Report Number 27

TIER II MUTAGENIC SCREENING OF 13 NIOSH PRIORITY COMPOUNDS

INDIVIDUAL COMPOUND REPORT N,N-DIMETHYLACETAMIDE

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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

CONTENTS

CONTENTS (continued)

CONTENTS (continued)

CONTENTS (continued)

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

1. Unscheduled DNA synthesis (UDS) assay in human diploid fibroblasts with exposures of 3 h duration and concentrations up to 9,370 µg/ml of culture medium.

2. Dominant lethal test in male rats with exposure to atmospheres containing 20 ppm or 700 ppm N,N-dimethylacetamide for 7 h per day for 5 consecutive days. Analysis of test atmospheres was by continuous infrared absorption monitoring at a wavelength of 9.9 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 conditons as in (2) or a single exposure of 7 h duration followed by sampling after 6 h, 24 h and 48 h.

5. Sex-linked recessive lethal (SLRL) test in Drosophila melanogaster with exposure to atmospheres of 200 ppm for 95 min.

The results obtained were as follows:

1. There was no increase in UDS in cells treated with N,N-dimethylacetamide.

2. There were no effects attributable to N,N-dimethylacetamide 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 significantly in the rat bone marrow cells.

5. SLRL frequency was not increased in Drosophila.

It was concluded that N,N-dimethylacetamide was devoid of genetic effects observable in these experiments.

INTRODUCTION

Properties

N,N-Dimethylacetamide (CAS No. 127-19-5) is a colourless liquid with solvent properties which have enabled it to be used in the formulation of parenterally administered drugs (Spiegel and Noseworthy, 1963). It is prepared from tris (dimethylamido) phosphate and acetic anhydride. A summary of its physical and chemical properties follows:

Formula	$CH_3CON(CH_3)_2$
Mol. wt.	87.12
Sp. gr. (25°C)	0.9366
M.P.	-20°C
B.P. (760 mm Hg)	163-165°C
Vapour pressure, mm Hg	
(25°C)	1.3
(60°C)	9.0
Refractive index (25°C)	0.9366
Flash point °F	15
Vapour density (air = 1)	3.01

It is infinitely soluble in water and most organic solvents.

Toxicology

N,N-Dimethylacetamide has an intraperitoneal LD_{50} in mice of 3,240 mg/kg (3.4 ml/kg). Tremors, depression, coma and delayed death were all observed. Liver damage occurred in dogs and rats during chronic exposures to greater than 0.1 ml/kg on skin or 40 ppm by inhalation. It may produce chronic liver and renal damage (Davis and Jenner, 1959; Horn, 1961). N,N-Dimethylacetamide is readily absorbed through skin, after which there is a somewhat greater tendency towards cumulative effects than is the case with dimethylformamide (Henderson and Haggard, 1943). There is also a tendency for greater weight loss after repeated intraperitoneal injections, when compared to dimethylformamide. At lethal doses (e.g. 2 ml/kg), N,N-dimethylacetamide causes an elevation in blood glucose (Grant, 1979).

In reproduction studies in rats, Thiersch (1971) found that giving pregnant animals 1.5 ml/kg on gestational Day 4 or 7 led to over 60% intrauterine deaths. The survivors were stunted, but no defects were found.

Reactions of humans to N,N-dimethylacetamide were unexpected. When given to humans in daily doses of 400 mg/kg for 3 days or more, depression, lethargy, confusion and disorientation were general consequences. In some patients there were visual and auditory hallucinations, perceptual distortions,

delusions and emotional detachment, all reminiscent of the reactions induced by mescaline and by lysergic acid derivatives (Weiss, 1962).

Details of its metabolism are not known, although oxidative demethylation seems a probable route, with release of formaldehyde.

The objective of the work described in this report is to investigate the mutagenic potential of N,N-dimethylacetamide in a variety of complex systems. The exposure conditions used were as follows:

human fibroblasts:	various concentrations up to 9,370 µg/ml for 3h.
rats and mice:	20 ppm or 700 ppm for 7 h/day for one or 5 days.
Drosophila:	200 ppm for 95 min.

MATERIALS AND METHODS

CHEMICALS

Test Substance

2 kg of N,N-dimethylacetamide, Batch No. 13737 (stated purity >99%) was received from Aldrich Chemical Company Limited, on 20 March 1979. The test material was a clear colourless liquid and was retained in the dark under ambient conditions in the company dispensary. A sample has been retained for analysis, should this be necessary.

Positive Control Substance

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

ANIMALS AND ANIMAL MANAGEMENT

Animals

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

$B6C3F_1$ hybrid mice were obtained from Charles River (USA) Limited.

These animals were obtained and used as follows.

Species	Date of Receipt	Age (Weeks)	Quarantine (Days)	Number (Sex)	Dates of Exposure	Comment
Rat	6 July 1979	9-10	10 or 17	220♂	16 July 1979 and 23 July 1979	
				176♀	16 July 1979 and 23 July 1979	
Mouse	3 July 1979	9-11	13	44♂	16 July 1979	
Rat	20 July 1979 etc.	8-10	None	80♀	None	DL mating

Pre-experiment Acceptance Tests

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

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

1. Ileal contents are incubated in selenite broth.
2. Lung, liver and kidney samples are incubated on blood agar plates.
3. Lung sample is plated on McConkey's medium.
4. Liver sample which was plated onto blood agar is then taken into a selenite tube.
5. All samples in selenite broth are incubated for 24 h, then plated on McConkey's medium for 24 h.

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

Animal Management

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

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

They were housed individually in cages in a room with a light intensity of approximately 200 lux, a 12 h light-dark cycle, approximately 10 air changes per hour, temperature maintained at ca 22°C with extreme limits of 19°C and 24.5°C, and relative humidity ca 50%, with extreme limits of 40% and 66%.

On completion of the dosing period, and as a precaution against accidental exposure to a different test compound, the dominant lethal test animals were relocated in a separate animal holding room for the duration of the test matings - approximately 12 weeks. This animal holding room had a light intensity of approximately 200 lux, a 12 h light-dark cycle, approximately 10 air changes per hour, temperature maintained at ca 22°C with extreme limits of 19.5°C and 24.5°C, and relative humidity ca 50% with extreme limits of 34% and 68%.

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

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

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

Diet

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

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

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

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

Allocation of Rats and Mice to Cages and Treatment Groups

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

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

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

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

Treatment groups were colour coded as follows:

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

Animal Identification

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

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

Animal Positioning in the Exposure Chambers

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

The treatment groups were constituted as follows:-

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

Observations

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

Body Weight

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

ATMOSPHERE GENERATION AND EXPOSURE

Exposure Chambers

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

Exposures to N,N-dimethylacetamide were carried out in 1.5 m^3 capacity chambers constructed of stainless steel and glass. The animals occupied a volume of 0.02 m^3 and were confined to a single tier of cages of 0.4 m^3 in volume (breathing zone). The breathing zone was ventilated at the rate of 12 air changes per hour. An additional chamber of 0.84 m^3 capacity was used for exposure of the air control group; the breathing zone in this chamber was ventilated at a 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 about 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-dimethylacetamide. 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 dimethylacetamide 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 for 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

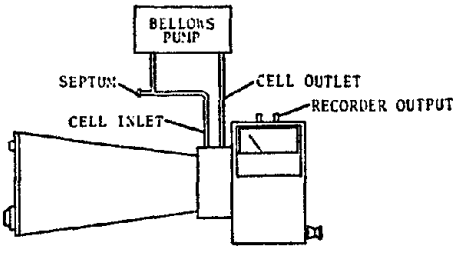
During preliminary analytical development, problems were encountered with the low level (originally 10 ppm) calibration procedure. It appeared that the 10 ppm atmosphere chart reading would be too close to the apparently unstable baseline, therefore, the principal investigator increased the low level exposure atmosphere concentration from 10 ppm to 20 ppm N,N-dimethylacetamide. If, following calibration, the Miran was 'purged' through the zero gas air filter and the original baseline was obtained, but, if the closed loop calibration pump was reconnected to the Miran the baseline was found to rise steadily over a 2 h period. It appeared that the N,N-dimethylacetamide was being adsorbed onto the internal surfaces of the infra-red spectrometer/ closed loop calibration apparatus and that the continual recycling of the atmosphere contained within the system caused the reversible adsorption of the compound, thereby giving an increased absorption reading and not a true baseline value. This desorption of N,N-dimethylacetamide was probably due to a temperature increase caused by the continuous operation of the calibration pump.

In order to check the infra-red absorption technique, the atmosphere was also analysed intermittently by gas-liquid chromatography. This parallel analysis indicated that components of the injection loop/calibration pump could reversibly adsorb the compound. It was necessary to purge this system after use with clean air for 4-5 h. Thus, the problem was confined to calibration (and this problem could be overcome) monitoring being unaffected, therefore, analysis of chamber atmospheres were routinely carried out using the infra-red absorption technique.

The infra-red gas analysers used to monitor chamber atmospheres of N,N-dimethylacetamide 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-dimethylacetamide were sequentially injected into the gas analyser via the closed loop calibration system through a rubber septum using a Hamilton glass micro syringe. After each injection the absorbence reading was allowed to stabilise as indicated on the chart recording.

The cumulative absorbence chart deflections for each injection were then measured and plotted against calculated concentrations to give a calibration graph used in subsequent determinations of chamber concentrations during atmospheric monitoring.



SCHEMATIC DIAGRAM OF CLOSED LOOP CALIBRATION SYSTEM

Analytical Conditions

Instrument Settings:

	Low Level	High Level
Wavelength :	9.9 µm	9.9 µm
Pathlength :	21.75 m	6.7 µm
Absorbence Range :	0.25 A	1 A
Slit Width :	0.5 mm	0.5 mm
Meter Response :	4	1
Recorder Voltage :	1 V	1 V
Chart Speed :	120 mm/h	120 mm/h

Calibration Data

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

Where:

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

Example of the Calculation for V

Compound: N,N-Dimethylacetamide

C = 700 ppm
ρ = 0.937 g/cm^3
M = 87.12

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

$$= \frac{700 \times 87.12}{0.937} \times \frac{5.64}{10^3 \times 24.06\ \mu l}$$

$$= 15.3\ \mu l$$

Therefore, to construct a calibration curve to cover the 700 ppm range, 4.0 µl samples of N,N-dimethylacetamide were injected into the analyser. An example of such a curve is given in Figure 2.

Atmosphere Generation

Schematic diagrams showing the vapour generating apparatus, exposure chambers and monitoring equipment are 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-dimethylacetamide contained in a glass gas washing, or Drechsel bottle immersed in a temperature controlled water bath at 50°C. The nitrogen/N,N-dimethylacetamide vapour mixture so generated was ducted through 7/16" ID stainless steel piping to a glass mixing vessel and diluted with filtered, compressed air. The resulting mixture of N,N-dimethylacetamide/air was ducted through 3/4" stainless steel piping to the top of the exposure chamber.

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

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

Homogeneity Data

Before starting the animal exposures, chamber concentrations at both the high and low levels were determined by continuous monitoring for periods of up to 6 h. In addition, samples were measured from different areas (at least 9) of the animal holding zone to confirm uniformity of N,N-dimethylacetamide concentration.

Measurement of Chamber Concentrations

Atmospheric concentrations of N,N-dimethylacetamide 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 (Figure 1a). Stainless steel sampling lines, fitted with a particulate filter (Whatman Mini-Filter, Grade 80) and positioned on a central reference point in each exposure chamber, were connected to the infra-red gas analysers. The sampling flow rate was approximately 4 l/min.

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

Test Compound Utilisation

At the beginning of each exposure day, the N,N-dimethylacetamide reservoir (gas washing or, Drechsel bottle) was replenished with test compound. Utilisation of test material was calculated on a daily basis by weighing the bottle before

vapour generation began and deducting the weight of the bottle and remaining test compound on completion of the exposure period.

Exposure Procedure

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

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

Animals exposed to N,N-dimethylacetamide were arranged in a single tier inside the exposure chamber. Air control animals were stacked in 2 tiers.

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

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

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

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

Positive Control Groups in Animal Tests

Preparation of Dosing Solutions

Dosing solutions were prepared daily 5 min before administration to the animals was started. The desired

amount of ethyl methanesulphonate was weighed into a volumetric flask and diluted with distilled water to obtain the correct concentration.

Treatment of Rats and Mice with Ethyl methanesulphonate

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

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

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

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

UNSCHEDULED DNA SYNTHESIS ASSAY

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

Chemicals

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

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

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

Test Solutions

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

Cells

Unscheduled DNA synthesis, following treatment with test compound, was measured in human embryonic intestinal cells (Flow 11,000 or Flow 2,002), passage 12-35 obtained from Flow Laboratories, Irvine, Scotland. These cell lines were chosen because of their higher permeability to some substrates than certain other human cell lines tested. Flow 2,002 line was used in some experiments because the growth characteristics of Flow 11,000 had deteriorated to such an extent that they were no longer suitable for these studies.

Culture Maintenance and Growth Media

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

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

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

Animals

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

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

Preparation of the 9,000 g Supernatant Fluid from Livers

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

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

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

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

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

co-factor solution	9 parts
liver preparation	1 part

Preliminary Toxicity Test

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

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

The compound was dissolved in dimethylsulphoxide, at concentrations of 100, 10, 1.0, 0.1 and 0.01 mg/ml and 10 µl samples were added to duplicate cell suspensions to give final concentrations of 1000, 100, 10, 1.0 and 0.1 µg/ml. To each control culture was added 10 µl of dimethyl-sulphoxide.

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

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

In fact no toxicity was observed, even at a concentration of 9.37 mg/µl which was selected as the highest in a series of 8 concentrations of N,N-dimethylacetamide 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% v/v) heat inactivated foetal bovine serum and the plates incubated for 24 h. The medium was then replaced with a further 2 ml of arginine-deficient DMEM and the incubation continued for a further 48 h. At the end of this time the cultures were divided into 2 groups and 100 µl of S-9 mix added to one of them. Solutions of hydroxyurea (250 mM) in sterile distilled water and 6-[^{3}H]-thymidine (21 Ci/mmol) were added to each culture giving final concentrations of 2.5 mM and 10 µCi/ml respectively. N,N-dimethylacetamide was added directly to the medium or dissolved in dimethylsulphoxide and dilutions were made from this to give a total of 8 test solutions. Triplicate wells, with and without S-9 mix, received 10 µl samples of test compound solution. 10 µl samples of dimethylsulphoxide were added to negative control cultures.

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

4-NQO	8 and 10 µg/ml
2-AAN	6.26 µg/ml

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

Autoradiography

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

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

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

Quantification of Repair Synthesis

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

DNA Repair Assay (Method 2)

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

groups and 500 µl of S-9 mix were added to one group. Solutions of hydroxyurea and [^{3}H]-deoxyguanosine (26 Ci/mmol) were added to each dish giving final concentrations of 2.5 mM and 10 µCi/ml respectively. 50 µl of undiluted N,N-dimethylacetamide was added to the cultures, both in the presence and absence of S-9 mix, to give a final concentration of 8,500 µg/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 µg/ml.

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

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

Incorporation of Label in the Presence of S-9 Mix

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

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

CYTOGENETIC ANALYSIS OF RAT BONE MARROW CELLS

Metaphase Cell Preparations

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

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

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

4-5 ml 0.075 M-KCl pre-heated to 37°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 were recorded using the microscope stage vernier. The slide number was always located on the right hand side.

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

DOMINANT LETHAL TESTING IN MALE RATS

Mating

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

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

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

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

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

Assessment

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

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

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

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

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

SPERM ABNORMALITIES TEST IN MICE

Preparation

Mice were killed 5 weeks from the last day of dosing (i.e., Friday 24 August 1979) by neck dislocation.

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

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

Assessment

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

The following types of sperm were not scored:

(1) separated tails and heads.

(2) clumps of sperm.

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

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

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

I Normal

II Abnormal

A. hook upturned or elongated.

B. banana-shaped head.

C. amorphous head.

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

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

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

SEX-LINKED RECESSIVE LETHAL TEST IN DROSOPHILA MELANOGASTER

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

Strains

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

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

Medium

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

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

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

Exposures

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

*Nipogen: bacteriostatic agent (BDH Limited).

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

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

Toxicity Test

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

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

Recessive Lethal Test

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

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

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

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

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

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

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

STATISTICAL EVALUATION

Cytogenetics Tests

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

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

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

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

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

Dominant Lethal Assay

The variates analysed were:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Sperm Abnormalities Test

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

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

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

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

Sex-linked Recessive Lethal Test

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

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

F_2 Generation

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

F_3 Generation

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

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

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

Calibration ranges adopted were 9.4-47.0 ppm (20 ppm target concentration), 94-376 ppm (200 ppm target concentration) and 188-940 ppm (700 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-dimethylacetamide was occurring. The results are shown in Table AT-4, where it can be seen that the maximum deviations encountered were -2.3% at the 20 ppm target concentration and $\pm$ 2.4% at the 700 ppm target concentration. No determinations were made on the fly exposure chamber (200 ppm target concentration), where the volume was less than one litre.

Chamber Atmospheres - Achieved Concentrations

A sample chart record taken during a day on which animals were exposed is shown in Figure 3. From charts such as this, deviations from the target concentrations of 20 ppm and 700 ppm were obtained and recorded in Tables AT-5 to 6.

Deviations from the target concentrations of more than $\pm$ 10% were limited, in the single exposure cytogenetic test, to 20 min high (20 ppm target concentration) and 40 min low, 30 min high (700 ppm target concentration). In the multiple exposure tests deviations were 45 min low, 45 min high (20 ppm target concentration) and 20 min low (700 ppm target concentration). There were no deviations in excess of $\pm$ 4% from 200 ppm during the exposure of the flies.

Animal Location

In Appendix Loc-1 and 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)

11 June 1979 Delivery: Four male mice were haphazardly selected for PEAT. There were no significant clinical observations, autopsy findings, histopathological, microbiological or parasitological findings.

6 July 1979 Delivery: Ten male and 10 female rats were haphazardly selected for PEAT. There were no significant clinical observations and autopsy findings of possible significance were restricted to irregular congestion of all lung lobes in one male. There were no microbiological or parasitological findings. Histopathology revealed peribronchial lymphoid cells in all female rats and all males except one where there were no abnormalities detected. The liver of one male rat showed mild centrilobular vacuolation and there were small lymphocytic foci in the liver of another male. There was a fine radial scar on a kidney of one male rat.

In the opinion of the pathologist these observations did not warrant rejection of the group.

Clinical Observations and Body Weights

No clinical signs of toxicity considered to be due to exposure to N,N-dimethylacetamide were observed in either rats or mice. Problems were encountered, however, with most rats from all treatment groups. They were seen to be sneezing and to have sublingual swellings before, during and after exposure to N,N-dimethylacetamide. These observations, which were consistent with sialodacryoadenitis, were also recorded for the dominant lethal test animals at various times during the 10 week mating period.

Rats treated with EMS showed occasional incidences of blood staining around the nose and other areas of the head during the dosing period.

Body weight values of the CD rats and B6C3F$_1$ mice taken at the time of dosing are given in Tables BW-1 to 4 and Appendix Tables BW-1 to 4. Weight gain was slightly depressed, when compared with the controls, in male and female CD rats exposed to 700 ppm N,N-dimethylacetamide atmospheres in the multiple exposure portion of the experiment. There was no effect upon mouse weight gain. EMS treated rats showed a marked loss in body weight over the dosing period and mouse body weight also was adversely affected to some extent.

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-dimethylacetamide (Table UDS-1). The highest concentration used in this test was 10 µl or 9.366 µg/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 greatly, this reduction being aggravated by the addition of S-9 mix to the incubation medium. Consequently, the measured incorporation of radioactivity was insufficient to provide any reasonable analysis of data produced in the presence of S-9 mix. (A more detailed description of these results is to be reported separately.) Results obtained in the absence of S-9 mix are given in Figure 4. There was no indication of deoxyguanosine incorporation into DNA.

