Report Number 26

TIER II MUTAGENIC SCREENING OF 13 NIOSH PRIORITY COMPOUNDS

INDIVIDUAL COMPOUND REPORT 1,1,2,2-TETRACHLOROETHANE

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AUTHENTICATION

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

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Principal Investigator

TABULATIONS

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

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

Example:

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

Abbreviations on Chromosomal Aberration Tables and Appendix Tables:

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

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LOCATION OF EXPERIMENT

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

DISCLAIMER

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

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SUMMARY

1,1,2,2-Tetrachloroethane 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 15,869 µg/ml of culture medium.

2. Dominant lethal test in male rats with exposure to atmospheres containing 5 ppm or 50 ppm 1,1,2,2-tetrachloroethane for 7 h per day for 5 consecutive days. Analysis of test atmospheres was by continuous infrared absorption monitoring at a wavelength of 8.3 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 5 ppm for 7 h or 50 ppm for 40 min.

The results obtained were as follows:

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

2. There were no signs of toxicity in either rats or mice exposed to 5 ppm or 50 ppm 1,1,2,2-tetrachloroethane atmospheres.

3. The frequency of aberrations other than gaps was increased in the females dosed with 50 ppm 1,1,2,2-tetrachloroethane for 7 h, then bone marrow cells sampled 6 h later ($P<0.05$). A similar increase was not seen in the males at that sampling time or in males or females in any other group.

4. There were no effects attributable to 1,1,2,2-tetrachloroethane in the dominant lethal test on pregnancy frequency, numbers of corpora lutea or implantations, or the frequency of early deaths. It is possible that a viral infection increased the numbers of early deaths in some groups, including the environmental control group.

5. The frequency of sperm with hook abnormalities was slightly increased in the 50 ppm 1,1,2,2-tetrachloroethane group ($P<0.05$).

6. Sex-linked recessive lethal mutation frequency was not increased by exposure conditions which produced sedation, but no decrease in survival.

It was concluded that 1,1,2,2-tetrachloroethane had not been tested to the practical limits of the mammalian assays and that the experiments should be repeated, in view of the low level of statistically significant increases in abnormal cells in the chromosome aberration and sperm abnormality tests.

INTRODUCTION

1,1,2,2-Tetrachloroethane (CAS No. 79-34-5) is a chlorinated, saturated hydrocarbon also known as acetylene tetrachloride. It is synthesised by chlorine reacting with acetylene in the presence of catalysts such as ferric chloride, antimony pentasulphide or a mixture of sulphur dichloride and ferric chloride (Hardie, 1964).

1,1,2,2-Tetrachloroethane is used in chlorinated solvent manufacture in many countries and may be used as a solvent itself.

Properties

1,1,2,2-Tetrachloroethane is a colourless liquid with a mild, sweetish odour similar to several other chlorinated hydrocarbons. It is neither explosive nor flammable. While some odour is detectable at 3 ppm (Lehmann and Schmidt-Kehl, 1936), this is very close to the TLV and does not give sufficient warning.

In the absence of air, water and light, 1,1,2,2-tetrachloroethane is stable, even at high temperatures, but in air dehydrochlorination occurs slowly giving trichloroethylene and traces of phosgene. Under pyrolytic conditions, hydrogen chloride and chlorine are released forming trichloroethylene, tetrachloroethylene, pentachloroethane and hexachloroethane (Hardie, 1964).

Structure:	$CHCl_2.CHCl_2$
M.W.	167.86
Sp. grav.	1.5869 ($25^{o}C$)
M.P.	$-42.5^{o}C$
B.P.	$146.3^{o}C$
Vap. dens.	5.79 (air = 1)
Vap. press.	6 mm Hg ($25^{o}C$)
Refr. index	1.4918 ($25^{o}C$)
% in "saturated" air	0.79 ($25^{o}C$)
Density in "saturated" air	1.04 (air = 1)
Solubility	very slightly soluble in water; soluble in ethanol and diethyl ether.

The current OSHA environmental standard is 5 ppm (35 mg/m^3) for an 8 h time weighted average.

Toxicology

The metabolism of 1,1,2,2-tetrachloroethane has not been thoroughly investigated. Its pulmonary and urinary

elimination rates are low, which may explain the recovery of appreciable amounts of the compound from body tissues in fatal or chronic poisoning (von Oettingen, 1955). There are very low levels of trichloroacetic acid and trichloroethane in the urine of dosed rodents, in contrast to the high levels found following 1,1,1,2-tetrachloroethane administration (Ikeda et al, 1972). In mice, 1,1,2,2-tetrachloroethane is subject to hydrolytic dehalogenation leading to a sequential loss of chlorine and terminating in the formation of carbon dioxide, which is exhaled as considerable proportion of the dose (Yllner, 1971).

1,1,2,2-Tetrachloroethane is one of the more toxic chlorohydrocarbons. The most significant injury from multiple or chronic exposure is to the liver, which becomes greatly enlarged and may progress to fatty degeneration and cirrhosis. Kidney damage also results from exposure. As with other chlorohydrocarbons, this compound induces CNS depression, dizziness, nervousness and incoordination. In very severe, acute exposures, unconsciousness and death may result, consequential to respiratory failure. The CNS effects, however, are uncommon industrially because of the low volatility of the compound. Respiratory damage is probably the result of respiratory irritation while irritation of the gastro-intestinal tract may result in nausea, vomiting and gastric pain.

Man seems to be much more susceptible than experimental animals to 1,1,2,2-tetrachloroethane toxicity. Lehmann and Flury (1943) exposed cats and rabbits to 100-160 ppm for 8-9 h/day for 4 weeks and found no typical organ changes. A lethal dose for dogs is 0.3 ml/kg, orally (Wright and Shaffer, 1930). Sherman (1953) reported that 8 people given 3 ml of 1,1,2,2-tetrachloroethane by mistake, along with 30 g $MgSO_4.7H_2O$ and water were comatose within 1.5-2.5 h. In these people, reflexes were absent and pulses were barely perceptible; respiration was shallow and rapid. All 8 people recovered with, apparently, no after effects.

Some carcinogenicity tests have been conducted. Orally administered technical grade 1,1,2,2-tetrachloroethane induced liver tumours in male and female $B6C3F_1$ mice, but no evidence of carcinogenicity emerged from studies with Osborne-Mendel rats (N.C.I., 1977). In the mice, 44/49 males given 108 mg/kg/day and 13/50 males given 62 mg/kg/day developed hepatocellular carcinomas by 78 weeks. The compound has also been reported as causing DNA damage in the E. coli $polA^+/A^-$ repair test system and being mutagenic in S. typhimurium TA 1530 and TA 1535, in Ames' test (Rosenkranz, 1977). It is important, however, when evaluating these studies, to consider the involvement of

impurities and stabilisers in the generation of the observed biological effects. Many chemicals used as stabilisers do have mutagenic properties.

The objective of the work described in this report is to collect information upon the genotoxicity of 1,1,2,2-tetrachloroethane in more complex test systems than bacteria. This information will be used to assess better the risks to man associated with exposure. Exposure conditions used were:

Human fibroblasts:	up to 15,869 µg/ml for 3 h.
Mice and rats:	5 ppm or 50 ppm for 7 h/day for one or 5 days.
Drosophila:	5 ppm for 7 h or 50 ppm for 40 min.

MATERIALS AND METHODS

CHEMICALS

Test Substance

3 kg of 1,1,2,2-Tetrachloroethane, Batch No. 11209 (stated purity 98%), was received from Aldrich Chemical Company Limited, on 16 February 1979. The test material was a clear liquid and was retained in the dark under ambient conditions in the company dispensary. 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, England and kept in a refrigerator in the company dispensary until used.

ANIMALS AND ANIMAL MANAGEMENT

Animals

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

B6C3F$_1$ hybrid mice were obtained from Charles River (USA) Limited.

These animals were obtained and used as follows.

Species	Date of Receipt	Age (Weeks)	Quarantine (Days)	Number (Sex)	Dates of Exposure	Comment
Rat	23 March 1979	9-10	5	220♂	28 March 1979	
			10	176♀	2-6 April 1979	
Mouse	21 March 1979	9-11	12	44♂	2-6 April 1979	
Rat	5 April 1979 etc	8-10	1	80♀	None	DL mating

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 15°C and 22.5°C, and relative humidity ca 50%, with extreme limits of 36% and 54%.

On completion of the dosing period, and as a precaution against accidental exposure to a different test compound, the dominant lethal test animals were relocated in a separate animal holding room for the duration of the test matings - approximately 12 weeks. This animal holding room had 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 27.5°C and 18°C, and relative humidity ca 50% with extreme limits of 62% and 32%.

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

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

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

Diet

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

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

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

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

Allocation of Rats and Mice to Cages and Treatment Groups

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

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

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

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

Treatment groups were colour coded as follows:

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

Animal Identification

The animals to be dosed were individually identified using brass ear tags bearing the animal number and the 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
	Multi-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	

Observations

All animals were inspected for clinical signs/mortality twice daily except at weekends when they were observed once only.

Body Weights

During the dosing period, all animals subjected to the exposure manipulations were weighed upon removal from the exposure chambers. Animals treated with the positive control substance (ethyl methanesulphonate) were weighed immediately prior to dosing.

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 1,1,2,2-tetrachloroethane 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 8 air changes per hour. An additional chamber of 0.84 m^3 capacity was used for exposure of the air control group; the breathing zone in this chamber was ventilated at the rate of 7 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 1,1,2,2-tetrachloroethane. Protective gloves and laboratory coats were worn and the test compound was handled in an extract hood at all times.

Monitoring Equipment

The atmospheres within the exposure chambers were analysed by infra-red spectroscopy using Miran-1A Portable Gas Analysers (Foxboro/Wilks Inc). This type of instrument is a single beam, variable wavelength spectrometer, scanning the infra-red spectrum between 2.5 and 14.5 μm. It is equipped with a gas cell having a variable pathlength of between 0.75 and 21.75 m. Samples of the chamber air were continuously pumped (4 l/min) through stainless steel sample lines of 1/8" ID, to the gas cell of the analyser. The concentration was measured and relayed to a chart recorder (Servoscribe RE 541) to provide a permanent record of the chamber concentrations.

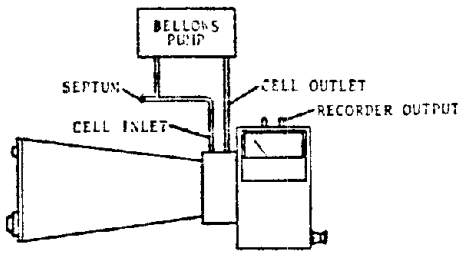
Calibration and Analytical Development

Most chemical compounds have characteristic infra-red spectra which can be used for identification and to quantify the amount present. The infra-red spectrum of 1,1,2,2-tetrachloroethane 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 1,1,2,2-tetrachloroethane were calibrated each day before vapour generation commenced.

The calibration was performed using a closed loop calibration system (see diagram below). Known volumes of 1,1,2,2-tetrachloroethane 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 :	8.3 µm	8.3 µm
Pathlength :	21.75 m	21.75 m
Absorbence Range :	0.1	0.25 A
Slit Width :	0.5	0.5 mm
Meter Response :	10	1
Recorder Voltage :	200 mV	0.5 V
Chart Speed :	600/300 mm/h	600 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^{o}C)
5.64 = Volume of Miran sample chamber (l)

Example of the Calculation for V

Compound: 1,1,2,2-Tetrachloroethane

C = 50 ppm
ρ = 1.5869 g/cm^3
M = 167.86

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

$$= \frac{50 \times 167.86}{1.5869} \times \frac{5.64}{10^3 \times 24.06\ \mu l}$$

$$= 1.24\ \mu l$$

Therefore, to construct a calibration curve to cover the 50 ppm range, 0.2 µl samples of 1,1,2,2-tetrachloroethane were injected into the analyser.

Atmosphere Generation

Schematic diagrams showing the vapour generating apparatus, exposure chambers and monitoring equipment is presented in Figures 1a and 1b. The test atmospheres were produced by bubbling dry, oxygen-free nitrogen (BOC Limited) through a liquid reservoir of 1,1,2,2-tetrachloroethane contained in a glass, gas washing or Drechsel bottle immersed in a temperature controlled water bath at 50°C. The nitrogen/1,1,2,2-tetrachloroethane 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 1,1,2,2-tetrachloroethane/air was ducted through 3/4" 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 6.5 h. In addition, samples were measured from different areas (at least 9) of the animal holding zone to confirm uniformity of 1,1,2,2-tetrachloroethane concentration.

Measurement of Chamber Concentrations

Atmospheric concentrations of 1,1,2,2-tetrachloroethane were monitored continuously during the 7 h exposure period from the breathing zone of the animals. A separate monitoring system was used for each concentration level. Stainless steel sampling lines, fitted with a particulate filter (Whatman Mini-Filter, Grade 80) and positioned on a central reference point in each exposure chamber, were connected to the infra-red gas analysers. The sampling flow rate was approximately 4 l/min.

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

During preliminary analytical development, difficulty was experienced in establishing a constant baseline whilst sampling from the low level exposure chamber. When sampling from the chamber with animals present a positive baseline deflection, from zero gas air, was obtained i.e. there was an increase in absorbence. This showed a steady decline as the humidity within the exposure chamber decreased. A constant "chamber" baseline was obtained once the chamber humidity had stabilised. Negative baseline deflections were obtained when sampling from the chambers without any animals being present, and flushing the chamber with dry, conditioned air only. The chamber humidity at this time was low (<40%). This seems to suggest that at the high instrument sensitivity settings necessary for analyses of such low levels of 1,1,2,2-tetrachloroethane at the selected wavelength (8.3 μm), there is too much interference from water in the chamber atmosphere to allow confidence in the results.

To overcome this problem it was decided that atmosphere generation should not be started until the chamber humidity had stabilised. Measurements of the chamber concentration of tetrachloroethane would then be calculated from this 'chamber baseline'. In addition, an alternative analytical method was developed for use with the 5 ppm 1,1,2,2-tetrachloroethane target concentration. This involved the use of charcoal absorption tubes located in an exit line from the exposure chamber.

Atmosphere Sampling Using Charcoal Tubes

During the 5 day exposure Sipin sampling tubes containing activated charcoal were inserted for specified lengths of time into the exhaust line from the low level chamber. The flow rate through the charcoal tubes was monitored by a rotameter and noted during the sampling periods.

At the end of each day the used tubes were stored in a refrigerator overnight and analysed next morning.

The charcoal tubes contain 2 sections of charcoal separated by a small piece of foam. The purpose of the second section is to remove any of the TCE which has not been adsorbed by the first section.

Treatment of the Charcoal Tubes

Each section of charcoal from each tube was tipped into a separate head space vial (6 ml) and labelled accordingly; the main charcoal being labelled 'a' and the second section 'b', i.e.

tube 1 gave samples 1a and 1b. The vials were cooled in an ice-salt bath.

Carbon disulphide (5 ml) was then added by pipette to the charcoal and the vials were sealed to prevent any significant loss of solvent. The vials were shaken and left for about 30 min before analysis.

Gas Chromatography

Standards

Standards in the appropriate range were prepared by dilutions of a stock w/v solution of 1,1,2,2-tetrachloroethane in carbon disulphide.

Analysis

Analysis was done using a Pye 104 gas chromatograph equipped with a flame ionisation detector.

Column:	18' glass column, 4 mm o.d.
Packing:	10% OV 101 on Diatomite CNAW 80-100 mesh
Oven Temperature:	140°C
Attenuation:	x 200
Carrier gas:	40 ml/min Nitrogen
Hydrogen:	40 ml/min
Air:	300 ml/min
Chart Recorder:	Servoscribe
Chart Speed:	60 cm/h
Injection Volume:	5 µl

Adsorption Efficiency

To prove that the tubes were capable of adsorbing considerably more 1,1,2,2-tetrachloroethane than required, a 100 ppm atmosphere of 1,1,2,2-tetrachloroethane was produced in the closed loop calibration system (CLCS) by straight injection of 2.5 µl liquid 1,1,2,2-tetrachloroethane. This means that the system contains in total 3.96 mg 1,1,2,2-tetrachloroethane. If all this were adsorbed and the tube treated as before, there would be 3.96 mg 1,1,2,2-tetrachloroethane in 5 ml carbon disulphide which gives a 792 µg/ml solution. By comparison with the values in the results table, it can be seen that this is far in excess of the experimental or expected results from the exposure samples.

The exit line from the CLCS pump was cut and an empty glass tube inserted. The system was then zeroed with zero gas air. The 1,1,2,2-tetrachloroethane was then injected into the CLCS and the recorder response noted. The pump was then switched off and the Miran cell isolated. The empty glass tube was replaced by a charcoal tube and the system was reset. The adsorption of the 1,1,2,2-tetrachloroethane onto the charcoal was monitored by a sharp decrease in recorder reading. The baseline returned to zero gas air level in 6 min, showing that all the 1,1,2,2-tetrachloroethane had been adsorbed.

It should also be noted that for all the animal exposure samples the second section of charcoal, i.e. the b samples, contained no detectable 1,1,2,2-tetrachloroethane.

Desorption Efficiency

Two methods were investigated:-

1. A direct injection of 1 µl 1,1,2,2-tetrachloroethane was made into 3 charcoal tubes which were desorbed into carbon disulphide. A recovery of 97% was found.

2. An atmosphere containing 514 µg 1,1,2,2-tetrachloroethane (equivalent to sampling a 5 ppm atmosphere at 1 l/min for 15 min) was generated in the CLCS as adapted for the adsorption efficiency experiment. The adsorption of the 1,1,2,2-tetrachloroethane was monitored on the Miran recorder. When apparently all the 1,1,2,2-tetrachloroethane had been adsorbed and the baseline had steadied at the previous zero gas air level the charcoal tube was removed and retained for analysis. This experiment was repeated 8 times. The contents of the tubes were then analysed by gas chromatography as described previously. The Miran was calibrated using 0.1 µl samples of 1,1,2,2-tetrachloroethane and the actual response of each of the above test atmospheres, measured before insertion of the charcoal tubes. The actual concentration of 1,1,2,2-tetrachloroethane in the tubes was calculated from the Miran calibration.

The percentage recovery was calculated as follows:

$$100 \times \frac{\text{The amount of TCE in } CS_2 \text{ solution } (\mu g/ml)}{\text{Calculated amount of TCE in } CS_2 \text{ solution } (\mu g/ml) \text{ from Miran Calibration}}$$

= 68.3%
Standard deviation = 8.3%
TCE = 1,1,2,2-tetrachloroethane

This value is considerably different from that obtained in 1.

As the conditions in this method reproduced as closely as possible the experimental conditions it is suggested that the recovery is 68.3%.

It must be noted that these desorption experiments were carried out under ambient conditions. The effect of a humid atmosphere (such as exists in the chambers) on the adsorption efficiency of the charcoal is not known.

Test Compound Utilisation

At the beginning of each exposure day, the 1,1,2,2-tetrachloroethane reservoir (a Drechsel bottle) was replenished with test compound. Utilisation of test material was calculated on a daily basis by weighing the bottle before vapour generation began and deducting the weight of the bottle and remaining test compound on completion of the exposure period.

Exposure Procedure

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

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

Animals exposed to 1,1,2,2-tetrachloroethane 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, multi-dose cytogenetic 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	100 mg/kg for 5 consecutive days.

UNSCHEDULED DNA SYNTHESIS ASSAY

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

Chemicals

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

6-[^{3}H]-thymidine (21 Ci/mmol) and 8-[^{3}H]-deoxyguanosine (26.4 Ci/mmol) were obtained from the Radiochemical Centre, Amersham, England.

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

Test Solutions

The test compound and positive controls were dissolved in dimethylsulphoxide ("AnalaR" grade from 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 or Flow 2,002), passage 12-35 obtained from Flow Laboratories, Irvine, Scotland. These cell lines were chosen because of their higher permeability to some substrates than certain other human cell lines tested. Flow 2,002 line was used in Method 2 because the growth characteristics of Flow 11,000 had deteriorated to such an extent that they are no longer usable for these studies.

Culture Maintenance and Growth Media

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

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

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

Animals

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

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

Preparation of the 9,000 g Supernatant Fluid from Livers

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

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

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

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

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

co-factor solution	9 parts
liver preparation	1 part

Preliminary Toxicity Test

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

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

The compound was dissolved in dimethylsulphoxide, at concentrations of 0.01, 0.1, 1.0, 10.0 and 100 mg/ml and 10 µl samples were added to duplicate cell suspensions to give final concentrations of 0.1, 1.0, 10, 100 and 1,000 µg/ml. To each control culture was added 10 µl of dimethyl-sulphoxide.

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

In fact no toxicity was observed, even at a concentration of 10,000 µg/ml. Consequently, it was decided that a concentration of 15,869 µg/ml (i.e. 10 µl/ml) should be tried as the highest dose in the UDS experiments.

DNA Repair Assay (Method 1)

The cells were harvested, sedimented, suspended in fresh culture medium at a density of 5 x 10^4 cells/ml and 2 ml samples of this suspension were pipetted into 35 mm tissue culture Petri dishes containing 3 sterile coverslips (Lux Scientific Corporation, California, U.S.A.). These were then incubated at 37°C in a humid atmosphere of 5% CO_2 in air for 72 h. The medium from each of the dishes was then replaced with 2 ml of arginine-deficient DMEM supplemented with 5% heat inactivated foetal bovine serum and the plates incubated for 24 h. The medium was then replaced with a further 2 ml of arginine-deficient DMEM and the incubation continued for a further 48 h. At the end of this time the cultures were divided into 2 groups and 100 µl of S-9 mix added to one of them. Solutions of hydroxyurea (250 mM) in sterile distilled water and 6-[^{3}H]-thymidine (21 Ci/mmol) were added to each culture giving final concentrations of 2.5 mM and 10 µCi/ml respectively. 1,1,2,2-Tetrachloroethane was added directly to the medium or dissolved in dimethylsulphoxide and dilutions were made from this to give solutions containing 124, 248, 496, 992, 1,984, 3,967, 7,935 and 15,859 µg/ml. 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	8 µg and 10 µg/ml
2-AAN	5 µg and 40 µg/ml

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

Autoradiography

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

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

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

Quantification of Repair Synthesis

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

DNA Repair Assay (Method 2)

Flow 11,000 or 2,002 cells were harvested, sedimented and suspended in fresh culture medium at a density of 5×10^4 cells/ml. 5 ml samples were dispensed into 60 mm tissue culture Petri dishes (Nunclon Delta) which were incubated in a humid atmosphere of 5% CO_2 in air at 37°C for 72 h. The medium from each of the Petri dishes was then replaced with 5 ml of arginine-deficient medium supplemented with 5% heat inactivated foetal bovine serum and the dishes incubated for 24 h. The medium was then replaced with a further 5 ml of arginine-deficient DMEM and the incubation continued for a further 48 h. The dishes were then randomly divided into

2 groups and 500 µl of S-9 mix were added to one group. Solutions of hydroxyurea and [^{3}H]-deoxyguanosine (26 Ci/mmol) were added to each dish giving final concentrations 2.5 mM and 10 µCi/ml respectively.

The positive control compounds, 4-nitroquinoline-N-oxide, for S-9 free cultures, and 2-aminoanthracene, for S-9 supplemented cultures, were dissolved in dimethylsulphoxide and diluted 1:100 in the culture medium to give final concentrations of 1 µg/ml.

10 µl of 1,1,2,2-tetrachloroethane in DMSO, at a concentration of 1.587 mg/ml, was added to the cultures, both in the presence and absence of S-9 mix, to give a final concentration of 15.87 µg/ml.

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

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

Incorporation of Label in the Presence of S-9 Mix

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

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

CYTOGENETIC ANALYSIS OF RAT BONE MARROW CELLS

Metaphase Cell Preparations

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

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

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

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

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

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

Slide Reading

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

Wherever possible, for each animal 50 cells with a minimum of 41 well spread chromosomes were examined and scored. The location of all spreads examined were 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 11 May 1979) by neck dislocation.

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

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

Assessment

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

The following types of sperm were not scored:

(1) separated tails and heads.

(2) clumps of sperm.

