Report Number 36

TIER II MUTAGENIC SCREENING OF 13 NIOSH PRIORITY COMPOUNDS

INDIVIDUAL COMPOUND REPORT N-METHYL DICYCLOHEXYLAMINE

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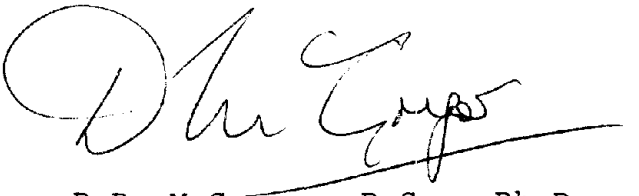
AUTHENTICATION

"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-Methyl dicyclohexylamine 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 912 µg/ml of culture medium.

2. Dominant lethal test in male rats with exposure to atmospheres containing 5 ppm or 25 ppm* N-methyl dicyclohexylamine for 7 h/day for 5 consecutive days. Analysis of test atmospheres was by gas chromatography of samples collected on absorption tubes.

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

4. Cytogenetic test in male and female rat bone marrow cells using the same exposure conditions as in (2) or a single exposure to 5 ppm or 20 ppm* 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 6 ppm for 4.5 min.

The results obtained were as follows:

1. There was no increase in UDS in cells treated with N-methyl dicyclohexylamine.

2. Mice and rats showed signs of severe systemic toxicity during exposure to 20 ppm or 25 ppm atmospheres.

3. N-Methyl dicyclohexylamine did not increase the frequency of aberrant cells in rat bone marrow.

4. There were no signs of dominant lethal mutation inducing potential or anti-fertility effects in male rats. In Week 5, 25 ppm atmosphere group, there were statistically significant increases in live implantations and live + late death implantations ($P<0.05$). These same 25 ppm atmosphere animals also showed significant increases in early death frequencies in Week 5 ($P<0.05$).

* Different concentrations were achieved in these separate tests (see p.41).

5. There were no effects upon the frequency of abnormal sperm in mice.

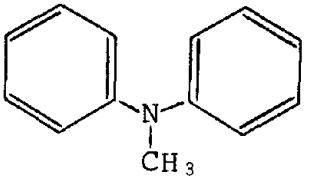
6. While N-methyl dicyclohexylamine was highly toxic to Drosophila, SLRL mutation frequency was not increased.

7. It was concluded that N-methyl dicyclohexylamine was devoid of genetic toxicity potential observable in these experiments.

INTRODUCTION

N-Methyl dicyclohexylamine (CAS No. 7560-83-0), (trade name Polycat 12) is a liquid, colourless tertiary amine with a characteristic amine odour. It is less than 0.1% soluble in water, but is soluble in organic solvents such as acetone, chloroform, alcohols, cyclohexane. A summary of its physical and chemical properties follows:

Formula



Mol. wt.	195.351
Sp. gr. (25°C)	0.9207
B.P.	265°C
Flash Point	214°F
Vapour density (air = 1)	6.728

Toxicology

Little information appears to be available on the toxicology of this compound. The oral LD_{50} (rat) is 521 mg/kg. The supplier's information is that animal studies indicate that the product presents a low, acute inhalation hazard, slight eye hazard and minimal topical hazard.

The purpose of the work described in this report was to investigate the genotoxic properties of N-methyl dicyclohexylamine in a variety of complex systems.

Conditions used in the various tests were as follows:

Human fibroblasts	up to 912 µg/ml for 3 h.
Mice and rats	5 ppm or 25 ppm for 7 h/day for 5 days; 5 ppm or 20 ppm for 7 h/day on one day.
Drosophila	6 ppm for 4.5 min.

MATERIALS AND METHODS

CHEMICALS

Test Substance

Four 750 g containers of N-methyl dicyclohexylamine (no batch number supplied) (stated purity 98%) were received from Abbott Laboratories, Chemical Division, North Chicago, Illinois 60064, U.S.A., through their outlet in the United Kingdom, Garrick Chemical and Equipment Company Limited, London, on 23 April 1980. The test material was a clear, colourless liquid and was retained in the dark under ambient conditions in the company dispensary until used. A sample has been held for analysis, should this be necessary.

Positive Control Substance

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

ANIMALS AND ANIMAL MANAGEMENT

Animals

Two hundred and thirty one male and 183 female CD rats (a remote Sprague-Dawley derived strain) were obtained from Charles River (U.K.) Limited, Manston, Kent, on 19 September 1980. They were ordered for delivery when they were 10-11 weeks old.

Forty four male B6C3F$_1$ mice were obtained from Charles River (U.S.A.) on 19 September 1980. They were ordered for delivery when they were 7-9 weeks old.

The animals were allowed to acclimatise to their new environment for at least 7 days before treatment began.

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 18°C and 24.5°C, and relative humidity ca 50%, with extreme limits of 30% and 68%.

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

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

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

Diet

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

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

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

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

Allocation of Rats and Mice to Cages and Treatment Groups

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

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

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

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

Treatment groups were colour coded as follows:

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

Animal Identification

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

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

Animal Positioning in the Exposure Chambers

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

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

The treatment groups were constituted as follows:-

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

ATMOSPHERE GENERATION AND EXPOSURE

Exposure Chambers

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

Exposures to N-methyl dicyclohexylamine were carried out in 1.5 m^3 capacity chambers constructed of stainless steel and glass. The animals occupied a volume of 0.02 m^3 and were confined to a single tier of cages of 0.4 m^3 in volume (the breathing zone). The breathing zone was ventilated at the rate of 12 air changes per hour. An additional chamber of 0.84 m^3 capacity was used for exposure of the air control group; the breathing zone in this chamber also was ventilated at the rate of 10 air changes per hour.

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

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

The generating apparatus and exposure chambers (Figures 1a and 1b) were positioned behind a screen in a room with a high efficiency exhaust system designed to ensure a safe working environment for laboratory personnel. The monitoring equipment was located on the outside of the screen at the opposite end of the room. Exposure personnel wore breathing apparatus with an external air supply. Protective gloves and laboratory coats were worn and the test compound was handled in an extract hood at all times.

Atmosphere Generation

Schematic diagrams showing the vapour generating apparatus, exposure chambers and monitoring equipment is presented in Figures 1a and 1b. The test atmospheres were produced by bubbling dry, oxygen-free nitrogen (BOC Limited) through a liquid reservoir of N-methyl dicyclohexylamine contained in

a 500 ml glass flask contained within a temperature controlled heating mantle. The nitrogen/N-methyl dicyclohexylamine vapour mixture so generated was ducted through 7/8" ID stainless steel piping to a glass mixing vessel and diluted with filtered, compressed air. The resulting mixture of N-methyl dicyclohexylamine/air was ducted through 7/8" stainless steel piping to the top of the exposure chamber.

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

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

Method Development

Infra-red Spectroscopy: The initial experiments to monitor N-methyl dicyclohexylamine were carried out using infra-red spectroscopy, employing a Miran-1A Portable Gas Analyser (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.

Most chemical compounds have characteristic infra-red spectra which can be used for identification and quantification. The infra-red spectrum of N-methyl dicyclohexylamine was recorded using a closed loop calibration system to generate the test atmosphere within the Miran gas cell. The only vibration possible to monitor in N-methyl dicyclohexylamine is that of the C-H stretch of the N-methyl group which can occur between 3.55-3.62 μm (m-s). For N-methyl dicyclohexylamine this band appears at 3.42 μm. Accordingly, the spectrometer was adjusted to monitor this wavelength. Suitable aliquots were injected in order to prepare a calibration curve.

Analytical Conditions

Instrument Settings:

Wavelength :	3.42	μm
Pathlength Setting :	13.9	m
Absorbence Range :	0.25	AUFS
Slit Width :	1	mm
Recorder Voltage :	1	v
Chart Speed :	300	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/cc)
M = Molecular weight of test sample
$\frac{(RT)}{(P)}$ = Molar volume of gas
5.64 = Volume of Miran sample chamber (l)

Example of the Calculation for V

Compound: N-Methyl dicyclohexylamine

C = 50 ppm
ρ = 0.9207
M = 195.351

$$V = \frac{50 \times 195.351 \times 5.64}{0.9207 \times 10^3 \times 24.4}$$

$$= 2.45\ \mu l$$

Therefore, to construct a calibration curve to cover the 50 ppm range 2 µl samples of N-methyl dicyclohexylamine would have to be injected into the analyser.

Results

It was not possible to prepare a calibration curve since, after every injection into the analyser (via the closed loop calibration system through a rubber septum using a Hamilton microlitre syringe), the absorbence did not stabilise but continued to rise. This suggested that the compound was being absorbed onto the cell and indeed, it was found necessary to pump 'zero gas air' through a filter into the system for a considerable period of time (usually overnight) before this absorbence was eliminated.

The difficulties experienced necessitated an alternative method of analysis being evaluated.

Gas Liquid Chromatography

Instrument Conditions:

Instrument:	Pye Model 104 fitted with a flame ionisation detector (FID).
Column:	6' glass column containing 3% OV-17 on chromosorb 80-100 mesh.
Column Temperature:	165°C
Injector Temperature Setting:	1.5
Detector Temperature:	175°C
Attenuation:	8×10^2
Chart Speed:	300 mm/h
Nitrogen Flow Rate:	30 ml/min
Hydrogen Pressure:	11 psi
Air Pressure:	9 psi

A typical chromatogram is shown in Figure 2.

Calibration

Calculation (Assuming use of a sampling loop)

A stock solution containing 9 mg/ml N-methyl dicyclohexylamine was prepared and then diluted in such a way as to produce a series of solutions where

Solution (i)	1 µl ≡ 22.4 ppm
Solution (ii)	1 µl ≡ 11.2 ppm
Solution (iii)	1 µl ≡ 5.6 ppm
Solution (iv)	1 µl ≡ 2.24 ppm
Solution (v)	1 µl ≡ 1.12 ppm

Since 195.351 g compound occupies 24.3×10^6 µl (at 23°C)

9.0 µg (1 µl) compound occupies

$$\frac{24.3 \times 10^6 \times 9 \times 10^{-6}}{195.351}$$

= 1.12 µl gas

If a 10 ml loop is used then

1.12 µl of gas is contained in 10 ml

i.e. 1.12 µl of gas is contained in one litre

i.e. 1 µl of stock solution ≡ 112 ppm v/v

Calibration

Calibration curves were prepared by injecting suitable aliquots directly onto the g.c. without using the sample loop.

Low Level

Concentration (µg)	Pk. Ht. 1 (cm)	Pk. Ht. 2 (cm)	Mean Pk. Ht. (cm)
0.09	0.85	0.90	0.875
0.18	2.0	2.3	2.15
0.45	7.15	7.4	7.28
0.72	11.6	10.7	11.15
0.90	15.1	15.9	15.5

This is shown graphically in Figure 3.

High Level

Concentration (µg)	Pk. Ht. (cm)
0.90	2.5
1.80	4.4
3.60	8.6
5.40	12.5
6.30	14.4

This is shown graphically in Figure 4.

Precision of Injection

Nine injections (5 µl) of solution (v) (p. 13) were made (directly onto the column) and the precision of injection calculated.

Sample No.	Peak Ht. (cm)	Sample No.	Peak Ht.
1	8.3	6	8.5
2	8.05	7	8.7
3	8.5	8	8.6
4	8.6	9	8.4
5	8.0		

Mean 8.4 $\pm$ 0.2 (3%)

Monitoring of Experimental Chambers

Initially, the atmospheres of N-methyl dicyclohexylamine within the exposure chambers were passed through stainless steel tubing into a gas chromatograph fitted with a gas sampling loop. When the loop was opened and the contents flushed onto the column no peaks were obtained. It was suspected that N-methyl dicyclohexylamine, which has a high boiling point and a low vapour pressure at normal ambient temperatures was condensing in the sample lines and absorbing onto the walls of the apparatus. Experiments whereby the N-methyl dicyclohexylamine was injected directly into the sampling loop system showed that even the loops were sufficiently large for the sample to be lost by absorption. For this reason, therefore, it was decided that the atmospheres generated in the exposure chambers should be monitored by the absorption of N-methyl dicyclohexylamine onto charcoal or silica tubes (Sipin) placed in the chambers.

Calculation of Concentration of N-Methyl dicyclohexylamine Using Charcoal or Silica Absorption Tubes

Total Volume (ml): Volume into which charcoal is extracted.

Volume Injected (µl): Volume injected onto gas chromatograph.

Peak Height/Area: Measure from chromatogram.

Weight Injected (µg): From calibration curve of standard solutions.

Weight Collected (mg): $\frac{\text{Wt injected x total volume}}{\text{volume injected}}$

Total Weight Collected (mg): Weight collected x efficiency of desorption.

Volume Collected (µl): Total weight x 124.4.

Volume Sampled (l): Sampling time x air flow.

Concentration ppm (v/v): $\frac{\text{Volume collected}}{\text{volume sampled}}$

Efficiency of Desorption

Since absorption tubes were to be used for the direct sampling of the atmosphere in the exposure chamber, it was necessary to determine the efficiency of desorption from charcoal and from silica gel.

N-Methyl dicyclohexylamine (9.31 mg) was added to 3 charcoal tubes. Air was sucked through each tube for one minute after which the tubes were allowed to stand (capped) for 2 h at room temperature. The tubes were broken, their contents transferred to 10 ml volumetric flasks and made to the mark with ethanol. The flasks were left at room temperature for 90 min before a suitable aliquot was analysed. The peak height was compared with that of a standard solution and it was found that 51% of the original material was recovered.

A similar experiment was carried out using absorption tubes containing silica gel. In this case the desorption efficiency was found to be 66%. Although this is considerably higher than for charcoal, the monitoring of the exposure chambers was carried out using charcoal since the latter was more readily available.

Effect of Time on Desorption

A known amount of N-methyl dicyclohexylamine was added to a charcoal tube and treated as for the desorption experiment. However, instead of the ethanol solution being allowed to stand at room temperature for 90 min before analysis, aliquots were removed at intervals and analysed.

Time (min)	Peak Ht. (cm)	Time (min)	Peak Ht. (cm)
0	3.0	60	4.2
15	3.5	90	4.5
30	3.9	120	4.5

This is shown graphically in Figure 5.

