Report Number 30

TIER II MUTAGENIC SCREENING OF 13 NIOSH PRIORITY COMPOUNDS

INDIVIDUAL COMPOUND REPORT 3-CHLOROPROPENE

Douglas B. McGregor

July 1, 1981

Supported by

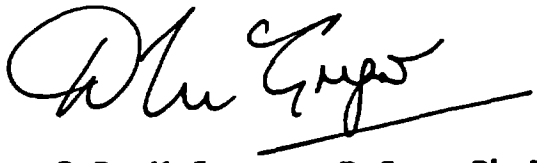
NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH
Division of Biomedical and Behavioural Science
Experimental Toxicology Branch
4676 Columbia Parkway, Cincinnati, Ohio 45226

Contract No. 210-78-0026

Inveresk Research International Limited
Musselburgh EH21 7UB, Scotland

AUTHENTICATION

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



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

TABULATIONS

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

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

Example:

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

Abbreviations on Chromosomal Aberration Tables and Appendix Tables:

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

CONTENTS

	Page
LOCATION OF EXPERIMENT	1
DISCLAIMER	1
PERSONNEL INVOLVED	2
SUMMARY	3
INTRODUCTION	5
Properties	6
Toxicology	6
MATERIALS AND METHODS	8
CHEMICALS	8
Test Substance	8
Positive Control Substance	8
ANIMALS AND ANIMAL MANAGEMENT	9
Animals	9
Pre-experimental Acceptance Tests	9
Animal Management	10
Diet	10
Allocation of Rats and Mice to Cages and Treatment Groups	11
Animal Identification	11
Animal Positioning in the Exposure Chambers	11
ATMOSPHERE GENERATION AND EXPOSURE	13
Exposure Chambers	13
Monitoring Equipment	14
Calibration and Analytical Development	14
Calibration	14
Analytical Conditions	15
Atmosphere Generation	17
Homogeneity Data	17
Measurement of Chamber Concentrations	17
Test Compound Utilisation	18
Exposure Procedure	18
Positive Control Groups in Animal Tests	19
Preparation of Dosing Solutions	19
Treatment of Rats and Mice with Ethyl methane ılphonate	19

CONTENTS (continued)

	Page
UNSCHEDULED DNA SYNTHESIS ASSAY	21
Chemicals	21
Test Solutions	21
Cells	21
Culture Maintenance and Growth Media	21
Animals	22
Preparation of the 9,000 g Supernatant Fluid from Livers	22
Preliminary Toxicity Test	23
DNA Repair Assay	24
Autoradiography	24
Quantification of Repair Synthesis	25
CYTOGENETIC ANALYSIS OF RAT BONE MARROW CELLS	26
Metaphase Cell Preparations	26
Slide Reading	26
DOMINANT LETHAL TESTING IN MALE RATS	28
Mating	28
Assessment	28
SPERM ABNORMALITIES TEST IN MICE	30
Preparation	30
Assessment	30
SEX-LINKED RECESSIVE LETHAL TEST IN DROSOPHILA MELANOGASTER	32
Strains	32
Medium	32
Exposures	32
Toxicity Test	33
Recessive Lethal Test	33
STATISTICAL EVALUATION	35
Cytogenetics Tests	35
Dominant Lethal Assay	35
Sperm Abnormalities Test	37
Sex-linked Recessive Lethal Test	37
RESULTS	39
Instrument Calibration	39
Chamber Atmospheres - Homogeneity	39
Chamber Atmospheres - Achieved Concentrations	39
Animal Location	39
Pre-experimental Acceptance Tests (PEAT)	40
Clinical Observations and Body Weights	40

CONTENTS (continued)

			Page
UNSCHEDULED DNA SYNTHESIS ASSAY			41
CYTOGENETIC ANALYSIS OF RAT BONE MARROW CELLS			42
DOMINANT LETHAL TEST			43
SPERM ABNORMALITY TEST			44
SEX-LINKED RECESSIVE LETHAL TEST IN DROSOPHILA			45
CONCLUSIONS			46
REFERENCES			47
TABLES			49
Atmospheric Analysis	-	AT-1 to 6	49
Body Weights	-	BW-1 to 4	57
UDS Assay	-	UDS-1	61
Chromosomal Analysis	-	CA-MD-M-1 to CA-F48-2	62
Dominant Lethal Test	-	DL-1 to 9	80
Sperm Abnormality Test	-	SA-1 and 2	89
Sex-linked Recessive Lethal Test	-	RL-1 and 2	91
FIGURES			97
1a - Schematic Lay-out of Exposure Area			97
1b - Schematic Lay-out of Vapour Generation Apparatus			98
2 - Calibration Graph for High Level			99
3 - Sample Record Chart of IR Absorption at 10.8 µm			100
APPENDICES			101
Diet Analysis	-	Diet	101
Animal Locations	-	Loc-1 and 2	102
Forms	-	Form-1 to 3	107
APPENDIX TABLES			110
Body Weights	-	BW-1 to 4	110
Chromosomal Analysis	-	CA-MD-M to CA-F48	130
Dominant Lethal Test	-	DL	178
Sperm Abnormality Test	-	SA	190
FINAL PAGE OF REPORT			191

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.

PERSONNEL INVOLVED

Principal Investigator:	D.B. McGregor, B.Sc., Ph.D.
Staff:	A. Campbell, B.Sc. C. Christie, B.Sc. K. D'Arcy-Burt, H.N.D. C. DeAngelis, B.Sc. E. Hall, F.I.M.L.S. M. Henderson, B.Sc., Ph.D. M. Holmström, B.Sc., M.Sc. G. Jones, B.Sc., Ph.D. A. Kirkwood M. Krowinski, B.Sc. C. Liddle S. Macdonald D. McDonald, B.Sc., Ph.D. P. McDonald, H.N.C., L.I.Biol. H. Narro A. Poole, B.Sc., Ph.D. P. Robinson, B.Sc. C. Ross, B.Sc., Ph.D. A. Spencer, B.V.M.S., Ph.D., M.R.C.V.S. A. Soden S. Souter K. Telfor D. Williams, M.A. M. Willins, B.Sc.
Quality Assurance:	E.M. Baxendine, B.Sc. A.W. Waddell, B.Sc., Ph.D.

SUMMARY

3-Chloropropene (allyl chloride) was subjected to a tier II mutagenic test screening programme. The assays used were the following:

1. Unscheduled DNA synthesis (UDS) assay in human diploid fibroblasts with exposures of 3 h duration and concentrations up to 9,900 µg/ml of culture medium.

2. Dominant lethal test in male rats with exposure to atmospheres containing 1 ppm or 25 ppm 3-chloropropene for 7 h per day for 5 consecutive days. Analysis of test atmospheres was by continuous infra-red absorption monitoring at a wavelength of 10.8 µm.

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

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

5. Sex-linked recessive lethal (SLRL) test in Drosophila melanogaster with exposure to atmospheres of 150 ppm for 7 h.

The results obtained were as follows:

1. There was no increase in UDS in cells treated with 3-chloropropene.

2. There were no increases in the frequencies of cells with aberrations seen as a result of examination of bone marrow metaphase cell analysis.

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

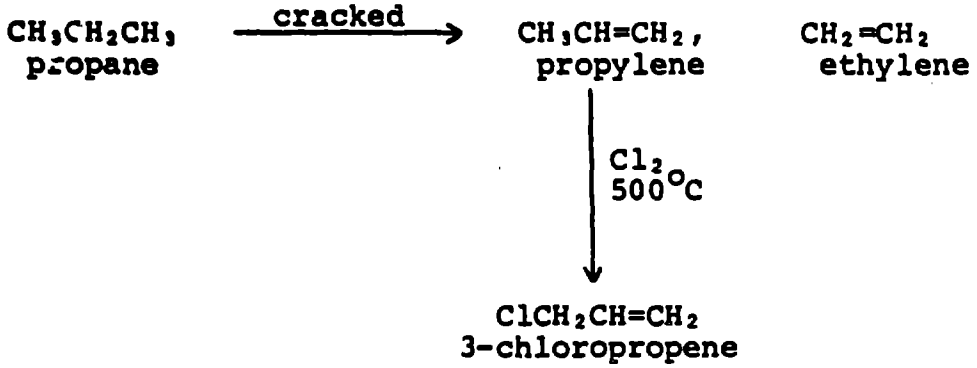
4. Sperm abnormality frequency was not affected by treatment.

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

It was concluded that 3-chloropropene was devoid of genetic effects detectable in these experiments, although it could have been tested at higher atmospheric concentrations.

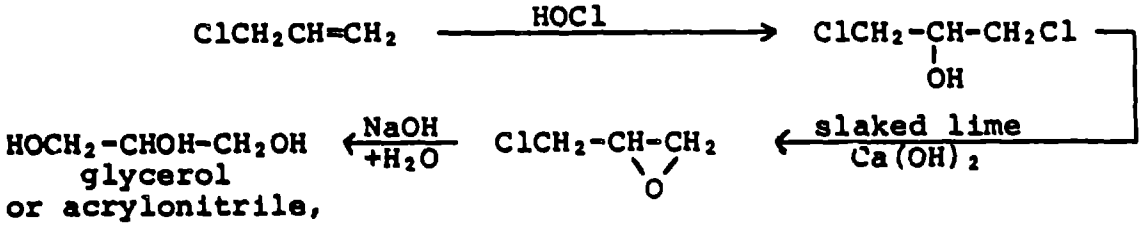
INTRODUCTION

3-Chloropropene (allyl chloride) (CAS No. 107-05-1) is a volatile, colourless liquid with an unpleasant, pungent odour which is manufactured from propane. Propane is cracked to give ethylene and propylene which undergoes high temperature chlorination to give 3-chloropropene.

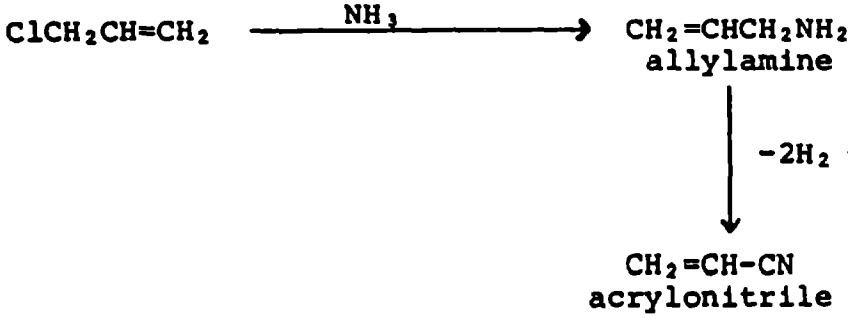


It can be used to manufacture allyl alcohol,

or glycerol,



or acrylonitrile,



(Acrylonitrile can, however, be formed directly from acetylene in the liquid state

Properties

Formula	$ClCH_2CH=CH_2$
Mol. wt.	76.53
Sp. gr. (20°C)	0.940
M.P.	-134.5°C
B.P.	45°C
Refractive index (20°C)	1.414
Flash point (open cup)	-28°C
Vapour density (air = 1)	2.64

It is insoluble in water, but infinitely miscible with alcohol, ether, acetone and benzene.

It is reactive, both as an organic halide and as an alkene and participates in both S_N1 and S_N2 reactions much more readily than the corresponding saturated compound. The double bond facilitates C-Cl bond cleavage in displacement and substitution reactions.

Toxicology

3-Chloropropene is a hepatotoxic agent which certainly can affect man. Serum bilirubin assay is a useful indicator of hepatotoxicity, particularly in its early diagnosis, so, it is significant that hyperbilirubinaemia occurs with high frequency among workers exposed to 3-chloropropene (Haüsler and Lenich, 1968). Certain serum enzymes (transaminases, lactate dehydrogenase and L-glutamate dehydrogenase) are elevated greatly in workers exposed to 1 ppm 3-chloropropene (Haüsler and Lenich, 1968). It can also affect the pancreas, as shown by the depression of trypsin and lipase activity, effects which are associated with the activation of enzyme inhibitors in the organ (Strusevich and Ehshtat, 1973).

In acute dosing experiments, the dermal LD_{50} in rabbits was 2,200 mg/kg, the oral LD_{50} in rats was 64 mg/kg. The human LC_{50} is about 3,000 ppm.

129 ppm 3-Chloropropene atmosphere inhaled for 1 h by mice produces considerable pulmonary damage, liver damage and slight kidney changes (NIOSH 1976).

Vapours of 3-chloropropene are quite irritating to the eyes nose and throat, and contact of the liquid with the skin, in addition to local vasoconstriction and numbness, may lead to rapid absorption and distribution throughout the body. Unless it is removed from skin, burns and internal injuries may result. Inhalation may cause headache, dizziness and, in high concentrations, loss of consciousness.

Chronically exposed animals show degenerative changes in the liver and kidneys. In a limited study, 3-chloropropene at a concentration of 3 ppm for 6 months failed to induce tumours in rats, guinea pigs or rabbits (Torkelson et al, 1959).

Because the compound has a very high vapour pressure at 37°C, the standard plate incorporation assay with Salmonella typhimurium is inappropriate and fails to show any mutagenic activity (Ames et al, 1975; McCoy et al, 1978). If steps are taken to reduce evaporation of 3-chloropropene from the plates, then mutagenicity can be demonstrated with S. typhimurium TA 1535 and TA 100. Rat liver preparations were not required for this activity (Ames et al, 1975; McCoy et al, 1978). Activity can also be demonstrated in the E. coli pol A^+/pol A^- system and gene conversion frequency is enhanced in Saccharomyces cerevisiae D_4 (McCoy et al, 1978; Rosenkranz et al, 1976).

In all of these microbial tests, 3-chloropropene does not require special activation or, if it does, the bacteria and yeast are capable of providing the conditions for the biotransformation. Rats also can metabolise this allyl halide, sulphur compounds being particularly important in this activity. Identified biliary metabolites are glutathione and cysteine conjugates while urinary metabolites include allyl mercapturic acid, its sulphoxide and 3-hydroxypropylmercapturic acid (Kaye et al, 1972).

The objective of the work reported here is to study the genotoxic potential of 3-chloropropene in more complex test systems than the microorganisms used previously. The data generated will be used to improve the evaluation of risks from exposure to this substance. The exposure conditions used were:

Human fibroblasts:	up to 9,900 µg/ml for 3 h.
Mice and Rats:	1 ppm and 25 ppm for 7 h/day for one or 5 days.
Drosophila:	150 ppm for 7 h.

MATERIALS AND METHODS

CHEMICALS

Test Substance

One kilogram of 3-chloropropene, Batch No. 17835 (stated purity 98%), was received from Aldrich Chemical Company 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	11 April 1980	10-11	10	220♂ 176♀	21-25 and 28 April 1980	Single dose cytogenetics slides not suitable.
Mouse	9 April 1980	10-12	12	44♂	21-25 April 1980	-
Rat	25 April 1980 etc	8-10	None	809 x 1C	None	DL matings.
Rat	12 June 1980	10-11	11	130♂ 130♀	23 June 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 19.5°C and 26°C, and relative humidity ca 50%, with extreme limits of 30% and 68%.

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 3-chloropropene 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 8 air changes per hour.

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

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

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

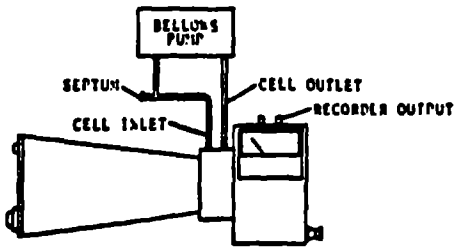
Calibration and Analytical Development

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 3-chloropropene 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 3-chloropropene were calibrated each day before vapour generation commenced.

The calibration was performed using a closed loop calibration system (see diagram below). For the high dose range, known volumes of 3-chloropropene 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 :	10.8 µm	10.8 µm
Pathlength :	20.25 m	20.25 m
Absorbence Range :	0.025 A	0.25 A
Slit Width :	1 mm	0.5 mm
Meter Response :	10	4
Recorder Voltage :	0.5 V	1 V
Chart Speed :	120 mm/h	120 mm/h

Calibration Data

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

Where:

C = Concentration (ppm)
V = Sample volume (µl)
ρ = Liquid density (g/cm^3)
M = Molecular weight of test sample
$\frac{(RT)}{(P)}$ = Molar volume of gas (24.06 at 20°C)
5.64 = Volume of Miran sample chamber (1)

Example of the Calculation for V

Compound: 3-Chloropropene

C = 25 ppm
ρ = 0.939 g/cm^3
M = 76.53

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

$$= \frac{25 \times 76.53}{0.939} \times \frac{5.64}{10^3 \times 24.06\ \mu l}$$

$$= 0.48\ \mu l$$

Therefore, to construct a calibration curve to cover the 25 ppm range, 0.2 µl samples of 3-chloropropene were injected into the analyser.

For the low concentration (1 ppm) atmosphere calibration curve, the instrument sensitivity had to be increased to a level where the baseline, although stable, was excessively noisy. Consequently, head-space analysis was adopted. An example of the calculations used follows.

$\log_{10} P = \left(-0.2185 \frac{A}{T}\right) + B$ (Schlessinger, 1972-3)

Where A = molar heat of vaporisation in calories per g-mole.

Temperature in laboratory = 21.5°C = 294.66°K
for 3-chloropropene, A = 7386.8 (Schlessinger,
and B = 7.992 1972-3)

$\therefore \log_{10} P = \left(\frac{-0.2185 \times 7386.8}{294.66}\right) + 7.992$

$= 7.992 - 5.478$
$= 2.514$
$\therefore \quad P = 326.59$

Using $\frac{P_1V_1}{T_1} = \frac{P_2V_2}{T_2}$ and

$P_1V_1 = nRT_1$

$n = \frac{P_2V_2}{T_2} \frac{T_1}{RT_1}$

$= \frac{P_2V_2}{T_2R}$

In which

$P_2 = 326.59$
$V_2 = 22.4$ l
$T_2 = 273.16°K$
$R = 62.4$ (l) (mm Hg)/(g-mole)(°K)

$\therefore \quad n = \frac{326.59 \times 22.4}{273.16 \times 62.4}$

$= 0.429$ ml
$= 429$ µl
$= 76.1$ ppm in 1 ml (Volume of Miran = 5.64 l)

$\therefore \quad 10$ µl $\equiv 0.74$ ppm

To construct a calibration curve to cover the 1 ppm range, 10 µl volumes of the head-space above 3-chloropropene in a sealed vial were injected into the IR analyser.

Atmosphere Generation

Schematic diagrams showing the vapour generating apparatus, exposure chambers and monitoring equipment is presented in Figures 1a and 1b. The high level atmosphere was produced by bubbling dry, oxygen-free nitrogen (BOC Limited) through a liquid reservoir of 3-chloropropene contained in a glass, gas washing or Drechsel bottle immersed in a temperature controlled water bath at 1°C. The low level atmosphere was generated by inserting a 100 µl glass syringe, through a rubber septum, into a glass T piece and feeding a constant volume of 3-chloropropene into a stream of nitrogen gas. The micro syringe was activated by a syringe driver. The nitrogen/3-chloropropene 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 3-chloropropene/air was ducted through 7/8" stainless steel piping to the top of the exposure chamber.

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

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

Homogeneity Data

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

Measurement of Chamber Concentrations

Atmospheric concentrations of 3-chloropropene 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.

Due to the highly sensitive spectrometer settings required to monitor the 1 ppm level, it was not possible to use the ZGA baseline from which to measure the chamber concentration. Sampling the exposure chamber air, without 3-chloropropene, gave an increase in absorbence of approximately 50% above ZGA. This was possibly due to the increased humidity inside the chamber. However, once the humidity within the chamber stabilised a constant absorbence reading was obtained. Therefore during the animal exposures a constant chamber baseline was obtained prior to commencement of atmosphere generation. Exposure chamber concentrations were then measured from this baseline.

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 3-chloropropene reservoir (a gas washing or Drechsel bottle), used for the high level generation, 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.

The micro syringe used for generating the low level atmosphere was refilled as necessary.

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 3-chloropropene were arranged in a single tier inside the exposure chamber. Air control animals were stacked in 2 tiers.

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

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

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

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

Positive Control Groups in Animal Tests

Preparation of Dosing Solutions

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

Treatment of Rats and Mice with Ethyl methanesulphonate

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

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

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

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

UNSCHEDULED DNA SYNTHESIS ASSAY

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

Chemicals

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

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

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

Test Solutions

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

Cells

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

Culture Maintenance and Growth Media

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

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

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

Animals

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

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

Preparation of the 9,000 g Supernatant Fluid from Livers

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

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

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

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

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

co-factor solution	9 parts
liver preparation	1 part

Preliminary Toxicity Test

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

The cells were harvested and suspended in growth medium as for sub-culture, sedimented by centrifugation at 200 g for 5 min and resuspended in fresh culture medium at a density of 5 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.

At a concentration of 9,900 µg/ml 75-100% of the cells were damaged, whereas at 990 µg/ml no cells were damaged.

DNA Repair Assay

The cells were harvested, sedimented, suspended in fresh culture medium at a density of 5 x 10^4 cells/ml and 2 ml samples of this suspension were pipetted into 35 mm tissue culture Petri dishes containing 3 sterile coverslips (Lux Scientific Corporation, California, U.S.A.). These were then incubated at 37°C in a humid atmosphere of 5% CO_2 in air for 72 h. The medium from each of the dishes was then replaced with 2 ml of arginine-deficient DMEM supplemented with 5% heat inactivated foetal bovine serum and the plates incubated for 24 h. The medium was then replaced with a further 1 ml of arginine-deficient DMEM and the incubation continued for a further 48 h. At the end of this time the cultures were divided into 2 groups and 100 µl of S-9 mix added to one of them. Solutions of hydroxyurea (250 mM) in sterile distilled water and 6-[^{3}H]-thymidine (21 Ci/mmol) were added to each culture giving final concentrations of 2.5 mM and 10 µCi/ml respectively. 3-Chloropropene was dissolved in dimethylsulphoxide and dilutions were made from this stock solution to give the required dose levels. 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 µg/ml
2-AAN	5 µg/ml

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

Autoradiography

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

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

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

Quantification of Repair Synthesis

The stained autoradiographs were examined with a Leitz Dialux 20 L microscope. Fifty nuclei were examined for each culture. The data are recorded as the average net grain counts for 3 coverslips $\pm$ 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), 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 30 May 1980) by neck dislocation.