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

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

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

I Normal

II Abnormal

A. hook upturned or elongated.

B. banana-shaped head.

C. amorphous head.

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

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

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

SEX-LINKED RECESSIVE LETHAL TEST IN DROSOPHILA MELANOGASTER

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

Strains

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

The Müller-5 (M-5) flies had the basc balancer X-chromosome, $In(1)\ sc^{S1L}\ sc^{8R} + s\ sc^{S1}\ sc^{8}\ w^{aB}$.

Medium

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

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

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

Exposures

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

*Nipogen: bacteriostatic agent (BDH Limited).

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

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

Toxicity Test

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

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

Recessive Lethal Test

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

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

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

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

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

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

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

STATISTICAL EVALUATION

Cytogenetics Tests

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

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

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

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

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

Dominant Lethal Assay

The variates analysed were:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Sperm Abnormalities Test

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

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

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

A one-sided t test was used on the transformed values. This analysis was performed on (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	0.06

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-2 and 3. The reproducibility of the calibration curve data from day to day is good.

Calibration ranges adopted were 2.06-12.36 ppm (5 ppm target concentration) and 8.25-66.0 ppm (50 ppm target concentration).

Chamber Atmospheres - Homogeneity

Prior to exposure of the animals, the chamber atmospheres were sampled at different positions to establish that adequate mixing of 1,1,2,2-tetrachloroethane was occurring. The results are shown in Table AT-4, where it can be seen that the maximum deviations encountered were +5% at the 5 ppm target concentration and +1% at the 50 ppm target concentration.

Chamber Atmospheres - Achieved Concentrations

A sample chart record taken during a day on which animals were exposed is shown in Figure 3. From charts such as this, deviations from the target concentrations of 5 ppm and 50 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.

Because of the interference of 1,1,2,2-tetrachloroethane absorption by water vapour, the 5 ppm exposure level results required special verification. This was provided by back-up g.c. analyses of the test atmospheres, as described in the Materials and Methods section. The results of these analyses are presented in Table AT-7. The sampling technique allowed a recovery efficiency of about 68% (see Table AT-1). Using this figure as a basis for calculations, the maximum deviation from the 5 ppm target concentration was -1.0 ppm (-20%) in the single exposure test and -1.5 ppm (-30%) on Day 2 of the multiple exposure test. It seemed unlikely that a significant improvement was obtainable, so, the experiment was 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 the typical examples of exposure location sheets as used during the study.

Pre-experimental Acceptance Tests (PEAT)

23 March 1979 Delivery: Ten male and 10 female rats and 4 male and 4 female mice were haphazardly selected for PEAT. In the mice there were no significant clinical observations, autopsy findings, microbiological or parasitological findings. Histologically, in 2 mice there were occasional, small peribronchial foci of lymphocytes. None of the rats showed clinical signs of infection or disease and there were no parasitological or microbiological findings.

At autopsy, a female rat showed emphysemic patches on the lungs. Irregular areas of congestion were seen on the kidneys of 6 male rats, while a 2 mm white nodule was seen on a kidney of another male rat.

Histologically, there were peribronchial foci of lymphocytes in 4/10 males and 4/10 females. Peribronchial and periportal lymphocytic foci were observed in 4/10 males and 5/10 females. The remaining female showed, besides peribronchial lymphocytic foci, a small area of interstitial nephritis. Of 2 remaining males one was without any signs of infection while the other showed peribronchial lymphocytic foci and a fine scar in the kidney.

Clinical Observations and Body Weights

No clinical signs of toxicity were observed which were considered to be due to exposure to 1,1,2,2-tetrachloroethane.

On the day prior to dosing, during the dosing period and during Week 3 of the dominant lethal test matings, some animals from all treatment groups were sneezing and had sub-lingual swellings. This condition was diagnosed as sialodacryoadenitis.

During the first week of the dominant lethal test matings, the 2 female rats mated with 375♂ had inflamed eyes. A female mated with 384♂ (50 ppm atmosphere exposure group) was found dead on 13 April 1979 (Week 3). Its lungs were severely congested and it was not pregnant. Another female, this time mated with 400♂ (ethyl methanesulphonate control group) was found dead on 17 June 1979 (Week 10). Its lungs were congested and the animal was not pregnant.

Sneezing was evident in some rats throughout the dominant lethal test. In such a test, there are no opportunities for isolation of animals, since virgin females have continually to be introduced. Once an infection is established, therefore, it is possible to infect the incoming animals which may be initially free from the responsible organism.

Body weight values of the CD rats and B6C3F$_1$ mice taken at the time of dosing are given in Tables BW-1 to 4 and Appendix Tables BW-1 to 4. Exposure to 5 or 50 ppm 1,1,2,2-tetrachloroethane atmospheres for 7 h/day on 5 consecutive days had no deleterious effects upon body weight gain. Dosing animals with ethyl methanesulphonate at 100 mg/kg/day caused substantial weight losses in the rats, but not in the mice.

UNSCHEDULED DNA SYNTHESIS ASSAY

In the initial 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 1,1,2,2-tetrachloroethane (Tables UDS-1 and 2). The experiment was partially repeated in the presence of S-9 mix because, in the first experiment, cells were killed when exposed to 1,1,2,2-tetrachloroethane concentrations in excess of 250 µg/ml. In the absence of S-9 mix, concentrations up to 15,869 µg/ml were possible without obvious signs of toxicity.

Treatment with 4-nitroquinoline-N-oxide at concentrations of 8 or 10 µg/ml gave a mean grain count of >100 per nucleus. Responses to 2-aminoanthracene, however, were variable and less satisfactory.

The tritiated deoxyguanosine incorporation assay was used to confirm the results of the first assay. During the course of these experiments, the permeability of both cell lines to deoxyguanosine decreased greatly, this reduction being aggravated by the addition of S-9 mix to the incubation medium. Consequently, the measured incorporation of radioactivity was insufficient to provide any reasonable analysis of data produced in the presence of S-9 mix. (A more detailed description of these results is being reported separately.) Results obtained in the absence of S-9 mix are given in Figure 4. There was no indication of deoxyguanosine incorporation into DNA.

CYTOGENETIC ANALYSIS OF RAT BONE MARROW CELLS

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

In the multiple exposure cytogenetic test, there were no indications of induction of chromosomal damage in either the male or female rats exposed to 5 ppm or 50 ppm 1,1,2,2-tetrachloroethane atmospheres. Responses to the positive control substance, ethyl methanesulphonate, were significant in both the females and in the males.

In the single exposure rats treated with ethyl methanesulphonate, the frequencies of all aberrations were increased in males at the 6 h and 24 h sample times and in females at the 24 h sample time only. When gaps were excluded from the computations, there were no increases in the males and, in the females, any increase in aberration frequency was restricted to the 6 h sample time.

The only significant increase in a 1,1,2,2-tetrachloroethane treated group was in females at the 6 h sample time, when aberrations excluding gaps were more frequent in the 50 ppm atmosphere exposure group. Robertsonian translocations* were particularly common in this group. This result perhaps suggests an effect due to 1,1,2,2-tetrachloroethane, but it was not seen also in the male rats either at the 6 h sample time or at any other time. Male and female rat slides were assessed by the same cytogeneticist. The possibility of a sex difference in the metabolism of the compound cannot be discounted on the available evidence. It is recommended, therefore, that this compound be re-examined for its clastogenic potential, particularly as no signs of toxicity were observed.

*Robertsonian translocations: two acrocentric chromosomes fuse to form a metacentric chromosome.

DOMINANT LETHAL TEST

Data are given in Tables DL-1 to 10 and Appendix Tables DL.

Pregnancy frequency was calculated in 2 ways; firstly, by considering as pregnant females with corpora lutea graviditatis (Table DL-1) and, secondly and more reliably, by considering as pregnant only females with implantations (Table DL-2). With neither method was there observed any effect upon pregnancy frequency due to 1,1,2,2-tetrachloroethane treatment, but there were reductions in Weeks 2 and 3 in the positive control groups. The 70% frequency recorded in Table DL-2 for Week 1, 5 ppm exposure group, is clearly not treatment related.

Corpora lutea graviditatis counts (Table DL-3) were not reduced in either of the 1,1,2,2-tetrachloroethane treated groups except in Week 1 of the 5 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 1,1,2,2-tetrachloroethane treatment, but were reduced in Weeks 1-4 of the positive control group. In the 5 ppm atmosphere group there was a reduction in Week 7 and increases in Weeks 1 and 10, but these variations are probably not treatment related.

The frequencies of live implantations (Table DL-5) and live implantations + late deaths (Table DL-6) followed very closely the pattern of total implantations per pregnancy. An exception was in Week 1 of the 5 ppm 1,1,2,2-tetrachloroethane exposure group where live implantations and live implantations + late deaths frequencies were low compared with the other groups.

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 1,1,2,2-tetrachloroethane treated groups, when compared with the air control group.

Analysis of the proportions of early deaths by various statistical methods (Tables DL-8 to 10) did not indicate any effects attributable to 1,1,2,2-tetrachloroethane treatment.

The proportion of early deaths was increased in Week 1 of the 5 ppm atmosphere group, but not in the 50 ppm atmosphere group.

It is considered that the high, sporadic frequencies - totally unrelated to treatment - of early deaths may have been due to the effects of the sialodacryoadenitis virus, clinical signs of which were definitely seen in Week 3 and may have been present earlier (see page 45).

SPERM ABNORMALITY TEST

Results of the sperm abnormality test are given in Tables SA-1 and 2 and Appendix Table SA. One mouse in the 5 ppm 1,1,2,2-tetrachloroethane atmosphere exposure group had very high proportions of various abnormalities (No. 337♂). This clearly abnormal and atypical animal was not taken into consideration when assessing the possible effects of the test compound.

There was a statistically significant increase in the frequency of Category A sperm (hook up-turned or hook elongated) in specimens taken from mice exposed to 50 ppm 1,1,2,2-tetrachloroethane. The increase over the concurrent control value was x 2.4, but it was, nevertheless, within the range normally expected of a control group.

Apart from this small increase of doubtful biological significance, there were no increases in the frequencies of abnormal sperm.

SEX-LINKED RECESSIVE LETHAL TEST IN DROSOPHILA

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

A dose ranging experiment was undertaken on 28 March 1979 in which flies were exposed to 5 ppm 1,1,2,2-tetrachloroethane for 1 h, 3 h or 7 h or to 50 ppm 1,1,2,2-tetrachloroethane for 10 min, 1 h, 2 h or 2.75 h. At 5 ppm 1,1,2,2-tetrachloroethane, there were no obvious signs of toxicity, whereas at 50 ppm 1,1,2,2-tetrachloroethane approximately 30% of the flies were sedated within 5 min of the beginning of exposure. All flies were sedated within 30 min.

Flies exposed for only 10 min were fully recovered within 2 min when in an air only atmosphere. Following exposing for 1 h, recovery was complete within 50 min and following exposing for 2.75 h recovery was complete within 1.75 h.

On the basis of these results, exposure conditions chosen for the main study on 9 April 1979 were:

> 5 ppm 1,1,2,2-tetrachloroethane for 7 h continuously, or 50 ppm 1,1,2,2-tetrachloroethane for 7 h in a total time of 8 h (chambers flushed with air only approximately every 45 min).

Two breeding stocks (A and B) were exposed (Table RL-2) in the main test. In the 5 ppm 1,1,2,2-tetrachloroethane exposure groups there were no signs of toxicity, but in the 50 ppm 1,1,2,2-tetrachloroethane exposure groups 80% of the flies were sedated after 40 min. After 1 h all flies were sedated. At the end of the exposure period, fly chambers were flushed with air. Sporadic movements were seen after 15 min and activity was generally increased after 1 h.

Analysis for SLRL revealed no effects which could be treatment related. At the 5 ppm atmosphere level, Stock A Brood 2 flies had 3 lethals (0.82%) which is suggestive of an effect, but was not seen in Stock B flies or in either Stock A or B following exposure to 50 ppm 1,1,2,2-tetrachloroethane.

It appeared that 1,1,2,2-tetrachloroethane had no effect upon the SLRL frequency of Drosophila.

CONCLUSIONS

In the UDS assay, only that portion of the experiment which involves incorporation of tritiated thymidine was completed. The concentrations of 1,1,2,2-tetrachloroethane used extended into the toxic range, but no increases in thymidine incorporation were noted. Hence, the compound was tested to the practical limits of the assay without any evidence accruing of DNA damage.

The mammalian *in vivo* assays were conducted at concentrations which did not extend into the toxic range. The *Drosophila* test exposure conditions certainly sedated the flies, but survival was not affected. It cannot be claimed therefore, that 1,1,2,2-tetrachloroethane has been tested to the practical limits of these assays.

Nevertheless, in 2 of these assays there were signs that the compound may possess some genotoxic potential. In the sperm abnormality test there was a statistically significant increase in sperm with hook abnormalities although the biological significance of such a small increase was doubtful. Also, in the 6 h sample of the cytogenetic assay, following a single 7 h exposure to 50 ppm, there was a small increase in aberrant cell frequency in female rats.

Neither of these results is convincing evidence of genotoxicity and it appears that the situation can be completely resolved only by further testing.

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TABLE AT-1

1,1,2,2-Tetrachloroethane
Results of the Desorption Efficiency Experiments using
the Miran Close Loop Calibration System

Run Number	Volume TCE Injected into Miran (µl)	Concentration in CS_2 Solution		Percentage Desorption Efficiency
		Theoretical From Miran Deflection µg/ml	Found µg/ml	
1	0.30	98.7	54.0	54.7
2	0.32	104.1	72.5	69.6
3	0.34	113.3	78.5	69.2
4	0.34	114.9	80.5	70.1
5	0.34	114.9	82.0	71.3
6	0.34	119.7	83.0	69.3
7	0.34	124.9	85.0	68.1
8	0.34	115.7	86.0	74.0

Mean and standard deviation value of desorption efficiency = 68.3% ± 5.8%

TABLE AT-2

1,1,2,2-Tetrachloroethane
Calibration Data for Low Level

Dose Level: 5 ppm v/v

Batch No.: 11209

Volume µl	Conc., ppm, (v/v)	Cumulative Chart Deflection, mm						
		28 March 1979	2 April 1979	3 April 1979	4 April 1979	5 April 1979	6 April 1979	9 April 1979
0	0	0	0	0	0	0	0	0
0.05	2.06	21.0	22.0	22.0	21.0	22.0	20.0	20.0
0.10	4.12	41.0	43.0	40.0	43.0	44.0	40.0	38.0
0.15	6.18	62.5	63.0	60.0	62.0	68.0	59.0	60.0
0.20	8.24	86.0	84.0	77.0	85.5	90.0	79.0	81.0
0.25	10.30	108.0	104.0	100.0	109.0	112.0	100.0	98.0
0.30	12.36	126.0	127.0	125.0	129.0	132.0	120.0	120.0
Chart deflection (mm) for 5 ppm		52.0	51.5	49.0	52.5	55.0	48.0	48.0

Instrument Setting

Pathlength: 21.75 m
Wavelength: 8.3 µm
Absorbence Range: 0.1
Slit Width: 0.5 mm
Recorder Voltage: 200 mV
Chart Speed: 600/300 mm/min

Calibration

Syringe: 1.0 µl Hamilton
Injection Volume: 0.05 µl
No. of Repeat Injections: x6

TABLE AT-3

1,1,2,2-Tetrachloroethane
Calibration Data for High Level

Dose Level: 50 ppm v/v

Batch No.: 11209

Volume µl	Conc., ppm, (v/v)	Cumulative Chart Deflection, mm						
		28 March 1979	2 April 1979	3 April 1979	4 April 1979	5 April 1979	6 April 1979	9 April 1979
0	0	0	0	0	0	0	0	0
0.2	8.25	23.0	22.0	26.0	23.0	24.0	23.0	24.0
0.4	16.5	46.5	45.0	49.0	47.0	48.0	47.0	48.0
0.6	24.75	70.0	69.0	72.0	70.0	71.0	70.0	72.0
0.8	33.0	93.0	92.0	93.0	93.0	94.0	92.0	93.5
1.0	41.25	115.5	114.0	114.0	115.0	115.0	114.0	116.0
1.2	49.5	138.0	136.0	135.0	137.0	137.5	137.0	138.0
1.4	57.75	159.5	157.5	153.0	159.0	159.0	160.0	159.0
1.6	66.0	181.0	179.0	177.0	182.0	179.0	181.0	179.0
Chart deflection (mm) for 50 ppm		138.0	136.0	137.0	138.0	139.0	138.0	140.0

Instrument Setting

Pathlength: 21.75 m
Wavelength: 8.3 µm
Absorbence Range: 0.25 A
Slit Width: 0.5 mm
Recorder Voltage: 0.5 V
Chart Speed: 600 mm/min

Calibration

Syringe: 1 µl Hamilton
Injection Volume: 0.2 µl
No. of Repeat Injections: x8

TABLE AT-4

1,1,2,2-Tetrachloroethane
Chamber Atmosphere Homogeneity Data

Dose Level: 5 ppm and 50 ppm

Concentration / Sample Location	% Deviation from Reference Sampling Point	
	Low	High
Reference Point (R)	0	0
Right Centre (RC)	+2.5	+1.0
Right Front (RF)	+5.0	0
Centre Front (CF)	0	+0.5
Left Front (LF)	-2.5	+0.5
Left Centre (LC)	0	+1.0
Left Back (LB)	0	0
Centre Back (CB)	0	0
Right Back (RB)	0	+0.5

Top view of exposure chamber

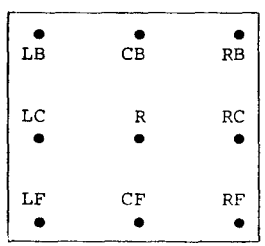


TABLE AT-5

1,1,2,2-Tetrachloroethane
Atmospheric Analysis by Infra-red Spectroscopy
Target Concentration 5 ppm

Date	Exposure Day	% Deviation from Target Concentration in Minutes									Time Averaged Concentration for 7 h (ppm)
		-15	-15	-10	-5	0	+5	+10	+15	+15	
28 March 1979	Single	34	36	86	78	92	36	28	4	20	4.8
2 April 1979	Multiple 1	-	-	-	5	55	55	45	95	165	5.8
3 April 1979	Multiple 2	-	-	-	120	105	115	80	-	-	5.1
4 April 1979	Multiple 3	60	90	70	105	35	55	5	-	-	4.6
5 April 1979	Multiple 4	-	-	10	5	75	35	75	70	150	5.6
6 April 1979	Multiple 5	60	145	110	25	20	35	20	5	-	4.5

TABLE AT-5 (continued)

1,1,2,2-Tetrachloroethane
Target Concentration 50 ppm

Date	Exposure Day	% Deviation from Target Concentration in Minutes							Time Averaged Concentration for 7 h (ppm)
		-7.5	-5	-2.5	0	+2.5	+5	+7.5	
28 March 1979	Single	-	-	80	250	90	-	-	50.0
2 April 1979	Multiple 1	-	10	-	185	210	15	-	51.6
3 April 1979	Multiple 2	2	25	35	158	140	35	25	50.6
4 April 1979	Multiple 3	-	15	80	265	60	-	-	49.9
5 April 1979	Multiple 4	-	-	115	165	95	45	-	50.2
6 April 1979	Multiple 5	10	35	105	215	55	-	-	49.6

TABLE AT-6

1,1,2,2-Tetrachloroethane
Atmospheric Analysis by Infra-red Spectroscopy
Target Concentrations 5 ppm and 50 ppm

Exposure Day	Target Concentration (ppm)	% Deviation from Target Concentration in Minutes										Total Time Exposed (h)	Time Averaged Concentration (ppm)
		-5.0	-2.5	0	+2.5	+5.0	+7.5	+10	+15	+20	+25		
9 April 1979	5	-	-	75	10	95	30	90	60	40	20	7	5.5
	50	-	50 (2)	296 (50)	74 (6)	-	-	-	-	-	-	⊛ 7(58)	50.1

⊛ Values in parenthesis indicate total time (min) during which Drosophila were exposed to fresh air

TABLE AT-7

1,1,2,2-Tetrachloroethane
Analysis of Charcoal Tubes

Date Sampled	Charcoal Tube Sample	Flow Rate l/min	Time Start/ Finish	TCE Conc. µg/ml CS_2	Conc. TCE in Atmosphere Assuming 100% Recovery	Atmosphere Assuming 68% Recovery	Mean
28 March 1979	1a	1	15.29 15.59	113	2.7	4.0	
	b			0			
	2a	1	16.05 16.36	120	2.9	4.2	
	b			0			
	3a	1	16.36 17.06	113	2.7	4.0	4.1 ppm
	b			0			
	4a	1	17.06 17.36	115	2.8	4.7	
	b			0			
	5a	1	18.20 18.50	113	2.7	4.5	
	b			0			
2 April 1979	1a	1	15.33 16.03	140	3.4	5.0	
	b			0			
	2a	1	16.25 16.55	178	4.3	6.2	
	b			0			
	3a	1	17.00 17.30	183	4.4	6.4	5.9 ppm
	b			0			
	4a	1	17.30 18.00	165	4.0	5.8	
	b			0			
	5a	1	18.00 18.30	175	4.2	6.1	
	b			0			
3 April 1979	1a	1	12.00 12.15	51	2.4	3.5	
	b			0			
	2a	1	13.00 13.15	52	2.5	3.7	
	b			0			
	3a	1	14.00 15.15	68	3.3	4.8	
	b			0			
	4a	1	14.30 14.45	60	2.9	4.2	
	b			0			4.6 ppm
	5a	1	15.10 15.25	67	3.2	4.7	
	b			0			
	6a	1	16.00 16.15	67	3.2	4.7	
	b			0			
	7a	1	16.15 16.35	75	3.6	5.2	
	b			0			
	8a	1	17.00 17.15	88	4.2	6.1	
	b			0			

TABLE AT-7 (continued)

1,1,2,2-Tetrachloroethane

Date Sampled	Charcoal Tube Sample	Flow Rate l/min	Time Start/ Finish	TCE Conc. µg/ml CS_2	Conc. TCE in Atmosphere Assuming 100% Recovery	Atmosphere Assuming 68% Recovery	Mean
4 April 1979	1a	1	11.0 11.15	68	3.3	4.8	
	b			0			
	2a	1	12.00 12.15	65	3.1	4.5	
	b			0			
	3a	1	13.00 13.15	54	2.6	3.8	
	b			0			
	4a	1	14.05 14.20	67	3.2	4.7	
	b			0			4.4 ppm
	5a	1	15.05 15.20	68	3.3	4.8	
	b			0			
	6a	1	16.05 16.20	55	2.6	3.8	
	b			0			
	7a	1-1.2	16.20 16.50	132	3.2	4.7	
	b			0			
	8a	0.75-1	17.00 17.50	58	2.8	4.1	
	b			0			
5 April 1979	1a	1	10.10 10.25	54	2.6	3.8	
	b			0			
	2a	1	11.10 11.30	97	3.5	5.1	
	b			0			
	3a	1	12.05 12.20	74	3.6	5.2	
	b			0			
	4a	1	13.10 13.25	74	3.6	5.2	5.1 ppm
	b			0			
	5a	1	14.20 14.35	82	3.9	5.7	
	b			0			
	6a	1	15.10 15.25	79	3.8	5.5	
	b			0			
	7a	1	16.01 16.18	85.5	3.7	5.4	
	b			0			

TABLE AT-7 (continued)

1,1,2,2-Tetrachloroethane

Date Sampled	Charcoal Tube Sample	Flow Rate l/min	Time Start/ Finish	TCE Conc. µg/ml CS_2	Conc. TCE in Atmosphere Assuming 100% Recovery	Atmosphere Assuming 68% Recovery	Mean
6 April 1979	1a	1	10.15 10.30	45	2.2	3.2	
	b			0			
	2a	1	11.14 11.34	49	2.4	3.5	
	b			0			
	3a	1	12.15 12.30	35	1.7	2.5	
	b			0			
	4a	1	13.20 13.35	45	2.2	3.2	3.1 ppm
	b			0			
	5a	1	14.15 14.30	44	2.1	3.1	
	b			0			
	6a	1	15.15 15.30	43	2.1	3.1	
	b			0			
	7a	1	16.05 16.20	41.5	2.0	3.0	
	b			0			
9 April 1979	1a	1	15.14 15.29	58	2.8	4.1	
	b			0			
	2a	1	16.01 16.21	63	2.3	3.4	
	b			0			
	3a	1	17.06 17.21	59	2.8	4.1	
	b			0			4.0 ppm
	4a	1	18.10 18.25	54	2.6	3.8	
	b			0			
	5a	1	19.10 19.25	58	2.8	4.1	
	b			0			
	6a	1	20.10 20.35	104	3.0	4.4	
	b			0			

