-4 (continued)

Hexachloro-1,3-butadiene

Multiple Dosing: 10 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	331M	29	27	28	29	28
	332M	29	27	28	29	30
	333M	27	25	27	26	26
	334M	27	25	27	27	28
	335M	27	26	27	26	26
	336M	27	26	28	27	26
	337M	28	28	29	28	28
	338M	28	27	29	29	29
	339M	28	27	29	28	28
	340M	30	29	31	30	29
	Mean ± S.D.	28.0 ± 1.1	26.7 ± 1.3	28.3 ± 1.3	27.9 ± 1.4	27.8 ± 1.4

APPENDIX TABLE BM-4 (continued)

Hexachloro-1,3-butadiene

Multiple Dosing: 50 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	341M	29	(a)	-	-	-
	342M	25	(a)	-	-	-
	343M	26	(a)	-	-	-
	344M	27	(a)	-	-	-
	345M	26	(a)	-	-	-
	346M	26	(b)	-	-	-
	347M	24	(a)	-	-	-
	348M	29	(b)	-	-	-
	349M	25	(b)	-	-	-
	350M	27	(a)	-	-	-
	Mean ± S.D.	26.4 ± 1.6	-	-	-	-

(a) = Animal found dead

(b) = Animal killed in extremis

APPENDIX TABLE BM-4 (continued)

Hexachloro-1,3-butadiene

Multiple Dosing: Ethyl methanesulphonate, 200 mg/kg

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	351M	26	25	26	26	25
	352M	29	28	28	28	27
	353M	29	28	27	27	26
	354M	27	27	28	27	27
	355M	29	29	29	29	28
	356M	27	27	26	27	27
	357M	26	26	27	27	26
	358M	26	26	25	25	23
	359M	26	26	26	26	24
	360M	30	29	30	31	29
	Mean	27.5	27.1	27.2	27.3	26.2
	± S.D.	± 1.6	± 1.4	± 1.5	± 1.7	± 1.8

APPENDIX TABLE CA-MD-M

Hexachloro-1,3-butadiene
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 May
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
125	156/1	50	25	25								
	156/2		25	24	1							32.3 x 109.2
126	154/2	50	25	25								
	154/4		25	25							1 Chromatid Fragment	62.1 x 110.1
129	22/1	50	25	24								
	22/2		25	25								
128	72/1	50	25	25								
	72/5		25	25								
127	38/1-5	0	0	0								
122	157/2	50	25	25								
	157/3		25	24		1						39.8 x 108.5
124	112/1		25	25								
	112/2		25	25								
130	65/1-5	0	0	0								
123	102/5	50	25	25								
	102/2		25	24	1							30.8 x 107.4
121	35/4	50	25	25								
	35/5		25	25								

APPENDIX TABLE CA-HD-M (continued)

Hexachloro-1,3-butadiene

Males

Multiple Dosing: 10 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	8/3	50	25	25								
	8/5		25	24							1 Chromatid Fragment	45.4 x 110.2
140	87/2	50	25	23							1 Chromatid Fragment	40.3 x 109.9
					1							47.8 x 108.9
	87/1		25	24	1							58.6 x 108.8
135	16/3	50	25	25								
	16/1		25	23	1							65.5 x 112.8
					1							60.9 x 111.0
136	137/1	50	25	25								
	137/2		25	24	1							51.0 x 112.3
131	103/1-5	0	0	0								
139	11/1	50	25	25								
	11/2		25	25								
137	82/2	50	25	23	1							38.8 x 112.2
					1							37.2 x 111.7
	82/3		25	25								
132	99/1	50	25	25								
	99/4		25	25								
134	149/2	50	25	25								
	149/4		25	24	1							30.5 x 112.8
133	33/2	50	25	25								
	33/3		25	25								

APPENDIX TABLE CA-MD-M (continued)

Hexachloro-1,3-butadiene
Males

Multiple Dosing: 50 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		
143	120/1	50	25	25								
	120/4		25	25								
144	64/2	50	25	25								
	64/3		25	23	1							38.1 x 109.2
141	115/3	50	25	25							1 Chromatid Fragment	59.8 x 106.3
	115/4		25	25								
150	155/1	50	25	25								
	155/3		25	24		1						
148	31/4	50	25	24	1							62.1 x 113.0
	31/2		25	25								35.1 x 109.8
145	57/1	50	25	25								
	57/2		25	25								
147	114/1	50	25	25								
	114/3		25	25								
146	113/3	50	25	24		1						
	113/4		25	25								30.7 x 111.5
142	118/4	50	25	25								
	118/5		25	23	1							
					1							54.8 x 111.0
149	134/5	50	25	25								53.4 x 108.6
	134/4		25	24	1							
												64.7 x 111.4

APPENDIX TABLE CA-MD-M (continued)

Hexachloro-1,3-butadiene

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				
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F	Miscellaneous	
157	143/2	50	25	23							1 Robertsonian Translocation 1 Pair of Minutes	38.7 x 112.2
											1 Chromatid Fragment 1 Chromosomal Fragment	32.6 x 110.6
	143/1		25	23	1							48.6 x 113.7
151	129/1	50	7	7							1 Exchange	45.7 x 113.9
	129/2		18	16			1					55.2 x 105.0
					1							35.3 x 101.4
	129/3		20	19	1							66.4 x 107.7
	129/4		5	5								
159	88/1	50	25	23	1							33.5 x 113.6
					1							32.6 x 114.4
	88/2		25	24		1					1 Exchange 1 Chromatid Fragment	60.1 x 107.5
160	28/1-5	0	0	0								
158	98/3	50	25	23	1							42.7 x 112.5
	98/2		25	22	1						1 Multi Aberration	40.3 x 112.3
					1							44.6 x 109.1
												39.9 x 109.1
154	51/3	50	25	24	1						1 Robertsonian Translocation	36.8 x 109.1
	51/4		25	25								47.7 x 112.0

APPENDIX TABLE CA-MD-M (continued)

Hexachloro-1,3-butadiene
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	39/3	50	25	25								
	39/5		25	25								
155	21/1	50	25	23	1							60.3 x 111.8
											1 Chromatid Fragment	53.6 x 109.0
	21/2		25	23		2						56.9 x 111.4
					1						2 Chromatid Fragments	32.2 x 106.3
152	77/4	50	25	25								
	77/1		25	25								
156	2/4	50	25	25								
	2/5		25	25								

APPENDIX TABLE CA-MD-F

Hexachloro-1,3-butadiene
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Females

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
282	317/3	50	25	25								
	317/1		25	25								
287	198/1	50	25	23	1							40.9 x 113.0
									1			39.4 x 112.6
	198/2		25	25								
289	182/1	50	25	25								
	182/2		25	24							2 Chromatid Fragments	34.9 x 114.1
281	195/3	50	25	25								
	195/2		25	25								
285	316/1	50	25	24	1							55.2 x 109.5
	316/2		25	24	1							54.5 x 110.6
288	232/2	50	25	25								
	232/4		25	24	1							53.3 x 110.2
286	314/5	50	25	23	1							52.1 x 112.4
					1							59.2 x 113.0
	314/2		25	25								
283	262/3	50	25	25								
	262/2		25	25								
284	272/2	50	25	25								
	272/3		25	24	1							63.5 x 106.2
290	225/1	50	25	24	2	1						39.2 x 112.5
	225/2		25	24	1							39.7 x 113.7

APPENDIX TABLE CA-MD-F (continued)