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

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

Assessment

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

The following types of sperm were not scored:

(1) separated tails and heads.

(2) clumps of sperm.

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

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

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

I Normal

II Abnormal

A. hook upturned or elongated.

B. banana-shaped head.

C. amorphous head.

D. abnormal tail (sharp, 180^{o} angle or tight coiling only).

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

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

SEX-LINKED RECESSIVE LETHAL TEST IN DROSOPHILA MELANOGASTER

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

Strains

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

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

Medium

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

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

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

Exposures

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

*Nipogen: bacteriostatic agent (BDH Limited).

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

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

Toxicity Test

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

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

Recessive Lethal Test

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

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

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

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

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

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

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

STATISTICAL EVALUATION

Cytogenetics Tests

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

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

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

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

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

Dominant Lethal Assay

The variates analysed were:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Sperm Abnormalities Test

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

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

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

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

Sex-linked Recessive Lethal Test

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

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

F_2 Generation

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

F_3 Generation

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

Against this background, the criteria for result assessment were:

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

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

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

RESULTS

Instrument Calibration

Calibration of the IR spectrometers was performed daily when atmosphere generation work was undertaken during the development phase and when animals were being exposed to test vapours. An example of a calibration curve is given in Figure 2. Data for the construction of such curves are given for various exposure dates in Tables AT-1 to 3. The reproducibility of the calibration curve data from day to day is good.

Calibration ranges adopted were 0.39-3.10 ppm (1 ppm target concentration), 9.8-78.4 ppm (25 ppm target concentration) and 49.0-245.0 ppm (150 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 3-chloropropene was occurring. The results are shown in Table AT-4, where it can be seen that the maximum deviations encountered was at the 1 ppm target concentration and at the 25 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 1 ppm, 25 ppm and 150 ppm were obtained and recorded in Tables AT-5 and 6.

Deviations from the target concentrations of more than $\pm$ 10% were limited to a few minutes, so, the exposures were considered to be acceptable and the remaining portions of the experiments allowed to proceed.

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)

9 April 1980 Delivery: Four mice were haphazardly selected for PEAT. None of the mice showed clinical signs of infection or disease, there were no abnormalities observed at autopsy and the microbiological/parasitological examination did not reveal any pathogens. One mouse showed mild, acute, focal inflammatory response in the liver during histopathological examination, but otherwise there were no significant observations.

11 April 1980 Delivery: Ten male and 10 female rats were haphazardly selected for PEAT. None of the rats showed clinical signs of infection or disease. No significant autopsy findings were made except in one case and there were no pathogens discovered in the microbiological/parasitological examinations. The exceptional finding at autopsy was an irregular reddening of the lungs in one female rat; histologically, this rat showed focal interstitial pneumonitis. The only histological findings in the other rats were mild interstitial reactions in the lungs of 9/10 males and 9/10 females; a small focus of cortical fibrosis in the kidney of one male rat; cystic space in the medulla of one female rat.

12 June 1980 Delivery: Ten male and 10 female rats were haphazardly selected for PEAT. One female rat had a lesion on the right shoulder, but otherwise there were no significant clinical observations. At autopsy, lungs of one female rat had many dark red, pin-point foci and the lungs of another female had a few 1-2 mm, clear, raised foci on the right median lobe. The microbiological/parasitological examination did not reveal any pathogens. Histology revealed bronchus associated lymphoid tissue in all the rats. In the opinion of the pathologist these were probably the result of an active Sendai virus infection (endemic in Charles River rats). Past experience shows that they recede and do not cause clinical signs. The only other histological observation of note was the presence of proteinaceous casts in the kidney of one male rat.

Clinical Observations and Body Weights

There were no significant clinical observations made as a result of exposure of mice or rats to 1 ppm or 25 ppm 3-chloropropene.

Body weights recorded during the exposure period are given in Tables BW-1 to 4. No reduction in body weight gain was seen over the 5 day dosing period. Treatment with EMS did evoke an adverse response, however, with body weights falling to values at the end of the dosing period considerably lower than they were at the beginning.

UNSCHEDULED DNA SYNTHESIS ASSAY

In the assay involving tritiated thymidine incorporation into non-S phase cells, there was no indication of any increase in the number of silver grains per nucleus at any concentration of 3-chloropropene (Table UDS-1). The substances used - 4-nitroquinoline-N-oxide and 2-amino-anthracene - in concurrent positive control groups evoked significant levels of unscheduled DNA synthesis from the cells.

CYTOGENETIC ANALYSIS OF RAT BONE MARROW CELLS

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

In the multiple exposure cytogenetic test, there were no indications of an increase in the frequency of cells with chromosomal aberrations, following exposure to 3-chloropropene. Female rats showed significant reductions in the frequencies of cells with gaps and other types of aberrations ($P<0.05$ at 1 ppm; $P<0.001$ at 25 ppm). EMS treatment also was without significant effect in this experiment.

The single exposure experiment was repeated because the quality of the prepared slides from the first experiment was not acceptable. The first experiment slides, although scanned for quality were not systematically examined. Consequently, remarks on the effects of a single exposure to 3-chloropropene are based only on the second experiment. There were no increases in the frequencies of aberrant cells in either male or female rat bone marrow at any sampling time. EMS treated groups did show frequencies higher than the control values in males at the 6 h sampling time (all types, $P<0.01$), 24 h sampling time (all types and after exclusion of gaps $P<0.001$) and 48 h sampling time (all types $P<0.01$; after exclusion of gaps, $P<0.001$). In female rats significant increases occurred at the 6 h sampling time (all types, $P<0.001$; after exclusion of gaps, $P<0.01$) and 24 h sampling time (all types and after exclusion of gaps, $P<0.001$), but not at the 48 h sampling time.

3-Chloropropene had no deleterious effects detectable in bone marrow metaphase chromosomes.

DOMINANT LETHAL TEST

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

Certain females in assessment Week 1 were pregnant before dosing. This deficiency was discovered retrospectively when some pregnancies came to term at the time of assessment or the foetuses were recognised as being too large for gestation days 14-17. Such pregnancies are indicated in the Appendix Tables and were not included in the statistical analyses.

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 any large effect upon pregnancy frequency due to 3-chloropropene treatment, but there was a reduction in Week 2 in the positive control group.

Corpora lutea graviditatis counts (Table DL-3) were not reduced in either of the 3-chloropropene treated groups except in Week 2 of the 25 ppm atmosphere group; these counts were greatly reduced, however, in Weeks 1-3 of the positive control group.

Implantations per pregnancy (Table DL-4) were unaffected by 3-chloropropene treatment, but were reduced in Weeks 1-3 of the positive control group.

The frequencies of live implantations (Table DL-5) and live implantations and late deaths (Table DL-6) followed very closely the pattern of total implantations per pregnancy.

A review of the data showing pregnancies with either (1) one or more early deaths or (2) two or more early deaths (Table DL-7) did not indicate any increase in these frequencies in the 3-chloropropene treated groups, when compared with the air control group.

Analysis of the proportions of early deaths by various statistical methods (Tables DL-8 and 9) did not indicate any effects attributable to 3-chloropropene treatment. On the other hand, there were significant increases in early death frequencies in Weeks 1, 2, 4 and 8 (poisson transformation) and Weeks 1-4 and 8 (binomial transformation).

SPERM ABNORMALITY TEST

There were no increases in the frequencies of abnormal sperm in any of the categories examined following exposure to 3-chloropropene atmospheres. There were small increases in aberrant sperm frequencies in Categories C and D (amorphous head and folded tail, respectively) (Table SA-1 and 2 and Appendix Table SA).

SEX-LINKED RECESSIVE LETHAL TEST IN DROSOPHILA

There was no information on the toxicity of 3-chloropropene to flies, so, a preliminary study was made (Table RL-1).

A dose ranging experiment was undertaken on 27 March 1980 in which flies were exposed to 50 ppm 3-chloropropene for 1 or 3 h. Within a few minutes of the start of exposure, the flies showed reduced activity but there were no further adverse reactions noted. It was decided to increase the concentration of 3-chloropropene for the third group of flies, so, these were exposed to 50 ppm for 3 h, 100 ppm for 50 min and 160 ppm for 70 min. Even at this high concentration, there were no signs of toxicity. Survival was good and so was egg hatchability after exposure.

In the main test on 9 April 1980 exposure conditions chosen were: 150 ppm for 7 h. Two breeding stocks of flies (A and B) were exposed to these conditions. The results of the SLRL test are given in Table RL-2.

The air control group gave 2 lethals in the F_2 generation, both of them being in the first brood of Stock A flies. The frequency in this brood was 0.35%, but no other lethals were detected in a total of 3,598 vials set up and 3,359 vials scored. No lethals were observed in the 562 vials scored in the F_3 generation.

Two lethals were also scored in the F_2 generation from flies exposed to 3-chloropropene. These also were in Brood 1 of Stock A and the frequency was 0.35%. No other F_2 generation lethals were scored out of 3,599 vials set up and 3,272 scored. No F_3 generation lethals were scored in a total of 1,325 vials.

Flies exposed to a solution of 0.4% EMS in sucrose (v/v) for 5 h gave 14% lethals in the F_2 generation.

It is concluded that no genetic effects of 3-chloropropene were observed in this experiment during which flies were exposed to very high levels of the compound (by mammalian standards).

CONCLUSIONS

None of the tests yielded results which indicated that 3-chloropropene might have genetic effects in complex biological systems.

In the mammalian *in vivo* tests, a concentration of 25 ppm for 7 h per day also failed to induce any signs of systemic toxicity. Similarly, 150 ppm for 7 h was non-toxic to *Drosophila*. The compound was not tested, therefore, to its biologically extreme limits, although the federal standard of 1 ppm 3-chloropropene as a time-weighted average for up to a 10 h work day, 40 h work week was adequately covered.

REFERENCES

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

(2) Ames, B.N., McCann, J. and Yamasaki, E., Mutation Res., 31, (1975), 347.

(3) Haüsler, M. and Lenich, R., Arch. Toxicol., 23, (1968), 209.

(4) Kaye, C.M., Clapp, J.J. and Young, L., Xenobiotica, 2, (1972), 129.

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

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

(7) McCoy, E.C., Burrows, L. and Rosenkranz, H.S., Mutation Res., 57, (1978), 11.

(8) NIOSH, Registry of Toxic Effects of Chemical Substances, (1976).

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

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

(11) Rosenkranz, H.S., Gutter, B. and Speck, W.T., Mutation Res., 41, (1976) 61.

(12) Schlessinger, G.G., C.R.C. Handbook of Chemistry and Physics, 53rd Edition, (1972-3), D-151.

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

(14) Strusevich, E.A. and Ehshtat, B., Gig. i Sanit., 8, (1973), 73.

(15) Torkelson, T.R., Wolf, M.A., Oyen, F. and Rowe, V.K., Am. Ind. Hyg. Assoc. J., 20, (1959), 217.

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

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

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

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

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

TABLE AT-1

3-Chloropropene
Calibration Data for Low Level

Dose Level: 1 ppm v/v

Batch No.: 17835

Volume µl	Conc., ppm, (v/v)	Cumulative Chart Deflection, mm						
		21 April 1980	22 April 1980	23 April 1980	24 April 1980	25 April 1980	28 April 1980	23 June 1980
0	0	0	0	0	0	0	0	0
5.0	0.39	-	-	-	-	-	-	20.0
10.0	0.78	31.0	37.5	38.0	34.5	40.0	38.0	44.0
15.0	1.17	-	-	-	-	-	-	67.0
20.0	1.55	70.5	75.0	77.0	73.0	77.0	77.0	88.0
25.0	1.95	-	-	-	-	-	-	105.0
30.0	2.33	106.0	113.0	114.5	108.0	109.0	117.0	-
40.0	3.10	143.0	151.0	148.0	142.0	147.0	156.0	-
Chart Deflection (mm) for 1 ppm		46.0	49.0	49.0	46.5	48.0	50.0	56.0

● = Headspace injected

Instrument Setting

Pathlength: 20.25 m
Wavelength: 10.8 µm
Absorbence Range: 0.025 A
Slit Width: 1 mm
Meter Response: 10
Recorder Voltage: 0.5 V
Chart Speed: 120 mm/h

Calibration

Syringe: 10 µl Hamilton
Injection Volume: 10 µl (5 µl)
No. of Repeat Injections: 4 (5)

TABLE AT-2

3-Chloropropene
Calibration Data for High Level

Dose Level: 25 ppm v/v Batch No.: 17835

Volume µl	Conc., ppm, (v/v)	Cumulative Chart Deflection, mm						
		21 April 1980	22 April 1980	23 April 1980	24 April 1980	25 April 1980	28 April 1980	23 June 1980
0	0	0	0	0	0	0	0	0
0.2	9.8	-	-	-	-	-	-	33.0
0.4	19.6	32.0	46.5	34.0	36.5	49.0	45.0	65.0
0.6	27.4	-	-	-	-	-	-	94.5
0.8	39.2	68.0	94.0	70.5	71.5	101.0	90.5	126.5
1.0	49.0	-	-	-	-	-	-	157.0
1.2	58.8	102.0	140.0	110.0	114.5	148.0	137.5	-
1.6	78.4	136.0	186.0	147.5	157.0	196.5	180.0	-
Chart Deflection (mm) for 25 ppm		44.0	59.0	47.0	49.0	62.5	58.0	84.0

Instrument Setting

Pathlength: 20.25 m (a)
Wavelength: 10.8 µm
Absorbence Range: 0.25 A
Slit Width: 0.5 mm
Meter Response: 4
Recorder Voltage: 1 V (b)
Chart Speed: 120 mm/h

Calibration

Syringe: 10 µl Hamilton (1 µl)
Injection Volume: 0.4 µl (0.2 µl)
No. of Repeat Injections: 4 (5)

(a) Pathlength set at 18.75 m on 22 April 1980.

(b) Recorder set at 2 V on 21 and 23 April 1980 and at 0.5 V on 24 and 28 April 1980.

TABLE AT-3

3-Chloropropene

Calibration Data for Drosophila Main Test

Dose Level: 150 ppm v/v
Batch No.: 17835

Volume, µl	Conc., ppm.(v/v)	Cumulative Chart Deflection, mm
		9 April 1980
0	0	0
1.0	49.0	30.0
2.0	98.0	60.0
3.0	147.0	90.0
4.0	196.0	118.5
5.0	245.0	146.5
Chart Deflection (mm) for 150 ppm		91.0

Instrument Setting

Pathlength: 20.25 mm
Wavelength: 10.8 µm
Absorbence Range: 1 A
Slit Width: 0.5 mm
Meter Response: 4
Recorder Voltage: 1 V
Chart Speed: 120 mm/h

Calibration

Syringe: 10 µl Hamilton
Injection Volume: 1.0 µl
No. of Repeat Injections: 5

TABLE AT-4

3-Chloropropene
Chamber Atmosphere Homogeneity Data

Dose Level: 1 ppm and 150 ppm

Sample Location	% Deviation from Reference Sampling Point	
	Low	High
Reference Point (R)	0	0
Right Centre (RC)	-6.3	-3.4
Right Front (RF)	-5.3	0
Centre Front (CF)	-5.3	0
Left Front (LF)	5.1	-3.4
Left Centre (LC)	0	-3.4
Left Back (LB)	-6.3	-5.2
Centre Back (CB)	9.1	1.7
Right Back (RB)	*17.3	-1.7

* Measured after generating syringe refilled.

Top view of exposure chamber

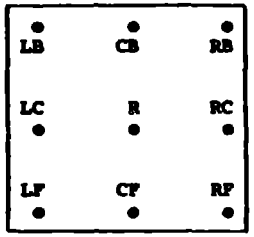


TABLE AT-5

3-Chloropropene
Atmospheric Analysis by Infra-red Spectroscopy
Target Concentration 1.0 ppm

Exposure Day		Single	Multiple 1	Multiple 2	Multiple 3	Multiple 4	Multiple 5	Repeat Single
Deviation from Target Concentration in Minutes	+57.5	-	-	-	-	-	5	-
	+50.0	-	-	-	-	-	-	35
	+47.5	-	-	-	-	-	-	15
	+45.0	-	-	10	-	-	-	-
	+42.5	-	-	-	-	-	15	15
	+40.0	-	-	30	-	-	-	-
	+37.5	-	-	45	-	-	-	5
	+35.0	10	-	5	15	10	15	10
	+32.5	-	-	-	15	10	-	15
	+30.0	40	-	-	-	80	-	-
	+27.5	-	-	5	10	-	25	-
	+25.0	-	-	-	45	-	-	17
	+22.5	-	-	30	-	35	15	-
	+20.0	10	-	35	15	10	75	13
	+17.5	55	-	10	15	-	40	-
	+15.0	10	5	10	25	15	5	30
	+12.5	-	15	-	-	-	15	10
	+10.0	60	-	5	35	5	80	-
	+ 7.5	-	-	10	25	-	30	-
	+ 5.0	-	15	110	75	5	10	25
	+ 2.5	20	-	80	10	-	-	-
	0	65	15	5	20	25	50	5

TABLE AT-5 (continued)

3-Chloropropene

Exposure Day		Single	Multiple 1	Multiple 2	Multiple 3	Multiple 4	Multiple 5	Repeat Single
Deviation from Target Concentration in Minutes	- 2.5	25	5	20	-	25	-	-
	- 5.0	20	-	-	10	15	-	-
	- 7.5	-	25	10	10	-	10	25
	-10.0	5	10	-	10	-	-	5
	-12.5	-	-	-	-	45	-	40
	-15.0	-	15	-	10	-	-	35
	-17.5	-	-	-	10	70	-	-
	-20.0	50	-	-	5	15	-	25
	-22.5	25	-	-	60	20	20	-
	-27.5	-	-	-	-	5	-	90
	-30.0	5	10	-	-	5	-	5
	-32.5	5	50	-	-	-	10	5
	-35.0	-	15	-	-	-	-	-
	-37.5	-	30	-	-	25	-	-
	-40.0	5	25	-	-	-	-	-
	-42.5	-	25	-	-	-	-	-
	-45.0	5	20	-	-	-	-	-
	-47.5	-	20	-	-	-	-	-
	-50.0	-	15	-	-	-	-	-
	-52.5	-	10	-	-	-	-	-
	-55.0	5	5	-	-	-	-	-
	-57.5	-	45	-	-	-	-	-
	-67.5	-	15	-	-	-	-	-
	-70.0	-	15	-	-	-	-	-
	-80.0	-	15	-	-	-	-	-
Time Averaged Concentration for 7 h (ppm)		1.02	0.64	1.15	1.05	1.01	1.12	1.01

TABLE AT-5 (continued)

3-Chloropropene
Target Concentration 25 ppm

Exposure Day		Single	Multiple 1	Multiple 2	Multiple 3	Multiple 4	Multiple 5	Repeat Single
Deviation from Target Concentration in Minutes	+30.0	-	-	-	-	-	5	-
	+20.0	-	-	-	-	-	10	-
	+15.0	-	-	20	10	-	-	-
	+12.5	-	-	-	-	-	-	5
	+10.0	10	-	35	20	5	20	
	+ 7.5	20	-	-	35	-	-	5
	+ 5.0	70	85	120	105	85	10	25
	+ 2.5	65	35	75	45	90	20	10
	0	90	110	10	170	185	30	215
	- 2.5	40	105	15	10	10	30	80
	- 5.0	105	80	120	20	40	95	55
	- 7.5	5	5	-	-	-	115	20
	-10.0	15	-	25	-	5	70	-
	-12.5	-	-	-	-	-	15	-
Time Averaged Concentration for 7 h (ppm)		25.0	24.9	25.3	25.7	25.3	24.0	24.8

TABLE AT-6

3-Chloropropene
Atmospheric Analysis by Infra-red Spectroscopy
Target Concentration 150 ppm

Exposure Day	% Deviation from Target Concentration in Minutes									Time Averaged Concentration for 7 h (ppm)
	-2.5	0	+1.5	+2.5	+5.0	+7.5	+10.0	+12.5	+15.0	
Drosophila Main Test	45	160	120	30	30	20	5	-	10	154.3

TABLE BW-1

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

Sex	Day	Air Control (0 ppm)	1 ppm	25 ppm	5 x 100 mg/kg EMS
Male	1	363.1 ± 14.6	360.2 ± 20.3	358.0 ± 20.1	357.8 ± 19.0
	2	367.3 ± 16.0	362.1 ± 20.7	361.6 ± 21.8	352.7 ± 18.3
	3	370.7 ± 12.9	363.2 ± 19.4	363.6 ± 21.8	340.8 ± 19.1
	4	373.4 ± 14.1	365.3 ± 19.5	367.2 ± 20.7	332.8 ± 19.5
	5	376.4 ± 12.7	366.8 ± 19.8	368.2 ± 21.3	323.4 ± 18.9
	Weight gain/ loss	13.3	6.6	10.2	-34.4
Female	1	240.0 ± 9.3	231.0 ± 10.8	237.2 ± 15.5	227.2 ± 13.0
	2	238.6 ± 11.1	230.6 ± 11.8	238.4 ± 17.9	222.1 ± 13.7
	3	241.5 ± 10.8	232.5 ± 11.4	243.0 ± 16.0	215.8 ± 13.2
	4	245.2 ± 12.4	234.7 ± 11.5	244.0 ± 16.7	209.5 ± 12.1
	5	246.8 ± 13.3	235.2 ± 11.4	245.7 ± 15.9	204.1 ± 11.8
	Weight gain/ loss	6.8	4.2	8.5	-23.1

