

Report Number 33

TIER II MUTAGENIC SCREENING OF
13 NIOSH PRIORITY COMPOUNDS

INDIVIDUAL COMPOUND REPORT
N,N-DIMETHYLFORMAMIDE

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May 1, 1981

Supported by

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

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AUTHENTICATION

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

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TABULATIONS

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

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

Example:

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

Abbreviations on Chromosomal Aberration Tables and Appendix Tables:

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

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LOCATION OF EXPERIMENT

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

DISCLAIMER

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

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SUMMARY

N,N-Dimethylformamide was subjected to a tier II mutagenic test screening programme. The assays used were the following:

1. Unscheduled DNA synthesis (UDS) assay in human diploid fibroblasts with exposure of 3 h duration and concentrations up to 9,220 µg/ml of culture medium.
2. Dominant lethal test in male rats with exposure to atmospheres containing 10 ppm or 400 ppm N,N-dimethylformamide for 7 h per day for 5 consecutive days. Analysis of test atmospheres was by continuous infra-red monitoring at a wavelength of 9.2 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 an atmosphere of 400 ppm for 2.25 h.

The results obtained were as follows:

1. There was no increase in UDS in cells treated with N,N-dimethylformamide.
2. There were no effects attributable to N,N-dimethylformamide in the dominant lethal test on pregnancy frequency, numbers of corpora lutea or implantations or the frequency of early deaths.
3. Abnormal sperm frequency was not affected.
4. The frequencies of chromosomal aberrations were not increased in rat bone marrow cells, except in the 24 h sample time males following a single exposure. This effect was almost entirely due to a single rat, which may have been abnormal.
5. SLRL frequency was not increased in Drosophila.
6. It was concluded that N,N-dimethylformamide was devoid of genetic effects observable in these experiments.

INTRODUCTION

N,N-Dimethylformamide (CAS No. 68-12-2) is a colourless liquid with solvent properties which has caused it to be used in several industries. It is a solvent for liquids and gases. In particular, it is used as a solvent for Orlon and similar polyacrylic fibres and wherever a solvent with a low rate of evaporation is required. It is prepared from dimethylamine and formic acid.

A summary of its physical and chemical properties follows:

Formula	HCON(CH ₃) ₂
Mol. wt.	73
Sp. gr. (25°C)	0.9445
Refractive index (25°C)	1.42803
M.P.	-61°C
B.P. (760 mm Hg)	153°C
Flash point	67°C
Vapour pressure, mm Hg (25°C)	3.7
Vapour density (air = 1)	2.51

It is infinitely soluble in water and most organic solvents.

Toxicology

N,N-Dimethylformamide is considered to be moderately hazardous by inhalation, moderately irritating to skin and a definite hazard by skin absorption (Massmann, 1956). Little is known about the metabolic fate of the compound. When administered intravenously into cats 10-15% is excreted unchanged (Massmann, 1956). It undergoes appreciable demethylation, with excretion of N-methylformamide and formamide in rat and human urine (Barnes and Ranta, 1972). Since N-methylformamide in urine correlates well with exposure to N,N-dimethylformamide, measuring the demethylated metabolite has been suggested as a monitor for human exposure (Yonemato and Suzuki, 1980). N-Methylformamide also accumulates in the blood of treated animals. The demethylation of N,N-dimethylformamide probably occurs in the liver, in a microsomal reaction (Barnes and Henry, 1974; Kimmerle and Eben, 1975).

Liver injury does occur in animals given prolonged inhalation of 100 ppm atmospheres (Kittila, 1963, 1973) and bromo-sulphthalein excretion is delayed, indicating hepatic incompetence (Clayton *et al*, 1963). Reduced Q_{O_2} values, with membranous lesions, have been observed at the onset of toxic symptoms (Carta *et al*, 1969). Associated with injury to the liver parenchyma, there are elevations of certain serum

enzymes (aldolase, glutamate-oxaloacetate transaminase, alkaline phosphatase, sorbital dehydrogenase, lactate dehydrogenase) (Zedda et al, 1969).

Adrenocortical function in man is affected by exposure, there being lowered mean excretion of 17-ketogenic steroids.

In reproductive studies on rats, Thiersch (1971) reports that 0.5-2.0 ml of N,N-dimethylformamide per kg had no effects on the litter exposed in utero.

There was no effect of N,N-dimethylformamide in an Ames' test conducted at this laboratory at dose levels up to 50 mg per plate.

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

Human fibroblasts:	up to 9,220 µg/ml for 3 h.
Mice and rats:	10 ppm or 400 ppm for 7 h/day for one or 5 days.
<u>Drosophila</u> :	400 ppm for 2.25 h.

REPETITION OF EXPOSURES

The initial exposures of rats and mice to N,N-dimethylformamide took place on 3-7, and 11 September 1979. The slides for both the multi-dose and single dose cytogenetics tests were prepared. However, these slides were found to be unreadable, so it was necessary to repeat the exposures on fresh rats. It was found that, again, the slides from the multi-dose rats were unsuitable for reading. This part of the experiment was repeated a second time. The following table summarises the dates of the various exposures and the tests to which the dates apply.

<u>Date</u>	<u>Test</u>	<u>Comments</u>
3-7 September 1979	Multi-dose cytogenetics	Slides prepared but not suitable for reading
	Dominant lethal	Completed
	Sperm abnormalities	Completed
11 September 1979	Single dose cytogenetics	Slides prepared but not suitable for reading
4-8 August 1980	Multi-dose cytogenetics	Slides prepared but not suitable for reading
11 August 1980	Single dose cytogenetics	Completed
18-22 August 1980	Multi-dose cytogenetics	Completed

MATERIALS AND METHODSCHEMICALSTest Substance

Two 1 kg bottles of N,N-dimethylformamide, Batch No. 15163 (stated purity 99%) were received from Aldrich Chemical Company Limited on 29 August 1978. A further 1 kg bottle, from the same batch, was received on 5 September 1979. The test material was a clear, colourless liquid and was retained in the dark under ambient conditions in the company dispensary until used. A sample has been retained for analysis, should this be necessary.

Positive Control Substance

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

ANIMALS AND ANIMAL MANAGEMENTAnimals

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

B6C3F₁ hybrid mice were obtained from Charles River (U.S.A.).

These animals were obtained on the following dates.

Species	Date of Receipt	Age (Weeks)	Quarantine (Days)	Number (Sex)	Dates of Exposure	Comment
Rat	24 August 1979	10-11	10	220♂ 176♀	3-7 and 11 September 1979	Cytogenetics slides not suitable.
Mouse				44♂	3-7 September 1979	
Rat	7 September 1979	8-10	None	80♂ x 10	None	D.L. matings.
Rat	25 July 1980	10-11	10	176♂ 176♀	4-8 and 11 August 1980	Multiple dose cytogenetics abandoned.
Rat	8 August 1980	10-11	10	44♂ 44♀	18-22 August 1980	Multiple dose cytogenetics only.

Pre-experiment Acceptance Tests

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

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

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

Animal Management

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

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

They were housed individually in cages in a room with a light intensity of approximately 200 lux, a 12 h light-dark cycle, approximately 10 air changes per hour, temperature maintained at ca 22°C and relative humidity ca 50%. Extreme limits of animal room conditions for the dominant lethal test rats and sperm abnormality test mice were 16.0°C and 24.5°C, and 30% and 67% R.H. For the single dose cytogenetic test rats, extreme limits were 18.5°C and 26.0°C, and 50% and 69% R.H. The limits for the multi-dose cytogenetic test rats were 19.0°C and 25.0°C, and 50% and 68% R.H.

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:-

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

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

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

Allocation of Rats and Mice to Cages and Treatment Groups

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

This complete process was repeated for the female rats and male B6C3F₁ mice. The mice were kept on a separate rack from the rats.

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

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

Treatment groups were colour coded as follows:

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

Animal Identification

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

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

Animal Positioning in the Exposure Chambers

Although homogeneity data were obtained which showed that there were no test compound concentration differences of any significance in the exposure chambers, animal positions were rotated on a daily basis to minimise any possible exposure location variations. Animal location charts for each day were drawn up, as shown in Appendix Loc-2.

The treatment groups were constituted as follows:-

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

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 N,N-dimethylformamide were carried out in 1.5 m³ capacity chambers constructed of stainless steel and glass. The animals occupied a volume of 0.02 m³ and were confined to a single tier of cages of 0.4 m³ in volume (the breathing zone). The breathing zone was ventilated at the rate of 12 air changes per hour. An additional chamber of 0.84 m³ capacity was used for exposure of the air control group; the breathing zone in this chamber also was ventilated at the rate of 10 air changes per hour.

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

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

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

Monitoring Equipment

The atmospheres within the exposure chambers were analysed by infra-red spectroscopy using Miran-1A Portable Gas Analysers (Foxboro/Wilks Inc). This type of instrument is a single beam, variable wavelength spectrometer, scanning the infra-red spectrum between 2.5 and 14.5 μm . It is equipped with a gas cell having a variable pathlength of between 0.75 and 21.75 m. Samples of the chamber air were continuously pumped (5 l/min) through nylon 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.

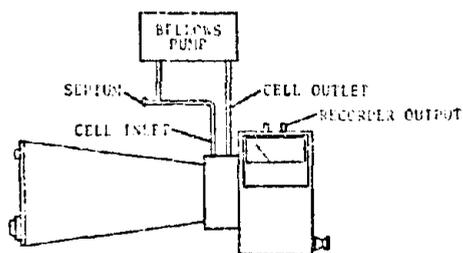
Calibration and Analytical Development

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 N,N-dimethylformamide 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 N,N-dimethylformamide were calibrated each day before vapour generation commenced.

The calibration was performed using a closed loop calibration system (see diagram below). Known volumes of N,N-dimethylformamide 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 absorbance reading was allowed to stabilise as indicated on the chart recording.



SCHEMATIC DIAGRAM OF CLOSED LOOP CALIBRATION SYSTEM

The cumulative absorbance 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:

	<u>Low Level</u>	<u>High Level</u>
Wavelength :	9.2 μm	9.2 μm
Pathlength :	14.25 m	(a) 3.75/5.25 m
Absorbance Range :	0.1 A	1 A
Slit Width :	0.5 mm	0.5 mm
Meter Response :	10	1
Recorder Voltage :	1 V	1 V
Chart Speed :	120 mm/h	120 mm/h

- (a) Pathlength increased to 5.25 m during repeat cytogenetics exposure in order to give a higher degree of sensitivity.

Calibration Data

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

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 (24.30 at 20°C)
 5.64 = Volume of Miran sample chamber (l)

Example of the Calculation for V

Compound:

$$\begin{aligned} C &= 10 \text{ ppm} \\ \rho &= 0.944 \text{ g}/\text{cm}^3 \\ M &= 73.1 \\ V &= \frac{C \times M \times 5.64}{\rho \times 10^3 \times 24.30 \mu\text{l}} \\ &= \frac{10 \times 73.1 \times 5.64}{0.944 \times 10^3 \times 24.30 \mu\text{l}} \\ &= 0.18 \mu\text{l} \end{aligned}$$

Therefore, to construct a calibration curve to cover the 10 ppm range, 0.1 μl samples of N,N-dimethylformamide 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 N,N-dimethylformamide contained in a glass gas washing, or Drechsel bottle immersed in a temperature controlled water bath at 50°C. The nitrogen/N,N-dimethylformamide vapour mixture so generated was ducted through 7/8" ID stainless steel piping to a glass mixing vessel and diluted with filtered, compressed air. The resulting mixture of N,N-dimethylformamide/air was ducted through 7/8" stainless steel piping to the top of the exposure chamber.

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

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

Homogeneity Data

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

Measurement of Chamber Concentrations

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

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

Test Compound Utilisation

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

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

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

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

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

Positive Control Groups in Animal Tests

Preparation of Dosing Solutions

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

Treatment of Rats and Mice with Ethyl methanesulphonate

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

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

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

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

UNSCHEDULED DNA SYNTHESIS ASSAY

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

Chemicals

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

6-[³H]-thymidine (21 Ci/mmol) and 8-[³H]-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 was used in Method 2 because the growth characteristics of Flow 11,000 had deteriorated to such an extent that they were no longer usable for these studies.

Culture Maintenance and Growth Media

Cells in 175 cm² Nunc flasks were routinely maintained at 37°C in Dulbecco's Minimum Essential Medium (DMEM) and in an atmosphere of 5% CO₂:95% air (v/v). The medium contained 2.0 g/l sodium bicarbonate and was supplemented with heat inactivated (65°C, 30 min) foetal calf serum, (10% v/v), gentamycin (50 µg/ml) and glutamine (2 mM). DMEM (10x concentrated) and antibiotics were obtained from Gibco Europe Limited, Paisley, Scotland, and serum from Flow Laboratories, Irvine, Scotland.

Arginine-deficient medium contained 3.70 g/l sodium bicarbonate and was supplemented with heat inactivated foetal calf

serum (5% v/v) and gentamycin (50 µg/ml). This medium was obtained from Flow Laboratories.

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

Animals

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

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

Preparation of the 9,000 g Supernatant Fluid from Livers

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

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

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

NADP-di-Na-salt	4 mM (= 3.366 mg/ml)
Glucose-6-phosphate-di-Na-salt	5 mM (= 1.521 mg/ml)
MgCl ₂ .6H ₂ O	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% CO₂ in air at 37°C for 72 h. The medium from each of the wells was then replaced with 1 ml of arginine-free DMEM supplemented with 5% (v/v) heat inactivated foetal bovine serum and the plate incubated for a further 48 h.

The compound was dissolved directly in culture medium and 10 µl samples added to duplicate cell suspension.

After incubation for 3 h at 37°C in a humid atmosphere of 5% CO₂ 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 the highest concentration of 9.663 mg/ml which was selected as the highest in a series of concentrations of N,N-dimethylformamide in the repair assay.

DNA Repair Assay (Method 1)

The cells were harvested, sedimented, suspended in fresh culture medium at a density of 5×10^4 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- ^3H -thymidine (21 Ci/mmol) were added to each culture giving final concentrations of 2.5 mM and 10 $\mu\text{Ci/ml}$ respectively. N,N-dimethylformamide was dissolved in dimethylsulphoxide and dilutions were made from this stock solution to give the required concentrations. Triplicate wells, with and without S-9 mix, received 10 μl samples of test compound solution. 10 μl samples of dimethylsulphoxide were added to negative control cultures.

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

4-NQO	1.25 $\mu\text{g/ml}$
2-AAN	3 $\mu\text{g/ml}$

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 ^3H -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 $20^\circ\text{C} \pm 2^\circ\text{C}$. Illumination was by a safelight fitted with a Kodak filter No. 1 (red) lit by a 25 watt bulb some 4-6 feet away from the working area.

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

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

Quantification of Repair Synthesis

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

DNA Repair Assay (Method 2)

Flow 11,000 cells were harvested, sedimented and suspended in fresh culture medium at a density of 5×10^4 cells/ml. 5 ml samples were dispensed into 60 mm tissue culture Petri dishes (Nunclon Delta) which were incubated in a humid atmosphere of 5% CO₂ 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 μ l of S-9 mix were added to one group. Solutions of hydroxyurea and [³H]-deoxyguanosine (26 Ci/mmol) were added to each dish giving final concentrations 2.5 mM and 10 μ Ci/ml respectively. N,N-dimethylformamide was added to give a concentration of 9.663 mg/ml. 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 $\mu\text{g/ml}$.

After a 4 h incubation at 37°C in an atmosphere of 5% CO₂ 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 fractionated 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 [³H]-deoxyguanosine was incorporated by any of the cultures treated. In the belief that some component of the S-9 was perhaps metabolising the [³H]-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. [³H]-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°C was added to the cells while they were agitated on a vortex mixer. Following incubation for 20 min in a 37°C water bath, the cells were centrifuged, the supernatant fluid decanted and the cells fixed in 4 ml freshly prepared fixative (methanol:glacial acetic acid; 3:1). The fixative was removed after centrifugation and replaced with 2 ml fresh fixative. Tubes containing fixed cells were stored in a 4°C refrigerator overnight.

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

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

Slide Reading

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

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

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

DOMINANT LETHAL TESTING IN MALE RATSMating

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 MICEPreparation

Mice were killed 5 weeks from the last day of dosing (i.e., Friday 12 October 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 base or Müller-5 test was used (Spencer and Stern, 1948; Würzler et al 1977). In this test, recessive lethal mutations induced in the X-chromosomes of treated male gametes are detected in the F₂ generation by the absence of wild-type males in the progeny of individual gametes. F₃ generation flies were also observed since this allows the detection of mosaics or delayed mutations which may not appear in the F₂ 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 base balancer X-chromosome, $1n(1) \text{SCS}1L \text{SC}8R + S \text{SCS}1 \text{SC}8 \text{waB}$.

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 F₂ generation and, where appropriate, the F₃ 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 F₁ generation flies from the pupae began about 10 days after mating.

Matings for the F₂ generation were set up 1-4 days later by mating brother with sisters.

Assessment of effects in the F₃ generation was undertaken in the same way as for the F₂ generation.

Experiments were normally scored 11-14 days after setting up the F₂ or F₃ 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 F₁ 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 F₂ generation were scored sterile.

STATISTICAL EVALUATIONCytogenetics 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 i th 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 j th female in the i th treatment group, then

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

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

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

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

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

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

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

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

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

Sperm Abnormalities Test

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

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

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

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

Sex-linked Recessive Lethal Test

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

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

F₂ 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

F₃ Generation

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

Against this background, the criteria for result assessment were:

- (a) a compound giving frequencies below 0.5% in duplicate experiments is considered to show no evidence of mutagenic activity.
- (b) a compound giving frequencies greater than 1.0% in the same brood in duplicate experiments is considered to show mutagenic potential.
- (c) a compound giving frequencies between 0.5% and 1.0% shows evidence of possibly being mutagenic. Although this evidence is not conclusive, the compound clearly would deserve further study.

RESULTS

Instrument Calibration

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

Calibration ranges adopted were 5.6-22.5 ppm (10 ppm target concentration) and 169-674 ppm (400 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 N,N-dimethylformamide was occurring. The results are shown in Table AT-3, where it can be seen that the maximum deviations encountered were +5.5% at the 10 ppm target concentration and -3.8% at the 400 ppm target concentration.

Chamber Atmospheres - Achieved Concentrations

A sample chart record taken during a day on which animals were exposed is shown in Figure 4. From charts such as this, deviations from the target concentrations of 10 ppm and 400 ppm were obtained and recorded in Tables AT-4 to 6.

Deviations from the target concentrations of more than $\pm 10\%$ were generally limited to a few minutes, so, the exposures were considered to be acceptable and the remaining portions of the experiments allowed to proceed. The longest deviations were in the single dose cytogenetics test, 400 ppm level, where only 76% of the time was within the prescribed target limits.

Animal Location

In Appendix Loc-1 and Appendix Loc-2 are shown respectively the locations of the cage racks in the holding room and typical examples of exposure location sheets as used during the study.

Pre-experimental Acceptance Tests (PEAT)

24 August 1979 Delivery: Ten male and 10 female rats and 4 male mice were haphazardly selected for PEAT. In the mice there were no significant clinical observations, autopsy, microbiological or parasitological findings. Histologically, there was a small focus of alveolar cell hyperplasia in one mouse.

None of the rats showed clinical signs of infection or disease and the only autopsy findings were a scar on the median lobe of the liver in one male rat and a congested thymus in one female rat. There were no microbiological or parasitological findings in male or female rats. Minor bronchus associated lymphoid tissue was observed in 4 male and 6 female rats. In one of the affected males there was a haemorrhagic focus in the liver, in one of the affected females there were sporadic intratubular casts of the kidney.

25 July 1980 Delivery: Ten male and 10 female rats were haphazardly selected for PEAT. There were no significant clinical observations or autopsy findings. One male rat lung culture developed colonies of Staphylococcus spp. and Streptococcus spp. All other cultures were sterile and no parasites were found. Histopathology revealed bronchus and vascular associated lymphoid tissue in all rats, this probably representing sub-clinical Sendai virus infection. In addition, a kidney of one female rat had a small cyst and lymphoid infiltration. No other notable features were seen.

8 August 1980 Delivery: Four male and 4 female rats were haphazardly selected for PEAT. There were no clinical signs of infection or disease. At autopsy, 2 female rats had grey foci on the lung lobes and one male had a flaccid, fluid-filled kidney. Microbiology and parasitology did not reveal any infections or infestations. Histopathology demonstrated bronchus associated lymphoid tissue in all males and 3 of the 4 female rats. These lung lesions probably reflected sub-clinical Sendai virus infection, which is endemic in this strain of rat, but the infection was not considered to impact upon the mutagenicity planned study.

Clinical Observations and Body Weights

No clinical signs of toxicity were seen in either rats or mice exposed to N,N-dimethylformamide.

Body weight values of the CD rats and B6C3F₁ mice taken at the time of dosing are given in Tables BW-1 to 4 and Appendix Tables BW-1 to 4. Exposure to 10 ppm or 400 ppm N,N-dimethylformamide atmospheres for 7 h/day on 5 consecu-

tive days had no deleterious effect upon mouse body weight changes, but there was quite clearly a reduction in weight gain in rats exposed to 400 ppm atmospheres.

Dosing with ethyl methanesulphonate at 100 mg/kg/day caused substantial weight losses to occur in rats and to a small extent, in 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 N,N-dimethylformamide (Table UDS-1). The highest concentration used in this test was 10 μ l or 9.445 mg/ml. The positive control substances used, 4-nitroquinoline-N-oxide and 2-aminoanthracene, induced significant responses in unscheduled DNA synthesis in these cells. These positive control substances, however are not appropriate for the demonstration of short patch repair when measured by Method 2.

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

CYTOGENETIC ANALYSIS OF RAT BONE MARROW CELLS

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

In the multiple dosing cytogenetic test, there were no indications of induction of chromosomal damage in either the male or female rats exposed to 10 ppm or 400 ppm N,N-dimethylformamide atmospheres. Responses to the positive control substance, ethyl methanesulphonate, were significant in both the males and the females.

In the single exposure rats treated with ethyl methanesulphonate, the frequencies of all aberrations were increased in males at the 24 h sample time only and in the females at the 6 h and 24 h sample times. When gaps were excluded from the computations, there were increases in aberrant cell frequency at the 24 h sample times in both males and females.

The only significant increase in a N,N-dimethylformamide treated group was in males at the 24 h sample time following a single dose. These were mainly gaps, although a Robertsonian translocation* and cells with unaligned chromatid fragments also were seen. A total of 13 aberrations (from 12 cells) were seen in this group compared with 3 aberrations in the air control group. However, 9 of the aberrations (8 cells) were observed in a single rat, which suggests that this particular rat was abnormal rather than an effect of the compound.

*Robertsonian translocation: 2 acrocentric chromosomes joined to form a simple metacentric chromosome.