CYTOGENETIC ANALYSIS OF RAT BONE MARROW CELLS

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

In the multiple exposure cytogenetic test, there were no indications of induction of chromosomal damage in either the male or female rats exposed to 20 ppm or 700 ppm N,N-dimethylacetamide atmospheres. Responses to the positive control substance, ethyl methanesulphonate, were significant in both the female and male rats.

A number of cells in all groups showed centromeric disjunction, indicating that the cells could be recovering from the metaphase block. The single exposure test rats did not show any significant increases in total aberration frequencies or in the frequencies of aberrations excluding gaps, when dosed with N,N-dimethlyacetamide.

Responses to EMS were generally significant, but particularly so at the 48 h sampling times from female rats. Large numbers of breaks with fragments, exchanges and multiple aberrations were found in this group.

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 of calculation was there any effect upon pregnancy frequency due to N,N-dimethylacetamide treatment, but there were reductions in Weeks 1-3 in the positive control group. These reductions were especially obvious when only females with implantations were considered to be pregnant.

Corpora lutea graviditatis counts (Tables DL-3) were not reduced in either of the N,N-dimethylacetamide treated groups; these counts were reduced, however, in Weeks 1-3 of the positive control group (Week 1, $P<0.01$; Week 2, $P<0.05$; Week 3, largest difference, but there was only one degree of freedom).

Implantations per pregnancy (Tables DL-4) were unaffected by N,N-dimethylacetamide treatment, but were reduced in Weeks 1-4 of the positive control group (Weeks 1 and 2, $P<0.001$; Week 3, $P<0.05$; Week 4, $P<0.01$).

The frequencies of live implantations (Table DL-5) and live implantations + late deaths (Table DL-6) followed closely the pattern of total implantations per pregnancy (Weeks 1, 2 and 3, $P<0.001$; Week 2, $P<0.05$).

A review of the data showing pregnancies with either one (1) or more early deaths or two (2) or more early deaths (Table DL-7) did not indicate any significant increase in these frequencies in the N,N-dimethylacetamide treated groups, when compared with the air control group. The frequency of 6/16 pregnancies with more than one early death observed in Week 6, 700 ppm dose group, is high in comparison with the concurrent air control group, but is seen in a different aspect when compared with a frequency of 7/19 recorded for Week 7, air control group.

Analysis of early death frequency, following Freeman-Tukey Poisson or binomial transformation failed to reveal any effects attributable to N,N-dimethylacetamide treatment (Tables DL-8 and 9). In the EMS treated group there were significant differences from the controls, following Poisson transformation, in Week 4 ($P<0.01$) and Week 5 ($P<0.05$). Analysis after transformation showed significant

increases in early death frequencies in the EMS treated group assessment Weeks 2-5 (Weeks 2 and 4, $P<0.01$; Weeks 3 and 5, $P<0.05$). It was concluded that the experiment was performed in a satisfactory manner and there was no evidence of effects due to N,N-dimethylacetamide treatment.

SPERM ABNORMALITY TEST

There were no increases in the frequencies of abnormal sperm in any of the categories examined (Tables SA-1 and 2 and Appendix Table SA). The categories C and D, amorphous head and folded tail respectively, showed significantly higher frequencies in the EMS treated groups than in the air control group.

Table SA-1 shows the high dose level group mean values both with and without data from 350♂. This animal was clearly abnormal, there being 22.2% abnormal sperm in the sample from this animal compared with a mean of 2.69% in the remainder of this group.

SEX-LINKED RECESSIVE LETHAL TEST IN DROSOPHILA

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

A dose ranging study was undertaken on 11 July 1979 in which flies were exposed to 700 ppm N,N-dimethylacetamide for 30 min or 95 min or to 1,200 ppm N,N-dimethylacetamide for 30 min or 70 min. At the higher concentration level, none of the flies survived; decreased activity was observed within 18 min and there were no signs of recovery within 2 h. Similarly, flies exposed to the 700 ppm atmosphere showed decreased activity after 15 min and none of the flies exposed for 95 min recovered. Exposure for 30 min was not lethal and there was no effect upon fertility. On the basis of these results, exposure conditions chosen for the main study on 24 July 1979 were 200 ppm N,N-dimethylacetamide for as long as it appeared that the flies would tolerate the substance. In reality this was 2.5 h.

Two breeding stocks (A and B) were exposed (Table RL-2) in the main test. The flies initially showed hyperactivity, followed by reduced activity after about 70 min. At 2.5 h a marked reduction in activity was noted, so, exposure was terminated. The fly chambers were flushed through with air for 20 min. While all flies were alive 30 min from the end of exposure, 15 min later some flies appeared to be dead. However, 3 h from the end of the exposure period it was evident that all flies were dead.

The experiment was re-started on 25 July 1979 using a 200 ppm N,N-dimethylacetamide atmosphere for 95 min. One hour from the beginning of exposure there was decreased activity and, by the time the exposure period was completed, activity of the flies was greatly reduced. The flies did recover, however, and when mated it was found that the number of sterile vials was lower than in the air exposed control groups (Table RL-2).

At least 600 F_2 vials were set up for each stock and brood, giving a total of 3,781. A total of 3,476 vials were scored. In stock A there were only 2 lethals, one in brood 1 and one in brood 2. This did not indicate a mutagenic response. Stock B flies, however, gave 3 lethals in brood 2, which was a frequency of 0.57%. Since all 3 lethals were derived from a single male, the high frequency of lethals in brood 2 was not interpreted as indicative of mutagenic response.

CONCLUSIONS

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

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TABLE AT-1

N,N-Dimethylacetamide
Calibration Data for Low Level

Dose Level: 20 ppm

Batch No. 13737

Volume µl	Conc., ppm, (v/v)	Cumulative Chart Deflection, mm					
		16 July 1979	17 July 1979	18 July 1979	19 July 1979	20 July 1979	23 July 1979
0	0	0	0	0	0	0	0
0.2	9.4	19.0	19.0	16.5	19.0	19.0	20.0
0.4	18.8	40.0	38.0	35.5	40.0	38.5	38.0
0.6	28.2	61.0	58.0	57.0	60.0	58.0	56.0
0.8	37.6	81.0	78.0	76.0	79.5	77.5	76.0
1.0	47.0	102.0	-	-	-	-	-
Chart deflection (mm) for 20 ppm		43.0	41.5	40.0	42.5	41.5	41.0

Instrument Setting

Pathlength: 21.75 m
Wavelength: 9.9 µm
Absorbence Range: 0.25 A
Slit Width: 0.5 mm
Meter Response: 4
Recorder Voltage: 1 V
Chart Speed: 120 mm/h

Calibration

Syringe: 1 µl Hamilton
Injection Volume: 0.2 µl
No. of Repeat Injections: x 5

TABLE AT-2

N,N-Dimethylacetamide
Calibration Date for High Level

Dose Level: 700 ppm v/v Batch No. 13737

Volume µl	Conc., ppm, (v/v)	Cumulative Chart Deflection, mm					
		16 July 1979	17 July 1979	18 July 1979	19 July 1979	20 July 1979	23 July 1979
0	0	0	0	0	0	0	0
4.0	188	32.0	36.0	38.0	30.0	32.0	32.5
8.0	376	60.0	67.0	68.0	59.0	61.0	61.5
12.0	564	84.0	92.0	88.0	83.5	86.0	86.0
16.0	752	106.0	115.0	107.0	103.5	109.0	109.0
20.0	940	122.0	134.0	124.0	120.0	126.0	126.0
Chart deflection (mm) for 700 ppm		98.0	109.0	104.0	98.0	102.5	102.0

Instrument Setting

Pathlength: 6.75 m
Wavelength: 9.9 µm
Absorbence Range: 1 A
Slit Width: 0.5 mm
Meter Response: 1
Recorder Voltage: 1 V
Chart Speed: 120 mm/h

Calibration

Syringe: 10 µl Hamilton
Injection Volume: 4.0 µl
No. of Repeat Injections: x 5

TABLE AT-3

N,N-Dimethylacetamide
Calibration for Drosophila Exposures

Dose Level: 200 ppm v/v Batch No. 13737

Volume µl	Conc., ppm, (v/v)	Cumulative Chart Deflection, mm	
		24 July 1979	25 July 1979
0	0	0	0
2.0	94.0	33.0	32.0
4.0	188.0	64.0	63.0
6.0	282.0	93.0	92.0
8.0	376.0	119.0	120.0
Chart deflection (mm) for 200 ppm		68.0	66.5

Instrument Setting

Pathlength: 6.75 m
Wavelength: 9.9 µm
Absorbence Range: 1 A
Slit Width: 0.5 mm
Meter Response: 1
Recorder Voltage: 0.5 V
Chart Speed: 120 mm/h

Calibration

Syringe: 10 µl Hamilton
Injection Volume: 2.0 µl
No. of Repeat Injections: x 4

TABLE AT-4

N,N-Dimethylacetamide
Chamber Atmosphere Homogeneity Data

Dose Level: 20 ppm and 700 ppm

Sample Location	% Deviation from Reference Sampling Point	
	Low	High
Reference Point (R)	-	-
Right Centre (RC)	-2.3%	+2.4%
Right Front (RF)	+1.1%	NDA
Centre Front (CF)	-1.2%	+2.4%
Left Front (LF)	-1.2%	+0.8%
Left Centre (LC)	-1.2%	-1.6%
Left Back (LB)	-2.3%	-1.6%
Centre Back (CB)	-1.2%	-2.4%
Right Back (RB)	0%	-2.4%

Top view of exposure chamber

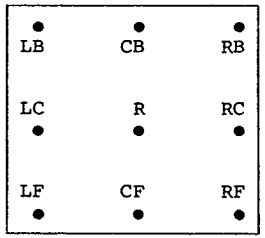


TABLE AT-5

N,N-Dimethylacetamide
Atmospheric Analysis by Infra-red Spectroscopy
Target Concentration 20 ppm (v/v)

Exposure Day	% Deviation from Target Concentration in Minutes										Time Averaged Concentration for 7 h (ppm)
	-10	-7.5	-5	-2.5	0	+2.5	+5	+7.5	+10	+12.5	
Single	-	20	30	20	175	25	50	30	20	20	20.4
Multiple 1	25	-	30	70	175	55	50	15	-	-	20.0
Multiple 2	-	10	5	-	10	-	30	150	170	45	21.6
Multiple 3	-	-	65	140	140	40	35	-	-	-	20.3
Multiple 4	-	-	15	-	25	30	200	75	75	-	21.1
Multiple 5	20	-	35	30	195	55	50	-	35	-	20.1

TABLE AT-5 (continued)

N,N-Dimethylacetamide
Target Concentration 700 ppm (v/v)

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	
Single	40	10	20	20	25	70	85	80	10	30	30	702.9
Multiple 1	20	20	35	130	95	15	40	-	-	-	-	659.7
Multiple 2	-	-	-	10	120	210	40	40	-	-	-	699.2
Multiple 3	-	-	-	20	60	125	65	90	60	-	-	713.5
Multiple 4	-	-	-	10	-	75	225	95	15	-	-	718.3
Multiple 5	-	-	10	10	125	190	65	15	5	-	-	697.3

TABLE AT-6

N,N-Dimethylacetamide
Atmospheric Analysis by Infra-red Spectroscopy
Target Concentration 200 ppm (v/v)

Exposure Day	% Deviation from Target Concentration in Minutes					Total Time Exposed	Time Averaged Concentration (ppm)
	-4	-2	0	+2	+4		
Drosophila Main Test	25	-	72.5	-	52.5	2 h 30 min	200
	25	15	26	10	19	1 h 35 min	199.3

TABLE BW-1

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

Sex	Day	Control (0 ppm)	20 ppm	700 ppm	5 x 100 mg/kg EMS
Male	1	339 ± 19	330 ± 22	335 ± 19	334 ± 23
	2	341 ± 20	330 ± 22	335 ± 18	328 ± 21
	3	346 ± 22	338 ± 22	338 ± 18	318 ± 23
	4	350 ± 22	340 ± 23	337 ± 20	306 ± 19
	5	353 ± 22	345 ± 23	338 ± 21	293 ± 19
	Weight gain/loss	14	15	3	-41
Female	1	209 ± 12	219 ± 12	212 ± 17	213 ± 20
	2	209 ± 12	218 ± 12	210 ± 18	207 ± 22
	3	212 ± 11	220 ± 13	212 ± 17	200 ± 21
	4	212 ± 10	221 ± 14	210 ± 16	193 ± 21
	5	213 ± 10	223 ± 14	211 ± 17	190 ± 21
	Weight gain/loss	4	4	-1	-23

TABLE BW-2

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

Sex	Sampling Time (h post exposure)	Control (0 ppm)	20 ppm	700 ppm	EMS 250 mg/kg
Male	6	345 ± 14	346 ± 30	352 ± 27	349 ± 18
	24	343 ± 17	341 ± 28	369 ± 19	349 ± 19
	48	346 ± 27	372 ± 32	362 ± 15	353 ± 27
Female	6	232 ± 19	225 ± 13	222 ± 22	237 ± 16
	24	222 ± 19	234 ± 16	224 ± 14	226 ± 15
	48	233 ± 13	224 ± 17	231 ± 18	220 ± 13

TABLE BW-3

N,N-Dimethylacetamide
Dominant Lethal Assay
Group Mean Body Weights (g) for the Dosing Period of Male CD Rats

Day	Air Control (0 ppm)	20 ppm	700 ppm	5 x 100 mg/kg EMS
1	342 ± 23	348 ± 25	334 ± 22	352 ± 21
2	344 ± 25	350 ± 24	336 ± 23	341 ± 18
3	347 ± 24	358 ± 27	337 ± 24	330 ± 16
4	351 ± 24	360 ± 29	337 ± 26	316 ± 17
5	353 ± 23	361 ± 30	334 ± 26	307 ± 16
Weight gain/loss	11	13	0	-45

TABLE BW-4

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

Day	Control (0 ppm)	20 ppm	700 ppm	5 x 100 mg/kg EMS
1	24 ± 1	23 ± 1	23 ± 1	24 ± 1
2	24 ± 1	24 ± 1	23 ± 1	24 ± 1
3	24 ± 1	24 ± 1	24 ± 1	23 ± 1
4	24 ± 1	24 ± 1	23 ± 1	22 ± 2
5	24 ± 1	25 ± 1	23 ± 1	22 ± 1
Weight gain/loss	0	2	0	-2

TABLE UDS-1

N,N-Dimethylacetamide
Unscheduled DNA Synthesis

Substance	Concentration (µg/ml)		Mean Number of Grains/Nucleus ± S.D.	
	With S-9	Without S-9	With S-9	Without S-9
Dimethylsulphoxide	1%	1%	9.3 ± 4.2	13.1 ± 6.5
4-Nitroquinoline-N-oxide	-	10	-	>100
	-	8	-	>100
2-Aminoanthracene	6.25		79.3 ± 21.1	-
N,N-Dimethylacetamide	70	73	7.6 ± 4.3	16.0 ± 6.9
	139	146	11.9 ± 4.1	17.3 ± 7.2
	279	293	8.8 ± 3.1	16.4 ± 6.8
	558	585	11.5 ± 3.8	14.9 ± 6.2
	1115	1171	2.0 ± 1.4	16.3 ± 8.1
	2230	2342	5.4 ± 5.6	14.3 ± 6.7
	4460	4683	11.4 ± 3.3	15.6 ± 7.3
	8920	9366	9.3 ± 2.8	14.7 ± 6.4

TABLE CA-MD-M-1

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

Multiple Dosing

Sampling Time: 6 h

Group	Number of Spreads Observed	Observed Aberrations						Miscellaneous
		Chromatid			Chromosome			
		Gap	B w F	B w/o F	Gap	B w F	B w/o F	
Air Control, 7 h/day	500	3	-	-	-	-	-	-
20 ppm, 7 h/day	500	4	-	1	-	-	-	1 Chromatid Fragment
700 ppm, 7 h/day	450	-	-	-	-	-	-	-
EMS, 100 mg/kg/day	450	22	18	-	1	-	1	5 Chromatid Fragments 5 Exchanges

TABLE CA-MD-M-2

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

Multiple Dosing

Sampling Time: 6 h

Treatment Group	Spreads with Aberrations					
	Total			Excluding Gaps		
	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t
Air Control	0.200	0.040		0.141	0.033	
20 ppm	0.230	0.040	0.53	0.180	0.033	0.86
700 ppm	0.141	0.042	-1.04	0.141	0.035	0.00
EMS, 100 mg/kg	0.614	0.042	7.20***	0.458	0.035	6.68***

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

***p<0.001

TABLE CA-MD-F-1

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

Multiple Dosing — Sampling Time: 6 h

Group	Number of Spreads Observed	Observed Aberrations						Miscellaneous
		Chromatid			Chromosome			
		Gap	B w F	B w/o F	Gap	B w F	B w/o F	
Air Control, 7 h/day	500	8	-	1	-	-	-	1 Chromatid Fragment
20 ppm, 7 h/day	400	3	-	1	-	-	-	1 Chromosomal Fragment
700 ppm, 7 h/day	400	3	-	-	-	-	-	1 Chromatid Fragment
EMS, 100 mg/kg/day	500	19	9	1	-	-	-	7 Chromatid Fragments 1 Exchange

TABLE CA-MD-F-1 (Supplement)

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

Multiple Dosing — Sampling Time: 6 h

Group	Rat No.	Description
20 ppm, 7 h/day	293	1 Chromosome split at centromere
	294	1 Chromosome split at centromere
	300	1 Chromosome split at centromere
700 ppm, 7 h/day	308	1 Chromosome split at centromere

TABLE CA-MD-F-2

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

Multiple Dosing — Sampling Time: 6 h

Treatment Group	Spreads with Aberrations					
	Total			Excluding Gaps		
	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t
Air Control	0.029	0.045		0.171	0.035	
20 ppm	0.253	0.050	-0.55	0.190	0.039	0.37
700 ppm	0.266	0.050	-0.36	0.165	0.039	-0.10
EMS, 100 mg/kg	0.499	0.045	3.28*	0.339	0.035	3.40**

S.E. of mean = Standard error of Freeman-Tukey binomial transformation mean
*P<0.0025
**P<0.001

TABLE CA-M6-1

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

Single Dosing — Sampling Time: 6 h

Group	Number of Spreads Observed	Observed Aberrations						Miscellaneous
		Chromatid			Chromosome			
		Gap	B w F	B w/o F	Gap	B w F	B w/o F	
Air Control, 7 h/day	500	-	-	-	-	-	-	1 Chromosomal Fragment 2 Chromatid Fragments
20 ppm, 7 h/day	500	3	-	-	-	-	-	1 Chromatid Fragment
700 ppm, 7 h/day	460	-	-	-	-	-	-	-
EMS, 250 mg/kg/day	450	4	4	-	-	-	-	1 Chromatid Fragment

TABLE CA-MF6-1 (Supplement)

N,N-Dimethylacetamide
Cytogenetic Analysis of Rat Bone Marrow Cells
Supplementary Observations
Males and Females

Single Dosing — Sampling Time : 6 h

Group	Rat No.	Description
Air Control, 7 h/day	7	Some chromosome split at centromere
	9	1 Chromosome split at centromere
20 ppm, 7 h/day	31	Some chromosome split at centromere
	33	Some chromosome split at centromere
	194	Some chromosome split at centromere
	200	Some chromosome split at centromere
700 ppm, 7 h/day	63	Chromosomes split at centromere
EMS, 250 mg/kg/day	93	Chromosomes split at centromere