TABLE EM-2

3-Chloropropene
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)	1 ppm	25 ppm	250 mg/kg EMS
Male	6	406.1 ± 20.2	389.5 ± 25.6	396.2 ± 24.4	396.6 ± 26.3
	24	390.9 ± 26.8	383.5 ± 31.6	393.9 ± 27.2	388.8 ± 24.2
	48	372.0 ± 23.4	384.6 ± 20.9	391.3 ± 29.2	385.6 ± 21.3
Female	6	251.0 ± 13.6	254.2 ± 15.1	253.3 ± 17.7	254.0 ± 14.8
	24	255.7 ± 18.9	267.0 ± 14.7	254.4 ± 21.3	257.9 ± 23.3
	48	252.3 ± 21.2	252.6 ± 20.0	251.0 ± 11.1	249.6 ± 17.2

TABLE BW-2 (Repeat)

3-Chloropropene
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)	1 ppm	25 ppm	250 mg/kg EMS
Male	6	394.6 ± 19.4	376.7 ± 18.7	384.4 ± 25.3	385.0 ± 27.7
	24	381.9 ± 12.8	374.6 ± 17.3	379.5 ± 27.2	391.4 ± 30.4
	48	373.5 ± 22.5	374.7 ± 17.2	387.1 ± 29.3	374.7 ± 15.0
Female	6	238.1 ± 15.0	239.2 ± 11.0	241.6 ± 15.0	232.2 ± 14.6
	24	236.4 ± 10.9	236.5 ± 19.9	233.3 ± 18.8	238.8 ± 12.3
	48	232.0 ± 14.9	236.0 ± 14.7	241.7 ± 15.9	240.2 ± 13.3

TABLE BW-3

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

Day	Air Control (0 ppm)	1 ppm	25 ppm	5 x 100 mg/kg EMS
1	356.2 ± 16.6	342.5 ± 16.1	361.9 ± 18.7	369.7 ± 27.9
2	360.0 ± 16.1	345.4 ± 16.1	366.6 ± 22.1	362.7 ± 29.0
3	363.8 ± 17.2	350.0 ± 18.6	369.5 ± 22.4	350.4 ± 29.1
4	369.9 ± 17.3	351.7 ± 20.4	371.9 ± 23.9	340.0 ± 30.4
5	373.2 ± 17.4	354.7 ± 20.7	375.9 ± 26.0	328.1 ± 30.4
Weight gain/loss	17.0	12.2	14.0	-41.6

TABLE BW-4

3-Chloropropene
Sperm Abnormalities Test
Group Mean Body Weights (g) for the Dosing Period of Male B6C3F1 Mice

Day	Air Control (0 ppm)	1 ppm	25 ppm	5 x 100 mg/kg EMS
1	26.2 ± 1.0	24.9 ± 1.2	25.3 ± 1.2	25.0 ± 0.7
2	26.2 ± 1.0	25.0 ± 1.2	25.8 ± 1.3	24.9 ± 0.7
3	26.4 ± 1.2	25.2 ± 0.9	25.9 ± 1.4	25.0 ± 0.7
4	26.4 ± 1.2	25.6 ± 0.8	26.0 ± 1.3	25.4 ± 1.0
5	26.4 ± 1.1	24.8 ± 1.0	25.9 ± 1.2	24.6 ± 1.4
Weight gain/loss	0.2	-0.1	0.6	-0.4

TABLE UDS-1

3-Chloropropene
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 ± 31.0
2-Aminoanthracene	5	-	78.9 ± 46.2	-
3-Chloropropene	77	77	4.7 ± 2.9	5.4 ± 4.1
	155	155	5.3 ± 3.6	5.0 ± 3.4
	310	310	3.9 ± 5.1	6.5 ± 5.0
	619	619	4.4 ± 2.9	5.1 ± 3.6
	1,238	1,238	7.2 ± 4.8	6.3 ± 3.0
	2,475	2,475	5.8 ± 4.3	5.4 ± 3.4
	4,950	4,950	4.6 ± 2.6	4.5 ± 2.7
	9,900	9,900	6.0 ± 3.3	4.7 ± 3.1

TABLE CA-MD-M-1

3-Chloropropene
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Males

Multiple Dosing

Sampling Time: 6 h

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

TABLE CA-MD-M-2

3-Chloropropene
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	0.191	0.041		0.160	0.028	
1 ppm	0.191	0.041	0.00	0.160	0.028	0.00
25 ppm	0.211	0.041	0.35	0.160	0.028	0.00
EMS, 100 mg/kg	0.303	0.041	1.95	0.219	0.028	1.50

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

TABLE CA-MD-F-1

3-Chloropropene
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Females

Multiple Dosing

Sampling Time: 6 h

Group	Number of Spreads Observed	Observed Aberrations						Miscellaneous
		Chromatid			Chromosome			
		Gap	B w F	B w/o F	Gap	B w F	B w/o F	
Air Control, 7 h/day	500	7	2	-	1	-	-	-
1 ppm, 7 h/day	500	1	-	-	3	-	-	-
25 ppm, 7 h/day	450	-	-	-	-	-	-	-
EMS, 100 mg/kg/day	500	14	-	1	-	-	-	1 Chromosomal Fragment

TABLE CA-MD-F-2

3-Chloropropene
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	0.302	0.038		0.180	0.019	
1 ppm	0.191	0.038	-2.04*	0.141	0.019	-1.48
25 ppm	0.141	0.041	-2.89**	0.141	0.029	1.44
EMS, 100 mg/kg	0.349	0.038	0.87	0.180	0.019	0.00

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

*P<0.05

**P<0.01

TABLE CA-M^{f}-1

3-Chloropropene
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Males

Single Dosing — Sampling Time: 6 h

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

TABLE CA-M6-2

3-Chloropropene
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	0.199	0.038		0.180	0.028	
1 ppm	0.180	0.038	-0.35	0.180	0.028	0.00
25 ppm	0.231	0.038	0.58	0.160	0.028	-0.50
EMS, 250 mg/kg	0.389	0.040	3.42**	0.219	0.030	0.93

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

TABLE CA-M24-1

3-Chloropropene
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	464	11	-	-	2	-	-	1 Robertsonian Translocation
1 ppm, 7 h/day	500	17	1	-	2	-	-	1 Chromatid Fragment
25 ppm, 7 h/day	473	9	2	-	1	-	-	1 Robertsonian Trars-location
EMS, 250 mg/kg/day	470	56	35	2	3	1	1	18 Chromatid Fragments 1 Translocation 1 Multi Aberration 3 Exchanges 2 Chromosomal Fragments

TABLE CA-M24-2

3-Chloropropene
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	0.338	0.058		0.173	0.042	
1 ppm	0.434	0.058	1.17	0.171	0.042	-0.03
25 ppm	0.328	0.058	-0.12	0.207	0.042	0.58
EMS, 250 mg/kg	0.819	0.058	5.85***	0.566	0.042	6.63***

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

TABLE CA-M48-1

3-Chloropropene
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	5	1	-	-	-	-	-
1 ppm, 7 h/day	450	7	-	-	-	-	-	1 Chromosomal Fragment
25 ppm, 7 h/day	450	4	6	-	-	-	-	-
EMS, 250 mg/kg/day	500	15	15	-	-	-	-	2 Exchanges 7 Chromatid Fragments 4 Chromosomal Fragments 1 Multi Aberration

TABLE CA-M48-2

3-Chloropropene
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	0.260	0.049		0.160	0.041	
1 ppm	0.284	0.052	0.33	0.163	0.043	0.04
25 ppm	0.317	0.052	0.79	0.241	0.043	1.36
EMS, 250 mg/kg	0.493	0.049	3.36**	0.406	0.041	4.28***

S.E. of mean = Standard error of Freeman-Tukey binomial transformation mean
**p<0.01
***p<0.001

TABLE CA-F6-1

3-Chloropropene
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Females

Single Dosing

Sampling Time: 6 h

Group	Number of Spreads Observed	Observed Aberrations						Miscellaneous
		Chromatid			Chromosome			
		Gap	B w F	B w/o F	Gap	B w F	B w/o F	
Air Control, 7 h/day	450	2	-	-	-	-	-	-
1 ppm, 7 h/day	450	4	-	-	-	-	-	-
25 ppm, 7 h/day	500	3	-	-	-	-	-	-
EMS, 250 mg/kg/day	500	17	3	1	-	-	-	-

TABLE CA-F6-2

3-Chloropropene
Cytogenetic Analysis of Rat Bone Marrow Cells
Summary of Observed Aberrations
Females

Single Dosing — Sampling Time: 6 h

Treatment Group	Spreads with Aberrations					
	Total			Excluding Gaps		
	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t
Air Control	0.185	0.044		0.141	0.020	
1 ppm	0.229	0.044	0.71	0.141	0.020	-0.00
25 ppm	0.200	0.042	0.26	0.141	0.019	-0.00
EMS, 250 mg/kg	0.400	0.042	3.55***	0.211	0.019	2.55*

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

*P<0.05

***P<0.001

TABLE CA-M6 and F6-1 (Supplementary)

3-Chloropropene
Cytogenetic Analysis of Rat Bone Marrow Cells
Supplementary Observations
Males and Females

Group	Animal Number/ Sex	Miscellaneous Observations
Air Control, 7 h/day	7♂	1 Chromosome split at centromere
	31♂	1 Chromosome split at centromere
	165♀	1 Chromosome split at centromere
1 ppm, 7 h/day	198♂	1 Chromosome split at centromere
EMS, 250 mg/kg/day	95♂	2 Chromosomes split at centromere
	258♀	2 Chromosomes split at centromere
	252♀	1 Chromosome split at centromere

TABLE CA-F24-1

3-Chloropropene
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	13	-	-	1	-	-	1 Robertsonian Translocation
1 ppm, 7 h/day	500	5	-	-	-	-	-	2 Robertsonian Translocations
25 ppm, 7 h/day	472	8	-	-	1	-	-	1 Chromosomal Fragment 1 Chromatid Fragment
EMS, 250 mg/kg/day	470	53	19	4	1	5	1	2 Exchanges 28 Chromatid Fragments 1 Chromosomal Fragment

TABLE CA-F24-2

3-Chloropropene
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	0.330	0.059		0.160	0.041	
1 ppm	0.247	0.059	-1.00	0.180	0.041	0.35
25 ppm	0.295	0.059	-0.42	0.187	0.041	0.47
EMS, 250 mg/kg	0.725	0.059	4.74***	0.492	0.041	5.76***

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

TABLE CA-P48-1

3-Chloropropene
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	404	5	2	-	-	-	-	1 Chromatid Fragment
1 ppm, 7 h/day	500	3	1	-	-	-	-	1 Chromatid Fragment
25 ppm, 7 h/day	500	8	3	-	-	-	-	-
EMS, 250 mg/kg/day	450	19	15	-	1	-	-	7 Exchanges 3 Chromatid Fragments 1 Chromosomal Fragment

TABLE CA-F48-2

3-Chloropropene
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	0.397	0.065		0.297	0.065	
1 ppm	0.220	0.062	-1.97	0.180	0.062	-1.30
25 ppm	0.331	0.062	-0.73	0.191	0.062	-1.19
EMS, 250 mg/kg	0.535	0.065	1.49	0.410	0.065	1.23

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

TABLE CA-M48-1 (Supplementary)

3-Chloropropene
Cytogenetic Analysis of Rat Bone Marrow Cells
Supplementary Observations
Males

Single Dosing — Sampling Time: 48 h

Animal Number	Sex	Group	Aberrations
82	♂	High	1 Chromosome split at centromere

TABLE DL-1

3-Chloropropene
Dominant Lethal Test in Rats
Pregnancy Frequency (Females with Corpora Lutea Graviditatis)

Assessment Week from Dosing	Air Control (0 ppm)	1 ppm	25 ppm	5 x 100 mg/kg EMS
1	88%	100%	75%	95%
2	85%	100%	100%	80%
3	95%	95%	100%	75%
4	85%	100%	95%	84%
5	100%	95%	100%	95%
6	90%	100%	95%	100%
7	95%	90%	90%	95%
8	85%	100%	100%	95%
9	95%	85%	90%	95%
10	95%	90%	100%	95%

TABLE DL-2

3-Chloropropene
Dominant Lethal Test in Rats
Pregnancy Frequency (Females with Implantations)

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)		1 ppm		25 ppm		5 x 100 mg/kg EMS	
1	15/17	88%	18/19	95%	15/20	75%	15/19	80%
2	17/20	85%	20/20	100%	20/20	100%	7/20	35%
3	18/20	90%	19/20	95%	20/20	100%	20/20	100%
4	17/20	85%	19/19	95%	19/20	95%	14/19	74%
5	19/20	95%	18/20	90%	20/20	100%	18/20	90%
6	17/20	85%	19/20	95%	19/20	95%	18/19	95%
7	19/20	95%	17/20	85%	18/20	90%	19/20	95%
8	16/20	80%	18/20	90%	20/20	100%	19/20	95%
9	18/20	90%	17/20	85%	18/20	90%	19/20	95%
10	17/19	89%	18/20	90%	18/20	90%	19/20	95%

TABLE DL-3

3-Chloropropene
Dominant Lethal Test in Rats
Total Number of Corpora Lutea per Pregnancy

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	1 ppm	25 ppm	5 x 100 mg/kg EMS
1	[1] 14.5 ± 0.75	14.1 ± 0.69	13.4 ± 0.75	9.0 ± 0.99***
2	13.3 ± 0.59	12.7 ± 0.55	11.2 ± 0.55*	2.4 ± 0.37***
3	12.0 ± 0.49	13.4 ± 0.47*	12.1 ± 0.46	6.0 ± 1.00
4	12.9 ± 0.63	13.2 ± 0.60	12.6 ± 0.60	12.1 ± 0.72
5	14.8 ± 0.69	13.0 ± 0.70	14.8 ± 0.67	15.2 ± 0.91
6	13.4 ± 0.68	13.1 ± 0.64	13.3 ± 0.64	13.4 ± 0.49
7	13.0 ± 0.46	12.2 ± 0.49	13.1 ± 0.48	13.0 ± 0.42
8	12.6 ± 0.58	12.8 ± 0.55	12.5 ± 0.52	13.0 ± 0.66
9	12.0 ± 0.62	12.5 ± 0.64	12.3 ± 0.62	13.1 ± 0.28
10	12.8 ± 0.75	12.7 ± 0.73	10.7 ± 0.73	13.2 ± 0.59

1 = Mean ± standard error of mean

***P<0.001

TABLE DL-4

3-Chloropropene
Dominant Lethal Test in Rats
Total Implantations per Pregnancy

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	1 ppm	25 ppm	5 x 100 mg/kg EMS
1	[1] 13.6 ± 0.73	13.8 ± 0.67	12.8 ± 0.73	8.3 ± 1.11**
2	13.0 ± 0.58	13.2 ± 0.54	11.9 ± 0.54	1.4 ± 0.20***
3	11.4 ± 0.40	12.4 ± 0.39	11.8 ± 0.38	1.5 ± 0.50*
4	12.8 ± 0.64	12.2 ± 0.60	12.8 ± 0.60	11.3 ± 0.71
5	12.9 ± 0.63	11.4 ± 0.64	13.3 ± 0.61	12.4 ± 0.71
6	12.5 ± 0.69	13.0 ± 0.65	12.4 ± 0.65	12.8 ± 0.42
7	12.5 ± 0.67	12.7 ± 0.71	12.4 ± 0.69	13.4 ± 0.38
8	12.8 ± 0.50	13.7 ± 0.47	13.3 ± 0.45	12.6 ± 0.66
9	12.1 ± 0.69	11.4 ± 0.71	12.5 ± 0.69	12.9 ± 0.40
10	12.4 ± 0.60	12.9 ± 0.58	11.8 ± 0.58	12.3 ± 0.47

1 = Mean ± standard error of mean

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

TABLE DL-5

3-Chloropropene
Dominant Lethal Test in Rats
Live Implantations per Pregnancy

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	1 ppm	25 ppm	5 x 100 mg/kg EMS
1	[1] 13.1 ± 0.70	13.1 ± 0.64	12.1 ± 0.70	3.5 ± 0.99***
2	12.4 ± 0.59	12.7 ± 0.54	11.5 ± 0.54	0.0 ± 0.00***
3	10.8 ± 0.43	12.0 ± 0.42	11.2 ± 0.41	0.0 ± 0.00*
4	11.7 ± 0.64	11.7 ± 0.61	12.4 ± 0.61	7.7 ± 1.07**
5	11.8 ± 0.62	10.6 ± 0.64	12.7 ± 0.61	11.6 ± 0.67
6	12.0 ± 0.70	12.8 ± 0.66	11.8 ± 0.66	12.1 ± 0.55
7	12.0 ± 0.66	12.3 ± 0.70	11.7 ± 0.68	12.8 ± 0.42
8	12.4 ± 0.71	13.3 ± 0.67	12.5 ± 0.64	11.5 ± 0.67
9	11.5 ± 0.78	10.6 ± 0.80	11.7 ± 0.78	12.6 ± 0.43
10	11.8 ± 0.68	11.9 ± 0.66	11.2 ± 0.66	11.5 ± 0.56

1 = Mean ± standard error of mean

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

TABLE DL-6

3-Chloropropene
Dominant Lethal Test in Rats
Live Implantations and Late Deaths per Pregnancy

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	1 ppm	25 ppm	5 x 100 mg/kg EMS
1	[1] 13.2 ± 0.72	13.2 ± 0.66	12.3 ± 0.72	3.5 ± 0.99***
2	12.5 ± 0.59	12.8 ± 0.54	11.6 ± 0.54	0.0 ± 0.00***
3	10.8 ± 0.44	12.0 ± 0.42	11.3 ± 0.41	0.0 ± 0.00*
4	11.7 ± 0.64	11.7 ± 0.61	12.4 ± 0.61	7.8 ± 1.07**
5	11.8 ± 0.62	10.6 ± 0.64	12.7 ± 0.61	11.7 ± 0.63
6	12.0 ± 0.70	12.8 ± 0.66	11.8 ± 0.66	12.1 ± 0.55
7	12.0 ± 0.67	12.3 ± 0.71	11.7 ± 0.69	12.8 ± 0.42
8	12.6 ± 0.56	13.3 ± 0.53	13.0 ± 0.50	11.6 ± 0.65
9	11.6 ± 0.78	10.6 ± 0.80	11.7 ± 0.78	12.6 ± 0.43
10	11.8 ± 0.68	12.1 ± 0.67	11.2 ± 0.67	11.6 ± 0.57

1 = Mean ± standard error of mean

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

TABLE DL-7

3-Chloropropene

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

TABLE DL-8

3-Chloropropene
Dominant Lethal Test in Rats
Early Death Frequency, Freeman-Tukey Poisson Transformation

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	1 ppm	25 ppm	5 x 100 mg/kg EMS
1	[1] 1.520 ± 0.2324	1.745 ± 0.2121	1.663 ± 0.2324	4.075 ± 0.5706***
2	1.668 ± 0.1857	1.532 ± 0.1712	1.495 ± 0.1712	2.728 ± 0.1479**
3	1.730 ± 0.2053	1.449 ± 0.1998	1.675 ± 0.1947	2.780 ± 0.3660
4	2.172 ± 0.2235	1.524 ± 0.2114*	1.560 ± 0.2114	3.580 ± 0.4735*
5	1.970 ± 0.2649	2.001 ± 0.2722	1.697 ± 0.2582	1.786 ± 0.2477
6	1.585 ± 0.1948	1.187 ± 0.1843	1.667 ± 0.1843	1.908 ± 0.2060
7	1.629 ± 0.1908	1.542 ± 0.2017	1.946 ± 0.1960	1.675 ± 0.2147
8	1.354 ± 0.1793	1.512 ± 0.1691	1.461 ± 0.1604	2.174 ± 0.2392*
9	1.626 ± 0.2515	1.899 ± 0.2515	1.709 ± 0.2515	1.411 ± 0.1658
10	1.566 ± 0.2485	1.859 ± 0.2415	1.742 ± 0.2415	1.763 ± 0.2541

1 = Mean ± standard error of mean

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

TABLE DL-9

3-Chloropropene
Dominant Lethal Test in Rats
Early Death Frequency, Freeman-Tukey Binomial Transformation

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	1 ppm	25 ppm	5 x 100 mg/kg EMS
1	[1] 0.407 ± 0.0609	0.465 ± 0.0556	0.459 ± 0.0609	1.716 ± 0.2443***
2	0.454 ± 0.0522	0.418 ± 0.0481	0.437 ± 0.0481	2.408 ± 0.0352***
3	0.507 ± 0.0609	0.410 ± 0.0592	0.432 ± 0.0577	2.421 ± 0.0870*
4	0.600 ± 0.0624	0.446 ± 0.0590	0.430 ± 0.0590	1.172 ± 0.1780**
5	0.544 ± 0.0731	0.588 ± 0.0751	0.464 ± 0.0712	2.496 ± 0.0642
6	0.450 ± 0.0581	0.323 ± 0.0550	0.482 ± 0.0550	0.533 ± 0.0644
7	0.468 ± 0.0526	0.423 ± 0.0556	0.549 ± 0.0541	0.453 ± 0.0587
8	0.379 ± 0.0538	0.405 ± 0.0507	0.405 ± 0.0481	0.607 ± 0.0652*
9	0.502 ± 0.0900	0.565 ± 0.0926	0.507 ± 0.0900	0.338 ± 0.0471
10	0.491 ± 0.0834	0.517 ± 0.0811	0.502 ± 0.0811	0.504 ± 0.0779