DOMINANT LETHAL TEST

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

Pregnancy frequency was calculated in 2 ways: firstly, by considering as pregnant females with corpora lutea graviditatis (Table DL-1) and, secondly and more reliably, by considering, as pregnant only females with implantations (Table DL-2). With neither method was there observed any effect upon pregnancy frequency clearly due to N,N-dimethylformamide, although in Week 1 the 400 ppm atmosphere exposed group frequency was only 70% compared with 80% in all the other groups (Table DL-2). In the EMS treated group, pregnancy frequency was reduced in Weeks 2 and 3.

Corpora lutea graviditatis counts (Table DL-3) were not reduced in either of the N,N-dimethylformamide exposed groups except in Week 5 of the 400 ppm atmosphere group ($P < 0.01$). Counts were particularly high in this week in the air control group (15.0 ± 0.75) and this seems to be the cause of the significant difference. In the EMS treated group, there were significant reductions in Weeks 1-3. Other relatively low values in the positive control group were observed in Weeks 5 and 6, but these were consequential to the very high air control values referred to above. Values of 12.0 (Week 5, 400 ppm atmosphere group), 12.1 (Week 5, EMS group) and 12.7 (Week 6, EMS group) certainly cannot be considered low for corpora lutea counts per pregnancy in this strain of rat, although they are statistically significantly lower, when compared with the Air Control group.

Implantations per pregnancy (Table DL-4) were reduced in Week 3 of the 400 ppm atmosphere group ($P < 0.05$) and Week 6 of the 10 ppm atmosphere group ($P < 0.001$). In the absence of a dose-related response, the latter result cannot be interpreted as having biological significance, while the former reduction from the air control value was slight (11.7 ± 0.54 compared with 13.4 ± 0.54). Significant reductions were observed in Weeks 1-4 of the EMS treated group.

Live implantations per pregnancy (Table DL-5) reduced throughout the experiment in all groups. In the air control group, for example, the frequency in Week 1 was 12.3 ± 0.63 whereas in Week 10 it was only 8.4 ± 1.04 . Compared with the concurrent air control group, there was a significant reduction in Week 5 ($P < 0.05$) and a significant increase in Week 8 ($P < 0.05$) in the 10 ppm N,N-dimethylformamide group, but no significant deviations occurred in the 400 ppm atmosphere group. In the EMS treated group there were significant reductions in Weeks 1-4 ($P < 0.001$).

Compilation of live implantations with late deaths (Table DL-6) and examination of the Appendix Table DL shows that there was a tendency for late deaths to increase in all groups throughout the experiment. This was probably due to some infection of the female rats passed from the males.

Compared with the concurrent air control group, the only significant reduction in the frequency of live implantations and late deaths was in Week 6 of the 10 ppm atmosphere group. This result cannot be attributed to treatment. In the EMS treated group significant decreases occurred in Weeks 1-4 ($P < 0.001$).

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) indicated increases in the first of these frequencies, only in Weeks 5-7 and 9 of the 10 ppm atmosphere group and in Weeks 4 and 9 of the 400 ppm atmosphere group. In the EMS treated group there were increases in both (1) and (2) in Weeks 1, 3, 4 and in (1) in Week 2.

Analysis of the proportions of early deaths (Tables DL-8 and 9) did not indicate any effects attributable to N,N-dimethylformamide treatment. The proportion of early deaths was increased in Week 5 of the 10 ppm atmosphere group ($P < 0.01$), but not in the 400 ppm atmosphere group. Increases were also seen in the EMS treated group in Weeks 1-4, although these sometimes failed to show statistical significance because of the small numbers of degrees of freedom.

It was concluded that N,N-dimethylformamide was devoid of effects recognizable in this dominant lethal assay which, unfortunately, was complicated by extraneous effects upon intrauterine survival unconnected with treatment.

SPERM ABNORMALITY TEST

There were no increases in the frequencies of abnormal sperm in any of the categories examined (Table SA-1 and 2 and Appendix Table SA). In the EMS treated group, only Category D (folded tail) showed any significant increase in frequency.

SEX-LINKED RECESSIVE LETHAL TEST IN DROSOPHILA

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

A dose ranging study was undertaken on 3 September 1979 in which flies were exposed to 10 ppm N,N-dimethylformamide for 1 h, 2 h or 6 h 20 min or to 400 ppm N,N-dimethylformamide for 1 h, 2 h or 4 h 20 min. At 10 ppm there were no signs of toxicity and neither was survival reduced at 400 ppm for 2 h. Exposure to 400 ppm for 4 h 20 min, however, reduced survival to 10%, although hatchability of eggs from females mated with these survivors was not adversely affected. Exposure conditions chosen for the main study were:

400 ppm N,N-dimethylformamide for 2 h 15 min.

Two breeding stocks (A and B) were exposed (Table RL-2) in the main test on 10 September 1979. The frequencies and numbers of lethals scored were not increased in either stock exposed to the test compound, as compared with the air control group flies. F₃ generation flies were set up and scored only for Stock A, but no lethals were observed.

The positive control group was exposed to 0.4% v/v EMS for 5 h. The lethal frequency in the F₂ generation was 11%.

CONCLUSIONS

There was no evidence to suggest that N,N-dimethylformamide induced damage which was detectable by the genetic tests employed in this programme.

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TABLE AT-1
 N,N-Dimethylformamide
 Calibration Data for Low Level

Batch No.: 15163

Volume μl	Conc., ppm, (v/v)	Cumulative Chart Deflection, mm										Instrument Setting				
		3 Sept. 1979	4 Sept. 1979	5 Sept. 1979	6 Sept. 1979	7 Sept. 1979	11 Sept. 1979	18 Aug. 1980	19 Aug. 1980	20 Aug. 1980	21 Aug. 1980	22 Aug. 1980	Pathlength:	Wavelength:		
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14.25 m	9.2 μm
0.1	5.62	42.0	42.5	38.0	45.0	41.0	40.5	49.0	52.0	51.0	54.0	52.0	50.0	50.0	Absorbance Range: 0.1	Slit Width: 0.5 mm
0.2	11.24	85.0	85.5	83.5	88.0	85.0	85.5	102.5	99.0	100.5	106.0	105.0	100.0	100.0	Meter Response: 1	Recorder Voltage: 1 V
0.3	16.86	132.0	123.5	126.0	131.0	123.0	130.0	152.0	150.0	150.0	158.0	156.0	151.0	151.0	Chart Speed: 120 mm/h	
0.4	22.48	177.0	166.0	167.0	176.0	166.0	174.0	200.0	198.0	200.0	-	-	199.0	199.0		
Chart Deflection (mm) for 10 ppm		78.0	74.0	74.0	78.0	74.0	77.0	89.0	88.0	90.0	94.0	93.0	89.0	89.0	Calibration	

Syringe: Hamilton 1 μl
 Injection Volume: 0.1 μl
 No. of Repeat Injections: 4

3-7 September 1979 - Multiple exposure cytogenetics (abandoned)
 Dominant lethal exposures
 Sperm abnormality exposures
 11 September 1979 - Single exposure cytogenetics (abandoned)
 11 August 1980 - Single exposure cytogenetics - repeat
 18-22 August 1980 - Multiple exposure cytogenetics - repeat

TABLE AT-2
 N,N-Dimethylformamide
 Calibration Data for High Level

Dose Level: 400 ppm v/v		Batch No.: 15163												
Volume μl	Conc., ppm, (v/v)	Cumulative Chart Deflection, mm												
		3 Sept. 1979	4 Sept. 1979	5 Sept. 1979	6 Sept. 1979	7 Sept. 1979	10 Sept. 1979	11 Sept. 1979	11 Aug. 1980	18 Aug. 1980	19 Aug. 1980	20 Aug. 1980	21 Aug. 1980	22 Aug. 1980
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3.0	168.6	39.0	39.0	39.0	40.0	36.5	39.0	38.5	40.5	36.0	36.5	35.0	37.0	37.0
6.0	337.2	73.5	74.0	73.0	74.5	72.5	74.0	73.5	68.5	68.5	67.0	69.0	72.5	65.5
9.0	505.8	102.5	103.0	103.0	103.0	102.5	102.0	102.0	96.5	93.5	95.0	96.0	97.0	91.0
12.0	674.4	127.0	128.0	127.5	128.0	128.0	126.0	126.0	114.0	115.0	115.0	118.0	115.0	112.0
Chart Deflection (mm) for 400 ppm		85.0	85.0	85.0	85.0	86.0	85.0	85.0	82.0	79.0	78.0	79.0	82.0	76.0

Instrument Setting

(a) Pathlength: 3.75 m
 Wavelength: 9.2 μm
 Absorbance Range: 1
 Slit Width: 0.5 mm
 Meter Response: 10
 Recorder Voltage: 1 V
 Chart Speed: 120 mm/h

Calibration

Syringe: Hamilton 10 μl
 Injection Volume: 3.0 μl
 No. of Repeat
 Injections: 4

(a) Pathlength: 5.25 m on
 11, 18-22 August 1980

3-7 September 1979 - Multiple exposure cytogenetics (abandoned)
 Dominant lethal exposures
 Sperm abnormality exposures
 10 September 1979 - Drosophila exposure
 11 September 1979 - Single exposure cytogenetics (abandoned)
 11 August 1980 - Single exposure cytogenetics - repeat
 18-22 August 1980 - Multiple exposure cytogenetics - repeat

TABLE AT-3

N,N-Dimethylformamide
Chamber Atmosphere Homogeneity Data

Dose Level: 10 ppm and 400 ppm

Sample Location	% Deviation from Reference Sampling Point	
	Low	High
Reference Point (R)	0	0
Right Centre (RC)	0	-1.3
Right Front (RF)	+2.7	-0.6
Centre Front (CF)	+3.4	+0.6
Left Front (LF)	+0.7	0
Left Centre (LC)	+1.4	-2.5
Left Back (LB)	+2.1	-1.9
Centre Back (CB)	+4.8	-3.8
Right Back (RB)	+5.5	-3.1

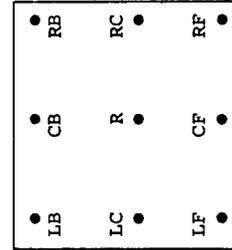


TABLE AT-4 (Abandoned)

N,N-Dimethylformamide
 Atmospheric Analysis by Infra-red Spectroscopy
 Target Concentration 10 ppm

Exposure Dates	Exposure Day	% Deviation from Target Concentration in Minutes											Time Averaged Concentration for 7 h. (ppm)		
		-17.5	-15.0	-12.5	-10.0	-7.5	-5.0	-2.5	0	+2.5	+5.0	+7.5		+10.0	+12.5
11 September 1979	Single	-	-	-	-	-	25	130	215	20	5	-	25	-	10.0
3 September 1979	Multiple 1	-	-	-	-	-	-	21	89	258	27	13	8	6	10.3
4 September 1979	Multiple 2	45	190	120	55	10	-	-	-	-	-	-	-	-	8.6
5 September 1979	Multiple 3	-	-	-	-	-	-	25	145	250	-	-	-	-	10.0
6 September 1979	Multiple 4	-	-	-	-	-	10	195	90	125	-	-	-	-	9.9
7 September 1979	Multiple 5	-	-	-	-	-	80	115	185	10	-	30	-	-	9.9

TABLE AT-4 (Abandoned continued)

N,N-Dimethylformamide
Target Concentration 400 ppm

Exposure Dates	Exposure Day	% Deviation from Target Concentration i Minutes											Time Averaged Concentration for 7 h (ppm)		
		-30	-20	-12.5	-10.0	-7.5	-5.0	-2.5	0	+2.5	+5.0	+7.5			
11 September 1979	Single	5	10	15	-	-	15	40	185	65	50	35			399.6
3 September 1979	Multiple 1	-	-	-	-	-	35	145	155	85	-	-			396.9
4 September 1979	Multiple 2	-	-	-	-	10	95	155	160	-	-	-			391.1
5 September 1979	Multiple 3	-	-	-	-	-	80	145	145	-	50	-			395.1
6 September 1979	Multiple 4	-	-	-	10	-	60	175	135	40	-	-			393.0
7 September 1979	Multiple 5	-	-	-	10	5	10	-	315	80	-	-			400.1

TABLE AT-5 (Repeat)

N,N-Dimethylformamide
 Atmospheric Analysis by Infra-red Spectroscopy
 Multiple Dosing Cytogenetic Test
 Target Concentration 10 ppm

Exposure Dates	Exposure Day	% Deviation from Target Concentration in Minutes												Time Averaged Concentration for 7 h (ppm)	
		-12.5	-10	-7.5	-5	-2.5	0	+2.5	+5	+7.5	+10	+12.5	+15		+65
11 August 1980	Single	7	3	10	52	88	120	45	90	-	-	-	-	5	10.05
18 August 1980	Multiple ₁	-	10	15	74	109	150	27	13	15	-	-	7	-	9.88
19 August 1980	Multiple ₂	-	-	30	15	25	230	95	25	-	-	-	-	-	10.00
20 August 1980	Multiple ₃	-	15	-	10	143	157	65	30	-	-	-	-	-	9.98
21 August 1980	Multiple ₄	-	-	-	15	60	105	140	80	-	5	15	-	-	10.18
22 August 1980	Multiple ₅	-	-	-	2.5	147.5	110	60	75	15	-	10	-	-	10.09

TABLE AT-6

N,N-Dimethylformamide
Atmospheric Analysis by Infra-red Spectroscopy
Drosophila Exposure - 2 h 12 min (10 September 1979)
Target Concentration 400 ppm

% Deviation from Target Concentration in Minutes				Time Averaged Concentration (ppm)
-5	-2.5	0	+2.5	+5
10	25	92	-	5
				397.3

TABLE BW-1 (Abandoned)

N,N-Dimethylformamide
Multiple Exposure Cytogenetics Test (3-7 September 1979)
Group Mean Body Weights (g) for the Dosing Period of Male and Female CD Rats

Sex	Day	Air Control (0 ppm)	10 ppm	400 ppm	5 x 100 mg/kg EMS
Male	1	351.3 ± 13.1	331.1 ± 21.3	339.7 ± 17.4	338.2 ± 28.4
	2	358.2 ± 12.7	334.3 ± 21.3	344.5 ± 16.7	334.6 ± 28.0
	3	361.8 ± 12.7	340.4 ± 23.0	344.4 ± 17.7	328.5 ± 29.4
	4	365.0 ± 12.1	348.8 ± 22.5	347.6 ± 20.9	319.5 ± 32.7
	5	371.2 ± 15.3	350.0 ± 23.3	349.2 ± 19.2	310.9 ± 32.5
	Weight gain/ loss	19.9	18.9	9.5	-27.3
Female	1	231.0 ± 8.5	226.6 ± 17.7	231.2 ± 21.2	221.9 ± 14.2
	2	231.6 ± 8.9	226.9 ± 18.8	229.4 ± 18.8	218.9 ± 13.4
	3	231.3 ± 8.8	227.6 ± 17.6	227.8 ± 18.2	216.3 ± 13.7
	4	232.3 ± 10.5	228.3 ± 17.0	227.5 ± 18.5	208.3 ± 13.9
	5	235.4 ± 9.8	227.9 ± 17.9	227.2 ± 18.3	202.1 ± 14.7
	Weight gain/ loss	4.4	1.3	-4.0	-19.8

TABLE BW-1 (Repeat)

N,N-Dimethylformamide
Multiple Exposure Cytogenetics Test (18-22 September 1979)
Group Mean Body Weights (g) for the Dosing Period of Male and Female CD Rats

Sex	Day	Air Control (0 ppm)	10 ppm	400 ppm	5 x 100 mg/kg EMS
Male	1	386.0 ± 17.8	378.2 ± 16.8	383.8 ± 16.4	371.3 ± 16.2
	2	388.8 ± 17.5	378.9 ± 17.4	383.6 ± 15.6	362.6 ± 19.0
	3	392.7 ± 19.6	383.2 ± 19.9	385.1 ± 15.9	351.2 ± 18.2
	4	398.5 ± 19.8	385.2 ± 18.4	383.3 ± 15.6	346.6 ± 19.9
	5	397.2 ± 21.8	390.5 ± 17.7	384.9 ± 16.8	342.1 ± 20.0
	Weight gain/ loss	11.2	12.3	1.1	-29.2
Female	1	248.5 ± 11.0	243.6 ± 11.8	254.4 ± 13.9	252.6 ± 10.7
	2	250.2 ± 12.6	243.2 ± 12.0	249.4 ± 12.2	245.4 ± 12.8
	3	251.7 ± 10.7	243.6 ± 10.3	252.9 ± 12.1	239.2 ± 15.1
	4	254.1 ± 11.2	245.4 ± 10.8	249.8 ± 11.2	237.1 ± 15.7
	5	252.1 ± 12.8	244.8 ± 13.1	247.6 ± 13.0	232.7 ± 16.6
	Weight gain/ loss	3.6	5.2	-6.8	-19.9

TABLE BW-2 (Abandoned)

N,N-Dimethylformamide
 Single Exposure Cytogenetics Test (11 September 1979)
 Group Mean Body Weights (g) for Male and Female CD Rats

Sex	Sampling Time (Hours Post Exposure)	Air Control (0 ppm)	10 ppm	400 ppm	250 mg/kg EMS
Male	6	394.3 ± 21.0	395.6 ± 18.3	399.9 ± 27.1	392.6 ± 26.1
	24	382.6 ± 13.3	394.7 ± 18.8	383.0 ± 22.8	393.2 ± 29.3
	48	386.4 ± 24.5	387.4 ± 21.4	382.1 ± 19.6	392.7 ± 24.8
Female	6	251.8 ± 20.3	249.5 ± 16.5	241.7 ± 26.5	252.4 ± 22.7
	24	248.6 ± 23.0	255.2 ± 14.9	234.5 ± 12.9	245.6 ± 15.8
	48	252.0 ± 28.0	254.4 ± 19.2	249.5 ± 14.8	242.4 ± 18.4

TABLE BW-2 (Repeat)

N,N-Dimethylformamide
 Single Exposure Cytogenetics Test (11 August 1981)
 Group Mean Body Weights (g) for Male and Female CD Rats

Sex	Sampling Time (Hours Post Exposure)	Air Control (0 ppm)	10 ppm	400 ppm	250 mg/kg EMS
Male	6	406.8 ± 22.0	404.4 ± 26.0	412.3 ± 28.8	414.0 ± 22.2
	24	402.8 ± 20.2	415.8 ± 23.3	394.8 ± 16.2	415.0 ± 19.8
	48	397.7 ± 28.0	407.1 ± 21.5	394.8 ± 25.6	427.3 ± 30.3
Female	6	252.6 ± 16.9	258.2 ± 13.6	259.8 ± 16.6	255.1 ± 15.5
	24	256.1 ± 17.9	255.6 ± 16.6	260.7 ± 15.2	256.1 ± 16.4
	48	260.2 ± 20.7	252.1 ± 18.8	262.1 ± 18.6	253.4 ± 17.1

TABLE BW-3

N,N-Dimethylformamide
 Dominant Lethal Assay (3-7 September 1979)
 Group Mean Body Weights (g) for the Dosing Period of Male CD Rats

Day	Air Control (0 ppm)	10 ppm	400 ppm	5 x 100 mg/kg EMS
1	344.5 ± 14.7	344.2 ± 12.1	343.4 ± 16.4	349.3 ± 16.4
2	352.8 ± 13.4	350.0 ± 12.1	346.7 ± 16.0	345.8 ± 16.7
3	356.5 ± 12.8	352.4 ± 14.3	344.0 ± 16.4	341.2 ± 16.2
4	361.2 ± 13.3	355.1 ± 13.3	345.0 ± 16.2	330.1 ± 17.5
5	365.3 ± 14.2	357.5 ± 16.1	344.8 ± 17.2	320.7 ± 18.1
Weight gain/loss	20.8	13.3	1.4	-28.6

TABLE BW-4

N,N-Dimethylformamide
 Sperm Abnormalities Test (3-7 September 1979)
 Group Mean Body Weights (g) for the Dosing Period of Male B6C3F₁ Mice

Day	Air Control (0 ppm)	10 ppm	400 ppm	5 x 200 mg/kg EMS
1	23.9 ± 0.7	23.0 ± 0.9	22.1 ± 0.9	24.3 ± 1.2
2	23.8 ± 1.1	23.9 ± 0.8	23.0 ± 1.1	24.3 ± 1.1
3	24.0 ± 1.0	24.2 ± 1.0	23.4 ± 0.8	24.6 ± 1.0
4	23.8 ± 1.2	24.0 ± 1.2	23.6 ± 1.1	24.3 ± 1.2
5	24.2 ± 1.0	24.3 ± 0.9	24.0 ± 1.2	24.1 ± 1.2
Weight gain/loss	0.3	1.3	1.9	-0.2

TABLE UDS-1

N,N-Dimethylformamide
Unscheduled DNA Synthesis

Substance	Concentration ($\mu\text{g/ml}$)		Mean Number of Grains/Nucleus \pm S.D.	
	With S-9	Without S-9	With S-9	Without S-9
Dimethylsulphoxide	10,000	10,000	12.1 \pm 7.6	6.7 \pm 3.1
4-Nitroquinoline-N-oxide	-	1.25	-	68.0 \pm 17.4
2-Aminoanthracene	3	-	65.0 \pm 27.8	-
N,N-Dimethylformamide	74	16	11.3 \pm 6.7	13.4 \pm 3.6
	148	31	12.6 \pm 7.6	8.4 \pm 6.1
	295	63	11.5 \pm 6.7	7.7 \pm 5.9
	590	125	19.0 \pm 10.8	7.1 \pm 3.8
	1,181	250	8.9 \pm 10.1	10.8 \pm 5.0
	2,361	500	9.4 \pm 3.7	9.3 \pm 3.5
	4,723	1,000	10.9 \pm 4.9	9.0 \pm 5.2
9,445	2,000	12.8 \pm 6.4	8.8 \pm 3.4	

TABLE CA-MD-M-1

N,N-Dimethylformamide
 Cytogenetic Analysis of Rat Bone Marrow Cells
 Chromatid/Chromosomal Aberrations Scored
 Males

Group	Number of Spreads Observed	Observed Aberrations							Miscellaneous			
		Chromatid			Chromosome							
		Gap	B	W/F	B	W/O	F	B		W/O	F	
Air Control, 7 h/day	500	4	1	-	-	2	-	-	-	-	-	-
10 ppm, 7 h/day	360	4	-	-	-	-	-	-	-	-	-	-
400 ppm, 7 h/day	424	4	2	-	-	-	-	-	-	-	-	-
EMS, 100 mg/kg/day	460	13	2	-	-	1	-	-	-	-	-	2 Exchanges

Multiple Dosing

Sampling Time: 6 h

TABLE CA-MD-M-2

N,N-Dimethylformamide
Cytogenetic Analysis of Rat Bone Marrow Cells
Summary of Observed Aberrations
Males