TABLE CA-M6-2

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

Single Dosing — Sampling Time: 6 h

Treatment Group	Spreads with Aberrations					
	Total			Excluding Gaps		
	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t
Air Control	0.200	0.035		0.200	0.030	
20 ppm	0.199	0.035	-0.02	0.160	0.030	-0.95
700 ppm	0.157	0.035	-0.86	0.157	0.030	-1.03
EMS, 250 mg/kg	0.303	0.037	1.99*	0.250	0.031	1.16

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

TABLE CA-M24-1

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

Single Dosing — Sampling Time: 24 h

Group	Number of Spreads Observed	Observed Aberrations						Miscellaneous
		Chromatid			Chromosome			
		Gap	B w F	B w/o F	Gap	B w F	B w/o F	
Air Control, 7 h/day	225	2	1	-	-	-	-	-
20 ppm, 7 h/day	351	-	-	-	-	-	-	-
700 ppm, 7 h/day	408	-	-	-	-	-	-	-
EMS, 250 mg/kg/day	357	6	2	-	-	-	-	8 Chromatid Fragments 2 Multi Aberrations

TABLE CA-M24-2

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

Single Dosing — Sampling Time: 24 h

Treatment Group	Spreads with Aberrations					
	Total			Excluding Gaps		
	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t
Air Control	0.271	0.064		0.220	0.060	
20 ppm	0.221	0.055	-0.59	0.221	0.052	0.01
700 ppm	0.159	0.052	-1.36	0.159	0.049	-0.79
EMS, 250 mg/kg	0.366	0.055	1.12	0.294	0.052	0.92

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

TABLE CA-M48-1

N,N-Dimethylacetamide
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosome Aberrations Scored
Males

Single Dosing — Sampling Time: 48 h

Treatment Group	Animals Observed	Spreads Observed	Observed Aberrations						Miscellaneous
			Chromatid			Chromosome			
			Gap	B w F	B w/o F	Gap	B w F	B w/o F	
Air Control, 7 h/day	10	500	3	2	-	-	-	-	-
20 ppm, 7 h/day	10	500	3	-	-	-	-	-	-
700 ppm, 7 h/day	10	500	1	-	-	-	-	-	-
EMS, 250 mg/kg/day	10	500	9	6	1	-	-	-	3 Multi Aberrations 7 Exchanges 3 Chromatid Fragments

TABLE CA-M48-1 (Supplement)

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

Single Dosing Sampling Time: 48 h

Group	Miscellaneous Observations
700 ppm, 7 h/day	3 Chromosomes split at centromere
EMS, 250 mg/kg/day	2 Chromosomes split at centromere

TABLE CA-M48-2

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

Single Dosing — Sampling Time: 48 h

Treatment Group	Spreads with Aberrations					
	Total			Excluding Gaps		
	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t
Air Control	0.219	0.052		0.180	0.040	
20 ppm	0.200	0.052	-0.26	0.141	0.040	-0.71
700 ppm	0.160	0.052	-0.80	0.141	0.040	-0.71
EMS, 250 mg/kg	0.389	0.052	2.31*	0.318	0.040	2.45**

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

TABLE CA-F6-1

N,N-Dimethylacetamide
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/chromosomal Aberrations Scored
Females

Single Dosing — Sampling Time: 6 h

Group	Number of Spreads Observed	Observed Aberrations						Miscellaneous
		Chromatid			Chromosome			
		Gap	B w F	B w/o F	Gap	B w F	B w/o F	
Air Control, 7 h/day	450	3	-	-	-	-	-	-
20 ppm, 7 h/day	450	2	1	-	1	-	-	-
700 ppm, 7 h/day	500	1	-	-	-	-	-	3 Chromatid Fragments
EMS, 250 mg/kg/day	405	19	5	-	1	1	-	-

TABLE CA-F6-2

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

Single Dosing — Sampling Time: 6 h

Treatment Group	Spreads with Aberrations					
	Total			Excluding Gaps		
	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t
Air Control	0.207	0.044		0.141	0.029	
20 ppm	0.229	0.044	0.35	0.163	0.029	0.53
700 ppm	0.220	0.042	0.22	0.200	0.028	1.48
EMS, 250 mg/kg	0.464	0.044	4.11***	0.306	0.029	3.98***

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

TABLE CA-F24-1

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

Single Dosing — Sampling Time: 6 h

Group	Number of Spreads Observed	Observed Aberrations						Miscellaneous
		Chromatid			Chromosome			
		Gap	B w F	B w/o F	Gap	B w F	B w/o F	
Air Control, 7 h/day	255	1	-	1	-	-	-	-
20 ppm, 7 h/day	151	-	-	-	-	-	-	-
700 ppm, 7 h/day	350	4	1	-	-	-	-	-
EMS, 250 mg/kg/day	287	14	4	2	-	-	-	4 Chromatid Fragments 1 Multi Aberration

TABLE CA-F24-2

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

Single Dosing — Sampling Time: 24 h

Treatment Group	Spreads with Aberrations					
	Total			Excluding Gaps		
	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t
Air Control	0.238	0.114		0.220	0.094	
20 ppm	0.302	0.139	0.36	0.302	0.115	0.55
700 ppm	0.258	0.114	0.12	0.174	0.094	-0.35
EMS, 250 mg/kg	0.631	0.098	2.62**	0.467	0.081	1.99*

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

TABLE CA-F48-1

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

Single Dosing

Sampling Time: 48 h

Treatment Group	Animals Observed	Spreads Observed	Observed Aberrations						Miscellaneous
			Chromatid			Chromosome			
			Gap	B w F	B w/o F	Gap	B w F	B w/o F	
Air Control, 7 h/day	10	500	7	5	-	-	-	-	1 Chromatid Fragment 1 Exchange
20 ppm, 7 h/day	10	500	5	-	-	-	-	-	1 Chromosomal Fragment
700 ppm, 7 h/day	10	500	3	1	-	-	-	-	-
EMS, 250 mg/kg/day	10	500	9	30	-	1	3	-	20 Multi Aberrations 8 Exchanges 5 Chromatid Fragments 1 Chromosomal Fragment

TABLE CA-F48-2

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

Single Dosing — Sampling Time: 48 h

Treatment Group	Spreads with Aberrations					
	Total			Excluding Gaps		
	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t
Air Control	0.330	0.063		0.261	0.049	
20 ppm	0.219	0.063	-1.25	0.160	0.049	-1.44
700 ppm	0.211	0.063	-1.34	0.160	0.049	-1.44
EMS, 250 mg/kg	0.613	0.063	3.20**	0.587	0.049	4.68***

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

TABLE DL-1

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

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	20 ppm	700 ppm	5 x 100 mg/kg EMS
1	90%	70%	80%	65%
2	90%	85%	90%	85%
3	90%	100%	100%	32%
4	90%	90%	100%	95%
5	100%	90%	94%	95%
6	95%	80%	100%	95%
7	90%	85%	85%	90%
8	100%	85%	100%	95%
9	100%	100%	95%	95%
10	95%	85%	90%	95%

TABLE DL-2

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

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)		20 ppm		700 ppm		5 x 100 mg/kg EMS	
1	17/20	85%	13/20	65%	16/20	80%	12/20	60%
2	18/20	90%	16/20	80%	18/20	90%	7/20	35%
3	16/20	80%	16/19	84%	20/20	100%	2/18	11%
4	20/20	100%	17/20	85%	20/20	100%	17/20	85%
5	19/20	95%	18/20	90%	16/18	89%	19/20	95%
6	19/20	95%	15/20	75%	20/20	100%	19/20	95%
7	18/20	90%	17/20	85%	17/20	85%	18/20	90%
8	20/20	100%	17/20	85%	19/20	95%	19/20	95%
9	20/20	100%	18/20	90%	19/20	95%	18/20	90%
10	19/20	95%	16/20	80%	18/20	90%	18/20	90%

TABLE DL-3

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

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	20 ppm	700 ppm	5 x 100 mg/kg EMS
1	[1] 13.06 ± 0.76	12.00 ± 0.87	12.12 ± 0.78	8.83 ± 0.68**
2	13.00 ± 0.46	13.44 ± 0.51	14.00 ± 0.48	7.57 ± 1.49*
3	12.87 ± 0.53	13.19 ± 0.53	12.30 ± 0.47	3.00 ± 3.00
4	12.65 ± 0.45	12.76 ± 0.49	13.40 ± 0.45	13.06 ± 0.43
5	12.79 ± 0.50	13.78 ± 0.52	13.25 ± 0.55	12.47 ± 0.50
6	13.00 ± 0.47	12.33 ± 0.52	13.35 ± 0.45	13.21 ± 0.55
7	12.67 ± 0.48	12.88 ± 0.50	12.76 ± 0.50	13.50 ± 0.58
8	14.05 ± 0.62	13.00 ± 0.68	13.42 ± 0.64	13.32 ± 0.42
9	13.60 ± 0.52	12.39 ± 0.55	14.00 ± 0.53	12.89 ± 0.45
10	12.95 ± 0.56	13.31 ± 0.61	13.39 ± 0.57	13.11 ± 0.50

[1] 1 = Mean ± standard error of mean

*P<0.05

**P<0.01

TABLE DL-4

N,N-Dimethylacetamide
Dominant Lethal Test in Rats
Total Implantations per Pregnancy

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	20 ppm	700 ppm	5 x 100 mg/kg EMS
1	[1] 13.18 ± 1.00	12.15 ± 1.14	11.94 ± 1.03	7.00 ± 0.87***
2	13.11 ± 0.53	13.56 ± 0.56	13.61 ± 0.53	1.14 ± 0.14***
3	12.44 ± 0.59	13.00 ± 0.59	12.50 ± 0.53	1.50 ± 0.50*
4	12.75 ± 0.39	12.41 ± 0.43	12.95 ± 0.39	9.77 ± 0.78**
5	11.79 ± 0.72	12.06 ± 0.74	12.81 ± 0.79	11.11 ± 0.64
6	12.16 ± 0.61	12.00 ± 0.68	12.30 ± 0.59	11.74 ± 0.37
7	12.28 ± 0.45	12.88 ± 0.46	12.65 ± 0.46	11.83 ± 0.54
8	12.70 ± 0.59	12.94 ± 0.64	13.53 ± 0.61	12.26 ± 0.55
9	12.75 ± 0.45	12.06 ± 0.48	13.74 ± 0.47	11.89 ± 0.46
10	12.89 ± 0.48	13.19 ± 0.52	12.94 ± 0.49	13.33 ± 0.46

1 = Mean ± standard error of mean

*P<0.05

**P<0.01

***P<0.001

TABLE DL-5

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

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	20 ppm	700 ppm	5 x 100 mg/kg EMS
1	[1] 12.71 ± 1.03	10.54 ± 1.18	11.62 ± 1.06	4.83 ± 0.84***
2	12.50 ± 0.54	12.81 ± 0.57	12.72 ± 0.54	0.43 ± 0.20***
3	11.69 ± 0.60	11.94 ± 0.60	11.95 ± 0.54	0.00 ± 0.00*
4	10.60 ± 0.79	10.76 ± 0.86	11.55 ± 0.79	6.29 ± 1.05**
5	10.89 ± 0.84	9.50 ± 0.87	10.25 ± 0.92	8.90 ± 0.90
6	10.58 ± 0.95	9.40 ± 1.07	9.85 ± 0.93	10.00 ± 0.72
7	10.39 ± 0.88	10.94 ± 0.90	11.18 ± 0.90	10.56 ± 0.77
8	10.45 ± 0.80	11.35 ± 0.87	11.74 ± 0.82	9.84 ± 0.90
9	10.75 ± 0.77	10.50 ± 0.82	11.58 ± 0.79	11.00 ± 0.50
10	10.05 ± 1.05	10.75 ± 1.14	9.72 ± 1.07	11.78 ± 0.64

1 = Mean ± standard error of mean

*P<0.05

**P<0.01

***P<0.001

TABLE DL-6

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

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	20 ppm	700 ppm	5 x 100 mg/kg EMS
1	[1] 12.82 ± 1.02	11.38 ± 1.16	11.75 ± 1.05	5.67 ± 0.67***
2	12.50 ± 0.55	13.12 ± 0.58	12.78 ± 0.55	0.43 ± 0.20***
3	11.69 ± 0.61	12.12 ± 0.61	12.05 ± 0.55	0.00 ± 0.00*
4	11.75 ± 0.56	11.24 ± 0.61	12.00 ± 0.56	6.53 ± 1.03***
5	11.26 ± 0.74	11.17 ± 0.76	11.12 ± 0.80	9.58 ± 0.76
6	10.84 ± 0.69	11.00 ± 0.78	11.05 ± 0.67	10.68 ± 0.48
7	11.56 ± 0.53	12.35 ± 0.55	11.94 ± 0.55	11.06 ± 0.66
8	11.65 ± 0.65	11.82 ± 0.70	12.63 ± 0.66	11.00 ± 0.81
9	11.90 ± 0.44	11.00 ± 0.46	12.95 ± 0.45	11.61 ± 0.45
10	10.84 ± 0.89	11.50 ± 0.97	11.11 ± 0.92	12.22 ± 0.59

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

TABLE DL-7

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

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)		20 ppm		700 ppm		5 x 100 mg/kg EMS	
	>0	>1	>0	>1	>0	>1	>0	>1
1	[1] 4/17	1/17	4/13	3/13	3/16	0/16	3/12	2/12
2	8/18	2/18	4/16	2/16	10/18	5/18	5/7	0/7
3	7/16	3/16	8/16	2/16	7/20	1/20	2/2	1/2
4	8/20	4/20	7/17	3/17	11/20	4/20	14/17	13/17
5	6/19	3/19	10/18	2/18	8/16	6/16	15/19	8/19
6	10/19	7/19	6/15	2/15	14/20	2/20	10/19	4/19
7	8/18	1/18	6/17	2/17	8/17	2/17	9/18	2/18
8	11/20	6/20	11/17	2/17	12/19	3/19	10/19	5/19
9	11/20	2/20	7/18	3/18	7/19	4/19	5/18	0/18
10	11/19	7/19	10/16	6/16	9/18	5/18	8/18	3/18

1 = Mean $\pm$ standard error of mean

TABLE DL-8

N,N-Dimethylacetamide
Dominant Lethal Test in Rats
Early Death Frequency, Freeman-Tukey Poisson Transformation

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	20 ppm	700 ppm	5 x 100 mg/kg EMS
1	[1] 1.410 ± 0.2126	1.733 ± 0.2431	1.265 ± 0.2191	1.893 ± 0.5205
2	1.742 ± 0.2180	1.482 ± 0.2313	1.989 ± 0.2180	2.010 ± 0.2608
3	1.829 ± 0.2513	1.920 ± 0.2513	1.561 ± 0.2248	2.780 ± 0.3660
4	1.892 ± 0.2968	1.987 ± 0.3219	2.022 ± 0.2968	3.490 ± 0.3971**
5	1.593 ± 0.2897	1.975 ± 0.2977	2.342 ± 0.3157	2.594 ± 0.2435*
6	2.213 ± 0.2976	1.881 ± 0.3349	2.326 ± 0.2900	2.063 ± 0.2802
7	1.777 ± 0.2362	1.620 ± 0.2430	1.816 ± 0.2430	1.874 ± 0.2455
8	2.104 ± 0.2427	2.180 ± 0.2633	2.070 ± 0.2490	2.182 ± 0.3122
9	1.957 ± 0.2694	1.926 ± 0.2840	1.790 ± 0.2764	1.393 ± 0.1536
10	2.602 ± 0.3978	2.471 ± 0.4335	2.347 ± 0.4087	1.985 ± 0.3335

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

TABLE DL-9

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

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	20 ppm	700 ppm	5 x 100 mg/kg EMS
1	[1] 0.400 ± 0.0756	0.504 ± 0.0864	0.413 ± 0.0779	0.688 ± 0.1720
2	0.478 ± 0.0600	0.400 ± 0.0640	0.534 ± 0.0600	1.782 ± 0.2780 **
3	0.510 ± 0.0705	0.531 ± 0.0705	0.433 ± 0.0631	2.421 ± 0.0870 *
4	0.532 ± 0.0913	0.575 ± 0.0991	0.562 ± 0.0913	1.268 ± 0.1856 **
5	0.454 ± 0.0814	0.570 ± 0.0837	0.662 ± 0.0887	0.810 ± 0.0951 *
6	0.654 ± 0.0907	0.541 ± 0.1020	0.658 ± 0.0884	0.600 ± 0.0851
7	0.504 ± 0.0713	0.446 ± 0.0733	0.516 ± 0.0733	0.556 ± 0.0802
8	0.616 ± 0.0705	0.604 ± 0.0765	0.563 ± 0.0723	0.686 ± 0.1384
9	0.542 ± 0.0720	0.543 ± 0.0759	0.477 ± 0.0739	0.395 ± 0.0418
10	0.786 ± 0.1477	0.701 ± 0.1609	0.724 ± 0.1517	0.543 ± 0.0936

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

TABLE SA-1

N,N-Dimethylacetamide
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	9635	16	14	181	41	113	365	0.16	0.14	1.81	0.41	1.13	3.65	
20 ppm, 7 h/day	9681	9	10	165	43	92	319	0.09	0.10	1.65	0.43	0.92	3.19	
700 ppm, 7 h/day	9509 [1])	21	22	259	74	115	491	0.21	0.22	2.59	0.74	1.15	4.91	[1])
	9731 [2])	7	10	121	48	83	269	0.08	0.11	1.34	0.53	0.92	2.69	[2])
EMS 200 mg/kg/day	8929	22	31	552	270	196	1071	0.22	0.31	5.52	2.70	1.96	10.71	

1) Including animal No. 350. 2) Excluding animal no. 350.