TABLE BW-1

1,1,2,2-Tetrachloroethane
Multiple Exposure Cytogenetics Test
Group Mean Body Weights (g) for the Dosing Period of
Male and Female CD Rats

Sex	Day	Air Control (0 ppm)	5 ppm	50 ppm	5 x 100 mg/kg EMS
Male	1	337.3 ± 32.8	317.2 ± 42.2	332.6 ± 26.3	327.5 ± 19.4
	2	337.7 ± 35.3	318.7 ± 42.8	336.0 ± 27.2	322.8 ± 21.4
	3	342.8 ± 35.0	322.8 ± 44.2	339.2 ± 29.0	308.9 ± 20.6
	4	350.6 ± 35.0	332.3 ± 43.3	344.2 ± 28.7	301.7 ± 20.1
	5	353.6 ± 36.3	334.2 ± 43.0	347.9 ± 28.5	293.9 ± 19.1
	Weight gain /loss	16.3	17.0	15.3	-33.6
Female	1	209.8 ± 18.7	210.3 ± 14.6	213.9 ± 12.8	214.4 ± 19.2
	2	211.4 ± 18.9	208.4 ± 15.5	212.5 ± 15.6	209.2 ± 18.0
	3	211.2 ± 17.1	208.3 ± 16.2	215.2 ± 15.6	195.7 ± 18.5
	4	213.2 ± 17.8	226.2 ± 28.6	215.8 ± 15.1	189.5 ± 18.9
	5	215.3 ± 17.2	214.6 ± 16.3	218.7 ± 15.2	185.0 ± 20.6
	Weight gain /loss	5.5	4.3	4.8	-29.4

TABLE BW-2

1,1,2,2-Tetrachloroethane
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)	5 ppm	50 ppm	250 mg/kg EMS
Male	6	309.5 ± 24.4	313.2 ± 25.3	306.9 ± 20.2	311.7 ± 18.3
	24	311.1 ± 25.7	318.9 ± 31.8	317.2 ± 21.2	307.0 ± 19.5
	48	316.0 ± 20.6	311.7 ± 28.4	302.1 ± 20.8	328.6 ± 27.6
Female	6	197.3 ± 12.6	206.2 ± 11.8	208.8 ± 13.7	208.8 ± 14.8
	24	204.3 ± 9.6	204.2 ± 11.4	204.9 ± 13.1	204.1 ± 7.5
	48	207.1 ± 15.6	212.7 ± 13.8	199.5 ± 14.2	210.6 ± 12.2

TABLE BW-3

1,1,2,2-Tetrachloroethane
Dominant Lethal Assay
Group Mean Dosing Weights (g) for the Dosing Period of Male CD Rats

Day	Air Control (0 ppm)	5 ppm	50 ppm	5 x 100 mg/kg EMS
1	329.5 ± 30.6	330.2 ± 13.9	323.4 ± 12.1	328.9 ± 20.6
2	332.2 ± 27.2	332.7 ± 17.5	327.6 ± 13.5	314.8 ± 23.7
3	338.8 ± 26.4	335.9 ± 16.5	333.1 ± 13.5	299.5 ± 25.1
4	344.0 ± 29.8	346.2 ± 14.9	338.2 ± 13.1	287.1 ± 25.3
5	346.5 ± 28.2	351.8 ± 14.0	343.3 ± 13.1	275.5 ± 24.5
Weight gain/loss	17.0	21.6	19.9	-53.4

TABLE BW-4

1,1,2,2-Tetrachloroethane
Sperm Abnormalities Test
Group Mean Body Weights (g) for the Dosing Period of Male B6C3F$_1$ Mice

Day	Air Control (0 ppm)	5 ppm	50 ppm	5 x 100 mg/kg EMS
1	24.8 ± 1.1	24.0 ± 0.7	24.4 ± 0.7	24.9 ± 1.1
2	25.2 ± 0.8	24.4 ± 0.8	24.9 ± 0.9	25.1 ± 1.0
3	24.9 ± 1.0	23.9 ± 0.9	24.8 ± 0.6	25.3 ± 1.2
4	24.7 ± 0.9	25.0 ± 0.8	25.0 ± 1.1	25.5 ± 1.1
5	25.7 ± 0.9	25.5 ± 1.2	25.3 ± 1.1	25.6 ± 1.3
Weight gain/loss	0.9	1.5	0.9	0.7

TABLE UDS-1

1,1,2,2-Tetrachloroethane
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	1%	1%	3.6 ± 9.6	13.1 ± 6.5
4-Nitroquinoline-N-oxide	-	10	-	>100
	-	8	-	>100
2-Aminoanthracene	40	-	4.3 ± 3.8	-
	5	-	78.9 ± 46.3	-
1,1,2,2-Tetrachloroethane	8	124	1.2 ± 1.1	1.9 ± 1.5
	16	248	2.3 ± 2.4	0.8 ± 1.0
	31	496	4.2 ± 2.5	16.6 ± 5.0
	63	992	4.5 ± 2.8	16.9 ± 5.3
	125	1,984	6.5 ± 4.4	14.0 ± 4.8
	250	3,967	5.0 ± 7.6	17.1 ± 5.8
	-	7,935	-	9.3 ± 4.6
	-	15,869	-	7.2 ± 3.0

TABLE UDS-2

1,1,2,2-Tetrachloroethane
Unscheduled DNA Synthesis

Group	Mean Number of Grains/Nucleus ± S.D.	
	with S-9 Mix	
Dimethylsulphoxide	1%	1.5 ± 2.5
2-Aminoanthracene	50 µg/ml	18.1 ± 8.6
1,1,2,2-Tetrachloroethane	1.3 µg/ml	2.5 ± 1.9
	2.5 µg/ml	2.5 ± 1.8
	5.0 µg/ml	1.7 ± 1.7
	10.0 µg/ml	0.7 ± 1.2

TABLE CA-MD-M-1

1,1,2,2-Tetrachloroethane
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	20	2	1	6	-	-	-
5 ppm, 7 h/day	500	20	2	-	9	1	1	-
50 ppm, 7 h/day	500	22	-	-	7	-	-	-
EMS, 100 mg/kg/day	500	42	11	1	7	-	-	1 Multi Aberration 1 Chromosomal Fragment 3 Exchanges

TABLE CA-MD-M-2

1,1,2,2-Tetrachloroethane
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	0.471	0.063		0.191	0.046	
5 ppm	0.514	0.063	0.49	0.220	0.046	0.45
50 ppm	0.495	0.063	0.27	0.141	0.046	-0.77
EMS, 100 mg/kg	0.660	0.063	2.11**	0.311	0.046	1.84*

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

*P<0.050

**P<0.025

TABLE CA-MD-F-1

1,1,2,2-Tetrachloroethane
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	29	-	-	4	-	-	3 Chromosomal Fragments
5 ppm, 7 h/day	500	24	1	-	2	-	-	-
50 ppm, 7 h/day	500	17	-	-	5	-	-	1 Chromosomal Fragment
EMS, 100 mg/kg/day	500	45	9	-	7	-	1	1 Chromatid Fragment 1 Chromosomal Fragment 1 Exchange 1 Dicentric

TABLE CA-MD-F-2

1,1,2,2-Tetrachloroethane
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	0.560	0.059		0.200	0.037	
5 ppm	0.469	0.059	-1.08	0.160	0.037	-0.76
50 ppm	0.439	0.059	-1.45	0.160	0.037	-0.76
EMS, 100 mg/kg	0.667	0.059	1.28	0.330	0.037	2.45

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

TABLE CA-M6-1

1,1,2,2-Tetrachloroethane
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	350	10	-	-	1	-	-	3 Chromatid Fragments
5 ppm, 7 h/day	500	10	3	-	-	1	-	1 Chromatid Fragment 1 Chromosomal Fragment 1 Robertsonian Translocation
50 ppm, 7 h/day	450	9	-	-	1	1	1	3 Chromatid Fragments 2 Chromosomal Fragments
EMS, 250 mg/kg/day	500	18	1	6	-	-	-	1 Robertsonian Translocation

TABLE CA-M6-2

1,1,2,2-Tetrachloroethane
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	0.384	0.074		0.212	0.059	
5 ppm	0.357	0.062	-0.28	0.271	0.049	0.76
50 ppm	0.422	0.065	0.38	0.266	0.052	0.68
EMS, 250 mg/kg	0.415	0.062	0.33	0.244	0.049	0.41

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

TABLE CA-M24-1

1,1,2,2-Tetrachloroethane
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	454	9	-	-	-	-	-	-
5 ppm, 7 h/day	469	10	1	-	-	-	-	-
50 ppm, 7 h/day	450	8	-	-	1	-	-	1 Misc Aberration
EMS, 250 mg/kg/day	450	45	-	-	-	-	-	-

TABLE CA-M24-2

1,1,2,2-Tetrachloroethane
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	0.301	0.057		0.173	0.022	
5 ppm	0.285	0.057	-0.19	0.169	0.022	-0.12
50 ppm	0.279	0.060	-0.27	0.163	0.023	-0.31
EMS, 250 mg/kg	0.598	0.060	3.57***	0.141	0.023	-1.00

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

TABLE CA-M48-1

1,1,2,2-Tetrachloroethane
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	22	5	-	3	-	-	-
5 ppm, 7 h/day	484	11	2	-	1	-	-	1 Chromatid Fragment
50 ppm, 7 h/day	457	12	-	-	1	-	-	-
EMS, 250 mg/kg/day	500	20	13	-	-	-	-	3 Chromatid Fragments

TABLE CA-M48-2

1,1,2,2-Tetrachloroethane
Cytogenetic Analysis of Rat Bone Marrow Cells
Summary of Observed Aberrations
Males

Single Dosing

Sampling Time: 48 h

Treatment Group	Spreads with Aberrations					
	Total			Excluding Gaps		
	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t
Air Control	0.433	0.062		0.220	0.037	
5 ppm	0.338	0.062	-1.08	0.203	0.037	-0.32
50 ppm	0.363	0.062	-0.80	0.163	0.037	-1.09
EMS, 250 mg/kg	0.506	0.062	0.83	0.298	0.037	1.47

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

TABLE CA-F6-1

1,1,2,2-Tetrachloroethane
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Females

Single Dosing

Sampling Time: 6 h

Group	Number of Spreads Observed	Observed Aberrations						Miscellaneous
		Chromatid			Chromosome			
		Gap	B w F	B w/o F	Gap	B w F	B w/o F	
Air Control, 7 h/day	500	18	3	1	2	-	-	1 Chromatid Fragment
5 ppm, 7 h/day	500	10	-	1	3	1	-	2 Chromatid Fragments 1 Translocation
50 ppm, 7 h/day	500	14	-	3	2	-	-	4 Chromatid Fragments 1 Chromosomal Fragment 1 Translocation 5 Robertsonian Translocations
EMS, 250 mg/kg/day	500	20	2	4	6	2	-	2 Chromatid Fragments 1 Chromosomal Fragment 2 Robertsonian Translocations

TABLE CA-F6-2

1,1,2,2-Tetrachloroethane
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	0.479	0.057		0.240	0.042	
5 ppm	0.385	0.057	-1.18	0.219	0.042	-0.35
50 ppm	0.485	0.057	0.08	0.359	0.042	1.99*
EMS, 250 mg/kg	0.546	0.057	0.82	0.341	0.042	1.69*

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

TABLE CA-F24-1

1,1,2,2-Tetrachloroethane
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	8	-	-	-	-	-	-
5 ppm, 7 h/day	471	8	-	-	-	-	-	-
50 ppm, 7 h/day	500	6	-	-	-	-	-	-
EMS, 250 mg/kg/day	500	44	-	-	1	-	-	-

TABLE CA-F24-2

1,1,2,2-Tetrachloroethane
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	0.291	0.054		0.141	0.037	
5 ppm	0.279	0.054	-0.15	0.148	0.037	1.41
50 ppm	0.241	0.054	-0.64	0.141	0.037	0.00
EMS, 250 mg/kg x 5	0.548	0.054	3.34***	0.141	0.037	0.00

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

TABLE CA-F48-1

1,1,2,2-Tetrachloroethane
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Females

Single Dosing

Sampling Time: 48 h

Group	Number of Spreads Observed	Observed Aberrations						Miscellaneous
		Chromatid			Chromosome			
		Gap	B w F	B w/o F	Gap	B w F	B w/o F	
Air Control, 7 h/day	500	16	-	-	-	1	-	-
5 ppm, 7 h/day	500	22	3	-	1	-	-	-
50 ppm, 7 h/day	500	12	2	-	4	-	-	1 Chromatid Fragment
EMS, 250 mg/kg/day	484	20	-	-	-	-	-	1 Chromatid Fragment

TABLE CA-F48-2

1,1,2,2-Tetrachloroethane
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	0.363	0.053		0.160	0.027	
5 ppm	0.446	0.053	1.12	0.191	0.027	0.80
50 ppm	0.397	0.053	0.45	0.200	0.027	1.05
EMS, 250 mg/kg	0.435	0.053	0.97	0.163	0.027	0.08

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

TABLE DL-1

1,1,2,2-Tetrachloroethane
Dominant Lethal Test in Rats
Pregnancy Frequency (Females with Corpora Lutea Graviditatis)

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	5 ppm	50 ppm	5 x 100 mg/kg EMS
1	100%	90%	89%	85%
2	100%	100%	90%	25%
3	90%	100%	80%	65%
4	90%	100%	100%	85%
5	95%	100%	90%	95%
6	90%	95%	100%	100%
7	100%	100%	95%	95%
8	80%	95%	100%	90%
9	95%	95%	95%	80%
10	95%	95%	85%	95%

TABLE DL-2

1,1,2,2-Tetrachloroethane
Dominant Lethal Test in Rats
Pregnancy Frequency (Females with Implantations)

Multiple Dosing

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

TABLE DL-3

1,1,2,2-Tetrachloroethane
Dominant Lethal Test in Rats
Total Number of Corpora Lutea per Pregnancy

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	5 ppm	50 ppm	5 x 100 mg/kg EMS
1	[1] 12.9 ± 0.49	11.4 ± 0.55*	12.1 ± 0.51	5.8 ± 0.86***
2	12.7 ± 0.62	11.9 ± 0.61	12.6 ± 0.64	4.0 ± 0.00
3	11.7 ± 0.40	12.0 ± 0.38	11.9 ± 0.42	3.2 ± 1.22***
4	13.0 ± 0.53	12.7 ± 0.50	13.0 ± 0.50	10.0 ± 0.92**
5	12.8 ± 0.47	12.4 ± 0.44	12.1 ± 0.50	11.8 ± 0.39*
6	12.6 ± 0.40	11.8 ± 0.39	12.6 ± 0.39	11.1 ± 0.55
7	13.0 ± 0.41	11.9 ± 0.41	12.9 ± 0.46	12.7 ± 0.46
8	12.8 ± 0.70	12.8 ± 0.66	12.5 ± 0.62	12.4 ± 0.68
9	12.5 ± 0.38	12.2 ± 0.38	12.6 ± 0.39	11.4 ± 0.57
10	11.7 ± 0.44	12.6 ± 0.41	11.9 ± 0.45	12.8 ± 0.35

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

TABLE DL-4

1,1,2,2-Tetrachloroethane
Dominant Lethal Test in Rats
Total Implantations per Pregnancy

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	5 ppm	50 ppm	5 x 100 mg/kg EMS
1	[1] 12.4 ± 0.70	11.1 ± 0.80	12.0 ± 0.74	5.4 ± 0.77***
2	12.7 ± 0.70	11.7 ± 0.68	12.7 ± 0.72	3.0 ± 0.00
3	12.1 ± 0.53	12.5 ± 0.50	11.8 ± 0.56	1.5 ± 0.22***
4	11.8 ± 0.57	12.7 ± 0.54	12.9 ± 0.54	7.6 ± 1.08**
5	12.8 ± 0.55	12.4 ± 0.52	12.3 ± 0.59	10.9 ± 0.72*
6	12.8 ± 0.52	12.3 ± 0.51	13.0 ± 0.51	12.0 ± 0.68
7	13.5 ± 0.45	11.9 ± 0.45	13.4 ± 0.49	13.1 ± 0.50
8	12.6 ± 0.82	12.1 ± 0.77	11.2 ± 0.73	11.7 ± 0.82
9	11.7 ± 0.49	11.8 ± 0.49	12.4 ± 0.50	11.6 ± 0.71
10	11.9 ± 0.41	13.3 ± 0.39	12.4 ± 0.43	12.3 ± 0.52

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

TABLE DL-5

1,1,2,2-Tetrachloroethane
Dominant Lethal Test in Rats
Live Implantations per Pregnancy

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	5 ppm	50 ppm	5 x 100 mg/kg EMS
1	[1] 12.1 ± 0.84	9.3 ± 0.96*	10.8 ± 0.89	2.3 ± 0.74***
2	11.3 ± 0.85	10.1 ± 0.83	11.9 ± 0.88	0.0 ± 0.00
3	11.3 ± 0.74	11.0 ± 0.70	10.6 ± 0.79	0.8 ± 0.40***
4	10.2 ± 1.00	11.6 ± 0.95	10.5 ± 0.95	6.4 ± 1.02**
5	11.7 ± 0.91	10.9 ± 0.87	10.8 ± 0.97	9.3 ± 1.04*
6	11.2 ± 0.75	10.6 ± 0.73	11.5 ± 0.73	9.8 ± 1.01
7	12.2 ± 0.58	10.1 ± 0.58	12.7 ± 0.63	11.5 ± 0.82
8	10.6 ± 0.99	11.1 ± 0.93	9.4 ± 0.88	10.3 ± 0.93
9	9.1 ± 0.82	10.5 ± 0.82	9.8 ± 0.85	9.5 ± 1.02
10	10.3 ± 0.71	12.7 ± 0.67	11.1 ± 0.72	11.2 ± 0.77

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

TABLE DL-6

1,1,2,2-Tetrachloroethane
Dominant Lethal Test in Rats
Live Implantations and Late Deaths per Pregnancy

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	5 ppm	50 ppm	5 x 100 mg/kg EMS
1	[1] 12.1 ± 0.85	9.4 ± 0.96*	10.8 ± 0.90	2.3 ± 0.74***
2	12.1 ± 0.78	10.7 ± 0.76	11.9 ± 0.80	0.0 ± 0.00
3	11.3 ± 0.74	11.0 ± 0.70	10.6 ± 0.78	0.8 ± 0.40***
4	10.3 ± 0.95	11.6 ± 0.90	11.1 ± 0.90	6.4 ± 1.02**
5	12.3 ± 0.83	11.0 ± 0.78	10.9 ± 0.88	9.3 ± 1.03*
6	12.0 ± 0.60	11.3 ± 0.59	11.5 ± 0.59	9.9 ± 0.99*
7	12.4 ± 0.49	10.7 ± 0.49	13.0 ± 0.53	11.5 ± 0.82
8	10.9 ± 0.90	11.3 ± 0.85	9.8 ± 0.81	10.4 ± 0.94
9	10.2 ± 0.70	11.1 ± 0.70	10.7 ± 0.72	10.5 ± 0.72
10	10.5 ± 0.55	12.7 ± 0.52	11.5 ± 0.57	11.3 ± 0.76

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

TABLE DL-7

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

TABLE DL-8

1,1,2,2-Tetrachloroethane
Dominant Lethal Test in Rats
Analysis of Proportions of Early Deaths Assuming Between
Female Variation (Logistic Scale)

Multiple Dosing

Assessment Week from Dosing	[1] Treatment Group	$\hat{\mu} \pm$ S.E. $\hat{\mu}$		Percentage Mean Probability of Early Deaths
		Total Implants a Linear Covariate	Assuming Extra-Binomial Variation	
1	1	-3.562 ± 0.8478	-3.603 ± 0.8478	2.7%
	2	-1.714 ± 0.4525	-1.621 ± 0.4291	16.5%
	3	-2.032 ± 0.4552	-2.024 ± 0.4522	11.7%
	4	-0.128 ± 0.0931		
2	1	-2.853 ± 0.3968	-2.879 ± 0.3917	5.3%
	2	-2.363 ± 0.3241	-2.153 ± 0.2898	10.4%
	3	-2.654 ± 0.3779	-2.677 ± 0.3697	6.4%
	4	-0.228 ± 0.0625		
3	1	-2.823 ± 0.5730	-2.771 ± 0.5596	5.9%
	2	-1.884 ± 0.3793	-1.914 ± 0.3757	12.9%
	3	-2.237 ± 0.4797	-2.147 ± 0.4602	10.5%
	4			
4	1	-2.190 ± 0.5971	-1.633 ± 0.4659	16.3%
	2	-2.306 ± 0.5665	-2.206 ± 0.5444	9.9%
	3	-1.631 ± 0.4554	-1.611 ± 0.4366	16.6%
	4	-0.351 ± 0.1242		

1. 1 = Air Control; 2 = 5 ppm 1,1,2,2-Tetrachloroethane; 3 = 50 ppm 1,1,2,2-Tetrachloroethane; 4 = 100 mg/kg, Ethyl methanesulphonate

TABLE DL-8 (continued)

1,1,2,2-Tetrachloroethane

Multiple Dosing

Assessment Week from Dosing	[1]Treatment Group	$\hat{\mu} \pm$ S.E. $\hat{\mu}$		Percentage Mean Probability of Early Deaths
		Total Implants a Linear Covariate	Assuming Extra-Binomial Variation	
5	1	-3.546 ± 0.7792	-3.192 ± 0.7249	3.9%
	2	-2.080 ± 0.4370	-1.790 ± 0.3834	14.3%
	3	-2.368 ± 0.5334	-1.937 ± 0.4531	12.6%
	4	-0.445 ± 0.1293		
6	1	-2.784 ± 0.4985	-2.708 ± 0.4899	6.3%
	2	-2.434 ± 0.4330	-2.469 ± 0.4321	7.8%
	3	-2.292 ± 0.4112	-2.140 ± 0.3760	10.5%
	4	0.163 ± 0.1144		
7	1	-2.541 ± 0.3576	-2.416 ± 0.3367	8.2%
	2	-2.169 ± 0.3246	-2.236 ± 0.3228	9.7%
	3	-3.595 ± 0.6005	-3.479 ± 0.5885	3.0%
	4	0.155 ± 0.1290		
8	1	-1.802 ± 0.3685	-1.835 ± 0.3649	13.8%
	2	-2.503 ± 0.4564	-2.517 ± 0.4559	7.5%
	3	-1.985 ± 0.3552	-1.964 ± 0.3511	12.3%
	4	-0.041 ± 0.0714		

1. 1 = Air Control; 2 = 5 ppm 1,1,2,2-Tetrachloroethane; 3 = 50 ppm 1,1,2,2-Tetrachloroethane; 4 = 100 mg/kg, Ethyl methanesulphonate

TABLE DL-8 (continued)

1,1,2,2-Tetrachloroethane

Multiple Dosing

Assessment Week from Dosing	[1] Treatment Group	$\hat{\mu} \pm$ S.E. $\hat{\mu}$		Percentage Mean Probability of Early Deaths
		Total Implants a Linear Covariate	Assuming Extra-Binomial Variation	
9	1	-1.955 ± 0.4077	-1.913 ± 0.4018	12.9%
	2	-2.650 ± 0.5361	-2.620 ± 0.5332	6.8%
	3	-1.734 ± 0.3926	-1.773 ± 0.3914	14.5%
	4	-0.109 ± 0.1161		
10	1	-2.137 ± 0.3268	-2.039 ± 0.3102	11.5%
	2	-3.035 ± 0.4616	-3.125 ± 0.4568	4.2%
	3	-2.523 ± 0.3866	-2.492 ± 0.3805	7.6%
	4	-0.139 ± 0.1217		