1 = Mean ± standard error of mean

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

TABLE SA-1

3-Chloropropene
Sperm Abnormality Test in Mice
Numbers and Proportions of Abnormalities

Multiple Dosing

Dose Group	Number Normal	Number Abnormal*						Percent Abnormal					
		A	B	C	D	E	Total	A	B	C	D	E	Total
Air Control, 7 h/day	9590	10	15	183	51	151	410	0.10	0.15	1.83	0.51	1.51	4.10
1 ppm, 7 h/day	9626	11	19	132	77	135	374	0.11	0.19	1.32	0.77	1.35	3.74
25 ppm, 7 h/day	9636	6	18	148	52	140	364	0.06	0.15	1.48	0.52	1.40	3.64
EMS, 200 mg/kg/day	9495	16	20	228	110	131	505	0.16	0.20	2.28	1.10	1.31	5.05

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

TABLE SA-2

3-Chloropropene
Sperm Abnormality Test in Mice
Means of Freeman-Tukey Binomial Transformation ± Standard Error

Multiple Dosing

Dose Group	Abnormality Category					
	A	B	C	D	E	Total
Air Control, 7 h/day	6.39 ± 1.163	8.43 ± 1.481	27.11 ± 1.463	14.59 ± 1.517	24.49 ± 1.598	40.83 ± 2.299
1 ppm, 7 h/day	6.69 ± 1.163	8.96 ± 1.481	23.04 ± 1.463	17.28 ± 1.517	22.91 ± 1.598	38.27 ± 2.299
2! ppm, 7 h/day	5.63 ± 1.163	9.02 ± 1.481	24.65 ± 1.463	14.61 ± 1.517	23.72 ± 1.598	38.40 ± 2.299
EMS, 200 mg/kg/day	8.08 ± 1.163	9.22 ± 1.481	30.17 ± 1.463	20.82** ± 1.517	24.07 ± 1.598	45.64 ± 2.299

A = Hook up-turned or hook elongated

B = Banana-shaped head

C = Amorphous head

D = Folded tail

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

**P<0.01

TABLE RL-1

3-Chloropropene

Drosophila Dose Ranging Experiment

Day		50 ppm						Date & Initial
		1 h		3 h		5 h		
0	No. of males exposed	109		100		100		27.3.80 KT
1	No. & % survival	109	100	100	100	100	100	28.3.80 KT
2	No. of eggs laid by 10 females	111		121		148		29.3.80 KT
3	No. & % hatched	105	94.5%	106	87.6%	129	87%	30.3.80 KT

Comments: Control:- eggs laid = 172
eggs hatched = 160 = 93%

Time chosen for test exposure: 7 h

TABLE RL-2

3-Chloropropene

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	10.4.80	13.4.80	18.4.80
F_2 set up	24.4.80	28.4.80	2.5.80
F_2 scored	6.5.80	13.5.80	14.5.80
F_2 repeats scored	19.5.80	-	-
F_3 set up	-	13.5.80	-
F_3 scored	-	26.5.80	-
F_3 repeats scored	-	-	-

RESULTS

	Brood 1	Brood 2	Brood 3	All Broods
No. of F_1 vials	100	100	93	293
No. of sterile F_1 vials	3	5	8	16
No. of F_1 vials used in F_2	97	95	85	277
No. of F_2 vials set up	600	598	600	1798
No. of F_2 vials scored	569	553	560	1682
No. of F_2 vials containing lethals	2	0	0	2
Frequency of F_2 lethals	0.35	0	0	0.11
No. of F_3 vials set up	-	600	-	600
No. of F_3 vials scored	-	562	-	562
No. of F_3 vials containing lethals	-	0	-	0
Frequency of F_3 lethals	-	0	-	0

TABLE RL-2 (continued)

3-Chloropropene
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	10.4.80	14.4.80	18.4.80
F_2 set up	24.4.80	28.4.80	2.5.80
F_2 scored	7.5.80	12.5.80	14.5.80
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	98	98	92	288
No. of sterile F_1 vials	2	4	7	13
No. of F_1 vials used in F_2	96	94	85	275
No. of F_2 vials set up	600	600	600	1800
No. of F_2 vials scored	554	561	562	1677
No. of F_2 vials containing lethals	0	0	0	0
Frequency of F_2 lethals	0	0	0	0
No. of F_3 vials set up	-	-	-	-
No. of F_3 vials scored	-	-	-	-
No. of F_3 vials containing lethals	-	-	-	-
Frequency of F_3 lethals	-	-	-	-

TABLE RL-2 (continued)

3-Chloropropene
Drosophila SLRL Procedure and Results

Compound: 3-Chloropropene **Concentration:** 100 ppm **Stock:** A
Length of Exposure: 7 h **Test exposure given:** 9.4.80

	Brood 1	Brood 2	Brood 3
F_1 set up	10.4.80	13.4.80	18.4.80
F_2 set up	23.4.80	25.4.80	1.5.80
F_2 scored	6.5.80	9.5.80	14.5.80
F_2 repeats scored	-	-	-
F_3 set up	6.5.80	9.5.80	14.5.80
F_3 scored	16.5.80	21.5.80	26.5.80
F_3 repeats scored	-	-	-

RESULTS

	Brood 1	Brood 2	Brood 3	All Broods
No. of F_1 vials	98	97	90	285
No. of sterile F_1 vials	4	5	9	18
No. of F_1 vials used in F_2	94	92	81	267
No. of F_2 vials set up	600	600	599	1799
No. of F_2 vials scored	559	496	557	1612
No. of F_2 vials containing lethals	2	0	0	2
Frequency of F_2 lethals	0.35%	0	0	0.12%
No. of F_3 vials set up	500	500	400	1400
No. of F_3 vials scored	469	478	378	1325
No. of F_3 vials containing lethals	0	0	0	0
Frequency of F_3 lethals	0	0	0	0

TABLE RL-2 (continued)

3-Chloropropene
Drosophila SLRL Procedure and Results

Compound: 3-Chloropropene **Concentration:** 150 ppm **Stock:** B
Length of Exposure: 7 h **Test exposure given:** 9.4.80

	Brood 1	Brood 2	Brood 3
F_1 set up	10.4.80	14.4.80	18.4.80
F_2 set up	23.4.80	28.4.80	1.5.80
F_2 scored	5.5.80	12.5.80	13.5.80
F_2 repeats scored	19.5.80	-	-
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	91	90	81	262
No. of sterile F_1 vials	5	11	7	23
No. of F_1 vials used in F_2	86	79	74	239
No. of F_2 vials set up	593	606	601	1800
No. of F_2 vials scored	529	569	562	1660
No. of F_2 vials containing lethals	0	0	0	0
Frequency of F_2 lethals	0	0	0	0
No. of F_3 vials set up	-	-	-	-
No. of F_3 vials scored	-	-	-	-
No. of F_3 vials containing lethals	-	-	-	-
Frequency of F_3 lethals	-	-	-	-

TABLE RL-2 (continued)

3-Chloropropene

Drosophila SLRL Procedure and Results

Compound: Ethyl methanesulphonate Concentration: 0.4% v/v Stock: A
Length of Exposure: 5 h Test exposure given: 9.4.80

	Brood 1	Brood 2	Brood 3
F_1 set up	10.4.80	-	-
F_2 set up	23.4.80	-	-
F_2 scored	6.5.80	-	-
F_2 repeats scored	19.5.80	-	-
F_3 set up	-	-	-
F_3 scored	-	-	-
F_3 repeats scored	-	-	-

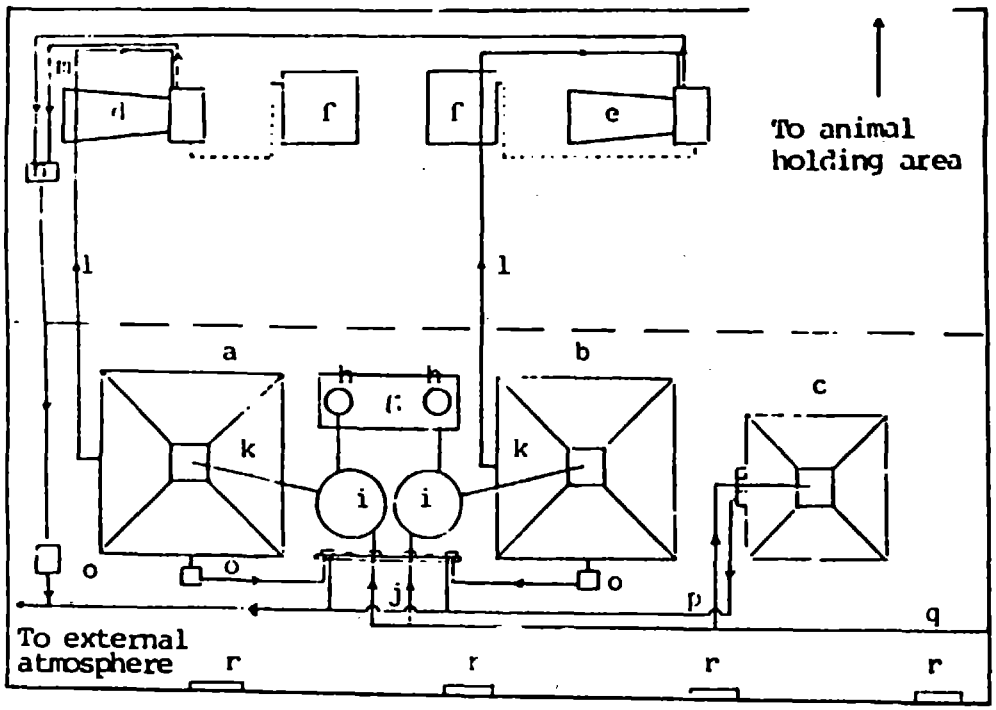
RESULTS

	Brood 1	Brood 2	Brood 3	All Broods
No. of F_1 vials	61	-	-	61
No. of sterile F_1 vials	0	-	-	0
No. of F_1 vials used in F_2	61	-	-	61
No. of F_2 vials set up	200	-	-	200
No. of F_2 vials scored	169	-	-	169
No. of F_2 vials containing lethals	25	-	-	25
Frequency of F_2 lethals	14%	-	-	14%
No. of F_3 vials set up	-	-	-	-
No. of F_3 vials scored	-	-	-	-
No. of F_3 vials containing lethals	-	-	-	-
Frequency of F_3 lethals	-	-	-	-

FIGURE 1a

3-Chloropropene

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

3-Chloropropene
Schematic Lay-out of Apparatus

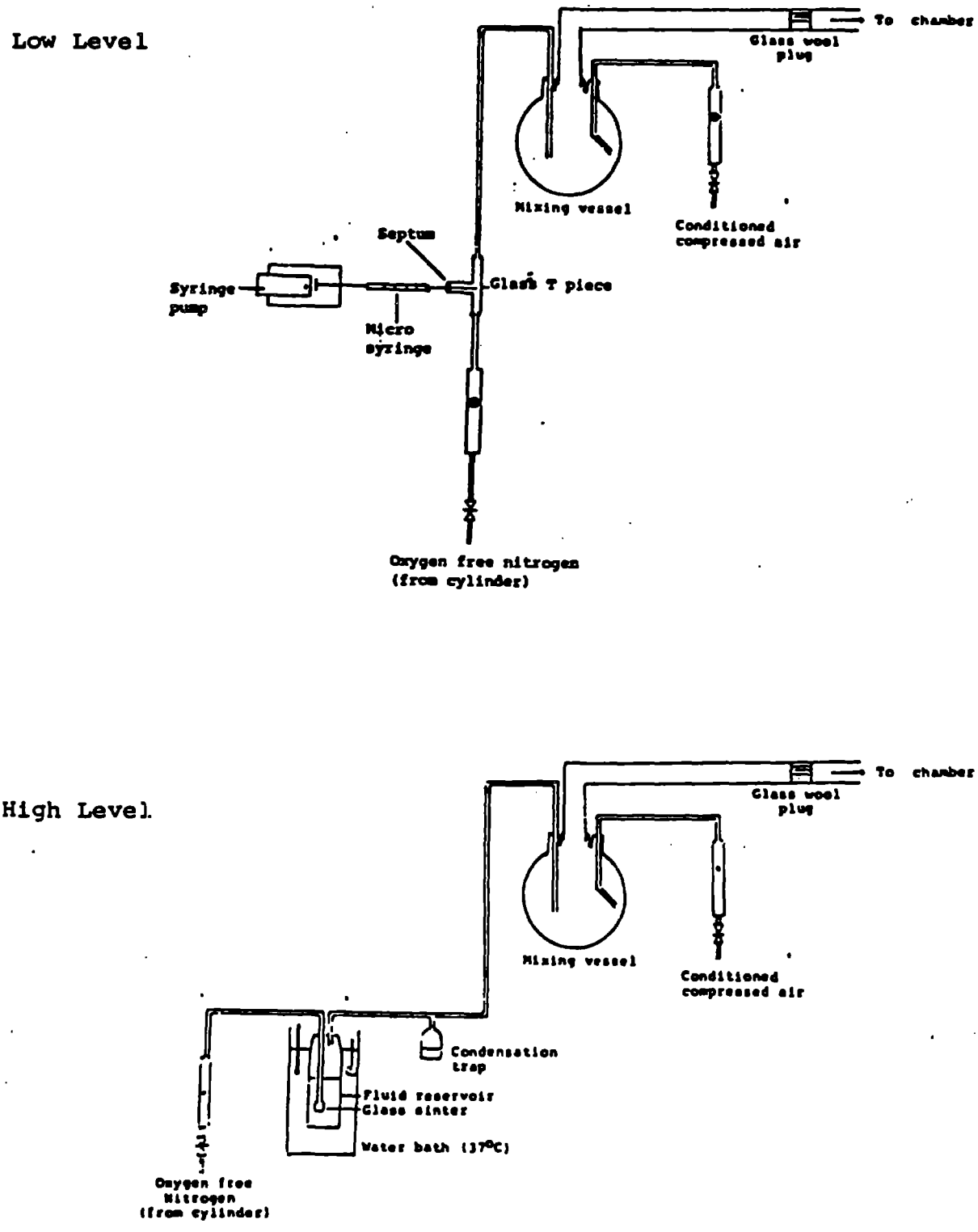


FIGURE 2

3-Chloropropene
Typical Calibration Graph for High Level
25 April 1980

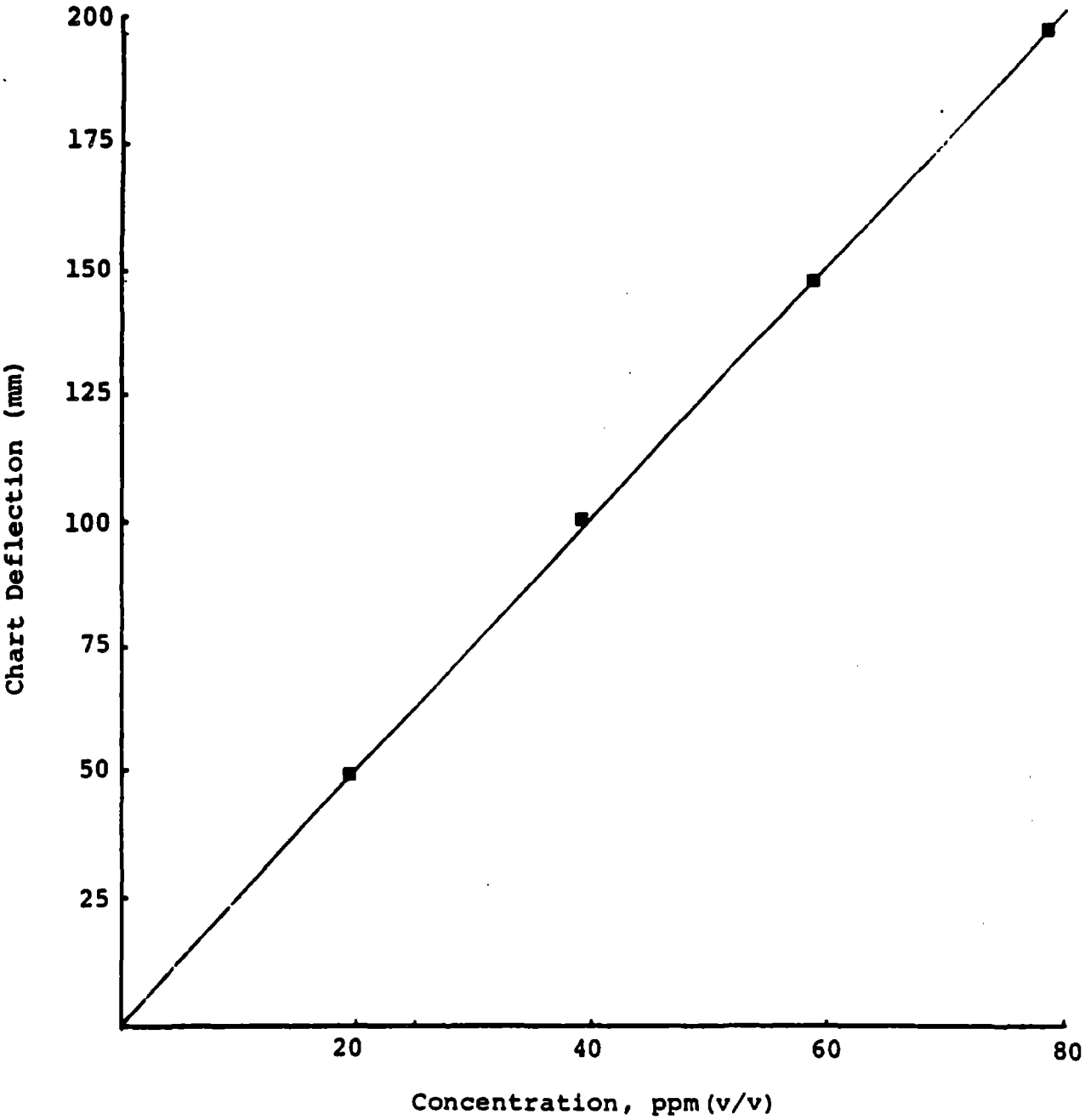
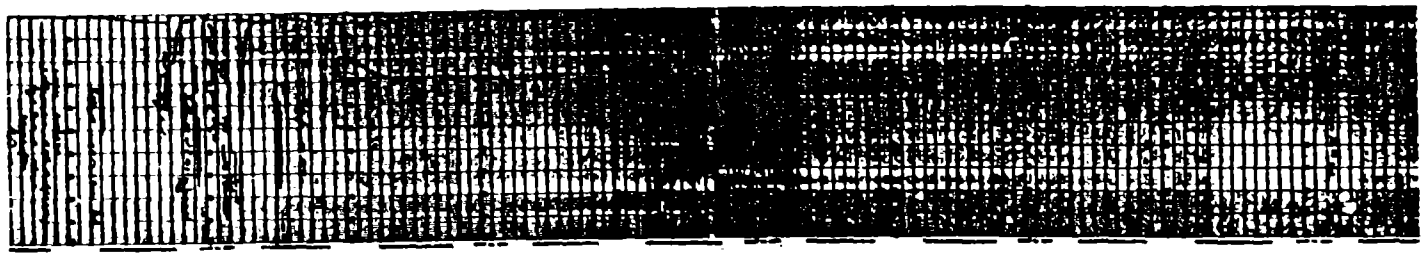
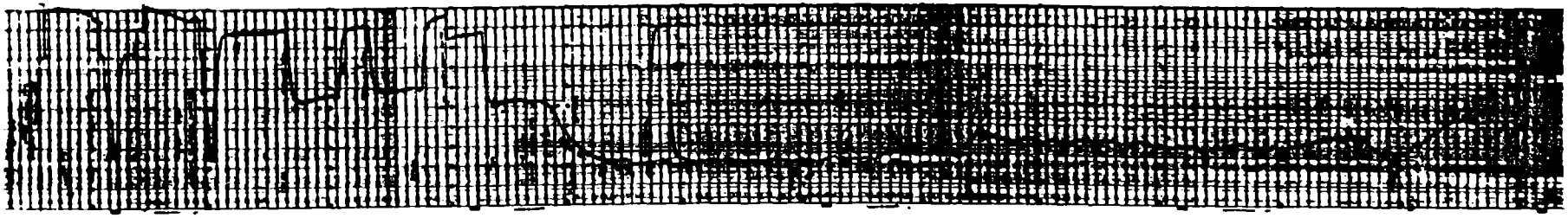