It can be seen that at the 90 min time interval in the previous experiment the desorption is at its maximum. When samples were left to stand for a longer period of time a second peak began to show on the chromatogram. This is in contrast to solutions of N-methyl dicyclohexylamine in ethanol (without either charcoal or silica gel) where only a single peak was observed even in "old" samples.

GC-MS Analysis of N-Methyl dicyclohexylamine Solutions

GC-MS was performed on a Finnigan 4000 mass spectrometer interfaced with a Finnigan 9600 gas chromatograph and controlled by a Finnigan 6110 data system. The instrument was operated in the chemical ionisation mode with ammonia as ioniser reagent gas. Specific gas chromatographic and mass spectrometric conditions are described below:

GC Column:	4' glass column packed with 1% OV-17 on Gas Chrom Q, 100-120 mesh.
Oven Temperature:	130^{o}C
Injector Setting:	1.5
Detector Temperature:	175^{o}C
Carrier Gas:	Helium
Flow Rate:	30 ml/min
Reagent Gas:	Ammonia
Ioniser Pressure:	0.4 Torr
Electron Energy:	70 e.v.

Commercial N-methyl dicyclohexylamine was chromatographed (see Figure 6) and gave a major peak at Scan 31 corresponding to N-methyl dicyclohexylamine and minor peaks at Scan 21

corresponding to dicyclohexylamine (<5%) and at Scan 39 (<2% and not interpreted). The mass spectra of these are shown in Figures 7-9.

It was observed that the N-methyl dicyclohexylamine contained in the heated reservoir through which nitrogen was passed during the generation of the test atmosphere tended to become dark brown in colour. A typical sample which had been in use for 14 h was analysed by GC-MS and showed 2 peaks in its chromatogram (Figure 10), the major peak at Scan 31 corresponding to N-methyl dicyclohexylamine. There was however a considerable (35%) amount of the N-demethylated compound. Minor products (<2%) were also present but not identified. The mass spectra are seen in Figures 11 and 12.

When desorbed samples in ethanol were allowed to stand in the presence of charcoal for long periods of time it was observed that 2 peaks appeared in the chromatograms. Two typical samples were later analysed by GC-MS (Figures 13 and 14) and it can be seen that they no longer contained any N-methyl dicyclohexylamine, but substantial amounts of dicyclohexylamine.

These results indicate that demethylation of N-methyl dicyclohexylamine can occur on standing in ethanol in the presence of charcoal. However, the analyses during the exposures were carried out normally within 90 min of sampling and it is considered that these analyses gave a reasonable indication of the concentration of N-methyl dicyclohexylamine in the exposure chambers. It is possible that small concentrations of dicyclohexylamine were present in the atmosphere but there was no evidence for this from the analysis of the desorbed solutions.

Homogeneity Data

Before starting the animal exposures, chamber concentrations at both the high and low levels were determined by frequent sampling/analysis for periods of up to 6½ h. In addition, samples were measured from different areas (at least 7) of the animal holding zone to confirm uniformity of N-methyl dicyclohexylamine concentration.

Measurement of Chamber Concentration

The gas chromatograph was calibrated at the beginning of each exposure day. Daily calibration data are presented in Tables AT-1 and 2. A typical calibration graph for the high level is presented in Figure 15.

Due to peak broadening effects observed during analysis of the desorbed atmospheric samples all measurements were converted to peak area (mm^2) for calculation of actual chamber concentrations.

Atmospheric concentrations of N-methyl dicyclohexylamine were sampled at regular intervals during the 7 h exposure period from the breathing zone of the animals. A separate sampling system was used for each concentration level (Figure 1a). The Sipin charcoal tube was connected to a vacuum pump, by means of PTFE tubing, and inserted into the exposure chamber through a sampling port located at the chamber side. The sampling flow rate was controlled using flow meters and varied from 2.5-3.0 l/min.

Test Compound Utilisation

At the beginning of each exposure day, the N-methyl dicyclohexylamine reservoir (a gas washing or Drechsel bottle) was replenished with test compound. If the test compound, remaining from the previous days' exposure, showed signs of discolouration it was discarded and fresh N-methyl dicyclohexylamine used. Utilisation of test material was calculated on a daily basis by weighing the glass flask before vapour generation began and deducting the weight of the flask 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-methyl dicyclohexylamine were arranged in a single tier inside the exposure chamber. Air control animals were stacked in 2 tiers.

During the multiple exposure period, rats designated for the dominant lethal test, cytogenetic multi-dose test and the mice for the sperm abnormality test were exposed together for 7 h/day for 5 consecutive days. The single dose cytogenetic test rats were exposed on a different day. Animal

positions within the exposure chambers were rotated on a daily basis to minimise any possible exposure location variations.

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

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

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

Positive Control Groups in Animal Tests

Preparation of Dosing Solutions

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

Treatment of Rats and Mice with Ethyl methanesulphonate

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

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

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

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

UNSCHEDULED DNA SYNTHESIS ASSAY

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

Chemicals

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

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

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

Test Solutions

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

Cells

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

Culture Maintenance and Growth Media

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

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

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

Animals

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

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

Preparation of the 9,000 g Supernatant Fluid from Livers

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

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

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

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

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

co-factor solution	9 parts
liver preparation	1 part

Preliminary Toxicity Test

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

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

The compound was dissolved in dimethylsulphoxide and 10 µl samples were added to duplicate cell suspensions to give final concentrations of 0.912 to 9,120 µg/ml. To each control culture was added 10 µl of dimethylsulphoxide.

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

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

Based on these results, 8 concentrations of N-methyl dicyclohexylamine were selected for use in the repair assay, the highest concentration tested being 912 µg/ml.

DNA Repair Assay

The cells were harvested, sedimented, suspended in fresh culture medium at a density of 5 x 10^4 cells/ml and 2 ml samples of this suspension were pipetted into 35 mm tissue culture Petri dishes containing 3 sterile coverslips (Lux Scientific Corporation, California, U.S.A.). These were then incubated at 37°C in a humid atmosphere of 5% CO_2 in air for 72 h. The medium from each of the dishes was then replaced with 2 ml of arginine-deficient DMEM supplemented with 5% heat inactivated foetal bovine serum and the plates incubated for 24 h. The medium was then replaced with a further 1 ml of arginine-deficient DMEM and the incubation continued for a further 48 h. At the end of this time the cultures were divided into 2 groups and 200 µl of S-9 mix added to one of them. Solutions of hydroxyurea (250 mM) in sterile distilled water and 6-[^{3}H]-thymidine (21 Ci/mmol) were added to each culture giving final concentrations of 2.5 mM and 10 µCi/ml respectively. N-Methyl dicyclohexylamine was dissolved in dimethylsulphoxide and dilutions were made from this to give concentrations indicated in the tables when mixed with culture medium. Triplicate wells, with and without S-9 mix, received 10 µl samples of test compound solution. 10 µl samples of dimethylsulphoxide were added to negative control cultures.

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

4-NQO	1.25 µg/ml
2-AAN	5.0 µg/ml

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

Autoradiography

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

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

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

Quantification of Repair Synthesis

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

CYTOGENETIC ANALYSIS OF RAT BONE MARROW CELLS

Metaphase Cell Preparations

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

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

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

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

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

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

Slide Reading

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

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

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

DOMINANT LETHAL TESTING IN MALE RATS

Mating

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

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

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

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

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

Assessment

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

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

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

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

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

SPERM ABNORMALITIES TEST IN MICE

Preparation

Mice were killed 5 weeks from the last day of dosing (i.e., Tuesday 11 November 1980) by neck dislocation.

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

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

Assessment

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

The following types of sperm were not scored:

(1) separated tails and heads.

(2) clumps of sperm.

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

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

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

I Normal

II Abnormal

A. hook upturned or elongated.

B. banana-shaped head.

C. amorphous head.

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

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

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

SEX-LINKED RECESSIVE LETHAL TEST IN DROSOPHILA MELANOGASTER

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

Strains

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

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

Medium

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

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

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

Exposures

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

*Nipogen: bacteriostatic agent (BDH Limited).

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

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

Toxicity Test

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

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

Recessive Lethal Test

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

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

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

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

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

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

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

STATISTICAL EVALUATION

Cytogenetics Tests

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

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

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

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

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

Dominant Lethal Assay

The variates analysed were:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Sperm Abnormalities Test

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

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

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

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

Sex-linked Recessive Lethal Test

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

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

F_2 Generation

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

F_3 Generation

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

Against this background, the criteria for result assessment were:

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

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

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

RESULTS

Instrument Calibration

Calibration of the GC apparatus was performed daily when atmosphere generation work was undertaken during the development phase and when animals were being exposed to test vapours. An example of a calibration curve is given in Figure 15. Data for the construction of such curves are given for various exposure dates in Tables AT-1 and 2.

Calibration ranges were adopted which would facilitate measurement of the 5 ppm and 20 or 25 ppm target concentrations.

Chamber Atmospheres - Homogeneity

Prior to exposure of the animals, the chamber atmospheres were sampled at different positions to establish that adequate mixing of N-methyl dicyclohexylamine was occurring. The results are shown in Table AT-3, where it can be seen that the maximum deviation encountered within a reference point concentration of 2.5 ppm was -0.9 ppm and, with a reference point concentration of 29.7 ppm, it was -6.5 ppm.

Chamber Atmospheres - Achieved Concentrations

Deviations from the target concentrations of 5 ppm and 25 ppm are recorded in Tables AT-4 and 5.

The large deviations recorded in Table AT-5 are not entirely due to problems of atmosphere instability. Toxicity to the animals was so severe that the Principal Investigator lowered the target concentration from the planned 50 ppm while the animals were still in the chambers. On Days 4 and 5 of the multiple exposure portion of the experiment, the target concentration was lowered to 15 ppm. Over the 5 days, the mean target was about 25 ppm. This figure is the one used (for convenience) in all subsequent tables and descriptions. The target concentration in the single exposure experiment was 20 ppm.

Animal Location

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

Pre-experimental Acceptance Tests (PEAT)

19 September 1980 Delivery: Four mice, 10 male and 10 female rats were haphazardly selected for PEAT. The mice showed no signs of infection, disease or abnormalities during clinical observation, autopsy, histology or microbiology/parasitology. Neither male nor female rats displayed notable clinical signs of infection and, at autopsy, the only finding was small, irregular red areas on all lung lobes of 3 male rats. The microbiological/parasitological assays did not reveal any infections. Histology revealed lymphoid cuffing of the blood vessels in all male rats and 4 female rats.

It was considered that these lung lesions represented the effects of Sendai virus infection, endemic in these rats, which could regress if given the opportunity. Therefore, 6 days later, a further 5 male and 5 female rats were examined for pulmonary lesions. Lymphocytic cuffing of blood vessels was observed in 2 male and 2 female rats and, even these lesions were of lesser severity than those observed previously. It was considered, therefore, that the rats would be suitable for the envisaged experiments, given a further period of recovery.

Seven days elapsed from the time of the second sample observation to the start of the inhalation experiments.

Clinical Signs

Sperm Abnormality Mice

No abnormalities were detected among the air control and EMS dosed animals.

Low

Reduced response to audio stimuli and subdued appearance were noted during exposure. Hyperkinesia and twitching was observed for all animals post exposure. At the end of the second days' exposure animal 337♂ had convulsions.

High

Day 1: During exposure animals exhibited a subdued appearance and diminished response to audio stimuli. All mice were observed to be dead after almost 7 h, when the exposure chamber concentration was approximately 34 ppm.

Day 2: Ten fresh male mice were dosed. During exposure the mice appeared subdued, ataxic and unresponsive to audio stimuli. After 4 h the mice

had laboured respiration and were lying on their sides, the animals were then removed from the exposure chamber. The concentration at this point was approximately 50 ppm. Four mice were found to be dead on removal and the remaining 6 died within 45 min.

Day 3: Another 10 fresh male mice were again placed in the exposure chamber. After 2 h 12 min the mice were removed from the chamber when they were observed lying on their sides with laboured respiration. The concentration had risen from an initial 22 ppm to approximately 50 ppm. Convulsions and shaking were observed upon removal from the chamber. Three mice (Nos. 427♂, 429♂ and 430♂) died and the remainder appeared very subdued.

Day 4: The 7 surviving mice appeared very subdued during exposure. At the end of the 7 h exposure period 4 mice (Nos. 422♂, 423♂, 424♂ and 426♂) were found dead. The concentration had not exceeded 22 ppm.

Dominant Lethal Test

No abnormalities were detected among the air control or EMS treated animals.

Animals exposed to N-methyl dicyclohexylamine were observed to be subdued and unresponsive to audio stimuli during exposure. Red encrustations were observed around the nostrils of some animals in both the high and low levels whilst body tremors, hypersensitivity and ataxia were noted for the high dose animals only. Loose faeces and a lesion on the right fore paw were observed for animal 381♂ on Day 3.

During the 5 day dosing period, animal 383♂ was observed to have damaged both fore paws whilst animal 386♂ had a damaged right fore paw; 383♂ also had lacerations on it's hind feet and 386♂ had abdominal abrasions. On Day 1 after exposure, animal 381♂ was found dead. This animal had red staining around the snout. No abnormalities were observed during the subsequent 10 week mating period.

Cytogenetics Tests

No abnormalities were observed among the air control animals. EMS treated animals had slight brown staining around their nostrils following dosing.

Low and high dose animals exposed to N-methyl dicyclohexylamine were observed to be subdued during the exposure period. Animals exposed to the high level were also unresponsive to audio stimuli and animal 147♂ had 'chewed' it's left fore paw. Following exposure both low and high dose animals were observed to have red staining around their nostrils. Ataxia, muscle tremors and convulsions were also observed for some high dose animals.

Animals 80♂ and 229♀, from the single exposure cytogenetics test and 302♀ and 304♀, from the multiple exposure, were found dead upon removal from the exposure chamber.