Multiple Dosing	Spreads with Aberrations						Excluding Gaps	
	Total			Spreads with Aberrations			Excluding Gaps	
	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t	S.E. of Mean	t
Air Control	0.250	0.055		0.160	0.027		0.027	
10 ppm	0.224	0.061	-0.31	0.161	0.030		0.030	0.02
400 ppm	0.286	0.055	0.47	0.214	0.027		0.027	1.41
EMS, 100 mg/kg	0.423	0.055	2.25*	0.237	0.027		0.027	2.00

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

*P<0.05

TABLE CA-MD-F-1

N,N-Dimethylformamide
 Cytogenetic Analysis of Rat Bone Marrow Cells
 Chromatid/Chromosomal Aberrations Scored
 Females

Multiple Dosing Group	Number of Spreads Observed	Observed Aberrations							Miscellaneous
		Chromatid			Chromosome				
		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
Air Control, 7 h/day	500	3	-	-	-	-	-	-	-
10 ppm, 7 h/day	465	5	-	-	1	-	-	-	-
400 ppm, 7 h/day	500	2	-	-	-	-	-	-	1 Exchange
EMS, 100 mg/kg/day	409	17	7	-	3	-	-	-	3 Exchanges

Sampling Time: 6 h

TABLE CA-MD-F-2

N,N-Dimethylformamide
Cytogenetic Analysis of Rat Bone Marrow Cells
Summary of Observed Aberrations
Females

Multiple Dosing	Spreads with Aberrations						Excluding Gaps	
	Total							
	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t	S.E. of Mean	t
Air Control	0.191	0.047		0.141	0.038		0.038	
10 ppm	0.271	0.047	1.22	0.152	0.038		0.038	0.21
400 ppm	0.200	0.047	0.14	0.160	0.038		0.038	0.37
EMS, 100 mg/kg	0.502	0.049	4.58***	0.337	0.040		0.040	3.54**

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

**P<0.01

***P<0.001

Sampling Time: 6 h

TABLE CA-M6-1

N,N-Dimethylformamide
 Cytogenetic Analysis of Rat Bone Marrow Cells
 Chromatid/Chromosomal Aberrations Scored
 Males

Single Dosing Group	Number of Spreads Observed	Observed Aberrations							Miscellaneous
		Chromatid		Chromosome			Miscellaneous		
		Gap	B W F	B w/o F	Gap	B W F		B w/o F	
Air Control, 7 h/day	350	-	-	-	-	-	-	-	
10 ppm, 7 h/day	400	1	-	-	-	-	-	-	
400 ppm, 7 h/day	400	1	-	-	-	-	-	-	
EMS, 250 mg/kg/day	168	1	-	-	-	-	-	-	

Sampling Time: 6 h

TABLE CA-M6-2

N,N-Dimethylformamide
Cytogenetic Analysis of Rat Bone Marrow Cells
Summary of Observed Aberrations
Males

Single Dosing	Spreads with Aberrations						Excluding Gaps	
	Total			Spreads with Aberrations			Excluding Gaps	
	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t	S.E. of Mean	t
Air Control	0.141	0.026		0.141	0.016		0.016	
10 ppm	0.165	0.024	0.70	0.141	0.015	0.00	0.015	0.00
400 ppm	0.165	0.024	0.70	0.141	0.015	-0.00	0.015	-0.00
EMS, 250 mg/kg	0.253	0.031	2.81**	0.214	0.018	3.03	0.018	3.03

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

**P<0.01

TABLE CA-M24-1

N,N-Dimethylformamide
 Cytogenetic Analysis of Rat Bone Marrow Cells
 Chromatid/Chromosomal Aberrations Scored
 Males

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	465	2	1	-	-	-	-	-	-
10 ppm, 7 h/day	500	1	-	-	-	-	-	-	2 Chromatid Fragments
400 ppm, 7 h/day	386	8	-	-	1	-	-	-	1 Robertsonian Translocation 3 Chromatid Fragments
EMS, 250 mg/kg/day	450	14	10	-	2	-	-	-	6 Chromatid Fragments 2 Multi Aberrations

Single Dosing

Sampling Time: 24 h

TABLE CA-M24-2

N,N-Dimethylformamide
Cytogenetic Analysis of Rat Bone Marrow Cells
Summary of Observed Aberrations
Males

Single Dosing	Spreads with Aberrations						Sampling Time: 24 h
	Total			Excluding Gaps			
	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t	
Air Control	0.191	0.057		0.172	0.036		
10 ppm	0.180	0.057	-0.13	0.180	0.036	0.17	
400 ppm	0.336	0.064	1.69***	0.201	0.040	0.54***	
EMS, 250 mg/kg	0.503	0.060	3.77***	0.370	0.038	3.77***	

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

***p<0.001

TABLE CA-M48-2

N,N-Dimethylformamide
Cytogenetic Analysis of Rat Bone Marrow Cells
Summary of Observed Aberrations
Males

Single Dosing

Sampling Time: 48 h

Treatment Group	Spreads with Aberrations				Excluding Gaps		t
	Total		t	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	S.E. of Mean	
	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean					
Air Control	0.277	0.047		0.190	0.039		
10 ppm	0.251	0.042	-0.42	0.191	0.035		0.01
400 ppm	0.263	0.044	-0.21	0.232	0.037		0.76
EMS, 250 mg/kg	0.244	0.042	-0.52	0.244	0.035		1.02

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

TABLE CA-F6-1

N,N-Dimethylformamide
 Cytogenetic Analysis of Rat Bone Marrow Cells
 Chromatid/Chromosomal Aberrations Scored
 Females

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	495	6	-	-	-	-	-	-
10 ppm, 7 h/day	450	2	2	-	-	-	1 Exchange	-
400 ppm, 7 h/day	450	1	1	-	-	-	-	-
EMS, 250 mg/kg/day	350	11	1	-	-	-	1 Chromatid Fragment	-

Single Dosing

Sampling Time: 6 h

TABLE CA-F6-2

N,N-Dimethylformamide
Cytogenetic Analysis of Rat Bone Marrow Cells
Summary of Observed Aberrations
Females

Single Dosing	Spreads with Aberrations						Sampling Time: 6 h
	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	0.252	0.044		0.141	0.022		
10 ppm	0.229	0.046	-0.36	0.185	0.023	1.35	
400 ppm	0.174	0.046	-1.22	0.163	0.023	0.66	
EMS, 250 mg/kg	0.355	0.053	1.50	0.197	0.027	1.62	

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

TABLE CA-P24-1
 N,N-Dimethylformamide
 Cytogenetic Analysis of Rat Bone Marrow Cells
 Chromatid/Chromosomal Aberrations Scored
 Females

Group	Number of Spreads Observed	Observed Aberrations										Miscellaneous			
		Chromatid				Chromosome									
		Gap	B	W	F	B	W	F	Gap	B	W		F		
Air Control, 7 h/day	500	4	-	-	1	1	-	-	1	-	-	-	-	-	1 Chromatid Fragment
10 ppm, 7 h/day	500	3	1	-	-	-	-	-	-	-	-	-	-	-	1 Chromatid Fragment
400 ppm, 7 h/day	500	7	1	-	2	-	-	-	-	-	-	-	-	-	1 Chromatid Fragment
EMS, 250 mg/kg/day	500	29	19	-	-	-	-	-	-	-	-	-	-	-	13 Chromatid Fragments 1 Robertsonian Translocation 5 Exchanges

Single Dosing

Sampling Time: 24 h

TABLE CA-F24-1 (Supplementary)

N,N-Dimethylformamide
Cytogenetic Analysis of Rat Bone Marrow Cells
Supplementary Observations
Females

Single Dosing		Sampling Time: 24 h	
Group	Animal Number	Miscellaneous Observations	
400 ppm, 7 h/day	235	1 Chromosome split at centromere	

TABLE CA-F24-2

N,N-Dimethylformamide
Cytogenetic Analysis of Rat Bone Marrow Cells
Summary of Observed Aberrations
Females

Single Dosing	Spreads with Aberrations						Excluding Gaps		Sampling Time: 24 h
	Total								
	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t			
Air Control	0.259	0.054		0.180	0.037		0.037		
10 ppm	0.231	0.054	-0.37	0.180	0.037	0.00	0.037	0.00	
400 ppm	0.257	0.054	-0.03	0.211	0.037	0.58	0.037	0.58	
EMS, 250 mg/kg	0.560	0.054	3.92**	0.424	0.037	4.63***	0.037	4.63***	

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

** P<0.01

*** P<0.001

TABLE CA-F48-1

N,N-Dimethylformamide
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Females

Group	Number of Spreads Observed	Observed Aberrations										Miscellaneous			
		Chromatid				Chromosome									
		Gap	B	W	F	Gap	B	W	F	B	w/o		F		
Air Control, 7 h/day	403	4	1	-	-	-	-	-	-	-	-	-	-	-	-
10 ppm, 7 h/day	450	5	3	-	-	-	-	-	-	-	-	-	-	-	-
400 ppm, 7 h/day	492	6	2	-	-	-	-	-	-	-	-	-	-	2 Robertsonian Translocations	-
EMS, 250 mg/kg/day	500	7	-	-	-	-	-	-	-	-	-	-	-	-	-

Single Dosing

Sampling Time: 48 h

TABLE CA-F48-2

N,N-Dimethylformamide
Cytogenetic Analysis of Rat Bone Marrow Cells
Summary of Observed Aberrations
Females

Single Dosing	Spreads with Aberrations						Sampling Time: 48 h
	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	
Treatment Group							
Air Control	0.283	0.045		0.205	0.033		
10 ppm	0.303	0.045	0.30	0.229	0.033	0.51	
400 ppm	0.301	0.043	0.29	0.222	0.031	0.36	
EMS, 250 mg/kg	0.280	0.043	-0.05	0.141	0.031	-1.43	

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

TABLE DL-1

N,N-Dimethylformamide
 Dominant Lethal Test in Rats
 Pregnancy Frequency (Females with Corpora Lutea Graviditatis)

Assessment Week from Dosing	Air Control (0 ppm)	10 ppm	400 ppm	5 x 100 mg/kg EMS
1	80%	80%	75%	85%
2	95%	95%	95%	35%
3	95%	95%	95%	53%
4	100%	80%	83%	84%
5	90%	100%	95%	100%
6	100%	95%	95%	85%
7	100%	95%	95%	95%
8	100%	95%	95%	90%
9	95%	100%	90%	95%
10	95%	84%	95%	95%

TABLE DL-2

N,N-Dimethylformamide
Dominant Lethal Test in Rats
Pregnancy Frequency (Females with Implantations)

Assessment Week from Dosing	Air Control (0 ppm)	10 ppm	400 ppm	5 x 100 mg/kg EMS
1	16/20	16/20	14/20	16/20
2	19/20	17/20	19/20	4/20
3	19/20	19/20	19/20	7/19
4	20/20	16/20	15/18	16/19
5	18/20	18/20	19/20	20/20
6	18/20	19/20	19/20	17/20
7	19/20	18/20	18/20	19/20
8	20/20	19/20	19/20	17/20
9	18/20	17/19	19/20	19/20
10	19/20	16/19	19/20	19/20

TABLE DL-3

N,N-Dimethylformamide
 Dominant Lethal Test in Rats
 Total Number of Corpora Lutea per Pregnancy

Assessment Week from Dosing	Air Control (0 ppm)	10 ppm	400 ppm	5 x 100 mg/kg EMS
1	13.4 ± 0.60	12.8 ± 0.60	13.6 ± 0.65	9.3 ± 1.16**
2	13.2 ± 0.47	13.1 ± 0.49	13.3 ± 0.48	2.0 ± 0.91**
3	13.1 ± 0.50	12.9 ± 0.50	12.4 ± 0.50	4.9 ± 2.18*
4	12.6 ± 0.56	13.4 ± 0.62	13.2 ± 0.64	11.3 ± 0.87
5	15.0 ± 0.75	13.9 ± 0.75	12.0 ± 0.73**	12.1 ± 0.94*
6	15.4 ± 0.75	13.7 ± 0.73	14.5 ± 0.73	12.7 ± 0.41**
7	13.2 ± 0.67	13.2 ± 0.69	12.9 ± 0.69	12.8 ± 0.48
8	13.4 ± 0.55	14.1 ± 0.57	13.4 ± 0.57	13.4 ± 0.43
9	13.4 ± 0.82	14.7 ± 0.84	13.4 ± 0.80	13.3 ± 0.61
10	13.3 ± 0.62	13.3 ± 0.67	12.4 ± 0.62	13.3 ± 0.38

1 = Mean ± standard error of mean

*P<0.05

**P<0.01

TABLE DL-4
 N,N-Dimethylformamide
 Dominant Lethal Test in Rats
 Total Implantations per Pregnancy

Assessment Week from Dosing	Air Control (0 ppm)	10 ppm	400 ppm	5 x 100 mg/kg EMS
1	12.9 ± 0.57	13.8 ± 0.57	13.9 ± 0.61	6.9 ± 0.98***
2	12.6 ± 0.48	12.5 ± 0.51	13.1 ± 0.48	1.5 ± 0.50***
3	13.4 ± 0.54	13.1 ± 0.54	11.7 ± 0.54*	5.4 ± 3.08*
4	12.5 ± 0.67	13.8 ± 0.75	13.1 ± 0.77	9.1 ± 1.08*
5	12.7 ± 0.92	13.0 ± 0.92	12.4 ± 0.90	11.5 ± 0.89
6	14.3 ± 0.46	12.0 ± 0.45***	13.7 ± 0.45	12.3 ± 0.51*
7	12.4 ± 0.70	13.3 ± 0.72	12.8 ± 0.72	12.7 ± 0.43
8	13.3 ± 0.43	13.5 ± 0.45	13.0 ± 0.45	13.5 ± 0.49
9	13.2 ± 0.84	13.4 ± 0.86	13.4 ± 0.82	12.3 ± 0.66
10	12.3 ± 0.54	12.3 ± 0.58	12.3 ± 0.54	13.0 ± 0.43

1 = Mean ± standard error of mean

*P<0.05

***P<0.001

TABLE DJ-5

N,N-Dimethylformamide
Dominant Lethal Test in Rats
Live Implantations per Pregnancy

Assessment Week from Dosing	Air Control (0 ppm)	10 ppm	400 ppm	5 x 100 mg/kg EMS
1	12.3 ± 0.63	13.1 ± 0.63	13.4 ± 0.67	3.2 ± 1.10***
2	11.9 ± 0.51	11.8 ± 0.54	12.3 ± 0.51	0.3 ± 0.25***
3	11.9 ± 0.78	12.4 ± 0.78	10.6 ± 0.78	0.0 ± 0.00***
4	11.7 ± 0.69	11.7 ± 0.77	11.3 ± 0.79	4.8 ± 0.87***
5	11.3 ± 0.95	7.8 ± 0.95*	11.3 ± 0.93	9.3 ± 1.04
6	10.0 ± 1.00	9.6 ± 0.97	10.6 ± 0.97	10.5 ± 0.84
7	10.8 ± 1.08	9.3 ± 1.11	11.3 ± 1.11	9.8 ± 0.91
8	9.6 ± 0.96	12.4 ± 0.98*	10.2 ± 0.98	10.9 ± 0.93
9	9.7 ± 1.22	10.7 ± 1.25	10.3 ± 1.19	9.79 ± 1.06
10	8.4 ± 1.04	10.5 ± 1.13	9.4 ± 1.04	9.4 ± 1.08

1 = Mean + standard error of mean

*p<0.05

***p<0.001

TABLE DL-6

N,N-Dimethylformamide
 Dominant Lethal Test in Rats
 Live Implantations and Late Deaths per Pregnancy

Assessment Week from Dosing	Air Control (0 ppm)	10 ppm	400 ppm	5 x 100 mg/kg EMS
1	12.3 ± 0.62	13.3 ± 0.62	13.4 ± 0.67	3.2 ± 1.10***
2	12.0 ± 0.50	11.9 ± 0.53	12.4 ± 0.50	0.3 ± 0.25***
3	12.3 ± 0.69	12.5 ± 0.69	11.2 ± 0.69	0.0 ± 0.00***
4	11.7 ± 0.65	12.8 ± 0.70	11.4 ± 0.73	4.9 ± 0.85***
5	12.4 ± 0.85	10.3 ± 0.85	11.6 ± 0.82	10.7 ± 0.82
6	13.0 ± 0.55	11.0 ± 0.53*	12.3 ± 0.53	11.3 ± 0.59*
7	11.3 ± 0.87	11.8 ± 0.90	11.8 ± 0.90	11.4 ± 0.63
8	12.5 ± 0.52	12.7 ± 0.53	12.0 ± 0.53	12.5 ± 0.50
9	11.8 ± 1.03	11.9 ± 1.06	11.6 ± 1.01	10.6 ± 0.97
10	11.0 ± 0.70	11.9 ± 0.76	10.8 ± 0.70	12.1 ± 0.39

1 = Mean ± standard error of mean

*P<0.05

***P<0.001

TABLE DL-7

N,N-Dimethylformamide
Dominant Lethal Test in Rats
Frequency of Pregnancies with One or More or Two or More Early Deaths

Assessment Week from Dosing	Air Control (0 ppm)		10 ppm		400 ppm		5 x 100 mg/kg EMS	
	>0	>1	>0	>1	>0	>1	>0	>1
1	7/16	3/16	5/16	1/16	6/14	1/14	12/16	10/16
2	8/19	3/19	8/17	1/17	9/19	3/19	3/4	1/4
3	5/19	2/19	9/19	1/19	7/19	3/19	7/7	4/7
4	9/20	4/20	6/16	4/16	11/15	5/15	15/16	12/16
5	5/18	0/18	14/18	6/18	8/19	5/19	11/20	5/20
6	8/18	6/18	12/19	4/19	9/19	5/19	6/17	3/17
7	9/19	1/19	12/18	5/18	8/18	4/18	11/19	6/19
8	10/20	3/20	7/19	3/19	8/19	5/19	9/17	6/17
9	5/18	4/18	11/17	6/17	12/19	7/19	8/19	3/19
10	10/19	4/19	4/16	2/16	9/19	5/19	10/19	5/19

TABLE DL-8
 N,N-Dimethylformamide
 Dominant Lethal Test in Rats
 Early Death Frequency, Freeman-Tukey Poisson Transformation

Assessment Week from Dosing	Air Control (0 ppm)	10 ppm	400 ppm	5 x 100 mg/kg EMS
1	1.793 ± 0.2231	1.524 ± 0.2231	1.658 ± 0.2385	3.632 ± 0.4835**
2	1.773 ± 0.2164	1.743 ± 0.2288	1.816 ± 0.2164	2.390 ± 0.5579
3	1.729 ± 0.2755	1.708 ± 0.2755	1.667 ± 0.2755	4.151 ± 1.0310
4	1.867 ± 0.2810	1.859 ± 0.3141	2.542 ± 0.3244	4.084 ± 0.3822
5	1.393 ± 0.3351	2.823 ± 0.3351**	1.819 ± 0.3261	1.990 ± 0.2193
6	2.137 ± 0.3216	2.105 ± 0.3130	2.207 ± 0.3130	1.824 ± 0.3519
7	1.930 ± 0.3154	2.395 ± 0.3240	1.940 ± 0.3240	2.264 ± 0.3024
8	1.875 ± 0.2584	1.775 ± 0.2651	1.948 ± 0.2651	2.042 ± 0.2576
9	1.907 ± 0.3755	2.404 ± 0.3864	2.531 ± 0.3655	2.169 ± 0.4479
10	2.162 ± 0.3152	1.445 ± 0.3435	2.196 ± 0.3152	2.025 ± 0.2524

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

TABLE DL-9

N,N-Dimethylformamide
Dominant Lethal Test in Rats
Early Death Frequency, Freeman-Tukey Binomial Transformation

Assessment Week from Dosing	Air Control (0 ppm)	10 ppm	400 ppm	5 x 100 mg/kg EMS
1	0.499 ± 0.0638	0.413 ± 0.0638	0.438 ± 0.0682	1.807 ± 0.2414
2	0.490 ± 0.0632	0.511 ± 0.0668	0.492 ± 0.0632	2.014 ± 0.4144*
3	0.489 ± 0.1059	0.471 ± 0.1059	0.552 ± 0.1059	2.534 ± 0.0905***
4	0.528 ± 0.0737	0.496 ± 0.0824	0.696 ± 0.0851	1.580 ± 0.1411***
5	0.382 ± 0.1083	0.833 ± 0.1083**	0.589 ± 0.1054	0.597 ± 0.0578
6	0.568 ± 0.0899	0.610 ± 0.0875	0.597 ± 0.0875	0.523 ± 0.1009
7	0.603 ± 0.1098	0.675 ± 0.1128	0.557 ± 0.1128	0.645 ± 0.0951
8	0.509 ± 0.0747	0.493 ± 0.0767	0.540 ± 0.0767	0.551 ± 0.0700
9	0.571 ± 0.1356	0.692 ± 0.1396	0.797 ± 0.1320	0.660 ± 0.1505
10	0.615 ± 0.1025	0.415 ± 0.1117	0.660 ± 0.1025	0.551 ± 0.0674

1 = Mean ± standard error or mean

*P<0.05

**P<0.01

***P<0.001

TABLE SA-1

N,N-Dimethylformamide
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	9699	7	14	173	24	83	301	0.07	0.14	1.73	0.24	0.83	3.01
10 ppm, 7 h/day	9336	7	10	155	19	74	264	0.07	0.10	1.61	0.20	0.77	2.75
400 ppm, 7 h/day	9688	6	23	171	22	90	312	0.06	0.23	1.71	0.22	0.90	3.12
EMS, 200 mg/kg/day	9632	9	6	187	48	118	368	0.09	0.06	1.87	0.48	1.18	3.68

*A = Hook up-turned or hook elongated

B = Banana-shaped head

C = Amorphous head

D = Folded tail

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

TABLE SA-2

N,N-Dimethylformamide
Sperm Abnormality Test in Mice
Mean of Freeman-Tukey Binomial Transformation
+ Standard Error

Dose Group	Abnormality Category					Total
	A	B	C	D	E	
Air Control, 7 h/day	5.86 + 0.953	7.28 + 1.222	25.52 + 1.932	9.67 + 1.275	18.48 + 1.652	33.96 + 2.472
10 ppm, 7 h/day	6.17 + 0.953	6.60 + 1.222	25.30 + 1.932	9.66 + 1.275	17.88 + 1.652	33.24 + 2.472
400 ppm, 7 h/day	5.41 + 0.953	9.62 + 1.222	26.26 + 1.932	9.14 + 1.275	18.98 + 1.652	35.29 + 2.472
EMS, 200 mg/kg/day	6.28 + 0.953	5.63 + 1.222	27.37 + 1.932	14.27* + 1.275	21.14 + 1.652	38.16 + 2.472

A = Hook up-turned or hook elongated

B = Banana-shaped head

C = Amorphous head

D = Folded tail

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

* = P < 0.05

TABLE RL-1

N,N-Dimethylformamide
Drosophila Dose Ranging Experiment

Day		10 ppm			400 ppm			Control
		1 h	2 h	6 h 20 min	1 h	2 h	4 h 20 min	
0	No. of males exposed	50	50	50	49	50	50	
1	No. and % survival	48/96%	50/100	50/100	48/97%	50/100%	5/10%	
2	No. of eggs laid by 5 ♀♀	23	27	37	18	13	31	43
3	No. and % hatched	20/86%	23/85%	34/91%	14/77%	10/76%	26/83%	40/93%