1. 1 = Air Control; 2 = 5 ppm 1,1,2,2-Tetrachloroethane; 3 = 50 ppm 1,1,2,2-Tetrachloroethane
4 = 100 mg/kg, Ethyl methanesulphonate

TABLE DL-9

1,1,2,2-Tetrachloroethane
Dominant Lethal Test in Rats
Early Death Frequency, Freeman-Tukey Poisson Transformation

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	5 ppm	50 ppm	5 x 100 mg/kg EMS
1	[1] 1.434 ± 0.3172	2.455 ± 0.3596*	1.955 ± 0.3364	3.167 ± 0.5198**
2	1.860 ± 0.2255	2.122 ± 0.2198	1.940 ± 0.2317	3.732 ± 0.000
3	1.811 ± 0.3191	2.218 ± 0.3027	2.161 ± 0.3384	1.829 ± 0.3863
4	2.275 ± 0.3781	1.903 ± 0.3587	2.614 ± 0.3587	2.054 ± 0.3728
5	1.618 ± 0.3186	2.195 ± 0.3023	2.214 ± 0.3380	2.107 ± 0.4266
6	1.902 ± 0.3161	1.929 ± 0.3077	2.196 ± 0.3077	2.527 ± 0.4571
7	2.034 ± 0.2570	2.222 ± 0.2570	1.542 ± 0.2787	2.335 ± 0.3660
8	2.541 ± 0.3468	1.940 ± 0.3270	2.116 ± 0.3102	2.164 ± 0.3476
9	2.377 ± 0.3228	1.739 ± 3.228	2.447 ± 0.3317	1.818 ± 0.3847
10	2.296 ± 0.2689	1.710 ± 0.2551	1.901 ± 0.2767	1.936 ± 0.3307

1 = Mean ± standard error of mean

*P<0.05

**P<0.01

TABLE DL-10

1,1,2,2-Tetrachloroethane
Dominant Lethal Test in Rats
Early Death Frequency, Freeman-Tukey Binomial Transformation

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	5 ppm	50 ppm	5 x 100 mg/kg EMS
1	[1] 0.401 ± 0.1221	0.812 ± 0.1384	0.613 ± 0.1295	1.741 ± 0.2667***
2	0.512 ± 0.0936	0.715 ± 0.0913	0.559 ± 0.0962	2.602 ± 0.000
3	0.518 ± 0.1136	0.682 ± 0.1078	0.644 ± 0.1205	1.532 ± 0.3863*
4	0.782 ± 0.1605	0.585 ± 0.1522	0.786 ± 0.1522	0.804 ± 0.1620
5	0.439 ± 0.1260	0.711 ± 0.1196	0.682 ± 0.1337	0.714 ± 0.1770
6	0.531 ± 0.0920	0.546 ± 0.0895	0.613 ± 0.0895	0.788 ± 0.1647
7	0.561 ± 0.0727	0.636 ± 0.0727	0.412 ± 0.0788	0.687 ± 0.1349
8	0.726 ± 0.1102	0.582 ± 0.1039	0.652 ± 0.0985	0.664 ± 0.1190
9	0.701 ± 0.1138	0.515 ± 0.1138	0.752 ± 0.1169	0.535 ± 0.1097
10	0.672 ± 0.0831	0.465 ± 0.0788	0.556 ± 0.0855	0.567 ± 0.1127

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

TABLE SA-1

1,1,2,2-Tetrachloroethane
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	9686	7	19	123	50	142	341	0.07	0.19	1.23	0.50	1.42	3.41
5 ppm, 7 h/day	9357[1])	34	50	221	101	237	643	0.34	0.50	2.21	1.01	2.37	6.43
	8671[2])	11	20	116	44	138	329	0.12	0.22	1.29	0.49	1.58	3.66
50 ppm, 7 h/day	9614	17	32	139	42	156	386	0.17	0.32	1.39	0.42	1.56	3.86
EMS, 100 mg/kg/day	9614	7	26	142	68	143	386	0.07	0.26	1.42	0.68	1.43	3.86

1) Including animal no. 337 2) Excluding animal number 337

*A = Hook up-turned or hook elongated

B = Banana-shaped head

C = Amorphous head

D = Folded tail

E = Miscellaneous (double head, double tail, triple tail, twisted neck, abaxial attachment, midpiece attachment, filamentous midpiece, enlarged midpiece, plier type)

TABLE SA-2

1,1,2,2-Tetrachloroethane
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	6.08 ± 0.904	9.55 ± 0.978	22.41 ± 1.466	13.91 ± 1.509	23.89 ± 1.693	37.27 ± 1.548
5 ppm, 7 h/day	7.06 ± 0.953	9.91 ± 1.030	22.89 ± 1.545	13.42 ± 1.591	24.79 ± 1.784	38.51 ± 1.632
50 ppm, 7 h/day	9.21* ± 0.904	11.32 ± 0.978	23.12 ± 1.466	13.25 ± 1.509	24.03 ± 1.693	38.62 ± 1.548
EMS, 100 mg/kg/day	5.86 ± 0.904	10.73 ± 0.978	23.89 ± 1.466	16.67 ± 1.509	23.56 ± 1.693	39.37 ± 1.548

A = Hook up-turned or hook elongated

B = Banana-shaped head

C = Amorphous head

D = Folded tail

E = Miscellaneous (double head, double tail, triple tail, twisted neck, abaxial attachment, midpiece attachment, filamentous midpiece, enlarged midpiece, plier type)

* = $P<0.05$

TABLE RL-1

1,1,2,2-Tetrachloroethane
Drosophila Dose Ranging Experiment

Day		5 ppm			50 ppm			
		1 h	3 h	7 h	10 min	1 h	2 h	2.75 h
0	No. of males exposed	100	100	100	100	100	100	100
1	No. and % survival	100/100%	100/100%	96/96%	96/96%	100/100%	95/95%	91/91%
2	No. of eggs laid by 5♀♀	111	125	78	71	111	28	82
3	No. and % hatched	99/89%	122/98%	66/85%	67/94%	106/96%	27/96%	77/94%

TABLE RL-2

1,1,2,2-Tetrachloroethane
Drosophila SLRL Procedure and Results

Compound: 1,1,2,2-Tetrachloroethane **Concentration:** 5 ppm **Stock:** A
Length of Exposure: 7 h **Test exposure given:** 9.4.79

	Brood 1	Brood 2	Brood 3
F_1 set up	10.4.79	13.4.79	18.4.79
F_2 set up	24.4.79	26.4.79	30.4.79
F_2 scored	7.5.79	9.5.79	12.5.79
F_2 repeats scored	-	-	-
F_3 set up	-	-	-
F_3 scored	-	-	-
F_3 repeats scored	-	-	-

RESULTS

	Brood 1	Brood 2	Brood 3	All Broods
No. of F_1 vials	97	97	95	289
No. of sterile F_1 vials	4	2	6	12
No. of F_1 vials used in F_2	93	95	89	277
No. of F_2 vials set up	400	400	400	1200
No. of F_2 vials scored	373	367	349	1089
No. of F_2 vials containing lethals	1	3	1	5
Frequency of F_2 lethals	0.27%	0.817%	0.287%	0.459%
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	-	-	-	-

Total No. of sterile vials = 113

TABLE RL-2 (continued)

1,1,2,2-Tetrachloroethane
Drosophila SLRL Procedure and Results

Compound: 1,1,2,2-Tetrachloroethane **Concentration:** 5 ppm **Stock:** B
Length of Exposure: 7 h **Test exposure given:** 9.4.79

	Brood 1	Brood 2	Brood 3
F_1 set up	10.4.79	13.4.79	18.4.79
F_2 set up	25.4.79	27.4.79	1.5.79
F_2 scored	9.5.79	10.5.79	14.5.79
F_2 repeats scored	22.5.79	22.5.79	-
F_3 set up	9.5.79	10.5.79	14.5.79
F_3 scored	22.5.79	24.5.79	30.5.79
F_3 repeats scored	-	-	-

RESULTS

	Brood 1	Brood 2	Brood 3	All Broods
No. of F_1 vials	91	90	90	271
No. of sterile F_1 vials	2	3	9	14
No. of F_1 vials used in F_2	89	87	80	256
No. of F_2 vials set up	400	400	400	1200
No. of F_2 vials scored	376	384	351	1111
No. of F_2 vials containing lethals	2	1	0	3
Frequency of F_2 lethals	0.531%	0.26%	0	0.27%
No. of F_3 vials set up	400	301	300	1001
No. of F_3 vials scored	395	290	292	977
No. of F_3 vials containing lethals	0	0	0	0
Frequency of F_3 lethals	0	0	0	0

Total No. of sterile vials = 127

TABLE RL-2 (continued)

1,1,2,2-Tetrachloroethane

Drosophila SLRL Procedure and Results

Compound: 1,1,2,2-Tetrachloroethane **Concentration:** 50 ppm **Stock:** A

Length of Exposure: 7 h (intermittent) **Test exposure given:** 9.4.79

	Brood 1	Brood 2	Brood 3
F_1 set up	10.4.79	13.4.79	18.4.79
F_2 set up	24.4.79	26.4.79	1.5.79
F_2 scored	7.5.79	9.5.79	14.5.79
F_2 repeats scored	-	-	-
F_3 set up	-	-	-
F_3 scored	-	-	-
F_3 repeats scored	-	-	-

RESULTS

	Brood 1	Brood 2	Brood 3	All Broods
No. of F_1 vials	95	88	75	258
No. of sterile F_1 vials	38	37	27	102
No. of F_1 vials used in F_2	57	51	48	156
No. of F_2 vials set up	392	400	400	1192
No. of F_2 vials scored	323	378	348	1049
No. of F_2 vials containing lethals	0	1	1	2
Frequency of F_2 lethals	0	0.264%	0.287%	0.19%
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	-	-	-	-

Total No. of sterile vials = 245

TABLE RL-2 (continued)

1,1,2,2-Tetrachloroethane
Drosophila SLRL Procedure and Results

Compound: 1,1,2,2-Tetrachloroethane **Concentration:** 50 ppm **Stock:** B
Length of Exposure: 7 h (intermittent) **Test exposure given:** 9.4.79

	Brood 1	Brood 2	Brood 3
F_1 set up	10.4.79	13.4.79	18.4.79
F_2 set up	25.4.79	27.4.79	30.4.79
F_2 scored	8.5.79	10.5.79	15.5.79
F_2 repeats scored	-	-	-
F_3 set up	8.5.79	10.5.79	15.5.79
F_3 scored	23.5.79	24.5.79	30.5.79
F_3 repeats scored	-	-	-

RESULTS

	Brood 1	Brood 2	Brood 3	All Broods
No. of F_1 vials	98	97	94	289
No. of sterile F_1 vials	4	2	4	10
No. of F_1 vials used in F_2	94	95	90	279
No. of F_2 vials set up	396	400	400	1196
No. of F_2 vials scored	378	376	362	1116
No. of F_2 vials containing lethals	1	0	1	2
Frequency of F_2 lethals	0.264%	0	0.276%	0.179%
No. of F_3 vials set up	396	300	300	996
No. of F_3 vials scored	384	294	289	967
No. of F_3 vials containing lethals	0	0	0	0
Frequency of F_3 lethals	0	0	0	0

Total No. of sterile vials = 119

TABLE RL-2 (continued)

1,1,2,2-Tetrachloroethane

Drosophila SLRL Procedure and Results

Compound: Ethyl methane-sulphonate **Concentration:** 0.4% v/v **Stock:** A

Length of Exposure: 5 h **Test exposure given:** 12.4.79

	Brood 1	Brood 2	Brood 3
F_1 set up	13.4.79	-	-
F_2 set up	25.4.79	-	-
F_2 scored	7.5.79	-	-
F_2 repeats scored	23.5.79	-	-
F_3 set up	-	-	-
F_3 scored	-	-	-
F_3 repeats scored	-	-	-

RESULTS

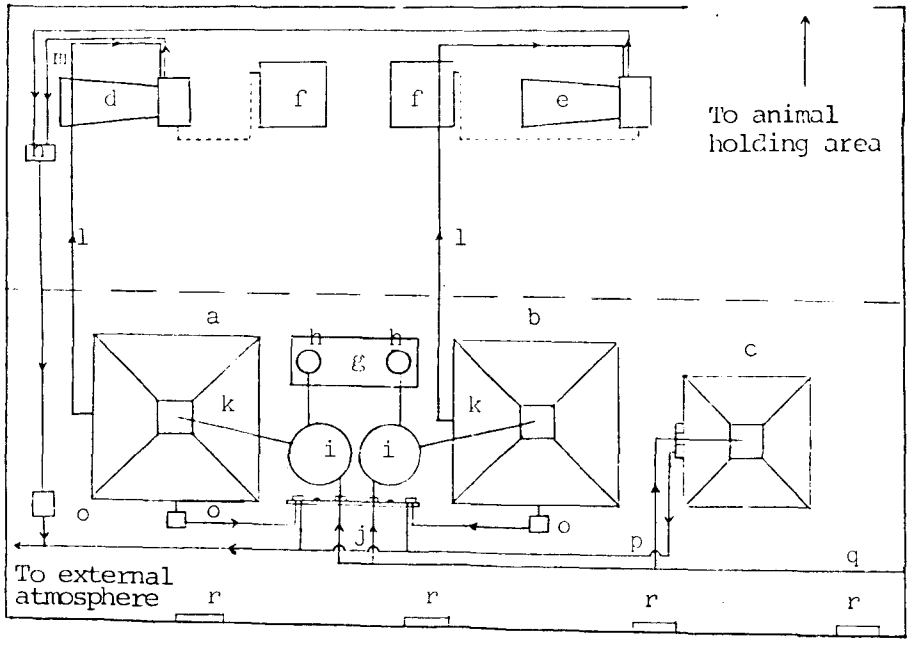
	Brood 1	Brood 2	Brood 3	All Broods
No. of F_1 vials	29	-	-	29
No. of sterile F_1 vials	9	-	-	9
No. of F_1 vials used in F_2	20	-	-	20
No. of F_2 vials set up	100	-	-	100
No. of F_2 vials scored	70	-	-	70
No. of F_2 vials containing lethals	11	-	-	11
Frequency of F_2 lethals	15.71%	-	-	15.71%
No. of F_3 vials set up	-	-	-	-
No. of F_3 vials scored	-	-	-	-
No. of F_3 vials containing lethals	-	-	-	-
Frequency of F_3 lethals	-	-	-	-

Total No. sterile vials = 39

FICURE 1a

1,1,2,2-Tetrachloroethane

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

1,1,2,2-Tetrachloroethane

Schematic Lay-out of Vapour Generation Apparatus

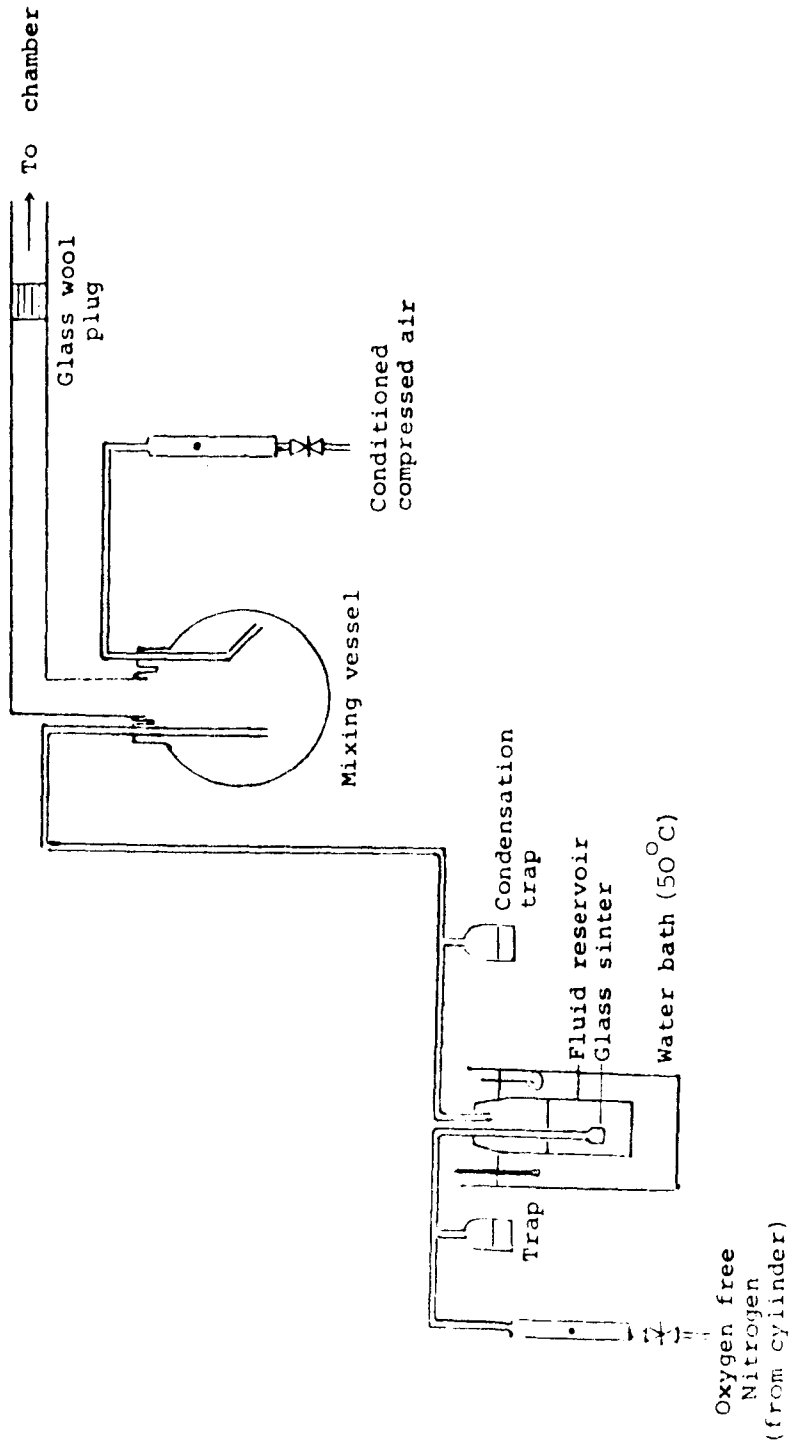


FIGURE 2

1,1,2,2-Tetrachloroethane
Typical Calibration Graph for High Level
5 April 1979

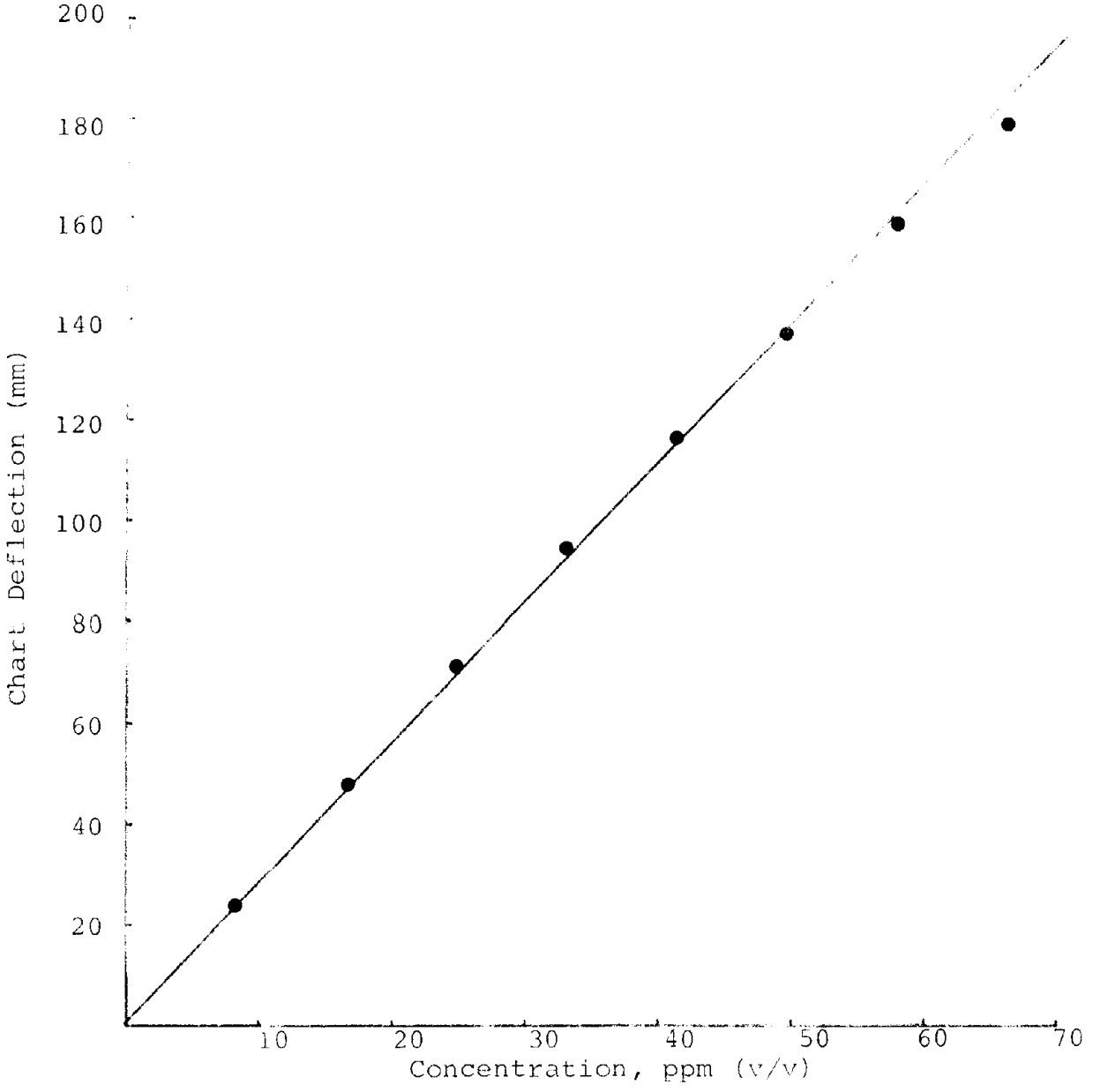
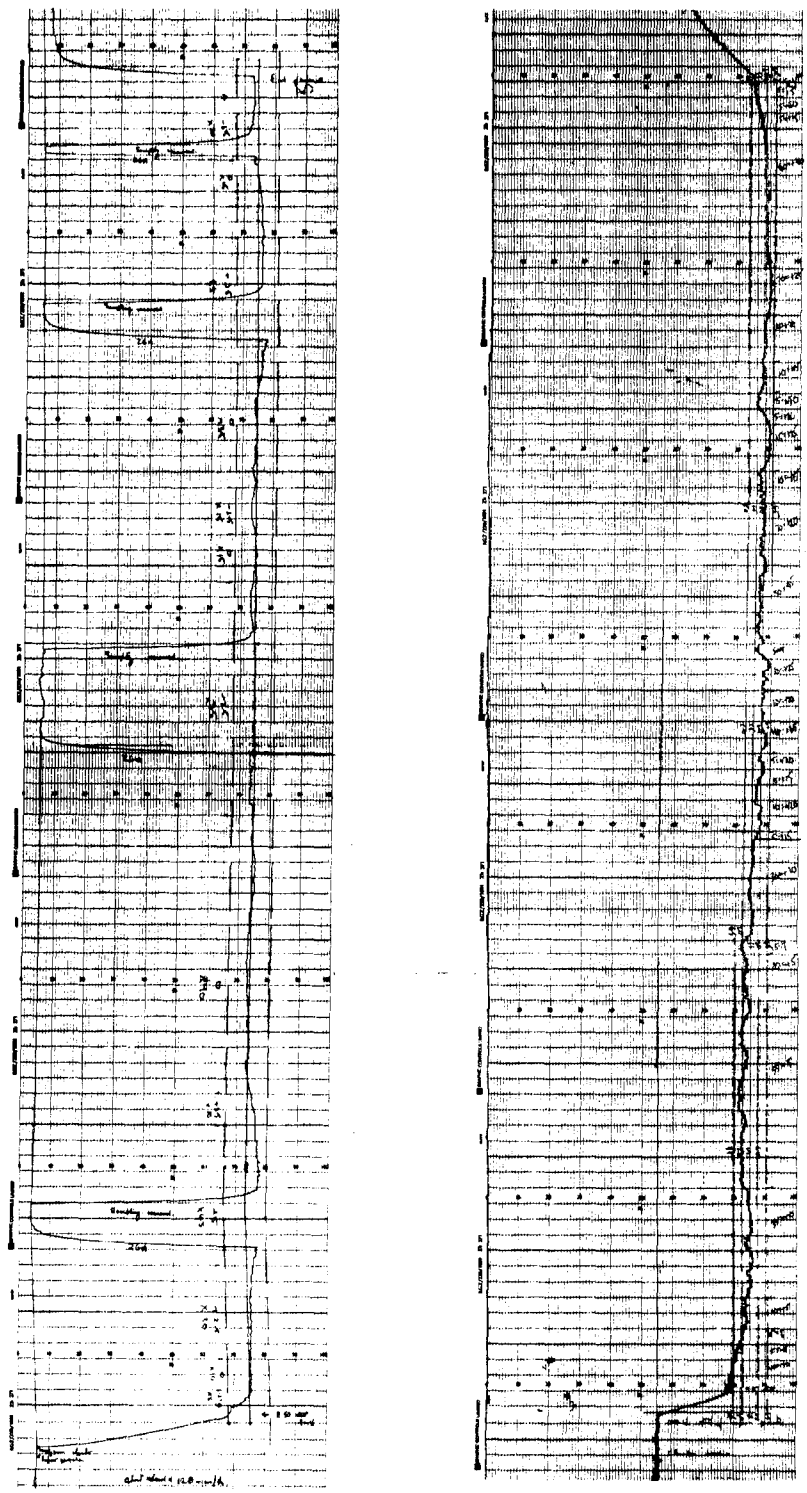


FIGURE 3

1,1,2,2-Tetrachloroethane

Sample Record Chart of IR Absorption at 8.3 μm



APPENDIX DIET

1,1,2,2-Tetrachloroethane
Diet Analysis

Spratt's Patent Ltd

Central House
Cambridge Road
Barking
Essex IG11 8NL

Telephone
01-594 7121
Telegrams
Spratt's Barking
Telex 897659

CERTIFICATE OF ANALYSIS

Product: L.A.D 1

Batch No: 147933

Date of Manufacture: 14TH MARCH, 1979.