Body Weight

Significant body weight losses were observed in male and female rats exposed to 25 ppm N-methyl dicyclohexylamine for 5 days. There was also slightly reduced body weight gain in the male rats in the cytogenetic test, but not in the dominant lethal test following exposure to 5 ppm atmospheres. There were no effects on body weight gain in the mice exposed to 5 ppm atmospheres and there were too few survivors in the group exposed to 25 ppm N-methyl dicyclohexylamine to allow any conclusions to be drawn. Rats and mice dosed with EMS for 5 days also showed a marked reduction in body weight.

UNSCHEDULED DNA SYNTHESIS ASSAY

In the assay involving tritiated thymidine incorporation into non-S phase cells, there was no indication of any increase in the number of silver grains per nucleus at any concentration of N-methyl dicyclohexylamine (Table UDS-1). An initial toxicity test indicated that the highest dose level to be used in the UDS assay should be 912 µg/ml. The substances - 4-nitroquinoline-N-oxide and 2-aminoanthracene - used in concurrent control groups evoked significant levels of unscheduled DNA synthesis from the cells.

It was intended to confirm these results, indicating a lack of effect due to N-methyl dicyclohexylamine, in further experiments which would have involved the incorporating of deoxyguanosine into the cells. It was found at about this time, however, that cell permeability to deoxyguanosine had decreased greatly, particularly in the presence of S-9 mix. This confirmation test was never attempted. (A more detailed account of these difficulties is being reported separately.)

CYTOGENETIC ANALYSIS OF RAT BONE MARROW CELLS

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

In the multiple dosing cytogenetic test, there were no indications of induction of chromosomal damage in either the male or female rats exposed to 5 ppm or 25 ppm N-methyl dicyclohexylamine atmospheres. Responses to the positive control substance, EMS, were significant in both male and female rats ($P<0.001$). Analysis using data from cells after the exclusion of gaps gave similar results and the same significance level.

In the single exposure rats treated with 5 ppm or 20 ppm N-methyl dicyclohexylamine, there was little chromosomal damage apart from chromatid gaps. Such damage that was observed occurred with similar frequency in cells from air control rats. Treatment with EMS did induce damage, however, particularly in the 24 h sample cells. At this time, damage induction was significant ($P<0.001$) in cells of male and female rats with any kind of aberration and in cells with aberrations excluding gaps only ($P<0.001$). Considering the other sampling times, male rats showed a statistically significant increase in all aberrant cells at the 6 h sample period ($P<0.05$), but not at the 48 h sample period. After exclusion of cells only with gaps, there were significant increases in aberrant male rat cells at the 6 h ($P<0.01$) and 48 h ($P<0.05$) sample periods. In female rats there was a significant increase in aberrant cells only after the exclusion of gaps at the 48 h ($P<0.05$) sample period.

It was concluded that N-methyl dicyclohexylamine did not increase the frequency of aberrant cells in rat bone marrow.

DOMINANT LETHAL TEST

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

Pregnancy frequency was calculated in 2 ways: firstly, by considering as pregnant females with corpora lutea graviditatis (Table DL-1) and, secondly and more reliably, by considering as pregnant only females with implantations (Table DL-2). With neither method was there observed any effect upon pregnancy frequency clearly due to N-methyl dicyclohexylamine. In the EMS treated group, pregnancy frequency was reduced in Weeks 1-4, but particularly in Weeks 2 and 3 (Table DL-2).

Corpora lutea graviditatis counts (Table DL-3) were not reduced in either of the N-methyl dicyclohexylamine exposed groups. A statistically significantly high value was observed in Week 2 of the 5 ppm exposure group. EMS treatment led to large reductions in corpora lutea counts in Weeks 1 and 3 ($P<0.001$) and 2 ($P<0.01$).

Implantations per pregnancy (Table DL-4) were unaffected by treatment with the test compound, but EMS induced large reductions in Weeks 1 ($P<0.01$), 2 and 3 ($P<0.001$).

Data for live implantations per pregnancy (Table DL-5) and live implantations + late deaths per pregnancy (Table DL-6) were very similar. There were no reductions from the air control group values in either of the N-methyl dicyclohexylamine treated groups. In Week 5, the 25 ppm atmosphere exposure group showed a statistically significant increase in both parameters ($P<0.05$), when compared with the concurrent air control group. EMS treatment greatly reduced both parameters in Weeks 1-3 ($P<0.001$) and 4 ($P<0.01$).

A review of the data showing pregnancies with either (1) one or more early deaths or (2) two or more early deaths (Table DL-7) did not indicate any increase in these frequencies in the N-methyl dicyclohexylamine treated groups. In the EMS treated group, the only indications of increased numbers of affected pregnancies were in Weeks 1-4 in pregnancies with one or more (>0) early deaths and Weeks 1 and 4 in pregnancies with 2 or more (>1) early deaths.

Analysis of early death frequencies, following the Freeman-Tukey poisson transformation (Table DL-8) and binomial transformation (Table DL-9) gave essentially the same results. There were no increases in N-methyl dicyclohexylamine treated groups except in Week 6 of the 25 ppm atmosphere exposed group ($P<0.05$). This increase was not due to the effects of any obviously abnormal litters and the possibility cannot be totally excluded that the test compound was responsible for

the result. However, only 18 early deaths were recorded and while this was high compared with the concurrent air control (4), it was within the range of results recorded for the air control group (4-19) in this experiment. In the EMS treated group, there were statistically significant increases in early death frequencies in Weeks 1-4 and 6.

It is concluded that N-methyl dicyclohexylamine probably has no dominant lethal mutation inducing potential and has no anti-fertility effect in male rats.

SPERM ABNORMALITY TEST

There were serious difficulties for the mice exposed to the higher concentration used of N-methyl dicyclohexylamine (Table SA-1) but 3 mice were exposed to, and survived, 15 ppm atmospheres for 2 days (Table SA-2). Formal statistical analysis was not performed on data from these 3 mice. Such analyses as were conducted did show, however, that 5 ppm atmospheres had no effect upon the frequency of abnormal sperm (Table SA-3). It is also concluded that the 2-day exposure to 15 ppm N-methyl dicyclohexylamine had no effect upon abnormal sperm frequency. EMS treatment, on the other hand, induced statistically significant increases in Category C sperm (amorphous head) ($P<0.001$) and Category E sperm (miscellaneous) ($P<0.01$).

SEX-LINKED RECESSIVE LETHAL TEST IN DROSOPHILA

There was no information on the toxicity of N-methyl dicyclohexylamine to flies, so, preliminary studies were made.

On 30 September 1980, flies were transferred to an exposure chamber and a 20.5 ppm atmosphere was introduced to the chamber. Activity was decreased after only 60 sec and at 78 sec all flies were immobile. Flies were removed after 5 min, but even after 4 h in air there were no vital signs. Clearly, this concentration was too high, so, a second group of flies was exposed, this time only to a 7.1 ppm atmosphere. Flies were immobile after 2.5 min.

On 1 October 1980, the fly exposure atmosphere was reduced further to 2.5 ppm and flies were immobile after 106 sec. Exposure continued for a total of 5 min, but all flies appeared to be dead and maintained this appearance for 2 h after which time they were discarded.

Further restrictions were placed upon exposure time and another batch of flies was exposed to a 3.4 ppm atmosphere for 2.5 min. These flies were still alive the following day. Other preliminary tests were conducted which confirmed that these last reported conditions were very close to the limit the flies could tolerate.

On 8 October, 2 breeding stocks (A and B) of flies were exposed to 6.0 ppm for 4.5 min. Flies had dropped to the bottom of the chamber within 1.5 min, but there were occasional twitching movements even at the end of this 4.5 min exposure period. The chamber was flushed through with air at this time. The Drosophila were immobile 4 h after exposure. Surprisingly, there was good recovery of these flies and fertility was normal. In neither stock were there any indications of sex-linked recessive lethal mutation induction (Table RL-1). In the air exposed flies, the F_2 generation exhibited no lethals in Stock B (out of a total of 1,596 vials set up) and only one in Stock A (out of a total of 1,617 vials set up). In the N-methyl dicyclohexylamine treated flies, only one lethal was recognised in the F_2 generation. There were none in Stock A (out of 1,715 vials set up) and one in Stock B (out of 1,718 vials set up).

In the F_3 generation, no lethals were recognised in the air controls (out of 558 Stock B vials) and no lethals were recognised in the N-methyl dicyclohexylamine treated group (out of 1,354 Stock B vials).

It was concluded, therefore, that while N-methyl dicyclohexylamine is highly toxic to Drosophila, it does not induce sex-linked recessive lethal mutations.

CONCLUSIONS

N-Methyl dicyclohexylamine posed 2 general problems in these experiments. Firstly, the planned, high level target concentration of 50 ppm was difficult to achieve and when it was achieved it was difficult to maintain. Consequently, the actual mean concentrations were about 25 ppm and 5 ppm in the multiple dosing experiment and about 20 ppm and 5 ppm in the single dose experiment. The high concentration level daily means ranged from 15.5 $\pm$ 2.2 ppm to 35.0 $\pm$ 16.3 ppm in the multiple dosing experiment. Secondly, the high dose level group animals were not tolerant of even these reduced achieved concentrations. Mice were particularly intolerant of the exposure conditions.

In spite of the rigorous conditions for the test animals, there were no indications that N-methyl dicyclohexylamine had genotoxic properties which could be detected using these protocols. Also, there was no indication that it could induce UDS in cultured mammalian cells.

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TABLE AT-1

N-Methyl dicyclohexylamine
Calibration Data for Low Level

Dose Level: 5 ppm v/v

Volume µl	Conc. µg	Peak Area (mm^2)					
		3 October 1980	4 October 1980	5 October 1980	6 October 1980	7 October 1980	8 October 1980
0	0	0	0	0	0	0	0
1	0.09	22.0	48.3	49.5	39.6	52.9	39.9
2	0.18	54.6	96.6	99.0	82.5	88.2 (105.6)	90.3
3	0.27	77.3	190.0	174.0	130.0	140.8 (170.8)	138.6
4	0.36	-	-	226.2	188.5	178.2 (223.6)	180.6
5	0.45	142.2	291.0	292.4	236.0	225.4	252.5
6	0.54	-	381.5	353.6	327.7	-	-
7	0.63	197.6	-	-	-	-	-

Values in parenthesis indicate repeat measurements, obtained following recalibration after GC malfunction.

Instrument Conditions:

Instrument:	Pye Model 104 fitted with a flame ionisation detector (FID).
Column:	6' glass column containing 3% OV-17 on chromosorb 80-100 mesh.
Column Temperature:	165°C
Injector Temperature Setting:	1.5
Detector Temperature:	175°C
Attenuation:	64 x 10^2
Chart Speed:	300 mm/h
Nitrogen Flow Rate:	30 ml/min
Hydrogen Pressure:	11 psi
Air Pressure:	9 psi

TABLE AT-2

N-Methyl dicyclohexlyamine
Calibration Data for High Level

Dose Level: 25 ppm v/v

Volume µl	Conc. µg	Peak Area (mm^2)					
		3 October 1980	4 October 1980	5 October 1980	6 October 1980	7 October 1980	8 October 1980
0	0	0	0	0	0	0	0
0.5	0.45	-	-	33.6	-	-	-
1	0.9	40.5	72.0	74.8	70.0	70.0 (70.4)	64.0
2	1.8	81.6	168.8	163.2	147.0	138.5 (145.2)	147.0
3	2.7	132.6	223.6	252.0	205.8	176.0 (248.4)	218.4
4	3.6	159.0	330.4	321.0	283.8	280.4	299.2
5	4.5	217.6	433.6	400.0	368.0	333.2 (387.5)	369.6
6	5.4	252.0	-	-	-	-	-

Values in parenthesis indicate repeat measurements obtained following recalibration after GC malfunction.

Instrument Conditions:

Instrument:	Pye Model 104 fitted with a flame ionisation detector (FID).
Column:	6' glass column containing 3% OV-17 on chromosorb 80-100 mesh.
Column Temperature:	165°C
Injector Temperature Setting:	1.5
Detector Temperature:	175°C
Attenuation:	8 x 10^2
Chart Speed:	300 mm/h
Nitrogen Flow Rate:	30 ml/min
Hydrogen Pressure:	11 psi
Air Pressure:	9 psi

TABLE AT-3

N-Methyl dicyclohexylamine
Chamber Atmosphere Homogeneity Data

Dose Level: 5 ppm and 25 ppm

Sample Location	Chamber Concentrations	
	5 ppm	25 ppm
Reference Point (R)	2.5	29.7
Right Centre (RC)	2.0	28.2
Right Front (RF)	3.0	26.0
Centre Front (CF)	-	28.4
Left Front (LF)	2.3	24.6
Left Centre (LC)	-	26.7
Left Back (LB)	1.9	23.2
Centre Back (CB)	2.5	24.9
Right Back (RB)	1.6	26.9

Top view of exposure chamber

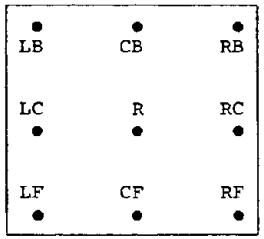


TABLE AT-4

N-Methyl dicyclohexylamine
Atmosphere Analysis of Target Concentration 5 ppm

	Exposure Day/Concentration (ppm)					
	1	2	3	4	5	Single Dose
	*(0.2)	4.5	5.7	6.4	3.8	4.2
	*(0.3)	5.5	4.3	5.0	4.9	4.0
	*(1.0)	6.5	5.9	5.8	5.6	5.3
	*(1.4)	4.9	6.4	6.8	4.2	6.0
	*(2.5)	4.7	8.2	5.5	5.1	5.0
	*(2.3)	6.4	4.5	6.5	4.7	5.1
	2.6	7.0	5.4	6.1	3.2	4.8
	2.8	4.6	5.1	5.9	3.9	3.6
	2.4	4.1	3.9	5.5	3.4	3.2
	3.3	6.3	6.3	5.4	3.9	3.5
	7.1	5.7	-	5.5	3.3	3.2
	2.7	6.1	-	6.5	3.6	2.7
	7.5	4.6	-	6.1	4.8	2.7
	13.8	5.0	-	4.7	4.2	3.3
	9.9	6.9	-	3.3	3.8	4.6
	7.1	-	-	-	-	-
	8.4	-	-	-	-	-
	6.6	-	-	-	-	-
	5.9	-	-	-	-	-
	7.8	-	-	-	-	-
	7.5	-	-	-	-	-
	5.7	-	-	-	-	-
Mean	*(5.3)6.3	5.5	5.6	5.7	4.16	4.1
± S.D.	±(4.0)3.1	± 1.0	± 1.2	± 0.9	± 0.7	± 1.0

* = Including work-up period

TABLE AT-5

N-Methyl dicyclohexylamine
Atmospheric Analysis by Gas Chromatography
Target Concentration 25 ppm (Multiple Exposure)
and 20 ppm (Single Exposure)

	Exposure Day/Concentration (ppm)					
	1	2	3	4*	5*	Single Dose
	7.0	12.0	22.0	10.6	19.7	12.1
	18.1	11.5	27.5	11.7	15.5	14.4
	17.0	17.9	38.4	14.7	16.3	19.9
	16.2	18.2	49.8	16.5	16.6	18.7
	14.6	16.3	44.4	15.4	17.4	15.9
	10.3	39.1	56.4	18.1	15.8	20.0
	14.5	21.3	54.0	18.5	17.4	20.4
	19.9	35.6	61.8	19.7	10.8	22.6
	12.9	54.0	23.6	19.2	14.3	19.7
	16.0	47.8	17.8	18.8	13.8	15.8
	15.1	40.4	20.9	17.4	13.8	18.9
	14.5	48.0	19.7	18.9	13.9	22.8
	9.9	46.4	19.2	18.6	12.7	17.3
	18.9	60.8	-	21.5	14.2	23.1
	27.9	56.1	-	20.9	15.1	20.6
	31.7	15.4	-	17.9	16.1	20.8
	29.2	19.4	-	17.4	16.4	22.7
	26.2	-	-	-	19.1	22.7
	33.7	-	-	-	-	11.0
Mean	18.6	33.0	35.0	17.4	15.5	18.9
± S.D.	± 7.6	± 17.2	± 16.3	± 2.9	± 2.2	± 3.6

* = Initially, the target concentration was 50 ppm, but this was difficult to achieve, then found toxic to mice. Consequently, the target was lowered to 15 ppm.