TABLE RL-2

N,N-Dimethylformamide
Drosophila SLRL Procedure and Results

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

	Brood 1	Brood 2	Brood 3
F ₁ set up	11.9.79	4.9.79	19.9.79
F ₂ set up	28.9.79	1.10.79	1.10.79
F ₂ scored	10.10.89	11.10.79	17.10.79
F ₂ repeats scored	-	-	-
F ₃ set up	-	-	17.10.79
F ₃ scored	-	-	31.10.79
F ₃ repeats scored	-	-	-

RESULTS

	Brood 1	Brood 2	Brood 3	All Broods
No. of F ₁ vials	96	89	92	277
No. of sterile F ₁ vials	11	5	8	24
No. of F ₁ vials used in F ₂	72	65	82	219
No. of F ₂ vials set up	612	602	744	1958
No. of F ₂ vials scored	569	533	610	1712
No. of F ₂ vials containing lethals	1	1	1	3
Frequency of F ₂ lethals	0.17%	0.18%	0.16%	0.17%
No. of F ₃ vials set up	0	0	389	389
No. of F ₃ vials scored	0	0	378	378
No. of F ₃ vials containing lethals	0	0	0	0
Frequency of F ₃ lethals	0	0	0	0

TABLE RL-2 (continued)

N,N-Dimethylformamide
Drosophila SLRL Procedure and Results

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

	Brood 1	Brood 2	Brood 3
F ₁ set up	11.9.79	14.9.79	19.9.79
F ₂ set up	27.9.79	4.10.79	3.10.79
F ₂ scored	8.10.79	18.10.79	16.10.79
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	62	56	42	160
No. of sterile F ₁ vials	37	35	26	98
No. of F ₁ vials used in F ₂	26	20	13	59
No. of F ₂ vials set up	594	375	272	1241
No. of F ₂ vials scored	528	333	253	1114
No. of F ₂ vials containing lethals	0	0	0	0
Frequency of F ₂ lethals	0	0	0	0
No. of F ₃ vials set up	-	-	-	-
No. of F ₃ vials scored	-	-	-	-
No. of F ₃ vials containing lethals	-	-	-	-
Frequency of F ₃ lethals	-	-	-	-

TABLE RL-2 (continued)

N,N-Dimethylformamide
Drosophila SLRL Procedure and Results

Compound: N,N-Dimethyl- Concentration: 400 ppm Stock: A
 formamide
 Length of Exposure: 2 1/4 h Test exposure given: 10.9.79

	Brood 1	Brood 2	Brood 3
F ₁ set up	11.9.79	14.9.79	19.9.79
F ₂ set up	28.9.79	1.10.79	4.10.79
F ₂ scored	9.10.79	15.10.79	19.10.79
F ₂ repeats scored	-	-	-
F ₃ set up	9.10.79	15.10.79	19.10.79
F ₃ scored	22.10.79	31.10.79	5.11.79
F ₃ repeats scored	-	-	-

RESULTS

	Brood 1	Brood 2	Brood 3	All Broods
No. of F ₁ vials	54	51	45	150
No. of sterile F ₁ vials	35	39	29	103
No. of F ₁ vials used in F ₂	18	15	8	41
No. of F ₂ vials set up	607	315	275	1197
No. of F ₂ vials scored	555	288	258	1101
No. of F ₂ vials containing lethals	1	0	0	1
Frequency of F ₂ lethals	0.18%	0	0	0.09%
No. of F ₃ vials set up	497	521	412	1430
No. of F ₃ vials scored	489	484	397	1370
No. of F ₃ vials containing lethals	0	0	0	0
Frequency of F ₃ lethals	0	0	0	0

TABLE RL-2 (continued)

N,N-Dimethylformamide
Drosophila SLRL Procedure and Results

Compound: N,N-Dimethyl- Concentration: 400 ppm Stock: B
 formamide
 Length of Exposure: 2 1/4 h Test exposure given: 10.9.79

	Brood 1	Brood 2	Brood 3
F ₁ set up	11.9.79	14.9.79	19.9.79
F ₂ set up	24.9.79	26.9.79	3.10.79
F ₂ scored	8.10.79	12.10.79	16.10.79
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	89	82	74	245
No. of sterile F ₁ vials	27	32	27	86
No. of F ₁ vials used in F ₂	61	46	46	153
No. of F ₂ vials set up	605	609	593	1807
No. of F ₂ vials scored	534	543	555	1632
No. of F ₂ vials containing lethals	1	0	0	1
Frequency of F ₂ lethals	0.18%	0	0	0.06%
No. of F ₃ vials set up				
No. of F ₃ vials scored				
No. of F ₃ vials containing lethals				
Frequency of F ₃ lethals				

TABLE RL-2 (continued)

N,N-Dimethylformamide
Drosophila SLRL Procedure and Results

Compound: Ethyl methanesulphonate Concentration: 0.4% v/v Stock: A
 Length of Exposure: 5 h Test exposure given: 21.9.79

	Brood 1	Brood 2	Brood 3
F ₁ set up	22.9.79		
F ₂ set up	5.10.79		
F ₂ scored	22.10.79		
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	17			17
No. of sterile F ₁ vials	1			1
No. of F ₁ vials used in F ₂	16			16
No. of F ₂ vials set up	178			178
No. of F ₂ vials scored	109			109
No. of F ₂ vials containing lethals	12			12
Frequency of F ₂ lethals	11.0%			11.0%
No. of F ₃ vials set up				
No. of F ₃ vials scored				
No. of F ₃ vials containing lethals				
Frequency of F ₃ lethals				

FIGURE 1a

N,N-Dimethylformamide
Schematic Lay-out of Exposure Area

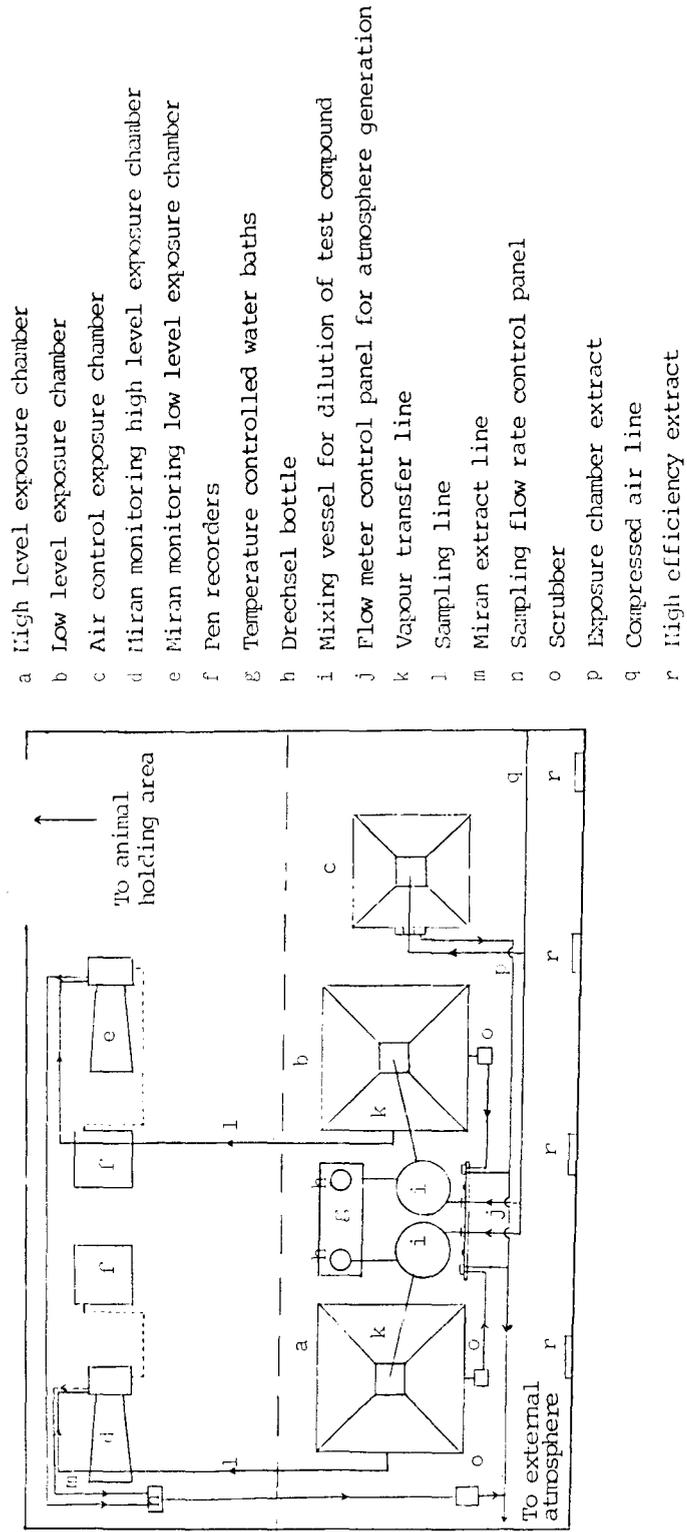


FIGURE 1b

N,N-Dimethylformamide

Schematic Lay-out of Vapour Generation Apparatus

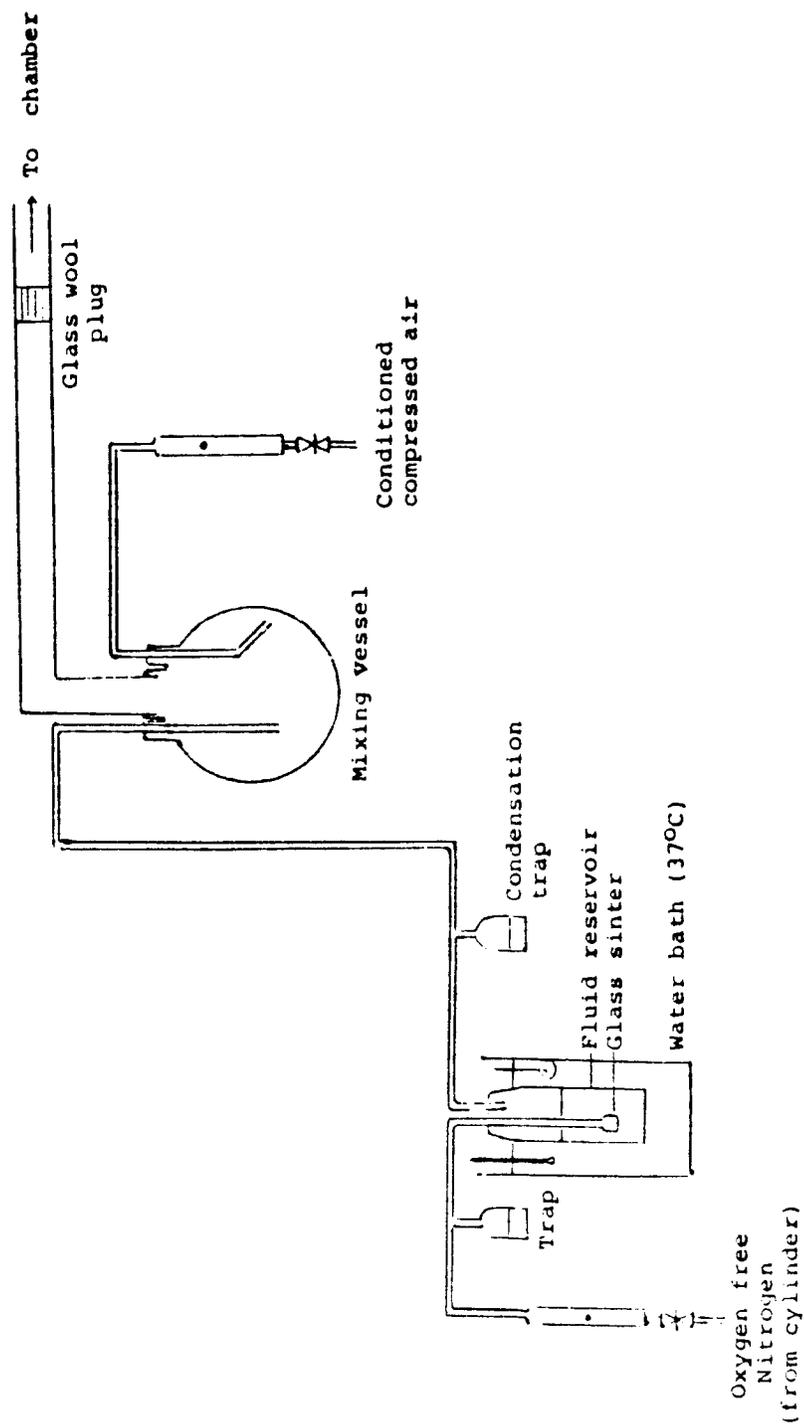


FIGURE 2

N,N-Dimethylformamide
Typical Calibration Graph for Low Level
19 August 1980

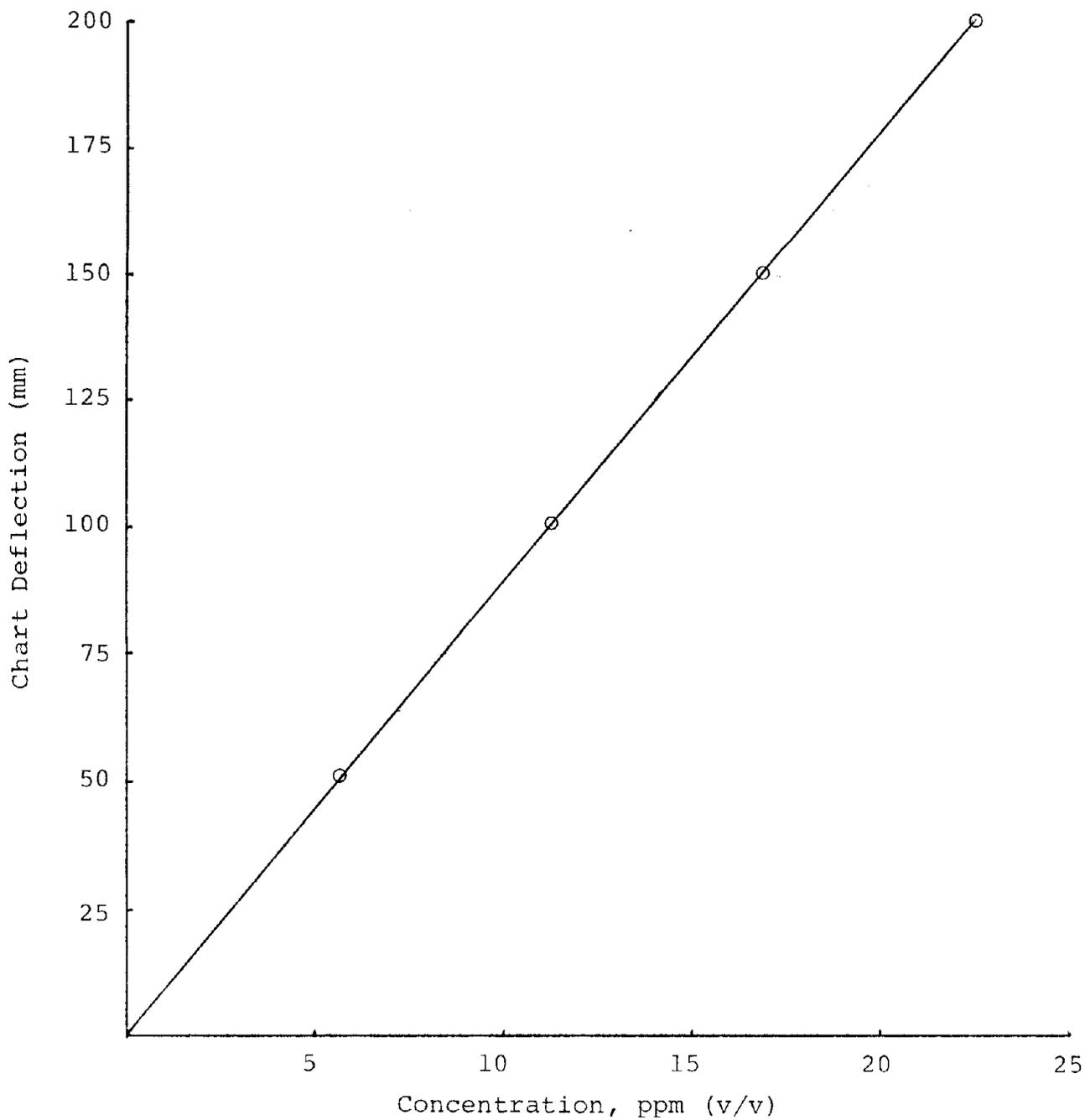


FIGURE 3

N,N-Dimethylformamide
Typical Calibration Graph for High Level

19 August 1980

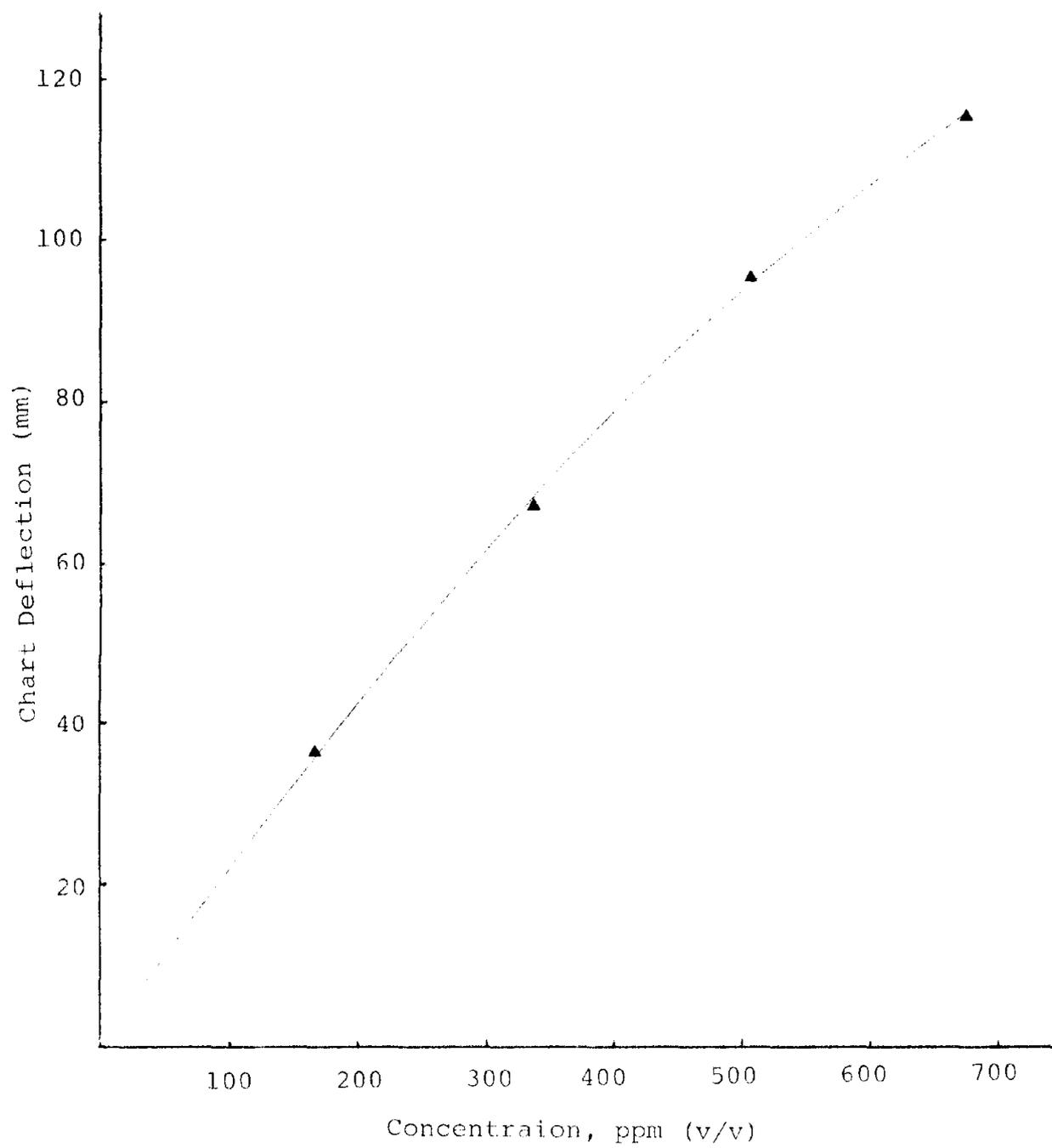
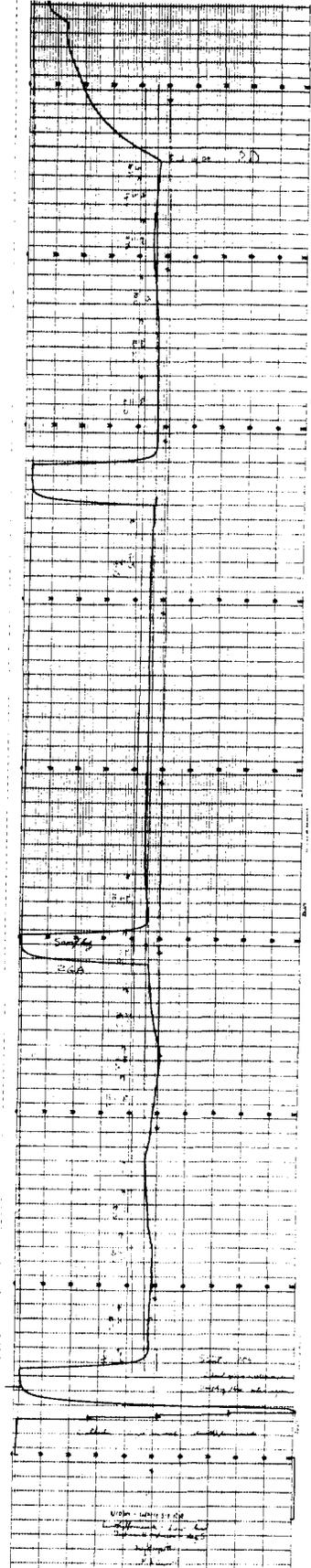
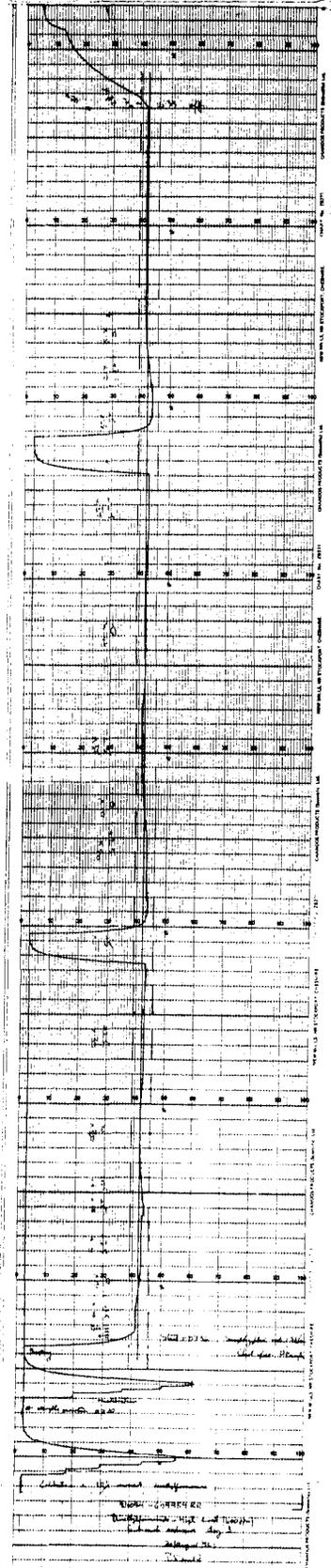


FIGURE 4

N,N-Dimethylformamide
Sample Record Chart of IR Absorption at 9.2 μm



APPENDIX DIET

N,N-Dimethylformamide
Diet Analysis



Spratt's Patent Ltd

Central House
Cambridge Road
Barking
Essex IG11 8NL

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

CERTIFICATE OF ANALYSIS

PRODUCT: LAD 1

BATCH NO: 027938

DATE OF MANUFACTURE: 2ND MY, 1979.