Hexachloro-1,3-butadiene
Females

Multiple Dosing: 10 ppm

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Variance Day
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
300	247/4	50	25	25								
	247/3		25	24	2							33.6 x 113.4
289	171/5	50	25	23	1							59.0 x 111.3
											1 Chromatid Fragment	41.6 x 111.7
	171/2		25	25								
291	263/3	50	25	24	1							33.8 x 107.4
	263/5		25	25								
292	259/1	50	25	25								
	259/4		25	25								
296	297/2	50	25	25								
	297/4		25	25								
295	176/3	50	25	24	1							27.5 x 108.7
	176/4		25	25								
297	242/3	50	25	24	1							30.2 x 112.2
	242/2		25	25								
298	168/1	50	25	24	1							30.9 x 111.3
	168/2		25	25								
294	309/3	50	25	25								
	309/4		25	24	1							37.5 x 110.8
293	193/3	50	25	25								
	193/4		25	25								

APPENDIX TABLE CA-MD-F (continued)

Hexachloro-1,3-butadiene
Females

Multiple Dosing: 50 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		
304	224/3	50	25	25								
	224/4		25	25								
310	315/1	50	25	25								
	315/4		25	25								
308	191/3	50	25	25								
	191/4		25	25								
301	275/3	50	25	25								
	275/4		25	25								
302	278/2	50	25	25								
	278/4		25	25								
303	280/1	50	25	23	1							46.1 x 110.5
						1						35.3 x 110.1
	280/4		25	25								
309	294/2	50	25	25								
	294/3		25	25								
307	274/4	50	25	25								
	274/3		25	25								
305	217/2	50	25	24	1							34.0 x 111.8
	217/4		25	25								
306	273/2	50	25	25								
	273/5		25	24	2							66.6 x 112.7

APPENDIX TABLE CA-MD-F (continued)

Hexachloro-1,3-butadiene
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							Version Key
					Chromatid			Chromosome				
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F	Miscellaneous	
313	199/1	50	25	24	1							42.5 x 111.5
	199/3		25	23		1						37.3 x 118.3
					1							29.4 x 107.7
315	181/1	14	7	7								
	181/2		5	5								
	181/3		2	2								
	181/4-5		0	0								
312	237/1	50	25	25								
	237/2		25	24	1							73.8 x 107.6
319	248/1	50	25	24							1 Multi Aberration	33.8 x 109.6
	248/2		25	25								
318	258/1	50	25	22	1							59.5 x 104.6
								1				47.3 x 104.2
					1							32.9 x 104.3
	258/2		25	24	1							31.8 x 108.5
317	303/4	50	25	22							1 Chromatid Fragment	28.4 x 111.2
					1							24.5 x 110.2
							1					26.6 x 110.2
	303/5		25	23		1						47.9 x 111.6
					1							43.5 x 111.8
311	289/1	50	25	24	1							58.5 x 108.2
	289/2		25	25								
316	162/3	50	25	24	1							39.5 x 110.6
	162/5		25	25								

APPENDIX TABLE CA-MD-F (continued)

Hexachloro-1,3-butadiene
Females

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
314	211/5	50	25	25								
	211/2		25	25								
320	188/3	50	25	23	1		1					29.6 x 114.3
					1							30.2 x 113.2
	188/5		25	22		1						68.0 x 113.4
					1							62.3 x 114.3
					1						1 Chromatid Fragment	59.6 x 113.1

APPENDIX TABLE CA-10G

Hexachloro-1,3-butadiene
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Males

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
5	97/1	50	25	23	1						1 Chromatid Fragment	27.0 x 112.1 37.9 x 112.0
	97/4		25	25								
10	69/2	50	25	25								
	69/3		25	25								
4	152/2	50	25	24							1 Chromatid Fragment	57.0 x 108.8
	152/5		25	25								
3	9/4	50	25	25								
	9/5		25	25								
6	129/2	50	25	25								
	129/4		25	25								
1	30/5	50	25	25								
	30/2		25	25								
8	110/3	50	25	25								
	110/4		25	25								
9	109/3	50	25	25								
	109/5		25	25								
7	112/3	50	11	11								
	112/1		15	15								
	112/2		8	8								
	112/4		16	16								
2	13/2	50	25	25								
	13/4		25	25								

APPENDIX TABLE CA-M6 (continued)

Hexachloro-1,3-butadiene
Males

Single Dosing: 10 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		
33	128/2	50	25	25								
	128/5		25	25								
36	56/3	50	25	23							1 Chromatid Fragment	67.0 x 113.8
									1			55.0 x 109.0
	56/4		25	25								
32	149/4	50	25	25								
	149/3		25	25								
35	131/3	18	14	14								
	131/4		4	4								
	131/1, 2,5		0	0								
34	143/3	50	25	25								
	143/4		25	25								
38	122/5	50	25	25								
	122/3		25	25								
40	55/1	50	25	25								
	55/4		25	25								
39	120/4	50	25	25								
	120/3		25	25								
31	116/3	50	25	25								
	116/4		25	25								
37	150/3	50	25	25								
	150/5		25	25								

APPENDIX TABLE CA-86 (continued)

Hexachloro-1,3-butadiene
Males

Single Dosing: 50 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		
62	86/2	50	25	25								
	86/4		25	25								
70	117/4	50	25	25								
	117/3		25	25								
66	37/3	50	25	25								
	37/4		25	24		1						69.0 x 102.2
64	75/3	50	25	25								
	75/5		25	24							1 Chromatid Fragment	54.2 x 113.8
63	165/5	50	25	25								
	165/2		25	23	1							64.2 x 108.9
					1							64.0 x 107.0
68	115/3	50	25	24	1							64.0 x 107.6
	115/4		25	24	1							61.5 x 115.8
65	43/4	50	25	25								
	43/3		25	25								
67	94/2	50	25	25								
	94/3		25	25								
69	14/4	50	25	25								
	14/5		25	25								
61	64/4	50	25	25								
	64/5		25	25								

APPENDIX TABLE CA-M6 (continued)

Hexachloro-1,3-butadiene

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		
100	48/2	50	25	25								
	48/5		25	25								
92	5/2	50	11	11								
	5/3		25	23	1							41.9 x 106.9
						1						50.7 x 98.3
	5/1		14	14								
96	157/4	50	25	24							1 Chromosomal Fragment	28.8 x 106.8
	157/5		25	23		1						33.9 x 108.0
					1							65.2 x 107.0
97	10/1-5	0	0	0								
93	52/2	50	25	25								
	52/5		25	25								
99	119/2	50	25	24	1							34.0 x 111.0
	119/4		25	24		1						33.8 x 112.3
95	47/2	50	25	25								
	47/1		25	23		1						62.1 x 108.0
					1							27.0 x 104.0
94	111/4	50	25	24	1							53.1 x 107.2
	111/5		25	24	1							61.0 x 114.9

APPENDIX TABLE CA-M6 (continued)

Hexachloro-1,3-butadiene

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				
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F	Miscellaneous	
98	132/3	50	23	20	1							59.7 x 113.9
					1							26.3 x 109.1
					1							31.0 x 104.0
	132/2		25	24	1							38.0 x 107.1
	132/1		2	2								
91	49/4	50	25	24	1							18.9 x 107.1
	49/2		25	25								

APPENDIX TABLE CA-M24

Hexachloro-1,3-butadiene
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		
13	121/2	50	25	25								
	121/3		25	25								
11	38/1	50	25	25								
	38/2		25	25								
20	58/4	50	25	25								
	58/5		25	25								
16	96/1	50	25	25								
	96/2		25	25								
15	23/4	50	25	25								
	23/5		25	24	1							70.5 x 106.1
19	95/2	50	25	25								
	95/4		25	24		1						34.7 x 110.6
14	90/2	50	25	25								
	90/4		25	25								
12	133/3	50	25	25								
	133/4		25	24	1							33.4 x 106.1
18	106/2	50	25	25								
	106/3		25	25								
17	80/2	50	25	25								
	80/3		25	25								