*A = Hook up-turned or hook elongated

B = Banana-shaped head

C = Amorphous head

D = Folded tail

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

TABLE SA-2

N,N-Dimethylacetamide
Sperm Abnormality Test
Means of Freeman-Tukey Binomial Transformation
± Standard Error

Multiple Dosing

Dose Group	Abnormality Category					
	A	B	C	D	E	Total
Air Control, 7 h/day	8.09	8.30	27.14	13.08	21.42	38.48
	± 0.969	± 1.040	± 1.510	± 1.601	± 1.078	± 1.958
20 ppm, 7 h/day	6.54	6.72	25.87	13.54	19.52	36.02
	± 0.969	± 1.040	± 1.510	± 1.601	± 1.078	± 1.958
700 ppm, 7 h/day	6.64	7.36	23.42	14.52	19.68	34.84
	± 1.022	± 1.097	± 1.592	± 1.688	± 1.136	± 2.064
EMS, 200 mg/kg/day	9.84	9.41	39.78*	28.16*	23.96	56.25*
	± 1.022	± 1.097	± 1.592	± 1.688	± 1.136	± 2.064

A = Hook up-turned or hook elongated

B = Banana-shaped head

C = Amorphous head

D = Folded tail

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

* = $P<0.01$

TABLE RL-1

N,N-Dimethylacetamide

Drosophila Dose Ranging Experiment

Day						Control
		30 min	95 min	30 min	70 min	
0	No. of males exposed	100	100	100	100	
1	No. and % survival	88/88%	0/0%	0/0%	0/0%	
2	No. of eggs laid by 5♀	324				90
3	No. and % hatched	263/81%				76/84%

TABLE RL-2

N,N-Dimethylacetamide
Drosophila SLRL Procedure and Results

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

	Brood 1	Brood 2	Brood 3
F_1 set up	30.7.79	2.8.79	8.8.79
F_2 set up	10.8.79	15.8.79	20.8.79
F_2 scored	23.8.79	28.8.79	5.9.79
F_2 repeats scored	-	-	-
F_3 set up	-	28.8.79	-
F_3 scored	-	13.9.79	-
F_3 repeats scored	-	25.9.79	-

RESULTS

	Brood 1	Brood 2	Brood 3	All Broods
No. of F_1 vials	98	87	64	249
No. of sterile F_1 vials	16	11	14	41
No. of F_1 vials used in F_2	82	75	50	207
No. of F_2 vials set up	598	600	506	1704
No. of F_2 vials scored	511	516	424	1451
No. of F_2 vials containing lethals	1	0	0	1
Frequency of F_2 lethals	0.19%	0	0	0.06%
No. of F_3 vials set up	-	600	-	600
No. of F_3 vials scored	-	577	-	577
No. of F_3 vials containing lethals	-	0	-	0
Frequency of F_3 lethals	-	0	-	0

TABLE RL-2 (continued)

N,N-Dimethylacetamide

Drosophila SLRL Procedure and Results

Compound: Air Concentration: - Stock: B

Length of Exposure: - Test exposure given:

	Brood 1	Brood 2	Brood 3
F_1 set up	30.7.79	2.8.79	8.8.79
F_2 set up	13.8.79	15.8.79	21.8.79
F_2 scored	29.8.79	27.8.79	4.9.79
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	92	87	69	248
No. of sterile F_1 vials	3	12	34	49
No. of F_1 vials used in F_2	89	75	35	199
No. of F_2 vials set up	601	600	351	1552
No. of F_2 vials scored	485	527	311	1323
No. of F_2 vials containing lethals	1	0	0	1
Frequency of F_2 lethals	0.20%	0	0	0.07%
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-Dimethylacetamide

Drosophila SLRL Procedure and Results

Compound: N,N-Dimethylacetamide Concentration: 200 ppm Stock: A
Length of Exposure: 95 min Test exposure given: 24.7.79

	Brood 1	Brood 2	Brood 3
F_1 set up	25.7.79	28.7.79	2.8.79
F_2 set up	7.8.79	9.8.79	16.8.79
F_2 scored	20.8.79	21.8.79	29.8.79
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	98	72	52	222
No. of sterile F_1 vials	33	12	13	58
No. of F_1 vials used in F_2	65	60	47	172
No. of F_2 vials set up	600	600	780	1980
No. of F_2 vials scored	560	575	705	1840
No. of F_2 vials containing lethals	1	0	1	2
Frequency of F_2 lethals	0.17%	0	0.14%	0.11%
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-Dimethylacetamide

Drosophila SLRL Procedure and Results

Compound: N,N-Dimethylacetamide Concentration: 200 ppm Stock: B
Length of Exposure: 95 min Test exposure given: 24.7.79

	Brood 1	Brood 2	Brood 3
F_1 set up	25.7.79	28.8.79	2.8.79
F_2 set up	7.8.79	8.8.79	17.8.79
F_2 scored	22.8.79	24.8.79	30.8.79
F_2 repeats scored	-	-	-
F_3 set up	22.8.79	24.8.79	30.8.79
F_3 scored	6.9.79	7.9.79	10.9.70
F_3 repeats scored	-	-	-

RESULTS

	Brood 1	Brood 2	Brood 3	All Broods
No. of F_1 vials	100	92	76	268
No. of sterile F_1 vials	20	18	15	53
No. of F_1 vials used in F_2	80	74	61	215
No. of F_2 vials set up	600	600	601	1801
No. of F_2 vials scored	563	520	553	1636
No. of F_2 vials containing lethals	0	*3	0	*3
Frequency of F_2 lethals	0	0.57%	0	0.18%
No. of F_3 vials set up	500	500	400	1400
No. of F_3 vials scored	484	484	393	1361
No. of F_3 vials containing lethals	0	0	0	0
Frequency of F_3 lethals	0	0	0	0

* All 3 lethals from one male

TABLE RL-2 (continued)

N,N-Dimethylacetamide

Drosophila SLRL Procedure and Results

Compound: EMS Concentration: 0.4% v/v Stock: A

Length of Exposure: 5 h Test exposure given: 23.4.79

	Brood 1	Brood 2	Brood 3
F_1 set up	24.7.79		
F_2 set up	6.8.79		
F_2 scored	21.8.79		
F_2 repeats scored			
F_3 set up			
F_3 scored			
F_3 repeats scored			

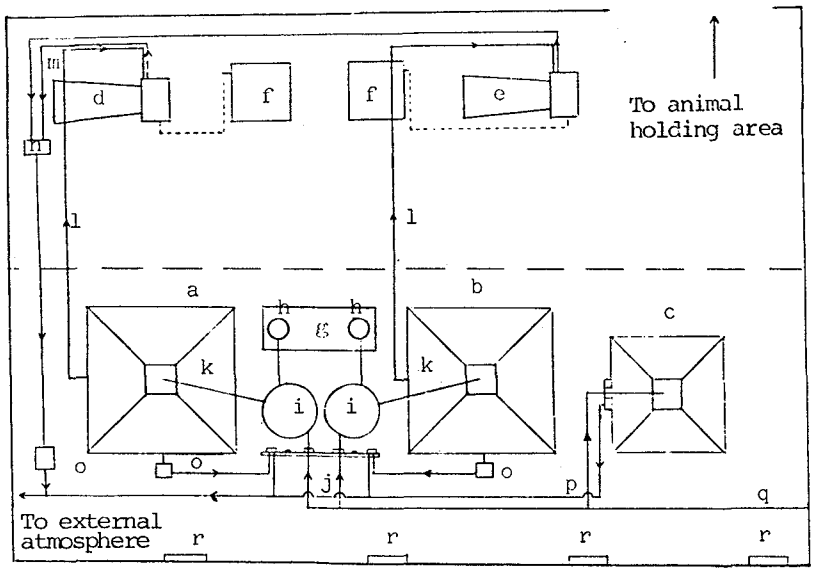
RESULTS

	Brood 1	Brood 2	Brood 3	All Broods
No. of F_1 vials	50			
No. of sterile F_1 vials	2			
No. of F_1 vials used in F_2	48			
No. of F_2 vials set up	144			
No. of F_2 vials scored	119			
No. of F_2 vials containing lethals	18			
Frequency of F_2 lethals	15.12%			
No. of F_3 vials set up				
No. of F_3 vials scored				
No. of F_3 vials containing lethals				
Frequency of F_3 lethals				

FIGURE 1a

N,N-Dimethylacetamide

Schematic Lay-out of Exposure Area



a High level exposure chamber
b Low level exposure chamber
c Air control exposure chamber
d Miran monitoring high level exposure chamber
e Miran monitoring low level exposure chamber
f Pen recorders
g Temperature controlled water baths
h Drechsel bottle
i Mixing vessel for dilution of test compound
j Flow meter control panel for atmosphere generation
k Vapour transfer line
l Sampling line
m Miran extract line
n Sampling flow rate control panel
o Scrubber
p Exposure chamber extract
q Compressed air line
r High efficiency extract

FIGURE 1b

N,N-Dimethylacetamide

Schematic Lay-out of Apparatus

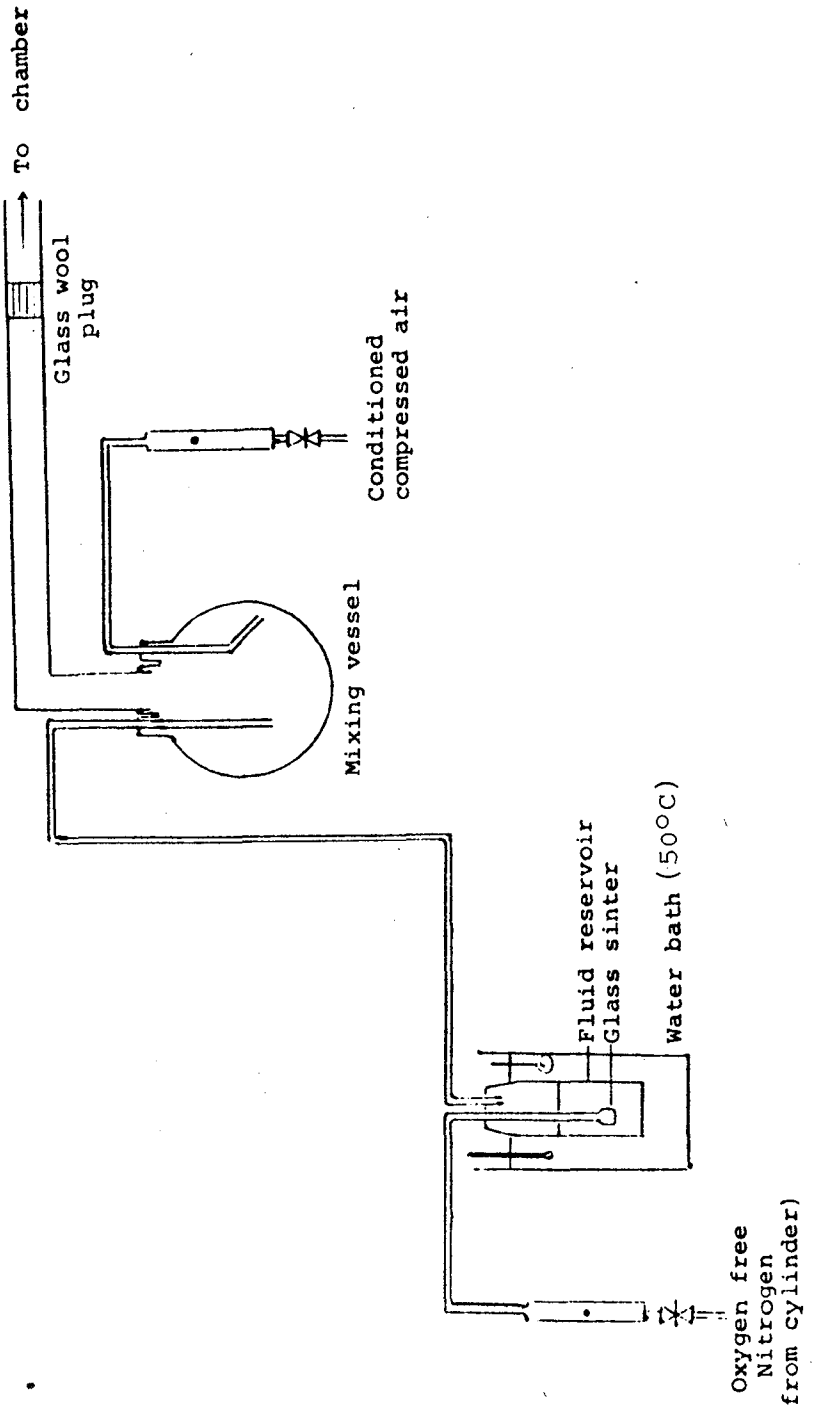


FIGURE 2

N,N-Dimethylacetamide
Typical Calibration Graph for High Level
18 July 1979

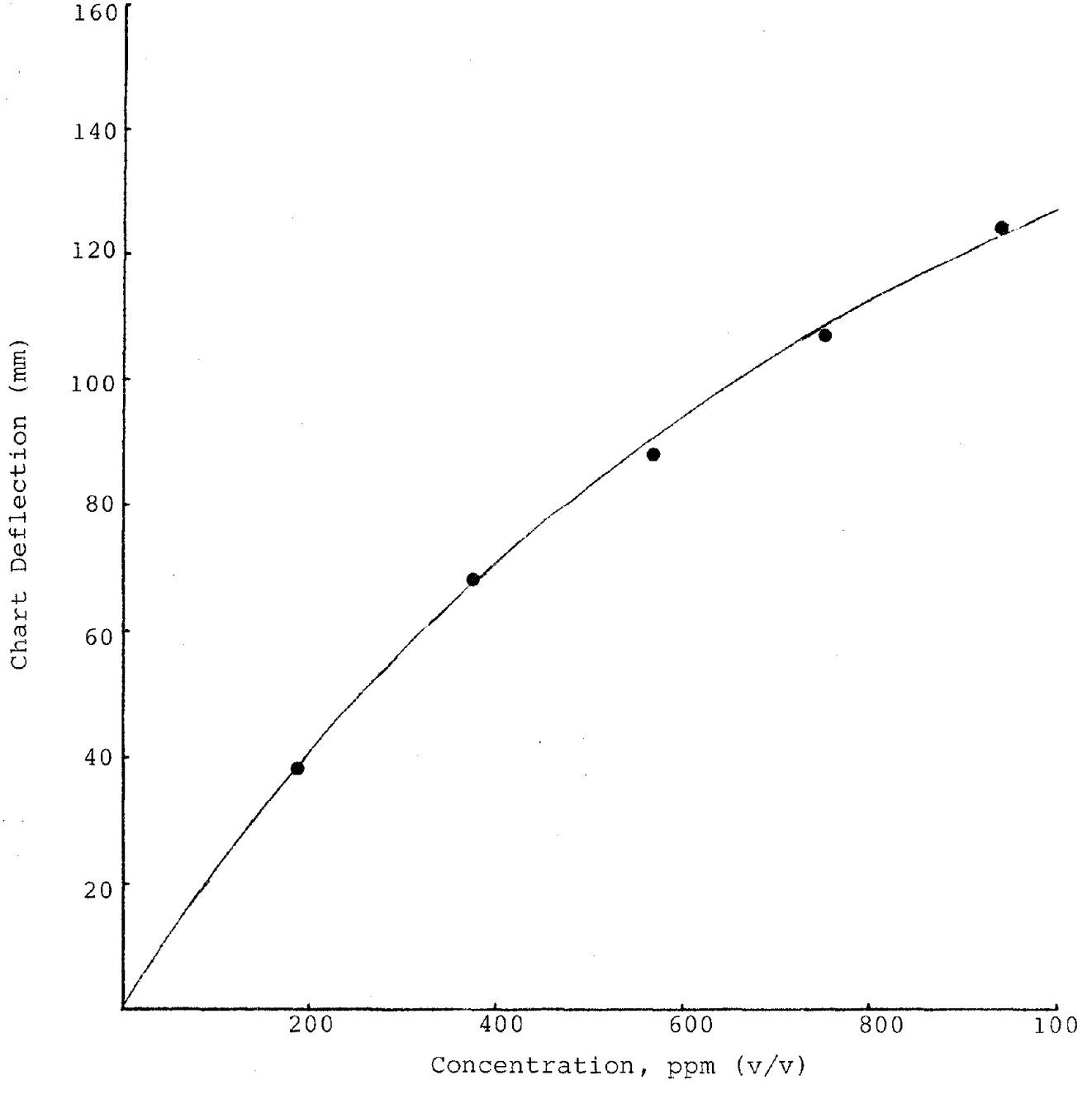


FIGURE 3

N,N-Dimethylacetamide

Sample Record Chart of IR Absorption at 9.9 μm

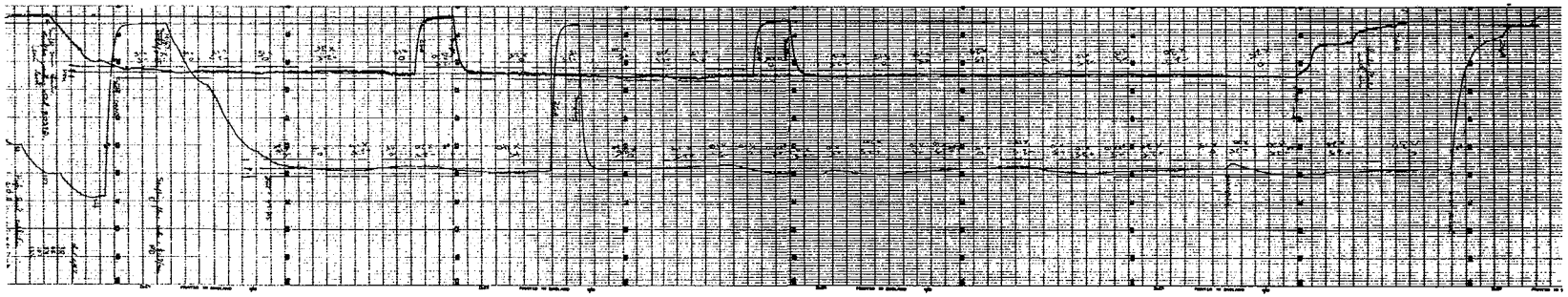


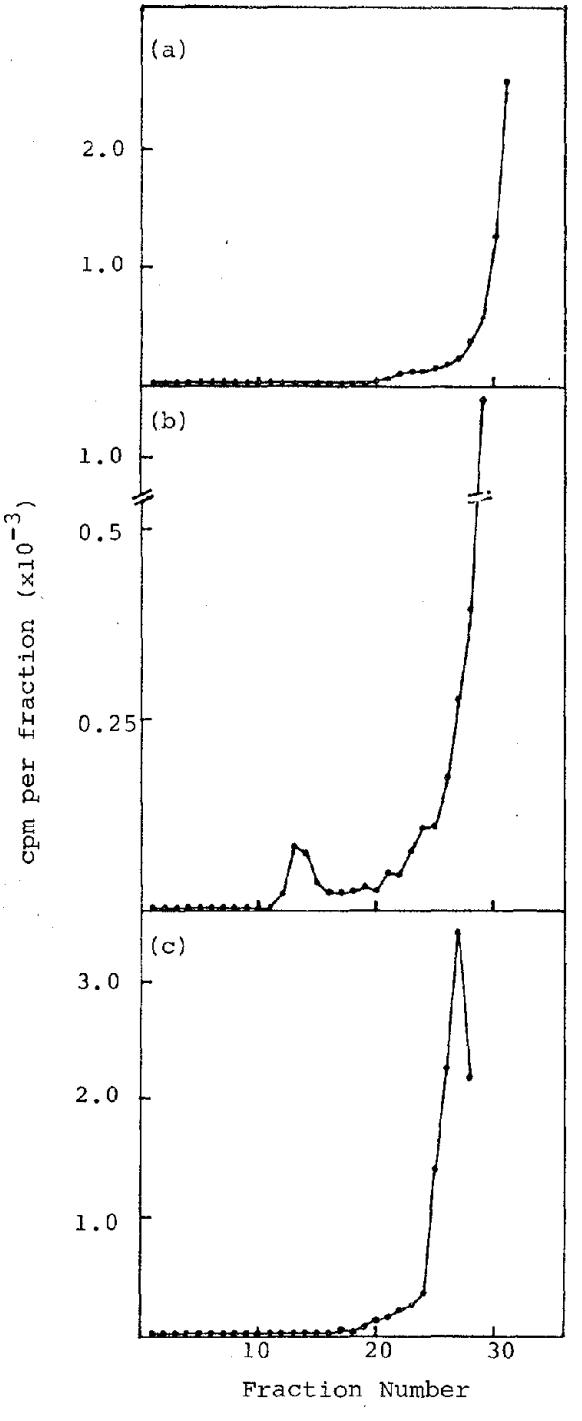
FIGURE 4

DNA Repair in the Absence of S-9
CsCl Density Gradient Centrifugation

Project No.:	409959
Contractor:	NIOSH
Substance:	N,N-Dimethylacetamide
Operator:	Carole Ross
Liver preparation:	None
Liver preparation date:	None
Cell culture batch:	Flow 2,002 P25
Date of test:	31 October 1980
Dates gradients run:	4-7 November 1980
Date of scintillation counting:	10-11 November 1980

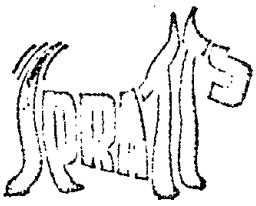
Flow 2,002 cells, arrested in arginine-deficient medium, were treated with (a) 9,4292 µg/ml of N,N-dimethylacetamide (b) 1 µg/ml of 4-NQO and (c) 1% DMSO in the presence of 10 µCi/ml of [3H]-deoxyguanosine for 4 h. Cell lysates were prepared and analysed by CsCl density gradient centrifugation at 100,000 g for 65 h at 20°C. Fractions were collected by upward displacement of the gradient and the total acid-insoluble radioactivity in each fraction analysed. DNA is detected in fractions 12-15 and RNA in fractions 21-30.