FOUND ANALYSIS

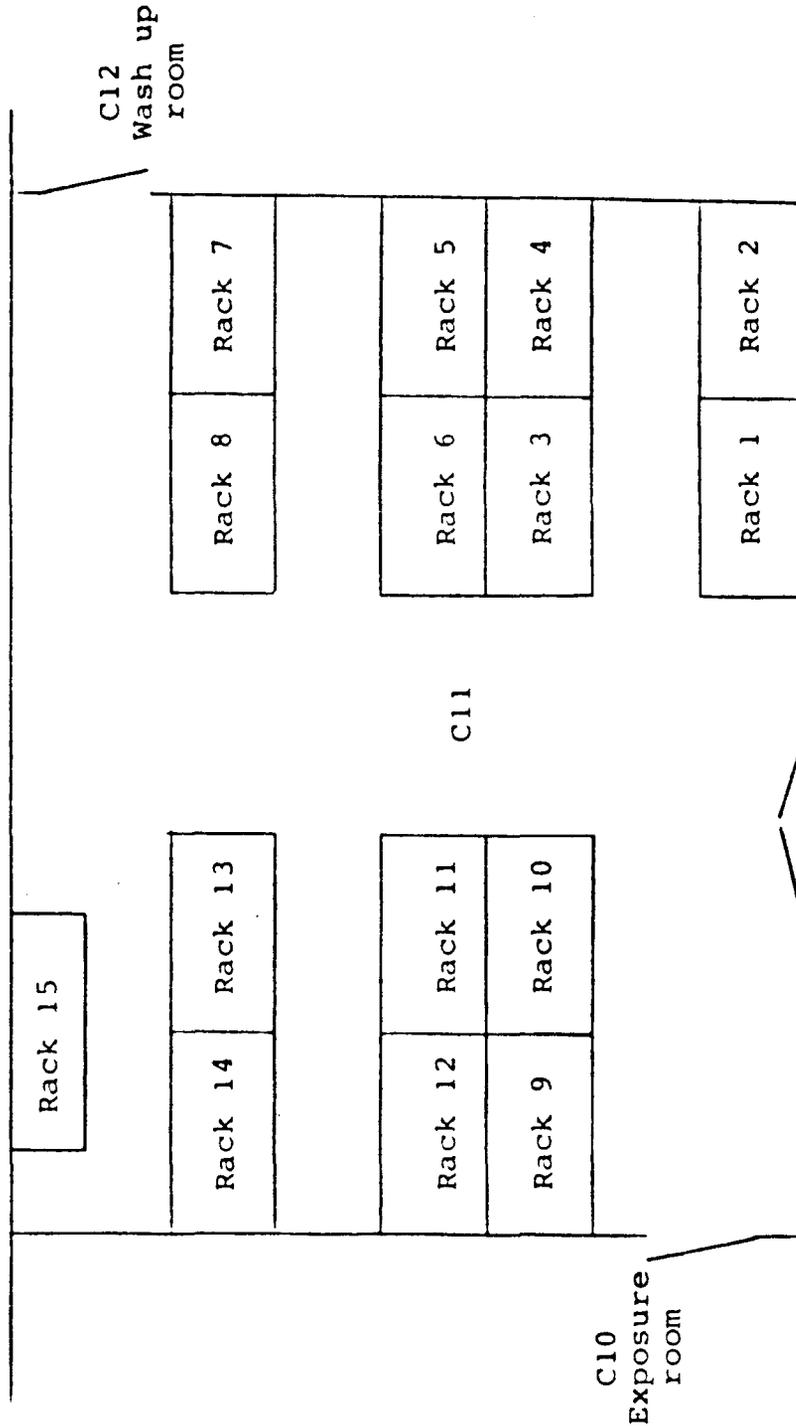
MOSITURE	9.6%
CRUDE FAT	4.0%
CRUDE PROTEIN	21.1%
ASH	5.7%
CALCIUM	1.08%
PHOSPHORUS	0.75%
NITRATE	< 1.0 mg/kg
NITRITE	2.6 mg/kg
SELENIUM	0.26 mg/kg
LEAD	4.0 mg/kg
ARSENIC	< 0.20 mg/kg
CADMIUM	< 0.20 mg/kg
MERCURY	0.023mg/kg
AFLATOXINS	NONE DETECTED
TOTAL P.C.B	NONE DETECTED
TOTAL D.D.T.	0.018 mg/kg
DIELDRIN	NONE DETECTED
LINDANE	0.13 mg/kg
HEPTACHLOR	NONE DETECTED
MALATHION	0.44 mg/kg
TOTAL VIABLE ORGANISMS	1.0 X 10 ³ /grm
E. COLI TYPE 1	NONE DETECTED
SALMONELLA SPECIES	NONE DETECTED
MOULDS.	NONE DETECTED

SIGNED *[Signature]*

DATE *[Signature]*

APPENDIX Loc-1

N,N-Dimethylformamide
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

N,N-Dimethylformamide
 Examples of Animal Location During Exposure
 Exposure Location Sheet

Project No: 409959Test Concentration: 0Test Compound: Air ControlTier No: 1Exposure Chamber No: 1

Multi-dose Cytogenetic ♂ and ♀

Day of Study: 2LEFT

Group Cage Treatment	1	281	285	289	-
		282	286	290	-
		283	287	-	-
	4	284	288	-	-

FRONTREAR

Group Cage Treatment	2	121	125	129	-
		122	126	130	-
		123	127	-	-
	0	124	128	-	-

RIGHT

SIGNBD: _____ DATE: _____

APPENDIX Loc-2 (continued)

N,N-Dimethylformamide
Exposure Location Sheet

Project No: 409959Test Concentration: 0Test Compound: Air ControlTier No: 2Exposure Chamber No: 1Dominant Lethal ♂
Sperm Ab. miceDay of Study: 2LEFT

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

FRONTREAR

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

RIGHT

SIGNED: _____ DATE: _____

APPENDIX LOC-2 (continued)N,N-Dimethylformamide
Exposure Location SheetProject No: 409959Test Concentration: LowTest Compound: N,N-DimethylformamideTier No: 1Exposure Chamber No: 2Day of Study: 2LEFT

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	-	-
-	-	-	-

FRONTREAR

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)N,N-Dimethylformamide
Exposure Location SheetProject No: 409959Test Concentration: HighTest Compound: N,N-DimethylformamideTier No: 1Exposure Chamber No: 3Day of Study: 2LEFT

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	-	-
-	-	-	-

FRONTREAR

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-2

N,N-Dimethylformamide

Contract No. 210-78-0026

DOMINANT LETHAL ASSESSMENT

NIOSH

Dose Group:

Assessors	Signature

Week No.	Male No.		Female No.		Corpora lutea		Total Implants		Live Implants		Early Deaths		Late Deaths		Corpora lutea		Total Implants		Live Implants		Early Deaths		Late Deaths		Total	Signature (s) and Date				
	1	2	1	2	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 TABLE BW-1 (Abandoned)

N,N-Dimethylformamide
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	350	355	359	364	372
	122	334	340	342	346	345
	123	373	380	383	388	397
	124	365	370	375	377	386
	125	359	361	366	368	377
	126	346	352	358	360	368
	127	343	354	357	362	369
	128	334	341	346	352	353
	129	362	370	373	372	381
	130	347	359	359	361	364
		Mean	351.3	358.2	361.8	365.0
	± S.D.	± 13.1	± 12.7	± 12.7	± 12.1	± 15.3

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	281	230	226	228	229	232
	282	245	240	240	243	242
	283	225	230	233	232	240
	284	242	244	241	245	248
	285	240	247	247	248	249
	286	231	232	230	236	238
	287	227	224	226	225	229
	288	226	225	224	225	228
	289	220	226	220	216	220
	290	224	222	224	224	226
		Mean	231.0	231.6	231.3	232.3
	± S.D.	± 8.5	± 8.9	± 8.8	± 10.5	± 9.8

APPENDIX TABLE BW-1 (Abandoned continued)

N,N-Dimethylformamide

Multiple Dosing: 10 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	131	335	336	345	351	550
	132	307	308	312	327	329
	133	310	312	316	329	326
	134	356	357	368	374	377
	135	361	362	366	375	377
	136	362	367	377	387	389
	137	318	325	332	344	343
	138	321	331	335	340	344
	139	326	332	333	339	342
	140	315	313	320	322	323
		Mean	331.1	334.3	340.4	348.8
	+ S.D.	+ 21.3	+ 21.3	+ 23.0	+ 22.5	+ 23.3

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	291	245	249	250	250	247
	292	229	230	230	230	231
	293	201	204	207	208	202
	294	223	220	220	224	222
	295	213	206	211	211	215
	296	251	257	258	254	257
	297	212	217	218	217	215
	298	247	243	238	243	243
	299	236	235	235	236	237
	300	209	208	209	210	210
		Mean	226.6	226.9	227.6	228.3
	+ S.D.	+ 17.7	+ 18.8	+ 17.6	+ 17.0	+ 17.9

APPENDIX TABLE BW-1 (Abandoned continued)

N,N-Dimethylformamide

Multiple Dosing: 400 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	141	322	328	324	329	332
	142	357	364	364	376	372
	143	350	356	355	359	359
	144	377	377	377	386	382
	145	321	327	322	322	323
	146	342	350	353	353	362
	147	334	339	342	342	350
	148	334	336	339	339	341
	149	333	334	332	330	331
	150	327	334	336	340	340
	Mean	339.7	344.5	344.4	347.6	349.2
	+ S.D.	+ 17.4	+ 16.7	+ 17.7	+ 20.9	+ 19.2

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	301	229	226	221	220	219
	302	237	236	235	236	236
	303	213	213	211	210	211
	304	203	207	205	206	204
	305	275	267	264	267	265
	306	231	231	228	225	227
	307	214	209	212	212	213
	308	255	250	249	246	247
	309	221	221	221	222	220
	310	234	234	232	231	230
	Mean	231.2	229.4	227.8	227.5	227.2
	+ S.D.	+ 21.2	+ 18.8	+ 18.2	+ 18.5	+ 18.3

APPENDIX TABLE BW-1 (Abandoned continued)

N,N-Dimethylformamide

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	151	374	370	369	364	357
	152	305	301	299	289	285
	153	359	351	345	336	325
	154	353	347	338	330	319
	155	281	278	274	261	253
	156	325	323	308	291	278
	157	334	330	323	318	309
	158	365	362	361	357	346
	159	341	347	348	342	337
	160	345	337	320	307	300
		Mean	338.2	334.6	328.5	319.5
	+ S.D.	+ 28.4	+ 28.0	+ 29.4	+ 32.7	+ 32.5

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	311	207	208	206	203	203
	312	225	225	223	212	203
	313	229	219	213	204	197
	314	198	197	197	187	177
	315	222	220	214	205	202
	316	239	235	236	228	222
	317	234	233	236	231	225
	318	203	199	198	191	183
	319	232	225	220	211	205
	320	230	228	220	211	204
		Mean	221.9	218.9	216.3	208.3
	+ S.D.	+ 14.2	+ 13.4	+ 13.7	+ 13.9	+ 14.7

APPENDIX TABLE BW-1 (Repeat)

N,N-Dimethylformamide
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 RR	344	348	348	351	346
	122 RR	411	413	421	427	423
	123 RR	396	394	396	402	404
	124 RR	373	373	377	384	375
	125 RR	396	400	402	408	409
	126 RR	389	392	396	400	402
	127 RR	391	394	402	407	408
	128 RR	386	389	385	398	393
	129 RR	393	397	405	406	407
	130 RR	381	388	395	402	405
		Mean	386.0	388.8	392.7	398.5
	± S.D.	± 17.8	± 17.5	± 19.6	± 19.8	± 21.8

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	281 RR	238	237	245	244	245
	282 RR	231	232	236	238	232
	283 RR	265	266	270	272	272
	284 RR	244	248	249	247	246
	285 RR	241	241	242	246	240
	286 RR	258	261	262	262	262
	287 RR	256	262	260	265	260
	288 RR	243	242	242	247	244
	289 RR	248	247	254	256	253
	290 RR	261	266	257	264	267
		Mean	248.5	250.2	251.7	254.1
	± S.D.	± 11.0	± 12.6	± 10.7	± 11.2	± 12.8

APPENDIX TABLE BW-1 (Repeat continued)

N,N-Dimethylformamide

Multiple Dosing: 10 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	131 RR	370	367	368	372	374
	132 RR	385	381	388	389	390
	133 RR	410	409	420	421	426
	134 RR	377	383	391	395	400
	135 RR	380	389	390	393	396
	136 RR	386	381	383	382	387
	137 RR	349	351	349	354	360
	138 RR	358	354	361	366	379
	139 RR	380	383	386	386	394
	140 RR	387	391	396	394	399
	Mean	378.2	378.9	383.2	385.2	390.5
	+ S.D.	+ 16.8	+ 17.4	+ 19.9	+ 18.4	+ 17.7

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	291 RR	230	230	230	232	228
	292 RR	249	244	247	249	251
	293 RR	241	242	238	240	240
	294 RR	250	252	252	255	256
	295 RR	244	250	250	251	250
	296 RR	231	227	230	232	231
	297 RR	242	241	243	245	240
	298 RR	249	248	248	245	245
	299 RR	269	267	264	269	272
	300 RR	231	231	234	236	235
	Mean	243.6	243.2	243.6	245.4	244.8
	+ S.D.	+ 11.8	+ 12.0	+ 10.3	+ 10.8	+ 13.1

APPENDIX TABLE BW-1 (Repeat continued)

N,N-Dimethylformamide

Multiple Dosing: 400 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	141 RR	388	392	397	389	394
	142 RR	378	374	379	375	377
	143 RR	382	382	382	376	380
	144 RR	401	399	398	396	394
	145 RR	360	362	360	364	363
	146 RR	380	383	384	387	391
	147 RR	364	362	364	359	356
	148 RR	417	413	413	412	414
	149 RR	382	385	382	383	385
	150 RR	386	384	392	392	395
		Mean	383.8	383.6	385.1	383.3
	+ S.D.	+ 16.4	+ 15.6	+ 15.9	+ 15.6	+ 16.8

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	301 RR	253	250	250	245	239
	302 RR	258	251	257	252	247
	303 RR	261	259	260	262	262
	304 RR	263	257	268	265	266
	305 RR	243	239	239	240	235
	306 RR	248	245	252	247	245
	307 RR	234	235	233	231	231
	308 RR	283	274	272	265	267
	309 RR	260	250	253	247	245
	310 RR	241	234	245	244	239
		Mean	254.4	249.4	252.9	249.8
	+ S.D.	+ 13.9	+ 12.2	+ 12.1	+ 11.2	+ 13.0

APPENDIX TABLE BW-1 (Repeat continued)

N,N-Dimethylformamide

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	151 RR	381	378	371	372	370
	152 RR	335	323	321	315	311
	153 RR	380	375	362	355	347
	154 RR	387	383	372	367	361
	155 RR	375	368	352	346	342
	156 RR	355	342	334	330	332
	157 RR	367	354	338	330	327
	158 RR	387	378	370	370	367
	159 RR	367	355	335	329	319
	160 RR	379	370	357	352	345
		Mean	371.3	362.6	351.2	346.6
	+ S.D.	+ 16.2	+ 19.0	+ 18.2	+ 19.9	+ 20.0

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	311 RR	275	272	271	270	269
	312 RR	257	246	237	240	233
	313 RR	248	245	241	237	236
	314 RR	237	226	223	219	215
	315 RR	252	246	243	242	234
	316 RR	249	242	235	231	225
	317 RR	240	231	217	217	216
	318 RR	257	254	249	242	240
	319 RR	251	239	229	224	215
	320 RR	260	253	247	249	244
		Mean	252.6	245.4	239.2	237.1
	+ S.D.	+ 10.7	+ 12.8	+ 15.1	+ 15.7	+ 16.6

APPENDIX TABLE BW-2 (Abandoned)

N,N-Dimethylformamide
 Single Exposure Cytogenetics Test
 Individual Body Weights (g)

Air Control (0 ppm)

Sex	Animal Number	6 h Sample	Animal Number	24 h Sample	Animal Number	48 h Sample
		Weight		Weight		Weight
Male	1	409	11	393	21	375
	2	390	12	387	22	388
	3	381	13	366	23	353
	4	380	14	375	24	378
	5	419	15	400	25	448
	6	414	16	368	26	394
	7	363	17	384	27	392
	8	422	18	367	28	379
	9	395	19	402	29	382
	10	370	20	384	30	375
	Mean	394.3		382.6		386.4
	± S.D.	± 21.0		± 13.3		± 24.5

Sex	Animal Number	6 h Sample	Animal Number	24 h Sample	Animal Number	48 h Sample	
		Weight		Weight		Weight	
Female	161	280	171	238	181	235	
	162	256	172	239	182	222	
	163	232	173	237	183	256	
	164	261	174	262	184	237	
	165	243	175	272	185	267	
	166	282	176	261	186	230	
	167	222	177	202	187	232	
	168	265	178	285	188	267	
	169	238	179	245	189	320	
	170	239	180	245	190	254	
		Mean	251.8		248.6		252.0
		± S.D.	± 20.3		± 23.0		± 28.0

APPENDIX TABLE BW-2 (Abandoned continued)

N,N-Dimethylformamide

Single Dosing: 10 ppm

Sex	Animal Number	6 h Sample	Animal Number	24 h Sample	Animal Number	48 h Sample
		Weight		Weight		Weight
Male	31	366	41	368	51	428
	32	408	42	431	52	384
	33	380	43	408	53	375
	34	418	44	379	54	405
	35	418	45	413	55	388
	36	385	46	395	56	376
	37	414	47	397	57	361
	38	399	48	390	58	368
	39	282	49	376	59	377
	40	386	50	390	60	412
		Mean	395.6		394.7	
	+ S.D.	+ 18.3		+ 18.8		+ 21.2

Sex	Animal Number	6 h Sample	Animal Number	24 h Sample	Animal Number	48 h Sample
		Weight		Weight		Weight
Female	191	238	201	281	211	251
	192	260	202	254	212	266
	193	257	203	256	213	288
	194	224	204	265	214	239
	195	262	205	250	215	236
	196	230	206	239	216	232
	197	278	207	274	217	261
	198	255	208	234	218	240
	199	239	209	256	219	281
	200	252	210	243	220	250
		Mean	249.5		255.2	
	+ S.D.	+ 16.5		+ 15.8		+ 19.2

APPENDIX TABLE BW-2 (Abandoned continued)

N,N-Dimethylformamide

Single Dosing: 400 ppm

Sex	Animal Number	6 h Sample	Animal Number	24 h Sample	Animal Number	48 h Sample
		Weight		Weight		Weight
Male	61	393	71	372	81	397
	62	450	72	399	82	399
	63	404	73	362	83	375
	64	370	74	364	84	404
	65	428	75	409	85	376
	66	391	76	359	86	384
	67	371	77	365	87	369
	68	417	78	408	88	398
	69	367	79	374	89	381
	70	408	80	418	90	338
	Mean	399.9		383.0		382.1
	± S.D.	± 27.1		± 22.8		± 19.6

Sex	Animal Number	6 h Sample	Animal Number	24 h Sample	Animal Number	48 h Sample
		Weight		Weight		Weight
Female	221	219	231	246	241	227
	222	300	232	238	242	259
	223	268	233	238	243	256
	224	216	234	243	244	252
	225	230	235	251	245	265
	226	216	236	227	246	232
	227	252	237	245	247	242
	228	229	238	211	248	275
	229	242	239	226	249	245
	230	245	240	220	250	242
		Mean	241.7		234.5	
	± S.D.	± 26.5		± 12.9		± 14.8

APPENDIX TABLE BW-2 (Abandoned continued)

N,N-Dimethylformamide

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sex	Animal Number	6 h Sample	Animal Number	24 h Sample	Animal Number	48 h Sample
		Weight		Weight		Weight
Male	91	429	101	449	401	342
	92	402	102	390	112	395
	93	416	103	351	113	424
	94	340	104	436	114	402
	95	363	105	382	115	375
	96	385	106	400	116	395
	97	406	107	391	117	420
	98	389	108	379	118	410
	99	387	109	370	119	394
	100	409	110	384	120	370
		Mean	392.6		393.2	
	± S.D.	± 26.1		± 29.3		± 24.8

Sex	Animal Number	6 h Sample	Animal Number	24 h Sample	Animal Number	48 h Sample
		Weight		Weight		Weight
Female	251	298	261	235	271	267
	252	228	262	236	272	238
	253	279	263	259	273	278
	254	250	264	258	274	222
	255	245	265	270	275	239
	256	234	266	238	403	230
	257	269	267	242	277	229
	258	239	267	245	278	226
	259	249	269	246	279	253
	260	233	270	277	402	242
		Mean	252.4		245.6	
	± S.D.	± 22.7		± 15.8		± 18.4

APPENDIX TABLE BW-2 (Repeat)

N,N-Dimethylformamide
 Single Exposure Cytogenetics Test
 Individual Body Weights (g)

Air Control (0 ppm)

Sex	Animal Number	6 h Sample	Animal Number	24 h Sample	Animal Number	48 h Sample
		Weight		Weight		Weight
Male	1 R	432	11 R	415	21 R	447
	2 R	401	12 R	430	22 R	368
	3 R	420	13 R	408	23 R	365
	4 R	412	14 R	395	24 R	435
	5 R	424	15 R	372	25 R	378
	6 R	416	16 R	380	26 R	381
	7 R	408	17 R	399	27 R	381
	8 R	385	18 R	421	28 R	400
	9 R	356	19 R	383	29 R	407
	10 R	414	20 R	425	30 R	415
		Mean	405.8		402.8	
	+ S.D.	+ 22.0		+ 20.2		+ 28.0