APPENDIX TABLE CA-M24 (continued)

Hexachloro-1,3-butadiene

Males

Single Dosing: 10 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		
44	92/1	50	25	25								
	92/2		25	25								
41	21/1	50	25	25								
	21/5		25	25								
49	54/1	50	25	25								
	54/2		25	25								
50	76/3	50	25	25								
	76/4		25	25								
47	59/1	50	25	25								
	59/5		25	25								
43	141/2	50	25	25								
	141/3		25	25								
46	124/1-5	0	0	0								
42	35/1-5	0	0	0								
45	50/5	50	25	25								
	50/4		25	25								
48	114/4	50	25	25								
	114/5		25	25								

APPENDIX TABLE CA-M24 (continued)

Hexachloro-1,3-butadiene
Males

Single Dosing: 50 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		
72	78/1	50	25	25								
	78/4		25	25								
79	104/2	50	25	25								
	104/3		25	25								
75	101/3	50	25	25								
	101/5		25	25								
80	91/1	50	25	25								
	91/2		25	24	1							61.9 x 111.9
76	74/1	50	25	24	1							64.0 x 110.9
	74/2		25	25								
73	70/1	50	25	24	1							61.9 x 111.4
	70/2		25	25								
77	65/2	50	25	25								
	65/5		25	25								
71	19/2	50	25	25								
	19/4		25	25								
74	34/1	50	25	25								
	34/3		25	25								
78	85/1	50	25	25								
	85/2		25	25								

APPENDIX TABLE CA-M24 (continued)

Hexachloro-1,3-butadiene

Males

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
105	44/1	50	25	24							1 Multi Aberration	55.7 x 111.5
	44/4		25	25								
102	159/5	50	25	24			1					29.1 x 115.1
	159/4		25	24							1 Exchange 2 Chromatid Fragments	64.2 x 114.1
107	41/1	50	25	25								
	41/4		25	25								
101	12/1	50	25	25								
	12/2		25	25								
106	140/2	50	25	24		1						47.3 x 110.9
	140/3		25	22							1 Chromatid Fragment	61.0 x 114.2
					1							59.4 x 114.2
						1					1 Exchange 2 Chromatid Fragments	57.2 x 114.2
104	27/3	50	25	24	1							37.5 x 111.1
	27/1		25	23	1	1						24.2 x 111.6
											1 Chromatid Fragment	26.9 x 111.4
109	154/1	50	25	25								
	154/2		25	25								
108	155/1	50	25	25								
	155/2		25	23		1						37.5 x 114.3
								1				25.5 x 114.9

APPENDIX TABLA CA-M24 (continued)

Hexachloro-1,3-butadiene

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		
110	107/1	50	25	25								
	107/3		25	23							1 Chromatid Fragment	30.5 x 112.9
											1 Multi Aberration	28.4 x 111.8
103	71/2	50	25	25								
	71/4		25	24		1						35.6 x 108.9

APPENDIX TABLE CA-M48

Hexachloro-1,3-butadiene
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
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
29	138/1	50	25	24							1 Chromatid Fragment	29.6 x 110.7
	138/4		25	25								
28	89/2	50	25	25								
	89/4		25	25								
24	8/1	50	25	25								
	8/4		25	25								
30	99/1	50	25	25								
	99/2		25	25								
26	160/1	50	25	25								
	160/2		25	25								
22	93/1	50	25	25								
	93/3		25	25								
27	89/1	50	25	25								
	89/4		25	25								
25	146/2	50	25	25								
	146/4		25	25								
21	125/4	50	25	25								
	125/3		25	25								
23	98/2	50	25	25								
	98/4		25	25								

APPENDIX TABLE CA-B48 (continued)

Hexachloro-1,3-butadiene
Males

Single Dosing: 10 ppm — Sampling Time: 48 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
59	126/2	50	25	25								
	126/3		25	25								
60	134/3	50	25	25								
	134/2		25	24	1							33.6 x 112.2
51	11/1	50	25	25								
	11/4		25	25								
54	24/1	50	25	25								
	24/3		25	25								
58	127/1	50	25	25								
	127/5		25	25								
56	158/1	50	25	25								
	158/5		25	25								
55	66/1	50	25	24		1						31.4 x 111.2
	66/4		25	25								
52	77/2	50	25	25								
	77/5		25	25								
53	25/1	50	25	25								
	25/2		25	24	1							38.3 x 113.9
57	130/1	50	25	25								
	130/4		25	25								

APPENDIX TABLE CA-N48 (continued)

Hexachloro-1,3-butadiene
Males

Single Dosing: 50 ppm — Sampling Time: 48 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
82	29/1	50	25	25								
	29/5		25	25								
90	144/4	50	25	25								
	144/1		25	25								
81	156/2	50	25	25								
	156/3		25	25								
87	40/4	50	25	25								
	40/3		25	24	1							
85	123/1-5	0	0	0								28.4 x 112.1
83	67/1	50	25	25								
	67/5		25	25								
86	108/1	50	25	25								
	108/3		25	25								
89	7/1	50	25	25								
	7/3		25	25								
88	45/1	50	25	25								
	45/5		25	25								
84	137/3	50	25	25								
	137/1		25	24	1							57.4 x 112.4

APPENDIX TABLE CA-M48 (continued)

Hexachloro-1,3-butadiene

Males

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
115	18/1	50	25	25								
	18/2		25	24							1 Robertsonian Translocation	61.7 x 111.7
112	2/3	50	25	24	1							56.8 x 111.8
	2/5		25	25								
120	118/2	50	25	25								
	118/3		25	25								
113	63/4	50	25	25								
	63/2		25	23	2							57.1 x 113.2
					1							61.4 x 113.8
114	15/2	50	25	25								
	15/4		25	25								
118	32/1	50	25	21		1						63.0 x 113.3
					1						1 Chromatid Fragment	62.1 x 112.2
					1	2					1 Exchange	61.7 x 112.2
					1							48.7 x 112.1
	32/4		25	24		1						58.8 x 113.8
116	72/2	50	25	23	1	2					2 Chromatid Fragments	32.1 x 114.4
					1							31.3 x 114.6
	72/5		25	24	1							58.1 x 113.5
117	136/4	50	25	25								
	136/2		25	25								
111	61/1	50	25	25								
	61/3		25	25								
119	103/2	50	25	25								
	103/3		25	25								

APPENDIX TABLE CA-F6

Hexachloro-1,3-butadiene
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Females

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
167	272/4	50	25	25								
	272/3		25	25								
168	270/4	50	25	25								
	270/5		25	25								
165	257/3	50	25	25								
	257/2		25	25								
163	169/2	50	25	25								
	169/4		25	25								
161	190/4	50	25	25								
	190/2		25	25								
170	229/2	50	25	25								
	229/4		25	25								
166	289/4	50	25	25								
	289/5		25	25								
164	312/2	29	12	12								
	312/4		6	6								
	312/5		11	11								
	312/1,3		0	0								
169	269/2	50	25	25								
	269/3		25	25								
162	173/3	50	25	25								
	173/1		25	25								

APPENDIX TABLE CA-P6 (continued)

Hexachloro-1,3-butadiene
Females

Single Dosing: 10 ppm

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
197	310/4	50	25	25								
	310/5		25	24							1 Chromatid Fragment	36.4 x 111.7
194	303/4	50	25	25								
	303/3		25	25								
192	309/2	50	25	25								
	309/3		25	25								
196	216/4	50	25	25								
	216/2		25	25								
191	276/1	50	25	25								
	276/2		25	24	1							23.0 x 110.0
199	280/2	50	25	25								
	280/5		25	25								
198	282/4	50	25	25								
	282/2		25	25								
195	291/3	50	25	24							1 Chromatid Fragment	52.7 x 112.0
	291/5		25	25								
1200	215/4	50	25	22	1							25.0 x 109.8
					1							65.1 x 107.6
					1							30.2 x 105.0
	215/5		25	25								
193	288/2	50	25	24	1							51.9 x 112.0
	288/3		25	25								