FIGURE 4


(a)
2.0
1.0
(b)
1.0
0.5
0.25
(c)
3.0
2.0
1.0
cpm per fraction (x10^{-3})
10
20
30
Fraction Number

APPENDIX DIET

N,N-Dimethylacetamide
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 MAY, 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	1.0×10^3 /grm
E. COLI TYPE 1	NONE DETECTED
SALMONELLA SPECIES	NONE DETECTED
MOULDS.	NONE DETECTED

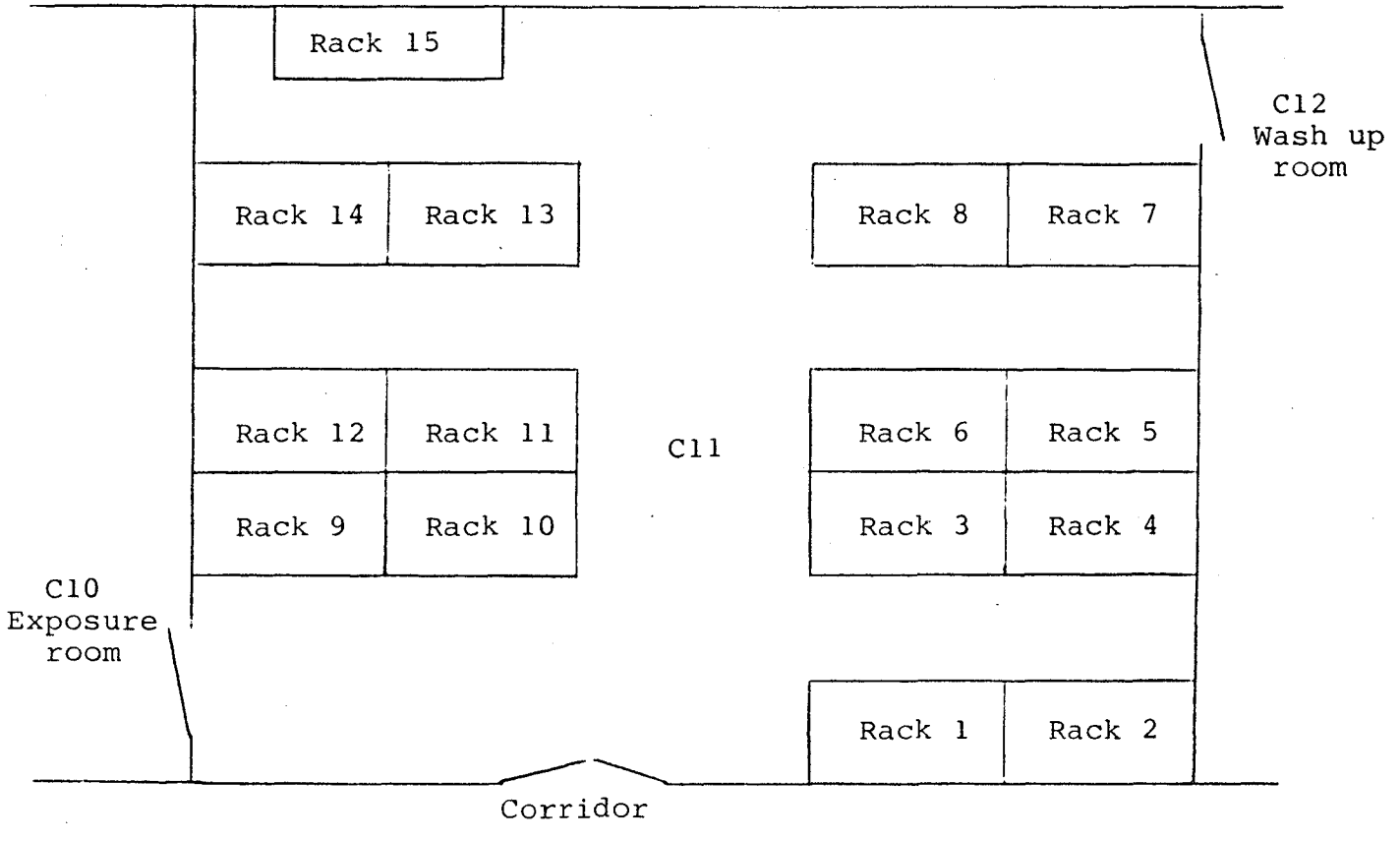
SIGNED .....

DATE 26.6.79

Registered in England Number 238748

APPENDIX Loc-1

N,N-Dimethylacetamide
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-Dimethylacetamide

Examples of Animal Location During Exposure

EXPOSURE LOCATION SHEET

Project No: 409959 Test Concentration: 0

Test Compound: Air Control Tier No: 1

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

Day of Study: 2

LEFT

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

FRONT REAR

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

RIGHT

SIGNED: _____ DATE: _____

APPENDIX Loc-2 (continued)

N,N-Dimethylacetamide
EXPOSURE LOCATION SHEET

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

Test Concentration: 0
Tier No: 2
Dominant Lethal ♂
Sperm Ab. mice

LEFT

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

FRONT REAR

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

RIGHT

SIGNED: _____ DATE: _____

APPENDIX Loc-2 (continued)

N,N-Dimethylacetamide
EXPOSURE LOCATION SHEET

Project No: 409959 Test Concentration: Low

Test Compound: N,N-Dimethylacetamide Tier No: 1

Exposure Chamber No: 2

Day of Study: 2

LEFT

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

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

FRONT REAR

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

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

RIGHT

Signed: _____ Date: _____

APPENDIX Loc-2 (continued)

N,N-Dimethylacetamide
EXPOSURE LOCATION SHEET

Project No: 409959 Test Concentration: High

Test Compound: N,N-Dimethylacetamide Tier No: 1

Exposure Chamber No: 3

Day of Study: 2

LEFT

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

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

FRONT REAR

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

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

RIGHT

Signed: _____ Date: _____

APPENDIX FORM-1

N,N-Dimethylacetamide

CYTOGENETIC SCORE SHEET NIOSH

Contract No. 210-78-0026 **Substance:**
Assessor: **Decoded group:**
Signature: **Slide No.:** **Decoded animal No.:**
Date(s) Single/Multi dose: **Time(h):**
Slide quality:
Excellent _____ **Good** _____ **Acceptable** _____ **Poor** _____ **Uncountable** _____

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

APPENDIX FORM-2

N,N-Dimethylacetamide

Assessors	Signature

Contract No. 210-78-0026

DOMINANT LETHAL ASSESSMENT

NIOSH

Dose Group:

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

APPENDIX FORM-3

N,N-Dimethylacetamide

Contract No. 210-78-0026 NIOSH

SPERM ABNORMALITIES ASSESSMENT

Assessor: De-coder

Date(s) assessed: Date de-coded:

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

APPENDIX TABLE BW-1

N,N-Dimethylacetamide
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	334	337	346	347	344
	122	337	340	346	350	353
	123	325	325	330	334	336
	124	300	300	300	308	300
	125	365	368	370	375	378
	126	360	365	371	380	384
	127	354	354	368	373	377
	128	348	351	352	357	366
	129	329	329	333	333	338
	130	338	336	340	343	351
	Mean ± S.D.	339 ± 19	341 ± 20	346 ± 22	350 ± 22	353 ± 25

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	281	210	212	210	212	218
	282	224	223	225	221	220
	283	209	210	212	214	212
	284	196	200	204	206	208
	285	224	224	225	226	226
	286	209	200	205	208	207
	287	204	204	207	211	213
	288	195	194	198	196	196
	289	195	200	200	202	200
	290	227	227	230	227	226
	Mean ± S.D.	209 ± 12	209 ± 12	212 ± 11	212 ± 10	213 ± 10

APPENDIX TABLE BW-1 (continued)

N,N-Dimethylacetamide

Multiple Dosing: 20 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	131	327	321	332	335	337
	132	336	337	344	350	356
	133	334	333	333	337	345
	134	335	336	340	345	348
	135	332	329	341	343	346
	136	371	375	388	387	391
	137	327	324	335	336	340
	138	291	293	301	298	300
	139	342	349	350	353	360
	140	303	307	320	319	327
	Mean	330	330	338	340	345
	± S.D.	± 22	± 22	± 22	± 23	± 23

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	291	216	208	212	211	213
	292	205	203	205	201	206
	293	232	233	233	232	235
	294	228	228	230	231	232
	295	230	229	234	236	240
	296	215	217	214	215	218
	297	230	227	233	236	234
	298	195	198	198	197	199
	299	219	218	221	223	221
	300	220	220	223	224	227
	Mean	219	218	220	221	223
	± S.D.	± 12	± 12	± 13	± 14	± 14

APPENDIX TABLE BW-1 (continued)

N,N-Dimethylacetamide

Multiple Dosing: 700 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	141	365	362	366	363	368
	142	344	338	339	332	341
	143	335	333	334	328	332
	144	345	346	352	355	354
	145	315	316	324	321	321
	146	308	314	316	316	314
	147	309	308	311	306	306
	148	339	338	339	337	334
	149	337	340	341	346	351
	150	352	355	360	363	363
	Mean ± S.D.	335 ± 19	335 ± 18	338 ± 18	337 ± 20	338 ± 21

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	301	233	228	234	230	232
	302	181	179	183	182	182
	303	207	214	212	212	213
	304	212	213	213	212	215
	305	233	231	231	230	233
	306	190	184	190	190	189
	307	210	204	210	207	211
	308	229	231	228	225	223
	309	210	206	209	206	206
	310	212	210	206	205	206
	Mean ± S.D.	212 ± 17	210 ± 18	212 ± 17	210 ± 16	211 ± 17

APPENDIX TABLE BW-1 (continued)

N,N-Dimethylacetamide

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	151	362	347	330	315	305
	152	312	305	295	285	274
	153	365	355	350	336	326
	154	364	361	355	337	322
	155	300	296	285	286	276
	156	330	326	316	308	297
	157	331	327	322	305	287
	158	327	320	302	291	277
	159	320	312	302	288	275
	160	332	330	318	307	287
	Mean	334	328	318	306	293
	± S.D.	± 23	± 21	± 23	± 19	± 19

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	311	203	193	187	187	186
	312	187	182	176	168	164
	313	220	218	210	202	200
	314	222	218	211	201	201
	315	218	213	201	194	192
	316	206	197	192	181	175
	317	241	240	235	232	228
	318	177	168	164	159	158
	319	219	211	200	193	190
	320	236	229	219	209	204
	Mean	213	207	200	193	190
	± S.D.	± 20	± 22	± 21	± 21	± 21

APPENDIX TABLE BW-2

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

Air Control (0 ppm)

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Male	1	345	11	326	21	342
	2	335	12	346	22	300
	3	352	13	370	23	370
	4	366	14	364	24	365
	5	345	15	333	25	397
	6	341	16	347	26	343
	7	367	17	318	27	337
	8	337	18	335	28	339
	9	324	19	356	29	349
	10	334	20	338	30	318
	Mean ± S.D.	345 ± 14		343 ± 17		346 ± 27

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Female	161	217	171	215	181	226
	162	260	172	215	182	222
	163	236	173	212	183	236
	164	231	174	216	184	230
	165	253	175	229	185	255
	166	248	176	211	186	231
	167	231	177	206	187	239
	168	197	178	222	188	220
	169	219	179	221	189	255
	170	224	180	273	190	220
	Mean ± S.D.	232 ± 19		222 ± 19		233 ± 13

APPENDIX TABLE BW-2 (continued)

N,N-Dimethylacetamide

Single Dosing: 20 ppm

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Male	31	344	41	319	51	400
	32	393	42	373	52	420
	33	300	43	297	53	355
	34	292	44	333	54	396
	35	366	45	362	55	350
	36	334	46	333	56	361
	37	352	47	345	57	365
	38	365	48	320	58	308
	39	356	49	334	59	389
	40	355	50	392	60	376
	Mean	346		341		372
	± S.D.	± 30		± 28		± 32

Sex	Animal Number	6 h Sample Weight	Animal Number	48 h Sample Weight	Animal Number	48 h Sample Weight
Female	191	217	201	219	211	228
	192	226	202	217	212	207
	193	228	203	231	213	216
	194	229	204	221	214	221
	195	227	205	258	215	253
	196	214	206	214	216	236
	197	223	207	231	217	235
	198	258	208	230	218	192
	199	210	209	230	219	229
	200	217	210	258	220	219
	Mean	225		234		224
	± S.D.	± 13		± 16		± 17

APPENDIX TABLE BW-2 (continued)

N,N-Dimethylacetamide

Single Dosing: 700 ppm

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Male	61	294	71	379	81	350
	62	353	72	330	82	348
	63	378	73	368	83	378
	64	387	74	366	84	364
	65	332	75	377	85	333
	66	363	76	362	86	355
	67	351	77	390	87	377
	68	367	78	359	88	365
	69	363	79	399	89	370
	70	328	80	360	90	376
	Mean	352		369		362
	± S.D.	± 27		± 19		± 15

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Female	251	271	231	228	241	228
	252	219	232	216	242	238
	253	215	233	225	243	217
	254	218	234	215	244	233
	255	208	235	253	245	237
	256	191	236	220	246	268
	257	231	237	231	247	216
	258	242	238	204	248	240
	259	215	239	235	249	204
	260	206	240	208	250	230
	Mean	222		224		231
	± S.D.	± 22		± 14		± 18

APPENDIX TABLE BW-2 (continued)

N,N-Dimethylacetamide

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Male	91	370	101	333	111	381
	92	335	102	340	112	310
	93	330	103	378	113	390
	94	335	104	331	114	346
	95	346	105	332	115	392
	96	361	106	382	116	345
	97	365	107	355	117	350
	98	337	108	364	118	339
	99	330	109	344	119	353
	100	377	110	333	120	325
	Mean	349		349		353
	± S.D.	± 18		± 19		± 27

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Female	221	227	261	236	271	243
	222	220	262	220	272	228
	223	215	263	195	273	209
	224	256	264	229	274	206
	225	233	265	213	275	213
	226	234	266	230	276	221
	227	268	267	226	277	236
	228	246	268	244	278	225
	229	242	269	227	279	208
	230	233	270	244	280	213
	Mean	237		226		220
	± S.D.	± 16		± 15		± 13

APPENDIX TABLE BW-3

N,N-Dimethylacetamide
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	352	356	358	368	359
	362	337	336	345	349	353
	363	301	299	305	312	317
	364	335	338	340	343	349
	365	340	346	343	341	341
	366	330	322	327	329	331
	367	329	336	340	340	347
	368	369	370	375	377	380
	369	384	392	393	397	397
	370	339	341	345	356	358
	Mean ± S.D.	342 ± 23	344 ± 25	347 ± 24	351 ± 24	353 ± 23

APPENDIX TABLE BW-3 (continued)

N,N-Dimethylacetamide

Multiple Dosing: 20 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	371	367	371	381	383	384
	372	345	348	350	353	339
	373	365	362	369	372	374
	374	363	358	366	377	357
	375	327	332	341	335	338
	376	307	313	315	315	319
	377	369	371	389	384	391
	378	363	363	371	379	391
	379	364	371	380	387	394
	380	305	310	314	311	318
	Mean	348	350	358	360	361
	± S.D.	± 25	± 24	± 27	± 29	± 30

APPENDIX TABLE BW-3 (continued)

N,N-Dimethylacetamide

Multiple Dosing: 700 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	381	300	300	302	301	299
	382	303	303	299	298	292
	383	335	336	338	333	333
	384	342	350	351	354	359
	385	360	361	354	352	342
	386	358	365	367	365	361
	387	329	334	335	332	330
	388	355	358	361	369	360
	389	316	316	315	313	313
	390	338	341	349	350	355
	Mean	334	336	337	337	334
	± S.D.	± 22	± 23	± 24	± 26	± 26

APPENDIX TABLE BW-3 (continued)

N,N-Dimethylacetamide

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	391	379	366	356	343	334
	392	335	332	326	317	312
	393	362	346	331	317	303
	394	369	362	346	337	322
	395	351	340	323	306	300
	396	376	350	331	315	308
	397	315	308	298	284	276
	398	335	327	319	305	295
	399	363	354	343	327	315
	400	336	327	322	313	302
	Mean	352	341	330	316	307
	± S.D.	± 21	± 18	± 16	± 17	± 16

APPENDIX TABLE BW-4

N,N-Dimethylacetamide
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	24	24	24	24
	322	24	25	24	25	25
	323	25	25	24	24	25
	324	22	22	23	23	24
	325	24	25	24	25	25
	326	23	23	23	24	24
	327	24	23	24	24	24
	328	24	25	25	25	26
	329	23	23	23	23	23
	330	23	23	22	23	23
	Mean	24	24	24	24	24
	± S.D.	± 1	± 1	± 1	± 1	± 1

APPENDIX TABLE BW-4 (continued)

N,N-Dimethylacetamide

Multiple Dosing: 20 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	331	23	23	22	22	23
	332	24	25	25	25	25
	333	23	24	25	25	25
	334	25	26	26	27	27
	335	22	23	24	24	24
	336	22	22	23	23	23
	337	23	24	25	24	25
	338	24	24	25	25	26
	339	23	24	25	24	24
	340	23	24	24	24	25
	Mean	23	24	24	24	25
	± S.D.	± 1	± 1	± 1	± 1	± 1

APPENDIX TABLE BW-4 (continued)

N,N-Dimethylacetamide

Multiple Dosing: 700 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	341	22	23	24	23	24
	342	23	24	24	24	24
	343	22	23	24	23	24
	344	22	22	23	23	24
	345	23	24	25	23	23
	346	23	23	24	24	24
	347	22	22	23	23	23
	348	25	25	25	24	25
	349	20	20	21	21	22
	350	23	23	23	22	21
	Mean	23	23	24	23	23
	± S.D.	± 1	± 1	± 1	± 1	± 1

APPENDIX TABLE BW-4 (continued)

N,N-Dimethylacetamide

Multiple Dosing: Ethyl methanesulphonate, 200 mg/kg/day

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	351	26	24	24	23	23
	352	25	25	24	24	24
	353	26	25	24	24	23
	354	24	24	24	23	23
	355	24	22	21	20	20
	356	24	24	23	22	23
	357	23	23	22	21	22
	358	24	24	23	21	22
	359	24	24	24	23	23
	360	23	24	23	20	20
	Mean	24	24	23	22	22
	± S.D.	± 1	± 1	± 1	± 2	± 1

APPENDIX TABLE CA-MD-M

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

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
130	140/2	50	25	25								
	140/3		25	25								
129	114/1	50	25	25								
	114/5		25	25								
128	67/3	50	25	25								
	67/5		25	25								
127	157/1	50	25	25								
	157/2		25	25								
122	10/1	50	25	25								
	10/4		25	24	1							57.0 x 107.8
121	69/2	50	25	25								
	69/5		25	25								
125	1/3	50	25	24	1							36.0 x 108.5
	1/4		25	25								
124	72/2	50	25	25								
	72/4		25	25								
126	36/1	50	25	25								
	36/3		25	25								
123	113/2	50	25	25								
	113/5		25	24	1							58.2 x 108.5

APPENDIX TABLE CA-MD-M (continued)

N,N-Dimethylacetamide

Males

Multiple Dosing: 20 ppm

Sampling Time : 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
140	5/1	50	22	20	1							56.1 x 107.2
					1							38.6 x 105.8
	5/4		3	3								
	5/2		25	24							1 Chromatid Fragment	66.3 x 99.0
133	55/2	50	25	25								
	55/1		25	25								
134	94/5	50	25	25								
	94/3		25	25								
132	148/1	50	25	25								
	148/5		25	25								
136	134/2	50	25	23	1							54.3 x 113.8
							1					55.2 x 104.9
	134/4		25	25								
131	71/3	50	25	25								
	71/2		25	25								
137	32/4	50	25	25								
	32/5		25	25								
139	59/3	50	25	25								
	59/1		25	24	1							24.0 x 110.2
135	30/1	50	25	25								
	30/3		25	25								
138	41/4	50	25	25								
	42/5		25	25								