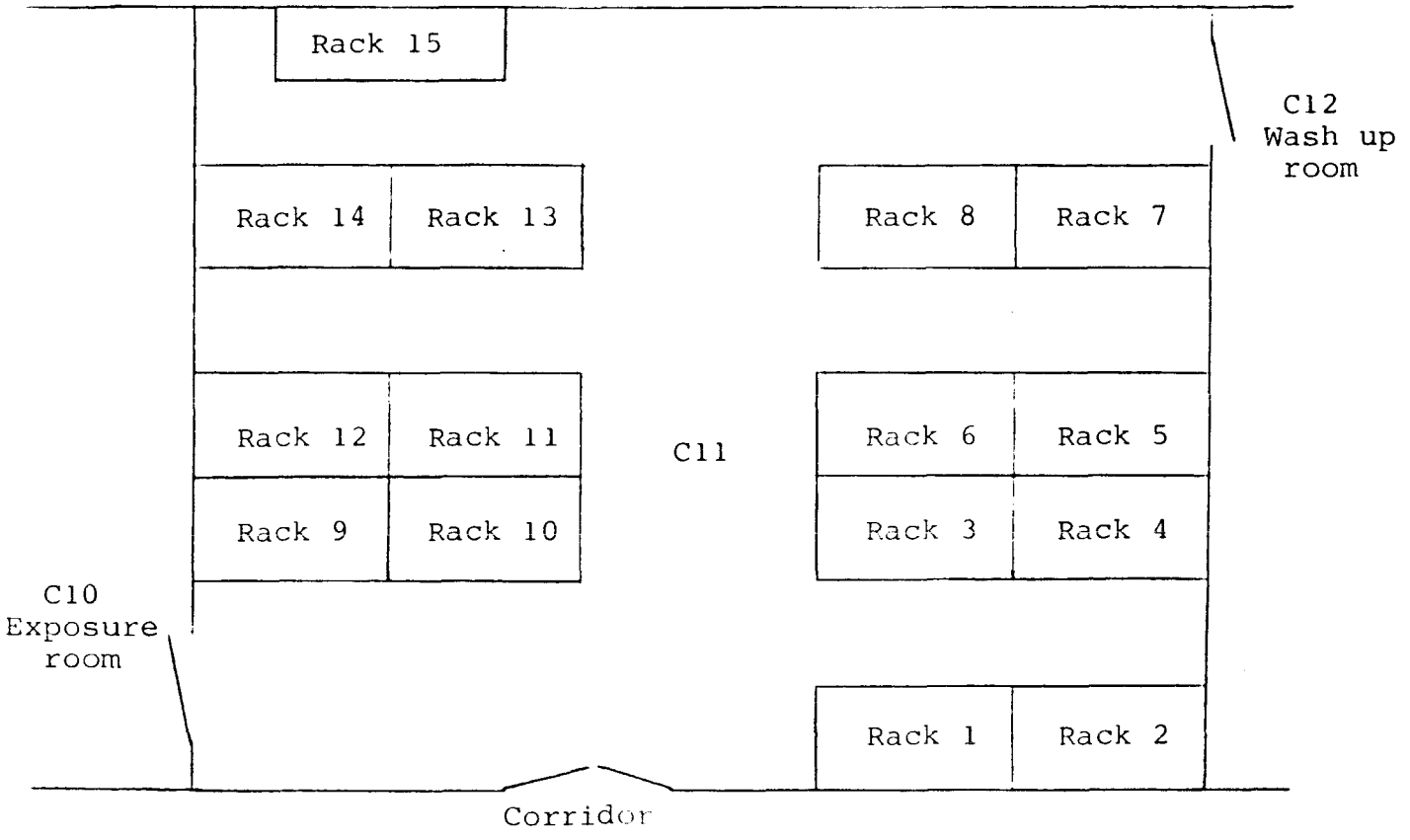
	Found Analysis
Moisture	10.2 %
Crude Fat	3.0 %
Crude Protein	21.1 %
Ash	4.2 %
Calcium	0.94 %
Phosphorus	0.75 %
Nitrate	1.0 mg/kg
Nitrite	1.6 mg/kg
Selenium	0.25 mg/kg
Lead	1.8 mg/kg
Arsenic	0.30 mg/kg
Cadmium	0.20 mg/kg
Mercury	0.041 mg/kg
Aflatoxins	NONE DETECTED
Total P.C.B.	NONE DETECTED
Total D.D.T.	NONE DETECTED
Dieldrin	0.010 mg/kg
Lindane	0.007 mg/kg
Heptachlor	0.009 mg/kg
Malathion	0.16 mg/kg
Total Viable Organisms	2.0 × 10^3/gm
E. Coli Type 1	NONE DETECTED
Salmonella Species	NONE DETECTED
moulds	NONE DETECTED

Signed .....

Date .....

APPENDIX LOC-1

1,1,2,2-Tetrachloroethane
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

1,1,2,2-Tetrachloroethane
Examples of Animal Location During Exposure
Exposure Location Sheet

Project No: 409959 Test Concentration: 0

Test Compound: Air Control Tier No: 1

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

Day of Study: 2

LEFT

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

FRONT REAR

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

RIGHT

SIGNED: _____ DATE: _____

APPENDIX Loc-2 (continued)

1,1,2,2-Tetrachloroethane
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)

1,1,2,2-Tetrachloroethane
Exposure Location Sheet

Project No: 409959 Test Concentration: Low

Test Compound: 1,1,2,2-Tetrachloroethane 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)

1,1,2,2-Tetrachloroethane

Exposure Location Sheet

Project No: 409959 Test Concentration: High

Test Compound: 1,1,2,2-Tetrachloroethane 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

1,1,2,2-Tetrachloroethane

CYTOGENETIC SCORE SHEET **NIOSH**

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

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

Signature: **Slide No.:**

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

Slide quality:

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

Vernier	Chromatid			Chromosomal			Markers			Multi-Abn.	Misc. Abn.	Obser-vation
	GAP	BwF	Bw/oF	GAP	BwF	Bw/oF	EXC.	DIC	RG			
1.												
2.												
3.												
4.												
5.												
6.												
7.												
8.												
9.												
10.												
11.												
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15.												
16.												
17.												
18.												
19.												
20.												
21.												
22.												
23.												
24.												
25.												

APPENDIX FORM-2

1,1,2,2-Tetrachloroethane

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

1,1,2,2-Tetrachloroethane

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

1,1,2,2-Tetrachloroethane
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	355	356	363	365	371
	122	337	339	346	347	352
	123	310	311	315	325	320
	124	358	375	372	390	392
	125	319	308	315	322	327
	126	370	373	377	382	385
	127	321	313	328	340	342
	128	400	397	404	411	417
	129	305	307	310	316	323
	130	298	298	298	308	307
	Mean	337.3	337.3	342.8	350.6	353.6
	± S.D.	± 32.8	± 35.3	± 35.0	± 35.0	± 36.3

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	281	195	197	200	205	206
	282	213	206	208	213	215
	283	226	228	227	227	234
	284	171	173	175	178	180
	285	215	220	218	222	218
	286	225	231	227	228	228
	287	222	224	221	224	224
	288	227	227	228	231	232
	289	215	215	213	216	221
	290	189	193	195	188	195
	Mean	209.8	211.4	211.2	213.2	215.3
	± S.D.	± 18.7	± 18.9	± 17.1	± 17.8	± 17.2

APPENDIX TABLE BW-1 (continued)

1,1,2,2-Tetrachloroethane

Multiple Dosing: 5 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	131	346	347	356	371	374
	132	356	355	360	366	361
	133	360	365	369	374	381
	134	348	352	352	365	363
	135	360	362	369	376	382
	136	296	293	301	310	312
	137	253	261	266	277	281
	138	271	269	269	286	288
	139	269	266	265	271	275
	140	313	317	321	327	325
	Mean ± S.D.	317.2 ± 42.2	318.7 ± 42.8	322.8 ± 44.2	332.3 ± 43.3	334.2 ± 43.0

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	291	223	225	222	232	230
	292	198	197	195	202	200
	293	211	210	208	216	214
	294	232	232	234	244	242
	295	218	217	218	225	223
	296	190	186	188	196	196
	297	216	214	209	219	217
	298	212	204	207	213	211
	299	217	214	220	224	224
	300	186	185	182	191	189
	Mean ± S.D.	210.3 ± 14.6	208.4 ± 15.5	208.3 ± 16.2	226.2 ± 28.6	214.6 ± 16.3

APPENDIX TABLE BW-1 (continued)

1,1,2,2-Tetrachloroethane

Multiple Dosing: 50 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	141	306	311	315	323	328
	142	368	375	379	384	389
	143	343	342	340	350	349
	144	315	321	325	325	326
	145	329	324	334	336	343
	146	346	351	359	362	366
	147	312	315	320	328	331
	148	292	296	289	295	300
	149	344	348	350	354	358
	150	371	377	381	386	389
	Mean	332.6	336.0	339.2	344.2	347.9
	± S.D.	± 26.3	± 27.2	± 29.0	± 28.7	± 28.5

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	301	219	213	220	216	218
	302	206	202	200	207	207
	303	220	220	219	212	220
	304	204	205	208	207	209
	305	205	201	207	209	211
	306	235	237	238	238	240
	307	209	209	212	212	213
	308	193	185	188	190	196
	309	230	233	237	237	245
	310	218	220	223	230	228
	Mean	213.9	212.5	215.2	215.8	218.7
	± S.D.	± 12.8	± 15.6	± 15.6	± 15.1	± 15.2

APPENDIX TABLE BW-1 (continued)

1,1,2,2-Tetrachloroethane

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	151	300	296	283	275	273
	152	320	316	303	295	289
	153	333	335	325	322	315
	154	348	340	316	307	298
	155	335	328	308	297	288
	156	310	300	290	285	275
	157	352	345	326	312	301
	158	325	321	313	306	300
	159	302	293	280	278	270
	160	350	354	345	340	330
	Mean ± S.D.	327.5 ± 19.4	322.8 ± 21.4	308.9 ± 20.6	301.7 ± 20.1	293.9 ± 19.1

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	311	210	209	198	187	182
	312	198	201	186	183	183
	313	201	194	178	170	163
	314	202	202	192	188	188
	315	225	222	210	200	195
	316	206	199	182	173	165
	317	244	236	223	217	211
	318	195	187	178	173	165
	319	213	201	187	179	173
	320	250	241	229	225	225
	Mean ± S.D.	214.4 ± 19.2	209.2 ± 18.0	195.7 ± 18.5	189.5 ± 18.9	185.0 ± 20.6

APPENDIX TABLE BW-2

1,1,2,2-Tetrachloroethane
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	278	11	315	21	332
	2	306	12	285	22	335
	3	280	13	362	23	330
	4	325	14	334	24	340
	5	323	15	301	25	288
	6	343	16	292	26	300
	7	313	17	318	27	315
	8	330	18	280	28	280
	9	303	19	294	29	315
	10	294	20	330	30	325
	Mean ± S.D.	309.5 ± 24.4		311.1 ± 25.7		316.0 ± 20.6

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Female	161	190	171	221	181	210
	162	204	172	202	182	217
	163	185	173	207	183	205
	164	181	174	201	184	225
	165	196	175	207	185	215
	166	206	176	197	186	202
	167	185	177	198	187	187
	168	195	178	219	188	175
	169	219	179	201	189	215
	170	212	180	190	190	220
	Mean ± S.D.	197.3 ± 12.6		204.3 ± 9.6		207.1 ± 15.6

APPENDIX TABLE BW-2 (continued)

1,1,2,2-Tetrachloroethane

Single Dosing: 5 ppm

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Male	31	296	41	273	51	336
	32	319	42	300	52	353
	33	291	43	347	53	338
	34	314	44	326	54	315
	35	336	45	323	55	268
	36	327	46	300	56	270
	37	335	47	309	57	292
	38	350	48	332	58	326
	39	267	49	293	59	309
	40	297	50	386	60	310
	Mean ± S.D.	313.2 ± 25.3		318.9 ± 31.8		311.7 ± 28.4

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Female	191	186	201	208	211	211
	192	211	202	191	212	213
	193	204	203	199	213	210
	194	200	204	219	214	230
	195	220	205	221	215	222
	196	198	206	192	216	194
	197	221	207	197	217	233
	198	206	208	218	218	207
	199	220	209	197	219	190
	200	196	210	200	220	217
	Mean ± S.D.	206.2 ± 11.8		204.2 ± 11.4		212.7 ± 13.8

APPENDIX TABLE BW-2 (continued)

1,1,2,2-Tetrachloroethane

Single Dosing: 50 ppm

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Male	61	298	71	277	81	276
	62	293	72	343	82	330
	63	297	73	300	83	338
	64	305	74	323	84	315
	65	300	75	316	85	299
	66	330	76	340	86	281
	67	271	77	300	87	300
	68	341	78	315	88	305
	69	322	79	316	89	297
	70	312	80	342	90	280
	Mean ± S.D.	306.9 ± 20.2		317.2 ± 21.2		302.1 ± 20.8

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Female	221	205	231	187	241	200
	222	184	232	214	242	190
	223	214	233	215	243	213
	224	210	234	197	244	202
	225	218	235	195	245	196
	226	228	236	189	246	198
	227	187	237	215	247	178
	228	217	238	225	248	228
	229	210	239	214	249	205
	230	215	240	198	250	185
	Mean ± S.D.	208.8 ± 13.7		204.9 ± 13.1		199.5 ± 14.2

APPENDIX TABLE BW-2 (continued)

1,1,2,2-Tetrachloroethane

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	303	101	318	111	300
	92	295	102	295	112	341
	93	318	103	300	113	375
	94	288	104	301	114	322
	95	324	105	274	115	334
	96	324	106	333	116	371
	97	315	107	301	117	296
	98	350	108	295	118	308
	99	301	109	313	119	309
	100	299	110	340	120	330
	Mean	311.7		307.0		328.6
	± S.D.	± 18.3		± 19.5		± 27.6

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Female	251	196	261	208	271	198
	252	177	262	200	272	219
	253	220	263	210	273	197
	254	211	264	195	274	222
	255	220	265	206	275	204
	256	207	266	199	276	226
	257	230	267	204	277	198
	258	210	268	193	278	211
	259	202	269	218	279	228
	260	215	270	208	280	203
	Mean	208.8		204.1		210.6
	± S.D.	± 14.8		± 7.5		± 12.2

APPENDIX TABLE BW-3

1,1,2,2-Tetrachloroethane
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	373	377	385	394	396
	362	300	310	314	316	315
	363	334	331	340	348	347
	364	380	372	377	391	390
	365	300	307	318	325	325
	366	328	329	330	335	339
	367	313	317	325	331	332
	368	354	356	360	365	365
	369	295	300	310	317	323
	370	318	323	329	318	333
	Mean	329.5	332.2	338.8	344.0	346.5
	± S.D.	± 30.6	± 27.2	± 26.4	± 29.8	± 28.2

APPENDIX TABLE BW-3 (continued)

1,1,2,2-Tetrachloroethane

Multiple Dosing: 5 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	371	336	319	321	336	343
	372	344	348	350	360	363
	373	307	317	319	330	337
	374	353	361	359	369	372
	375	337	341	347	356	360
	376	336	348	350	360	361
	377	321	318	324	334	343
	378	327	330	335	342	344
	379	314	306	310	325	330
	380	327	339	344	350	365
	Mean ± S.D.	330.2 ± 13.9	332.7 ± 17.5	335.9 ± 16.5	346.2 ± 14.9	351.8 ± 14.0

APPENDIX TABLE BW-3 (continued)

1,1,2,2-Tetrachloroethane

Multiple Dosing: 50 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	381	317	318	330	332	341
	382	335	338	350	353	365
	383	343	344	350	354	356
	384	332	340	343	350	350
	385	316	318	321	321	327
	386	326	334	335	340	346
	387	300	301	306	314	320
	388	326	332	334	342	346
	389	324	334	337	340	345
	390	315	317	325	336	337
	Mean ± S.D.	323.4 ± 12.1	327.6 ± 13.5	333.1 ± 13.5	338.2 ± 13.1	343.3 ± 13.1

APPENDIX TABLE BW-3 (continued)

1,1,2,2-Tetrachloroethane

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	391	316	302	287	277	263
	392	340	326	308	294	279
	393	335	327	318	308	296
	394	359	346	329	316	307
	395	295	276	256	244	233
	396	324	308	295	282	270
	397	349	345	331	318	303
	398	316	293	275	262	252
	399	348	331	317	306	299
	400	307	294	279	264	253
	Mean	328.9	314.8	299.5	287.1	275.5
	± S.D.	± 20.6	± 23.7	± 25.1	± 25.3	± 25.4

APPENDIX TABLE BW-4

1,1,2,2-Tetrachloroethane
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	25	25	26
	322	23	24	24	24	25
	323	27	26	25	26	26
	324	24	24	23	23	24
	325	25	25	25	25	26
	326	26	26	26	26	27
	327	24	25	24	24	25
	328	25	26	26	25	27
	329	25	25	25	24	25
	330	24	26	26	25	26
	Mean ± S.D.	24.8 ± 1.1	25.2 ± 0.8	24.9 ± 1.0	24.7 ± 0.9	25.7 ± 0.9

APPENDIX TABLE BW-4 (continued)

1,1,2,2-Tetrachloroethane

Multiple Exposure: 5 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	331	24	25	24	25	26
	332	24	23	22	23	24
	333	23	24	24	25	25
	334	25	25	25	26	26
	335	24	24	24	25	25
	336	24	25	24	25	26
	337	25	26	25	26	28
	338	24	24	24	25	24
	339	24	24	23	25	25
	340	23	24	24	25	26
	Mean ± S.D.	24.0 ± 0.7	24.4 ± 0.8	23.9 ± 0.9	25.0 ± 0.8	25.5 ± 1.2

APPENDIX TABLE BW-4 (continued)

1,1,2,2-Tetrachloroethane

Multiple Dosing: 50 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	341	24	25	25	25	26
	342	25	25	25	25	25
	343	25	25	24	24	25
	344	25	26	26	27	27
	345	24	25	25	26	27
	346	25	25	25	25	25
	347	25	26	25	25	25
	348	24	25	24	25	25
	349	24	24	25	25	24
	350	23	23	24	23	24
	Mean ± S.D.	24.4 ± 0.7	24.9 ± 0.9	24.8 ± 0.6	25.0 ± 1.1	25.3 ± 1.1

APPENDIX TABLE BW-4 (continued)

1,1,2,2-Tetrachloroethane

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	351	24	24	24	24	24
	352	25	25	25	26	25
	353	24	25	26	26	26
	354	24	25	25	25	26
	355	26	26	26	26	26
	356	25	25	25	25	25
	357	27	27	27	27	28
	358	26	26	27	27	27
	359	24	24	24	24	24
	360	24	24	24	25	25
	Mean ± S.D.	24.9 ± 1.1	25.1 ± 1.0	25.3 ± 1.2	25.5 ± 1.1	25.6 ± 1.3

APPENDIX TABLE CA-MD-M

1,1,2,2-Tetrachloroethane
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Males

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
126	42/4	50	25	25								
	42/5		25	24	1							14.3 x 110.2
121	4/4	50	25	24				1				13.2 x 117.1
	4/2		25	23				1				14.2 x 117.0
					1							13.8 x 109.3
124	33/3	50	25	25								
	33/1		25	25								
125	52/2	50	25	22	1							13.0 x 118.5
								1				11.3 x 118.0
					1							10.1 x 98.1
	52/3		25	25								
130	98/3	50	25	25								
	98/4		25	24			1					14.4 x 113.2
127	124/4	50	25	20	1							14.9 x 103.2
					1							14.9 x 98.9
					1							14.3 x 114.0
								1				14.1 x 94.8
					1							14.0 x 82.2

APPENDIX TABLE CA-MD-M (continued)

1,1,2,2-Tetrachloroethane
Males

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
127	124/5		25	23	1							14.8 x 116.8
								1				14.2 x 99.8
128	75/5	50	25	25								
	75/1		25	24	1							14.1 x 107.8
122	136/4	50	25	23	1							10.0 x 88.9
						1						9.3 x 99.4
	136/1		25	21	1							8.5 x 117.3
						1		1				7.8 x 97.8
					1							7.8 x 120.1
					1							7.6 x 86.1
123	89/2	50	25	23	1							10.4 x 96.2
					1							9.8 x 95.8
	89/1		25	24	1							8.2 x 114.3
129	16/2	50	25	23	2							9.3 x 102.3
					1							8.9 x 93.5
	16/3		25	25								

APPENDIX TABLE CA-MD M (continued)

1,1,2,2-Tetrachloroethane
Males

Multiple Dosing: 5 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		
133	67/2	50	25	24	1							12.3 x 92.9
	67/3		25	25								
135	153/5	50	25	25								
	153/2		25	23	1							14.8 x 96.5
									1			14.5 x 117.0
139	54/5	50	25	24	1							12.5 x 115.3
	54/4		25	21	1							10.3 x 86.9
					1							10.2 x 90.0
								1				9.3 x 87.5
					1							8.9 x 95.2
138	114/4	50	25	21				1				12.0 x 109.9
					1							12.1 x 107.1
								1				10.9 x 112.5
								1				10.8 x 94.6
	114/2		25	23	1							9.3 x 117.0
					1							8.8 x 98.3
137	113/1	50	25	24	1							14.1 x 115.0
	113/2		25	24		1						12.1 x 108.2

APPENDIX TABLE CA-MD-M (continued)