APPENDIX TABLE CA-P$ (continued)

Hexachloro-1,3-butadiene
Females

Single Dosing: 50 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		
228	275/3	50	25	25								
	275/4		25	25								
222	246/2	50	25	25								
	246/1		25	25								
230	277/3	50	25	24	1							
	277/5		25	25								29.9 x 112.9
223	305/3	50	25	25								
	305/5		25	25								
1221	224/1-5	0	0	0								
227	254/2	50	25	25								
	254/3		25	25								
224	235/4	50	25	24		1						
	235/3		25	25								61.9 x 107.8
225	203/3	50	25	25								
	203/2		25	25								
226	197/3	50	25	25								
	197/1		25	25								
229	174/2	50	25	25								
	174/4		25	25								

APPENDIX TABLE CA-P6 (continued)

Hexachloro-1,3-butadiene
Females

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome				
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F	Miscellaneous	
251	209/3	50	25	24	1							31.5 x 104.2
	209/4		25	25								
256	317/2	50	25	24				1				35.4 x 106.9
	317/3		25	24		1						61.2 x 113.2
259	279/4	50	25	25								
	279/3		25	23		1						62.0 x 112.0
					1							23.4 x 108.8
258	292/3	50	25	23	1							70.2 x 105.9
					1							33.0 x 103.0
	292/2		25	25								
255	207/4	50	15	15								
	207/5		15	15								
	207/2		9	9								
	207/1		11	11								
253	212/1-5	0	0	0								
260	208/1-5	0	0	0								
252	165/4	50	25	23		1						59.2 x 113.8
						1						26.0 x 104.0
	165/5		25	25								
257	170/1-5	0	0	0								
254	271/3	50	25	24	1							68.8 x 110.6
	271/2		25	25								

APPENDIX TABLE CA-F24

Hexachloro-1,3-butadiene
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Females

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
179	255/1	50	25	25								
	255/2		25	25								
171	198/2	50	25	25								
	198/4		25	25								
180	218/1	50	25	25								
	218/3		25	24	1							62.0 x 112.5
174	250/4	50	25	25								
	250/5		25	25								
173	281/3	50	25	25								
	281/4		25	25								
175	183/4	50	25	25								
	183/5		25	25								
177	240/4	50	25	25								
	240/3		25	25								
176	256/2	50	25	25								
	256/3		25	25								
178	266/3	50	25	25								
	266/5		25	25								
172	293/1	50	25	25								
	293/4		25	25								

APPENDIX TABLE CA-F24 (continued)

Hexachloro-1,3-butadiene
Females

Single Dosing: 10 ppm

Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome				
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F	Miscellaneous	
208	274/1	50	25	25								
	274/2		25	25								
204	252/2	50	25	25								
	252/4		25	25								
205	210/1	50	25	25								
	210/3		25	25								
210	236/3	50	25	25								
	236/2		25	25								
209	214/1	50	25	25								
	214/2		25	25								
202	195/1	50	25	23							1 Exchange	44.9 x 104.7
	195/2		25	24	1						1 Chromatid Fragment	69.9 x 103.2
207	219/2	50	25	25								34.7 x 106.4
	219/4		25	24		1						
206	284/3	50	25	25							1 Pair of Minutes	42.6 x 109.8
	284/5		25	25								
203	301/1	50	25	25								
	301/3		25	25								
201	181/2	50	25	25								
	181/4		25	25								

APPENDIX TABLE CA-F24 (continued)

Hexachloro-1,3-butadiene
Females

Single Dosing: 50 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		
240	251/1	50	25	25								
	251/3		25	25								
239	264/1	50	25	25								
	264/4		25	25								
235	261/1	50	21	21								
	261/2		12	12								
	261/4		12	12								
	261/5		5	5								
236	234/2	50	25	25								
	234/3		25	25								
233	230/5	50	25	25								
	230/3		18	18								
	230/2		7	7								
231	179/1	50	25	25								
	179/2		25	25								
234	194/1	50	25	25								
	194/3		25	24	1							33.1 x 111.1
238	245/2	50	25	25								
	245/3		22	22								
	245/1		3	3								

APPENDIX TABLE CA-F24 (continued)

Hexachloro-1,3-butadiene
Females

Single Dosing: 50 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		
237	225/2	50	25	25								
	225/3		25	25								
232	238/5	50	25	25								
	238/3		25	25								

APPENDIX TABLE CA-F24 (continued)

Hexachloro-1,3-butadiene
Females

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
263	231/1	50	25	24		1						66.8 x 113.1
	231/2		25	21	1							26.5 x 113.9
											1 Multi Aberration	31.3 x 113.5
											1 Exchange	63.6 x 113.5
											1 Multi Aberration	32.7 x 112.3
269	314/1	50	25	24	1							32.8 x 114.0
	314/5		25	23	1	1						32.8 x 112.0
						1						32.1 x 112.3
266	300/1	50	25	24	1							38.9 x 112.0
	300/2		25	23							1 Exchange	21.0 x 113.0
					2	1					1 Exchange	69.2 x 112.0
268	315/2	50	25	23		1						70.9 x 109.2
								1				70.1 x 109.0
	315/3		25	23				1				29.3 x 112.4
					1							31.6 x 110.8
265	204/3	50	25	24		1						26.7 x 101.3
	204/2		25	23		1						59.5 x 107.8
								1				65.4 x 103.0
267	201/2	50	25	24	1						1 Chromatid Fragment	29.7 x 110.0
	201/4		25	25								
264	187/5	50	25	24	1							64.5 x 112.6
	187/4		25	25								

APPENDIX TABLE CA-F24 (continued)

Hexachloro-1,3-butadiene
Females

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

Animal Number	Slide Number	Spreads Examined: Per Animal	Spreads Examined: Per Slide	Number of Spreads Without Aberrations	Observed Aberrations per Spread: Chromatid: Gap	Chromatid: B w F	Chromatid: B w/o F	Chromosome: Gap	Chromosome: B w F	Chromosome: B w/o F	Miscellaneous	Vernier Key
270	267/1	50	25	25								33.7 x 113.6
	267/2		25	22	1	1						28.9 x 108.5
					1							29.2 x 108.4
					1							39.9 x 112.6
262	319/2	50	25	23							1 Multi Aberration	45.6 x 112.0
											1 Exchange	38.1 x 112.4
	319/4		25	24		2					2 Chromatid Fragments	31.5 x 112.9
261	172/2	50	25	20							1 Multi Aberration	29.6 x 112.6
					1							29.4 x 112.7
						2						31.1 x 111.2
					1							33.1 x 111.1
					1							33.9 x 111.6
	172/4		25	24							1 Multi Aberration	

APPENDIX TABLE C4-F48

Hexachloro-1,3-butadiene
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Females

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
189	290/1	50	25	25								
	290/5		25	25								
183	258/5	50	25	25								
	258/2		25	25								
186	320/2	50	25	25								
	320/3		25	25								
187	242/5	50	25	25								
	242/4		25	25								
182	253/1	50	25	25								
	253/2		25	25								
181	285/1-5	0	0	0								
184	168/1	50	25	25								
	168/3		25	25								
190	259/3	50	25	24		1						34.1 x 113.3
	259/4		25	25								
185	306/1-5	0	0	0								
188	249/1	50	25	25								
	249/3		25	24							1 Chromatid Fragment	31.7 x 110.9