FIGURE 3

3-Chloropropene

Sample Record Chart of IR Absorption at 10.8 μm





APPENDIX DIET

3-Chloropropene
Diet Analysis

Spratt's Patent Ltd

Central House
Cambridge Road
Barking
Essex IG11 8NL

Telephone
01-594 7121
Telegrams
Spratt's Barking
Telex 897888

CERTIFICATE OF ANALYSIS

Product: LAD 2

Batch No: 028067

Date of Manufacture: 2nd April 1980

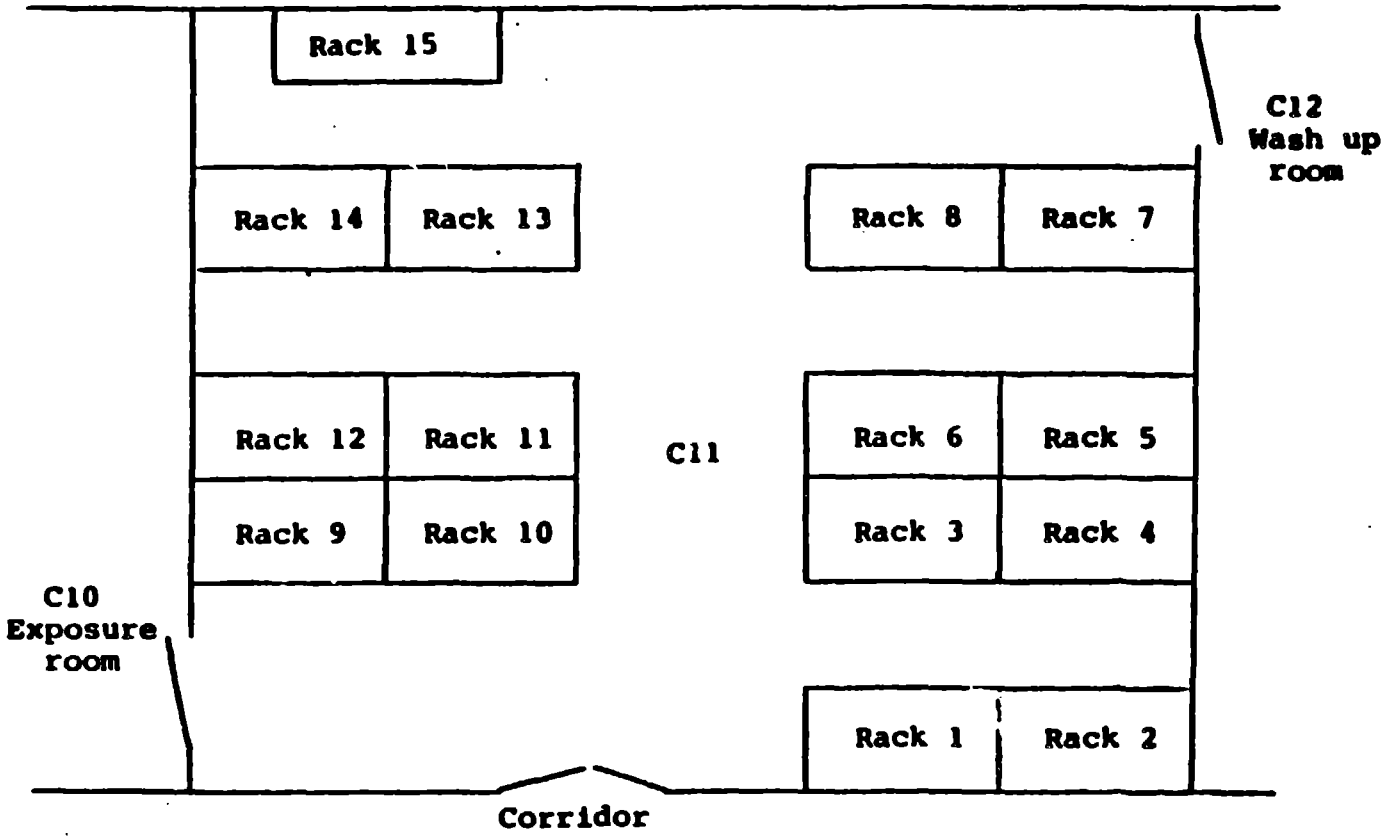
	Found Analysis
Moisture	8.0 %
Crude Fat	4.0 %
Crude Protein	21.4 %
Ash	5.2 %
Calcium	0.92 %
Phosphorus	0.76 %
Nitrate	11.0 mg/kg
Nitrite	< 1.0 mg/kg
Selenium	0.19 mg/kg
Lead	< 1.0 mg/kg
Arsenic	0.31 mg/kg
Cadmium	< 0.20 mg/kg
Mercury	0.03 mg/kg
Aflatoxins	NONE DETECTED
Total P.C.B.	NONE DETECTED
Total D.D.T.	0.006 mg/kg
Dieldrin	NONE DETECTED
Lindane	NONE DETECTED
Heptachlor	0.002 mg/kg
Malathion	NONE DETECTED
Total Viable Organisms	41.0×10^3/grm
E. Coli Type 1	NONE DETECTED
Salmonella Species	NONE DETECTED
Moulds	50/grm

Signed .....

Date .....

APPENDIX Loc-1

3-Chloropropene
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

3-Chloropropene
Examples of Animal Location During Exposure
Exposure Location Sheet

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

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

LEFT

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

FRONT REAR

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

RIGHT

SIGNED: _____ DATE: _____

APPENDIX Loc-2 (continued)

3-Chloropropene
Exposure Location Sheet

Project No: 409959

Test Compound: Air Control

Exposure Chamber No: 1

Day of Study: 2

Test Concentration: 0

Tier No: 2

Dominant Lethal ♂
Sperm Ab. mice

LEFT

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

FRONT REAR

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

RIGHT

SIGNED: _____ DATE: _____

APPENDIX LOC-2 (continued)

3-Chloropropene
Exposure Location Sheet

Project No: 409959 Test Concentration: Low

Test Compound: 3-Chloropropene Tier No: 1

Exposure Chamber No: 2

Day of Study: 2

LEFT

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

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

FRONT REAR

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

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

RIGHT

Signed: _____ Date: _____

APPENDIX Loc-2 (continued)

3-Chloropropene
Exposure Location Sheet

Project No: 409959 Test Concentration: High

Test Compound: 3-Chloropropene 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

3-Chloropropene

CYTOGENETIC SCORE SHEET

Contract No. 210-78-0026 Substance:

Assessor: Decoded group:

Signature: Slide No.: Decoded animal No.:

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

Slide quality:

Excellent _____ Good _____ Acceptable _____ Poor _____ Uncountable _____

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

APPENDIX FORM-2

3-Chloropropene

Assessors	Signature

Contract No. 210-78-0026

DOMINANT LETHAL ASSESSMENT

NIOSH

Dose Group:

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

APPENDIX FORM-3

3-Chloropropene

Contract No. 210-78-0026 NIOSH

SPERM ABNORMALITIES ASSESSMENT

Assessor: De-coder

Date(s) assessed: Date de-coded:

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

APPENDIX TABLE BW-1

3-Chloropropene
Multiple Exposure Cytogenetics Test
Individual Body Weights (g)

Air Control (0 ppm)

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	121	357	359	368	367	374
	122	375	371	381	376	376
	123	375	386	380	386	389
	124	376	381	384	388	387
	125	344	349	350	353	356
	126	360	372	372	375	379
	127	346	346	354	353	358
	128	383	387	385	391	393
	129	370	375	376	383	384
	130	345	347	357	362	368
	Mean ± S.D.	363.1 ± 14.6	367.3 ± 16.0	370.7 ± 12.9	373.4 ± 14.1	376.4 ± 12.7

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	281	255	262	259	269	272
	282	238	230	237	240	239
	283	239	239	240	246	253
	284	225	227	225	230	233
	285	235	231	235	235	234
	286	231	232	234	237	240
	287	253	254	259	264	266
	288	239	234	237	240	238
	289	247	238	246	246	248
	290	238	239	243	245	245
	Mean ± S.D.	240.0 ± 9.3	238.6 ± 11.1	241.5 ± 10.8	245.2 ± 12.4	246.8 ± 13.3

APPENDIX TABLE BW-1 (continued)

3-Chloropropene

Multiple Dosing: 1 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	131	323	324	331	331	332
	132	368	369	366	368	370
	133	380	380	383	389	390
	134	379	382	386	383	384
	135	357	358	363	365	367
	136	374	377	377	382	384
	137	384	387	384	384	387
	138	346	350	353	351	353
	139	354	356	346	355	355
	140	339	338	343	345	346
	Mean ± S.D.	360.2 ± 20.3	362.1 ± 20.7	363.2 ± 19.4	365.3 ± 19.5	366.8 ± 19.8

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	291	223	225	226	226	226
	292	250	247	251	250	246
	293	229	227	222	228	227
	294	215	215	219	220	219
	295	236	228	229	232	234
	296	238	246	249	251	251
	297	216	212	221	220	222
	298	237	241	241	246	249
	299	236	232	233	238	239
	300	230	233	234	236	239
	Mean ± S.D.	231.0 ± 10.8	230.6 ± 11.8	232.5 ± 11.4	234.7 ± 11.5	235.2 ± 11.4

APPENDIX TABLE BW-1 (continued)

3-Chloropropene

Multiple Dosing: 25 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	141	331	332	336	340	341
	142	369	369	376	380	383
	143	357	351	354	349	353
	144	382	389	392	389	387
	145	367	365	363	376	368
	146	335	342	346	350	345
	147	330	333	334	342	346
	148	370	376	380	385	387
	149	384	394	395	396	402
	150	355	365	360	365	370
	Mean ± S.D.	358.0 ± 20.1	361.6 ± 21.8	363.6 ± 21.8	367.2 ± 20.7	368.2 ± 21.3

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	301	248	258	261	265	263
	302	238	237	243	245	247
	303	249	253	252	254	255
	304	204	203	213	210	213
	305	255	255	259	260	260
	306	236	247	254	254	254
	307	217	214	219	223	223
	308	242	246	246	248	252
	309	243	237	245	244	246
	310	240	234	236	241	244
	Mean ± S.D.	237.2 ± 15.5	238.4 ± 17.9	243.0 ± 16.0	244.4 ± 16.7	245.7 ± 15.9

APPENDIX TABLE BW-1 (continued)

3-Chloropropene

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	151	343	343	333	331	322
	152	347	337	318	307	297
	153	380	381	369	361	354
	154	354	353	346	338	324
	155	375	367	359	355	344
	156	396	384	369	356	343
	157	340	335	320	308	306
	158	343	336	326	319	306
	159	350	346	333	329	327
	160	350	345	335	324	311
	Mean ± S.D.	357.8 ± 19.0	352.7 ± 18.3	340.8 ± 19.1	332.8 ± 19.5	323.1 ± 18.9

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	311	237	237	231	226	218
	312	246	239	232	224	220
	313	223	213	200	191	185
	314	241	237	228	218	211
	315	205	200	199	195	194
	316	223	222	211	210	205
	317	224	223	222	215	210
	318	224	218	216	208	205
	319	212	204	198	196	188
	320	237	228	221	212	205
	Mean ± S.D.	227.2 ± 13.0	222.1 ± 13.7	215.8 ± 13.2	209.5 ± 12.1	204.1 ± 11.8

APPENDIX TABLE BW-2

3-Chloropropene
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	376	11	401	21	395
	2	424	12	425	22	327
	3	379	13	383	23	378
	4	407	14	411	24	356
	5	393	15	379	25	370
	6	429	16	419	26	367
	7	412	17	343	27	407
	8	393	18	376	28	351
	9	415	19	361	29	379
	10	433	20	411	30	390
	Mean ± S.D.	406.1 ± 20.2		390.0 ± 26.8		372.0 ± 23.4

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Female	161	253	171	265	181	262
	162	275	172	243	182	234
	163	270	173	276	183	245
	164	245	174	261	184	275
	165	242	175	286	185	236
	166	242	176	247	186	226
	167	240	177	248	187	297
	168	232	178	233	188	250
	169	254	179	228	189	254
	170	257	180	270	190	244
	Mean ± S.D.	251.0 ± 13.6		255.7 ± 18.9		252.3 ± 21.2

APPENDIX TABLE BW-2 (continued)

3-Chloropropene

Single Dosing: 1 ppm

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Male	31	400	41	355	51	425
	32	402	42	393	52	396
	33	406	43	410	53	373
	34	381	44	375	54	357
	35	386	45	355	55	388
	36	346	46	442	56	384
	37	419	47	415	57	410
	38	370	48	386	58	375
	39	425	49	359	59	373
	40	360	50	345	60	365
	Mean ± S.D.	389.5 ± 25.6		383.5 ± 31.6		384.6 ± 20.9

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Female	191	248	201	280	211	237
	192	275	202	285	212	256
	193	248	203	263	213	285
	194	251	204	252	214	245
	195	245	205	280	215	242
	196	235	206	245	216	275
	197	265	207	250	217	235
	198	235	208	276	218	225
	199	263	209	279	219	276
	200	277	210	260	220	250
	Mean ± S.D.	254.2 ± 15.1		267.0 ± 14.7		252.6 ± 20.0

APPENDIX TABLE BW-2 (continued)

3-Chloropropene

Single Dosing: 25 ppm

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Male	61	404	71	400	81	424
	62	375	72	402	82	406
	63	415	73	403	83	381
	64	406	74	420	84	363
	65	358	75	386	85	400
	66	367	76	420	86	426
	67	381	77	378	87	392
	68	418	78	407	88	423
	69	406	79	326	89	345
	70	432	80	397	90	357
	Mean	396.2		393.9		391.3
	± S.D.	± 24.4		± 27.2		± 29.2

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Female	221	250	231	239	241	256
	222	240	232	296	242	262
	223	290	233	263	243	237
	224	265	234	251	244	234
	225	270	235	255	245	250
	226	250	236	223	246	237
	227	227	237	235	247	265
	228	253	238	242	248	257
	229	242	239	267	249	255
	230	246	240	273	250	257
	Mean	253.3		254.4		251.0
	± S.D.	± 17.7		± 21.3		± 11.1

APPENDIX TABLE BW-2 (continued)

3-Chloropropene

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	435	101	366	111	423
	92	421	102	415	112	421
	93	400	103	362	113	377
	94	373	104	402	114	376
	95	400	105	398	115	373
	96	410	106	412	116	387
	97	363	107	377	117	356
	98	372	108	350	118	390
	99	367	109	419	119	381
	100	425	110	387	120	372
	Mean	396.6		388.8		385.6
	± S.D.	± 26.3		± 24.2		± 21.3

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Female	251	269	261	238	271	220
	252	266	262	238	272	279
	253	281	263	264	273	246
	254	246	264	300	274	251
	255	258	265	253	275	247
	256	243	266	234	276	246
	257	242	267	264	277	245
	258	233	268	293	278	277
	259	245	269	258	279	245
	260	257	270	237	280	240
	Mean	254.0		257.9		249.6
	± S.D.	± 14.8		± 23.3		± 17.2

APPENDIX TABLE BW-2 (Repeat)

3-Chloropropene
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	386	11	383	21	402
	2	415	12	398	22	352
	3	374	13	389	23	365
	4	420	14	378	24	341
	5	403	15	378	25	397
	6	420	16	400	26	351
	7	393	17	375	27	382
	8	390	18	355	28	369
	9	365	19	385	29	407
	10	380	20	378	30	363
	Mean ± S.D.	394.6 ± 19.4		381.9 ± 12.8		373.5 ± 22.5

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Female	161	247	171	230	181	252
	162	237	172	239	182	241
	163	227	173	222	183	216
	164	265	174	250	184	226
	165	260	175	219	185	225
	166	235	176	242	186	213
	167	233	177	245	187	247
	168	228	178	250	188	224
	169	217	179	231	189	223
	170	232	180	236	190	253
	Mean ± S.D.	238.1 ± 15.0		236.4 ± 10.9		232.0 ± 14.9

APPENDIX TABLE BW-2 (Repeat continued)

3-Chloropropene

Single Dosing: 1 ppm

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Male	31	367	41	347	51	360
	32	378	42	378	52	385
	33	352	43	353	53	360
	34	377	44	367	54	370
	35	407	45	395	55	350
	36	375	46	362	56	372
	37	369	47	380	57	391
	38	397	48	375	58	399
	39	395	49	397	59	397
	40	350	50	392	60	363
	Mean ± S.D.	376.7 ± 18.7		374.6 ± 17.3		374.7 ± 17.2

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Female	191	243	201	210	211	259
	192	249	202	242	212	235
	193	239	203	223	213	246
	194	255	204	230	214	230
	195	232	205	246	215	233
	196	227	206	240	216	229
	197	254	207	258	217	222
	198	232	208	226	218	230
	199	223	209	215	219	260
	200	238	210	275	220	216
	Mean ± S.D.	239.2 ± 11.0		236.5 ± 19.9		236.0 ± 14.7

APPENDIX TABLE BW-2 (Repeat continued)

3-Chloropropene

Single Dosing: 25 ppm

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Male	61	370	71	369	81	352
	62	421	72	370	82	382
	63	336	73	411	83	413
	64	405	74	415	84	420
	65	380	75	356	85	365
	66	405	76	334	86	356
	67	390	77	400	87	385
	68	397	78	407	88	372
	69	356	79	373	89	442
	70	384	80	360	90	384
	Mean ± S.D.	384.4 ± 25.3		379.5 ± 27.2		387.1 ± 29.3

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Female	221	236	231	277	241	272
	222	254	232	223	242	231
	223	250	233	248	243	245
	224	240	234	217	244	255
	225	234	235	218	245	223
	226	233	236	242	246	243
	227	216	237	226	247	246
	228	251	238	215	248	246
	229	270	239	234	249	216
	230	232	240	233	250	240
	Mean ± S.D.	241.6 ± 15.0		233.3 ± 18.8		241.7 ± 15.9

APPENDIX TABLE BW-2 (Repeat continued)

3-Chloropropene

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	340	101	430	111	375
	92	376	102	408	112	369
	93	396	103	398	113	368
	94	429	104	355	114	361
	95	390	105	350	115	383
	96	361	106	397	116	393
	97	405	107	374	117	366
	98	396	108	442	118	369
	99	410	109	392	119	406
	100	379	110	368	120	367
	Mean ± S.D.	385.0 ± 27.7		391.4 ± 30.4		374.7 ± 15.0

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Female	251	234	261	252	271	224
	252	235	262	233	272	246
	253	216	263	240	273	249
	254	229	264	230	274	260
	255	219	265	231	275	214
	256	258	266	262	276	243
	257	226	267	248	277	233
	258	227	268	239	278	247
	259	257	269	220	279	240
	260	221	270	233	280	246
	Mean ± S.D.	232.2 ± 14.6		238.8 ± 12.3		240.2 ± 13.3

APPENDIX TABLE BW-3

3-Chloropropene
Dominant Lethal Assay
Individual Body Weights (g)

Multiple Dosing: Air Control (0 ppm)

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	361	355	356	364	366	364
	362	373	370	376	382	390
	363	365	372	376	384	389
	364	352	356	365	367	374
	365	390	397	398	405	403
	366	351	356	359	367	372
	367	334	342	343	351	353
	368	352	351	355	358	358
	369	336	344	338	346	350
	370	354	356	364	373	379
	Mean ± S.D.	356.2 ± 16.6	360.0 ± 16.1	363.8 ± 17.2	369.9 ± 17.3	373.2 ± 17.4

APPENDIX TABLE BW-3 (continued)

3-Chloropropene

Multiple Dosing: 1 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	371	333	333	333	334	337
	372	350	351	356	357	359
	373	360	360	367	373	376
	374	314	320	322	318	322
	375	353	355	361	364	367
	376	319	320	320	322	325
	377	351	355	355	357	356
	378	336	340	350	350	352
	379	357	363	370	374	378
	380	352	357	366	368	375
	Mean ± S.D.	342.5 ± 16.1	345.4 ± 16.1	350.0 ± 18.6	351.7 ± 20.4	354.7 ± 20.7

APPENDIX TABLE BW-3 (continued)

3-Chloropropene

Multiple Dosing: 25 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	381	330	331	335	334	337
	382	364	366	369	368	369
	383	342	341	343	344	343
	384	387	398	398	404	406
	385	385	392	397	400	409
	386	355	358	358	359	363
	387	349	351	353	357	356
	388	358	364	368	372	383
	389	376	386	391	394	397
	390	373	379	383	387	396
	Mean ± S.D.	361.9 ± 18.7	366.6 ± 22.1	369.5 ± 22.4	371.9 ± 23.9	375.9 ± 26.0

APPENDIX TABLE BW-3 (continued)

3-Chloropropene

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	391	382	372	361	358	347
	392	375	360	347	337	327
	393	386	384	371	362	356
	394	345	336	323	311	300
	395	356	353	335	321	306
	396	316	306	299	286	275
	397	380	373	352	337	320
	398	350	347	337	329	319
	399	410	407	400	388	373
	400	397	389	379	371	358
	Mean ± S.D.	369.7 ± 27.9	362.7 ± 29.0	350.4 ± 29.1	340.0 ± 30.4	328.1 ± 30.4

APPENDIX TABLE BW-4

3-Chloropropene
Sperm Abnormality Test
Individual Body Weights (g)

Multiple Dosing: Air Control (0 ppm)

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	321	25	25	26	26	26
	322	27	27	27	27	27
	323	25	26	26	26	26
	324	27	27	27	27	27
	325	25	25	25	25	26
	326	27	27	27	27	26
	327	28	28	29	29	29
	328	26	26	26	26	26
	329	26	26	25	25	25
	330	26	25	26	26	26
	Mean	26.2	26.2	26.4	26.4	26.4
	± S.D.	± 1.0	± 1.0	± 1.2	± 1.2	± 1.1

APPENDIX TABLE BW-4 (continued)

3-Chloropropene

Multiple Dosing: 1 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	331	23	23	24	25	23
	332	24	24	24	25	25
	333	25	26	25	25	25
	334	25	25	26	26	26
	335	26	26	26	26	26
	336	23	24	24	24	23
	337	26	26	26	26	25
	338	25	24	25	26	25
	339	26	26	26	26	25
	340	26	26	26	27	25
	Mean ± S.D.	24.9 ± 1.2	25.0 ± 1.2	25.2 ± 0.9	25.6 ± 0.8	24.8 ± 1.0

APPENDIX TABLE BW-4 (continued)

3-Chloropropene

Multiple Dosing: 25 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	341	24	25	25	25	25
	342	26	26	27	27	26
	343	25	25	25	25	25
	344	25	25	25	25	25
	345	25	26	26	26	26
	346	27	28	28	28	28
	347	27	28	28	28	28
	348	24	25	25	25	25
	349	24	24	24	25	25
	350	26	26	26	26	26
	Mean ± S.D.	25.3 ± 1.2	25.8 ± 1.3	25.9 ± 1.4	26.0 ± 1.3	25.9 ± 1.2