1,1,2,2-Tetrachloroethane
Males

Multiple Dosing: 5 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		
132	3/2	50	25	21	1			2				12.3 x 117.2
					1							12.3 x 108.0
						1						12.1 x 99.5
								1				10.4 x 98.0
	3/4		25	25								
140	117/5	50	25	24	1							10.0 x 108.2
	117/1		25	23	1							10.9 x 101.9
					1							10.0 x 108.2
131	35/1	50	25	25								
	35/2		25	23	1							14.0 x 98.8
										1		13.5 x 111.8
134	45/3	50	25	25								
	45/2		25	24	1							8.8 x 110.2
136	63/2	50	25	25								
	63/4		25	21	1							14.9 x 100.6
					1							13.9 x 114.5
					1							13.0 x 107.6
								2				7.8 x 104.8

APPENDIX TABLE CA-MD-M (continued)

1,1,2,2-Tetrachloroethane
Males

Multiple Dosing: 50 ppm

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
141	24/3	50	25	24	1							14.5 x 107.0
	24/2		25	22	1							9.2 x 104.7
								1				9.2 x 90.3
					1							9.2 x 100.4
144	7/2	50	25	22	1							12.6 x 104.5
					1							12.2 x 104.3
					1							9.0 x 118.2
	7/3		25	23	1							8.0 x 92.0
					1							6.9 x 99.2
148	149/2	50	25	25								
	149/5		25	24	1							13.7 x 96.9
149	109/5	50	25	23	1							8.4 x 99.2
								1				8.1 x 89.2
	109/1		25	21	1							11.1 x 90.0
					1							10.7 x 119.5
								1				10.7 x 117.7
					1							10.8 x 90.0

APPENDIX TABLE CA-MD-M (continued)

1,1,2,2-Tetrachloroethane
Males

Multiple Dosing: 50 ppm

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
142	107/1	50	25	24	1							9.1 x 90.0
	107/2		25	24				1				8.2 x 95.3
145	36/1	50	25	24	1							9.8 x 117.2
	36/2		25	24	1							7.0 x 113.1
147	50/5	50	25	23				1				9.5 x 103.5
								1				7.9 x 105.5
	50/3		25	24	1							10.4 x 102.2
143	8/1	50	25	25								
	8/4		25	25								
150	86/5	50	25	23	1							12.4 x 92.0
					1							11.9 x 95.5
	86/3		25	25								
146	108/3	50	25	24	1							14.8 x 109.8
	108/2		25	22	1							14.8 x 86.4
					1							14.3 x 98.8
								1				14.3 x 111.0

APPENDIX TABLE CA-MD-M (continued)

1,1,2,2-Tetrachloroethane

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		
157	72/2	50	25	24	1							15.0 x 102.0
	72/3		25	25								
159	76/1	50	25	24	1							10.9 x 101.0
	76/4		25	25								
151	102/1	50	25	23	1							13.5 x 108.5
					1							13.5 x 107.3
	102/4		25	20	1							14.8 x 104.9
					1							14.8 x 101.0
					1							14.6 x 95.5
					1							14.4 x 88.0
					2							14.4 x 89.0
152	126/5	50	25	25								
	126/3		25	22				1				11.9 x 116.5
					1							11.5 x 119.1
					1							11.0 x 101.0
153	111/5	50	25	20				1				12.6 x 106.4
					2							13.2 x 103.2
					1	1						12.2 x 95.1
								1				11.8 x 112.8
								1				11.7 x 102.0

APPENDIX TABLE CA-MD-M (continued)

1,1,2,2-Tetrachloroethane
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	111/3		25	21	1							13.0 x 128.3
					1							11.6 x 111.8
					1							11.3 x 108.8
					1						1 Exchange	10.8 x 112.0
156	31/2	50	25	25								
	31/3		25	22	1							13.3 x 102.2
					1	1						13.2 x 112.1
					1							13.1 x 118.1
158	141/1	50	25	22	1							13.2 x 108.3
					1		1					12.3 x 113.8
					1							11.5 x 106.5
	141/4		25	25								
154	12/4	50	25	17	1							11.2 x 111.1
											1 Exchange	11.1 x 108.3
					1							11.0 x 108.2
								1				11.2 x 97.6
						2						10.6 x 104.5
						1						10.6 x 109.1
											Multi Aberration	10.8 x 110.0
					1	1					Chromosomal Fragment	10.1 x 106.9

APPENDIX TABLE CA-MD-M (continued)

1,1,2,2-Tetrachloroethane
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		
154	12/3		25	16		1						14.2 x 103.2
						1						0.5 x 105.2
											1 Exchange	33.2 x 109.5
					1							33.2 x 110.0
					2							33.2 x 110.4
						1						33.1 x 111.2
					1							32.3 x 106.5
						1						32.0 x 101.0
								1				31.8 x 104.2
155	154/5	50	25	22	1							6.4 x 104.5
						1						6.3 x 101.6
					1							25.7 x 94.8
	154/4		25	23	2							10.8 x 101.0
					1							10.8 x 98.2
160	20/5	50	25	23	1							13.0 x 111.1
								1				12.1 x 97.4
	20/1		25	21	1							11.6 x 101.0
					1							11.5 x 105.2
					1							10.9 x 107.1
					1							10.5 x 107.0

APPENDIX TABLE CA-MD-F

1,1,2,2-Tetrachloroethane
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		
285	212/3	50	25	25								
	212/5		25	22	1							13.8 x 113.8
					1							13.5 x 102.5
					1							12.8 x 103.0
290	258/1	50	25	24	1							7.3 x 105.8
	258/2		25	22				1				8.3 x 101.5
					1							8.4 x 98.1
											1 Chromosomal Fragment	8.3 x 117.6
286	202/5	50	25	24	1							15.0 x 110.2
	202/2		25	25								
287	284/2	50	25	22	1							11.8 x 107.5
					1							11.2 x 114.9
					1							10.4 x 99.0
	284/3		25	23	1							10.2 x 101.4
											1 Chromosomal Fragment	10.1 x 98.6
289	176/3	50	25	24	1							11.7 x 104.2
	176/4		25	23	1							9.9 x 104.0
					1							9.9 x 100.5
283	249/4	50	25	24							1 Chromosomal Fragment	10.6 x 90.3
	249/5		25	22	1							10.3 x 91.6
					1							10.3 x 98.3
					1							8.4 x 93.1

APPENDIX TABLE CA-MD-F (continued)

1,1,2,2-Tetrachloroethane
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		
282	296/4	50	25	22	1							14.2x 114.2
					1							14.2 x 113.0
					1							14.0 x 110.3
	296/2		25	25								
284	193/1	50	25	23	1							5.5 x 105.3
					1							4.9 x 114.1
	193/2		25	22				1				9.7 x 96.9
					1							9.6 x 100.6
					1							9.0 x 105.0
288	235/4	50	25	24	1							12.7 x 110.8
	235/3		25	25								
281	164/1	50	25	22	1							11.7 x 97.5
								1				11.2 x 95.0
					1							11.2 x 92.6
	164/2		25	21				1				14.5 x 117.9
					1							14.7 x 99.8
					1							13.9 x 116.2
					1							13.6 x 103.4

APPENDIX TABLE CA-MD-F (continued)

1,1,2,2-Tetrachloroethane
Females

Multiple Dosing: 5 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		
293	227/4	50	25	22	1							11.4 x 104.2
					1							11.5 x 106.6
								1				11.3 x 115.2
	227/1		25	24	1							14.6 x 97.8
295	313/4	50	25	22	1							12.8 x 91.7
					1							11.7 x 103.0
					1							11.4 x 121.5
	313/5		25	22	1							14.6 x 115.4
					2							13.8 x 91.8
					1							13.5 x 92.0
297	273/2	50	25	25								
	273/5		25	24	1							8.4 x 105.3
296	223/2	50	25	24	1							11.0 x 97.2
	223/1		25	22	1							12.3 x 119.3
					1							11.5 x 106.8
					1							11.5 x 97.5

APPENDIX TABLE CA-MD-F (continued)

1,1,2,2-Tetrachloroethane
Females

Multiple Dosing: 5 ppm

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
299	214/2	50	25	25								
	214/5		25	24	1							12.0 x 86.2
298	274/2	50	25	22		1						9.0 x 110.1
					1							9.1 x 109.0
					1							8.0 x 101.9
	274/1		25	24	1							9.7 x 107.2
292	163/2	50	25	24	1							12.9 x 98.4
	163/3		25	24	1							13.5 x 104.1
294	205/3	50	25	24	1							13.1 x 99.1
	205/4		25	24	1							13.2 x 94.2
291	195/2	50	25	23	1							10.3 x 119.0
								1				9.7 x 116.6
	195/3		25	25								
300	277/2	50	25	25								
	277/5		25	25								

APPENDIX TABLE CA-MD-F (continued)

1,1,2,2-Tetrachloroethane
Females

Multiple Dosing: 50 ppm

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
301	184/2	50	25	23				1				8.9 x 88.6
					1							8.9 x 88.3
	184/3		25	24	1							10.2 x 98.2
305	196/5	50	25	25								
	196/4		25	22	1							9.2 x 94.0
					1							8.9 x 92.3
								1				7.6 x 88.7
306	268/3	50	25	23	1							13.8 x 108.1
								1				13.6 x 104.1
	268/4		25	23	1							12.9 x 116.2
					1							12.8 x 103.9
302	267/3	50	25	23				1				12.0 x 100.6
					1							12.0 x 97.7
	267/4		25	24	1							8.9 x 101.5

APPENDIX TABLE CA-MD-F (continued)

1,1,2,2-Tetrachloroethane
Females

Multiple Dosing: 50 ppm

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
308	305/5	50	25	25								
	309/3		25	25								10.9 x 112.0
310	246/2	50	25	24	1							
	246/3		25	25								
307	210/2	50	25	24				1				10.4 x 105.1
	210/3		25	23	1							10.8 x 112.0
					1							8.7 x 112.4
303	168/5	50	25	25								
	168/3		25	25								
309	269/3	50	25	24	1							13.5 x 112.9
	269/2		25	22	1							12.4 x 109.5
					1							10.8 x 97.0
					1							10.4 x 107.5
304	167/4	50	25	24							1 Chromosomal Fragment	10.1 x 96.9
	167/1		25	24	1							8.9 x 120.1

APPENDIX TABLE CA-MD-F (continued)

1,1,2,2-Tetrachloroethane
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		
319	236/2	50	25	22	1			1				11.1 x 108.6
					1							9.2 x 115.2
					1							9.3 x 105.5
	236/1		25	17	1							14.1 x 104.9
					2							14.2 x 100.0
					1							13.6 x 94.0
										1		13.3 x 98.4
								1				13.5 x 101.5
					1							13.3 x 105.3
					1							13.1 x 100.0
					1							13.2 x 96.0
311	262/2	50	25	23	1							12.2 x 100.2
					1							11.4 x 97.1
	262/4		25	23	1							10.4 x 110.0
					1							10.1 x 110.0
317	232/2	50	25	24							1 Chromatid Fragment	10.1 x 97.0
	232/4		25	23		1						10.3 x 89.0
					1							10.4 x 90.1
320	180/3	50	25	24	1							11.7 x 107.0
	180/2		25	22	1							12.9 x 116.1
								1				13.0 x 105.3
					1							12.8 x 108.6

APPENDIX TABLE CA-MD-F (continued)

1,1,2,2-Tetrachloroethane
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		
315	314/4	50	25	24	1							10.0 x 111.9
	314/2		25	25								
318	301/4	50	25	24	1							13.7 x 102.0
	301/3		25	24	1							13.4 x 100.4
313	271/3	50	25	19		1						11.1 x 116.0
					1							11.2 x 115.0
					1			1			1 Exchange 1 Chromosomal Fragment	11.2 x 106.5
					1						1 Chromatid Fragment	10.4 x 90.4
						1						10.5 x 100.8
					1							10.1 x 116.7
	271/2		25	19	1							10.8 x 99.0
					1							10.1 x 99.0
					1						1 Chromatid Fragment	9.8 x 114.9
					1							9.4 x 96.2
					1							9.8 x 94.6
					1	2		1				9.7 x 93.0
312	286/3	50	25	21				1				11.7 x 117.9
					1							10.1 x 114.7
					1							10.0 x 115.9
					1							9.0 x 101.7

APPENDIX TABLE CA-MD-F (continued)

1,1,2,2-Tetrachloroethane
Females

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
312	286/5		25	20							1 Chromatid Fragment	11.7 x 114.8
								1				11.1 x 94.2
					1							11.0 x 94.0
					2							10.2 x 92.7
					1							10.0 x 117.2
314	172/2	50	25	23		1						11.3 x 96.0
					1							11.0 x 98.1
	172/4		25	25								
316	191/5	50	25	22	2	1						10.3 x 101.2
						1						10.1 x 88.4
					1							7.8 x 108.8
	191/4		25	21	1							12.2 x 104.0
						1					1 Dicentric	10.7 x 105.0
					1							9.8 x 112.0
					1							5.8 x 86.9

APPENDIX TABLE CA-M6

1,1,2,2-Tetrachloroethane
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		
2	95/1	50	25	24	1							115.5 x 9.0
	95/2		25	23				1				105.0 x 10.0
					1							117.6 x 10.8
4	6/1	50	25	25								
	6/2		25	24	1							98.8 x 7.0
5	26/1	50	25	25								
	26/2		25	22	1							120.8 x 4.0
											1 Chromatid Fragment	123.5 x 8.5
											1 Chromatid Fragment	92.2 x 8.5
9	101/5	50	25	25								
	101/4		25	25								
7	135/1	50	25	21	1							121.4 x 3.5
					1							125.5 x 3.5
											1 Chromatid Fragment	117.2 x 4.0
					1							104.3 x 4.8
	135/3		25	24	1							125.4 x 8.5
10	65/1	50	25	24	2							106.2 x 4.8
	65/2		25	25								
3	121/2	50	25	25								
	121/1		25	25								

APPENDIX TABLE CA-M6 (continued)

1,1,2,2-Tetrachloroethane

Males

Single Dosing: 5 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		
37	83/1	50	25	23	1							136.1 x 5.0
					1							111.8 x 8.0
	83/2		25	24	1							116.0 x 7.0
38	122/5	50	25	25								
	122/1		25	25								
31	84/2	50	17	16		1						27.0 x 69.5
	84/1		8	8								
	84/4		25	24	1							26.0 x 66.6
32	62/5	50	25	24					1			24.5 x 72.6
	62/1		25	25								
39	47/1	50	25	24	1							33.8 x 105.8
	47/4		25	24		1						38.4 x 100.3
35	91/1	50	25	25								
	91/3		25	25								
34	93/1	50	25	25								
	93/4		25	25								
33	150/1	50	25	24	1							109.0 x 6.9
	150/2		25	24							1 Chromatid Fragment	
40	1/1	50	25	24	1							113.4 x 16.1
	1/2		25	21	1							122.1 x 21.0
					1	1						117.8 x 18.0
											1 Chromosomal Fragment	131.2 x 18.0
					1							129.4 x 18.5
36	58/4	50	25	24							1 Robertsonian Translocation	28.3 x 105.5
	58/5		25	25								

APPENDIX TABLE CA-M6 (continued)

1,1,2,2-Tetrachloroethane

Males

Single Dosing: 50 ppm

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
63	71/4	50	25	24							1 Chromatid Fragment	141.4 x 18.0
	71/1		25	21							1 Chromosomal Fragment	118.9 x 6.2
										1		108.2 x 8.2
									1			137.8 x 19.0
											1 Chromatid Fragment	95.5 x 22.5
61	43/1	50	25	24	1							139.4 x 6.5
	43/3		25	24	1							87.8 x 10.5
69	48/2	50	25	25								
	48/4		25	24	1							120.1 x 7.5
62	148/1	50	25	24				1				22.5 x 101.2
	148/2		25	23	1							26.2 x 96.2
					1							36.2 x 83.0
68	70/2	50	25	25								
	70/4		25	24	1							130.2 x 7.3
67	133/2	50	25	25								
	133/5		25	25								
70	142/1	50	25	25								
	142/2		25	23	1							131.0 x 5.0
											1 Chromatid Fragment	109.2 x 5.0
65	29/1	50	25	24	1							134.8 x 8.2
	29/5		25	24		1						140.1 x 8.3
66	85/4	50	25	24	1							25.3 x 77.0
	85/1		15	14							1 Chromosomal Fragment	21.2 x 99.0
	85/2		10	10								

APPENDIX TABLE CA-M6 (continued)

1,1,2,2-Tetrachloroethane
Males

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
95	77/1	50	25	25								
	77/2		25	25								
91	131/2	50	25	24	1							126.0 x 7.5
	131/3		25	25								
98	106/1	50	25	19			1					103.1 x 4.7
							1					95.8 x 4.8
					1							132.5 x 6.1
					1							98.1 x 8.2
							1					131.2 x 8.9
					1							120.2 x 11.5
	106/2		25	22			1					127.5 x 7.8
					1							130.5 x 17.0
						1						100.0 x 19.2
93	80/2	50	22	21			1					117.1 x 11.1
	80/1		3	3								
	80/3		25	24	1							107.9 x 3.1

APPENDIX TABLE CA-M6 (continued)

1,1,2,2-Tetrachloroethane

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	82/1	50	25	22	1							116.0 x 3.5
					1							108.7 x 8.0
					1							137.1 x 11.3
	82/2		25	23	1							122.5 x 4.3
					1							131.4 x 8.5
99	87/1	50	25	25								
	87/2		23	23								
	87/3		2	2								
100	41/4	50	25	25								
	41/5		25	25								
97	38/3	50	25	24	1							22.5 x 85.2
	38/5		25	25								
92	156/2	50	25	23	1						1 Robertsonian Translocation	105.0 x 6.2
					2							105.0 x 8.2
	156/3		25	23			1					105.2 x 6.2
					1							103.6 x 15.2
96	139/1	50	25	23	1							133.0 x 16.0
					1							124.5 x 17.2
	139/2		25	25								

APPENDIX TABLE CA-M24

1,1,2,2-Tetrachloroethane
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		
16	10/5	50	25	24	1							52.8 x 103.8
	10/1		25	25								
20	119/5	50	25	25								
	119/2		25	25								
17	71/5	50	25	25								
	71/1		25	25								
15	116/5	50	25	25								
	116/1		25	25								
19	74/2	50	25	25								
	74/4		25	25								
11	18/1	50	25	23	1							34.1 x 109.0
					1							28.6 x 107.0
	18/4		25	24	1							52.2 x 108.2
12	127/2	50	25	25								
	127/5		25	25								
14	130/3	50	25	23	1							56.0 x 109.0
					1							60.0 x 104.6
	130/5		25	25								

APPENDIX TABLE CA-M24 (continued)

1,1,2,2-Tetrachloroethane

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	55/3	4	2	2								
	55/2		1	1								
	55/1		1	1								
	55/4		0	0								
	55/5		0	0								
13	152/4	50	25	24	1							40.2 x 107.8
	152/5		25	23	1							41.0 x 107.0
					1							67.0 x 102.8

APPENDIX TABLE CA-M24 (continued)

1,1,2,2-Tetrachloroethane

Males

Single Dosing: 5 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		
46	57/1	50	25	24	1							40.8 x 105.1
	57/3		25	25								
49	137/3	50	25	25								
	137/5		25	25								
45	118/1	50	25	25								
	118/4		25	23	2							35.9 x 108.6
					1							64.7 x 103.8
43	159/5	50	25	25								
	159/4		25	25								
41	78/1	50	25	25								
	78/2		25	25								
44	40/3	50	25	23	2							56.6 x 110.1
					1							41.8 x 105.8
	40/2		25	23	1							31.5 x 109.0
					1							65.3 x 107.6
50	34/4	50	25	24		1						87.5 x 103.6
	34/1		25	25								

APPENDIX TABLE CA-M24 (continued)

1,1,2,2-Tetrachloroethane
Males

Single Dosing: 5 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	19/2	19	5	5								
	19/4		7	7								
	19/3		1	1								
	19/5		4	4								
	19/1		2	2								
47	151/2	50	25	25								
	151/4		25	25								
48	143/4	50	25	24	1							26.4 x 103.7
	143/5		25	25								

APPENDIX TABLE CA-M24 (continued)

1,1,2,2-Tetrachloroethane

Males

Single Dosing: 50 ppm

Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
72	138/5	50	25	25								
	138/4		25	25								
75	105/5	50	25	25								
	105/2		25	25								
78	27/5	50	25	25								
	27/4		25	25								
76	104/2	50	25	24	1							59.3 x 107.8
	104/3		25	25								
73	44/2	50	25	25								
	44/3		25	25								
79	15/3	50	25	25								
	15/5		25	24	1							36.8 x 110.0
77	46/2	50	25	25								
	46/5		25	25								
74	134/1	50	25	25								
	134/4		25	24	1							26.0 x 105.6
71	99/4	50	25	21	1							60.5 x 109.8
					1							63.4 x 108.6
								1				65.1 x 107.0
					1							29.0 x 106.0
	99/3		25	22	1							62.7 x 108.7
					1							53.6 x 107.0
											1 Misc Aberration	61.4 x 104.0

APPENDIX TABLE CA-M24 (continued)

1,1,2,2-Tetrachloroethane

Males

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
104	86/4	50	25	20	1							60.0 x 109.0
					1							55.0 x 108.5
					2							57.0 x 107.6
					1							29.0 x 106.8
					1							35.5 x 105.0
	86/1		25	21	1							54.0 x 106.7
					1							45.0 x 107.0
					1							40.2 x 102.4
					1							38.0 x 101.8
108	132/5	50	25	23	1							54.8 x 105.6
					1							55.4 x 105.5
	132/4		25	23	1							40.6 x 105.8
					3							59.9 x 102.7
105	69/5	50	25	25								
	69/4		25	24	2							37.7 x 106.8
109	14/5	50	25	20	1							62.3 x 110.1
					1							66.0 x 109.8
					1							63.6 x 109.3
					1							61.0 x 105.5
					1							63.7 x 102.0
	14/2		25	22	1							59.2 x 109.4
					2							59.0 x 108.0
					1							29.1 x 102.0

APPENDIX TABLE CA-M24 (continued)

1,1,2,2-Tetrachloroethane

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		
103	155/4	50	25	23	1							32.5 x 110.0
					1							33.7 x 108.1
	155/4		25	24	1							41.0 x 104.9
107	92/3	50	25	24	1							53.1 x 104.3
	92/2		25	23	2							60.9 x 108.7
					1							66.5 x 107.7
101	49/2	50	25	22	2							40.7 x 109.9
					1							40.5 x 109.3
					1							38.6 x 107.9
	49/4		25	24	1							40.3 x 107.0
102	56/3	50	25	24	1							28.8 x 104.5
	56/2		25	24	1							28.3 x 103.6
110	145/4	50	25	22	1							34.7 x 109.8
					1							54.8 x 108.0
					1							47.9 x 107.2
	145/5		25	24	1							38.3 x 106.6

APPENDIX TABLE CA-M48

1,1,2,2-Tetrachloroethane
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		
22	51/2	50	25	24	1							19.1 x 101.0
	51/5		25	23	1							10.4 x 104.9
					1							10.0 x 105.8
24	32/2	50	25	25								
	32/3		25	22		2						20.9 x 95.9
					1							20.0 x 96.4
					1							18.2 x 121.2
21	66/1	50	25	25								
	66/3		25	25								
25	144/1	50	25	21	1							22.9 x 99.9
					1							22.1 x 91.1
					1							22.0 x 96.8
					1							21.2 x 106.1
	144/5		25	21	1							25.1 x 100.4
						1		1				18.9 x 105.5
					1							17.9 x 98.9
					1			1				15.8 x 111.5
28	160/3	50	25	23	1							21.2 x 106.2
						1						21.5 x 105.8
	160/5		25	23	1							18.0 x 86.9
					1							16.8 x 103.6
26	158/2	50	25	25								
	158/5		25	25								

APPENDIX TABLE CA-M48 (continued)

1,1,2,2-Tetrachloroethane

Males

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
30	68/2	50	25	21	1							20.9 x 123.5
					1							20.1 x 119.0
					1							18.9 x 113.0
						1						16.1 x 107.0
	68/5		25	25								
27	28/1	50	25	21	1							25.0 x 90.0
					1							22.9 x 96.0
								1				17.9 x 103.0
					1							7.9 x 74.0
	28/4		25	24	1							25.5 x 107.1
29	140/2	50	25	25								
	140/5		25	25								
23	157/3	50	25	25								
	157/4		25	25								

APPENDIX TABLE CA-M48 (continued)