TABLE BW-1

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

Sex	Day	Air Control (0 ppm)	5 ppm	25 ppm	EMS 5 x 100 mg/kg
Male	1	382.7 ± 17.8	385.6 ± 27.3	362.2 ± 20.6	400.4 ± 20.7
	2	384.8 ± 19.7	387.7 ± 27.3	350.6 ± 22.2	390.9 ± 24.5
	3	389.7 ± 20.9	387.1 ± 28.2	336.8 ± 20.5	383.7 ± 26.7
	4	390.9 ± 20.9	387.1 ± 28.4	334.7 ± 22.1	371.7 ± 27.9
	5	392.4 ± 21.4	388.5 ± 28.4	332.6 ± 24.1	361.1 ± 29.3
	Weight gain/loss	9.7	2.9	-29.6	-39.3
Female	1	246.8 ± 21.5	241.7 ± 20.3	246.7 ± 10.2	249.5 ± 17.0
	2	245.1 ± 22.2	238.7 ± 21.8	240.1 ± 10.4	244.4 ± 15.9
	3	245.5 ± 21.7	237.7 ± 21.7	234.9 ± 9.8	240.6 ± 14.5
	4	247.0 ± 22.1	238.3 ± 21.5	228.7 ± 9.0	234.3 ± 11.6
	5	245.8 ± 22.4	237.9 ± 21.9	223.6 ± 8.8	230.4 ± 13.1
	Weight gain/loss	-1	-3.8	-23.1	-19.1

TABLE BW-2

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

Sex	Sampling Time (Hours Post Exposure)	Air Control (0 ppm)	5 ppm	20 ppm	250 mg/kg EMS
Male	6	400.3 ± 22.8	395.1 ± 23.8	400.7 ± 30.9	411.0 ± 25.1
	24	412.0 ± 23.8	415.7 ± 30.4	407.8 ± 11.4	418.7 ± 38.4
	48	388.8 ± 27.1	413.0 ± 20.1	389.7 ± 18.2	404.7 ± 37.9
Female	6	258.2 ± 22.3	248.5 ± 24.2	254.3 ± 18.9	269.5 ± 14.2
	24	254.1 ± 14.1	260.2 ± 18.3	262.4 ± 26.8	261.6 ± 18.2
	48	255.8 ± 17.7	264.2 ± 17.7	257.8 ± 22.6	260.9 ± 16.7

TABLE BW-3

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

Day	Air Control (0 ppm)	5 ppm	25 ppm	5 x 100 mg/kg EMS
1	386.2 ± 27.7	380.4 ± 35.6	357.6 ± 15.0	390.8 ± 25.9
2	389.0 ± 28.2	382.6 ± 37.6	338.4 ± 10.4	382.1 ± 26.2
3	389.5 ± 28.6	382.0 ± 38.9	329.7 ± 11.7	374.6 ± 25.8
4	391.7 ± 26.9	382.2 ± 38.2	321.6 ± 12.1	363.2 ± 24.0
5	391.8 ± 29.9	385.4 ± 38.4	319.3 ± 14.4	354.3 ± 22.4
Weight gain/loss	5.6	5.0	-38.3	-36.4

TABLE BW-4

N-Methyl dicyclohexylamine
Sperm Abnormalities Test
Group Mean Body Weights (g) for the Dosing Period of Male $B6C3F_1$ Mice

Day	Air Control (0 ppm)	5 ppm	⊛ 25 ppm	5 x 200 mg/kg EMS
1	26.3 ± 1.2	23.1 ± 1.7	24.7 ± 23.3	25.5 ± 1.4
2	26.4 ± 1.2	23.1 ± 1.4	23.3 ± 0.6	25.4 ± 1.2
3	26.8 ± 0.8	24.2 ± 1.5	-	25.8 ± 0.8
4	26.6 ± 1.0	24.3 ± 1.8	-	24.6 ± 1.0
5	26.8 ± 0.9	24.9 ± 1.7	-	23.5 ± 1.2
Weight gain/loss	0.5	1.8		-2.0

⊛ Original group of animals and first replacement group all died, second replacement group had only 3 survivors after 2 days dosing.

TABLE UDS-1

N-Methyl dicyclohexylamine
Unscheduled DNA Synthesis

Substance	Concentration (µg/ml)		Mean Number of Grains/Nucleus ± S.D.	
	With S-9	Without S-9	With S-9	Without S-9
Dimethylsulphoxide	10,000	10,000	3.6 ± 9.6	7.1 ± 4.3
4-Nitroquinoline-N-oxide	-	1.25	-	97.0 ± 31.1
2-Aminoanthracene	5	-	78.9 ± 46.2	-
N-Methyl dicyclohexylamine	7	7	6.1 ± 3.2	2.5 ± 2.7
	14	14	5.5 ± 3.4	1.8 ± 2.4
	29	29	4.8 ± 2.7	9.5 ± 5.8
	57	57	3.9 ± 3.0	11.3 ± 9.5
	114	114	7.5 ± 4.5	5.4 ± 3.9
	228	228	5.7 ± 3.7	6.4 ± 3.7
	456	456	4.5 ± 3.1	4.2 ± 4.2
	912	912	5.1 ± 3.5	5.3 ± 3.5

TABLE CA-MD-M-1

N-Methyl dicyclohexylamine
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	9	3	-	-	-	-	2 Chromatid Fragments
5 ppm, 7 h/day	500	9	2	-	-	-	-	1 Chromatid Fragment 1 Ring Chromosome
25 ppm, 7 h/day	500	9	1	-	-	-	-	2 Dicentrics
EMS, 100 mg/kg/day	500	31	24	4	3	-	-	9 Chromatid Fragments 1 Exchange

TABLE CA-MD-M-1 (Supplementary)

N-Methyl dicyclohexylamine
Cytogenetic Analysis of Rat Bone Marrow Cells
Supplementary Observations
Males

Multiple Dosing

Sampling Time: 6 h

Group	Animal No.	Sex	Miscellaneous Observations
EMS, 100 mg/kg/day	153	♂	2 Chromosomes split at centromere

TABLE CA-MD-M-2

N-Methyl dicyclohexylamine
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	0.322	0.052		0.219	0.039	
5 ppm	0.321	0.052	-0.02	0.211	0.039	-0.15
25 ppm	0.320	0.052	-0.03	0.200	0.039	-0.34
EMS, 100 mg/kg	0.606	0.052	3.88***	0.450	0.039	4.14***

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

TABLE CA-MD-F-1

N-Methyl dicyclohexylamine
Cytogenetic Analysis of Rat Bone Marrow Cell
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	12	3	-	-	-	-	1 Chromatid Fragment
5 ppm, 7 h/day	500	16	3	-	-	-	-	1 Chromatid Fragment 1 Dicentric
25 ppm, 7 h/day	350	26	2	-	-	-	-	4 Chromatid Fragments
EMS, 100 mg/kg/day	500	43	25	1	5	-	-	3 Chromatid Fragments 2 Multi Aberrations

TABLE CA-MD-F-2

N-Methyl dicyclohexylamine
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	0.327	0.057		0.211	0.041	
5 ppm	0.346	0.057	0.24	0.221	0.041	0.18
25 ppm	0.549	0.068	2.51	0.284	0.049	1.16
EMS, 100 mg/kg	0.681	0.057	4.41***	0.438	0.041	3.92***

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

TABLE CA-M6-1

N-Methyl dicyclohexylamine
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	25	-	-	3	-	-	1 Robertsonian Translocation
5 ppm, 7 h/day	455	18	1	-	1	1	-	1 Robertsonian Translocation
20 ppm, 7 h/day	500	7	-	-	2	-	-	-
EMS, 100 mg/kg/day	451	61	2	-	5	1	1	3 Chromosomal Fragments 1 Chromatid Fragment

TABLE CA-M6-2

N-Methyl dicyclohexylamine
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	0.428	0.071		0.160	0.041	
5 ppm	0.424	0.071	-0.04	0.207	0.041	0.81
20 ppm	0.280	0.071	-1.48	0.141	0.041	-0.35
EMS, 250 mg/kg	0.667	0.071	2.40*	0.326	0.041	2.87**

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

TABLE CA-M24-1

N-Methyl dicyclohexylamine
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	457	8	-	-	5	-	-	-
5 ppm, 7 h/day	500	3	-	-	-	-	-	1 Chromosomal Fragment
20 ppm, 7 h/day	450	4	-	-	1	-	-	1 Exchange
EMS, 250 mg/kg/day	500	40	24	3	4	-	-	2 Chromatid Fragments 6 Multi Aberrations 19 Exchanges 1 Pair of Minutes 3 Minutes

TABLE CA-M24-2

N-Methyl dicyclohexylamine
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	0.351	0.059		0.163	0.038	
5 ppm	0.220	0.059	-1.58	0.160	0.038	-0.04
20 ppm	0.263	0.062	-1.04	0.163	0.040	0.00
EMS, 250 mg/kg	0.677	0.059	3.93***	0.525	0.038	6.78***

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

TABLE CA-M48-1

N-Methyl dicyclohexylamine
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Males

Single Dosing — Sampling Time: 48 h

Group	Number of Spreads Observed	Observed Aberrations						Miscellaneous
		Chromatid			Chromosome			
		Gap	B w F	B w/o F	Gap	B w F	B w/o F	
Air Control, 7 h/day	467	3	-	-	-	-	-	1 Pair of Minutes
5 ppm, 7 h/day	500	9	1	-	-	-	-	-
20 ppm, 7 h/day	500	8	-	-	-	-	-	1 Chromatid Fragment
EMS, 250 mg/kg/day	500	15	5	-	-	-	-	2 Multi Aberrations 2 Exchanges 1 Chromatid Fragment

TABLE CA-M48-1 (Supplementary)

N-Methyl dicyclohexylamine
Cytogenetic Analysis of Rat Bone Marrow Cells
Supplementary Observations
Males

Single Dosing

Sampling Time: 48 h

Group	Animal No.	Sex	Miscellaneous Observations
Air Control, 7 h/day	25	♂	1 Chromosome split at centromere
EMS, 250 mg/kg/day	112	♂	1 Chromosome split at centromere
	111	♂	1 Chromosome split at centromere

TABLE CA-M48-2

N-Methyl dicyclohexylamine
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	0.221	0.061		0.170	0.031	
5 ppm	0.260	0.061	0.45	0.160	0.031	-0.22
20 ppm	0.239	0.061	0.22	0.160	0.031	-0.22
EMS, 250 mg/kg	0.329	0.061	1.24	0.260	0.031	2.06*

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

TABLE CA-F6-1

N-Methyl dicyclohexylamine
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Females

Single Dosing

Sampling Time: 6 h

Group	Number of Spreads Observed	Observed Aberrations						Miscellaneous
		Chromatid			Chromosome			
		Gap	B w F	B w/o F	Gap	B w F	B w/o F	
Air Control, 7 h/day	500	15	1	-	2	-	-	-
5 ppm, 7 h/day	462	6	-	-	1	-	-	-
20 ppm, 7 h/day	414	9	-	-	2	-	-	2 Chromatid Fragments
EMS, 250 mg/kg/day	500	35	4	-	3	2	-	1 Chromatid Fragment

TABLE CA-F6-2

N-Methyl dicyclohexylamine
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	0.369	0.061		0.160	0.034	
5 ppm	0.264	0.061	-1.21	0.155	0.034	-0.12
20 ppm	0.395	0.065	0.29	0.218	0.036	1.14
EMS, 250 mg/kg	0.509	0.061	1.62	0.231	0.034	1.44

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

TABLE CA-F24-1

N-Methyl dicyclohexylamine
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Females

Single Dosing

Sampling Time: 24 h

Group	Number of Spreads Observed	Observed Aberrations						Miscellaneous
		Chromatid			Chromosome			
		Gap	B w F	B w/o F	Gap	B w F	B w/o F	
Air Control, 7 h/day	500	7	-	-	1	-	-	-
5 ppm, 7 h/day	500	13	1	-	1	-	-	-
20 ppm, 7 h/day	499	10	-	-	3	-	-	-
EMS, 250 mg/kg/day	500	56	34	1	4	-	-	2 Chromatid Fragments 8 Multi Aberrations 21 Exchanges 6 Minutes