APPENDIX TABLE CA-F48 (continued)

Hexachloro-1,3-butadiene
Females

Single Dosing: 10 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		
1219	286/1-5	0	0	0								
216	318/1	50	25	24	1							38.3 x 112.6
	318/4		25	25								
220	294/5	50	25	25								
	294/2		25	25								
212	237/3	50	25	25								
	237/5		25	25								
214	184/3	50	25	25								
	184/4		25	25								
211	171/4	50	25	25								
	171/1		25	25								
213	185/5	50	25	25								
	185/3		25	25								
217	290/2	50	25	25								
	290/5		25	25								
215	226/1-5	0	0	0								
218	287/1-5	0	0	0								

APPENDIX TABLE CA-F48 (continued)

Hexachloro-1,3-butadiene

Females

Single Dosing: 50 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		
241	316/1-5	0	0	0								
1245	283/1	50	25	25								
	283/4		25	25								
244	297/1	50	25	25								
	297/5		25	25								
242	189/1	50	25	25								
	189/3		25	25								
243	227/3	50	25	25								
	227/5		25	25								
250	304/4	50	25	25								
	304/1		25	25								
249	167/3	50	25	25								
	167/1		25	25								
248	205/2	50	25	25								
	205/3		25	24	1							57.8 x 109.5
246	268/3	50	25	24	1							56.3 x 112.1
	268/4		25	25								
247	200/1-5	0	0	0								

APPENDIX TABLE CA-F48 (continued)

Hexachloro-1,3-butadiene
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		
277	296/1	50	25	24	1							61.1 x 106.7
	296/3		25	23	1						1 Chromatid Fragment	38.6 x 113.2
					1							34.6 x 109.5
273	223/2	50	25	24	1							28.1 x 110.2
	223/4		25	25								
271	221/1	50	25	25								
	221/2		25	25								
280	278/2	50	25	22							1 Chromatid Fragment	54.7 x 112.1
						1						55.1 x 112.5
						2						59.2 x 112.2
	278/5		25	22							1 Chromatid Fragment	30.4 x 110.4
					1							30.0 x 110.7
						1						28.8 x 110.2
274	175/3	50	25	24	1							37.4 x 113.0
	175/5		25	24	1							65.1 x 112.3
278	192/1	50	25	24							1 Exchange	24.4 x 114.1
	192/2		25	22				1				32.1 x 113.7
											1 Multi Aberration	31.7 x 112.8
											1 Chromatid Fragment	34.5 x 112.3

APPENDIX TABLE CA-F48 (continued)

Hexachloro-1,3-butadiene
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	263/1	50	25	25								63.2 x 109.3
	263/3		25	24		1						33.8 x 112.3
275	178/5	50	25	24							1 Multi Aberration	30.5 x 112.8
	178/1		25	24							1 Exchange	
272	162/1	50	25	25								
	162/4		25	25								
276	232/4	50	25	25								
	232/5		25	25								

APPENDIX TABLE D6

Hexachloro-1,3-butadiene
Dominant Lethal Assessment

Multiple Dosing: Air Control (0 ppm)

Week No.	Male No.	361		362		363		364		365		366		367		368		369		370		Total
	Female	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
1	Corpora lutea	11	0	18	17	16	12	10	15	20	16	13	13	14	13	17	13	15	0	25	15	271
	Total Implants	12	0	16	15	13	12	15	13	12	16	10	12	14	14	14	13	14	0	17	14	246
	Live Implants	12	0	16	15	13	12	15	12	12	15	9	12	12	14	13	11	13	0	17	14	237
	Early Deaths	0	0	0	0	0	0	0	0	0	1	1	0	2	0	1	2	1	0	0	0	8
	Late Deaths	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
2	Corpora lutea	6	14	13	16	12	0	10	15	16	14	12	14	12	12	0	12	14	13	17	16	238
	Total Implants	0	14	13	17	12	0	11	15	11	13	10	15	13	12	0	13	14	11	15	15	224
	Live Implants	0	13	13	17	12	0	11	15	10	12	10	14	12	12	0	13	14	8	13	14	213
	Early Deaths	0	1	0	0	0	0	0	0	1	1	0	1	1	0	0	0	0	3	2	1	11
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	Corpora lutea	18	14	0	18	16	10	15	15	13	13	12	13	11	12	9	14	12	13	11	13	252
	Total Implants	19	13	0	15	16	9	13	17	10	12	14	12	11	13	13	15	10	14	12	13	251
	Live Implants	18	13	0	14	15	9	10	15	10	12	14	11	11	13	11	14	10	13	12	13	238
	Early Deaths	1	0	0	1	1	0	3	2	0	0	0	1	0	0	2	1	0	1	0	0	13
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	Corpora lutea	16	12	13	14	13	14	14	13	13	13	3	14	10	12	14	11	19	14	12	13	257
	Total Implants	11	11	14	14	13	8	13	13	14	14	0	16	12	13	14	12	15	16	12	14	249
	Live Implants	11	11	14	13	13	8	13	12	14	12	0	16	10	12	13	12	15	14	11	14	238
	Early Deaths	0	0	0	1	0	0	0	1	0	2	0	0	2	1	1	0	0	2	1	0	11
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

APPENDIX TABLE DL (continued)

Hexachloro-1,3-butadiene

Multiple Dosing: Air Control (0 ppm)

Week No.	Male No.	361		362		363		364		365		366		367		368		369		370		Total
	Female	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
5	Corpora lutea	2	15	15	13	0	14	0	11	15	14	10	17	12	11	14	12	11	11	13	12	222
	Total Implants	0	15	15	13	0	13	0	13	15	14	10	17	14	11	13	12	11	11	11	15	223
	Live Implants	0	14	14	13	0	13	0	13	13	12	10	15	14	11	13	12	11	10	11	12	211
	Early Deaths	0	1	1	0	0	0	0	0	2	2	0	2	0	0	0	0	0	0	0	3	11
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
6	Corpora lutea	12	15	13	14	17	11	15	12	11	14	12	0	12	13	13	17	13	15	10	13	252
	Total Implants	13	15	12	13	14	10	18	12	10	14	11	0	12	15	12	14	13	15	9	11	243
	Live Implants	12	15	12	12	13	10	16	12	8	14	11	0	12	14	11	14	13	15	9	11	234
	Early Deaths	1	0	0	1	1	0	1	0	2	0	0	0	0	1	1	0	0	0	0	0	8
	Late Deaths	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
7	Corpora lutea	11	13	10	12	15	13	12	0	15	14	12	10	14	14	13	12	12	10	13	11	236
	Total Implants	11	13	11	12	14	15	13	0	14	14	12	10	12	15	14	11	4	5	13	12	225
	Live Implants	11	13	11	11	13	15	12	0	14	14	11	10	12	14	13	11	2	5	12	12	216
	Early Deaths	0	0	0	1	1	0	1	0	0	0	1	0	0	1	1	0	2	0	1	0	9
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	Corpora lutea	10	14	13	15	13	17	13	14	12	13	12	14	14	14	15	16	5	13	14	15	266
	Total Implants	13	13	13	15	10	14	13	9	14	12	12	13	13	16	13	14	2	13	13	15	250
	Live Implants	12	13	13	13	10	14	12	9	14	11	11	11	13	16	13	14	2	13	12	15	241
	Early Deaths	1	0	0	2	0	0	1	0	0	1	1	2	0	0	0	0	0	0	1	0	9
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

APPENDIX TABLE DL (continued)

Hexachloro-1,3-butadiene

Multiple Dosing: Air Control (0 ppm)