APPENDIX TABLE CA-MD-M (continued)

N,N-Dimethylacetamide

Males

Multiple Dosing: 700 ppm

Sampling Time : 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
145	84/4	50	25	25								
	84/2		25	25								
147	76/3	50	25	25								
	76/2		25	25								
144	52/1	50	25	25								
	52/5		25	25								
150	75/5	50	25	25								
	75/1		25	25								
149	83/5	50	25	25								
	83/4		25	25								
141	136/1-5	0	0	0								
148	28/2	50	25	25								
	28/1		25	25								
146	56/3	50	25	25								
	56/1		25	25								
142	60/4	50	25	25								
	60/2		25	25								
143	19/5	50	25	25								
	19/3		25	25								

APPENDIX TABLE CA-MD-M (continued)

N,N-Dimethylacetamide

Males

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
160	70/4	50	25	23	1							53.9 x 108.5
						2						32.0 x 107.0
	70/5		25	24	1							62.1 x 111.2
155	96/3	50	25	22	1							57.0 x 109.7
						1						68.1 x 104.1
											1Chromatid Fragment	64.0 x 104.0
	96/4		25	24	1							52.3 x 107.0
153	93/1-5	0	0	0								
152	92/1	50	25	20							1Chromatid Fragment	64.1 x 111.9
						1						60.0 x 112.2
								1				55.0 x 112.1
						3						54.6 x 110.8
					1					1		67.0 x 107.9
	92/4		25	20	1						1Chromatid Fragment	23.9 x 108.9
					1	1						22.8 x 108.3
											1 Exchange	36.9 x 106.8
					1							50.0 x 107.0
					1	1					1 Exchange	50.0 x 107.2
151	54/4	50	20	17	1							61.2 x 106.9
						1						63.0 x 99.5
					1							37.1 x 98.2
	54/1		25	22	1	1						31.2 x 106.0
					1							64.0 x 106.0
					1							66.0 x 100.0
	54/5		5	5								

APPENDIX TABLE CA-MD-M (continued)

N,N-Dimethylacetamide

Males

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
159	102/5	50	25	23							2 Exchanges	65.0 x 107.2
						1						67.2 x 106.0
	102/4		25	23	2							34.1 x 110.8
											1Chromatid Fragment	53.1 x 105.5
156	78/2	50	25	25								
	78/1		25	23		1						66.7 x 107.5
						1						61.2 x 107.2
154	144/2	50	25	23	1							33.6 x 108.0
											1Chromatid Fragment	33.9 x 106.6
	144/3		25	25								
157	122/3	50	25	20		1						59.3 x 110.8
					1							40.0 x 110.1
					1							38.5 x 110.0
					1	1					1 Exchange	32.9 x 108.8
						1						37.5 x 108.8
	122/1		25	23		1						52.9 x 107.9
					1							59.3 x 107.7
158	151/2	50	25	24	1							57.9 x 106.2
	151/5		25	24	1							65.5 x 108.3

APPENDIX TABLE CA-MD-F

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

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
283	273/3	50	25	24	1							59.5 x 109.0
	273/4		25	24	1							37.0 x 107.7
290	300/3	50	25	25								
	300/2		25	25								
282	170/3	50	25	25								
	170/1		25	24	1							64.0 x 107.0
284	232/5	50	25	25								
	232/4		25	25								
287	317/4	50	25	25								
	317/3		25	25								
285	161/4	50	25	25								
	161/2		25	23	1							63.0 x 108.0
					1							62.2 x 105.8
286	196/1	50	25	25								
	196/3		25	25								
288	227/4	50	25	24	1							28.9 x 104.5
	227/2		25	24	1							57.2 x 108.3
289	274/3	50	25	23			1					62.2 x 109.3
											1Chromatid Fragment	50.1 x 109.8
	274/1		25	24	1							37.4 x 109.4
281	229/3	50	25	25								
	229/2		25	25								

APPENDIX TABLE CA-MD-F (continued)

N,N-Dimethylacetamide

Females

Multiple Dosing: 20 ppm

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
293	215/2	50	25	25								
	215/1		25	24	1							36.5 x 110.3
294	254/3	50	25	25								
	254/1		25	25								
292	308/1-5	0	0	0								
291	231/1-5	0	0	0								
295	190/5	50	25	25								
	190/4		25	24							1 Chromosomal Fragment	52.0 x 109.8
300	165/1	50	25	25								
	165/3		25	25								
296	294/4	50	25	25								
	294/3		25	25								
299	219/5	50	25	25								
	219/3		25	24			1					33.9 x 106.0
298	201/3	50	25	25								
	201/4		25	25								
297	192/1	50	25	24	1							64.0 x 107.0
	192/2		25	24	1							49.8 x 105.8

APPENDIX TABLE CA-MD-F (continued)

N,N-Dimethylacetamide
Females

Multiple Dosing: 700 ppm

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
302	220/1-5	0	0	0								
303	179/3	50	25	25								
	179/4		25	25								
304	212/5	50	25	24	1							57.5 x 112.8
	212/3		25	25								60.1 x 111.0
308	188/5	50	25	25								
	188/3		25	23	1							34.0 x 108.0
											1 Chromatid Fragment	**32.2 x 108.2**
307	236/2	50	25	25								
	236/5		25	24	1							30.0 x 106.0
305	244/1-5	0	0	0								
306	216/3	50	25	25								
	216/1		25	25								
301	296/4	50	25	25								
	296/5		25	25								
309	243/1	50	25	24	1							28.8 x 106.1
	243/5		25	25								
310	235/1	50	25	25								
	235/2		25	25								

APPENDIX TABLE CA-MD-F (continued)

N,N-Dimethylacetamide

Females

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Sampling Time : 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
312	252/1	50	25	24	1							32.9 x 108.4
	252/2		25	25								
318	311/2	50	25	23	1							31.0 x 106.8
					1							33.0 x 109.2
	311/1		25	25								
319	262/1	50	25	25								
	262/3		25	24	1							25.8 x 107.0
317	282/1	50	25	24		1						56.1 x 109.3
	282/2		25	24	1							38.0 x 106.7
315	256/2	50	25	25								
	256/3		25	22	1							30.3 x 107.1
											1 Chromatid Fragment	29.0 x 107.1
											1 Chromatid Fragment	27.0 x 106.8
320	230/2	50	25	23		1						33.8 x 111.4
					1							61.9 x 112.4
	230/4		25	23		1					3 Chromatid Fragments	62.6 x 110.9
						2					1 Chromatid Fragment	58.9 x 111.0

APPENDIX TABLE CA-MD-F (continued)

N,N-Dimethylacetamide

Females

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
316	238/1	50	25	25								
	238/2		14	11	1	1						60.4 x 106.9
						1						42.1 x 99.5
					1							62.6 x 94.0
	238/4		11	9	1							64.6 x 108.8
					1							69.0 x 105.8
313	253/5	50	25	22	1							28.0 x 106.7
					1							52.3 x 107.0
					2							28.0 x 106.5
	253/1		25	21							1 Exchange	53.6 x 110.3
											1 Chromatid Fragment	59.6 x 108.8
						1						36.9 x 107.5
					1							33.7 x 107.9
311	214/5	50	25	24			1					56.8 x 111.5
	214/4		25	24	1							55.7 x 108.8
314	304/4	50	25	22	1							59.1 x 106.9
					1							58.1 x 106.9
						1						58.1 x 104.0
	304/2		25	25								

APPENDIX TABLE CA-M6

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

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
10	133/2	50	25	25								
	133/5		25	25								
1	149/1	50	25	25								
	149/2		25	25								
4	121/1	50	25	25								
	121/5		25	25								
8	23/3	50	25	24							1 Chromosomal Fragment	38.3 x 109.3
	23/2		25	25								
6	49/1	50	25	25								
	49/2		25	25								
5	126/3	50	25	24							1 Chromatid Fragment	35.8 x 111.4
	126/4		25	25								
7	142/1	50	25	25								
	142/3		25	25								
3	6/1	50	25	25								
	6/3		25	25								
2	87/3	50	25	24							1 Chromatid Fragment	29.2 x 110.4
	87/4		25	25								
9	129/1	50	25	25								
	129/2		25	25								

APPENDIX TABLE CA-M6 (continued)

N,N-Dimethylacetamide

Males

Single Dosing: 20 ppm

Sampling Time : 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
34	53/1	50	25	25								
	53/3		25	25								
36	97/4	50	25	24	1							61.1 x 112.0
	97/2		25	23	1							35.3 x 112.0
											1 Chromatid Fragment	30.3 x 110.3
38	68/3	50	25	25								
	68/4		25	25								
40	160/1	50	25	25								
	160/2		25	25								
31	22/2	50	25	25								
	22/3		25	25								
32	99/1	50	25	25								
	99/3		25	25								
35	98/1	50	25	25								
	98/3		25	25								
33	37/1	50	25	25								
	37/4		25	25								
39	73/1	50	25	25								
	73/2		25	25								
37	124/2	50	25	25								
	124/3		25	24	1							32.3 x 110.4

APPENDIX TABLE CA-M6 (continued)

N,N-Dimethylacetamide

Males

Single Dosing: 700 ppm

Sampling Time : 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
65	89/3	50	25	25								
	89/4		25	25								
61	42/1	50	25	25								
	42/4		25	25								
66	61/1	50	25	25								
	61/2		25	25								
64	11/1	50	25	25								
	11/2		25	25								
63	51/1	50	25	25								
	51/4		25	25								
68	66/1	10	5	5								
	66/2		5	5								
	66/3		0	0								
	66/4		0	0								
	66/5		0	0								
67	109/1	50	25	25								
	109/2		25	25								
69	15/1	50	25	25								
	15/3		25	25								
70	8/1	50	25	25								
	8/2		25	25								
62	46/1	50	25	25								
	46/2		25	25								

APPENDIX TABLE CA-M6 (continued)

N,N-Dimethylacetamide

Males

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
91	106/3	50	25	24	1							64.4 x 110.6
	106/4		25	25								
92	130/1	50	25	25								
	130/4		25	25								
100	64/1	50	25	25								
	64/3		25	24	1							35.6 x 113.3
93	50/1	50	25	25								
	50/2		25	24	1	1						33.6 x 107.9
99	12/1	50	25	25								
	12/3		25	25								
95	159/1	50	25	24		1						62.4 x 110.8
	159/2		25	25								
96	63/1	0	0	0								
	63/2		0	0								
	63/3		0	0								
	63/4		0	0								
	63/5		0	0								
97	115/3	50	25	25								
	115/4		25	24							1 Chromatid Fragment	33.4 x 112.4
94	143/1	50	25	25								
	143/4		25	25								
98	156/2	50	25	23	1							37.1 x 111.7
						1						54.5 x 111.3
	156/4		25	23		1						26.2 x 112.3
						1						23.2 x 110.5

APPENDIX TABLE CA-M24

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

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
11	29/1	50	25	24	1							63.1 x 104.2
	29/2		25	25								
13	154/3	50	25	25								
	154/2		25	25								
14	139/3	5	2	2								
	139/2		1	1								
	139/4		1	1								
	139/1		0	0								
	139/5		1	1								
17	150(1-5)	0	0	0								
18	111/3	50	25	25								
	111/4		25	25								
12	9(1-5)	0	0	0								
19	86/4	50	25	25								
	86/1		25	25								
20	57(1-5)	0	0	0								
15	25/4	50	25	23		1						58.5 x 108.4
					1							66.4 x 108.4
	25/5		25	25								
16	125(1-5)	0	0	0								

APPENDIX TABLE CA-M24 (continued)

N,N-Dimethylacetamide
Males

Single Dosing: 20 ppm

Sampling Time : 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
43	74/3	50	25	25								
	74/5		25	25								
46	45/2	50	25	25								
	45/5		25	25								
42	58/4	50	25	25								
	58/1		25	25								
50	40/3	50	25	25								
	40/4		25	25								
45	24/2	50	25	25								
	24/5		25	25								
48	43/4	1	1	1								
	43/1		0	0								
	43/2		0	0								
	43/3		0	0								
	43/5		0	0								
49	39(1-5)	0	0	0								
44	17/5	50	25	25								
	17/1		25	25								
41	118(1-5)	0	0	0								
47	77/1	50	25	25								
	77/3		12	12								
	77/2		5	5								
	77/4		8	8								

APPENDIX TABLE CA-M24 (continued)

N,N-Dimethylacetamide

Males

Single Dosing: 700 ppm

Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
72	3/3	50	25	25								
	3/4		25	25								
77	31/3	50	25	25								
	31/2		25	25								
75	14/2	50	25	25								
	14/1		25	25								
79	90/5	50	25	25								
	90/4		25	25								
71	16/4	50	25	25								
	16/3		25	25								
80	135/3	50	25	25								
	135/1		25	25								
76	105/2	50	25	25								
	105/1		25	25								
73	117/4	10	3	3								
	117/3		5	5								
	117/2		2	2								
	117/1		0	0								
	117/5		0	0								
74	147(1-5)	0	0	0								
78	137/4	50	25	25								
	137/1		25	25								

APPENDIX TABLE CA-M24 (continued)

N,N-Dimethylacetamide
Males

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
103	13/2	50	25	25								
	13/4		25	25								
101	101/4	50	25	25								
	101/2		25	25								
110	34(1-5)	0	0	0								
105	116/5	50	25	24	1							62.9 x 102.9
	116/3		25	25								
102	35/1	50	25	25								
	35/4		17	15		1						58.5 x 106.4
											1 Chromatid Fragment	36.7 x 97.6
	35/2		8	8								
106	21(1-5)	0	0	0								
107	145/4	50	25	23	1						1 Chromatid Fragment	67.2 x 105.0
					1							34.7 x 102.8
	145/5		25	25								
109	20/1	7	4	4								
	20/2		3	3								
	20(3-5)		0	0								
104	110/2	50	25	23	1							58.4 x 108.2
											Multiple Aberration	33.4 x 108.4
	110/4		25	24	1						6 Chromatid Fragments	58.4 x 110.3
108	127/4	50	25	22		1						28.2 x 106.2
											Multiple Aberration	28.7 x 105.0
					1							64.5 x 105.0
	127/2		25	25								

APPENDIX TABLE CA-M48

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

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
30	44/1	50	18	18								
	44/2		10	10								
	44/5		9	9								
	44/3		10	10								
	44/4		3	3								
27	120/1	50	25	25								
	120/2		25	25								
24	26/5	50	20	19	1							31.5 x 112.7
	26/4		15	13		1						28.4 x 108.1
					1							32.3 x 106.0
	26/2		15	15								
25	80/2	50	25	25								
	80/3		25	25								
29	18/5	50	25	24		1						66.2 x 108.9
	18/4		25	25								
22	132/1	50	25	25								
	132/2		25	25								
28	4/2	50	25	25								
	4/3		25	25								
21	155/3	50	25	24	1							63.6 x 112.2
	155/4		25	25								

APPENDIX TABLE CA-M48 (continued)

N,N-Dimethylacetamide

Males

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
23	2/1	50	25	25								
	2/2		25	25								
26	131/5	50	25	25								
	131/4		25	25								

APPENDIX TABLE CA-M48 (continued)

N,N-Dimethylacetamide

Males

Single Dosing: 20 ppm

Sampling Time: 48 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
57	65/4	50	25	25								
	65/3		25	25								
55	38/1	50	25	25								
	38/5		25	25								
60	88/1	50	25	24	1							31.7 x 112.0
	88/2		25	25								
58	91/3	50	25	25								
	91/4		25	25								
51	82/1	50	25	25								
	82/4		25	25								
59	141/5	50	23	23								
	141/4		22	21	1							69.2 x 104.4
	141/3		5	5								
54	85/1	50	25	25								
	85/2		25	25								
52	107/1	50	25	24	1							33.4 x 108.5
	107/2		25	25								
56	47/5	50	25	25								
	47/4		25	25								
53	138/1	50	25	25								
	138/2		25	25								

APPENDIX TABLE CA-M48 (continued)

N,N-Dimethylacetamide

Males

Single Dosing: 700 ppm

Sampling Time: 48 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
88	103/1	50	22	22								
	103/3		25	25								
	103/4		3	3								
81	104/1	50	25	25								
	104/2		25	25								
87	119/1	50	25	25								
	119/2		25	25								
84	100/1	50	25	25								
	100/2		25	24	1							51.5 x 108.9
89	108/1	50	25	25								
	108/2		25	25								
83	158/1	50	25	25								
	158/4		25	25								
85	79/2	50	25	25								
	79/3		25	25								
90	62/2	50	25	25								
	62/3		25	25								
86	128/1	50	25	25								
	128/2		25	25								
82	48/5	50	25	25								
	48/4		25	25								

APPENDIX TABLE CA-M48 (continued)

N,N-Dimethylacetamide
Males

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
118	81/1	50	25	21							1 Multi Aberration	37.0 x 112.3
						1						28.3 x 110.4
					1							26.7 x 110.6
											1 Exchange	36.6 x 109.7
	81/2		25	20							1 Multi Aberration	61.1 x 113.1
					1							34.4 x 111.0
					1							63.4 x 111.1
						2	1					63.7 x 111.0
					2							66.3 x 111.0
111	27/5	50	25	25								
	27/4		25	25								
113	153/1	50	25	23							1 Chromatid Fragment	34.4 x 111.0
					1							27.0 x 110.9
	153/2		25	25								
119	152/1	50	25	25								
	152/2		25	24		1						35.4 x 109.5
116	112/1	50	25	25								
	112/4		25	25								
115	7/3	50	25	22							1 Exchange	26.8 x 110.9
					1							37.7 x 111.0
					1							34.6 x 109.7
	7/4		25	25								
112	146/2	50	25	25								
	146/3		25	25								

APPENDIX TABLE CA-M48 (continued)

N,N-Dimethylacetamide
Males

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sampling Time: 48 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
120	95/4	50	25	25								
	95/5		25	25								
114	33/2	50	25	25								
	33/3		25	24	1							60.1 x 112.5
117	123/1	50	25	24							1 Exchange	35.2 x 110.9
	123/4		25	19							1 Multi Aberration	63.1 x 111.8
											1 Exchange 1 Chromatid Fragment	61.6 x 111.9
											1 Chromatid Fragment	57.8 x 111.8
						1						37.3 x 111.9
											1 Exchange	32.2 x 111.9
						1					2 Exchanges	31.1 x 111.9

APPENDIX TABLE CA-F6

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

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
168	183/1	50	25	25								
	183/4		25	25								
165	286/1	50	25	25								
	286/2		25	25								
162	247/4	50	25	25								
	247/5		25	25								
167	302/2	50	25	24	1							32.2 x 110.2
	302/1		25	25								
170	293/3	50	25	25								
	293/4		25	25								
163	166/3	50	25	25								
	166/5		25	24	1							30.1 x 107.8
161	309/1	50	25	25								
	309/2		25	25								
169	289/1	50	25	25								
	289/2		25	25								
166	209/1	0	0	0								
	209/2		0	0								
	209/3		0	0								
	209/4		0	0								
	209/5		0	0								
164	281/1	50	25	25								
	281/4		25	24	1							63.4 x 107.3

APPENDIX TABLE CA-F6 (continued)