TABLE CA-F24-1 (Supplementary)

N-Methyl dicyclohexylamine
Cytogenetic Analysis of Rat Bone Marrow Cels
Supplementary Observatons
Females

Single Dosing — Sampling Time 24 h

Group	Animal No.	Sex	Miscellaneous Observations
Ethyl methanesulphonate	263	♀	Telomeric stickiness

TABLE CA-F24-2

N-Methyl dicyclohexylamine
Cytogenetic Analysis of Rat Bone Marrow Cells
Summary of Observed Aberrations
Females

Single Dosing — Sampling Time: 24 h

Treatment Group	Spreads with Aberrations					
	Total			Excluding Gaps		
	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t	Mean of Freeman-Tukey Binomial Transformation	S.E. of Mean	t
Air Control	0.291	0.063		0.141	0.033	
5 ppm	0.350	0.063	0.68	0.160	0.033	0.43
20 ppm	0.314	0.063	0.26	0.141	0.033	0.00
EMS, 250 mg/kg	0.734	0.063	5.00***	0.590	0.033	9.61***

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

TABLE CA-F48-1

N-Methyl dicyclohexylamine
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Females

Single Dosing

Sampling Time: 48 h

Group	Number of Spreads Observed	Observed Aberrations						Miscellaneous
		Chromatid			Chromosome			
		Gap	B w F	B w/o F	Gap	B w F	B w/o F	
Air Control, 7 h/day	450	4	-	-	-	-	-	1 Pair of Minutes
5 ppm, 7 h/day	500	3	-	-	-	-	-	1 Exchange
20 ppm, 7 h/day	415	5	-	-	-	-	-	-
EMS, 250 mg/kg/day	420	6	1	-	-	-	-	2 Multi Aberrations 2 Exchanges

TABLE CA-F48-2

N-Methyl dicyclohexylamine
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	0.251	0.048		0.162	0.029	
5 ppm	0.211	0.045	-0.61	0.160	0.028	-0.05
20 ppm	0.253	0.048	0.03	0.153	0.029	-0.23
EMS, 250 mg/kg	0.297	0.045	0.70	0.258	0.028	2.36*

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

TABLE DL-1

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

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	5 ppm	25 ppm	5 x 100 mg/kg EMS
1	85%	100%	83%	75%
2	90%	95%	94%	55%
3	100%	100%	100%	75%
4	95%	100%	94%	85%
5	85%	100%	94%	90%
6	95%	100%	100%	100%
7	100%	100%	89%	85%
8	95%	100%	100%	90%
9	95%	100%	94%	95%
10	90%	100%	94%	95%

TABLE DL-2

N-Methyl dicyclohexylamine
Dominant Lethal Test in Rats
Pregnancy Frequency (Females with Implantations)

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)		5 ppm		25 ppm		5 x 100 mg/kg EMS	
1	17/20	85%	20/20	100%	15/18	83%	14/20	70%
2	18/20	90%	19/20	95%	17/20	85%	4/20	20%
3	20/20	100%	20/20	100%	18/18	100%	8/20	40%
4	19/20	95%	20/20	100%	16/18	89%	15/20	75%
5	17/20	85%	20/20	100%	16/18	89%	18/20	90%
6	19/20	95%	20/20	100%	18/18	100%	18/20	90%
7	20/20	100%	20/20	100%	16/18	89%	17/20	85%
8	19/19	100%	19/19	100%	18/18	100%	18/20	90%
9	19/20	95%	20/20	100%	17/18	94%	18/20	90%
10	18/20	90%	20/20	100%	17/18	94%	19/20	95%

TABLE DL-3

N-Methyl dicyclohexylamine
Dominant Lethal Test in Rats
Total Number of Corpora Lutea per Pregnancy

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	5 ppm	25 ppm	5 x 100 mg/kg EMS
1	[1] 12.7 ± 0.490	12.2 ± 0.451	13.3 ± 0.521	8.8 ± 0.547***
2	12.5 ± 0.519	14.0 ± 0.505*	13.2 ± 0.534	2.5 ± 0.646**
3	12.4 ± 0.475	13.1 ± 0.475	11.9 ± 0.501	3.9 ± 0.639***
4	13.3 ± 0.647	14.4 ± 0.631	12.8 ± 0.705	12.9 ± 0.954
5	13.1 ± 0.513	12.9 ± 0.473	14.4 ± 0.529	13.2 ± 0.521
6	12.7 ± 0.457	12.8 ± 0.445	12.4 ± 0.470	13.6 ± 0.611
7	13.4 ± 0.571	14.4 ± 0.571	13.5 ± 0.638	14.1 ± 0.419
8	13.3 ± 0.598	13.8 ± 0.598	13.1 ± 0.615	13.1 ± 0.565
9	13.8 ± 0.505	13.3 ± 0.492	13.4 ± 0.533	13.4 ± 0.573
10	13.1 ± 0.567	13.1 ± 0.538	12.0 ± 0.583	13.2 ± 0.414

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

TABLE DL-4

N-Methyl dicyclohexylamine
Dominant Lethal Test in Rats
Total Implantations per Pregnancy

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	5 ppm	25 ppm	5 x 100 mg/kg EMS
1	[1] 13.4 ± 0.582	12.7 ± 0.537	13.4 ± 0.620	9.7 ± 0.997**
2	13.0 ± 0.502	12.2 ± 0.489	13.5 ± 0.517	1.3 ± 0.250***
3	12.4 ± 0.674	13.5 ± 0.674	12.3 ± 0.710	1.4 ± 0.183***
4	12.6 ± 0.533	13.3 ± 0.519	12.6 ± 0.581	11.1 ± 0.946
5	12.5 ± 0.562	12.8 ± 0.518	13.8 ± 0.579	12.3 ± 0.600
6	12.3 ± 0.477	13.5 ± 0.465	13.4 ± 0.490	13.2 ± 0.459
7	12.8 ± 0.584	14.4 ± 0.584	13.7 ± 0.653	13.6 ± 0.486
8	13.8 ± 0.322	13.2 ± 0.322	13.1 ± 0.331	13.1 ± 0.457
9	13.8 ± 0.566	12.3 ± 0.551	13.0 ± 0.598	12.6 ± 0.776
10	12.4 ± 0.644	12.8 ± 0.611	12.9 ± 0.662	13.2 ± 0.327

1 = Mean ± standard error of mean

**P<0.01

***P<0.001

TABLE DL-5

N-Methyl dicyclohexylamine
Dominant Lethal Test in Rats
Live Implantations per Pregnancy

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	5 ppm	25 ppm	5 x 100 mg/kg EMS
1	[1] 12.7 ± 0.626	12.1 ± 0.578	13.0 ± 0.667	2.8 ± 0.833***
2	12.3 ± 0.559	11.1 ± 0.544	13.0 ± 0.575	0.0 ± 0.000***
3	11.7 ± 0.654	12.9 ± 0.654	11.9 ± 0.690	0.0 ± 0.000***
4	12.1 ± 0.568	12.9 ± 0.553	12.0 ± 0.619	7.6 ± 1.041**
5	11.7 ± 0.542	12.4 ± 0.500	13.5 ± 0.559*	11.6 ± 0.638
6	12.1 ± 0.521	12.9 ± 0.507	12.6 ± 0.535	12.3 ± 0.510
7	12.2 ± 0.586	13.7 ± 0.586	12.9 ± 0.656	12.4 ± 0.665
8	12.8 ± 0.318	12.5 ± 0.318	12.3 ± 0.326	12.4 ± 0.390
9	13.2 ± 0.590	11.7 ± 0.575	12.1 ± 0.623	12.1 ± 0.782
10	11.8 ± 0.640	12.4 ± 0.608	12.6 ± 0.659	12.7 ± 0.342

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

TABLE DL-6

N-Methyl dicyclohexylamine
Dominant Lethal Test in Rats
Live Implantations and Late Deaths per Pregnancy

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	5 ppm	25 ppm	5 x 100 mg/kg EMS
1	[1] 12.7 ± 0.620	12.1 ± 0.572	13.0 ± 0.660	2.8 ± 0.833***
2	12.3 ± 0.565	11.3 ± 0.550	13.0 ± 0.581	0.0 ± 0.000***
3	11.7 ± 0.652	12.9 ± 0.652	11.9 ± 0.688	0.0 ± 0.000***
4	12.2 ± 0.562	12.9 ± 0.548	12.1 ± 0.612	7.6 ± 1.041**
5	11.7 ± 0.542	12.4 ± 0.500	13.5 ± 0.559*	11.6 ± 0.638
6	12.1 ± 0.520	12.9 ± 0.507	12.6 ± 0.534	12.3 ± 0.511
7	12.3 ± 0.593	13.7 ± 0.593	13.1 ± 0.663	12.5 ± 0.681
8	12.8 ± 0.320	12.6 ± 0.320	12.3 ± 0.329	12.6 ± 0.406
9	13.2 ± 0.588	11.7 ± 0.573	12.2 ± 0.622	12.1 ± 0.782
10	11.8 ± 0.642	12.4 ± 0.609	12.6 ± 0.661	12.7 ± 0.342

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

TABLE DL-7

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

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)		5 ppm		25 ppm		5 x 100 mg/kg EMS	
	>0	>1	>0	>1	>0	>1	>0	>1
1	9/17	3/17	9/20	3/20	6/15	0/15	12/14	12/14
2	8/18	1/18	11/19	3/19	6/17	2/17	4/4	1/4
3	11/20	4/20	9/20	3/20	6/18	1/18	8/8	3/8
4	6/19	2/19	6/20	2/20	7/16	1/16	13/15	12/15
5	8/17	3/17	7/20	1/20	5/16	0/16	10/18	4/18
6	4/19	0/19	6/20	2/20	11/18	4/18	11/18	4/18
7	7/20	2/20	7/20	4/20	8/16	2/16	8/17	4/17
8	12/19	6/19	8/19	3/19	9/18	4/18	5/18	4/18
9	7/19	2/19	9/20	2/20	8/17	3/17	7/18	3/18
10	7/18	3/18	6/20	1/20	3/17	2/17	8/19	1/19

TABLE DL-8

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

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	5 ppm	25 ppm	5 x 100 mg/kg EMS
1	[1] 1.878 ± 0.2038	1.746 ± 0.1879	1.566 ± 0.2170	5.105 ± 0.5179***
2	1.755 ± 0.2361	2.053 ± 0.2298	1.620 ± 0.2430	2.597 ± 0.1830
3	1.924 ± 0.1905	1.746 ± 0.1905	1.512 ± 0.2008	2.689 ± 0.1340*
4	1.524 ± 0.1850	1.497 ± 0.1803	1.664 ± 0.2016	3.703 ± 0.3989***
5	1.859 ± 0.2029	1.532 ± 0.1870	1.442 ± 0.2091	1.948 ± 0.2157
6	1.298 ± 0.1999	1.574 ± 0.1948	2.059 ± 0.2053*	2.027 ± 0.2096*
7	1.597 ± 0.2096	1.696 ± 0.2096	1.799 ± 0.2343	2.039 ± 0.3285
8	2.155 ± 0.2141	1.711 ± 0.2141	1.870 ± 0.2200	1.588 ± 0.2361
9	1.655 ± 0.2162	1.739 ± 0.2107	1.829 ± 0.2285	1.672 ± 0.2115
10	1.705 ± 0.1945	1.461 ± 0.1845	1.336 ± 0.2001	1.634 ± 0.1791

1 = Mean ± standard error of mean

*P<0.05

***P<0.001

TABLE DL-9

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

Multiple Dosing

Assessment Week from Dosing	Air Control (0 ppm)	5 ppm	25 ppm	5 x 100 mg/kg EMS
1	[1] 0.510 ± 0.0630	0.506 ± 0.0580	0.420 ± 0.0670	1.960 ± 0.1895***
2	0.486 ± 0.0695	0.588 ± 0.0676	0.440 ± 0.0715	2.377 ± 0.0435***
3	0.551 ± 0.0518	0.462 ± 0.0518	0.440 ± 0.0546	2.399 ± 0.0318***
4	0.050 ± 0.0783	0.403 ± 0.0763	0.463 ± 0.0853	1.269 ± 0.1697**
5	0.524 ± 0.0544	0.421 ± 0.0501	0.382 ± 0.0561	0.564 ± 0.0690
6	0.369 ± 0.0574	0.432 ± 0.0560	0.553 ± 0.0590*	0.556 ± 0.0595*
7	0.456 ± 0.0588	0.445 ± 0.0588	0.483 ± 0.0657	0.558 ± 0.0977
8	0.570 ± 0.0569	0.464 ± 0.0569	0.505 ± 0.0585	0.427 ± 0.0605
9	0.442 ± 0.0617	0.515 ± 0.0601	0.494 ± 0.0652	0.472 ± 0.0582
10	0.483 ± 0.0534	0.404 ± 0.0507	0.374 ± 0.0550	0.443 ± 0.0486

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

TABLE SA-1

N-Methyl dicyclohexylamine
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	9602	7	31	162	68	130	398	0.07	0.31	1.62	0.68	1.30	3.98
5 ppm, 7 h/day	9517[1]	5	17	182	174	105	483	0.05	0.17	1.82	1.74	1.05	4.83
	[2]	5	16	163	96	98	378	0.06	0.18	1.81	1.07	1.09	4.20
25 ppm, 7 h/day	†-	-	-	-	-	-	-	-	-	-	-	-	-
EMS, 200 mg/kg/day	9184	13	52	443	106	202	816	0.13	0.52	4.43	1.06	2.02	8.16

† All animals dead as a result of dosing

1) Including animal No. 331

2) Excluding animal No. 331

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

TABLE SA-2

N-Methyl dicyclohexylamine
Sperm Abnormality Test in Mice
Numbers and Proportions of Abnormalities
Supplementary Dose Results

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†	2876	1	4	55	30	25	115	0.03	0.13	1.83	1.00	0.83	3.83
15 ppm, 7 h/day††	2883	0	4	50	29	34	117	0.00	0.13	1.67	0.97	1.13	3.90