Week No.	Male No.	361		362		363		364		365		366		367		368		369		370		Total
	Female	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
9	Corpora lutea	14	13	13	12	10	12	15	9	12	13	12	12	20	10	12	11	13	12	11	11	247
	Total Implants	14	13	14	12	13	13	15	13	11	12	13	13	12	10	13	11	11	11	9	11	244
	Live Implants	13	13	14	11	12	11	13	13	10	9	11	13	12	10	13	11	11	11	8	11	230
	Early Deaths	1	0	0	1	1	1	2	0	1	3	1	0	0	0	0	0	0	0	1	0	12
	Late Deaths	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	2
10	Corpora lutea	12	14	1	13	16	12	15	14	11	14	11	13	14	13	16	16	13	14	13	11	258
	Total Implants	12	12	0	13	17	15	16	14	11	10	10	13	15	17	11	15	13	16	13	14	259
	Live Implants	10	11	0	12	16	15	15	14	11	10	10	11	15	17	10	15	13	14	11	12	242
	Early Deaths	2	1	0	1	1	0	1	0	0	0	0	0	0	0	1	0	0	4	2	2	15
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	2
I	Corpora lutea	112	124	109	144	128	115	119	118	138	138	109	120	133	124	125	134	125	115	139	130	2499
	Total Implants	105	119	108	139	122	109	127	119	122	131	102	121	128	136	117	130	107	114	124	136	2414
	Live Implants	99	116	107	131	117	107	117	115	116	121	97	113	123	133	110	127	104	103	116	128	2300
	Early Deaths	6	3	1	8	5	1	9	3	6	10	4	6	5	3	7	3	3	10	8	6	107
	Late Deaths	0	0	0	0	0	1	1	1	0	0	1	2	0	0	0	0	0	1	0	0	7

APPENDIX TABLE IX. (continued)

Hexachloro-1,3-butadiene

Multiple Dosing: 10 ppm

Week No.	Male No.	371		372		373		374		375		376		377		378		379		380		Total
	Female	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
1	Corpora lutea	13	14	14	16	14	12	10	11	14	14	12	10	0	13	16	15	12	14	0	16	246
	Total Implants	13	12	13	16	14	12	12	11	14	14	12	12	0	11	14	14	12	14	15	14	249
	Live Implants	13	12	13	16	14	12	11	10	14	14	11	10	0	11	14	13	12	14	15	14	243
	Early Deaths	0	0	0	0	0	0	1	1	0	0	1	2	0	0	0	1	0	0	0	0	6
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	Corpora lutea	13	12	11	12	11	15	15	12	13	*-	11	12	15	16	11	14	8	14	15	13	243
	Total Implants	14	13	10	13	12	14	14	12	14	*-	13	12	14	11	11	13	4	15	15	14	238
	Live Implants	14	12	10	12	12	13	14	11	14	*-	13	9	14	11	10	13	3	15	15	14	229
	Early Deaths	0	0	0	1	0	1	0	1	0	*-	0	3	0	0	1	0	1	0	0	0	8
	Late Deaths	0	1	0	0	0	0	0	0	0	*-	0	0	0	0	0	0	0	0	0	0	1
3	Corpora lutea	18	14	0	13	11	17	12	14	15	14	11	12	13	10	8	16	13	9	16	13	249
	Total Implants	19	16	0	14	6	17	12	14	15	15	13	15	13	11	7	16	14	10	16	13	256
	Live Implants	19	15	0	14	6	17	12	14	14	14	12	14	11	10	4	15	13	10	12	13	239
	Early Deaths	0	1	0	0	0	0	0	0	1	1	1	1	2	1	3	1	1	0	4	0	17
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	Corpora lutea	12	13	12	7	15	12	9	12	14	12	11	11	13	0	11	12	14	12	13	16	231
	Total Implants	15	13	13	2	14	10	12	12	15	13	11	14	13	0	11	13	15	12	13	16	237
	Live Implants	15	13	11	2	14	10	10	12	14	12	10	13	13	0	10	11	15	12	13	16	226
	Early Deaths	0	0	2	0	0	0	2	0	1	1	1	1	0	0	1	2	0	0	0	0	11
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

*- Ambiguous record of result

APPENDIX TABLE D6 (continued)

Hexachloro-1,3-butadiene

Multiple Dosing: 10 ppm

Week No.	Male No.	371		372		373		374		375		376		377		378		379		380		Total
	Female	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
5	Corpora lutea	14	11	12	12	12	15	13	12	14	11	15	14	13	10	15	15	10	11	15	15	259
	Total Implants	13	10	10	8	11	15	13	13	14	13	16	16	13	10	16	14	9	10	17	14	255
	Live Implants	12	10	10	7	11	14	13	13	14	13	14	15	13	10	16	13	9	10	17	12	246
	Early Deaths	1	0	0	1	0	1	0	0	0	0	2	1	0	0	0	0	0	0	0	2	8
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
6	Corpora lutea	14	13	0	0	12	15	12	13	15	18	15	14	18	14	13	12	12	13	11	12	246
	Total Implants	15	13	0	0	11	16	13	12	11	19	13	15	12	17	13	13	12	14	13	15	247
	Live Implants	14	13	0	0	11	16	13	12	11	18	10	15	12	17	13	13	11	13	13	15	240
	Early Deaths	1	0	0	0	0	0	0	0	0	1	3	0	0	0	0	0	1	1	0	0	7
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	Corpora lutea	13	3	14	11	20	12	20	13	9	13	12	10	13	17	12	16	14	13	14	13	261
	Total Implants	13	0	15	12	15	12	12	15	8	14	15	7	11	15	13	16	13	14	14	13	247
	Live Implants	13	0	15	12	14	12	11	15	8	13	15	7	9	15	12	15	11	12	14	13	236
	Early Deaths	0	0	0	0	1	0	1	0	0	1	0	0	2	0	1	1	2	2	0	0	11
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	Corpora lutea	15	13	9	13	11	13	14	10	15	10	14	12	11	15	12	15	14	14	15	13	258
	Total Implants	10	12	8	13	10	13	10	10	14	12	14	12	11	14	13	13	14	12	15	11	241
	Live Implants	10	8	8	13	10	11	8	10	14	11	13	12	11	14	12	13	14	12	15	11	230
	Early Deaths	0	4	0	0	0	2	2	0	0	1	1	0	0	0	1	0	0	0	0	0	11
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

APPENDIX TABLE DL (continued)

Hexachloro-1,3-butadiene

Multiple Dosing: 10 ppm

Week No.	Male No.	371		372		373		374		375		376		377		378		379		380		Total
	Female	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
9	Corpora lutea	12	16	10	2	12	10	14	11	15	15	12	13	21	13	12	10	14	13	12	6	245
	Total Implants	11	16	11	0	7	10	12	10	12	13	12	14	15	13	13	12	13	13	14	11	232
	Live Implants	10	15	10	0	7	10	12	10	12	12	12	14	12	13	13	12	13	13	14	11	225
	Early Deaths	1	1	1	0	0	0	0	0	0	1	0	0	3	0	0	0	0	0	0	0	7
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	Corpora lutea	15	14	16	17	14	10	16	16	13	14	14	12	0	11	14	13	0	13	13	14	249
	Total Implants	17	17	16	15	13	15	16	16	12	14	13	13	0	16	14	12	0	15	15	8	257
	Live Implants	16	16	16	15	10	14	16	16	10	14	12	11	0	15	14	10	0	15	14	8	242
	Early Deaths	1	1	0	0	3	1	0	0	2	0	1	2	0	1	0	2	0	0	1	0	15
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Σ	Corpora lutea	139	123	98	103	132	131	135	124	137	121	127	120	117	119	124	138	110	126	132	131	2487
	Total Implants	140	122	96	93	113	134	126	125	129	127	132	130	102	118	125	136	106	129	147	129	2459
	Live Implants	136	114	93	91	109	129	120	123	125	121	122	120	95	116	110	128	101	126	142	127	2356
	Early Deaths	4	7	3	2	4	5	6	2	4	6	10	10	7	2	7	7	5	3	5	2	101
	Late Deaths	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2