Sex	Animal Number	6 h Sample	Animal Number	24 h Sample	Animal Number	48 h Sample	
		Weight		Weight		Weight	
Female	161 R	267	171 R	292	181 R	257	
	162 R	260	172 R	275	182 R	263	
	163 R	259	173 R	240	183 R	298	
	164 R	250	174 R	257	184 R	270	
	165 R	285	175 R	254	185 R	262	
	166 R	247	176 R	263	186 R	274	
	167 R	233	177 R	259	187 R	235	
	168 R	231	178 R	240	188 R	224	
	169 R	235	179 R	231	189 R	250	
	170 R	263	180 R	250	190 R	269	
		Mean	252.6		256.1		260.2
		+ S.D.	+ 16.9		+ 17.9		+ 20.7

APPENDIX TABLE BW-2 (Repeat continued)

N,N-Dimethylformamide

Single Dosing: 10 ppm

Sex	Animal Number	6 h Sample	Animal Number	24 h Sample	Animal Number	48 h Sample
		Weight		Weight		Weight
Male	31 R	383	41 R	408	51 R	433
	32 R	373	42 R	402	52 R	397
	33 R	411	43 R	403	53 R	415
	34 R	448	44 R	426	54 R	414
	35 R	415	45 R	413	55 R	393
	36 R	414	46 R	419	56 R	377
	37 R	413	47 R	406	57 R	397
	38 R	410	48 R	474	58 R	443
	39 R	419	49 R	387	59 R	382
	40 R	358	50 R	420	60 R	420
		Mean	404.4		415.8	
	+ S.D.	+ 26.0		+ 23.3		+ 21.5

Sex	Animal Number	6 h Sample	Animal Number	24 h Sample	Animal Number	48 h Sample
		Weight		Weight		Weight
Female	191 R	247	201 R	260	211 R	276
	192 R	268	202 R	242	212 R	213
	193 R	254	203 R	256	213 R	246
	194 R	258	204 R	237	214 R	279
	195 R	280	205 R	257	215 R	244
	196 R	234	206 R	295	216 R	243
	197 R	250	207 R	244	217 R	247
	198 R	275	208 R	252	218 R	251
	199 R	261	209 R	246	219 R	261
	200 R	255	210 R	267	220 R	261
		Mean	258.2		255.6	
	+ S.D.	+ 13.6		+ 16.6		+ 18.8

APPENDIX TABLE BW-2 (Repeat continued)

N,N-Dimethylformamide

Single Dosing: 400 ppm

Sex	Animal Number	6 h Sample	Animal Number	24 h Sample	Animal Number	48 h Sample
		Weight		Weight		Weight
Male	61 R	431	71 R	595	81 R	414
	62 R	388	72 R	418	82 R	385
	63 R	392	73 R	388	83 R	357
	64 R	437	74 R	383	84 R	437
	65 R	455	75 R	365	85 R	415
	66 R	377	76 R	407	86 R	422
	67 R	425	77 R	418	87 R	378
	68 R	398	78 R	392	88 R	390
	69 R	378	79 R	388	89 R	373
	70 R	442	80 R	394	90 R	377
		Mean	412.3		394.8	
	+ S.D.	+ 28.8		+ 16.2		+ 25.6

Sex	Animal Number	6 h Sample	Animal Number	24 h Sample	Animal Number	48 h Sample
		Weight		Weight		Weight
Female	221 R	260	231 R	272	241 R	265
	222 R	268	232 R	236	242 R	228
	223 R	275	233 R	260	243 R	234
	224 R	276	234 R	282	244 R	273
	225 R	276	235 R	265	245 R	264
	226 R	273	236 R	244	246 R	262
	227 R	242	237 R	278	247 R	266
	228 R	254	238 R	269	248 R	273
	229 R	244	239 R	247	249 R	292
	230 R	230	240 R	254	250 R	264
		Mean	259.8		260.7	
	+ S.D.	+ 16.6		+ 15.2		+ 18.6

APPENDIX TABLE BW-2 (Repeat continued)

N,N-Dimethylformamide

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sex	Animal Number	6 h Sample	Animal Number	24 h Sample	Animal Number	48 h Sample
		Weight		Weight		Weight
Male	91 R	430	101 R	411	111 R	440
	92 R	408	102 R	401	112 R	445
	93 R	393	103 R	384	113 R	447
	94 R	384	104 R	433	114 R	453
	95 R	417	105 R	417	115 R	416
	96 R	444	106 R	400	116 R	469
	97 R	421	107 R	415	117 R	390
	98 R	440	108 R	424	118 R	369
	99 R	381	109 R	409	119 R	420
	100 R	422	110 R	456	120 R	424
		Mean	414.0		415.0	
	+ S.D.	+ 22.2		+ 19.8		+ 30.3

Sex	Animal Number	6 h Sample	Animal Number	24 h Sample	Animal Number	48 h Sample
		Weight		Weight		Weight
Female	251 R	238	261 R	269	271 R	269
	252 R	232	262 R	233	272 R	250
	253 R	255	263 R	270	273 R	231
	254 R	275	264 R	240	274 R	242
	255 R	240	265 R	258	275 R	246
	256 R	260	266 R	275	276 R	286
	257 R	255	267 R	272	277 R	249
	258 R	281	268 R	232	278 R	239
	259 R	258	269 R	262	279 R	249
	260 R	257	270 R	250	280 R	273
		Mean	255.1		256.1	
	+ S.D.	+ 15.5		+ 16.4		+ 17.1

APPENDIX TABLE BW-3

N,N-Dimethylformamide
 Dominant Lethal Assay
 Individual Body Weights (g)

Multiple Dosing: Air Control (0 ppm)

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	361	355	340	364	368	372
	362	354	359	361	363	369
	363	323	361	364	372	377
	364	343	353	352	355	355
	365	322	331	335	340	340
	366	329	335	333	337	343
	367	355	367	364	369	374
	368	351	358	365	372	379
	369	363	371	370	374	376
	370	350	353	357	362	368
		Mean	344.5	352.8	356.5	361.2
	± S.D.	± 14.7	± 13.4	± 12.8	± 13.3	± 14.2

APPENDIX TABLE BW-3 (continued)

N,N-Dimethylformamide

Multiple Dosing: 10 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	371	341	344	343	347	348
	372	345	351	353	350	357
	373	350	359	357	359	362
	374	348	351	354	357	358
	375	341	346	353	355	358
	376	348	348	354	357	359
	377	368	373	383	385	392
	378	348	360	360	363	368
	379	330	338	335	341	333
	380	323	330	332	337	340
	Mean	344.2	350.0	352.4	355.1	357.5
	+ S.D.	+ 12.1	+ 12.1	+ 14.3	+ 13.3	+ 16.1

APPENDIX TABLE BW-3 (continued)

N,N-Dimethylformamide

Multiple Dosing: 400 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	381	330	335	334	334	325
	382	361	360	360	360	360
	383	352	356	356	354	360
	384	365	372	367	370	370
	385	350	352	348	349	352
	386	345	348	351	350	352
	387	322	330	321	322	319
	388	339	338	342	338	342
	389	354	357	345	353	343
	390	316	319	316	320	325
	Mean	343.4	346.7	344.0	345.0	344.8
	+ S.D.	+ 16.4	+ 16.0	+ 16.4	+ 16.2	+ 17.2

APPENDIX TABLE BW-3 (continued)

N,N-Dimethylformamide

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	391	354	353	349	338	335
	392	343	335	326	315	302
	393	345	342	345	337	329
	394	354	353	347	330	316
	395	313	309	308	292	284
	396	359	360	358	354	344
	397	338	335	328	320	310
	398	352	349	339	330	321
	399	360	353	352	342	334
	400	372	369	360	343	332
		Mean	349.3	345.8	341.2	330.1
	+ S.D.	+ 16.4	+ 16.7	+ 16.2	+ 17.5	+ 18.1

APPENDIX TABLE BW-4

N,N-Dimethylformamide
Sperm Abnormality Test
Individual Body Weights (g)

Multiple Dosing: Air Control (0 ppm)

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	321	24	25	25	25	25
	322	25	25	25	25	26
	323	24	24	24	24	24
	324	24	24	24	25	24
	325	24	24	24	24	24
	326	23	22	23	22	23
	327	23	23	23	22	23
	328	23	22	22	22	23
	329	25	25	25	25	25
	330	24	24	25	24	25
		Mean	23.9	23.8	24.0	23.8
	+ S.D.	+ 0.7	+ 1.1	+ 1.0	+ 1.2	+ 1.0

APPENDIX TABLE BW-4 (continued)

N,N-Dimethylformamide

Multiple Dosing: 10 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	331	23	24	24	25	25
	332	23	24	25	24	24
	333	23	23	23	23	24
	334	22	23	24	23	24
	335	23	24	24	24	24
	336	24	25	25	25	25
	337	21	23	23	22	23
	338	24	25	26	25	25
	339	23	23	23	23	23
	340	24	25	25	26	26
		Mean	23.0	23.9	24.2	24.0
	\pm S.D.	\pm 0.9	\pm 0.8	\pm 1.0	\pm 1.2	\pm 0.9

APPENDIX TABLE BW-4 (continued)

N,N-Dimethylformamide

Multiple Dosing: 400 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	341	24	25	25	25	26
	342	21	22	22	22	22
	343	21	22	24	22	23
	344	23	23	24	25	24
	345	22	24	23	23	23
	346	22	22	23	23	23
	347	22	23	23	24	24
	348	22	22	24	24	25
	349	22	24	23	24	25
	350	22	23	23	24	25
		Mean	22.1	23.0	23.4	23.6
	\pm S.D.	\pm 0.9	\pm 1.1	\pm 0.8	\pm 1.1	\pm 1.2

APPENDIX TABLE BW-4 (continued)

N,N-Dimethylformamide

Multiple Dosing: Ethyl Methanesulphonate, 200 mg/kg

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	351	23	24	23	23	24
	352	26	26	26	26	26
	353	25	25	25	25	25
	354	26	26	26	26	25
	355	23	24	24	23	23
	356	24	24	25	25	25
	357	23	23	24	23	22
	358	24	24	24	24	24
	359	24	24	24	24	23
	360	25	23	25	24	24
		Mean	24.3	24.3	24.6	24.3
	\pm S.D.	\pm 1.2	\pm 1.1	\pm 1.0	\pm 1.2	\pm 1.2

APPENDIX TABLE CA-MD-M

N,N-Dimethylformamide
 Cytogenetic Analysis of Rat Bone Marrow Cells
 Chromatid/Chromosomal Aberrations Scored
 Males

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				
129	64/1	50	25	25																				
	64/2		25	25																				
127	126/5	50	25	25																				
	126/2		25	25																				
123	93/3	50	25	23	1																			10.6 x 129.0 8.8 x 115.9 9.8 x 114.9
	93/5		25	24																				
126	67/2	50	25	25																				
	67/3		25	24	1																			
122	131/2	50	25	23																				
	131/3		25	25																				
130	89/4	50	25	25																				
	89/2		25	25																				
125	32/5	50	25	25																				
	32/4		25	25																				
124	78/5	50	25	25																				
	78/2		25	25																				
121	142/3	50	25	25																				
	142/5		25	25																				
128	139/1	50	25	24	1																			
	139/3		25	25																				

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

APPENDIX TABLE CA-MD-M (continued)

N,N-Dimethylformamide
Males

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	Gap	B w F B w/o F	
141	51/5	50	25	24	1						14.8 x 121.9
	51/2	50	25	25							
148	88/4	50	25	25							1.9 x 90.8
	88/3	50	25	25	1						
145	35/5	50	25	24							2.8 x 118.1
	35/4	50	25	25							
146	59/5	50	25	25							7.8 x 88.8
	59/4	50	25	25							
149	50/3	18	4	3	1						7.6 x 91.2
	50/1	3	3	3							
150	50/4	7	7	7							10.9 x 90.9
	50/2	1	1	1							
142	50/5	3	3	3							7.8 x 88.8
	63/5	50	25	23	1						
143	63/1	25	25	24							7.6 x 91.2
	155/2	50	25	25							
144	155/3	50	25	25							10.9 x 90.9
	19/3	50	25	25							
144	19/4	50	25	25							7.8 x 88.8
	18/5	50	25	25							
	18/3	25	25	25							

Multiple Dosing: 400 ppm

Sampling Time: 6 h

APPENDIX TABLE CA-MD-M (continued)

N,N-Dimethylformamide
Males

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		
147	129/5	6	4	4								
	129/2		1	1								
	129/1, 3, 4		0	0								

Multiple Dosing: 400 ppm

Sampling Time: 6 h

APPENDIX TABLE CA-MD-M (continued)

N,N-Dimethylformamide
Males

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			
158	4/2	50	25	25							13.7 x 124.1 14.7 x 121.1
	4/3	50	25	25	1						
154	118/5	50	25	24							7.3 x 121.3
	118/1	50	25	24	1						
155	21/5	50	25	25							7.9 x 86.0
	21/4	50	25	25							
159	138/5	50	25	25							8.1 x 113.0 1.1 x 119.0
	138/4	50	25	24							
151	157/3	10	1	1							11.2 x 92.5 8.8 x 86.4 8.8 x 90.3 8.0 x 110.1 12.0 x 87.1
	157/5	8	8	8							
157	157/4		1	0							1 Exchange
	157/1,2		0	0							
160	132/5	50	25	23	1				1		11.8 x 124.5
	132/4		25	21							
152	91/3	50	25	24							11.8 x 124.5
	91/4	50	25	25							
152	77/2	50	25	25							11.8 x 124.5
	77/5	50	25	24							

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Sampling Time: 6 h

APPENDIX TABLE CA-MD-M (continued)

N,N-Dimethylformamide
Males

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread								Vernier Key		
		Per Animal	Per Slide		Chromatid		Chromosome				Miscellaneous				
					B	F	Gap	B	F	B		F		w/o	F
156	60/1	50	25	24	1										13.8 x 108.0
	60/3		25	25											3.7 x 98.9
153	8/1	50	25	24	1										10.5 x 97.4
	8/2		25	21										1 Exchange	9.1 x 102.9
					1										7.4 x 94.5
					1										7.8 x 94.5

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

APPENDIX TABLE CA-MD-F

N,N-Dimethylformamide
 Cytogenetic Analysis of Rat Bone Marrow Cells
 Chromatid/Chromosomal Aberrations Scored
 Females

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			F	B
289	224/4	50	25	25										
	224/1		25	25										
283	253/4	50	25	25										
	253/5		25	25										
288	299/1	50	25	25										
	299/3		25	25										
281	302/3	50	25	25										
	302/2		25	25										
287	286/2	50	25	25										10.3 x 120.9 9.3 x 122.7
	286/5		25	23	1									
286	227/1	50	25	25										
	227/2		25	25										
285	192/4	50	25	25										
	192/2		25	25										
282	291/5	50	25	25										
	291/3		25	25										
284	238/2	50	25	25										
	238/3		25	25										
290	249/2	50	25	24	1									6.3 x 123.0
	249/3		25	25										

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

APPENDIX TABLE CA-MD-F (continued)

N,N-Dimethylformamide
Females

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/o F	Gap	B w/o F			
292	194/4	50	25	24	1						7.2 x 113.1
	194/5	25	25								
300	303/2	50	25	25							14.3 x 117.6
	303/4	50	25		1						
295	199/4	50	25	24	1						13.2 x 109.0
	199/5	50	25								
293	300/2	50	25	25							8.8 x 81.2
	300/4	50	25								
294	182/3	50	25	25							9.3 x 127.2
	182/5	50	25		1						
297	201/1	50	25	25							34.0 x 100.2
	201/2	50	25		1						
298	285/3	15	3	3							34.0 x 100.2
	285/1	9	9								
296	285/2	3	3	0							34.0 x 100.2
	285/4,5	0	0								
291	184/3	50	25	25							34.0 x 100.2
	184/4	50	25								
299	310/2	50	25	25							34.0 x 100.2
	310/3	50	25								
299	207/1	50	22	22							34.0 x 100.2
	207/2	25	25		1						
	207/3	3	3	3							

Multiple Dosing: 10 ppm

Sampling Time: 6 h

APPENDIX TABLE CA-MD-F (continued)

N,N-Dimethylformamide
Females

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread						Vernier Key	
		Per Animal	Per Slide		Chromatid		Chromosome		Miscellaneous			
					B	w/o F	Gap	B w/o F		B		w/o F
301	211/4	50	25	25								
	211/5		25	25								
302	315/3	50	25	25								
	315/4		25	25								
304	178/4	50	25	25								
	178/5		25	25								
308	248/4	50	25	25								
	248/5		25	24								
309	210/5	50	25	25								
	210/4		25	24	1							10.3 x 105.3
305	195/2	50	25	25								6.6 x 95.0
	195/3		25	25								
307	289/1	50	25	25								
	289/3		25	25								
303	189/5	50	25	25								
	179/3		25	25								
310	223/5	50	25	25								
	223/1		25	25								
306	219/2	50	25	25								
	219/5		25	25								7.8 x 126.5

Multiple Dosing: 400 ppm

Sampling Time: 6 h

APPENDIX TABLE CA-MD-F (continued)

N,N-Dimethylformamide
Females

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread								Vernier Key	
		Per Animal	Per Slide		Chromatid		Chromosome			Miscellaneous				
					Gap	B w F	B w/o F	Gap	B w F	B w/o F	1 Exchange	1 Exchange		
311	317/4	50	25	23	1									7.5 x 93.1
	317/5		25	25	1									7.8 x 97.2
317	292/1	50	25	21			1							8.3 x 88.2
							1							9.0 x 81.0
							1							7.5 x 85.4
315	292/2		25	22	1									5.3 x 92.0
	181/4	50	25	25	2							1 Exchange		7.8 x 93.2
314	181/3		25	25										7.5 x 85.2
	278/1-5	0	0	0								1 Exchange		7.3 x 81.6

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Sampling Time: 6 h

APPENDIX TABLE CA-M6

N,N-Dimethylformamide
 Cytogenetic Analysis of Rat Bone Marrow Cells
 Chromatid/Chromosomal Aberrations Scored
 Males

Single Dosing: Air Control (0 ppm)			Observed Aberrations per Spread							Vernier Key		
Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w/o F		Gap	B w/o F			
						B	w/o F		B	w/o F		
9	81/2	50	25	25								
	81/4		25	25								
2	109/1	50	25	25								
	109/5		25	25								
7	92/1	50	25	25								
	92/2		25	25								
3	153/1-5	0	0	0								
4	31/1	50	25	25								
	31/2		25	25								
6	120/1	50	25	25								
	120/2		25	25								
8	113/1-5	0	0	0								
1	14/1	50	25	25								
	14/2		25	25								
5	61/2	50	25	25								
	61/4		25	25								
10	121/1-5	0	0	0								

Sampling Time: 6 h

APPENDIX TABLE CA-M6 (continued)

N,N-Dimethylformamide
Males

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	F	B			W	F	
96	1/1	50	25	25													
	1/5		25	25													
92	130/1-5	0	0	0													
99	108/2	8	8	8													
	108/1,3-5		0	0													
94	95/2	10	10	10													
	95/1,3-5		0	0													
100	103/1-5	0	0	0													
93	28/1-5	0	0	0													
98	99/1	50	25	25													
	99/2		25	25													
95	76/1-5	0	0	0													
97	105/1	50	25	24													
	105/2		25	25													
91	37/1-5	0	0	0													66.8 x 105.0

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sampling Time: 6 h

APPENDIX TABLE CA-M24

N,N-Dimethylformamide
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Males

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	F	B			w	F		
14	144/4	50	25	25														
	144/1		25	25														
15	49/3	50	25	25														
	49/4		25	25														
19	57/5	15	10	10														
	57/4		5	5														
	57/1-3		0	0														
16	75/2	50	25	25														
	75/3		25	25														
18	73/2	50	25	25														
	73/5		25	25														
20	127/2	50	25	25														
	127/5		25	25														
13	86/1	50	25	25														
	86/2		25	25														
12	112/3	50	25	25														
	112/5		25	25														
11	20/3	50	25	24														
	20/5		15	14														
	20/2		10	9														
17	6/2	50	25	25														
	6/3		25	25														

62.3 x 105.5
28.4 x 99.4
56.0 x 104.4

APPENDIX TABLE CA-M24 (continued)

N,N-Dimethylformamide
Males

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/o F	Gap	B w/o F	B w/o F	B w/o F	1 Chromatid Fragment	1 Chromatid Fragment		
43	46/1	50	25	25										
	46/2		25	25										
45	74/3	50	19	19										
	74/4		7	7										
50	74/5		11	11										
	74/1		13	13										
47	10/2	50	25	25										
	10/5		25	25										
46	151/5	50	25	25										
	151/4		25	25										
48	5/2	50	25	25										
	5/5		25	25										
49	107/4	50	25	25										
	107/1		25	25										
42	80/2	50	25	25										
	80/1		25	25										
44	149/1	50	25	25										
	149/4		25	24									1 Chromatid Fragment	38.6 x 106.4
41	15/4	50	25	25										
	15/3		25	24	1									
	152/2	50	25	25										
	152/3		25	25										

Multiple Dosing: 10 ppm

Sampling Time: 24 h

APPENDIX TABLE CA-M24 (continued)

N,N-Dimethylformamide
Males

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	F	B	w/o	F	Gap	B	F	B		w/o	F						
77	122/2	50	25	25																			
	122/5		25	25																			
74	101/5	50	25	25																			
	101/4		25	25																			
72	124/1-5	0	0	0																			
75	85/1-5	0	0	0																			
73	100/4	50	25	25																			
	100/5		25	25																			
71	133/1	50	25	24	1																		37.2 x 104.6
	133/2		25	25																			
78	154/3	50	25	25																			58.9 x 105.0
	154/4		25	23	1																		36.5 x 104.9
79	55/1	50	25	25																			45.8 x 96.1
	55/3		25	24	1																		
76	147/4	50	25	25																			29.2 x 102.8
	147/2		25	25																			32.2 x 103.5
80	62/3	36	14	13																			31.6 x 103.4
	62/4		6	3																			37.8 x 101.4

Single Dosing: 400 ppm

Sampling Time: 24 h

1 Robertsonian Translocation

APPENDIX TABLE CA-M24 (continued)

N,N-Dimethylformamide
Males

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/o F	Gap	B	w	F	B	w/o		F		
109	40/3	50	25	23	1											54.3 x 100.9
	40/4		25	23	1											56.4 x 101.0
104	141/3	50	25	25												42.3 x 109.9
	141/4		25	23												59.2 x 109.8
110	12/1	50	25	24	1											55.4 x 107.9
	12/4		25	23	1											31.1 x 108.8
108	87/3	50	25	25												61.9 x 104.1
	87/4		25	23												62.0 x 99.8
101	65/4	50	25	24												56.6 x 97.9
	65/2		25	25												59.1 x 107.8
105	114/4	50	25	24												44.3 x 108.0
			25	24												63.5 x 107.2
106	114/5		25	25												25.1 x 110.4
	11/3	50	25	24												64.7 x 112.4
102	11/1		25	25												55.0 x 108.0
	38/1-5	0	0	0												
103	16/4	50	25	24	2											45.1 x 107.0
	16/5		25	24	1											54.3 x 109.5