APPENDIX TABLE BW-4 (continued)

3-Chloropropene

Multiple Dosing: Ethyl methanesulphonate, 200 mg/kg

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	351	25	25	25	25	24
	352	25	25	25	26	26
	353	24	24	24	24	23
	354	25	26	25	26	25
	355	25	26	25	26	25
	356	26	25	26	27	27
	357	26	25	24	24	24
	358	24	25	25	25	23
	359	25	24	26	26	26
	360	25	24	25	25	23
	Mean ± S.D.	25.0 ± 0.7	24.9 ± 0.7	25.0 ± 0.7	25.4 ± 1.0	24.6 ± 1.4

APPENDIX TABLE CA-MD-K

3-Chloroprovene
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Males

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
124	87/3	50	25	25								
	87/5		25	25								
126	7/2	50	25	25								
	7/4		25	25								
129	93/1	50	25	25								
	93/2		25	23							1 Fragment	58.5 x 109.9
					1							50.4 x 104.2
121	22/1	50	25	25								
	22/2		25	24	1							37.9 x 124.7
130	27/2	50	25	25								
	27/4		25	25								
125	77/1	50	25	25								
	77/3		25	25								
122	13/2	50	25	25								
	13/4		25	25								
123	62/2	50	25	25								
	62/3		25	25								
128	73/2	50	25	25								
	73/3		25	25								
127	20/1	50	25	25								
	20/2		25	25								

APPENDIX TABLE CA-MD-M (continued)

3-Chloropropene
Males

Multiple Dosing: 1 ppm

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
136	141/1	50	25	23	1							36.0 x 110.0
											2 Chromosomal Fragments	37.9 x 110.0
	141/2		25	25								
134	38/2	50	25	24				1				58.4 x 122.4
	38/3		25	25								
137	55/3	50	25	25								
	55/4		25	25								
132	143/3	50	25	25								
	143/5		25	25								
138	106/1	50	25	25								
	106/2		25	25								
135	1/1	50	25	25								
	1/2		25	25								
131	3/2	50	25	25								
	3/4		25	25								
133	137/2	50	25	25								
	137/4		25	25								
139	133/1	50	25	25								
	133/2		25	25								
140	81/2	50	25	25								
	81/3		25	25								

APPENDIX TABLE CA-MD-M (continued)

3-Chloropropene

Males

Multiple Dosing: 25 ppm

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
147	97/1	50	25	23	1						1 Chromatid Fragment	63.8 x 112.2
												67.7 x106.4
	97/5		25	25								
145	59/1	50	25	25								
	59/4		25	25								
149	121/2	50	25	25								
	121/3		25	25								
144	108/1	50	25	25								
	108/2		25	25								
142	40/1	50	25	25								
	40/2		25	25								
143	86/1	50	25	25								
	86/3		25	25								
141	128/2	50	25	25								
	128/3		25	25								
150	82/4	50	25	25								
	82/5		25	25								
148	122/1	50	25	25								
	122/3		25	24				1				60.1 x 104.2
146	131/4	50	25	24	1							31.6 x 109.2
	131/5		25	25								

APPENDIX TABLE CA-MD-M (continued)

3-Chloropropene

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	90/2	50	25	25								
	90/3		25	25								
156	23/1	50	25	25								
	23/3		25	23	1							61.5 x 112.0
					1						1 Exchange	64.5 x 110.3
154	111/1	50	25	24	1							57.5 x 122.5
	111/3		25	25								
160	75/1	50	25	25								
	75/4		25	25								
152	145/2	50	25	24		1						22.0 x 97.7
	145/3		25	25								
151	49/1	50	25	25								
	49/3		25	24	1							52.9 x 111.4
158	11/1	50	25	23			1					30.1 x 121.8
							1					28.3 x 135.8
	11/2		25	22	1							53.5 x 109.5
											1 Exchange	56.0 x 109.2
					1							56.2 x 102.2
157	46/2	50	25	25								
	46/3		25	25								

APPENDIX TABLE CA-MN-M (continued)

3-Chloropropene
Males

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
158	135/1	50	25	25								
	135/2		25	25								
159	12/1	50	25	25								
	12/2		25	24	1							62.1 x 106.3

APPENDIX TABLE CA-ND-F

3-Chloropropene
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Females

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
289	253/2	50	25	25								
	253/1		25	25								
283	222/1	50	25	24				1				55.0 x 122.0
	222/3		25	24	1							60.5 x 106.5
281	182/1	50	25	24		1						57.8 x 109.0
	182/2		25	24	1							51.5 x 104.8
288	233/1	50	25	24		1						56.0 x 121.5
	233/2		25	25								
287	180/1	50	25	24	1							30.3 x 121.0
	180/2		25	24	1							46.3 x 125.9
282	173/1	50	25	25								
	173/4		25	25								
286	167/2	50	25	25								
	167/4		25	25								
284	247/2	50	25	25								
	247/5		25	24	1							28.8 x 108.8
290	187/2	50	25	23	1							56.1 x 128.8
					1							29.4 x 118.5
	187/4		25	25								
285	237/3	50	25	25								
	237/5		25	25								

APPENDIX TABLE CA-MD-F (continued)

3-Chloropropene

Females

Multiple Dosing: 1 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		
298	266/3	50	25	25								
	266/1		25	25	1							20.0 x 112.3
								2				42.5 x 110.0
291	163/2	50	25	25								
	163/1		25	25								
299	293/1	50	25	25								
	293/3		25	24				1				48.5 x 108.9
295	161/1	50	25	25								
	161/2		25	25								
294	198/1	50	25	25								
	198/3		25	25								
297	215/1	50	25	25								
	215/3		25	25								
300	241/1	50	25	25								
	241/2		25	25								
296	301/2	50	25	25								
	301/3		25	25								
292	303/3	50	25	25								
	303/4		25	25								
293	297/2	50	25	25								
	297/3		25	25								

APPENDIX TABLE CA-MD-F (continued)

3-Chloropropene

Females

Multiple Dosing: 25 ppm

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
301	286/1	50	25	25								
	288/2		25	25								
304	268/2	50	25	25								
	268/3		25	25								
306	291/2	50	25	25								
	291/3		25	25								
302	200/3	50	25	25								
	200/4		25	25								
303	246/1-5	0	0	0								
305	219/2	50	25	25								
	219/4		25	25								
308	282/3	50	25	25								
	282/4		25	25								
307	257/2	50	25	25								
	257/3		25	25								
310	242/2	50	25	25								
	242/3		25	25								
309	281/2	50	25	25								
	281/4		25	25								

APPENDIX TABLE CA-MD-F (continued)

3-Chloropropene

Females

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
317	206/3	50	25	25								
	206/4		25	25								
314	271/3	50	25	24	1							62.4 x 124.6
	271/5		25	25								
320	235/1	50	25	24	1							49.0 x 105.5
	235/3		25	24	1		1					59.5 x 109.2
318	171/1	50	25	24	1							58.0 x 109.1
	171/2		25	24	2							33.0 x 104.8
316	183/1	50	25	23	1							51.9 x 124.3
					1							63.4 x 128.0
	183/2		25	24	1							68.5 x 106.7
312	305/1	50	25	25								
	305/4		25	25								
311	209/2	50	25	24	1							64.9 x 120.1
	209/4		25	24	1							51.7 x 123.3
313	250/3	50	25	25								
	250/4		25	25								
315	295/3	50	25	24	1							27.9 x 126.3
	295/5		25	25								
319	172/4	50	25	24	1							42.5 x 119.5
			25	23	1							26.6 x 117.9
											1 Chromosomal Fragment	37.8 x 118.6

APPENDIX TABLE CA-M6

3-Chloropropene
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Males

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
10	130/4	50	25	23	1						2 Chromatid Fragments	66.8 x 113.5
												60.4 x 112.9
	130/3		25	24				1				38.4 x 108.7
5	34/2	50	25	25								
	34/1		25	25								
1	28/3	50	25	25								
	28/4		25	25								
3	138/5	50	25	25								
	138/3		25	25								
7	156/1	50	25	25								
	156/4		25	25								
4	141/5	50	25	25								
	141/4		25	25								
2	144/5	50	25	25								
	144/1		25	25								
9	99/5	50	25	25								
	99/4		25	25								
6	32/1	50	25	25								
	32/3		25	24		1						60.1 x 109.0
8	95/1	50	25	25								
	95/5		25	25								

APPENDIX TABLE CA-M6 (continued)

3-Chloropropene

Males

Single Dosing: 1 ppm

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	
38	61/1	50	25	25								
	61/3		25	25								
36	42/3	50	25	25								
	42/5		25	25								
39	10/1	50	25	25								
	10/2		25	25								
35	44/2	50	25	24		1						42.3 x 111.0
	44/3		25	25								
32	135/3	50	25	25								
	135/5		25	24							1 Chromatid Fragment	30.9 x 111.2
33	145/1	50	25	25								
	145/2		25	25								
34	151/1	50	25	25								
	151/3		25	25								
31	114/1	50	25	25								
	114/3		25	25								
40	59/2	50	25	25								
	59/3		25	25								
37	132/3	50	25	25								
	132/4		25	25								

APPENDIX TABLE CA-M6 (continued)

3-Chloropropene

Males

Single Dosing: 25 ppm

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
66	85/1	50	25	24					1			29.6 x 110.1
	85/3		25	25								
63	157/1	50	25	24	1							34.2 x 113.7
	157/2		25	25								
62	1/3	50	25	24	1							43.8 x 110.7
	1/4		25	25								
67	100/1	50	25	25								
	100/2		25	25								
65	152/1	50	25	25								
	152/2		25	25								
64	129/2	50	25	23	1							61.4 x 112.0
					1							57.9 x 108.8
	129/1		25	25								
68	102/2	50	25	25								
	102/5		25	25								
69	26/4	50	25	25								
	26/2		25	25								
70	3/3	50	25	25								
	3/5		25	25								
61	58/1	50	25	25								
	58/2		25	25								

APPENDIX TABLE CA-M6 (continued)

3-Chloropropene

Males

Single Dosing: Ethyl methanesulphonate, 250 mg/kg — Sampling Time: 5 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		
92	112/4	50	25	24	1							35.3 x 111.6
	112/2		25	22	2							61.5 x 109.0
									1			46.1 x 108.7
							1					35.9 x 108.8
98	80/1-5	0	0	0								
100	15/1	50	25	24	3							33.5 x 115.1
	15/4		25	25								
96	146/2	50	25	25								
	146/1		25	24	1							30.6 x 111.2
93	123/1	50	25	24	1							59.0 x 110.5
	123/3		25	25								
91	153/1	50	25	25								
	153/3		7	7								
	153/5		7	6	1							43.4 x 105.0
	153/4		11	11								
95	54/1	50	25	22	1							66.1 x 113.2
						1						63.1 x 109.3
					1							42.8 x 109.0
	54/2		25	25								
97	140/3	50	25	25								
	140/5		25	25								
99	142/5	50	25	24	1							60.1 x 112.0
	142/3		25	23	1							66.0 x 113.3
						1						60.8 x 112.9

APPENDIX TABLE CA-M6 (continued)

3-Chloropropene
Males

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
94	46/3	50	25	25								
	46/4		25	24	1							35.7 x 114.2

APPENDIX TABLE CA-M24

3-Chloropropene
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Males

Single Dosing: Air Control (0 ppm)

Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
18	134/2	50	25	25								
	134/4		25	25								
19	64/1	50	25	25								
	64/3		25	25								
14	143/1	14	2	2								
	143/2		0	0								
	143/3		4	4								
	143/4		6	6								
	143/5		2	2								
13	18/4	50	17	16	1							114.9 x 17.5
	18/5		25	23	1							94.5 x 22.5
					1							111.1 x 19.5
	18/3		8	7	1							115.0 x 21.9
16	104/1	50	25	23	1							120.8 x 20.9
								1				85.6 x 19.1
	104/2		25	25								
12	136/4	50	25	24	1							77.2 x 17.1
	136/5		25	25								
17	127/5	50	25	24	1							77.2 x 22.0
	127/4		25	25								
20	98/2	50	25	25								
	98/3		25	25								

APPENDIX TABLE CA-M24 (continued)

3-Chloropropene

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		
11	111/4	50	25	22	2							107.1 x 20.1
					1							107.1 x 20.0
											1 Robertsonian Translocation	119.9 x 18.2
	111/2		25	25								
15	6/1	50	15	14	1							95.0 x 17.0
	6/2		8	8								
	6/3		4	4								
	6/4		3	3								
	6/5		20	19				1				117.0 x 22.2

APPENDIX TABLE CA-M24 (continued)

3-Chloropropene

Males

Single Dosing: 1 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		
47	55/3	50	25	23				1				73.2 x 20.9
					1							107.8 x 20.2
	55/4		25	25								
44	62/5	50	25	23				1				74.1 x 16.0
					1							122.0 x 15.2
	62/3		25	23	1							103.2 x 20.1
					1							108.5 x 12.0
50	83/4	50	25	25								
	83/2		25	23	1							112.0 x 19.0
					1							85.2 x 11.1
46	78/5	50	18	18								
	78/2		25	23	1							104.0 x 15.2
					1							78.1 x 8.9
	78/3		7	6	1							93.8 x 18.2
48	45/2	50	25	23		1						106.1 x 19.9
											1 Chromatid Fragment	83.0 x 17.4
	45/3		25	24	1							110.5 x 18.0
49	23/4	50	25	25								
	23/5		25	24	1							84.9 x 16.4
45	35/3	50	25	23	1							105.8 x 21.6
					1							101.9 x 19.9
	35/4		25	25								
43	37/4	50	25	25								
	37/5		25	24	1							109.1 x 20.1

APPENDIX TABLE CA-M24 (continued)

3-Chloropropene

Males

Single Dosing: 1 ppm

Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
42	147/1	50	25	25								
	147/2		25	25								
41	67/2	50	25	23	1							105.9 x 19.1
					1							99.9 x 10.9
	67/5		25	24	1							85.9 x 16.8

APPENDIX TABLE CA-M24 (continued)

3-Chloropropene

Males

Single Dosing: 25 ppm

Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
73	36/3	50	25	25								
	36/4		25	25								
74	39/4	50	25	25								
	39/3		25	24	2						1 Robertsonian Translocation	114.2 x 19.6
75	50/2	50	25	25								
	50/3		25	25								
77	92/2	50	25	23	1							91.9 x 15.8
					1							89.9 x 15.8
	92/4		25	25								
79	20/2	50	25	25								
	20/4		25	25								
71	77/5	50	25	24		1						82.2 x 17.8
	77/4		25	25								
78	107/3	50	25	25								
	107/2		25	24	1							82.0 x 12.0
76	86/4	50	25	22	1							96.1 x 19.9
					1							98.8 x 18.0
								1				90.9 x 16.8
	86/3		25	24		1						84.9 x 9.0
80	8/4	50	25	25								
	8/5		25	25								

APPENDIX TABLE CA-M24 (continued)

3-Chloropropene

Males

Single Dosing: 25 ppm

Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromacid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
72	84/4	23	4	3	1							105.9 x 17.0
	84/3		7	6	1							112.2 x 8.8
	84/2		2	2								
	84/1		5	5								
	84/5		5	5								

APPENDIX TABLE CA-M24 (continued)

3-Chloropropane

Males

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
101	53/1	50	25	19	1							83.6 x 19.8
					2						2 Chromatid Fragments	74.2 x 18.9
											1 Multi Aberration	89.9 x 18.2
											2 Chromatid Fragments	78.0 x 18.3
									1		1 Exchange	82.2 x 18.3
						1						85.1 x 18.5
	53/5		25		1							106.1 x 18.4
					1							88.9 x 18.1
106	17/4	50	25	21		1						102.1 x 23.5
						1						111.5 x 21.9
					1							103.1 x 21.9
					1	3		1			1 Chromatid Fragment	88.2 x 13.0
	17/5		25	17	1							102.4 x 19.9
					1							104.0 x 18.0
					2							82.1 x 17.5
										1	2 Chromatid Fragments	106.5 x 16.9
					1	2						116.5 x 13.3
					1							112.4 x 10.9
					1		1				2 Chromatid Fragments	101.1 x 10.0
					2						1 Chromatid Fragment	115.0 x 8.5

APPENDIX TABLE CA-M24 (continued)

3-Chloropropene

Males

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
110	16/3	50	25	18		1						109.5 x 21.2
					2							118.0 x 18.5
						1						117.0 x 17.4
					1							87.5 x 17.2
						1					1 Exchange	87.2 x 17.4
						3						104.1 x 16.1
					1							104.9 x 15.5
	16/4		25	20	2	1						114.2 x 18.5
					1							90.0 x 16.0
						1						116.1 x 16.0
					2							118.0 x 16.0
					1							73.0 x 15.1
102	7/2	50	25	22		3						105.1 x 19.9
					1							107.0 x 17.9
					1							109.0 x 13.4
	7/3		25	23	2							102.0 x 9.8
					1							104.0 x 9.1
105	51/2	50	25	23	1	3					1 Chromatid Fragment	112.0 x 16.4
						2						116.5 x 11.8
	51/5		25	20	1							75.1 x 19.5
					1							99.8 x 17.9
					1							108.5 x 16.2
					1	1						81.2 x 15.8
						1					2 Chromatid Fragments	106.8 x 15.9

APPENDIX TABLE CA-M24 (continued)

3-Chloropropene

Males

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
108	106/1	50	25	21	1							83.8 x 21.1
					1							90.9 x 19.1
					1	3					1 Chromatid Fragment	78.9 x 18.1
					1							100.0 x 13.2
	106/4		25	24	1	3					1 Exchange	87.5 x 20.8
107	73/1	20	4	4								
	73/2		3	1	1	1						102.8 x 14.0
											1 Chromatid Fragment	107.5 x 12.0
	73/3		3	1		1						117.5 x 19.0
					1							90.9 x 13.2
	73/4		8	5				1			1 Chromosomal Fragment	102.0 x 16.9
					1							109.0 x 16.1
								1				82.2 x 11.2
	73/5		2	2								
104	66/1	50	25	23	1							77.2 x 22.1
					1							108.2 x 15.2
	66/2		25	22		1					1 Chromatid Fragment	83.1 x 20.0
					1							117.8 x 15.2
											1 Chromatid Fragment	104.8 x 13.1
103	65/2	50	25	24	1							107.5 x 19.2
	65/3		25	22	1							84.2 x 22.0
					1							88.1 x 20.8
											1 Chromosomal Fragment	
					1						1 Chromatid Fragment 1 Translocation	110.0 x 16.8

APPENDIX TABLE CA-M24 (continued)

3-Chloropropene
Males

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
109	154/2	50	25	25								
	154/4		25	21	1		1					111.9 x 21.8
					1							114.9 x 21.8
					2							102.1 x 21.0
					1							113.5 x 20.2

APPENDIX TABLE CA-M48

3-Chloropropene
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Males

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
21	159/1	50	25	25								
	159/3		25	25								
23	14/3	50	25	25								
	14/4		25	24	1							40.1 x 107.2
28	158/2	50	25	25								
	158/4		25	24	1							30.5 x 109.4
29	63/3	50	25	24	1							65.4 x 111.7
	63/1		25	25								
27	19/3	50	25	25								
	19/2		25	25								
22	97/3	50	25	25								
	97/2		25	25								
30	155/1	50	25	25								
	155/5		25	25								
26	47/2	50	25	24	1							59.4 x 106.9
	47/1		25	25								
25	101/3	50	25	24	1							60.0 x 109.1
	101/2		25	25								
24	27/3	50	25	25								
	27/4		25	24	1							36.6 x 110.1

APPENDIX TABLE CA-M48 (continued)

3-Chloropropene
Males

Single Dosing: 1 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		
58	60/3	50	25	25								
	60/2		25	24	1							41.6 x 111.2
53	113/3	50	25	23	1							29.4 x 108.6
					1							32.0 x 108.5
	113/4		25	25								
60	119/3	50	25	25								
	119/5		25	25								
54	96/3	50	25	25								
	96/2		25	25								
55	81/2	50	25	23	1							64.8 x 111.0
					1							26.5 x 111.2
	81/4		25	24	1							31.9 x 109.2
51	30/2	50	25	25								
	30/1		25	24	1							64.1 x 109.2
52	21/1-5	0	0	0								
56	124/2	50	25	25								
	124/3		25	25								
59	115/1	50	25	25								
	115/2		25	24							1 Chromosomal Fragment	60.2 x 111.6
57	40/2	50	25	25								
	40/1		25	25								

APPENDIX TABLE CA-H48 (continued)

3-Chloropropene

Males

Single Dosing: 25 ppm

Sampling Time: 48 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
82	13/2	50	25	25								
	13/3		25	24	1							41.6 x 105.0
84	148/1	50	25	25								
	148/2		25	24	1							48.0 x 108.6
87	75/1	50	25	24		1						32.9 x 111.0
	75/2		25	25								
83	38/2	50	25	23		1						33.1 x 112.6
						2						63.5 x 108.5
	38/1		25	24	1							65.3 x 113.1
90	120/3	50	25	25								
	120/2		25	24		1						44.5 x 108.5
81	109/3	50	25	25								
	109/2		25									
88	68/1-5	0	0									
86	43/3	50	25	25								
	43/4		25	24	1							43.4 x 104.6
89	79/2	50	25	25								
	79/4		25	24		1						30.4 x 110.0
85	9/3	50	25	25								
	9/4		25	25								

APPENDIX TABLE CA-M48 (continued)