† Control animals (Nos. 411, 412 and 413) not exposed in inhalation chambers

†† First batch of supplementary high dose animals (Nos. 401-410) all dead as a result of dosing. These results are for surviving animals (Nos. 421, 425 and 428) of second replacement batch (Nos. 421-430, exposed for 2 days only)

* A = Hook up-turned or hook elongated

B = Banana-shaped head

C = Amorphous head

D = Folded tail

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

TABLE SA-3

N-Methyl dicyclohexylamine
Sperm Abnormality Test in Mice
Means of Freeman-Tukey Binomial Transformation ± Standard Error

Multiple Dosing

Dose Group	Abnormality Category					
	A	B	C	D	E	Total
Air Control, 7 h/day	5.86 ± 1.038	10.76 ± 1.491	25.40 ± 2.731	16.52 ± 1.702	22.94 ± 1.153	39.92 ± 2.561
5 ppm, 7 h/day	5.41 ± 1.094	8.88 ± 1.571	26.93 ± 2.879	20.25 ± 1.794	21.05 ± 1.216	41.27 ± 2.700
25 ppm, 7 h/day	†-	-	-	-	-	-
EMS, 200 mg/kg/day	7.30 ± 1.038	14.31 ± 1.491	41.07*** ± 2.731	20.58 ± 1.702	28.75** ± 1.153	57.28*** ± 2.561

A = Hook up-turned or hook elongated

B = Banana-shaped head

C = Amorphous head

D = Folded tail

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

† = All animals dead as a result of dosing

**P<0.01

***P<0.001

TABLE RL-1

N-Methyl dicyclohexylamine
Drosophila SLRL Procedure and Results

Compound: N-Methyl dicyclohexylamine **Concentration:** 6.0 ppm **Stock:** A
Length of Exposure: 4.5 min **Test exposure given:** 8.10.80

	Brood 1	Brood 2	Brood 3
F_1 set up	9.10.80	12.10.80	17.10.80
F_2 set up	22.10.80	28.10.80	30.10.80
F_2 scored	4.11.80	11.11.80	13.11.80
F_2 repeats scored	-	-	-
F_3 set up	-	-	-
F_3 scored	-	-	-
F_3 repeats scored	-	-	-

RESULTS

	Brood 1	Brood 2	Brood 3	All Broods
No. of F_1 vials	65	57	56	178
No. of sterile F_1 vials	11	4	8	23
No. of F_1 vials used in F_2	54	53	48	155
No. of F_2 vials set up	600	603	600	1803
No. of F_2 vials scored	577	575	563	1715
No. of F_2 vials containing lethals	0	0	0	0
Frequency of F_2 lethals	0	0	0	0
No. of F_3 vials set up	-	-	-	-
No. of F_3 vials scored	-	-	-	-
No. of F_3 vials containing lethals	-	-	-	-
Frequency of F_3 lethals	-	-	-	-

TABLE RL-1 (continued)

N-Methyl dicyclohexylamine
Drosophila SLRL Procedure and Results

Compound: N-Methyl dicyclohexylamine **Concentration:** 6 ppm **Stock:** B
Length of Exposure: 4.5 min **Test exposure given:** 8.10.80

	Brood 1	Brood 2	Brood 3
F_1 set up	9.10.80	12.10.80	17.10.80
F_2 set up	22.10.80	28.10.80	31.10.80
F_2 scored	5.11.80	11.11.80	12.11.80
F_2 repeats scored	-	-	-
F_3 set up	5.11.80	11.11.80	12.11.80
F_3 scored	18.11.80	25.11.80	25.11.80
F_3 repeats scored	-	-	-

RESULTS

	Brood 1	Brood 2	Brood 3	All Broods
No. of F_1 vials	98	95	81	274
No. of sterile F_1 vials	12	9	10	31
No. of F_1 vials used in F_2	86	86	71	243
No. of F_2 vials set up	600	615	592	1807
No. of F_2 vials scored	571	594	553	1718
No. of F_2 vials containing lethals	0	1	0	1
Frequency of F_2 lethals	0	0.16%	0	0.058%
No. of F_3 vials set up	509	500	400	1409
No. of F_3 vials scored	486	485	383	-
No. of F_3 vials containing lethals	10	0	0	-
Frequency of F_3 lethals	-	0	0	-

Comments: Male number 80, $B1F_3$ - 10/10 vials lethal - re-tested all lethal.

TABLE RL-1 (continued)

N-Methyl dicyclohexylamine
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	9.10.80	12.10.80	17.10.80
F_2 set up	21.10.80	24.10.80	30.10.80
F_2 scored	4.11.80	6.11.80	13.11.80
F_2 repeats scored	-	-	-
F_3 set up	-	-	-
F_3 scored	-	-	-
F_3 repeats scored	-	-	-

RESULTS

	Brood 1	Brood 2	Brood 3	All Broods
No. of F_1 vials	91	87	83	261
No. of sterile F_1 vials	10	7	18	35
No. of F_1 vials used in F_2	81	80	65	226
No. of F_2 vials set up	600	598	601	1799
No. of F_2 vials scored	571	472	574	1617
No. of F_2 vials containing lethals	1	0	0	1
Frequency of F_2 lethals	0.175%	0	0	0.061%
No. of F_3 vials set up	-	-	-	-
No. of F_3 vials scored	-	-	-	-
No. of F_3 vials containing lethals	-	-	-	-
Frequency of F_3 lethals	-	-	-	-

TABLE RL-J (continued)

N-Methyl dicyclohexylamine

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	9.10.80	12.10.80	17.10.80
F_2 set up	21.10.80	24.10.80	30.10.80
F_2 scored	4.11.80	6.11.80	14.11.80
F_2 repeats scored	-	-	-
F_3 set up	-	-	14.11.80
F_3 scored	-	-	26.11.80
F_3 repeats scored	-	-	-

RESULTS

	Brood 1	Brood 2	Brood 3	All Broods
No. of F_1 vials	95	95	85	275
No. of sterile F_1 vials	9	18	18	45
No. of F_1 vials used in F_2	86	77	67	230
No. of F_2 vials set up	669	569	600	1838
No. of F_2 vials scored	616	423	557	1596
No. of F_2 vials containing lethals	0	0	0	0
Frequency of F_2 lethals	0	0	0	0
No. of F_3 vials set up	-	-	600	600
No. of F_3 vials scored	-	-	558	558
No. of F_3 vials containing lethals	-	-	0	0
Frequency of F_3 lethals	-	-	0	0

Comments: In Brood $2F_2$ there were 600 F_2 vials set up originally but due to an accident some were lost.

TABLE RL-1 (continued)

N-Methyl dicyclohexylamine

Drosophila SLRL Procedure and Results

Compound: Ethyl methanesulphonate **Concentration:** 0.4% v/v **Stock:** B
Length of Exposure: 7 h **Test exposure given:** 8.10.80

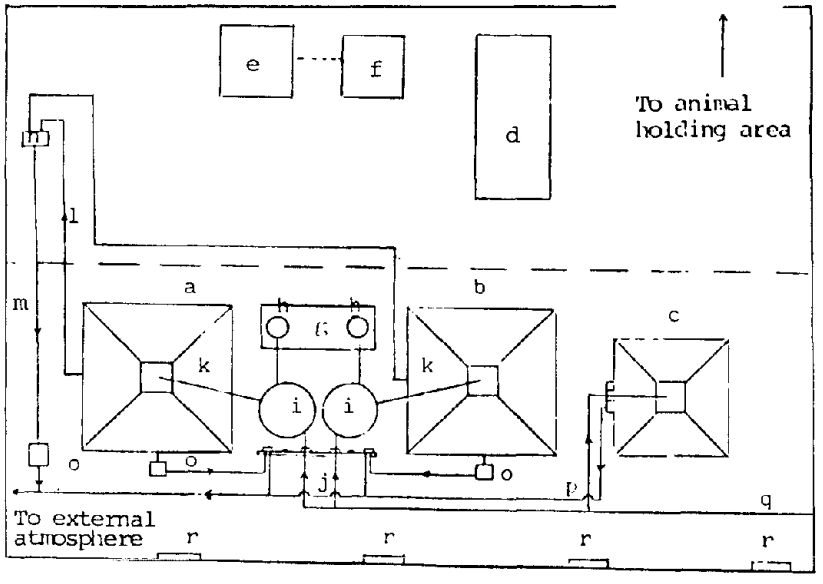
	Brood 1	Brood 2	Brood 3
F_1 set up	9.10.80	-	-
F_2 set up	23.10.80	-	-
F_2 scored	7.11.80	-	-
F_2 repeats scored	-	-	-
F_3 set up	-	-	-
F_3 scored	-	-	-
F_3 repeats scored	-	-	-

RESULTS

	Brood 1	Brood 2	Brood 3	All Broods
No. of F_1 vials	74	-	-	-
No. of sterile F_1 vials	7	-	-	-
No. of F_1 vials used in F_2	67	-	-	-
No. of F_2 vials set up	199	-	-	-
No. of F_2 vials scored	166	-	-	-
No. of F_2 vials containing lethals	34	-	-	-
Frequency of F_2 lethals	20.5%	-	-	-
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-Methyl dicyclohexylamine
Schematic Lay-out of Exposure Area



a High level exposure chamber
b Low level exposure chamber
c Air control exposure chamber
d Work bench
e Gas chromatograph
f Pen recorder
g Temperature controlled
h Test compound reservoir and heating mantle
i Mixing vessel for dilution of test compound
j Flow meter control panel for atmosphere generation
k Vapour transfer line
l Sampling line
m Vacuum line
n Sampling flow rate control panel
o Scrubber
p Exposure chamber extract
q Compressed air line
r High efficiency extract

FIGURE 1b

N-Methyl dicyclohexylamine
Schematic Lay-out of Apparatus

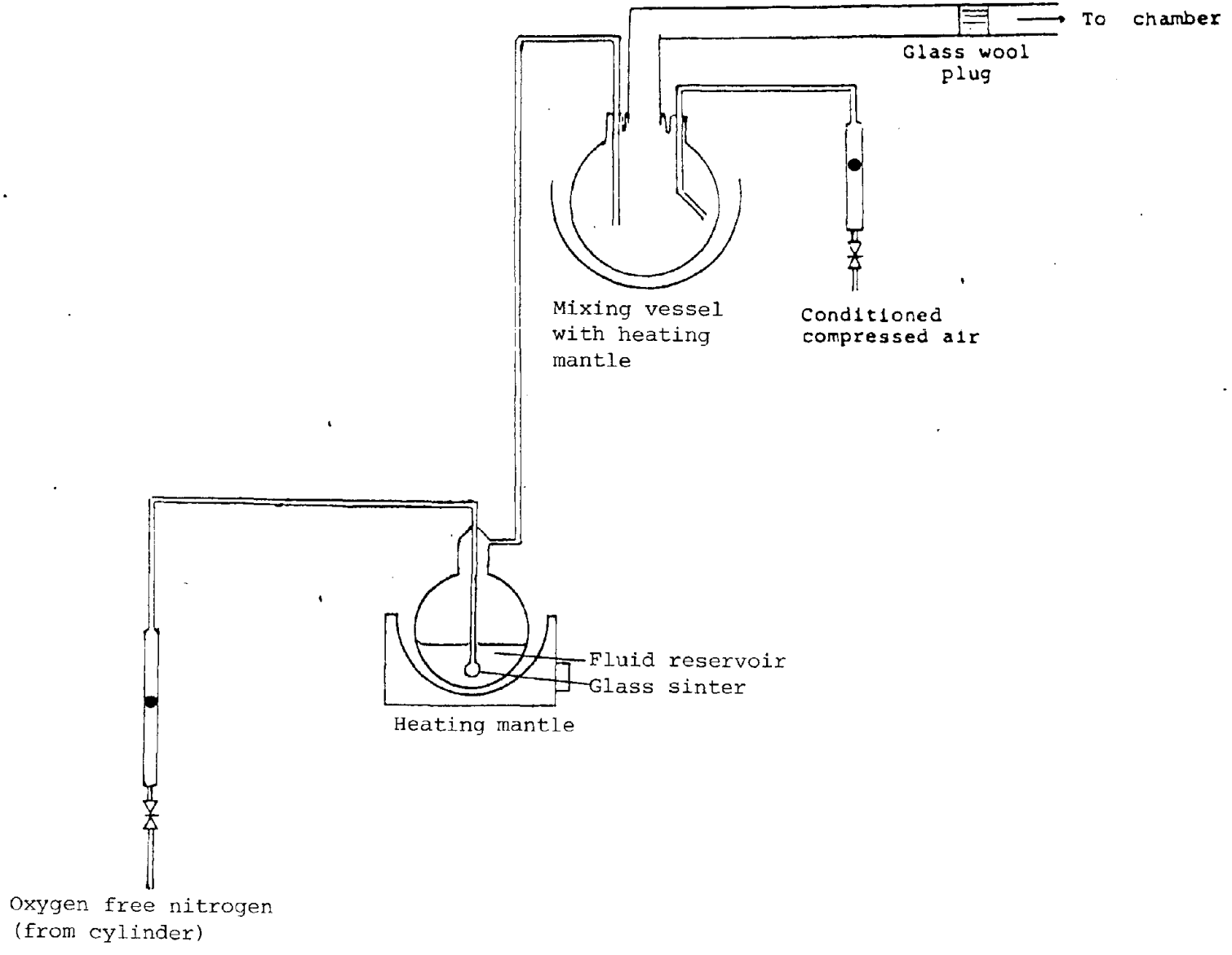


FIGURE 2

N-Methyl dicyclohexylamine
Gas Chromatogram

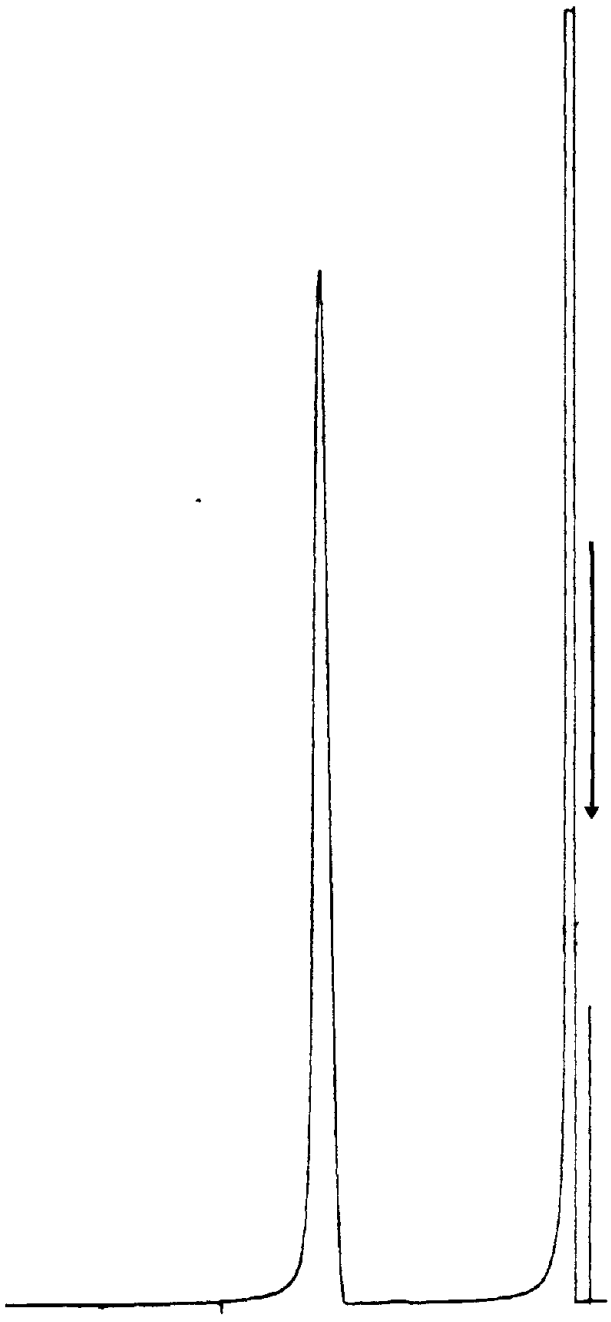


FIGURE 3

N-Methyl dicyclohexylamine
Typical Calibration Curve (Low Level)