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sampling Time: 24 h

APPENDIX TABLE CA-M48

N,N-Dimethylformamide
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Males

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	F	B	w/o	F			Gap	B	F	B
23	29/5	50	25	24	1											124.9 x 36.0
	29/1		25	24	1											146.0 x 39.8
29	54/2	50	25	25												
	54/3		11	11												
	54/4		14	14												
	23/3	50	12	12												
25	23/2		13	13												
	23/5		16	16												
24	23/4		9	9												
	66/5	50	20	19	1											125.5 x 31.8
	66/1		5	4							1					113.1 x 45.0
	66/2		25	24	1											143.1 x 40.0
22	69/2	50	25	25												
	69/3		25	25												
21	70/4	50	25	24	1											116.5 x 35.0
	70/5		25	25												
28	135/1	50	25	25												
	135/3		25	25												
27	134/5	50	25	24							1					
	134/2		25	25												
26	128/1-5	0	0	0												
30	84/1-5	0	0	0												

Single Dosing: Air Control (0 ppm)

Sampling Time: 48 h

APPENDIX TABLE CA-M48 (continued)

N,N-Dimethylformamide
Males

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/o F	B w/o F	Gap	B w/o F		B w/o F		
59	17/2	50	18	18									
	17/4		7										
	17/3		13										
	17/1		12										
60	26/2	50	15	15									
	26/4		10										
	26/3		16										
	26/1		9										
56	30/1	50	25	23	1								149.8 x 46.0 106.0 x 45.5
	30/4		18										
	30/3		7										
	33/2		25										
52	33/5	50	25	25								1 Robertsonian translocation	
	123/2		25										
55	123/2	50	25	25									
	123/3		25										
	117/3		7										
	117/1		10										
58	117/5	50	8	8									124.3 x 45.6
	117/2		11										
	117/4		14										
	117/3		1										

Single Dosing: 10 ppm

Sampling Time: 48 h

APPENDIX TABLE CA-M48 (continued)

N,N-Dimethylformamide
Males

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key	
		Per Animal	Per Slide		Chromatid		Chromosome			Miscellaneous			
					B	w/o F	Gap	B	w/o F		B w/o F		
57	68/2	50	20	20									
	68/5		5										
51	68/3	50	25	25									
	79/3		25										
53	79/4	50	25	24	1								112.1 x 34.3
	148/1		25										
54	148/3	50	25	24	1								118.2 x 32.0
	136/1		25										
	136/4		25										

Single Dosing: 10 ppm

Sampling Time: 48 h

APPENDIX TABLE CA-M48 (continued)

N,N-Dimethylformamide
Males

Animal Number	Slide Number	400 ppm		Number of Spreads Without Aberrations	Observed Aberrations per Spread								Vernier Key					
		Spreads Examined			Chromatid				Chromosome					Miscellaneous				
		Per Animal	Per Slide		Gap	B	W	F	B	W	F	B			W	F		
90	13/1	42	8	8														
	13/2		7	7														
	13/4		10	10														
	13/5		14	14														
	13/3		3	3														
85	25/2	50	25	25														
	25/3		25	22	1													110.5 x 46.1
82	48/5	50	25	23						1								121.9 x 42.8
																		135.8 x 36.7
87	48/4	50	25	25														136.2 x 43.3
	36/4		25	25														144.1 x 30.8
83	36/2	50	25	25														1 Fragment
	116/4		25	25														
81	116/5		14	14														
	116/3		11	11														
	102/5	50	10	10														
	102/4		15	15														
	102/2		12	12														
	102/3		13	13														

Single Dosing: 400 ppm

Sampling Time: 48 h

APPENDIX TABLE CA-M48 (continued)

N,N-Dimethylformamide
Males

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	F	B			W	F
89	71/3	50	13	13												
	71/1		12	12												
	71/4		23	23												
	71/2		2	2												
84	160/2	50	25	24	1											118.8 x 40.2
	160/3		25	25												
86	56/4	50	25	24	1											
	56/3		25	25												
88	52/1-5	0	0	0												

Single Dosing: 400 ppm

Sampling Time: 48 h

APPENDIX TABLE CA-M48 (continued)

N,N-Dimethylformamide
Males

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread						Vernier Key
		Per Animal	Per Slide		Chromatid		Chromosome		Miscellaneous		
					Gap	B W F B w/o F	Gap	B W F B w/o F			
111	2/2	50	9	9							112.2 x 40.2
	2/1		16								
	2/5		25								
113	9/1	50	25	24							114.8 x 40.3
	9/2		25								
119	27/2	50	25	24							144.0 x 47.0
	27/3		25								
116	44/4	50	25	25							1 Ring Chromosome
	44/5		25								
114	96/1	50	25	24							1 Ring Chromosome
	96/2		25								
120	110/5	30	6	6							1 Ring Chromosome
	110/3		4								
115	110/2		7	7							1 Ring Chromosome
	110/1		8								
115	110/4		5	13							1 Ring Chromosome
	82/1	50	13								
	82/5		12								
	82/4		19								
	82/3		6	6							

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sampling Time: 48 h

APPENDIX TABLE CA-M48 (continued)

N,N-Dimethylformamide
Males

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread								Vernier Key	
		Per Animal	Per Slide		Chromatid		Chromosome			Miscellaneous				
					B	w/o F	Gap	B	w	F	B	w/o		F
112	146/1	50	25	24										143.6 x 42.0
	146/3		25	25										
	159/5	50	25	25										
117	159/4		25	25										124.2 x 36.7
	145/1	50	21	21										
118	145/3		4	4										143.6 x 42.0
	145/5		20	19										
	145/2		5	5										

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sampling Time: 48 h

APPENDIX TABLE CA-F6

N,N-Dimethylformamide
 Cytogenetic Analysis of Rat Bone Marrow Cells
 Chromatid/Chromosomal Aberrations Scored
 Females

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/o	F	Gap	B	W	F	B	W/o		F
166	280/1	45	11	11												32.4 x 97.2
	280/2		8	7	1											
	280/3		8	8												
	280/4		3	3												
	280/5		15	15												
165	221/1	50	25	25												60.8 x 106.7
	221/2		25	25												
162	269/1	50	25	25												66.7 x 105.0
	269/2		25	25												
164	191/1	50	25	25												57.2 x 107.9
	191/2		25	24	1											
163	313/1	50	25	24												41.6 x 108.5
	313/2		25	25	1											
167	252/2	50	25	25												62.0 x 105.0
	252/4		25	25												
161	174/1	50	25	23												57.2 x 107.9
	174/3		25	25												
169	241/1	50	25	24												41.6 x 108.5
	241/2		25	25												
168	273/1	50	25	25												62.0 x 105.0
	273/2		25	25												
170	281/2	50	25	25												62.0 x 105.0
	281/3		25	25												

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

APPENDIX TABLE CA-F6 (continued)

N,N-Dimethylformamide
Females

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread								Vernier Key	
		Per Animal	Per Slide		Chromatid		Gap	Chromosome		Miscellaneous				
					B	W/O F		B	W/O F					
193	163/2	50	25	25										
	163/3		25	25										
196	243/1	50	25	25										
	243/2		25	25										
199	264/1	50	25	25										
	264/5		25	24	1									72.3 x 108.1
198	318/1-5	0	0	0										
192	213/1	50	25	25										
	213/2		25	24		1								59.4 x 109.5
200	167/1	50	25	25										
	167/2		25	25										
194	316/1	50	25	24										
	316/2		25	25										
191	254/1	50	25	25										
	254/2		25	25										
195	271/1	50	25	25										
	271/2		25	25										
197	205/1	50	25	25										
	205/2		25	24	1									42.0 x 105.5

Single Dosing: 10 ppm

Spreads Examined

Per Animal

Per Slide

Number of Spreads Without Aberrations

Observed Aberrations per Spread

Chromatid

B

W/O F

Gap

Chromosome

B

W/O F

Miscellaneous

Vernier Key

Sampling Time: 6 h

APPENDIX TABLE CA-F6 (continued)

N,N-Dimethylformamide
Females

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key		
		Per Animal	Per Slide		Chromatid		Gap	Chromosome		Miscellaneous				
					B	W/O		F	B		W		O	F
224	279/1	50	25	25										
	279/2		25	25										
223	232/1	50	25	25										
	232/2		25	25										
229	218/1	50	25	25										
	218/3		25	25										
226	266/2	50	25	25										
	266/3		25	25										
225	203/1-5	0	0	0										
228	275/1	50	25	25										
	275/2		25	25										
222	250/1	50	25	25										
	250/2		25	25										
221	297/2	50	25	23	1									72.0 x 110.4 71.7 x 110.4
	297/3		25	25										
230	202/2	50	25	25										
	202/3		25	25										
227	257/1	50	25	25										
	257/2		25	25										

Single Dosing: 400 ppm

Sampling Time: 6 h

APPENDIX TABLE CA-F6 (continued)

N,N-Dimethylformamide
Females

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			
255	236/1-5	0	0	0										
260	263/1	50	25	24	1									27.0 x 110.5
254	263/4		25	25										58.2 x 106.0
	255/1	50	25	22		1								54.6 x 105.1
					1									36.8 x 104.1
259	255/5		25	25										
256	268/1-5	0	0	0										
	161/1	50	25	25										
	161/3		25	25										
252	290/1	50	13	13										
	290/2		16	16										
	290/3		21	21										
258	259/1-5	0	0	0										
253	188/1	50	25	25										
	188/2		25	25										
251	197/1	50	25	24										
	197/2		25	21										
													1 Chromatid Fragment	59.1 x 103.0
														69.5 x 103.3
														26.7 x 100.2
														35.7 x 96.4
														37.3 x 96.7
257	265/1	50	25	24										55.9 x 106.7
	265/2		25	23										63.0 x 99.0
														42.9 x 100.9

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sampling Time: 6 h

APPENDIX TABLE CA-F24

N,N-Dimethylformamide
 Cytogenetic Analysis of Rat Bone Marrow Cells
 Chromatid/Chromosomal Aberrations Scored
 Females

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	w/o	F	Gap	B	w	F			w/o	F						
173	246/2	50	25	25																				
	246/4		25	25																				
177	166/1	50	25	25																				
	166/3		25	25																				
176	235/3	50	25	25																				
	235/2		25	25																				
179	217/1	50	25	24																				
	217/2		25	25																				
174	304/3	50	25	25																				
	304/4		25	24																				
172	272/1	50	25	24																				
	272/3		25	25																				
180	287/2	50	25	25																				
	287/5		25	25																				
178	233/4	50	25	25																				
	233/3		25	24																				
171	180/2	50	17	16																				
	180/3		24	23																				
	180/4		9	8																				
175	209/4	50	25	25																				
	209/3		25	25																				

Sampling Time: 24 h

APPENDIX TABLE CA-F24 (continued)

N,N-Dimethylformamide
Females

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	F	B	w/o	F			Gap	B	w/o
201	312/5	50	25	23	1										39.1 x 109.9
					1										39.4 x 107.8
203	312/4	50	25	25	1										67.1 x 106.8
	206/4	50	25	24											
	206/3	50	25	25											
202	309/1	50	25	25											
	309/4	50	25	24											
209	240/3	50	25	25											
	240/5	50	25	25											
208	267/5	50	25	25											
	267/2	50	25	25											
205	234/3	50	25	25											
	234/2	50	25	25											
206	165/5	50	25	25											
	165/3	50	25	25											
204	175/1	50	25	25											
	175/3	50	25	25											
207	311/3	50	25	25											
	311/2	50	25	25	1										
210	170/3	50	25	24											28.3 x 109.7
	170/2	50	25	25											

Single Dosing: 10 ppm

Sampling Time: 24 h

APPENDIX TABLE CA-F24 (continued)

N,N-Dimethylformamide
Females

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	F	B			W	F	
240	222/2	50	25	25													
	222/3	50	25														
236	307/3	50	25	25													
	307/5	50	25														
231	293/5	50	25	25													
	293/4	50	25														
233	260/5	50	25	25													
	260/4	50	25														
239	215/3	50	25	25													
	215/1	50	25														
232	284/1	50	25	25													
	284/5	50	25														
238	314/2	50	25	23	1												54.7 x 112.5
					1												
234	314/3	50	25	24									1				58.8 x 108.4
	261/2	50	25														
237	261/1	50	25	23													52.6 x 104.8
	282/3	50	25											1			
235	282/2	50	25	24													63.3 x 112.2
	245/4	50	25											1			
	245/2	50	25	25													28.4 x 101.4

1 Chromatid Fragment

APPENDIX TABLE CA-F24 (continued)

N,N-Dimethylformamide
Females

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					
263	176/1	50	9	8	1						31.2 x 108.5		
	176/2		22	21							68.2 x 107.3		
	176/3		19	19									
264	301/3	50	25	24	1						65.9 x 106.2		
	301/4		25	25									
261	225/2	50	25	25									
	225/3		25	24									
269	200/5	50	25	24	1					1 Robertsonian Translocation	69.0 x 101.6		
	200/1		25	22									
266	171/5	50	25	22	1							53.9 x 100.4	
													62.6 x 107.6
													36.4 x 104.2
					2								49.9 x 101.6
262	198/2	50	25	20	1							68.1 x 100.9	
													33.0 x 99.5
													49.0 x 108.3
265	274/2	50	25	25	1							29.0 x 109.2	
													59.2 x 104.2
													63.9 x 104.0
					3								41.2 x 97.4
	274/5		25	24							58.7 x 104.9		
			25	24							60.9 x 109.4		

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sampling Time: 24 h

APPENDIX TABLE CA-F24 (continued)

N,N-Dimethylformamide
Females

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						
270	172/3	50	16	13	2												56.5 x 110.3			
					1														37.8 x 102.7	
					1															68.4 x 99.4
					3															60.9 x 99.8
267	172/4	50	12	9	1												63.0 x 98.9			
					1													40.0 x 96.9		
					1														56.3 x 106.5	
					3														33.4 x 107.9	
268	258/1	50	25	21	1												64.1 x 103.0			
					1													55.6 x 101.2		
					2														39.0 x 100.3	
					3														47.0 x 105.5	
268	240/3	50	25	22	1												48.7 x 103.6			
					1													55.0 x 105.0		
					1														55.0 x 103.8	
					2														58.5 x 97.5	
268	240/4	50	25	20	3												58.0 x 109.4			
					1														63.1 x 107.3	
					1															68.0 x 106.2
					1															30.3 x 102.0
					1											64.1 x 100.5				

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sampling Time: 24 h

APPENDIX TABLE CA-F48

N,N-Dimethylformamide
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Females

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	F	Gap	B	w	F	B			w	F						
183	189/2	50	21	21																				
	189/4		4	4																				
	189/1		9	9																				
	189/5		12	12																				
	189/3		4	4																				
181	230/3	50	25	25																				
	230/2		25	25																				
189	214/3	50	25	25																				
	214/1		25	25																				
190	244/2	50	25	25																				
	244/3		25	24																				
184	226/4	50	17	17																				
	226/1		8	8																				
	226/2		20	20																				
	226/3		5	5																				
187	294/5	50	25	24																				
	294/3		25	25																				
185	183/5	50	17	16																				
	183/4		8	8																				
	183/3		25	25																				

Single Dosing: Air Control (0 ppm)

Sampling Time: 48 h

140.9 x 41.1

43.1 x 109.1

121.2 x 59.5

APPENDIX TABLE CA-F48 (continued)

N,N-Dimethylformamide
Females

Animal Number		Slide Number		Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread						Vernier Key									
				Per Animal	Per Slide		Chromatid			Chromosome				Miscellaneous								
							Gap	B	W	F	B	w/o	F	Gap	B	W	F	B	w/o	F		
186		288/2		50	19	18	1															120.8 x 53.2
		288/4			6	6																
		288/3			19	18				1												
188		288/3			6	6																
		295/5		3	3	3																
		295/1-4		0	0	0																
182		229/1-5		0	0	0																

Single Dosing: Air Control (0 ppm)

Sampling Time: 48 h

APPENDIX TABLE CA-F48 (continued)

N,N-Dimethylformamide
Females

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread						Vernier Key	
		Per Animal	Per Slide		Chromatid		Chromosome			Miscellaneous		
					B	w/o F	Gap	B w F	B w/o F			
214	296/4	50	25	25								116.0 x 46.5
	296/3		25	24	1							
	277/5	50	25	25								
218	277/1		19	18	1							122.2 x 36.5
	277/4		6	6								
	283/4	50	16	16								
215	283/2		9	8								119.1 x 65.0
	283/5		17	17		1						
	283/3		8	8								
211	239/1-5	0	0	0								

Single Dosing: 10 ppm

Sampling Time: 48 h

APPENDIX TABLE CA-F48 (continued)

N,N-Dimethylformamide
Females

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/o	F	Gap	B	w/o	F	1	Robertsonian Translocation		
250	173/1	50	25	25												
	173/2		14	14												120.0 x 39.1
	173/3		11	10	1											
245	185/1	50	25	25												
	185/2		25	25												
246	216/1	50	25	25												
	216/3		25	24												152.8 x 40.0
247	196/1	50	25	25												
	196/5		25	25												
241	262/3	50	25	25												
	262/2		25	24												122.0 x 41.9
248	212/2	50	25	25												
	212/3		25	23									1			146.8 x 46.0 115.5 x 31.2
244	320/3	42	12	12												
	320/2		13	13												
	320/4		7	7												
	320/1		8	8												
	320/5		2	2												

Single Dosing: 400 ppm

Spreads Examined

Number of Spreads Without Aberrations

Observed Aberrations per Spread

Vernier Key

APPENDIX TABLE CA-F48 (continued)

N,N-Dimethylformamide
Females

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key			
		Per Animal	Per Slide		Chromatid			Chromosome							
					Gap	B	W	F	B	W	F		B	W	F
249	231/3	50	25	24	1										109.8 x 38.2
	231/5		25	23	1										110.8 x 30.0
243	276/1	50	25	24	1										114.0 x 27.5
	276/2		25	24	1										120.8 x 40.8
242	208/2	50	25	25											115.1 x 31.6
	208/1		25	25											

Single Dosing: 400 ppm

Sampling Time: 48 h

APPENDIX TABLE CA-F48 (continued)

N,N-Dimethylformamide
Females

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key	
		Per Animal	Per Slide		Chromatid		Chromosome			Miscellaneous			
					B	w/o F	Gap	B	w/o F		B		w/o F
273	169/1	50	25	25									125.1 x 32.7 114.0 x 44.9
	169/2		25	24	1								
271	162/3	50	25	24	1								117.6 x 41.8 126.2 x 39.5
	162/2		25	25									
275	242/1	50	25	25									125.3 x 42.0
	242/3		25	24	1								
274	256/1	50	25	24	1								118.2 x 64.6 114.5 x 26.8
	256/2		25	25									
280	270/4	50	25	25									
	270/5		25	25									
272	306/5	50	25	25									
	306/3		25	24	1								
279	187/1	50	25	25									
	187/2		25	25									
276	204/4	50	25	25									
	204/5		25	24	1								
278	305/2	50	25	24	1								
	305/3		25	25									
277	319/2	50	25	25									
	319/4		25	25									

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sampling Time: 48 h

APPENDIX TABLE DL

N,N-Dimethylformamide
Dominant Lethal Assessment

Multiple Dosing: Air Control (0 ppm)

Week No.	Male No.		361		362		363		364		365		366		367		368		369		370		Total	
	Female		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2		
1	Corpora lutea		15	13	17	13	15	11	0	12	14	11	0	17	11	13	10	0	15	13	0	14	214	
	Total Implants		11	13	14	13	17	12	0	13	14	11	0	17	6	14	10	0	15	14	0	13	207	
	Live Implants		11	11	14	12	17	12	0	13	13	10	0	17	6	14	9	0	15	11	0	11	196	
	Early Deaths		0	2	0	1	0	0	0	0	0	1	1	0	0	0	1	0	0	0	3	0	2	11
	Late Deaths		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	Corpora lutea		15	12	15	12	13	10	14	16	16	14	12	10	11	13	15	12	0	11	15	15	251	
	Total Implants		12	11	14	11	12	10	13	16	15	11	12	9	14	14	15	12	0	11	14	14	240	
	Live Implants		10	11	14	11	12	9	10	13	13	10	11	9	13	14	15	12	0	11	14	14	226	
	Early Deaths		1	0	0	0	0	1	3	3	2	1	1	0	1	0	0	0	0	0	0	0	13	
	Late Deaths		1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	
3	Corpora lutea		12	14	16	9	11	11	13	13	13	0	13	13	14	15	16	11	16	15	10	13	248	
	Total Implants		12	14	16	12	13	14	13	12	13	0	13	12	15	16	14	10	16	13	13	13	254	
	Live Implants		12	14	16	12	13	13	13	12	13	0	13	11	14	15	9	10	0	13	13	10	226	
	Early Deaths		0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	15	0	0	3	21	
	Late Deaths		0	0	0	0	0	1	0	0	0	0	0	0	0	0	5	0	1	0	0	0	7	
4	Corpora lutea		11	12	17	11	11	15	10	16	10	12	11	15	8	10	12	15	14	19	11	12	252	
	Total Implants		12	13	13	16	2	15	9	16	11	16	11	15	8	10	11	16	16	13	14	13	250	
	Live Implants		8	13	13	16	2	13	8	14	11	16	11	14	8	10	10	15	13	12	14	13	234	
	Early Deaths		4	0	0	0	0	2	1	2	0	0	0	1	0	0	1	1	3	1	0	0	16	
	Late Deaths		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
5	Corpora lutea		14	14	12	13	13	18	15	12	24	15	15	15	15	17	0	12	18	15	13	0	270	
	Total Implants		13	15	13	14	14	6	13	11	14	12	12	15	12	13	0	13	13	12	14	0	215	
	Live Implants		12	9	13	14	14	6	13	11	13	12	11	13	11	3	0	12	13	10	13	0	190	
	Early Deaths		1	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	1	5	
	Late Deaths		0	6	0	0	0	0	0	0	0	0	1	1	0	10	0	1	0	2	0	0	21	

APPENDIX TABLE DL (continued)