3-Chloropropene

Males

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
113	24/2	50	25	21							1 Exchange	30.9 x 110.0
					1						1 Chromatid Fragment	60.3 x 108.3
					1							65.2 x 108.6
											1 Chromosomal Fragment	70.8 x 107.3
	24/3		25	25								
111	72/2	50	25	22	2	1						38.4 x 111.0
											2 Chromatid Fragments	44.0 x 107.7
											1 Chromatid Fragment	44.2 x 107.6
	72/4		25	25								
117	5/2	50	25	25								
	5/5		25	25								
119	70/1	50	25	20		2					2 Chromosomal Fragments	67.8 x 106.9
						1					1 Chromatid Fragment	65.6 x 107.0
						1						64.7 x 106.7
						1						63.7 x 106.7
					1							62.1 x 107.0
	70/2		25	23								
116	91/2	50	25	25								
	91/5		25	25								
114	41/2	50	25	23	1							67.6 x 109.0
											1 Chromatid Fragment	69.3 x 109.3
	41/3		25	22	1							72.3 x 108.7
											1 Chromatid Fragment	65.1 x 107.4
						1						70.5 x 106.5

APPENDIX TABLE CA-F6

3-Chloropropene
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		
170	290/5	50	25	25								
	290/4		25	24	1							37.5 x 110.3
167	316/2	50	25	24	1							62.5 x 111.4
	316/3		25	25								
168	255/1	50	25	25								
	255/5		25	25								
161	188/2	50	25	25								
	188/3		25	25								
163	298/3	50	25	25								
	298/5		25	25								
166	192/2	50	25	25								
	192/4		25	25								
164	301/2	50	25	25								
	301/4		25	25								
169	259/2	50	25	25								
	259/5		25	25								
165	194/2	50	25	25								
	194/3		25	25								

APPENDIX TABLE CA-F6 (continued)

3-Chloropropene
Females

Single Dosing: 1 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		
199	170/2	50	25	25								
	170/5		25	25								
197	292/5	50	25	25								
	292/3		25	24	1							58.6 x 112.6
198	221/1	50	25	25								
	221/2		25	25								
191	274/3	50	25	24	1							60.3 x 106.1
	274/4		24	24								
	274/5		1	1								
192	295/1	50	25	25								
	295/4		25	25								
193	305/2	50	25	25								
	305/3		25	25								
196	202/5	50	25	24	1							35.2 x 108.0
	202/2		25	25								
194	311/1	50	25	24	1							62.8 x 113.6
	311/4		25	25								
200	219/2	50	25	25								
	219/3		25	25								

APPENDIX TABLE CA-P6 (continued)

3-Chloropropene
Females

Single Dosing: 25 ppm

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
222	161/3	50	25	25								
	161/1		25	25								
225	312/2	50	25	25								
	312/3		25	25								
223	317/5	50	25	25								
	317/1		23	22	1							53.2 x 102.2
	317/2		2	2								
229	186/2	50	25	25								
	186/3		25	25								
228	262/5	50	25	25								
	262/4		25	25								
230	163/2	50	25	25								
	163/5		25	25								
224	289/1	50	10	10								
	289/4		25	24	1							69.7 x 114.2
	289/5		15	15								
226	245/1	50	25	25								
	245/2		22	22								
	245/3		3	2	1							47.6 x 110.8
221	218/3	50	25	25								
	218/2		25	25								
227	260/2	50	25	25								
	260/3		25	25								

APPENDIX TABLE CA-P6 (continued)

3-Chloropropene

Females

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
259	302/4	50	25	24	1							51.0 x 114.8
	302/5		25	25								
254	206/3	50	25	25								
	206/5		25	24	1		1					33.2 x 104.4
252	272/3	50	25	24	1							69.4 x 108.2
	272/4		25	25								
256	306/1	50	25	23	1							37.0 x 113.4
					1							48.1 x 113.5
	306/2		25	24	1							40.0 x 115.7
257	300/5	50	25	25								
	300/4		25	25								
253	283/4	50	25	24	1							68.6 x 109.6
	283/5		25	22		1						41.0 x 108.2
					1							42.8 x 107.9
					1							61.9 x 106.8
[illegible]	313/1	50	25	23	1							38.4 x 110.0
					1							35.4 x 110.3
	313/4		25	25								
255	214/1	50	25	21	1							37.6 x 113.2
						1						41.4 x 109.7
					1							40.6 x 109.7
					1							51.1 x 106.5

APPENDIX TABLE CA-F6 (continued)

3-Chloropropene

Males

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
255	214/3		25	22	1							61.8 x 110.3
						1						38.3 x 108.5
					1							3C.8 x 106.1
260	175/3	50	25	24								28.7 x 114.1
	175/4		25	25								
258	240/2	50	25	25								
	240/3		25	25								

APPENDIX TABLE CA-F24

3-Chloropropene
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	224/1	50	25	24							1 Robertsonian Translocation	111.9 x 19.8
	224/2		25	24				1				102.1 x 15.5
172	296/2	50	25	24	1							104.9 x 17.1
	296/3		25	24	1							95.1 x 19.8
176	264/1	50	25	25								
	264/2		23	23								
	264/3		2	2								
175	166/1	50	25	25								
	166/2		25	25								
171	271/1	50	25	25								
	271/2		25	25								
174	303/1	50	25	21	1							110.0 x 19.1
					2							107.2 x 17.2
					1							106.9 x 15.8
					1							91.1 x 13.9
	303/4		25	23	1							103.1 x 19.9
					1							99.0 x 17.8
180	258/2	50	25	25								
	258/4		25	24	1							118.2 x 19.1
173	178/3	50	25	25								
	178/4		25	25								

(APPENDIX TABLE CA-F24 (continued)

3-Chloropropene
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		
177	287/2	50	25	24	1							87.5 x 21.1
	287/3		25	24	1							105.9 x 19.0
178	294/3	50	25	24	1							102.4 x 15.2
	294/5		25	25								

APPENDIX TABLE CA-F24 (continued)

3-Chloropropene

Females

Single Dosing: 1 ppm

Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
205	195/5	50	25	25								
	195/4		25	24							1 Robertsonian Translocation	120.9 x 18.9
203	197/1	50	25	25								
	197/2		25	25								
202	307/4	50	25	25								
	307/2		25	24	1							118.0 x 14.1
209	183/2	50	25	25								
	183/5		25	25								
207	215/1	50	25	25								
	215/2		25	25								
204	222/2	50	25	24							1 Robertsonian Translocation	85.1 x 18.0
	222/4		25	25								
208	205/2	50	25	25								
	205/3		25	25								
206	238/2	50	25	25								
	238/3		25	25								
210	243/3	50	25	25								
	243/4		25	25								
201	227/1	50	25	24	1							108.9 x 17.9
	227/2		25	22	1							110.0 x 21.9
					1							104.4 x 19.8
					1							107.9 x 19.2

APPENDIX TABLE CA-F24 (continued)

3-Chloropropene

Females

Single Dosing: 25 ppm

Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
236	246/2	50	25	25								
	246/5		25	25								
240	168/4	50	25	24							1 Chromatid Fragment	84.9 x 17.6
	168/5		25	25								
238	267/4	50	25	25								
	267/5		17	16	1							79.8 x 17.2
	267/2		8	8								
237	252/1	50	15	14	1							105.8 x 12.0
	252/2		22	21	1							103.9 x 7.9
	252/3		13	13								
235	210/3	50	25	25								
	210/4		25	25								
232	244/1	50	25	25								
	244/4		25	25								
233	196/2	50	25	23	2							107.9 x 17.5
											1 Chromosomal Fragment	78.1 x 15.0
	196/3		25	24	1							111.2 x 20.0
231	237/1	50	25	25								
	237/5		25	25								

APPENDIX TABLE CA-F24 (continued)

3-Chloropropene

Females

Single Dosing: 25 ppm

Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
234	199/1	22	1	1								
	199/2		3	3								
	199/3		8	8								
	199/4		7	7								
	199/5		3	3								
239	180/3	50	25	23	1							101.0 x 18.9
					1							113.9 x 19.0
	180/4		25	24				1				91.1 x 11.9

APPENDIX TABLE CA-F24 (continued)

3-Chloropropene

Females

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
267	233/1	50	25	23	1							86.9 x 19.2
											1 Chromatid Fragment	110.1 x 16.6
	233/2		25	25								
268	266/1	50	25	22			1					86.1 x 20.0
						1						111.1 x 20.0
						1						80.0 x 19.1
	266/2		25	23	1	1					1 Chromatid Fragment	80.1 x 19.8
									1			109.2 x 16.0
269	314/2	50	25	25								
	314/3		25	19		1						111.5 x 19.1
					1	1					1 Chromatid Fragment	111.8 x 19.1
					3							111.0 x 18.0
					2				1		1 Exchange 1 Chromatid Fragment	88.0 x 16.9
					1	3					1 Exchange 1 Chromatid Fragment	114.1 x 16.9
						1					3 Chromatid Fragments	115.9 x 16.9
266	177/1	41	6	6								
	177/2		6	6								
	177/3		12	10	1							83.0 x 17.8
					1							74.1 x 12.6
	177/4		10	10								
	177/5		7	6	2	1						87.8 x 89.1

APPENDIX TABLE CA-F24 (continued)

3-Chloropropene
Females

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
261	213/1	29	3	3								
	213/2		7	7								
	213/3		5	4	2							113.8 x 18.0
	213/4		8	7	1							115.0 x 17.0
	213/5		6	6								
270	176/3	50	25	21	1							110.0 x 18.2
					3	1					1 Chromatid Fragment	88.1 x 16.9
											1 Chromatid Fragment	112.5 x 13.0
					2						2 Chromatid Fragments	111.0 x 10.2
	176/5		25	22	1							86.4 x 19.2
					1						2 Chromatid Fragments	80.9 x 15.0
						1						96.9 x 11.2
264	226/1	50	4	4								
	226/2		20	15			2			1	3 Chromatid Fragments	84.1 x 17.8
					1							107.1 x 15.1
					1							77.2 x 13.1
					1	1						112.0 x 7.0
											2 Chromatid Fragments 1 Chromosomal Fragment	109.8 x 85.8

APPENDIX TABLE CA-F24 (continued)

3-Chloropropene
Females

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
264	226/3		20	14		1						90.0 x 21.4
					1							109.8 x 20.1
									1			102.0 x 20.2
					1	1					3 Chromatid Fragments	116.5 x 16.0
					1							112.9 x 14.2
											1 Chromatid Fragment	113.0 x 11.1
	226/4		6	4		1						117.0 x 19.9
						1			2		3 Chromatid Fragments	91.0 x 16.6
263	225/1	50	18	17		1						114.8 x 10.1
	225/2		8	7		1						83.1 x 16.9
	225/3		15	15								
	225/4		9	9								
265	211/4	50	25	20	1							112.0 x 20.0
											1 Chromatid Fragment	101.5 x 17.0
					1							110.1 x 16.1
					1							104.0 x 11.6
					1							88.1 x 11.0
	211/1		15	13	1							107.0 x 18.0
					1							102.2 x 13.0
	211/2		10	7	1							108.5 x 19.2
					1							99.9 x 18.2
					1							85.8 x 17.9

APPENDIX TABLE CA-F24 (continued)

3-Chloropropene
Females

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
262	167/3	50	25	20	3							109.2 x 22.2
					1							115.0 x 20.2
					1							90.0 x 20.2
								1				109.1 x 18.8
					3							101.9 x 18.8
	167/5		24	18	1							86.0 x 21.0
					1							106.9 x 18.2
					1							108.0 x 17.4
					1							112.1 x 15.1
					1	1	1				1 Chromatid Fragment	86.2 x 12.9
					1							86.4 x 12.9
	167/1		1	1								

APPENDIX TABLE CA-F48

3-Chloropropene
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		
186	207/3	4	4	3		1						35.7 x 97.2
	207/1,2,4,5		0	0								
185	261/3	50	25	25								
	261/1		25	24	1							54.8 x 105.5
184	187/4	50	25	24	1							67.5 x 108.6
	187/3		25	25								
188	318/2	50	25	25								
	318/3		25	25								
182	257/2	50	25	24	1							69.7 x 106.6
	257/3		25	24	1							34.0 x 109.2
189	223/3	50	25	25								
	223/1		25	24							1 Chromatid Fragment	38.2 x 107.3
183	174/1-5	0	0	0								
181	319/1	50	25	24		1						50.8 x 112.1
	319/3		25	25								
190	315/3	50	25	25								
	315/5		25	25								
187	179/1	50	25	24	1							73.6 x 103.8
	179/2		25	25								

APPENDIX TABLE CA-F48 (continued)

3-Chloropropene
Females

Single Dosing: 1 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		
211	190/3	50	25	25								
	190/5		25	25								
215	241/3	50	25	25								
	241/2		25	24	1						1 Chromatid Fragment	35.6 x 109.0
214	256/1	50	25	25								
	256/4		25	24		1						37.7 x 105.0
212	181/1	50	25	25								
	181/3		25	25								
213	273/1	50	25	24								66.3 x 114.8
	273/3		25	25								
218	220/1	50	25	25								
	220/2		25	25								
216	284/3	50	25	25								
	284/4		25	25								
217	200/3	50	25	24	1							34.1 x 112.0
	200/4		25	25								
220	279/3	50	25	25								
	279/4		25	25								
219	275/2	50	25	25								
	275/5		25	25								

APPENDIX TABLE CA-F48 (continued)

3-Chloropropene
Females

Single Dosing: 25 ppm

Sampling Time: 48 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
249	239/3	50	25	25								
	239/4		25	24	1							65.4 x 105.9
247	235/2	50	25	25								
	235/1		25	24	1							34.4 x 109.8
244	308/4	50	25	25								
	308/1		25	25								
241	269/2	50	25	23	1							62.5 x 111.9
					1							30.8 x 109.1
	269/4		25	25								
248	228/1	50	25	25								
	228/4		25	24	1							42.4 x 108.6
246	203/2	50	25	24		1						42.6 x 105.0
	203/4		25	24		1						51.7 x 102.1
245	169/3	50	25	24	1							73.0 x 109.6
	169/4		25	25								
243	198/1	50	25	24	1							34.0 x 107.5
	198/4		25	25								
250	280/4	50	25	24		1						59.7 x 108.2
	280/3		25	24	1							38.1 x 112.0
242	173/4	50	25	25								
	173/3		25	25								

APPENDIX TABLE CA-F48 (continued)

3-Chloropropene

Females

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
115	139/1	50	25	21	1							60.6 x 109.0
					1							65.0 x 107.6
					1							39.9 x 106.4
					2							33.4 x 106.7
	139/3		25	23	1							39.6 x 109.6
					1							36.3 x 109.6
118	105/4	50	25	24		1					1 Exchange 1 Chromatid Fragment	65.6 x 108.1
	105/3		25	21	1	3					1 Chromosomal Fragment	52.0 x 114.1
											1 Multi Aberration	62.8 x 112.5
						1						68.0 x 111.4
						1						68.0 x 111.3
112	125/2	50	25	24		1						27.6 x 110.2
	125/3		25	24		1						65.0 x 109.0
120	71/1	50	25	25								
	71/5		25	24		1						60.5 x 109.3

APPENDIX TABLE CA-F48 (continued)

3-Chloropropene

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		
271	232/4	50	25	25								
	232/2		25	22							1 Chromosomal Fragment 1 Chromatid Fragment	61.5 x 108.9
						1						31.5 x 107.1
											1 Exchange	63.7 x 107.0
278	265/3	50	-	20	1							29.8 x 109.0
					1							60.1 x 107.9
					1							28.7 x 106.5
					1							31.1 x 107.0
					2							60.0 x 107.2
	265/2		25	24				1				59.2 x 105.2
275	299/2	50	25	24		1						29.7 x 110.5
	299/3		25	24		1						57.1 x 103.4
277	165/3	50	25	22							2 Exchanges	58.0 x 109.0
					1							59.0 x 108.3
					1							61.6 x 108.2
	165/2		25	20		1						40.5 x 110.0
					1	1						34.0 x 107.3
					1							57.7 x 107.8
					1							61.3 x 108.7
					2	2					2 Exchanges	55.8 x 106.7
279	230/2	50	25	25								
	230/3		25	24		1						35.9 x 111.8

APPENDIX TABLE CA-F48 (continued)

3-Chloropropene
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		
280	231/2	50	25	24	1	1					1 Exchange 1 Chromatid Fragment	37.5 x 108.7
	231/3		25	23	1							74.0 x 111.0
											1 Chromatid Fragment	60.4 x 109.5
274	201/4	50	25	23	1							68.5 x 112.4
						1						60.4 x 110.6
	201/1		25	24							1 Chromatid Fragment	36.5 x 111.5
276	251/2	50	25	22							1 Exchange	63.8 x 109.6
						1						30.3 x 109.4
					1							36.4 x 105.5
	251/3		25	22		1						28.3 x 108.1
					1	3						27.7 x 107.8
					1							28.8 x 106.7
272	285/1-5	0	0	0								
273	184/2	50	25	25								
	184/3		25	25								

APPENDIX TABLE DL

3-Chloropropene
Dominant Lethal Assessment

Multiple Dosing: Air Control (0 ppm)

Week No.	Male No.	361		362		363		364		365		366		367		368		369		370		Total
	Female	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
1	Corpora lutea	15	18	16	14	0	12	18	17	12	11	L	15	11	11	P/15	15	17	0	L	15	232
	Total Implants	15	11	16	15	0	11	14	15	15	4	L	15	15	11	P/12	15	15	0	L	17	216
	Live Implants	14	11	14	15	0	11	13	14	13	4	L	15	15	11	P/12	15	15	0	L	17	209
	Early Deaths	1	0	1	0	0	0	1	1	2	0	L	0	0	0	P/0	0	0	0	L	0	6
	Late Deaths	0	0	1	0	0	0	0	0	0	0	L	0	0	0	P/0	0	0	0	L	0	1
2	Corpora lutea	16	16	0	18	0	15	15	15	10	12	9	15	15	12	0	14	12	11	9	12	226
	Total Implants	13	13	0	15	0	15	13	15	11	13	9	13	14	19	0	14	12	11	8	13	221
	Live Implants	13	12	0	15	0	13	12	13	11	12	9	12	12	19	0	14	11	11	8	13	210
	Early Deaths	0	1	0	0	0	2	0	1	0	1	0	1	2	0	0	0	1	0	0	0	9
	Late Deaths	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	2
3	Corpora lutea	10	14	12	14	0	9	14	15	10	12	10	10	10	13	11	15	11	13	3	13	219
	Total Implants	11	14	11	14	0	9	12	15	10	11	8	9	10	12	11	14	9	13	0	13	206
	Live Implants	10	13	11	14	0	9	12	14	10	10	7	8	10	12	11	10	8	13	0	13	195
	Early Deaths	1	1	0	0	0	0	0	1	0	1	1	1	0	0	0	4	1	0	0	0	11
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	Corpora lutea	14	16	18	16	0	15	16	12	0	0	12	15	12	8	13	10	10	10	13	10	220
	Total Implants	14	11	14	11	0	15	16	13	0	0	12	14	13	10	13	14	10	15	14	9	218
	Live Implants	14	9	13	11	0	15	14	12	0	0	9	13	12	9	13	12	10	10	14	9	199
	Early Deaths	0	2	1	0	0	0	2	1	0	0	3	1	1	1	0	2	0	5	0	0	19
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

P = Probably Pregnant before dosing i.e. nearly full term

L = Littered at time of assessment, therefore certainly pregnant before dosing

APPENDIX TABLE DL (continued)

3-Chloropropene

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	11	16	13	16	18	8	16	9	12	13	14	19	15	17	22	21	14	10	14	12	285
	Total Implants	11	16	14	16	16	0	14	9	9	8	14	13	14	13	9	19	13	10	13	14	240
	Live Implants	10	16	14	15	16	0	14	9	9	8	13	10	13	13	7	10	13	10	10	14	224
	Early Deaths	1	0	0	1	0	0	0	0	0	0	1	3	1	0	2	9	0	0	3	0	16
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	Corpora lutea	10	11	19	17	11	0	15	13	10	2	15	14	12	14	13	14	11	17	12	0	230
	Total Implants	11	12	12	16	11	0	12	14	2	0	12	13	12	15	14	14	13	17	12	0	212
	Live Implants	11	12	11	16	11	0	12	12	2	0	12	12	12	15	12	13	12	17	12	0	204
	Early Deaths	0	0	1	0	0	0	0	0	0	0	0	1	0	0	2	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	0	0
7	Corpora lutea	12	15	14	12	13	14	14	14	0	10	14	12	16	14	14	12	12	8	11	15	246
	Total Implants	12	15	13	11	13	15	14	13	0	2	14	11	15	17	14	7	12	12	12	16	238
	Live Implants	12	15	12	10	13	15	14	13	0	2	13	10	13	16	14	7	12	9	11	16	227
	Early Deaths	0	0	1	1	0	0	0	0	0	0	1	1	2	0	0	0	0	3	1	0	10
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1

APPENDIX TABLE DL (continued)