*- Ambiguous record of result

APPENDIX TABLE IX (continued)

Hexachloro-1,3-butadiene

Multiple Dosing: 50 ppm

Week No.	Male No.	381		382		383		384		385		386		387		388		389		390		Total
	Female	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
1	Corpora lutea	0	0	0	9	13	16	11	14	15	13	14	*-	12	13	15	15	11	18	13	13	215
	Total Implants	0	0	0	11	13	14	10	14	13	13	14	*-	13	12	11	12	11	12	9	13	195
	Live Implants	0	0	0	0	13	14	10	14	13	13	13	*-	13	11	10	10	11	8	9	13	175
	Early Deaths	0	0	0	11	0	0	0	0	0	0	1	*-	0	1	1	2	0	4	0	0	20
	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	M	D	10	14	12	0	16	0	13	8	13	16	16	17	10	11	12	12	11	10	199
	Total Implants	A	E	11	12	13	0	14	0	11	3	12	13	13	14	11	11	12	13	13	9	185
	Live Implants	L	A	10	11	13	0	13	0	9	3	12	13	13	13	11	10	11	13	13	9	177
	Early Deaths	E	D	1	1	0	0	1	0	2	0	0	0	0	1	0	1	1	0	0	0	8
	Late Deaths			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	Corpora lutea	M	D	14	10	0	7	13	12	11	14	12	14	13	13	15	11	17	11	0	12	199
	Total Implants	A	E	15	10	0	11	14	13	11	13	12	14	13	15	12	10	13	12	0	12	200
	Live Implants	L	A	14	10	0	11	14	13	10	12	10	14	13	14	11	10	13	11	0	12	192
	Early Deaths	E	D	1	0	0	0	0	0	1	1	2	0	0	1	1	0	0	1	0	0	8
	Late Deaths			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	Corpora lutea	M	D	14	12	13	15	11	12	14	13	13	15	11	12	8	14	20	15	14	2	228
	Total Implants	A	E	17	15	13	15	13	12	15	14	14	8	15	13	11	13	14	14	14	0	230
	Live Implants	L	A	17	8	13	15	12	12	14	13	14	7	14	12	11	12	13	14	14	0	215
	Early Deaths	E	D	0	7	0	0	1	0	1	1	0	1	1	1	0	1	1	0	0	0	15
	Late Deaths			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

*- Ambiguous record of result

APPENDIX TABLE DL (continued)

Hexachloro-1,3-butadiene

Multiple Dosing: 50 ppm

Week No.	Male No.	381		382		383		384		385		386		387		388		389		390		Total
	Female	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
5	Corpora lutea	M	D	17	16	11	15	10	0	13	15	12	15	10	10	15	13	14	16	13	8	223
	Total Implants	A	E	17	16	12	15	11	0	15	13	11	15	11	12	16	12	14	15	13	6	224
	Live Implants	L	A	16	14	12	15	10	0	15	13	11	15	9	12	16	12	11	15	13	6	215
	Early Deaths	E	D	1	2	0	0	1	0	0	0	0	0	2	0	0	0	3	0	0	0	9
	Late Deaths			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	Corpora lutea	M	D	13	14	7	14	15	14	12	13	14	0	10	19	12	13	14	11	12	3	200
	Total Implants	A	E	13	12	5	15	16	15	14	10	13	0	10	11	10	16	15	12	10	1	198
	Live Implants	L	A	13	12	5	15	15	14	11	10	12	0	9	11	10	16	15	12	10	1	191
	Early Deaths	E	D	0	0	0	0	1	1	3	0	1	0	1	0	0	0	0	0	0	0	7
	Late Deaths			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	Corpora lutea	M	D	14	13	0	0	9	13	11	13	12	19	13	12	12	16	13	12	11	13	206
	Total Implants	A	E	14	13	0	0	8	14	12	13	13	15	13	9	12	12	14	11	11	13	197
	Live Implants	L	A	14	11	0	0	7	12	11	12	12	15	13	9	12	10	14	11	11	13	187
	Early Deaths	E	D	0	2	0	0	1	2	1	1	1	0	0	0	0	2	0	0	0	0	10
	Late Deaths			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	Corpora lutea	M	D	15	11	0	14	13	11	12	13	14	13	12	12	14	12	12	15	13	10	216
	Total Implants	A	E	16	11	0	14	13	11	15	12	14	13	14	12	14	12	13	15	13	12	224
	Live Implants	L	A	15	10	0	13	13	10	15	12	14	12	14	12	13	12	13	15	13	12	218
	Early Deaths	E	D	1	1	0	1	0	1	0	0	0	1	0	0	1	0	0	0	0	0	6
	Late Deaths			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

APPENDIX TABLE DL (continued)

Hexachloro-1,3-butadiene

Multiple Dosing: 50 ppm

Week No.	Male No.	381		382		383		384		385		386		387		388		389		390		Total
	Female	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
9	Corpora lutea	M	D	15	10	12	11	13	12	18	12	12	15	14	16	11	13	13	13	15	10	235
	Total Implants	A	E	15	11	12	11	13	11	12	14	12	15	15	15	10	13	11	12	14	6	224
	Live Implants	L	A	13	11	11	10	12	10	11	14	11	15	15	14	9	13	10	12	14	6	213
	Early Deaths	E	D	2	0	1	1	1	1	1	0	1	0	0	1	1	0	1	0	0	0	11
	Late Deaths			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	Corpora lutea	M	D	14	14	11	10	4	11	15	11	14	16	21	0	13	14	13	12	16	15	224
	Total Implants	A	E	13	14	11	11	5	15	14	13	14	15	14	0	13	14	16	13	16	13	224
	Live Implants	L	A	13	14	11	11	2	14	14	12	13	14	13	0	13	13	16	12	16	13	214
	Early Deaths	E	D	0	0	0	0	3	1	0	1	1	1	1	0	0	1	0	1	0	0	10
	Late Deaths			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
I	Corpora lutea	0*	0*	126	123	79	102	115	99	134	125	130	123	130	124	125	132	139	135	118	96	2155
	Total Implants	0*	0*	131	125	79	106	117	105	132	118	129	108	131	113	120	125	133	129	113	87	2101
	Live Implants	0*	0*	125	101	78	104	108	99	123	114	122	105	126	108	116	118	127	123	113	87	1997
	Early Deaths	0*	0*	6	24	1	2	9	6	9	4	7	3	5	5	4	7	6	6	0	0	104
	Late Deaths	0*	0*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

* - male died after first mating

APPENDIX TABLE DL (continued)