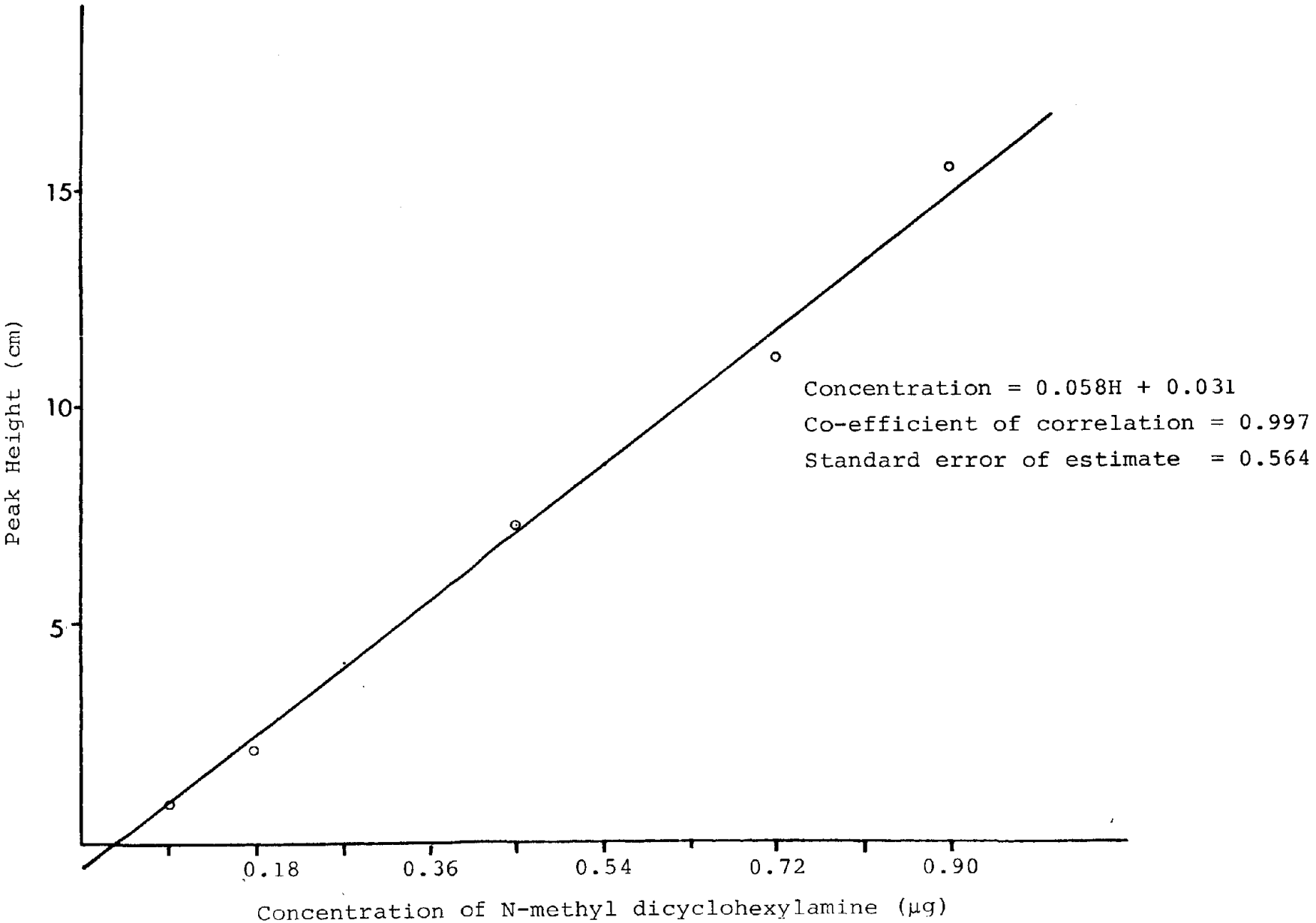


FIGURE 4

N-Methyl dicyclohexylamine
Typical Calibration Curve (High Level)

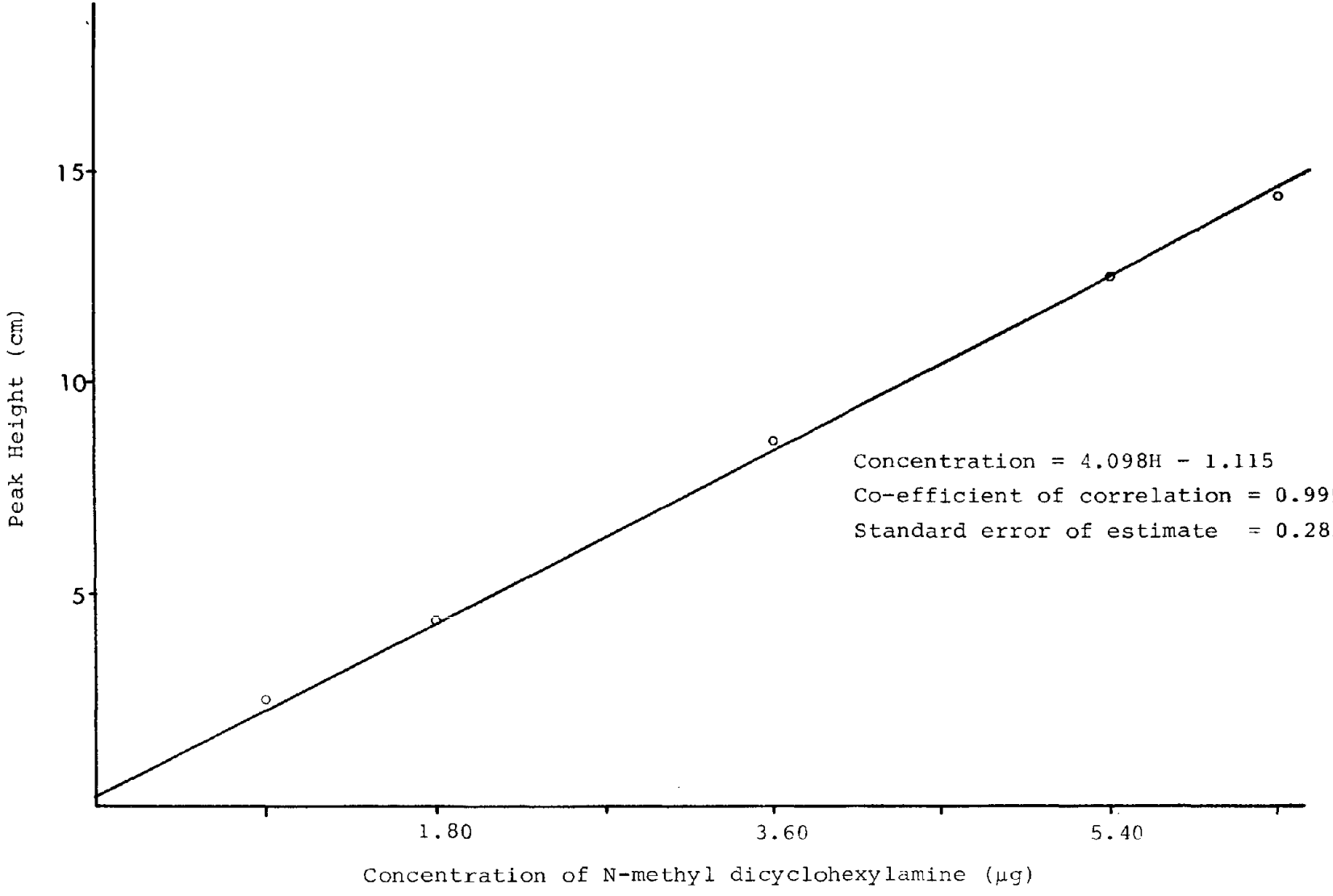


FIGURE 5

N-Methyl dicyclohexylamine
Effect of Time on Desorption

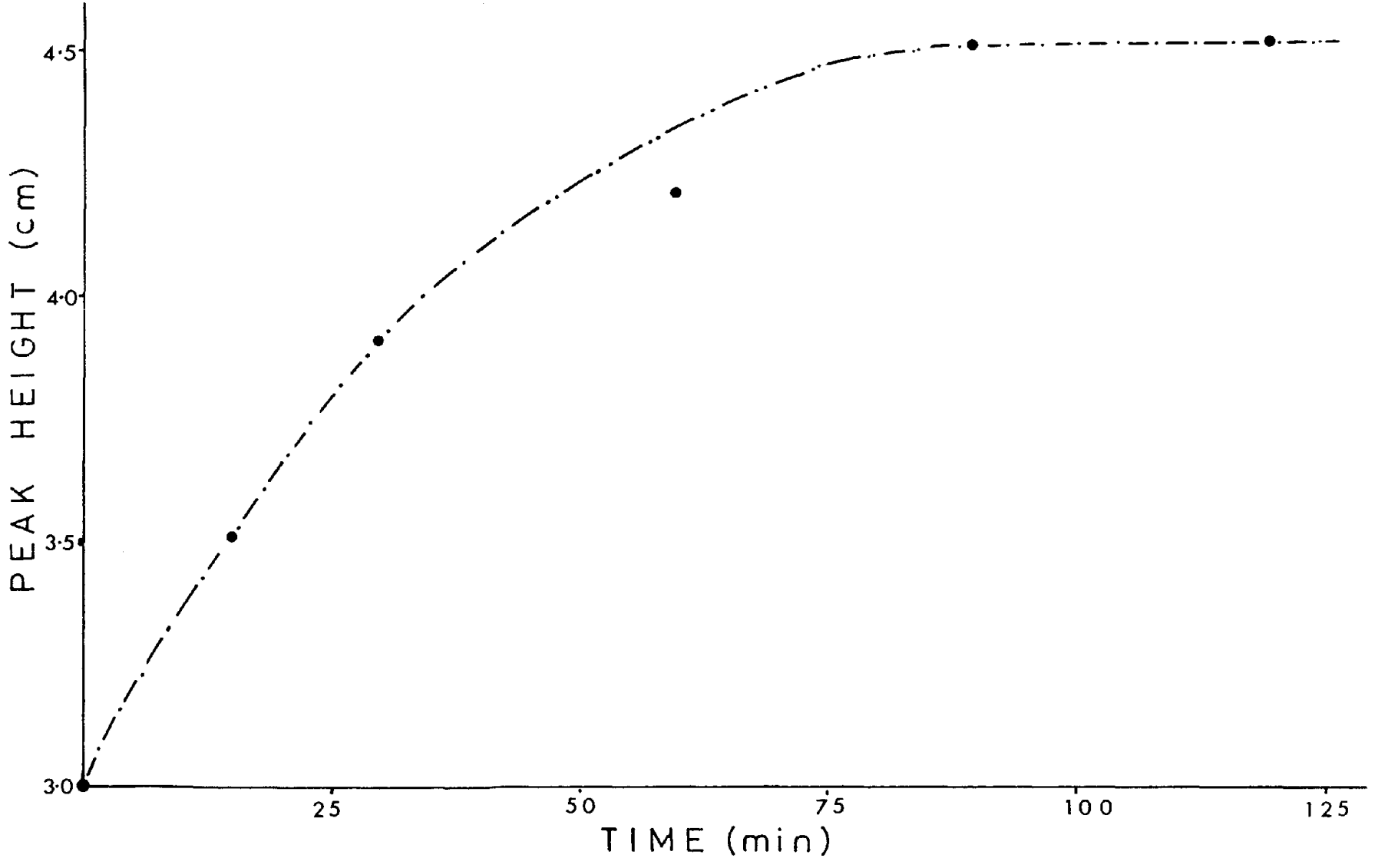


FIGURE 6

N-Methyl dicyclohexylamine
Gas Chromatogram of Commercial Sample

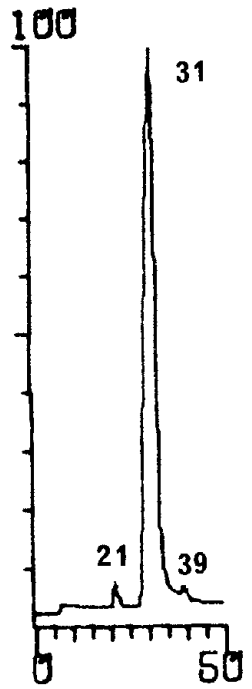


FIGURE 7

N-Methyl dicyclohexylamine
GC-MS of Commercial Sample
Mass Spectrum at Scan 21 (Dicyclohexylamine)