1,1,2,2-Tetrachloroethane

Males

Single Dosing: 5 ppm

Sampling Time: 48 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
52	112/1	50	25	25								
	112/4		25	25								
51	39/3	50	25	24	1							20.1 x 105.8
	39/5		25	24		1						17.0 x 121.2
58	11/2	50	25	25								
	11/4		25	25								
56	59/1	50	25	24	1							19.1 x 76.6
	59/3		25	23	1							19.9 x 96.9
						1						19.8 x 86.9
60	100/1	50	25	25								
	100/5		25	25								
54	115/1	50	25	25								
	115/2		25	23	1							19.8 x 98.1
											1 Chromatid Fragment	19.8 x 106.6
59	129/4	50	25	25								
	129/3		25	25								
57	22/1	34	12	11	1							6.1 x 102.0
	22/2		5	4	1							5.8 x 119.9
	22/3		9	9								
	22/4		3	2	1							19.2 x 96.1
	22/5		5	4				1				19.2 x 118.2
53	120/5	50	25	25								
	120/3		25	25								
55	103/2	50	25	24	1							24.0 x 114.0
	103/4		25	23	2							24.6 x 90.0
					1							24.6 x 93.5

APPENDIX TABLE CA-M48 (continued)

1,1,2,2-Tetrachloroethane

Males

Single Dosing: 50 ppm

Sampling Time: 48 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
81	2/2	50	25	23	1							18.1 x 101.8
					1							17.1 x 101.0
	2/4		25	25								
82	128/3	50	25	25								
	128/5		25	23	1							25.2 x 118.0
					1							23.4 x 91.8
86	147/1	7	0	0								
	147/2		0	0								
	147/3		0	0								
	147/4		7	7								
	147/5		0	0								
89	64/1	50	25	25								
	64/5		25	25								
84	94/4	50	25	24	1							23.2 x 103.0
	94/5		25	25								
83	73/1	50	25	24	1							19.0 x 76.1
	73/5		25	25								
90	9/3	50	25	24	1							18.1 x 104.5
	9/4		25	24	1							23.8 x 108.0

APPENDIX TABLE CA-M48 (continued)

1,1,2,2-Tetrachloroethane
Males

Single Dosing: 50 ppm

Sampling Time: 48 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
87	146/2	50	25	24	1							16.1 x 98.8
	146/3		25	23	1							18.1 x 115.1
								1				18.0 x 115.1
88	88/3	50	25									
	88/4		25	23	1							15.2 x 113.0
					1							12.1 x 99.1
85	60/4	50	25	25								
	60/5		25	25								

APPENDIX TABLE CA-M48 (continued)

1,1,2,2-Tetrachloroethane

Males

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
118	96/1	50	25	24	1							21.9 x 112.3
	96/2		25	22	1							18.1 x 82.1
					1							17.2 x 88.5
						1						17.2 x 101.2
115	30/3	50	25	23	1	1						20.1 x 98.0
					1							16.0 x 75.2
	30/5		25	23		1						17.2 x 100.1
					1							16.1 x 78.2
120	23/3	50	25	23	1							19.1 x 100.9
						1						16.8 x 105.9
	23/4		25	24		1						17.0 x 92.9
111	79/2	50	25	24		1						16.0 x 85.1
	79/4		25	21		3					1 Chromatid Fragment	21.5 x 90.4
						1					1 Chromatid Fragment	19.1 x 109.2
						1					1 Chromatid Fragment	16.6 x 112.5
					1							16.5 x 111.4
116	61/3	50	25	24	1							20.8 x 96.0
	61/4		25	24	1							19.1 x 112.0
119	110/1	50	25	24	1							18.2 x 86.0
	110/2		25	25								
112	17/3	50	25	24	1							17.9 x 90.0
	17/5		25	25								

APPENDIX TABLE CA-M48 (continued)

1,1,2,2-Tetrachloroethane
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		
114	90/2	50	25	22		1						18.9 x 103.1
					1	1						16.4 x 94.2
					1							16.8 x 100.1
	90/5		25	23	1							20.1 x 113.9
					1							17.0 x 106.5
117	53/1	50	25	24	1							21.0 x 111.1
	53/4		25	25								
113	13/2	50	25	23	1							20.9 x 96.2
					1							17.0 x 99.1
	13/5		25	24	1							23.1 x 92.0

APPENDIX TABLE CA-F6

1,1,2,2-Tetrachloroethane
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Females

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
163	281/1	50	25	24	1							133.0 x 14.7
	281/2		25	25								
164	166/2	50	25	22	1							126.5 x 5.0
					1							97.8 x 6.0
						1						104.0 x 6.0
	166/3		25	24	1							106.1 x 6.8
169	261/3	50	25	25								
	261/4		25	23	1							134.5 x 4.8
					1							135.3 x 5.8
170	225/3	50	25	24	1							26.8 x 89.0
	225/2		21	18		1						25.4 x 80.0
					1							25.2 x 95.6
					1							30.5 x 70.8
	225/5		4	4								
161	165/2	50	25	25								
	165/3		25	23	1							23.3 x 102.5
								1				24.8 x 78.5

APPENDIX TABLE CA-F6 (continued)

1,1,2,2-Tetrachloroethane
Females

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
165	186/1	50	25	24	1							96.1 x 1.0
	186/3		25	23							1 Chromatid Fragment	120.1 x 3.9
					1							133.3 x 3.2
162	255/1	50	25	23	1							123.0 x 11.1
					1							128.0 x 19.1
	255/2		25	23			1					104.2 x 5.1
					1							137.5 x 7.5
168	283/1	50	25	24	1							37.5 x 82.3
	283/2		25	24	1	1						26.2 x 92.2
166	285/4	50	25	25								
	285/5		25	23	1							141.7 x 8.2
								1				99.9 x 9.8
167	295/5	50	25	24	1							116.7 x 5.0
	295/4		25	25								

APPENDIX TABLE CA-F6 (continued)

1,1,2,2-Tetrachloroethane
Females

Single Dosing: 5 ppm

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
198	282/1	50	25	22					1			135.0 x 8.5
					1							95.2 x 10.1
											1 Chromatid Fragment	130.1 x 13.5
	282/4		21	18				1				139.2 x 11.0
											1 Chromatid Fragment	138.0 x 18.0
								1				102.0 x 21.0
	282/5		4	3	1							138.0 x 8.8
193	310/1	50	25	24							1 Translocation	99.1 x 9.0
	310/2		25	24	1							121.1 x 12.8
197	243/1	50	20	19				1				33.0 x 68.9
	243/2		5	5								
	243/3		25	25								
199	207/2	50	25	25								
	207/4		25	25								

APPENDIX TABLE CA-F6 (continued)

1,1,2,2-Tetrachloroethane
Females

Single Dosing: 5 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		
192	222/1	50	25	25								
	222/3		25	24	1							26.0 x 88.5
194	253/1	50	25	24	1							37.3 x 95.8
	253/5		17	17								
	253/2		8	8								
195	251/1	50	25	24			1					128.9 x 21.0
	251/1		25	25								
200	161/5	50	20	20								
	161/3		5	5								
	161/1		25	25								
196	218/1	50	25	24	1							27.5 x 100.0
	218/5		25	24	1							30.3 x 68.8
191	244/1	50	25	25								
	244/2		25	22	1							121.1 x 5.1
					1							106.3 x 4.9
					1							96.7 x 5.2

APPENDIX TABLE CA-F6 (continued)

1,1,2,2-Tetrachloroethane
Females

Single Dosing: 50 ppm

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
226	245/2	50	25	21	1							133.5 x 7.0
											1 Translocation	133.8 x 7.1
					1							129.0 x 9.0
							1					122.2 x 11.9
	245/3		25	24	1							102.0 x 7.0
229	185/2	50	25	23							1 Chromatid Fragment	115.5 x 6.5
					1							119.2 x 7.0
	185/3		25	23	2							127.2 x 3.8
					1							112.4 x 5.0
221	203/2	50	25	23	1							120.1 x 5.2
											1 Chromatid Fragment	118.5 x 4.5
	203/3		25	23	1							105.0 x 5.0
					1							131.0 x 6.8
227	293/1	50	25	22	1							103.8 x 2.1
					1							138.0 x 5.0
					1							116.0 x 5.5
	293/2		25	23			1					129.2 x 5.0
											1 Chromatid Fragment	111.0 x 7.5

APPENDIX TABLE CA-F6 (continued)

1,1,2,2-Tetrachloroethane
Females

Single Dosing: 50 ppm

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
222	308/1	50	21	21				1				110.0 x 12.2
	308/2		4	4								
	308/3		25	24			1					118.1 x 7.0
230	302/1	50	25	25								
	302/2		25	25								
228	230/1	50	25	23				1				97.7 x 4.1
											1 Chromatid Fragment	97.2 x 5.6
	230/2		25	24	1							120.1 x 4.2
225	189/1	50	25	22							1 Robertsonian Translocation	109.9 x 5.1
											1 Robertsonian Translocation	137.5 x 8.2
											1 Robertsonian Translocation	123.0 x 9.9
	189/2		25	25								
223	181/1	50	25	22							1 Chromosomal Fragment	133.0 x 6.5
											1 Robertsonian Translocation	135.0 x 7.2
											1 Robertsonian Translocation	134.8 x 7.2
	181/2		25	25								
229	208/1	50	25	25								
	208/2		25	25								

APPENDIX TABLE CA-F6 (continued)

1,1,2,2-Tetrachloroethane

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		
257	198/1	50	25	22			1					130.9 x 6.1
					1							128.5 x 7.0
					1							127.0 x 7.8
	198/2		25	24							1 Chromatid Fragment	103.6 x 11.0
258	266/2	50	25	23	1							116.1 x 8.2
					1							124.6 x 7.7
	266/4		25	20					1			107.2 x 5.1
					1	1						96.4 x 6.2
					1							111.9 x 6.2
					1							119.7 x 7.2
								1	1			114.3 x 6.7
259	247/3*	50	11	11								
	247/3*		14	14								
	247/4		13	13								
	247/2		12	12								
252	316/1	50	25	24	1							28.2 x 98.2
	316/3		25	24							1 Robertsonian Translocation	33.2 x 69.8
260	201/1	50	22	21	1							36.2 x 71.6
	201/2		3	3								
	201/4		25	24		1						27.5 x 106.0
254	242/1	50	25	23	1							32.1 x 68.2
					1							34.1 x 94.2
	242/4		25	25								

* 2 Slides numbered 247/3

APPENDIX TABLE CA-F6 (continued)

1,1,2,2-Tetrachloroethane
Females

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
253	240/1	50	25	24			1					108.1 x 7.5
	240/3		25	23				1				110.2 x 7.0
											1 Chromatid Fragment	133.2 x 6.9
256	299/1	50	25	22	1							129.8 x 3.0
							1					118.5 x 6.0
					1							121.5 x 6.9
	299/2		25	22							1 Robertsonian Translocation	112.0 x 3.9
					1							115.0 x 3.9
					1							124.8 x 3.7
251	291/1	50	25	21	1							104.2 x 13.5
					1							108.8 x 13.2
					1							126.8 x 15.1
								1				132.0 x 19.1
	291/2		25	20				1				133.6 x 3.2
							1					119.0 x 3.2
											1 Chromosomal Fragment	112.2 x 3.0
					1							114.0 x 4.0
								1				135.1 x 4.0
255	237/1	50	25	23	1							23.2 x 88.0
								1				23.8 x 98.5
	237/2		25	25								

APPENDIX TABLE CA-F24

1,1,2,2-Tetrachloroethane
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Females

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
176	170/5	50	25	24	1							62.5 x 104.8
	170/1		25	25								
179	234/2	50	25	25								
	234/4		25	24	1							60.7 x 105.0
174	290/3	50	25	25								
	290/5		25	25								
178	215/3	50	25	25								
	215/4		25	25								
172	287/4	50	25	24	1							62.0 x 103.1
	287/3		25	24	1							35.9 x 103.6
180	279/1	50	25	24	1							50.1 x 107.5
	279/3		25	25								
173	312/5	50	25	25								
	312/1		25	24	1							61.6 x 105.7
171	178/3	50	25	24	1							65.2 x 102.0
	178/5		25	25								
175	276/2	50	25	25								
	276/4		25	24	1							40.8 x 101.7
177	231/3	50	25	25								
	231/1		25	25								

APPENDIX TABLE CA-F24 (continued)

1,1,2,2-Tetrachloroethane
Females

Single Dosing: 5 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		
208	303/2	50	25	25								
	303/1		25	24	1							59.3 x 103.7
210	194/1	50	25	25								
	194/2		25	25								
201	238/5	50	25	25								
	238/3		25	25								
207	311/2	50	25	25								
	311/4		25	25								
203	319/4	50	25	24	1							35.8 x 104.2
	319/2		25	25								
205	278/5	50	25	24	2							32.6 x 109.1
	278/4		25	24	1							56.0 x 104.7
204	200/1	50	25	25								
	200/4		25	25								
209	293/5	50	25	25								
	293/4		25	25								
202	179/2	21	4	3	1							62.6 x 108.0
	179/1		0	0								
	179/3		5	5								
	179/5		9	9								
	179/4		3	3								
206	217/1	50	25	24	1							31.0 x 107.0
	217/2		25	24	1							56.4 x 108.0

APPENDIX TABLE CA-F24 (continued)

1,1,2,2-Tetrachloroethane
Females

Single Dosing: 50 ppm

Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
234	294/3	50	25	25								
	294/2		25	25								
237	206/1	50	25	24	1							64.2 x 104.6
	206/2		25	25								
240	257/2	50	25	25								
	257/5		25	25								
239	175/3	50	25	24	1							44.0 x 104.1
	175/2		25	25								
236	264/3	50	25	23	1							60.0 x 110.8
					1							61.8 x 109.0
	264/2		25	25								
235	265/2	50	20	20								
	265/3		25	25								
	265/1		5	5								
238	187/3	50	25	25								
	187/4		25	25								
232	298/5	50	25	25								
	298/2		25	25								
231	259/2	50	25	25								
	259/1		25	25								
233	204/4	50	25	23	1							33.0 x 107.8
					1							40.0 x 106.4
	204/1		25	25								

APPENDIX TABLE CA-F24 (continued)

1,1,2,2-Tetrachloroethane

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		
267	252/3	50	25	24	1							61.0 x 107.0
	252/5		25	24	1							35.8 x 103.6
264	241/5	50	25	23	1							35.0 x 103.6
					1							35.4 x 103.4
	241/4		25	24	1							45.0 x 102.3
262	216/4	50	25	21	1							36.5 x 107.5
					1							61.0 x 107.7
					1			1				62.4 x 106.8
					1							64.0 x 102.0
	216/1		25	24	1							68.0 x 109.7
266	197/5	50	14	13	1							62.9 x 102.7
	197/4		25	22	1							55.7 x 108.4
					1							57.0 x 106.7
					1							41.9 x 101.1
	197/2		11	8	1							58.2 x 108.7
					1							32.5 x 108.6
					1							29.5 x 104.5
263	315/2	50	25	25								
	315/4		25	25								
261	209/3	50	25	24	1							52.1 x 103.0
	209/2		25	25								
268	292/2	50	25	23	2							35.1 x 105.7
					1							56.7 x 106.0
	292/1		25	25								

APPENDIX TABLE CA-F24 (continued)

1,1,2,2-Tetrachloroethane
Females

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
269	174/2	50	25	23	3							55.1 x 107.0
					1							63.0 x 104.6
	174/5		25	18	1							59.7 x 108.5
					1							50.0 x 105.0
					1							33.2 x 103.8
					2							62.2 x 103.7
					1							38.0 x 102.9
					1							35.6 x 101.8
					1							60.6 x 100.6
270	305/2	50	25	24	2							65.3 x 109.0
	305/4		25	25								
265	229/1	50	25	22	1							57.3 x 108.8
					1							32.1 x 105.3
					1							41.0 x 105.2
	229/4		25	19	1							65.3 x 110.0
					1							65.6 x 109.5
					1							69.0 x 109.4
					1							61.4 x 107.6
					1							66.9 x 106.6
					1							56.8 x 102.7

APPENDIX TABLE CA-F48

1,1,2,2-Tetrachloroethane
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		
182	211/3	50	25	22	1							21.0 x 103.1
					1							17.8 x 86.8
					1							14.9 x 107.9
	211/4		25	25								
183	317/3	50	25	23	1							20.9 x 102.0
					1							20.9 x 105.1
	317/4		25	24	1							20.0 x 102.9
188	320/3	50	25	25								
	320/4		25	25								
190	228/3	50	25	25								
	228/5		25	25								
181	226/1	50	25	24	1							14.0 x 106.5
	226/3		25	25								
184	192/3	50	25	25								
	192/5		25	25								
186	318/2	50	25	24	1							23.2 x 107.8
	318/5		25	25								

APPENDIX TABLE CA-F48 (continued)

1,1,2,2-Tetrachloroethane
Females

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
189	300/2	50	25	22	1							24.2 x 108.8
					1							22.2 x 109.5
					2							21.5 x 95.0
	300/4		25	24	1							25.5 x 77.5
185	304/3	50	25	24	1							18.9 x 105.1
	304/4		25	25								
187	188/1	50	25	24	1							8.1 x 93.4
	188/2		25	23					1			21.0 x 106.2
					1							19.9 x 118.2

APPENDIX TABLE CA-F48 (continued)

1,1,2,2-Tetrachloroethane
Females

Single Dosing: 5 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		
218	171/1	50	25	25								
	171/4		25	23		1						15.1 x 110.5
					1							13.1 x 88.6
217	182/2	50	25	22	1							19.9 x 87.1
					1							18.8 x 104.5
					1							17.4 x 118.0
	182/3		25	23	1							21.8 x 91.5
					1							20.2 x 108.0
212	272/1	50	25	22	1							23.2 x 106.0
					1							21.1 x 99.9
					2							18.5 x 102.8
	272/5		25	25								
214	275/1	50	25	22	1							23.0 x 108.0
								1				18.0 x 119.0
					2							15.0 x 103.0
	275/4		25	25								
213	280/3	50	25	25								
	280/5		25	25								
216	219/3	50	25	25								
	219/4		22	21	1							9.0 x 113.0
	219/5		3	3								

APPENDIX TABLE CA-F48 (continued)

1,1,2,2-Tetrachloroethane

Females

Single Dosing: 5 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		
219	289/4	50	25	25								
	289/3		25	24	1							20.9 x 99.4
211	199/2	50	25	24	1							20.2 x 100.1
	199/4		25	24	1							21.6 x 108.8
220	260/1	50	25	24		1						17.0 x 106.1
					1							15.5 x 116.2
	260/2		25	22	1							19.5 x 77.0
					1							16.1 x 79.2
						1						15.6 x 82.0
215	263/2	50	25	24	1							18.1 x 99.1
	263/3		25	24	1							20.6 x 103.2

APPENDIX TABLE CA-F48 (continued)

1,1,2,2-Tetrachloroethane
Females

Single Dosing: 50 ppm — Sampling Time: 48 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
247	306/2	50	25	25								
	306/3		25	25								
243	233/2	50	25	25								
	233/3		25	24		1						20.8 x 108.1
244	254/3	50	25	25								
	254/4		25	24	1							20.0 x 112.0
246	307/2	50	25	22	1							25.0 x 85.0
					1							15.2 x 88.1
					1							12.9 x 79.1
	307/3		25	24				1				21.1 x 100.8
241	162/2	50	25	25								
	162/3		25	25								
249	224/1	50	25	25								
	224/3		25	23	1							23.1 x 106.1
						1						20.4 x 112.5
250	169/1	50	25	20	1							23.0 x 114.9
								1				22.1 x 111.9
					1							18.2 x 107.8
											1 Chromatid Fragment	16.1 x 81.8
								1				12.9 x 77.1
	169/2		25	24	1							18.1 x 116.1
245	220/1	50	25	25								
			25	23	1							24.0 x 111.2
					1							22.6 x 92.2
242	288/1	50	25	24 4	1							18.0 x 106.0
	288/5		25	24 4	1							19.5 x 114.9
248	284/1	50	25	25 5								
	284/5		25	24 4				1				17.1 x 96.2

APPENDIX TABLE CA-F48 (continued)

1,1,2,2-Tetrachloroethane

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		
275	190/2	50	25	25								
	190/3		25	24	1							19.2 x 102.0
276	221/3	50	25	24	1							21.8 x 82.0
	221/4		25	24							1 Chromatid Fragment	21.9 x 110.1
278	256/1	34	6	6								
	256/2		10	10								
	256/3		6	4	1							15.4 x 88.0
					1							13.0 x 112.1
	256/4		7	7								
	256/5		5	3	1							15.6 x 77.1
					1							11.4 x 115.0
273	173/1	50	25	24	1							23.0 x 108.9
	173/4		25	25								
279	270/2	50	25	24	1							21.2 x 98.4
	270/4		25	25								
272	177/1	50	25	25								
	177/3		25	24	1							19.8 x 98.8
280	183/3	50	25	25								
	183/4		25	21	2							24.1 x 76.0
					1							24.1 x 83.4
					2							23.8 x 109.2
					1							22.9 x 109.2
277	213/2	50	25	25								
	213/3		25	24	1							21.1 x 111.0

APPENDIX TABLE CA-F48 (continued)

1,1,2,2-Tetrachloroethane

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		
274	250/1	50	25	25								
	250/3		25	22	1							20.9 x 99.9
					1							19.2 x 91.1
					1							18.9 x 102.9
271	239/1	50	25	25								
	239/4		25	24	1							22.5 x 95.0

APPENDIX TABLE DL

1,1,2,2-Tetrachloroethane
Dominant Lethal Assessment

Multiple Dosing: Air Control (0 ppm)

Week No.	Male No.	361		362		363		364		365		366		367		368		369		370		Total
	Female	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
1	Corpora lutea	4	11	12	15	10	16	11	13	*-	16	15	11	13	14	11	11	14	10	17	13	237
	Total Implants	0	11	12	15	10	15	11	11	*-	14	15	11	13	14	11	11	15	9	15	10	223
	Live Implants	0	11	12	13	9	15	11	11	*-	13	15	11	13	13	11	11	15	9	15	9	217
	Early Deaths	0	0	0	2	1	0	0	0	*-	1	0	0	0	1	0	0	0	0	0	1	6
	Late Deaths	0	0	0	0	0	0	0	0	*-	0	0	0	0	0	0	0	0	0	0	0	0
2	Corpora lutea	13	15	12	16	13	13	14	12	2	11	12	13	16	14	10	11	15	12	11	9	244
	Total Implants	14	16	11	16	14	13	12	14	0	12	12	14	12	14	6	10	14	10	14	14	242
	Live Implants	12	15	10	15	13	12	10	13	0	12	11	14	10	14	6	10	2	10	12	13	214
	Early Deaths	2	1	1	1	1	0	2	1	0	0	1	0	2	0	0	0	1	0	0	0	13
	Late Deaths	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	11	0	2	1	15
3	Corpora lutea	10	7	14	15	13	10	11	11	14	13	0	11	0	11	12	12	13	11	10	12	209
	Total Implants	10	14	14	11	15	10	10	9	13	18	0	13	0	10	12	12	12	12	10	12	217
	Live Implants	9	14	14	10	15	9	10	7	12	14	0	13	0	10	11	12	12	12	10	10	204
	Early Deaths	1	0	0	1	0	1	0	2	1	4	0	0	0	0	1	0	0	0	0	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
4	Corpora lutea	12	14	13	11	15	13	7	13	17	9	0	11	15	12	14	14	14	13	17	0	234
	Total Implants	12	12	13	12	15	7	7	13	15	7	0	14	14	7	14	12	14	9	15	0	212
	Live Implants	10	12	13	12	15	6	0	13	14	0	0	13	14	7	14	11	13	8	8	0	183
	Early Deaths	1	0	0	0	0	1	7	0	1	7	0	1	0	0	0	1	1	1	6	0	27
	Late Deaths	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2
5	Corpora lutea	12	13	13	13	2	15	11	14	8	15	13	17	11	11	15	11	12	11	0	15	232
	Total Implants	12	13	13	13	0	15	11	14	8	16	9	17	9	12	12	16	13	13	0	15	231
	Live Implants	12	12	13	13	0	10	11	11	1	16	9	16	9	11	12	15	12	13	0	15	211
	Early Deaths	0	0	0	0	0	3	0	3	0	0	0	1	0	1	0	1	1	0	0	0	10
	Late Deaths	0	1	0	0	0	2	0	0	7	0	0	0	0	0	0	0	0	0	0	0	10