Hexachloro-1,3-butadiene

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Week No.	Male No.	391		392		393		394		395		396		397		398		399		400		Total
	Female	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
1	Corpora lutea	7	0	11	25	9	0	3	24	0	12	8	12	2	11	5	18	10	6	8	11	172
	Total Implants	6	0	7	15	11	0	2	15	0	11	0	10	0	9	0	13	5	0	7	10	121
	Live Implants	0	0	6	11	6	0	0	8	0	1	0	2	0	1	0	7	0	0	7	5	55
	Early Deaths	6	0	1	4	5	0	2	7	0	9	0	8	0	8	0	6	5	0	0	4	65
	Late Deaths	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
2	Corpora lutea	5	0	3	0	6	2	0	2	5	2	4	0	14	6	6	1	3	6	0	8	73
	Total Implants	1	0	2	0	1	0	0	1	2	0	1	0	0	0	0	0	1	1	0	0	16
	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	1	0	2	0	1	0	0	1	2	0	1	0	0	0	0	0	1	1	0	0	10
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	Corpora lutea	1	0	1	3	0	0	1	0	0	0	3	5	1	0	0	1	0	4	0	0	20
	Total Implants	0	0	1	3	0	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	8
	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	1	3	0	0	0	0	0	1	0	1	0	0	1	0	0	1	0	0	8
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	Corpora lutea	5	15	8	0	1	5	16	9	13	13	6	16	9	12	14	4	12	12	12	9	191
	Total Implants	3	16	13	0	0	3	11	11	12	10	4	13	8	14	15	1	11	8	12	6	171
	Live Implants	1	13	10	0	0	1	10	6	10	3	1	10	2	11	12	0	5	3	11	0	109
	Early Deaths	2	3	3	0	0	2	1	4	2	7	3	3	6	3	3	1	6	5	1	6	61
	Late Deaths	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1

APPENDIX TABLE DL (continued)

Hexachloro-1,3-butadiene

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Week No.	Male No.	391		392		393		394		395		396		397		398		399		400		Total
	Female	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
5	Corpora lutea	1	14	8	14	*-	13	11	13	13	*-	13	13	12	0	15	14	13	14	15	15	211
	Total Implants	0	14	8	14	*-	14	10	14	12	*-	13	9	13	0	15	14	14	12	15	15	206
	Live Implants	0	14	8	12	*-	13	7	13	11	*-	13	8	13	0	15	14	14	12	12	13	192
	Early Deaths	0	0	0	2	*-	1	3	1	1	*-	0	1	0	0	0	0	0	0	2	2	13
	Late Deaths	0	0	0	0	*-	0	0	0	0	*-	0	0	0	0	0	0	0	0	1	0	1
6	Corpora lutea	11	10	13	13	13	14	9	20	14	20	13	11	13	11	11	16	10	16	2	13	293
	Total Implants	11	10	14	13	12	14	10	13	14	19	13	12	13	11	10	12	12	16	0	12	241
	Live Implants	11	8	14	13	10	12	10	12	13	19	13	12	13	11	10	12	12	16	0	12	233
	Early Deaths	0	2	0	0	2	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	7
	Late Deaths	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
7	Corpora lutea	18	13	10	10	15	12	13	9	15	2	13	12	5	16	12	15	15	15	14	13	246
	Total Implants	13	14	13	9	13	12	12	11	15	0	13	12	0	16	14	16	15	15	13	10	236
	Live Implants	13	14	13	8	12	12	11	11	14	0	11	11	0	16	14	14	15	14	13	8	224
	Early Deaths	0	0	0	1	1	0	1	0	1	0	1	1	0	0	0	0	0	1	0	2	9
	Late Deaths	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2	0	0	0	0	3
8	Corpora lutea	12	0	16	14	13	12	10	13	14	11	10	12	15	14	12	0	10	12	11	13	224
	Total Implants	12	0	8	14	13	12	9	12	12	9	13	13	10	3	15	0	10	12	11	14	202
	Live Implants	10	0	8	13	13	12	8	11	12	8	12	13	10	3	13	0	10	11	10	14	191
	Early Deaths	2	0	0	1	0	0	1	1	0	1	1	0	0	0	2	0	0	1	1	0	11
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

*- Ambiguous record of result

APPENDIX TABLE DL (continued)

Hexachloro-1,3-butadiene

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Week No.	Male No.	391		392		393		394		395		396		397		398		399		400		Total
	Female	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
9	Corpora lutea	13	18	1	14	14	13	12	12	14	13	12	12	16	13	13	13	12	14	12	14	255
	Total Implants	13	15	0	13	14	13	8	12	13	13	12	13	7	14	12	16	12	14	13	13	240
	Live Implants	12	14	0	12	13	12	6	11	12	13	11	12	7	14	11	13	10	14	13	12	222
	Early Deaths	1	1	0	1	1	1	2	1	1	0	1	1	0	0	1	2	1	0	0	1	16
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	2
10	Corpora lutea	15	17	0	12	13	*-	18	13	15	14	13	14	13	15	15	18	4	12	15	15	251
	Total Implants	11	15	0	12	14	*-	16	17	12	15	14	14	14	16	13	15	1	14	15	15	243
	Live Implants	11	15	0	12	13	*-	15	16	12	15	14	13	12	16	11	14	1	13	15	14	232
	Early Deaths	0	0	0	0	1	*-	1	1	0	0	0	1	2	0	2	1	0	1	0	1	11
	Late Deaths	0	0	0	0	0	*-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Σ	Corpora lutea	86	87	71	105	84	71	93	115	103	87	95	107	100	98	103	100	89	111	89	11	1905
	Total Implants	70	84	66	93	78	68	78	106	92	78	83	97	65	83	95	87	81	93	86	95	1678
	Live Implants	58	78	59	81	67	62	67	88	84	59	75	81	57	72	86	74	67	83	81	75	1458
	Early Deaths	12	6	7	12	11	5	11	17	8	18	7	16	8	11	9	10	13	10	4	14	211
	Late Deaths	0	0	0	0	0	1	0	1	0	1	1	0	0	0	0	3	1	0	1	0	9

*- Ambiguous record of result

APPENDIX TABLE 8A

Hexachloro-1,3-butadiene
Sperm Abnormality Assessment

Multiple Dosing: Air Control (0 ppm)
Low, 10 ppm
High, 50 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
349	98	0	1	0	0	1	2	100*	321	Air
327	987	0	0	12	4	7	23	1000	322	Air
332	96	0	0	3	0	1	4	100*	323	Air
336	929	0	2	21	13	35	71	1000	324	Air
358	920	0	3	12	57	8	80	1000	325	Air
344	977	0	1	8	5	9	23	1000	326	Air
353	950	0	3	21	9	17	50	1000	327	Air
321	960	0	1	21	16	2	40	1000	328	Air
335	972	0	1	13	4	10	28	1000	329	Air
351	975	0	1	8	5	11	25	1000	330	Air
325	971	1	0	18	3	7	29	1000	331	Low
360	968	0	2	8	11	11	32	1000	332	Low
338	485	0	0	5	3	7	15	500*	333	Low
334	95	0	0	2	1	2	5	100*	334	Low
356	970	0	1	15	5	9	30	1000	335	Low
359	**	**	**	**	**	**	**	**	336	Low
328	941	1	2	24	16	16	59	1000	337	Low
331	191	0	0	3	1	5	9	200*	338	Low
342	963	0	1	13	6	17	37	1000	339	Low
339	470	2	1	17	4	6	30	500*	340	Low

* 1000 Sperm counts not obtainable

** No sperm observed on slides

APPENDIX TABLE 8A (continued)

Hexachloro-1,3-butadiene

Multiple Dosing: Air Control (0 ppm)
Low, 10 ppm
High, 50 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
All animals dead as a result of dosing									341	High
									342	High
									343	High
									344	High
									345	High
									346	High
									347	High
									348	High
									349	High
									350	High
333	956	0	2	23	8	11	44	1000	351	+
352	926	0	1	41	16	16	74	1000	352	+
326	459	0	1	19	8	13	41	500*	353	
350	460	0	0	5	7	8	20	1000	354	+
343	953	2	0	25	11	9	47	1000	355	+
330	950	0	3	23	10	14	50	1000	356	+
324	965	1	2	11	2	19	35	1000	357	+
329	914	0	3	31	25	27	86	1000	358	+
323	946	1	3	28	9	13	54	1000	359	+
348	949	0	1	29	7	14	51	1000	360	+

* 1000 Sperm counts not obtainable