N,N-Dimethylacetamide
Females

Single Dosing: 20 ppm

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
196	257/1	50	25	25								
	257/2		25	25								
197	284/2	50	25	24				1				38.4 x 110.5
	284/1		25	25								
199	233/1	50	25	25								
	233/2		25	25								
192	259/2	50	25	25								
	259/4		25	25								
191	182/1	0	0	0								
	183/2		0	0								
	182/3		0	0								
	182/4		0	0								
	182/5		0	0								
194	213/1	50	25	25								
	213/4		25	24		1						59.3 x 106.5
198	228/1	50	19	19								
	228/2		9	9								
	228/3		12	12								
	228/4		10	10								
200	320/2	50	25	25								
	320/3		25	24	1							33.3 x 111.0
193	197/1	50	25	24	1							29.5 x 109.8
	197/3		25	25								
195	258/2	50	25	25								
	258/3		25	25								

APPENDIX TABLE CA-F6 (continued)

N,N-Dimethylacetamide
Females

Single Dosing: 700 ppm

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
257	269/2	50	25	25								
	269/5		25	25								
260	168/5	50	25	25								
	168/4		25	25								
254	171/1	50	25	24							1 Chromatid Fragment	43.6 x 107.1
	171/2		25	25								
255	249/1	50	25	25								
	249/2		25	25								
252	206/3	50	25	24	1							33.9 x 109.8
	206/4		25	25								
259	175/3	50	25	25								
	175/5		25	25								
253	211/3	50	25	25								
	211/5		25	25								
258	226/1	50	25	25								
	226/2		25	25								
251	202/3	50	25	24							1 Chromatid Fragment	37.6 x 111.7
	202/4		25	25								
256	221/3	50	25	24							1 Chromatid Fragment	41.8 x 111.2
	221/4		25	25								

APPENDIX TABLE CA-F6 (continued)

N,N-Dimethylacetamide
Females

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
226	223/1	50	25	22		1						62.2 x 110.0
					1							60.0 x 110.2
					2							60.7 x 110.0
	223/2		25	22	1	1						68.9 x 111.6
					1							61.1 x 111.6
					1							42.6 x 111.4
227	275/2	50	25	25								
	275/1		25	23	1							63.5 x 108.4
					1							34.5 x 108.5
228	316/1	50	25	22	1							58.1 x 109.0
					1							53.9 x 106.3
						1						30.7 x 105.6
	316/3		25	21	1							55.1 x 109.1
					1							33.3 x 108.5
								1	1			32.2 x 107.7
					2							42.9 x 104.6
229	172/1	5	4	4								
	172/2		1	1								
	172/3		0	0								
	172/4		0	0								
	712/5		0	0								

APPENDIX TABLE CA-F6 (continued)

N,N-Dimethylacetamide
Females

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
222	290/1	50	25	25								
	290/2		25	25								
224	303/1	50	25	23	1							58.9 x 111.2
					1							54.9 x 110.5
	303/2		20	20								
	303/3		5	4	1							60.7 x 107.6
221	366/1	50	25	25								
	266/2		25	24	1							41.6 x 109.5
230	224/1	0	0	0								
	224/2		0	0								
	224/3		0	0								
	224/4		0	0								
	224/5		0	0								
225	319/2	50	25	25								
	319/4		25	23	1							58.8 x 110.6
						1						63.9 x 109.4
223	210/1	50	25	25								
	210/3		25	24		1						31.8 x 108.3

APPENDIX TABLE CA-F24

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

Single Dosing: Air Control (0 ppm)

Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
179	246/2	50	25	25								
	246/3		25	25								
178	271/1	50	25	25								
	271/5		25	25								
175	185(1-5)	0	0	0								
173	314(1-5)	0	0	0								
180	217/4	5	5	5								
	217/1		0	0								
	217/2		0	0								
	217/3		0	0								
	217/5		0	0								
171	189/5	50	25	25								
	189/4		25	25								
177	310(1-5)	0	0	0								
172	169(1-5)	0	0	0								
176	285/1	50	25	23	1							59.5 x 104.3
							1					30.4 x 103.3
	285/3		25	25								
174	299/4	50	25	25								
	299/5		8	8								
	299/3		7	7								
	299/2		10	10								

APPENDIX TABLE CA-F24 (continued)

N,N-Dimethylacetamide
Females

Single Dosing: 20 ppm

Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
209	199(1-5)	0	0	0								
208	203(1-5)	0	0	0								
204	177(1-5)	0	0	0								
201	278(1-5)	0	0	0								
205	184(1-5)	0	0	0								
200	218(1-5)	0	0	0								
203	234/3	50	25	25								
	234/1		25	25								
206	205/4	50	25	25								
	205/3		25	25								
207	237/3	1	1	1								
	237/1		0	0								
	237/2		0	0								
	237/4		0	0								
	237/5		0	0								
210	200/2	50	25	25								
	200/5		25	25								

APPENDIX TABLE CA-F24 (continued)

N,N-Dimethylacetamide
Females

Single Dosing: 700 ppm

Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
234	307/2	50	25	25								
	307/5	50	25	25								
240	295/4	50	25	25								
	295/2		25	25								
239	191/5	50	25	24	1							61.7 x 108.2
	191/4		25	25								
231	176(1-5)	0	0	0								
239	250/5	50	25	25								
	250/4		25	24		1						33.3 x 109.0
238	297(1-5)	0	0	0								
232	163(1-5)	0	0	0								
236	265/2	50	25	25								
	265/1		25	25								
233	277(1-5)	0	0	0								
235	174/1	50	25	24	1							73.7 x 105.4
	174/5		25	24	2							65.3 x 110.5

APPENDIX TABLE CA-F24 (continued)

N,N-Dimethylacetamide
Females

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
264	270/2	50	25	25								
	270/5		25	25								
261	261/2	50	25	22	1		1					33.1 x 110.5
											1 Chromatid Fragment	61.4 x 102.5
					1	1						29.0 x 102.3
	261/5		25	23	1							62.4 x 105.6
					1							37.3 x 104.9
262	195/1	50	25	25								
	195/2		25	24	1		1				2 Chromatid Fragments	67.2 x 107.3
267	305/1	50	25	25								
	305/5		25	23	1							68.1 x 104.2
					1							46.1 x 101.8
263	173/5	10	4	3							Multiple Aberration	38.0 x 105.7
	173/4		1	0	1							59.6 x 95.5
	173/3		1	1								
	173/2		3	1	1	1					1 Chromatid Fragment	27.2 x 110.7
					1							55.0 x 97.0
	173/1		1	1								

APPENDIX TABLE CA-F24 (continued)

N,N-Dimethylacetamide
Females

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
269	180(1-5)	0	0	0								
268	287/1	1	1	1								
	287(2-5)		0	0								
270	194(2-5)	0	0	0								
265	276/2	26	9	9								
	276/4		8	5	1							39.0 x 114.4
					1							39.0 x 114.3
					1							70.4 x 112.0
	276/3		9	8		1						55.0 x 105.6
266	181/4	50	25	24	1							42.5 x 103.4
	181/3		25	24		1						63.0 x 110.9

APPENDIX TABLE CA-F48

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

Single Dosing: Air Control (0 ppm)

Sampling Time: 48 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
190	204/5	50	25	24		1						57.6 x 112.2
	204/4		25	25								
184	186/1	50	25	25								
	186/2		25	25								
186	291/1	50	25	25								
	291/2		25	25								
188	164/1	50	25	25								
	164/4		25	25								
187	280/2	50	25	23	1							26.8 x 109.8
					1							35.9 x 108.9
	280/3		25	23		1						63.4 x 111.5
						1						40.0 x 105.3
182	292/1	50	25	25								
	292/2		25	25								
183	162/4	50	25	24	1							23.0 x 107.1
	162/3		25	24							1 Chromatid Fragment	33.2 x 113.5
189	178/2	50	25	25								
	178/3		25	24							1 Exchange	67.2 x 112.9
185	240/1	50	25	25								
	240/2		25	24	1							31.6 x 113.0
181	315/1	50	25	23	1							56.0 x 111.7
					1							33.8 x 110.3
	315/3		25	22	1							61.5 x 111.8
						1						56.7 x 111.0
						1						36.4 x 111.5

APPENDIX TABLE CA-F48 (continued)

N,N-Dimethylacetamide
Females

Single Dosing: 20 ppm

Sampling Time: 48 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
217	225/4	50	25	24							1 Chromosomal Fragment	58.5 x 110.5
	225/5		25	25								
213	298/1	50	25	25								
	298/4		25	25								
212	267/1	50	25	25								
	267/2		25	24	1							52.9 x 103.1
219	301/5	50	25	25								
	301/3		25	25								
215	198/1	50	25	25								
	198/5		25	25								
211	242/5	50	25	25								
	242/4		25	25								
220	248/5	50	25	25								
	248/4		2	1	1							57.3 x 113.3
	248/1		23	21	1							36.1 x 108.5
					2							35.3 x 108.3
218	251/1	50	25	25								
	251/2		25	25								
216	207/1	50	25	25								
	207/2		25	25								
214	245/1	50	25	25								
	245/5		25	25								

APPENDIX TABLE CA-F48 (continued)

N,N-Dimethylacetamide

Females

Single Dosing: 700 ppm

Sampling Time: 48 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
246	288/1	50	25	25								
	288/2		25	25								
249	268/1	50	25	25								
	268/3		25	25								
241	264/1	50	25	24	1							58.8 x 108.2
	264/2		25	25								
248	263/1	50	25	25								
	263/2		25	24	1							57.9 x 105.6
245	239/4	50	25	25								
	239/5		25	25								
247	279/1	50	25	24	1							68.6 x 109.4
	279/5		7	6		1						60.0 x 109.2
	279/2		18	18								
250	222/1	50	25	25								
	222/3		25	25								
244	260/1	50	25	25								
	260/2		25	25								
243	318/2	50	25	25								
	318/3		25	25								
242	208/1	50	25	25								
	208/2		25	25								

APPENDIX TABLE CA-F48 (continued)

N,N-Dimethylacetamide
Females

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sampling Time: 48 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
276	272/5	50	25	25								
	272/4		25	23	1						1 Chromatid Fragment	67.3 x 107.7
						1					1 Chromatid Fragment	68.3 x 106.7
279	312/2	50	25	22	1						1 Exchange	51.4 x 110.1
						1						31.6 x 110.0
						1						49.0 x 109.3
	312/3		25	25								
280	255/5	50	25	22							1 Chromosomal Fragment	62.2 x 111.5
						1					1 Exchange	61.5 x 110.0
											1 Multi Aberration	63.9 x 110.1
	255/3		25	21							1 Multi Aberration	33.4 x 110.4
						3					1 Exchange	37.8 x 110.5
											1 Multi Aberration	37.1 x 109.7
						2			1		1 Exchange	30,4 x 109.0
278	241/3	50	25	18		2			1			40.2 x 110.9
											1 Multi Aberration	36.7 x 111.1
								1				31.6 x 107.1
											1 Multi Aberration	63.4 x 106.7
								1				42.6 x 104.5
								1			1 Chromatid Fragment	62.2 x 104.5
						1						71.8 x 107.1
	241/2		25	23	1							27.9 x 107.7
						1			1			42.1 x 104.7
277	283/5	50	25	25								
	283/4		25	24							1 Multi Aberration	29.6 x 110.1

APPENDIX TABLE CA-F48 (continued)

N,N-Dimethylacetamide
Females

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sampling Time: 48 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
274	193/2	50	25	21							1 Multi Aberration	32.9 x 109.6
						1					1 Exchange	32.0 x 109.4
											1 Multi Aberration	29.8 x 109.3
											1 Multi Aberration	54.6 x 108.5
	193/4		25	19							1 Multi Aberration	27.4 x 111.6
					2	1					1 Exchange	36.4 x 110.9
						1						57.2 x 110.2
											1 Multi Aberration	61.5 x 110.4
											1 Multi Aberration	63.6 x 109.7
					1							63.7 x 109.6
275	167/3	50	25	22							1 Multi Aberration	62.6 x 112.8
						1						64.2 x 112.9
											1 Multi Aberration	66.9 x 112.6
	167/2		25	17	1							63.1 x 112.7
						1						63.3 x 112.4
											1 Multi Aberration	65.1 x 112.8
						3					1 Exchange	66.1 x 112.0
						1						64.1 x 112.1
											1 Multi Aberration	63.0 x 112.0
											1 Chromatid Fragment	62.3 x 112.1
											1 Multi Aberration	61.6 x 112.0

APPENDIX TABLE CA-F48 (continued)

N,N-Dimethylacetamide
Females

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sampling Time: 48 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
272	306/2	50	25	22							1 Multi Aberration	59.4 x 111.5
											1 Multi Aberration	29.7 x 111.4
											1 Exchange	28.5 x 111.4
	306/4		25	22		2						53.7 x 111.5
											1 Multi Aberration	57.5 x 111.5
						4					1 Chromatid Fragment	59.5 x 110.9
273	313/5	50	25	25								
	313/4		25	25								
271	197/2	50	25	25								
	197/5		25	24		2	1					65.1 x 111.5

APPENDIX TABLE DL

N,N-Dimethylacetamide
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	13	14	11	11	16	12	13	18	12	12	0	15	10	13	0	16	3	15	7	14	225
	Total Implants	15	17	9	4	17	9	16	16	11	12	0	15	14	15	0	16	0	18	5	15	224
	Live Implants	15	17	8	4	16	8	15	16	11	12	0	15	14	12	0	16	0	18	5	14	216
	Early Deaths	0	0	1	0	1	1	0	0	0	0	0	0	0	3	0	0	0	0	0	0	6
	Late Deaths	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	2
2	Corpora lutea	15	14	12	0	11	15	14	14	13	9	15	11	9	11	15	11	15	15	15	0	234
	Total Implants	15	14	15	0	12	16	12	14	12	10	15	12	8	11	15	11	15	15	14	0	236
	Live Implants	14	13	12	0	10	15	12	14	12	10	15	12	7	11	14	10	15	15	14	0	225
	Early Deaths	1	1	3	0	2	1	0	0	0	0	0	0	1	0	1	1	0	0	0	0	11
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	Corpora lutea	10	14	13	12	13	15	15	14	0	10	12	0	4	14	11	11	15	13	*	14	210
	Total Implants	11	13	11	8	13	13	14	13	0	13	13	0	0	14	12	11	14	12		14	199
	Live Implants	11	13	10	8	10	13	14	13	0	12	12	0	0	14	12	11	13	10		11	187
	Early Deaths	0	0	1	0	3	0	0	0	0	1	1	0	0	0	0	0	1	2		3	12
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0
4	Corpora lutea	13	14	12	19	11	12	16	13	11	13	13	12	14	13	11	10	14	11	11	10	253
	Total Implants	13	15	12	11	11	13	14	13	13	14	13	13	13	13	12	12	14	13	12	11	255
	Live Implants	13	15	12	10	11	12	9	0	10	13	13	13	13	11	12	12	0	10	12	11	212
	Early Deaths	0	0	0	1	0	1	1	9	2	1	0	0	0	0	0	0	3	2	0	0	20
	Late Deaths	0	0	0	0	0	0	4	4	1	0	0	0	0	2	0	0	11	1	0	0	23
5	Corpora lutea	17	13	12	10	13	12	12	16	11	12	12	12	12	16	16	13	13	14	12	12	260
	Total Implants	0	16	10	6	11	4	12	14	11	12	10	11	11	14	14	13	13	15	14	13	224
	Live Implants	0	10	10	6	10	4	12	12	10	12	10	11	11	13	12	13	13	14	13	11	207
	Early Deaths	0	3	0	0	1	0	0	2	1	0	0	0	0	0	2	0	0	0	0	1	10
	Late Deaths	0	3	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1	7

* unassessable brown mass

APPENDIX TABLE DL (continued)

N,N-Dimethylacetamide

Multiple Dosing: Air Control (0 ppm)

Week No.	Male No. / Female	361		362		363		364		365		366		367		368		369		370		Total
		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
6	Corpora lutea	12	13	11	10	13	13	14	11	12	16	0	13	11	10	14	17	14	13	15	15	247
	Total Implants	12	13	11	10	12	12	14	11	14	16	0	14	11	11	6	10	14	13	13	14	231
	Live Implants	12	12	7	8	12	12	14	9	13	14	0	12	0	8	6	8	14	13	13	14	201
	Early Deaths	0	1	4	2	0	0	0	1	1	2	0	2	8	2	0	2	0	0	0	0	25
	Late Deaths	0	0	0	0	0	0	0	1	0	0	0	0	3	1	0	0	0	0	0	0	5
7	Corpora lutea	16	10	14	12	10	13	12	11	0	12	0	12	14	15	11	12	11	15	15	13	228
	Total Implants	16	12	16	11	10	12	12	11	0	13	0	13	13	8	11	13	11	13	15	11	221
	Live Implants	16	12	15	10	10	0	11	10	0	11	0	11	12	0	10	12	9	13	14	11	187
	Early Deaths	0	0	1	1	0	6	1	1	0	0	0	0	1	0	0	1	0	0	1	0	13
	Late Deaths	0	0	0	0	0	6	0	0	0	2	0	2	0	8	1	0	2	0	0	0	21
8	Corpora lutea	22	16	10	11	11	12	15	18	15	14	8	16	12	14	15	13	20	12	12	15	281
	Total Implants	14	17	7	10	11	12	14	15	13	13	5	14	12	14	15	13	16	12	12	14	253
	Live Implants	4	17	6	8	9	11	13	15	5	12	1	14	11	9	15	10	16	11	8	14	209
	Early Deaths	2	0	1	2	2	1	0	0	1	0	2	0	1	5	0	3	0	1	0	0	21
	Late Deaths	8	0	0	0	0	0	1	0	7	1	2	0	0	0	0	0	0	0	4	0	23
9	Corpora lutea	22	13	17	9	11	14	13	15	10	19	12	14	14	12	12	14	10	10	15	16	272
	Total Implants	13	14	13	9	11	14	13	16	11	17	10	15	13	11	11	14	11	11	12	16	255
	Live Implants	6	14	12	8	0	12	13	16	10	17	10	9	12	11	11	9	10	11	12	12	215
	Early Deaths	1	0	1	1	1	1	0	0	1	0	0	5	1	0	0	1	1	0	0	3	17
	Late Deaths	6	0	0	0	10	1	0	0	0	0	0	1	0	0	0	4	0	0	0	1	23
10	Corpora lutea	13	8	15	14	15	15	10	10	11	11	13	14	14	17	16	15	15	11	9	0	246
	Total Implants	14	4	15	13	16	12	12	16	11	12	15	15	14	13	15	11	15	11	11	0	245
	Live Implants	13	4	12	4	12	10	9	16	0	0	15	14	14	12	13	6	15	11	11	0	191
	Early Deaths	1	0	3	4	3	0	3	0	11	8	0	1	0	1	1	3	0	0	0	0	39
	Late Deaths	0	0	0	5	1	2	0	0	0	4	0	0	0	0	1	2	0	0	0	0	15

APPENDIX TABLE DL (continued)