*-Missing value : ambiguous record of result

APPENDIX TABLE DL (continued)

1,1,2,2-Tetrachloroethane

Multiple Dosing: Air Control (0 ppm)

Week No.	Male No.	361		362		363		364		365		366		367		368		369		370		Total
	Female	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
6	Corpora lutea	13	0	14	14	11	12	12	0	15*	13	13	10	12	16	10	11	16	12	11	12	227
	Total Implants	12	0	15	15	13	11	13	0	15	13	13	11	13	16	10	9	16	13	10	12	230
	Live Implants	12	0	14	15	12	11	11	0	0	13	12	9	13	15	8	9	16	10	10	11	201
	Early Deaths	0	0	1	0	1	0	2	0	0	0	1	2	0	1	2	0	0	3	0	1	14
	Late Deaths	0	0	0	0	0	0	0	0	15	0	0	0	0	0	0	0	0	0	0	0	15
7	Corpora lutea	15	13	15	9	9	12	14	16	13	15	9	10	16	13	13	15	14	12	14	12	259
	Total Implants	15	14	14	12	9	14	14	14	13	13	11	14	15	14	13	16	13	14	13	15	270
	Live Implants	15	13	14	9	8	13	12	11	13	13	10	4	15	13	13	13	13	14	13	15	244
	Early Deaths	0	1	0	3	1	1	2	1	0	0	1	9	0	1	0	2	0	0	0	0	22
	Late Deaths	0	0	0	0	0	0	0	2	0	0	0	1	0	0	0	1	0	0	0	0	4
8	Corpora lutea	12	11	13	14	0	11	15	9	12	11	12	0	15	14	0	0	16	15	12	12	227
	Total Implants	7	11	12	14	0	13	17	9	11	11	13	0	15	14	0	0	17	14	12	12	202
	Live Implants	7	10	12	13	0	13	9	0	11	8	13	0	14	12	0	0	15	14	9	10	170
	Early Deaths	0	1	0	1	0	0	8*	5*	0	3	0	0	1	2	0	0	2	0	3	2	28
	Late Deaths	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	4
9	Corpora lutea	14	10	12	11	12ø	12	13	14	13	13	10	13	12	14	14*	11*	14	14	0	11	226
	Total Implants	8	9	13	11	12	13	7	14	11	13	10	13	12	14	14	10	12	15	0	11	222
	Live Implants	5	6	13	0	8	9	7	13	10	13	10	9	12	14	6	4	12	14	0	8	173
	Early Deaths	1	0	0	1	3	3	0	1	1	0	0	3	0	0	7	5	0	1	0	3	29
	Late Deaths	2	3	0	10*	1	1	0	0	0	0	0	1	0	0	1	1	0	0	0	0	20
10	Corpora lutea	12	9	12	10	3	14	12	12	13	11	0	11	13	12	11	6	12	12	14	14	234
	Total Implants	12	10	14	10	0	14	12	12	14	10	0	11	15	12	10	13	10	12	12	11	214
	Live Implants	11	9	12	8	0	13	12	12	10	9	0	11	15	12	10	0	8	11	12	10	185
	Early Deaths	1	1	2	2	0	1	0	0	4	1	0	0	0	0	0	9	2	1	0	1	25
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	4

* haemorrhaged

ø very large foetuses

APPENDIX TABLE DL (continued)

1,1,2,2-Tetrachloroethane

Multiple Dosing: 5 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	0	9	0	13	10	13	15	12	1	10	10	13	2	10	13	2	1	10	12	9	165
	Total Implants	0	2	0	13	13	17	15	12	0	12	8	14	0	10	13	0	0	11	12	3	155
	Live Implants	0	2	0	12	12	14	15	12	0	12	0	11	0	9	11	0	0	6	11	3	130
	Early Deaths	0	0	0	1	1	3	0	0	0	0	8	3	0	1	2	0	0	5	0	0	24
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
2	Corpora lutea	7	15	13	8	10	17	13	13	14	12	11	14	4	14	19	14	13	12	7	8	238
	Total Implants	6	17	14	10	13	16	8	11	14	12	15	14	3	14	16	11	14	12	7	7	234
	Live Implants	4	17	10	9	13	16	5	11	14	10	10	13	0	14	14	4	14	12	4	7	201
	Early Deaths	2	0	3	1	0	0	3	0	0	1	1	1	3	0	2	2	0	0	2	0	21
	Late Deaths	0	0	1	0	0	0	0	0	0	1	4	0	0	0	0	5	0	0	1	0	12
3	Corpora lutea	14	11	10	12	13	10	12	14	11	11	13	11	12	10	13	11	12	14	11	15	240
	Total Implants	14	11	7	16	14	11	12	15	12	13	13	11	11	10	12	12	13	14	14	15	250
	Live Implants	14	11	7	14	14	4	11	15	12	12	13	10	0	7	12	11	12	14	14	13	220
	Early Deaths	0	0	0	2	0	7	1	0	0	1	0	1	11	3	0	1	1	0	0	2	30
	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	14	11	14	12	13	15	14	13	10	14	15	12	12	11	12	11	13	9	14	254
	Total Implants	15	15	11	14	11	8	13	14	11	13	17	15	13	12	13	12	11	13	10	13	254
	Live Implants	15	15	11	14	11	6	13	12	11	10	16	15	12	9	13	12	11	13	0	13	232
	Early Deaths	0	0	0	0	0	2	0	2	0	3	1	0	1	3	0	0	0	0	10	0	22
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	Corpora lutea	13	15	11	10	10	9	14	13	13	12	13	14	10	12	14	12	12	15	14	11	247
	Total Implants	12	15	12	12	10	7	14	12	16	11	14	14	9	12	14	13	13	13	14	11	248
	Live Implants	12	15	10	12	8	0	14	12	16	9	12	14	0	12	14	12	13	13	10	10	218
	Early Deaths	0	0	2	0	2	6	0	0	0	2	2	0	9	0	0	1	0	0	4	1	29
	Late Deaths	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1

APPENDIX TABLE DL (continued)

1,1,2,2-Tetrachloroethane

Multiple Dosing: 5 ppm

Week No.	Male No.	371		372		373		374		375		376		377		378		379		380		Total
	Female	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
6	Corpora lutea	11	0	11	12	13	11	15	12	12	11	11	12*	10	12	13	10	12	13	11	12	224
	Total Implants	11	0	11	12	14	12	12	12	13	11	11	10	9	12	14	13	13	15	14	14	233
	Live Implants	11	0	11	11	14	11	10	12	13	11	11	10	9	8	12	2	12	15	12	7	202
	Early Deaths	0	0	0	1	0	1	2	0	0	0	0	0	0	4	1	0	1	0	2	7	19
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	11	0	0	0	0	12
7	Corpora lutea	9	12	10	16	12	13	11	10	13	12	13	13	12	11	15	11	11	12	10	11	237
	Total Implants	9	13	14	16	12	15	11	12	14	13	4	12	11	12	14	12	11	9	9	14	237
	Live Implants	9	11	12	11	11	11	11	11	13	12	4	12	10	12	14	11	10	6	9	1	201
	Early Deaths	0	1	2	5	1	4	0	1	0	1	0	0	1	0	0	1	1	3	0	3	24
	Late Deaths	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	10	12
8	Corpora lutea	13	13	13	3	16	12	10	11	10	16	16	13	12	20	14	11	14	14	0	≠	231
	Total Implants	13	6	12	4	15	12	10	11	10	17	16	13	11	12	15	12	14	14	0	≠	217
	Live Implants	11	3	11	4	15	12	8	10	8	15	16	13	10	12	15	11	13	13	0	≠	200
	Early Deaths	1	3	1	0	0	0	2	1	2	1	0	0	0	0	0	1	1	1	0	≠	14
	Late Deaths	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	≠	3
9	Corpora lutea	0	13	12	11	13	13	12	11	10	11	14	15	10	11	12	14	16	13	10	11	232
	Total Implants	0	13	12	13	13	11	11	10	10	11	13	10	9	11	11	16	15	13	10	12	224
	Live Implants	0	13	12	12	13	10	10	10	10	11	13	7	3	11	11	14	10	8	10	12	200
	Early Deaths	0	0	0	1	0	1	0	0	0	0	0	2	6	0	0	1	1	2	0	0	14
	Late Deaths	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	4	3	0	0	10
10	Corpora lutea	12	13	10	11	12	15	13	12	10	14	12	13	13	11	13	12	14	14	13	15	252
	Total Implants	12	15	13	13	13	13	14	12	14	14	11	15	14	12	12	10	14	15	14	15	265
	Live Implants	11	15	12	9	12	13	14	11	14	14	11	15	14	12	12	9	12	14	14	14	252
	Early Deaths	1	0	1	2	1	0	0	1	0	0	0	0	0	0	0	1	2	1	0	1	11
	Late Deaths	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2

≠ uncountable - haemorrhaged * Haemorrhaged

APPENDIX TABLE DL (continued)

1,1,2,2-Tetrachloroethane

Multiple Dosing: 50 ppm

Week No.	Male No.	381		382		383		384		385		386		387		388		389		390		Total
	Female	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
1	Corpora lutea	15	0	0	14	10	17	1	D	10	13	10	13	10	10	13	11	12	12	12	11	193
	Total Implants	15	0	0	14	10	18	-	E	11	11	12	13	7	10	14	10	11	12	12	12	192
	Live Implants	15	0	0	14	1	15	-	A	11	11	12	13	4	10	14	9	11	12	8	12	172
	Early Deaths	0	0	0	0	9	3	-	D	0	0	0	0	3	0	0	1	0	0	4	0	20
	Late Deaths	0	0	0	0	0	0	-		0	0	0	0	0	0	0	0	0	0	0	0	0
2	Corpora lutea	16	11	13	14	0	11	12	14	16	13	14	13	11	13	11	0	12	14	7	11	226
	Total Implants	16	10	12	13	0	12	13	14	16	13	12	16	12	14	12	0	12	15	4	12	228
	Live Implants	16	10	11	12	0	12	13	14	15	12	12	16	10	13	10	0	12	14	3	9	214
	Early Deaths	0	0	1	1	0	0	0	0	1	1	0	0	2	1	2	0	0	1	1	3	14
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	Corpora lutea	11	12	11	12	15	10	0	12	10	15	15	10	12	0	0	12	0	12	12	10	191
	Total Implants	11	13	11	12	17	10	0	14	10	16	7	10	13	0	0	12	0	11	12	10	189
	Live Implants	9	13	10	12	17	10	0	13	9	16	5	10	6	0	0	8	0	10	11	10	169
	Early Deaths	2	0	1	0	0	0	0	1	1	0	2	0	7	0	0	3	0	1	1	0	19
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
4	Corpora lutea	11	18	15	12	10	11	13	13	15	13	12	8	13	12	16	14	10	12	17	14	259
	Total Implants	12	17	16	12	10	9	11	13	15	14	14	10	14	12	15	12	11	12	14	14	257
	Live Implants	11	17	13	11	10	0	10	8	14	13	12	10	14	12	15	0	11	4	14	10	209
	Early Deaths	1	0	3	1	0	4	1	5	1	1	2	0	0	0	0	12	0	6	0	4	41
	Late Deaths	0	0	0	0	5	5	0	0	0	0	0	0	0	0	0	0	0	2	0	0	7
5	Corpora lutea	2	15	9	9	12	15	11	11	0	10	11	12	0	15	13	13	1	11	12	15	197
	Total Implants	0	13	11	10	11	15	11	12	0	11	11	13	0	16	14	14	0	13	6	15	196
	Live Implants	0	13	10	8	11	15	10	3	0	11	10	11	0	16	14	12	0	11	2	15	172
	Early Deaths	0	0	1	2	0	0	1	8	0	0	1	1	0	0	0	1	0	2	4	0	21
	Late Deaths	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	3

384/1, wk 1 uncountable brown mass in uterus

APPENDIX TABLE DL (continued)

1,1,2,2-Tetrachloroethane

Multiple Dosing: 50 ppm

Week No.	Male No.	381		382		383		384		385		386		387		388		389		390		Total
	Female	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
6	Corpora lutea	12	15	9	12	11	10	11	14	15	11	14	14	13	11	17	*-	13	12	14	12	240
	Total Implants	11	14	11	8	12	8	11	14	15	12	17	15	12	11	19	*-	14	15	15	13	247
	Live Implants	9	14	11	8	12	8	9	13	14	12	15	3	11	11	15	*-	14	15	14	10	218
	Early Deaths	2	0	0	0	0	0	2	1	0	0	2	12	1	0	4	*-	0	0	1	3	28
	Late Deaths	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	*-	0	0	0	0	1
7	Corpora lutea	13	15	11	13	0	11	15	13	12	11	≠	15	3	13	13	12	12	13	1	14	210
	Total Implants	16	16	14	14	0	12	14	13	13	12	11	12	0	14	15	15	11	13	0	13	228
	Live Implants	16	13	14	14	0	12	13	11	11	12	11	11	0	13	15	14	11	11	0	13	215
	Early Deaths	0	2	0	0	0	0	1	1	1	0	0	1	0	0	0	1	0	0	0	0	7
	Late Deaths	0	1	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	2	0	0	6
8	Corpora lutea	14	15	15	16	7	12	14	12	14	13	14	15	9	13	7	15	9	12	13	11	250
	Total Implants	14	15	16	5	7	13	14	12	12	13	15	9	7	12	8	17	5	11	12	6	223
	Live Implants	13	14	16	5	7	13	14	12	12	0	9	7	5	12	7	15	5	4	11	6	187
	Early Deaths	1	0	0	0	0	0	0	0	0	7	6	2	2	0	1	2	0	7	0	0	28
	Late Deaths	0	1	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	1	0	8
9	Corpora lutea	0	*-	11	11	15	12	10	13	10	14	12	15	16*	13	11	15	13	12	12	11	226
	Total Implants	0	*-	15	7	15	13	10	11	12	11	14	17	12	13	10	14	13	12	15	10	224
	Live Implants	0	*-	15	6	14	13	6	10	4	9	13	7	0	12	10	14	13	7	15	8	176
	Early Deaths	0	*-	0	1	1	0	4	1	2	1	0	4	12	1	0	0	0	2	0	2	31
	Late Deaths	0	*-	0	0	0	0	0	0	6	1	1	6	0	0	0	0	0	3	0	0	17
10	Corpora lutea	0	13	15	9	14	0	13	8	12	12	11	9	0	12	10	12	15	10	15	12	202
	Total Implants	0	12	15	8	14	0	14	12	14	14	10	13	0	15	11	12	12	12	15	8	211
	Live Implants	0	9	15	0	14	0	14	12	14	14	10	13	0	13	11	10	11	7	15	7	189
	Early Deaths	0	3	0	3	0	0	0	0	0	0	0	0	0	2	0	2	1	3	0	1	15
	Late Deaths	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	7

* haemorrhaged ≠ one ovary lost - no count

*- Missing value: ambiguous record of result

APPENDIX TABLE DL (continued)

1,1,2,2-Tetrachloroethane

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Week No.	Male No.	391		392		393		394		395		396		397		398		399		400		Total
	Female	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
1	Corpora lutea	12	4	0	6	2	8	4	6	6	3	3	0	6	8	12	10	1	0	8	2	101
	Total Implants	11	3	0	6	2	8	4	6	8	9	4	0	6	9	1	6	1	0	0	2	86
	Live Implants	2	0	0	6	0	1	4	6	0	0	0	0	0	9	0	6	0	0	0	2	36
	Early Deaths	9	3	0	0	2	7	0	0	8	9	4	0	6	0	1	0	1	0	0	0	50
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	Corpora lutea	0	0	0	0	0	0	0	0	2	0	0	4	3	0	0	0	0	6	2	0	17
	Total Implants	0	0	0	0	0	0	0	0	0	0	0	3	0	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	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	3
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	Corpora lutea	0	2	12	14	1	0	0	0	0	1	1	0	7	2	0	2	1	4	7	8	62
	Total Implants	0	0	0	0	1	0	0	0	0	0	2	0	2	0	0	2	1	0	1	0	9
	Live Implants	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	2	1	0	0	0	5
	Early Deaths	0	0	0	0	1	0	0	0	0	0	2	0	0	0	0	0	0	0	1	0	4
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	Corpora lutea	4	9	0	13	5	12	2	10	0	1	14	7	12	0	11	9	13	11	9	14	156
	Total Implants	0	9	0	12	3	6	0	13	0	1	14	4	12	0	2	5	9	5	9	10	114
	Live Implants	0	6	0	12	3	6	0	13	0	1	9	4	10	0	2	0	8	5	7	10	96
	Early Deaths	0	3	0	0	0	0	0	0	0	0	5	0	2	0	0	5	1	0	2	0	18
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	Corpora lutea	13	12	12	10	10	15	14	12	15	13	11	11	11	10	12	10	0	10	*-	12	213
	Total Implants	13	13	12	8	9	15	13	12	15	11	10	14	5	10	12	10	0	4	-	10	196
	Live Implants	13	13	11	8	1	12	13	12	15	11	10	14	5	10	8	0	0	4	-	7	167
	Early Deaths	0	0	1	0	8	3	0	0	0	0	0	0	0	0	3	10	0	0	-	3	28
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	-	0	1

*-Missing value : ambiguous record of result

APPENDIX TABLE DL (continued)

1,1,2,2-Tetrachloroethane

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Week No.	Male No.	391		392		393		394		395		396		397		398		399		400		Total
	Female	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
6	Corpora lutea	3	12	12	11	9	12	11	13	*-	7*	7	13	12	11	12	11	12	16	7	12	203
	Total Implants	0	12	12	12	11	12	11	14	*-	15	6	12	16	10	14	10	13	17	6	13	216
	Live Implants	0	7	11	10	10	12	10	14	*-	0	6	12	12	2	14	10	13	16	5	13	177
	Early Deaths	0	5	1	2	1	0	1	0	*-	15	0	0	4	7	0	0	0	1	1	0	38
	Late Deaths	0	0	0	0	0	0	0	0	*-	0	0	0	0	1	0	0	0	0	0	0	1
7	Corpora lutea	12	12	11	14	11	16	15	10	12	16	0	8	13	14	12	13	14	12	12	14	241
	Total Implants	12	6	11	13	14	14	16	11	14	15	0	15	14	14	12	13	14	12	14	14	248
	Live Implants	12	5	10	12	12	14	15	11	12	15	0	0	12	14	11	12	14	11	12	14	218
	Early Deaths	0	1	1	1	2	0	1	0	2	0	0	15	2	0	1	1	0	1	2	0	30
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	Corpora lutea	13	0	6	13	12	10	11	0	15	15	16	11	13	13	13	7	11	18	13	13	223
	Total Implants	9	0	3	13	12	7	14	0	15	16	15	12	13	13	15	6	11	13	11	13	211
	Live Implants	6	0	3	13	11	2	14	0	14	13	15	11	7	11	15	6	11	10	11	13	186
	Early Deaths	3	0	0	0	0	5	0	0	1	3	0	1	6	2	0	0	0	3	0	0	24
	Late Deaths	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
9	Corpora lutea	12	15	0	13	12	12	10	0	12	12	13	0	12	11	11	11	11	12	0	4	183
	Total Implants	12	15	0	11	11	14	11	0	11	13	13	0	12	12	13	13	10	12	0	2	185
	Live Implants	12	0	0	11	11	5	11	0	11	12	12	0	11	10	12	12	10	12	0	0	152
	Early Deaths	0	5	0	0	0	9	0	0	0	1	0	0	0	0	1	1	0	0	0	0	17
	Late Deaths	0	10	0	0	0	0	0	0	0	0	1	0	1	2	0	0	0	0	0	2	16
10	Corpora lutea	14	13	12	10	10	14	14	11	13	13	0	14	12	14	15	13	13	11	D	14	216
	Total Implants	14	13	12	9	6	12	13	11	13	12	0	15	13	14	14	13	13	10	E	15	222
	Live Implants	14	13	11	9	6	12	10	10	13	2	0	14	12	13	13	13	13	8	A	15	201
	Early Deaths	0	0	1	0	0	0	2	0	0	10	0	1	1	1	1	0	0	2	D	0	19
	Late Deaths	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0		0	2

* haemorrhaged *- Missing value: ambiguous record of result

APPENDIX TABLE SA

1,1,2,2-Tetrachloroethane
Sperm Abnormality Assessment

Multiple Dosing: Air Control (0 ppm)
Low, 5 ppm
High, 50 ppm
Positive, Ethyl methanesulphonate, 100 mg/kg

Slide No.	Normal	Abnormality					Total Abnormal	Total Examined	De-coded Information	
		A	B	C	D	E			Animal No.	Group
331	975	0	1	10	5	9	25	1000	321	AIR
353	969	1	2	13	4	11	31	1000	322	AIR
357	967	0	4	14	1	14	33	1000	323	AIR
333	968	0	1	11	3	17	32	1000	324	AIR
352	965	0	3	13	12	7	35	1000	325	AIR
332	960	1	2	22	2	13	40	1000	326	AIR
338	964	2	1	8	13	12	36	1000	327	AIR
358	965	1	1	9	5	19	35	1000	328	AIR
323	953	1	2	15	1	28	47	1000	329	AIR
351	973	1	2	8	4	12	27	1000	330	AIR
359	950	4	3	16	4	23	50	1000	331	LOW
329	966	0	2	7	20	5	34	1000	332	LOW
357	967	0	4	14	1	14	33	1000	333	LOW
346	967	0	1	19	1	12	33	1000	334	LOW
348	978	2	0	6	3	11	22	1000	335	LOW
350	954	2	4	15	4	21	46	1000	336	LOW

APPENDIX TABLE SA (continued)

1,1,2,2-Tetrachloroethane

Multiple Dosing: Air Control (0 ppm)
Low, 5 ppm
High, 50 ppm
Positive, Ethyl methanesulphonate, 100 mg/kg

Slide No.	Normal	Abnormality					Total Abnormal	Total Examined	De-coded Information	
		A	B	C	D	E			Animal No.	Group
326	686*	23	30	105	57	99	314*	1000	337*	LOW
342	964	0	2	15	5	14	36	1000	338	LOW
345	961	2	2	11	4	20	39	1000	339	LOW
321	966	1	2	13	2	18	36	1000	340	LOW
360	965	3	7	18	1	6	35	1000	341	HIGH
343	976	1	2	2	4	15	24	1000	342	HIGH
330	970	2	2	10	4	12	30	1000	343	HIGH
325	966	2	4	10	4	14	34	1000	344	HIGH
339	953	1	3	20	6	27	57	1000	345	HIGH
336	974	2	1	10	2	11	26	1000	346	HIGH
349	963	1	0	13	7	16	37	1000	347	HIGH
341	960	2	3	12	4	19	40	1000	348	HIGH
347	953	1	5	22	3	16	47	1000	349	HIGH
334	961	2	4	19	6	8	39	1000	350	HIGH

* Animal no. 337 : 31.4% abnormal sperms

APPENDIX TABLE SA (continued)

1,1,2,2-Tetrachloroethane

Multiple Dosing: Air Control (0 ppm)
Low, 5 ppm
High, 50 ppm
Positive, Ethyl methanesulphonate, 100 mg/kg

Slide No.	Normal	Abnormality					Total Abnormal	Total Examined	De-coded Information	
		A	B	C	D	E			Animal No.	Group
354	968	0	1	10	11	10	32	1000	351	+
328	959	1	3	13	10	14	41	1000	352	+
324	973	1	1	8	9	8	27	1000	353	+
344	970	0	6	12	4	8	30	1000	354	+
356	972	1	3	11	2	11	28	1000	355	+
337	934	0	1	30	10	25	66	1000	356	+
332	946	0	3	15	3	33	54	1000	357	+
355	959	0	3	13	6	19	41	1000	358	+
327	972	2	1	11	7	7	28	1000	359	+
340	961	2	4	19	6	8	39	1000	360	+