N,N-Dimethylformamide

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	18	13	13	4	17	8	18	17	18	19	12	14	14	18	25	11	2	16	283	
	Total Implants		15	13	12	14	0	16	11	14	17	18	15	12	13	15	14	16	12	0	17	257	
	Live Implants		14	13	11	13	0	16	0	6	17	6	13	6	11	15	0	3	8	0	14	180	
	Early Deaths		0	0	1	0	0	0	1	0	0	4	2	5	2	0	0	0	5	0	0	3	23
7	Late Deaths		1	0	0	0	0	0	10	8	0	8	0	1	0	0	14	8	4	0	0	54	
	Corpora lutea		16	13	14	1	14	14	13	14	11	13	14	19	13	12	12	3	17	12	13	251	
	Total Implants		13	9	14	0	17	15	16	14	15	9	14	13	14	14	13	12	1	13	8	236	
	Live Implants		13	8	13	0	16	15	15	14	13	6	13	11	13	14	12	12	1	0	5	205	
8	Early Deaths		0	1	1	0	1	0	1	0	0	1	1	0	0	1	0	0	0	13	1	0	21
	Late Deaths		0	0	0	0	0	0	0	0	2	3	0	1	1	0	0	0	0	0	2	1	10
	Corpora lutea		15	12	17	11	16	14	14	16	13	14	10	12	12	10	13	11	13	14	14	17	268
	Total Implants		15	13	17	11	14	13	14	17	13	12	10	12	13	12	13	11	12	14	13	16	265
9	Live Implants		12	0	16	0	14	13	15	2	9	10	12	12	11	13	10	12	0	12	16	192	
	Early Deaths		0	3	1	0	0	1	1	3	0	0	0	1	1	0	1	0	2	1	0	15	
	Late Deaths		3	10	0	11	0	10	0	1	8	3	0	0	0	0	0	0	0	12	0	0	58
	Corpora lutea		15	11	13	12	14	14	5	11	12	15	9	23	11	0	16	12	15	13	17	13	251
10	Total Implants		18	11	14	14	15	1	9	16	12	0	16	12	0	14	12	0	14	12	15	16	237
	Live Implants		15	10	2	14	5	15	1	0	16	12	0	14	12	0	4	11	14	2	16	12	175
	Early Deaths		3	0	0	0	0	0	5	0	0	0	2	0	0	0	1	0	0	13	0	0	24
	Late Deaths		0	1	12	0	9	0	4	0	0	0	0	0	0	0	9	1	1	0	0	1	38
10	Corpora lutea		0	9	12	12	18	15	18	11	12	13	11	12	13	12	20	15	12	14	10	14	253
	Total Implants		0	12	11	12	7	15	12	14	12	13	12	17	14	12	9	13	12	13	11	13	234
	Live Implants		0	11	11	11	7	0	12	0	6	5	12	15	14	9	5	1	12	11	8	9	159
	Early Deaths		0	0	0	1	0	1	0	10	4	1	0	1	0	1	0	1	0	2	0	3	25
Late Deaths		0	1	0	0	0	14	0	4	2	7	0	1	0	2	4	11	0	0	3	1	50	

APPENDIX TABLE DL (continued)

N,N-Dimethylformamide

Multiple Dosing: 10 ppm

Week No.	Male No.		371		372		373		374		375		376		377		378		379		380		Total
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
1	Female	16	13	10	11	0	15	14	10	0	14	0	16	14	12	17	10	13	7	205			
	Corpora lutea	16	13	14	12	0	16	14	12	0	14	0	16	16	14	16	10	10	15	220			
	Total Implants	15	13	14	12	0	15	12	11	0	12	0	16	16	14	16	10	7	15	210			
	Live Implants	1	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	3	0	7			
	Early Deaths	0	0	0	0	0	0	1	0	0	2	0	0	0	0	0	0	0	0	3			
2	Female	4	1	12	14	18	13	12	11	10	13	0	11	15	12	11	14	16	12	14	227		
	Corpora lutea	0	0	13	15	11	10	13	11	4	14	0	15	14	11	10	14	16	13	14	212		
	Total Implants	0	0	11	15	10	10	12	8	3	14	0	15	12	10	10	14	15	13	14	200		
	Live Implants	0	0	1	0	1	0	1	3	1	0	0	0	1	1	0	0	1	0	0	10		
	Early Deaths	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	2		
3	Female	15	12	14	11	13	10	15	13	12	13	0	10	14	16	11	11	16	13	14	245		
	Corpora lutea	14	7	15	11	13	12	12	15	12	13	0	15	14	16	12	12	14	15	12	248		
	Total Implants	13	6	15	10	13	12	11	12	12	12	0	15	13	15	12	12	13	14	13	235		
	Live Implants	1	1	0	1	0	0	1	0	0	1	0	0	1	1	0	0	1	0	2	10		
	Early Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3		
4	Female	14	12	10	11	0	12	15	15	14	19	0	15	12	15	12	11	15	0	13	215		
	Corpora lutea	13	12	10	11	0	13	17	13	14	19	0	13	16	15	11	13	15	0	15	220		
	Total Implants	13	10	10	11	0	13	17	11	14	2	0	11	9	15	11	13	12	0	15	187		
	Live Implants	0	1	0	0	0	0	0	2	0	1	0	2	7	0	0	0	2	0	0	15		
	Early Deaths	0	1	0	0	0	0	0	0	0	16	0	0	0	0	0	0	1	0	0	18		
5	Female	12	12	20	13	15	7	11	17	12	16	20	16	2	14	12	13	14	0	17	252		
	Corpora lutea	11	13	15	11	14	6	10	16	12	15	29	14	0	15	12	14	13	0	13	234		
	Total Implants	10	8	7	10	13	1	8	15	10	0	0	13	0	14	9	11	10	0	1	140		
	Live Implants	1	1	0	1	0	4	2	1	1	1	0	28	1	0	1	2	1	2	0	48		
	Early Deaths	0	4	8	0	1	1	0	0	1	15	1	0	0	0	1	2	1	0	10	1	46	

APPENDIX TABLE DL (continued)

N,N-Dimethylformamide

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		
6	Corpora lutea	22	16	13	16	14	0	13	12	15	10	15	11	17	17	12	12	10	11	12	13	261
	Total Implants	14	13	12	14	13	0	13	11	15	11	14	11	10	14	11	11	6	11	12	11	227
	Live Implants	1	11	11	12	13	0	9	10	8	10	13	10	8	12	11	9	5	7	11	11	182
	Early Deaths	0	2	1	2	0	0	4	1	0	1	1	1	0	0	0	1	1	2	1	0	18
	Late Deaths	13	0	0	0	0	0	0	0	0	7	0	0	2	2	0	1	0	2	0	0	27
7	Corpora lutea	15	13	13	6	15	16	13	9	11	0	11	17	12	12	13	10	18	15	15	12	246
	Total Implants	15	14	10	11	14	14	14	0	12	0	11	17	12	16	13	11	16	14	13	12	239
	Live Implants	13	13	0	0	9	14	14	0	11	0	10	16	4	0	13	3	13	12	11	11	167
	Early Deaths	2	1	0	6	3	0	0	0	1	0	1	1	0	1	0	6	0	1	2	1	26
	Late Deaths	0	0	10	5	2	0	0	0	0	0	0	0	8	15	0	2	3	1	0	0	46
8	Corpora lutea	15	15	18	9	0	14	14	14	13	13	16	12	15	18	15	14	10	12	14	16	267
	Total Implants	12	15	16	9	0	13	13	14	13	14	13	14	14	16	15	13	9	15	12	16	256
	Live Implants	10	10	16	8	0	11	12	14	13	14	13	14	11	16	15	13	5	15	11	14	235
	Early Deaths	2	5	0	0	0	1	1	0	0	0	0	0	1	0	0	0	4	0	0	1	15
	Late Deaths	0	0	0	1	0	1	0	0	0	0	0	0	2	0	0	0	0	0	1	1	6
9	Corpora lutea	13	16	15	15	1	15	16	2	2	12	13	17	14	12	21	15	11	12	16	17	253
	Total Implants	14	17	12	15	0	*	13	16	0	15	14	14	13	11	13	15	12	12	7	15	228
	Live Implants	12	16	7	12	0	13	13	0	13	0	8	13	13	0	9	13	11	12	4	13	182
	Early Deaths	1	1	1	2	0	0	0	3	0	2	0	1	0	10	0	1	0	0	2	2	26
	Late Deaths	1	0	4	1	0	0	0	0	0	0	6	0	0	1	4	1	1	0	1	0	20
10	Corpora lutea	14	14	14	20	0	12	11	11	D	13	0	19	16	12	11	11	12	0	14	8	212
	Total Implants	13	13	16	9	0	13	13	15	E	11	0	13	14	12	13	11	14	0	14	2	196
	Live Implants	11	13	16	9	0	13	11	12	A	0	0	13	14	12	13	11	14	0	4	2	168
	Early Deaths	2	0	0	0	0	0	2	1	D	0	0	0	0	0	0	0	0	0	0	1	6
	Late Deaths	0	0	0	0	0	0	0	2	11	0	0	0	0	0	0	0	0	0	9	0	22

* Unassessable, extensive haemorrhage

APPENDIX TABLE DL (continued)

N,N-Dimethylformamide

Multiple Dosing: 400 ppm

Week No.	Male No.	381		382		383		384		385		386		387		388		389		390		Total
		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
1	Female	2	11	11	0	0	16	0	13	10	11	0	13	12	14	16	0	15	18	15	16	193
	Corpora lutea	0	14	13	0	0	15	0	14	11	12	0	12	12	14	12	0	16	17	16	17	195
	Total Implants	0	13	13	0	0	13	0	14	11	12	0	11	11	13	11	0	16	16	16	17	187
	Live Implants	0	1	0	0	0	2	0	0	0	0	0	0	0	1	1	0	0	0	1	0	7
	Early Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
2	Female	11	16	19	12	15	13	12	11	14	13	11	*	14	11	0	12	15	14	14	13	240
	Corpora lutea	12	14	15	12	14	12	11	11	13	15	12	14	12	12	0	12	16	13	14	14	248
	Total Implants	12	11	14	10	13	11	11	11	13	14	12	14	10	12	0	12	15	11	14	13	233
	Live Implants	0	3	1	2	1	1	0	0	0	1	0	0	0	0	0	0	0	1	2	0	13
	Early Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	2
3	Female	13	10	12	10	12	12	14	14	15	12	14	14	13	15	14	0	14	4	14	10	236
	Corpora lutea	14	4	12	10	13	14	14	13	12	13	14	13	11	12	13	0	15	3	13	10	223
	Total Implants	14	4	12	10	13	14	14	12	12	12	13	11	11	10	12	0	15	0	13	0	202
	Live Implants	0	0	0	0	0	0	0	1	0	1	2	0	0	2	1	0	0	3	0	0	11
	Early Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10
4	Female	10	10	12	16	10	13	13	17	15	13	13	13	13	0	16	0	12	16	12	13	198
	Corpora lutea	7	11	13	16	11	14	13	17	14	**	**	11	0	19	0	12	12	12	14	0	196
	Total Implants	6	11	13	15	9	13	11	14	12			11	0	9	0	10	11	12	13	0	170
	Live Implants	1	0	0	1	2	1	1	3	2			0	0	10	0	2	1	0	1	0	25
	Early Deaths	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
5	Female	1	16	16	11	0	11	12	12	15	11	11	12	13	14	12	11	14	13	11	12	228
	Corpora lutea	1	9	16	14	0	12	13	12	16	8	11	11	15	16	13	16	14	12	14	12	235
	Total Implants	0	9	15	14	0	10	13	7	16	8	9	11	14	14	11	16	11	11	14	11	214
	Live Implants	1	0	0	0	0	2	0	1	0	0	2	0	1	2	2	0	3	0	0	0	14
	Early Deaths	0	0	1	0	0	0	0	4	0	0	0	0	0	0	0	0	0	1	0	0	7

* c.l.l. counts not done

** Unassessable brown mass

APPENDIX TABLE DL (continued)

N,N-Dimethylformamide

Multiple Dosing: 400 ppm

Week No.	Male No. Female	381		382		383		384		385		386		387		388		389		390		Total
		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
6	Corpora lutea	14	16	11	16	18	13	13	0	16	13	17	14	19	13	13	13	11	19	17	276	
	Total Implants	15	15	11	15	15	13	14	0	12	14	13	19	13	13	12	14	12	15	14	261	
	Live Implants	12	14	3	14	15	4	14	0	12	6	12	5	13	11	12	14	8	4	15	13	201
	Early Deaths	3	1	1	0	0	8	0	0	0	3	1	8	0	2	0	0	0	0	0	1	28
	Late Deaths	0	7	1	1	0	1	0	0	0	5	0	6	0	0	0	0	4	8	0	0	32
7	Corpora lutea	11	14	15	13	9	15	11	5	16	13	2	15	13	14	0	16	17	12	12	235	
	Total Implants	14	14	15	14	11	15	12	2	16	12	0	14	13	8	0	15	16	12	14	230	
	Live Implants	12	14	10	12	11	13	12	2	15	12	0	14	12	7	0	15	16	11	14	204	
	Early Deaths	0	2	2	0	2	0	0	0	1	0	0	0	1	1	0	0	0	1	0	8	18
	Late Deaths	2	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	8
8	Corpora lutea	8	16	10	12	10	13	21	12	12	18	15	11	15	15	0	14	13	13	14	254	
	Total Implants	11	16	12	12	11	12	17	13	12	15	13	10	14	13	0	14	12	10	14	246	
	Live Implants	7	13	10	12	9	11	11	11	10	15	13	0	14	13	0	0	9	10	12	193	
	Early Deaths	0	2	0	0	1	0	4	0	0	0	0	2	0	0	0	6	2	0	1	19	
	Late Deaths	4	3	0	0	1	1	2	2	2	0	0	8	0	0	0	8	1	0	1	34	
9	Corpora lutea	14	8	11	14	20	16	0	0	15	11	14	15	14	13	14	14	17	15	16	254	
	Total Implants	17	4	12	15	16	15	1	0	13	13	12	16	14	14	14	13	18	14	17	255	
	Live Implants	16	3	1	12	13	13	0	0	3	1	12	12	14	12	14	12	13	13	17	196	
	Early Deaths	1	1	5	2	0	1	1	0	1	10	0	3	0	2	0	0	5	0	0	2	34
	Late Deaths	0	0	6	1	3	1	0	0	9	2	0	1	0	0	0	1	0	1	0	0	25
10	Corpora lutea	14	17	9	11	15	10	14	15	11	12	13	11	14	12	13	0	11	9	11	13	235
	Total Implants	14	15	13	12	12	12	14	11	12	10	13	14	16	11	13	0	10	10	14	13	233
	Live Implants	10	15	12	11	0	12	14	11	12	1	12	11	10	10	12	0	10	2	2	11	178
	Early Deaths	1	0	0	0	11	0	0	0	0	5	1	3	0	1	1	0	0	3	2	0	28
	Late Deaths	3	0	1	1	1	0	0	0	4	0	0	0	0	0	0	0	0	5	10	2	27

APPENDIX TABLE DL (continued)

N,N-Dimethylformamide

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Week No.	Male No.	391		392		393		394		395		396		397		398		399		400		Total
		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
1	Female	13	10	9	5	10	0	4	4	0	11	6	12	2	14	1	15	18	11	0	6	151
	Corpora lutea	12	8	6	6	9	0	3	2	0	12	2	7	0	12	1	10	9	2	0	10	111
	Total Implants	7	0	1	0	9	0	0	1	0	12	0	0	0	12	0	5	2	2	0	0	51
	Live Implants	5	8	5	6	0	0	3	1	0	0	2	7	0	0	1	5	7	0	0	10	60
	Early Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	Female	3	8	0	1	0	0	2	0	0	2	0	0	0	0	1	0	4	0	0	0	21
	Corpora lutea	3	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	6
	Total Implants	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	Live Implants	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	5
	Early Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	Female	2	**	1	0	1	3	0	0	1	1	2	0	0	0	0	11	2	15	0	0	39
	Corpora lutea	1	0	0	0	0	2	0	0	0	0	2	0	0	0	0	8	0	23	0	1	38
	Total Implants	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Live Implants	1	1	0	0	0	2	0	0	0	0	2	0	0	0	0	8	0	23	0	1	38
	Early Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	Female	16	13	15	11	0	12	11	12	4	12	3	12	12	0	13	0	10	10	15	11	180
	Corpora lutea	16	10	11	9	0	14	*	12	3	1	1	12	11	0	12	0	9	7	9	9	146
	Total Implants	12	8	2	2	0	5	6	1	0	0	0	3	6	0	7	0	3	4	8	9	76
	Live Implants	4	2	7	7	0	9	6	2	1	1	1	9	5	0	5	0	6	3	1	0	68
	Early Deaths	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
5	Female	8	14	15	12	11	4	15	12	13	13	17	14	9	6	5	22	13	14	13	12	242
	Corpora lutea	14	11	15	13	10	2	15	7	15	12	13	12	11	13	1	15	15	13	11	12	230
	Total Implants	13	10	14	11	9	1	12	6	13	12	12	9	11	1	14	13	12	11	1	1	196
	Live Implants	0	1	1	2	1	0	3	1	2	0	0	0	0	0	0	1	2	1	0	2	17
	Early Deaths	1	0	0	0	0	1	0	0	0	0	1	3	0	12	0	0	0	0	0	0	27

* Unassessable brown mass

** No result recorded

APPENDIX TABLE DL (continued)

N,N-Dimethylformamide

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Week No.	Male No. Female	391		392		393		394		395		396		397		398		399		400		Total
		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
6	Corpora lutea	14	9	11	12	14	13	11	13	12	0	16	0	13	14	11	12	15	13	13	216	
	Total Implants	12	11	12	13	13	12	12	11	12	0	14	0	11	14	6	13	16	13	14	209	
	Live Implants	10	11	11	13	13	12	11	11	12	0	14	0	11	14	5	10	6	13	1	178	
	Early Deaths	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	2	10	0	2	17	
	Late Deaths	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	11	14
7	Corpora lutea	13	13	12	11	16	14	11	12	9	12	13	10	12	11	16	14	0	12	16	16	243
	Total Implants	13	11	12	12	16	14	13	12	11	11	10	10	14	11	13	14	0	13	17	14	241
	Live Implants	13	11	11	12	15	12	5	8	0	1	10	10	13	10	11	12	0	12	8	13	187
	Early Deaths	0	0	0	0	1	2	3	1	5	6	0	0	1	1	2	2	0	1	0	0	25
	Late Deaths	0	0	1	0	0	0	5	3	6	4	0	0	0	0	0	0	0	0	0	9	1
8	Corpora lutea	0	16	15	16	15	13	11	11	0	13	11	12	2	14	15	13	15	13	11	13	229
	Total Implants	0	14	17	15	17	13	12	10	0	13	13	11	0	14	14	14	12	11	13	16	229
	Live Implants	0	13	17	13	15	12	10	10	0	10	12	5	0	9	12	13	11	0	12	12	186
	Early Deaths	0	0	0	2	2	0	2	0	0	0	0	2	0	3	1	0	0	1	1	2	16
	Late Deaths	0	1	0	0	0	1	0	0	0	3	1	4	0	2	1	1	1	10	0	2	27
9	Corpora lutea	12	14	6	0	14	17	13	17	15	13	13	11	14	14	13	18	10	13	13	13	253
	Total Implants	11	14	2	0	14	14	11	14	14	13	13	13	13	13	10	13	14	13	10	15	234
	Live Implants	10	0	0	0	11	10	10	14	13	13	13	11	8	12	10	12	13	12	10	14	186
	Early Deaths	1	13	0	0	1	12	1	0	0	0	0	0	3	0	0	0	1	0	0	1	33
	Late Deaths	0	1	2	0	2	2	0	0	1	0	0	2	2	1	0	1	0	1	0	0	15
10	Corpora lutea	13	17	14	13	0	13	12	13	15	12	11	13	13	12	12	11	15	15	13	16	253
	Total Implants	14	13	15	14	0	14	12	14	12	12	13	11	13	12	10	12	15	10	13	18	247
	Live Implants	13	0	15	11	0	13	12	8	12	1	12	11	13	0	10	11	14	8	10	4	178
	Early Deaths	1	4	0	2	0	0	0	2	0	0	1	0	0	0	0	1	1	1	1	2	18
	Late Deaths	0	9	0	1	0	1	0	4	0	11	0	0	0	12	0	0	0	1	1	11	51

APPENDIX TABLE SA

N,N-Dimethylformamide
Sperm Abnormality Assessment

Multiple Dosing: Air Control (0 ppm)
 Low, 10 ppm
 High, 400 ppm
 Positive, Ethyl methanesulphonate, 200 mg/kg

Slide No.	Normal	Abnormality					Total Abnormal	Total Examined	De-coded Information	
		A	B	C	D	E			Animal No.	Group
333	977	0	0	15	1	7	23	1000	321	Air
324	911	2	7	58	6	16	89*	1000	322	Air
349	983	1	0	7	0	9	17	1000	323	Air
326	978	2	2	10	3	5	22	1000	324	Air
340	967	1	1	17	5	9	33	1000	325	Air
338	970	1	0	16	5	8	30	1000	326	Air
334	980	0	1	13	2	4	20	1000	327	Air
327	980	0	1	13	0	6	20	1000	328	Air
328	978	0	0	13	1	8	22	1000	329	Air
337	975	0	2	11	1	11	25	1000	330	Air
322	946	2	3	31	3	15	54	1000	331	Low
331	981	0	2	10	0	7	19	1000	332	Low
336	983	0	1	9	1	7	17	1001	333	Low
325	971	1	2	21	1	4	29	1000	334	Low
329	977	1	2	14	2	4	23	1000	335	Low
352	983	1	0	10	3	3	17	1000	336	Low
321	980	1	0	12	2	5	20	1000	337	Low
350	973	0	0	15	1	11	27	1000	338	Low
359	961	1	0	25	3	10	39	1000	339	Low
335	581	0	0	8	3	8	19	600*	340	Low

APPENDIX TABLE SA (continued)

N,N-Dimethylformamide

Multiple Dosing: Air Control (0 ppm)
 Low, 10 ppm
 High, 400 ppm
 Positive, Ethyl methanesulphonate, 200 mg/kg

Slide No.	Normal	Abnormality					Total Abnormal	Total Examined	De-coded Information	
		A	B	C	D	E			Animal No.	Group
330	976	1	0	10	5	8	24	1000	341	High
336	985	0	1	11	0	3	15	1000	342	High
351	972	0	1	20	1	6	28	1000	343	High
339	962	0	9	21	0	8	38	1000	344	High
353	979	0	1	13	1	6	21	1000	345	High
343	965	2	3	19	3	8	35	1000	346	High
345	975	1	1	12	3	8	25	1000	347	High
342	946	2	3	27	0	22	54	1000	348	High
341	959	0	1	24	4	12	41	1000	349	High
360	969	0	3	14	5	9	31	1000	350	High
332	964	3	0	17	7	9	36	1000	351	+
323	966	1	0	14	4	15	34	1000	352	+
346	970	0	0	19	4	7	30	1000	353	+
348	955	0	0	26	5	14	45	1000	354	+
344	969	2	0	16	6	7	31	1000	355	+
354	981	0	1	8	5	5	19	1000	356	+
347	919	1	1	33	9	37	81	1000	357	+
358	970	0	1	23	2	4	30	1000	358	+
357	964	2	2	17	4	11	36	1000	359	+
355	974	0	1	14	2	9	26	1000	360	+