N,N-Dimethylacetamide

Multiple Dosing: 20 ppm

Week No.	Male No. / Female	371		372		373		374		375		376		377		378		379		380		Total
		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
1	Corpora lutea	0	0	4	12	0	11	12	14	15	12	0	13	11	15	0	11	9	17	0	4	160
	Total Implants	0	0	4	12	0	11	14	15	15	12	0	11	8	14	0	14	12	16	0	0	158
	Live Implants	0	0	4	11	0	11	13	11	14	12	0	11	2	14	0	14	12	8	0	0	137
	Early Deaths	0	0	0	0	0	0	1	4	0	0	0	0	3	0	0	0	0	2	0	0	10
	Late Deaths	0	0	0	1	0	0	0	0	1	0	0	0	3	0	0	0	0	6	0	0	11
2	Corpora lutea	14	12	14	17	14	13	13	10	13	10	16	13	14	14	16	0	12	11	0	0	226
	Total Implants	13	14	15	14	13	14	13	6	14	14	16	13	15	15	15	0	13	0	0	0	217
	Live Implants	12	14	14	14	11	13	13	6	14	11	13	13	15	14	15	0	13	0	0	0	205
	Early Deaths	1	0	1	0	2	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	7
	Late Deaths	0	0	0	0	0	1	0	0	0	0	3	0	0	1	0	0	0	0	0	0	5
3	Corpora lutea	17	13	13	10	13	11	15	11	12	10	12	*-	15	15	16	13	13	13	14	13	249
	Total Implants	12	13	13	12	14	14	15	0	13	10	12	*-	16	14	16	13	7	14	0	0	208
	Live Implants	12	13	12	6	13	12	13	0	12	10	12	*-	15	13	15	13	7	13	0	0	191
	Early Deaths	0	0	1	6	1	2	1	0	1	0	0	*-	0	1	0	0	0	1	0	0	14
	Late Deaths	0	0	0	0	0	0	1	0	0	0	0	*-	1	0	1	0	0	0	0	0	3
4	Corpora lutea	13	11	13	12	13	13	0	13	12	16	16	10	13	11	14	11	12	14	0	1	218
	Total Implants	16	11	13	12	13	9	0	13	13	16	14	7	14	12	12	10	12	14	0	0	211
	Live Implants	12	10	12	11	11	9	0	13	13	16	14	7	8	1	12	9	11	14	0	0	183
	Early Deaths	3	1	1	1	0	0	0	0	0	0	0	0	4	9	0	1	0	0	0	0	20
	Late Deaths	1	0	0	0	2	0	0	0	0	0	0	0	2	2	0	0	1	0	0	0	8
5	**Corpora lutea**	12	12	15	18	18	15	13	7	13	12	14	18	13	15	15	15	11	12	0	0	248
	Total Implants	13	3	15	12	14	14	12	7	13	14	7	16	13	13	15	13	11	12	0	0	217
	Live Implants	13	3	14	3	13	14	9	6	12	8	7	13	13	12	14	6	11	0	0	0	171
	Early Deaths	0	0	1	1	1	0	2	1	1	6	0	0	0	1	1	0	0	1	0	0	16
	Late Deaths	0	0	0	8	0	0	1	0	0	0	0	3	0	0	0	7	0	11	0	0	30

* missing value : ambiguous record of result

APPENDIX TABLE DL (continued)

N,N-Dimethylacetamide

Multiple Dosing: 20 ppm

Week No.	Male No. / Female	371		372		373		374		375		376		377		378		379		380		Total
		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
6	Corpora lutea	12	0	10	10	12	14	12	0	11	14	11	12	14	11	14	14	14	14	0	0	199
	Total Implants	14	0	8	9	12	15	11	0	13	9	12	12	15	11	12	14	13	0	0	0	180
	Live Implants	14	0	7	9	11	13	9	0	11	8	12	12	15	11	5	0	4	0	0	0	141
	Early Deaths	0	0	1	0	1	1	0	0	1	0	0	0	0	0	0	8	3	0	0	0	15
	Late Deaths	0	0	0	0	0	1	2	0	1	1	0	0	0	0	7	6	6	0	0	0	24
7	Corpora lutea	15	16	10	16	16	10	0	13	11	14	12	11	12	13	12	15	12	11	0	0	219
	Total Implants	11	12	12	16	16	15	0	13	12	14	14	10	12	12	13	13	12	12	0	0	219
	Live Implants	11	9	10	16	0	12	0	12	12	9	14	10	12	11	13	13	11	11	0	0	186
	Early Deaths	0	2	0	0	0	1	0	0	0	3	0	0	0	1	0	0	1	1	0	0	9
	Late Deaths	0	1	2	0	16	2	0	1	0	2	0	0	0	0	0	0	0	0	0	0	24
8	Corpora lutea	15	14	12	11	14	15	5	12	14	14	14	13	10	14	17	15	12	0	0	0	221
	Total Implants	15	14	12	11	14	15	2	12	15	14	15	14	10	14	15	16	12	0	0	0	220
	Live Implants	14	11	11	10	12	13	2	11	15	14	15	13	10	13	9	8	12	0	0	0	193
	Early Deaths	1	1	1	1	1	1	0	1	0	0	0	1	0	1	4	6	0	0	0	0	19
	Late Deaths	0	2	0	0	1	1	0	0	0	0	0	0	0	0	2	2	0	0	0	0	8
9	Corpora lutea	10	12	13	13	12	13	13	14	12	10	12	11	14	10	14	13	14	13	6	2	231
	Total Implants	10	12	15	15	13	12	10	13	12	10	13	11	14	9	14	15	12	7	0	0	217
	Live Implants	10	12	9	15	12	11	8	13	12	4	12	11	9	8	14	10	12	7	0	0	189
	Early Deaths	0	0	6	0	1	1	0	0	0	0	1	0	4	1	0	5	0	0	0	0	19
	Late Deaths	0	0	0	0	0	0	2	0	0	6	0	0	1	0	0	0	0	0	0	0	9
10	Corpora lutea	14	0	0	16	13	14	11	12	18	13	13	14	9	13	9	14	14	16	3	0	216
	Total Implants	14	0	0	16	15	13	14	10	16	12	12	13	10	12	13	14	13	14	0	0	211
	Live Implants	10	0	0	14	10	13	14	9	13	0	12	10	9	9	10	14	11	14	0	0	172
	Early Deaths	4	0	0	2	1	0	0	0	3	10	0	1	1	2	1	0	2	0	0	0	27
	Late Deaths	0	0	0	0	4	0	0	1	0	2	0	2	0	1	2	0	0	0	0	0	12

APPENDIX TABLE DL (continued)

N,N-Dimethylacetamide

Multiple Dosing: 700 ppm

Week No.	Male No. / Female	381		382		383		384		385		386		387		388		389		390		Total
		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
1	Corpora lutea	17	0	16	13	10	13	12	7	15	10	0	14	14	0	0	14	7	16	5	11	194
	Total Implants	18	0	16	15	10	13	14	2	15	12	0	14	14	0	0	14	7	16	5	6	191
	Live Implants	18	0	15	15	10	13	13	1	15	11	0	13	14	0	0	14	7	16	5	6	186
	Early Deaths	0	0	0	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	3
	Late Deaths	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2
2	Corpora lutea	16	16	14	14	16	0	15	15	15	14	13	10	0	12	11	14	17	15	11	14	252
	Total Implants	9	14	15	14	18	0	15	13	14	14	13	13	0	14	11	10	17	16	11	14	245
	Live Implants	8	13	14	13	17	0	15	11	14	14	11	13	0	13	11	8	15	16	11	12	229
	Early Deaths	0	1	1	1	1	0	0	2	0	0	2	0	0	1	0	2	2	0	0	2	15
	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	13	13	12	12	12	12	8	12	11	17	10	15	9	14	10	11	14	12	18	11	246
	Total Implants	12	15	14	15	9	13	8	15	11	15	15	15	9	6	10	13	14	13	17	11	250
	Live Implants	12	15	13	15	9	12	7	14	11	14	15	12	9	6	9	12	13	13	17	11	239
	Early Deaths	0	0	0	0	0	1	0	1	0	1	0	3	0	0	1	1	1	0	0	0	9
	Late Deaths	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2
4	Corpora lutea	12	14	15	11	14	11	14	13	13	14	18	10	16	10	16	15	14	10	15	13	268
	Total Implants	13	14	11	14	13	11	16	11	12	14	15	11	15	9	14	13	14	12	15	12	259
	Live Implants	13	14	11	14	13	10	14	10	11	14	14	9	13	9	14	12	12	0	14	10	231
	Early Deaths	0	0	0	0	0	0	1	1	1	0	1	2	2	0	0	1	1	6	1	2	19
	Late Deaths	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	6	0	0	9
5	Corpora lutea	14	15	15	12	14	3	15	*	13	13	0	11	*	15	15	13	13	7	14	13	215
	Total Implants	14	16	15	4	14	0	15		12	11	0	10		15	16	14	13	8	15	13	205
	Live Implants	11	16	15	2	11	0	3		12	7	0	8		13	13	11	13	8	13	8	164
	Early Deaths	1	0	0	0	3	0	11		0	2	0	0		2	2	0	0	0	1	5	27
	Late Deaths	2	0	0	2	0	0	1		0	2	0	2		0	1	3	0	0	1	0	14

* unassessable brown mass

APPENDIX TABLE DL (continued)

N,N-Dimethylacetamide

Multiple Dosing: 700 ppm

Week No.	Male No.	381		382		383		384		385		386		387		388		389		390		Total
	Female	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
6	Corpora lutea	12	10	15	12	14	13	13	11	15	9	17	11	13	16	15	19	13	12	13	14	267
	Total Implants	12	3	14	12	10	11	13	11	14	9	15	8	13	16	17	17	12	12	12	15	246
	Live Implants	9	3	12	9	9	8	11	7	14	9	10	0	12	15	16	16	12	11	0	14	197
	Early Deaths	3	0	1	3	1	0	1	1	0	0	5	0	1	1	1	1	0	1	4	1	25
	Late Deaths	0	0	1	0	0	3	1	3	0	0	0	8	0	0	0	0	0	0	8	0	24
7	Corpora lutea	12	0	13	0	14	13	15	13	12	0	12	12	12	15	17	15	11	14	8	9	217
	Total Implants	12	0	14	0	15	11	13	12	11	0	12	13	12	17	15	14	12	13	10	9	215
	Live Implants	11	0	13	0	15	10	13	12	11	0	12	13	12	16	15	13	9	4	3	8	190
	Early Deaths	1	0	1	0	0	1	0	0	0	0	0	0	0	1	0	1	2	0	4	1	12
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	9	3	0	13
8	Corpora lutea	14	10	16	11	2	14	16	14	14	13	13	14	15	11	9	14	11	15	14	17	257
	Total Implants	14	12	16	13	0	15	17	14	13	13	14	14	15	11	9	14	11	13	14	15	257
	Live Implants	14	11	15	11	0	15	16	14	12	3	14	14	9	10	8	12	10	10	12	13	223
	Early Deaths	0	1	1	2	0	0	1	0	1	0	0	0	3	1	1	0	1	3	1	1	17
	Late Deaths	0	0	0	0	0	0	0	0	0	10	0	0	3	0	0	2	0	0	1	1	17
9	Corpora lutea	16	16	15	15	13	15	13	13	13	15	10	17	0	13	11	16	12	16	13	14	266
	Total Implants	16	17	15	15	15	13	13	15	13	13	12	14	0	13	12	11	11	14	14	15	261
	Live Implants	16	12	3	15	15	12	13	0	13	13	10	12	0	13	11	11	11	11	14	15	220
	Early Deaths	0	4	0	0	0	1	0	4	0	0	2	2	0	0	1	0	0	1	0	0	15
	Late Deaths	0	1	12	0	0	0	0	11	0	0	0	0	0	0	0	0	0	2	0	0	26
10	Corpora lutea	15	13	18	0	11	12	13	0	13	12	15	8	16	14	14	11	13	17	12	14	241
	Total Implants	12	14	15	0	10	12	13	0	13	13	14	12	14	12	14	11	13	15	12	14	233
	Live Implants	8	14	15	0	10	10	0	0	13	12	7	12	13	0	13	0	11	15	10	12	175
	Early Deaths	3	0	0	0	0	2	11	0	0	0	0	0	1	1	1	11	2	0	1	0	33
	Late Deaths	1	0	0	0	0	0	2	0	0	1	7	0	0	11	0	0	0	0	1	2	25

APPENDIX TABLE DL (continued)

N,N-Dimethylacetamide

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Week No.	Male No.	391		392		393		394		395		396		397		398		399		400		Total
	Female	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
1	Corpora lutea	8	12	10	0	0	0	12	6	0	0	9	8	10	6	0	7	0	9	6	12	115
	Total Implants	7	9	6	0	0	0	12	6	0	0	5	3	5	4	0	7	0	0	7	13	84
	Live Implants	7	2	6	0	0	0	11	6	0	0	5	3	5	4	0	0	0	0	7	2	58
	Early Deaths	0	7	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	8	16
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	3	10
2	Corpora lutea	12	9	5	1	12	4	13	8	11	11	8	10	0	0	12	0	15	12	6	8	157
	Total Implants	1	1	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	8
	Live Implants	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	3
	Early Deaths	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	5
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	Corpora lutea	0	0	9	0	6	0	9	0	0	3	0	**	0	0	0	0	0	0	*-	0	27
	Total Implants	0	2	0	0	1	0	0	0	0	0	0		0	0	0	0	0	0	*-	0	3
	Live Implants	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	*-	0	0
	Early Deaths	0	2	0	0	1	0	0	0	0	0	0		0	0	0	0	0	0	*-	0	3
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	*-	0	0
4	Corpora lutea	14	13	0	15	15	15	15	10	14	15	11	11	4	13	12	13	10	2	14	12	228
	Total Implants	9	7	0	7	13	7	15	12	14	14	8	10	0	8	13	9	6	0	10	4	166
	Live Implants	3	3	0	3	11	7	13	0	12	12	8	9	0	8	9	4	0	0	3	2	107
	Early Deaths	5	4	0	4	2	0	2	12	2	2	0	1	0	0	4	2	6	0	7	2	55
	Late Deaths	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	4
5	Corpora lutea	14	13	12	10	13	15	10	12	15	12	12	9	0	14	18	14	10	11	11	12	237
	Total Implants	12	14	16	10	13	11	6	12	15	12	11	8	0	8	11	14	10	8	13	7	211
	Live Implants	10	13	8	9	11	10	4	11	13	12	11	8	0	6	10	14	6	1	12	0	169
	Early Deaths	2	1	3	1	2	1	1	1	2	0	0	0	0	2	1	0	2	5	1	4	29
	Late Deaths	0	0	5	0	0	0	1	0	0	0	0	0	0	0	0	0	2	2	0	3	13

** unassessable brown mass

* missing value : ambiguous record of result

APPENDIX TABLE DL (continued)

N,N-Dimethylacetamide

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Week No.	Male No.	391		392		393		394		395		396		397		398		399		400		Total
	Female	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
6	Corpora lutea	13	11	15	12	12	14	14	12	10	13	11	10	0	18	18	16	11	14	15	12	251
	Total Implants	14	10	15	10	11	12	13	12	11	12	10	12	0	12	13	13	8	13	11	11	223
	Live Implants	12	10	15	8	11	9	12	12	10	12	7	0	0	11	13	10	8	11	8	11	190
	Early Deaths	1	0	0	0	0	1	1	0	1	0	3	6	0	1	0	3	0	2	1	0	20
	Late Deaths	1	0	0	2	0	2	0	0	0	0	0	6	0	0	0	0	0	0	2	0	13
7	Corpora lutea	13	16	12	10	0	14	14	18	16	12	11	12	0	16	14	12	17	9	12	15	243
	Total Implants	11	16	12	8	0	13	13	15	12	12	11	13	0	11	13	10	7	9	13	14	213
	Live Implants	9	16	11	3	0	11	13	14	12	12	9	12	0	6	11	10	6	8	13	14	190
	Early Deaths	1	0	1	1	0	1	0	1	0	0	0	0	0	5	2	0	1	1	0	0	14
	Late Deaths	1	0	0	4	0	1	0	0	0	0	2	1	0	0	0	0	0	0	0	0	9
8	Corpora lutea	13	13	11	12	13	17	13	12	13	13	14	9	13	16	0	15	14	12	15	15	253
	Total Implants	13	14	11	13	13	14	12	9	13	11	14	6	11	11	0	15	9	14	15	15	233
	Live Implants	10	7	11	12	13	14	8	8	13	5	13	0	8	4	0	13	8	14	14	12	187
	Early Deaths	3	5	0	0	0	0	2	1	0	0	1	6	3	0	0	1	1	0	1	0	24
	Late Deaths	0	2	0	1	0	0	2	0	0	6	0	0	0	7	0	1	0	0	0	3	22
9	Corpora lutea	14	11	0	12	13	11	13	13	12	10	14	14	3	15	17	14	12	14	14	9	235
	Total Implants	10	13	0	11	13	9	13	13	12	11	13	13	0	12	14	14	8	14	13	8	214
	Live Implants	8	11	0	10	12	9	13	13	12	10	13	11	0	12	14	12	8	14	9	7	198
	Early Deaths	0	1	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	5
	Late Deaths	2	1	0	0	0	0	0	0	0	1	0	1	0	0	0	2	0	0	3	1	11
10	Corpora lutea	11	12	0	10	13	15	11	12	14	14	13	12	16	*-	11	14	16	16	10	16	221
	Total Implants	10	12	0	10	15	15	12	12	13	14	13	10	15	*-	14	14	16	15	14	16	230
	Live Implants	10	12	0	9	5	14	11	12	9	9	13	10	12	*-	14	14	16	15	14	13	208
	Early Deaths	0	0	0	1	8	1	1	0	2	5	0	0	1	*-	0	0	0	0	0	1	16
	Late Deaths	0	0	0	0	2	0	0	0	2	0	0	0	2	*-	0	0	0	0	0	2	6

* Missing value : ambiguous record of result

APPENDIX TABLE SA

N,N-Dimethylacetamide

Sperm Abnormality Assessment

Multiple Dosing: Air Control (0 ppm)
Low, 20 ppm
High, 700 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
343	960	1	2	26	4	7	40	1000	326	AIR
337	971	0	1	13	4	11	29	1000	328	AIR
358	970	1	2	19	1	7	30	1000	330	AIR
326	963	1	1	14	7	14	37	1000	329	AIR
345	964	2	1	13	2	18	36	1000	327	AIR
354	973	0	0	15	4	8	27	1000	325	AIR
352	962	4	3	19	3	9	38	1000	323	AIR
329	961	0	1	24	4	10	39	1000	321	AIR
335	969	4	1	15	2	9	31	1000	324	AIR
323	942	3	2	23	10	20	58	1000	322	AIR
341	975	2	0	13	4	6	25	1000	337	LOW
331	963	1	1	26	3	6	37	1000	340	LOW
356	970	2	2	13	2	11	30	1000	335	LOW
346	975	2	3	11	3	6	25	1000	339	LOW
330	974	0	0	13	3	10	26	1000	334	LOW
340	967	0	0	13	5	15	33	1000	332	LOW

APPENDIX TABLE SA (continued)

N,N-Dimethylacetamide

Multiple Dosing: Air Control (0 ppm)
Low, 20 ppm
High, 700 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
348	962	1	1	23	3	10	38	1000	338	LOW
344	959	0	2	20	10	9	41	1000	336	LOW
351	975	0	0	13	4	8	25	1000	333	LOW
360	961	1	1	20	6	11	39	1000	331	LOW
328	973	1	1	15	2	8	27	1000	347	HIGH
327	778	14	12	138	26	32	222	1000	350	HIGH
324	964	1	2	15	7	11	36	1000	349	HIGH
347	970	1	1	13	3	12	30	1000	348	HIGH
336	978	1	1	7	4	9	22	1000	344	HIGH
325	973	0	1	11	7	8	27	1000	345	HIGH
339	978	1	0	10	2	9	22	1000	343	HIGH
353	973	1	1	13	3	9	27	1000	346	HIGH
357	959	0	0	15	16	10	41	1000	342	HIGH
322	963	1	3	22	4	7	37	1000	341	HIGH

APPENDIX TABLE SA (continued)

N,N-Dimethylacetamide

Multiple Dosing: Air Control (0 ppm)
Low, 20 ppm
High, 700 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
359	922	4	4	44	13	13	78	1000	357	+
350	923	2	0	43	18	14	77	1000	358	+
321	640	3	11	195	83	68	360	1000	355	+
349	947	1	0	26	14	12	53	1000	360	+
355	913	1	1	46	22	17	87	1000	356	+
342	939	1	1	33	16	10	61	1000	352	+
332	945	1	3	22	11	18	55	1000	354	+
338	948	2	6	26	10	5	52	1000	353	+
334	861	5	3	67	44	20	139	1000	351	+
333	891	2	2	50	36	19	109	1000	359	+