3-Chloropropene

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	
8	Corpora lutea	20	12	12	11	0	15	12	11	0	4	12	13	11	12	14	13	10	11	13	0	206
	Total Implants	10	12	12	13	0	18	12	14	0	0	11	13	13	13	13	12	14	10	15	0	205
	Live Implants	8	12	11	12	0	18	12	14	0	0	11	12	12	13	13	12	14	9	15	0	198
	Early Deaths	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	4
	Late Deaths	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	3
9	Corpora lutea	1	13	12	13	15	11	9	10	3	0	15	13	10	13	13	14	10	16	15	11	217
	Total Implants	0	12	11	13	15	11	10	10	3	0	15	13	12	14	13	15	10	16	14	11	218
	Live Implants	0	12	10	12	12	11	10	10	1	0	15	10	12	14	13	15	10	16	1[illegible]	11	207
	Early Deaths	0	0	1	1	2	0	0	0	2	0	0	3	0	0	0	0	0	0	1	0	10
	Late Deaths	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
10	Corpora lutea	12	19	1	12	12	12	17	*-	0	12	13	11	12	15	12	14	12	10	12	11	219
	Total Implants	12	13	0	13	13	9	13	*-	0	2	15	11	13	16	12	16	13	15	13	11	210
	Live Implants	12	11	0	13	13	8	13	*-	0	1	15	11	12	16	8	16	13	15	13	11	201
	Early Deaths	0	2	0	0	0	1	0	*-	0	1	0	0	1	0	4	0	0	0	0	0	9
	Late Deaths	0	0	0	0	0	0	0	*-	0	0	0	0	0	0	0	0	0	0	0	0	0
Σ	Corpora lutea	121	150	117	143	69	111	146	116	57	76	114	152	124	129	127	142	119	106	102	99	2300
	Total Implants	109	129	103	137	68	103	130	118	50	40	110	125	131	140	111	135	121	119	101	104	2184
	Live Implants	104	123	96	133	65	100	126	111	46	37	104	113	123	138	103	124	118	110	96	104	2074
	Early Deaths	4	6	6	3	2	1	3	6	4	3	6	11	8	1	8	11	3	9	5	0	102
	Late Deaths	1	0	1	1	1	1	0	1	0	0	0	1	0	1	0	0	0	0	0	0	8

*- = Missing value: ambiguous record of result

APPENDIX TABLE DL (continued)

3-Chloropropene

Multiple Dosing: 1 ppm

Week No.	Male No.	371		372		373		374		375		376		377		378		379		380		Total
	Female	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
1	Corpora lutea	14	13	16	12	12	12	10	16	15	18	14	4	L	10	15	21	17	13	12	13	257
	Total Implants	14	14	14	11	14	14	14	12	13	15	14	10	L	12	14	16	16	12	15	15	249
	Live Implants	13	14	12	11	14	11	14	12	15	15	14	0	L	10	13	14	16	11	14	15	236
	Early Deaths	0	0	2	0	0	3	0	0	0	0	0	0	L	2	1	2	0	1	1	0	12
	Late Deaths	1	0	0	0	0	0	0	0	0	0	0	0	L	0	0	0	0	0	0	0	1
2	Corpora lutea	13	13	13	12	16	13	11	11	12	15	8	11	9	15	13	13	15	15	12	13	253
	Total Implants	14	13	13	13	12	14	10	14	12	15	12	14	14	13	13	19	15	14	11	13	263
	Live Implants	13	13	13	12	12	13	8	13	12	15	[illegible]	14	14	13	12	14	14	14	11	12	254
	Early Deaths	1	0	0	1	0	1	2	0	0	0	0	0	0	0	1	0	0	1	0	1	8
	Late Deaths	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
3	Corpora lutea	14	11	14	14	0	14	14	12	13	17	10	13	14	14	13	11	12	17	13	15	255
	Total Implants	11	12	10	11	0	13	11	13	13	13	11	14	13	14	14	12	12	15	10	13	235
	Live Implants	11	11	8	11	0	13	11	13	13	13	10	13	13	14	14	10	12	15	10	13	228
	Early Deaths	0	1	2	0	0	0	0	0	0	0	1	1	0	0	0	2	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
4	Corpora lutea	15	12	14	14	11	13	12	11	*-	22	12	10	10	15	12	11	10	16	17	14	251
	Total Implants	13	13	13	11	13	13	13	12	*-	16	13	1	10	14	14	9	12	13	14	14	231
	Live Implants	13	12	11	11	13	13	12	10	*-	16	13	1	10	14	13	9	12	13	13	14	223
	Early Deaths	0	1	2	0	0	0	1	2	*-	0	0	0	0	0	1	0	0	0	1	0	8
	Late Deaths	0	0	0	0	0	0	0	0	*-	0	0	0	0	0	0	0	0	0	0	0	0

*- = Missing value: ambiguous record of result

L = Littered at time of assessment, therefore certainly pregnant before dosing

APPENDIX TABLE DL (continued)

3-Chloropropene

Multiple Dosing: 1 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	1	12	14	13	18	6	15	0	14	13	17	11	15	8	15	14	11	11	14	13	235
	Total Implants	0	11	14	13	15	9	14	0	13	11	4	9	15	1	15	13	11	9	11	12	206
	Live Implants	0	11	11	12	13	9	14	0	12	11	4	8	15	6	14	12	11	8	11	8	190
	Early Deaths	0	0	3	1	2	0	0	0	1	0	0	1	0	1	1	1	0	1	0	4	16
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	Corpora lutea	13	1	13	14	19	12	12	8	16	11	11	16	13	11	11	14	14	16	12	13	250
	Total Implants	13	0	14	14	17	11	11	11	15	11	9	15	15	11	9	15	15	17	12	11	246
	Live Implants	13	0	14	14	17	11	11	11	15	11	9	13	15	11	9	15	14	17	12	11	243
	Early Deaths	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	1	0	0	0	3
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	Corpora lutea	14	11	11	12	12	11	16	13	9	13	1	0	0	11	11	13	12	12	13	13	207
	Total Implants	14	11	13	13	7	12	17	15	12	14	0	0	0	12	14	12	12	12	13	13	216
	Live Implants	14	11	13	13	7	11	16	13	12	14	0	0	0	12	14	11	12	11	12	13	209
	Early Deaths	0	0	0	0	0	1	1	2	0	0	0	0	0	0	0	1	0	1	1	0	7
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

APPENDIX TABLE DL (continued)

3-Chloropropene

Multiple Dosing: 1 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	
8	Corpora lutea	11	9	12	9	14	13	13	16	13	13	19	4	12	0	10	13	12	20	12	10	235
	Total Implants	12	9	15	10	16	14	14	15	15	13	10	0	14	0	14	15	15	17	15	14	247
	Live Implants	12	9	14	10	16	14	12	15	15	12	9	0	13	0	14	15	14	17	15	14	240
	Early Deaths	0	0	1	0	0	0	2	0	0	1	1	0	1	0	0	0	1	0	0	0	7
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	Corpora lutea	13	10	15	16	11	15	11	0	12	14	0	0	11	10	10	16	12	11	14	11	212
	Total Implants	10	2	14	16	12	14	10	0	11	14	0	0	11	9	9	12	12	11	15	11	193
	Live Implants	10	2	13	15	11	13	6	0	10	12	0	0	11	9	8	11	12	11	15	11	180
	Early Deaths	0	0	1	1	1	1	4	0	1	2	0	0	0	0	1	1	0	0	0	0	13
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	Corpora lutea	12	13	14	11	14	18	12	12	11	9	0	0	10	12	16	8	13	11	18	15	229
	Total Implants	12	16	15	7	13	15	12	11	13	12	0	0	10	13	13	11	13	13	15	18	232
	Live Implants	6	16	14	7	13	14	11	11	13	11	0	0	10	13	11	11	13	10	14	17	215
	Early Deaths	6	0	0	0	0	1	1	0	0	1	0	0	0	0	2	0	0	2	1	1	15
	Late Deaths	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2
Σ	Corpora lutea	120	105	136	127	127	127	126	99	115	145	92	69	94	106	126	134	128	141	137	130	2384
	Total Implants	113	101	135	119	119	129	126	103	117	134	73	53	102	105	129	133	133	133	131	134	2318
	Live Implants	105	99	123	116	116	122	115	98	115	130	71	49	101	102	122	122	130	127	127	128	2218
	Early Deaths	7	2	11	3	3	7	11	4	2	4	2	4	1	3	7	7	3	5	4	6	96
	Late Deaths	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	4

APPENDIX TABLE DL (continued)

3-Chloropropene

Multiple Dosing: 25 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	21	11	0	13	17	15	15	0	0	11	0	10	7	10	15	15	13	14	14	0	201
	Total Implants	13	12	0	15	17	12	18	0	0	10	0	10	5	12	15	15	7	15	16	0	192
	Live Implants	12	10	0	14	15	12	15	0	0	10	0	9	5	12	15	15	7	15	16	0	182
	Early Deaths	1	2	0	1	1	0	2	0	0	0	0	1	0	0	0	0	0	0	0	0	8
	Late Deaths	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2
2	Corpora lutea	12	11	10	12	5	14	10	11	12	10	7	10	13	12	11	14	10	11	11	18	234
	Total Implants	10	13	13	14	15	13	11	11	7	14	2	13	16	13	11	13	10	12	13	14	238
	Live Implants	9	13	13	14	14	13	10	11	7	14	2	13	16	12	11	11	9	12	12	14	230
	Early Deaths	1	0	0	0	1	0	1	0	0	0	0	0	0	1	0	1	1	0	1	0	7
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
3	Corpora lutea	12	11	12	15	15	14	12	10	12	15	14	7	13	11	12	14	12	14	8	8	241
	Total Implants	11	10	13	13	14	13	12	12	8	12	13	11	11	11	14	10	12	13	10	13	236
	Live Implants	10	10	13	12	13	13	12	12	7	11	13	11	9	11	12	10	10	12	10	13	224
	Early Deaths	1	0	0	1	0	0	0	0	1	1	0	0	2	0	2	0	2	1	0	0	11
	Late Deaths	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
4	Corpora lutea	18	10	15	13	13	11	12	14	12	11	12	12	11	0	11	13	10	12	14	15	239
	Total Implants	13	12	14	13	14	12	13	14	14	6	13	13	12	0	11	15	9	12	19	15	239
	Live Implants	12	10	14	13	14	12	12	14	13	6	13	12	12	0	11	15	9	11	19	14	231
	Early Deaths	1	2	0	0	0	0	1	0	1	0	0	1	0	0	0	0	0	1	0	1	8
	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)

3-Chloropropene

Multiple Dosing: 25 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	13	20	13	12	15	17	12	18	12	14	17	14	16	14	13	14	18	13	12	18	295
	Total Implants	15	12	13	12	12	16	11	14	11	14	11	12	15	14	11	11	18	14	11	18	265
	Live Implants	14	12	13	9	11	15	11	14	8	14	11	11	14	14	11	11	18	14	11	17	253
	Early Deaths	1	0	0	3	1	1	0	0	3	0	0	1	1	0	0	0	0	0	0	1	12
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	Corpora lutea	11	12	12	15	11	11	0	12	19	18	17	10	11	9	12	15	20	15	11	12	258
	Total Implants	11	13	15	12	12	12	0	12	11	16	16	7	13	5	12	16	13	16	12	12	236
	Live Implants	11	13	15	12	11	12	0	12	10	14	15	4	11	5	12	16	12	16	12	12	225
	Early Deaths	0	0	0	0	1	0	0	0	1	2	1	3	2	0	0	0	1	0	0	0	11
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	Corpora lutea	11	10	13	12	12	8	14	13	0	14	0	9	13	16	10	12	16	17	12	13	235
	Total Implants	5	13	13	6	12	4	13	8	0	13	0	12	12	17	13	14	15	15	13	15	223
	Live Implants	5	13	12	6	10	13	13	7	0	12	0	11	11	17	13	13	14	15	12	13	210
	Early Deaths	0	0	1	0	2	1	0	1	0	1	0	1	1	0	0	1	1	0	1	2	13
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

APPENDIX TABLE DL (continued)

3-Chloropropene

Multiple Dosing: 25 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	
8	Corpora lutea	14	15	12	15	12	13	12	14	13	11	13	12	13	14	12	12	10	11	10	12	250
	Total Implants	15	15	12	15	13	12	13	17	14	11	14	15	11	13	16	11	10	14	12	13	266
	Live Implants	15	15	12	15	13	11	13	17	14	0	13	15	10	13	16	10	9	14	12	13	250
	Early Deaths	0	0	0	0	0	1	0	0	0	2	1	2	1	0	0	1	1	0	0	0	7
	Late Deaths	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	9
9	Corpora lutea	12	15	14	0	15	10	14	16	6	10	13	0	12	14	12	9	11	11	16	12	222
	Total Implants	12	15	14	0	17	10	15	16	8	8	9	0	12	14	13	11	11	12	16	13	225
	Live Implants	11	14	14	0	17	10	15	16	5	8	5	0	8	14	13	11	11	12	15	11	210
	Early Deaths	0	1	0	0	0	0	0	0	3	0	4	0	4	0	0	0	0	0	0	2	14
	Late Deaths	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
10	Corpora lutea	11	11	11	13	13	11	3	12	11	12	10	19	11	11	10	12	10	12	2	9	214
	Total Implants	12	12	13	10	14	11	14	13	13	13	13	11	10	12	11	9	0	12	0	10	213
	Live Implants	12	11	12	7	14	10	14	12	13	12	13	11	10	12	10	9	0	10	0	10	202
	Early Deaths	0	1	1	3	0	1	0	1	0	1	0	0	0	0	1	0	0	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
T	Corpora lutea	135	126	117	120	138	134	114	120	97	126	103	103	120	111	118	130	120	130	110	117	2389
	Total Implants	117	127	120	110	140	125	120	117	25	117	91	104	117	111	127	125	105	135	116	123	2333
	Live Implants	111	121	118	102	132	121	115	115	77	101	85	97	106	110	124	121	99	131	114	117	2217
	Early Deaths	5	6	2	8	6	4	4	2	9	7	6	7	11	1	3	3	6	4	2	6	102
	Late Deaths	1	0	0	0	2	0	1	0	0	9	0	0	0	0	0	1	0	0	0	0	14

APPENDIX TABLE DL (continued)

3-Chloropropene

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Week No.	Male No.	391		392		393		394		395		396		397		398		399		400		Total
	Female	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
1	Corpora lutea	11	11	7	9	10	1	10	13	L	0	11	2	5	7	11	9	1	10	1	15	147
	Total Implants	13	7	0	9	9	0	3	13	L	0	12	3	2	7	9	9	2	12	0	15	125
	Live Implants	6	7	0	9	0	0	3	2	L	0	9	0	2	1	1	0	0	1	0	11	52
	Early Deaths	7	0	0	0	9	0	0	11	L	0	3	3	0	6	8	9	2	11	0	4	73
	Late Deaths	0	0	0	0	0	0	0	0	L	0	0	0	0	0	0	0	0	0	0	0	0
2	Corpora lutea	2	0	2	6	0	2	2	1	2	1	1	0	1	3	4	2	4	0	3	4	40
	Total Implants	1	0	0	0	0	2	2	0	0	0	1	0	0	2	0	0	0	0	1	1	10
	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	0	0	0	2	2	0	0	1	0	0	0	2	0	0	0	0	1	1	10
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	Corpora lutea	3	5	4	0	0	3	5	2	2	5	0	5	7	0	4	6	3	2	1	0	57
	Total Implants	0	2	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	3
	Live Implants	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Early Deaths	0	2	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	3
	Late Deaths																					
4	Corpora lutea	10	11	10	0	15	10	14	18	13	8	2	*-	9	11	2	13	13	14	0	0	173
	Total Implants	9	12	11	0	15	10	13	15	13	9	0	*-	6	12	0	8	11	14	0	0	158
	Live Implants	7	12	10	0	11	3	6	15	9	2	0	*-	1	9	0	7	11	5	0	0	108
	Early Deaths	2	0	1	0	4	7	7	0	4	7	0	*-	5	3	0	1	0	8	0	0	49
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	*-	0	0	0	0	0	1	0	0	1

*- = Missing value: ambiguous record result.

L = Littered at time of assessment, therefore certainly pregnant before dosing

APPENDIX TABLE DL (continued)

3-Chloropropene

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	10	13	14	14	16	15	13	16	15	14	14	0	15	14	30	14	15	12	14	16	284
	Total Implants	0	13	13	13	13	13	11	12	13	8	9	0	5	13	19	13	14	12	14	16	224
	Live Implants	0	12	13	13	11	13	10	12	13	6	8	0	5	13	17	11	12	12	14	13	208
	Early Deaths	0	1	0	0	2	0	0	0	0	1	0	0	0	0	2	2	2	0	0	3	13
	Late Deaths	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	3
6	Corpora lutea	15	*-	18	12	11	10	11	13	13	14	13	13	14	12	17	15	14	14	14	11	244
	Total Implants	15	*-	14	12	12	12	12	14	0	15	3	14	15	13	10	10	14	11	15	10	231
	Live Implants	15	*-	14	12	11	12	11	13	0	15	11	13	15	13	8	8	13	10	15	9	218
	Early Deaths	0	*-	0	0	1	0	1	1	0	0	2	1	0	0	2	2	1	1	0	1	13
	Late Deaths	0	*-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	Corpora lutea	15	14	14	14	10	13	12	13	12	12	14	12	12	16	11	11	0	11	14	17	247
	Total Implants	14	15	10	14	13	12	14	14	11	14	14	12	12	14	13	11	0	15	13	17	254
	Live Implants	14	14	10	14	13	12	12	12	11	14	12	11	14	14	13	10	0	15	11	17	243
	Early Deaths	0	1	0	0	0	0	2	2	0	0	2	1	0	0	0	1	0	0	2	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

*- = Missing value: ambiguous record result.

APPENDIX TABLE DL (continued)

3-Chloropropene

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	
♂	Corpora lutea	10	12	12	10	16	12	13	16	14	10	10	15	21	12	12	10	11	14	0	16	246
	Total Implants	13	13	13	12	13	10	13	18	14	11	13	14	15	15	13	11	13	3	0	13	240
	Live Implants	13	11	12	12	8	10	12	17	12	9	12	13	14	15	13	11	10	3	0	11	218
	Early Deaths	0	2	1	0	4	0	1	1	2	2	1	1	1	0	0	0	3	0	0	1	20
	Late Deaths	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2
9	Corpora lutea	12	11	11	16	13	0	14	13	12	13	13	13	13	12	15	13	13	13	14	14	248
	Total Implants	13	10	11	16	13	0	15	14	12	11	14	13	13	15	14	13	9	12	14	13	245
	Live Implants	13	10	11	16	13	0	15	14	12	10	13	12	13	15	13	13	9	10	14	13	239
	Early Deaths	0	0	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0	2	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
10	Corpora lutea	11	12	0	16	16	12	11	14	12	10	17	16	15	13	9	14	12	18	10	12	250
	Total Implants	10	15	0	16	11	12	9	14	9	13	15	12	15	12	10	13	13	12	11	12	234
	Live Implants	10	12	0	16	11	11	9	13	6	13	15	8	15	12	10	12	13	12	11	10	219
	Early Deaths	0	2	0	0	0	1	0	1	3	0	0	4	0	0	0	1	0	0	0	2	14
	Late Deaths	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Σ	Corpora lutea	89	89	92	97	107	78	105	119	85	87	95	76	111	103	113	107	86	109	74	104	1926
	Total Implants	68	87	72	92	99	71	92	114	72	82	90	68	86	103	88	88	76	91	68	97	1724
	Live Implants	78	78	70	92	78	61	78	98	63	69	80	57	79	92	75	72	68	68	65	84	1505
	Early Deaths	10	8	2	0	20	10	13	16	9	12	9	11	7	11	13	16	8	22	3	12	212
	Late Deaths	0	1	0	0	1	0	1	0	0	1	1	0	0	0	0	0	0	1	0	1	7

APPENDIX TABLE 8A

3-Chloropropene
Sperm Abnormality Assessment

Multiple Dosing: Air Control (0 ppm)
Low, 1 ppm
High, 25 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
341	974	0	0	11	1	14	26	1000	321	Air
356	966	0	1	12	5	16	34	1000	322	Air
352	964	1	0	20	3	12	36	1000	323	Air
324	964	0	1	18	4	13	36	1000	324	Air
328	947	1	6	18	9	19	53	1000	325	Air
329	969	0	0	20	7	4	31	1000	326	Air
340	972	0	0	14	3	11	28	1000	327	Air
353	956	3	1	14	6	20	44	1000	328	Air
334	957	4	1	19	6	13	43	1000	329	Air
335	921	1	5	37	7	29	79	1000	330	Air
348	931	3	2	25	8	31	69	1000	331	Low
358	979	0	0	11	3	7	21	1000	332	Low
338	968	3	0	12	9	8	32	1000	333	Low
342	959	1	2	15	12	11	41	1000	334	Low
336	974	0	4	8	2	12	26	1000	335	Low
330	939	2	1	20	18	20	61	1000	336	Low
350	983	0	1	8	4	4	17	1000	337	Low
327	970	0	2	10	4	14	30	1000	338	Low
331	977	0	2	8	4	9	23	1000	339	Low
346	946	2	5	15	13	19	54	1000	340	Low

APPENDIX TABLE 5A (continued)

3-Chloropropene

Multiple Dosing: Air Control (0 ppm)
Low, 1 ppm
High, 25 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
357	960	1	1	19	4	15	40	1000	341	High
332	958	0	2	13	10	17	42	1000	342	High
326	953	0	2	14	8	23	47	1000	343	High
339	956	2	5	13	2	22	44	1000	344	High
360	971	1	1	16	1	10	29	1000	345	High
349	963	1	1	18	6	11	37	1000	346	High
343	977	1	0	10	6	6	23	1000	347	High
323	970	0	1	16	3	10	30	1000	348	High
345	973	0	2	10	5	10	27	1000	349	High
347	955	0	3	19	7	16	45	1000	350	High
337	950	3	1	16	10	20	50	1000	351	*
359	932	5	7	42	14	10	68	1000	352	*
333	950	0	0	20	16	14	50	1000	353	*
325	955	2	1	25	2	15	45	1000	354	*
350	947	3	1	25	6	18	53	1000	355	*
344	932	1	3	27	21	16	68	1000	356	*
351	965	1	2	11	10	11	35	1000	357	*
322	957	0	3	19	9	12	43	1000	358	*
354	966	1	1	15	7	10	34	1000	359	*
321	941	0	1	28	15	15	59	1000	360	*