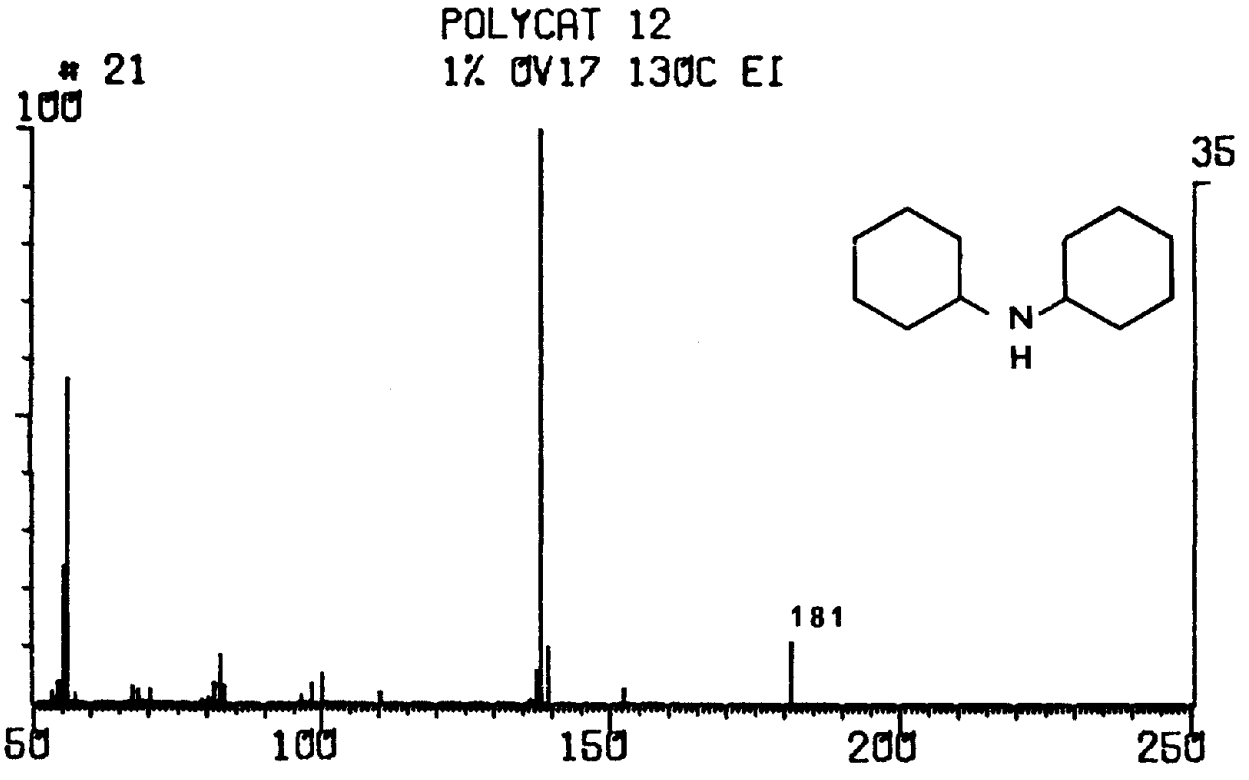


FIGURE 8

N-Methyl dicyclohexylamine
GC-MS of Commercial Sample
Mass Spectrum at Scan 31 (N-Methyl dicyclohexylamine)

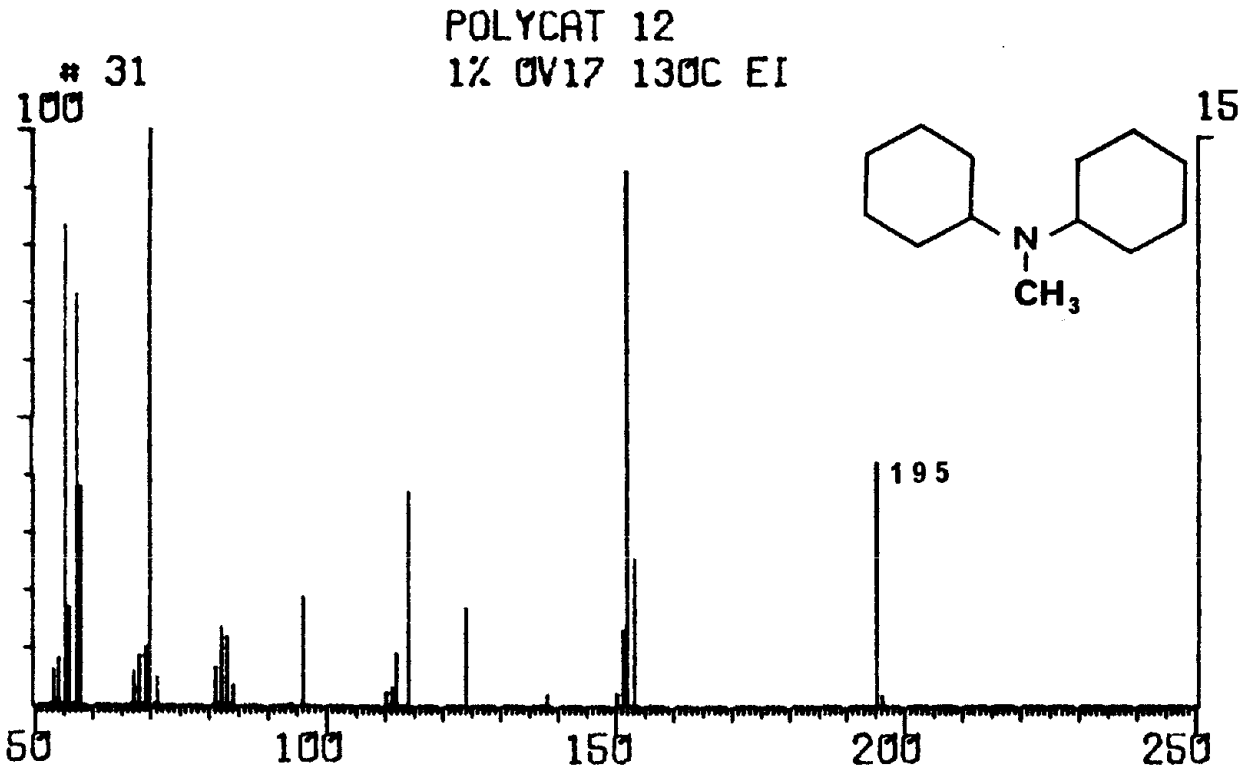


FIGURE 9

N-Methyl dicyclohexylamine
GC-MS of Commercial Sample
Mass Spectrum at Scan 39

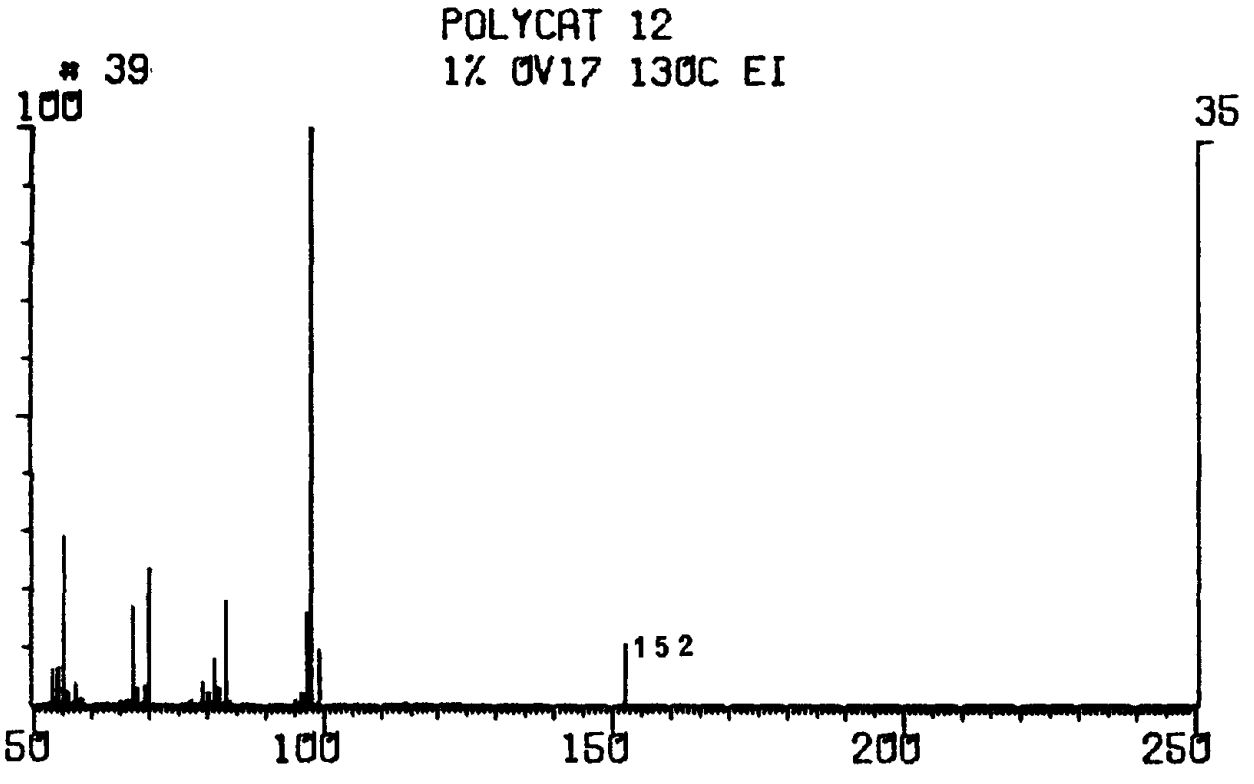


FIGURE 10

N-Methyl dicyclohexylamine
Gas Chromatogram of Sample After 14 h
in Use for Vapour Generation

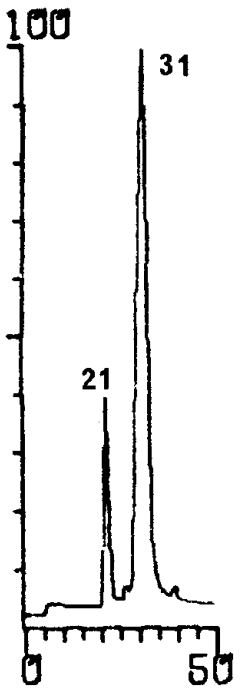


FIGURE 11

N-Methyl dicyclohexylamine
GC-MS of Sample After 12 h in Use for Vapour Generation
Mass Spectrum at Scan 21 (Dicyclohexylamine)

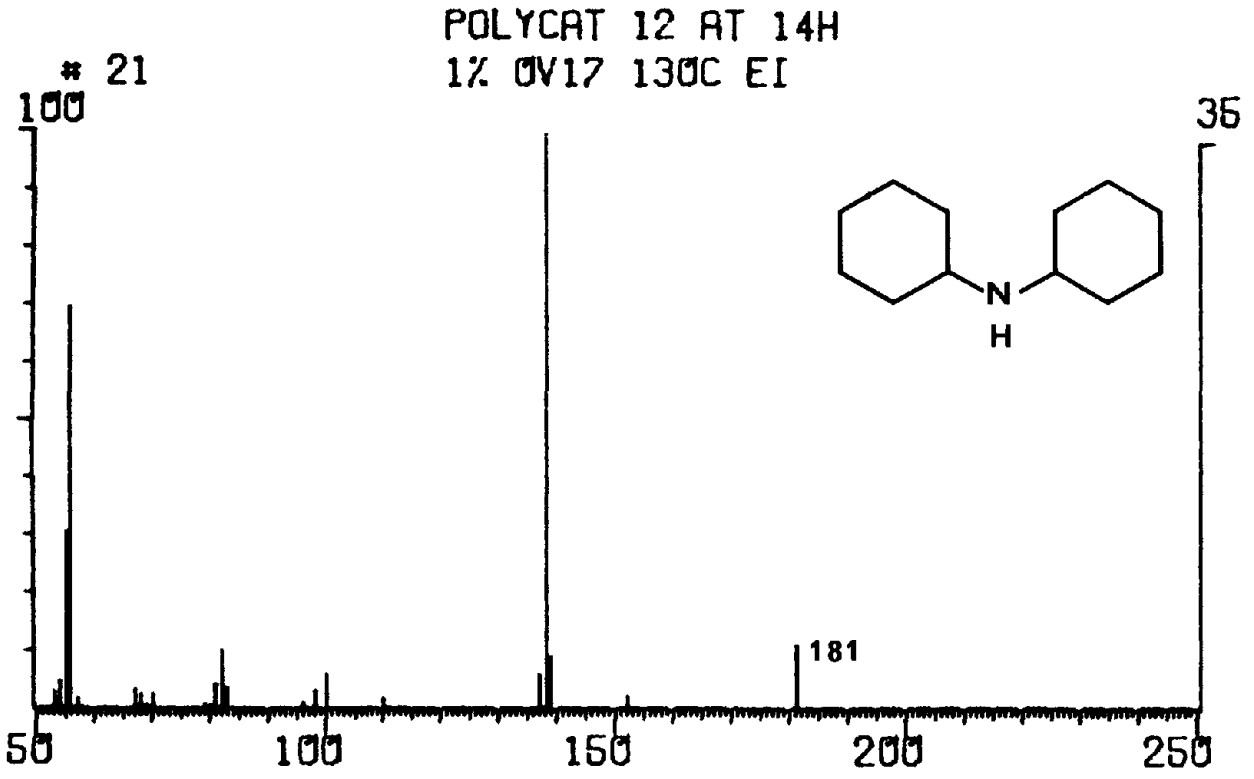


FIGURE 12

N-Methyl dicyclohexylamine
GC-MS of Sample After 12 h in Use for Vapour Generation
Mass Spectrum at Scan 31 (N-Methyl dicyclohexylamine)

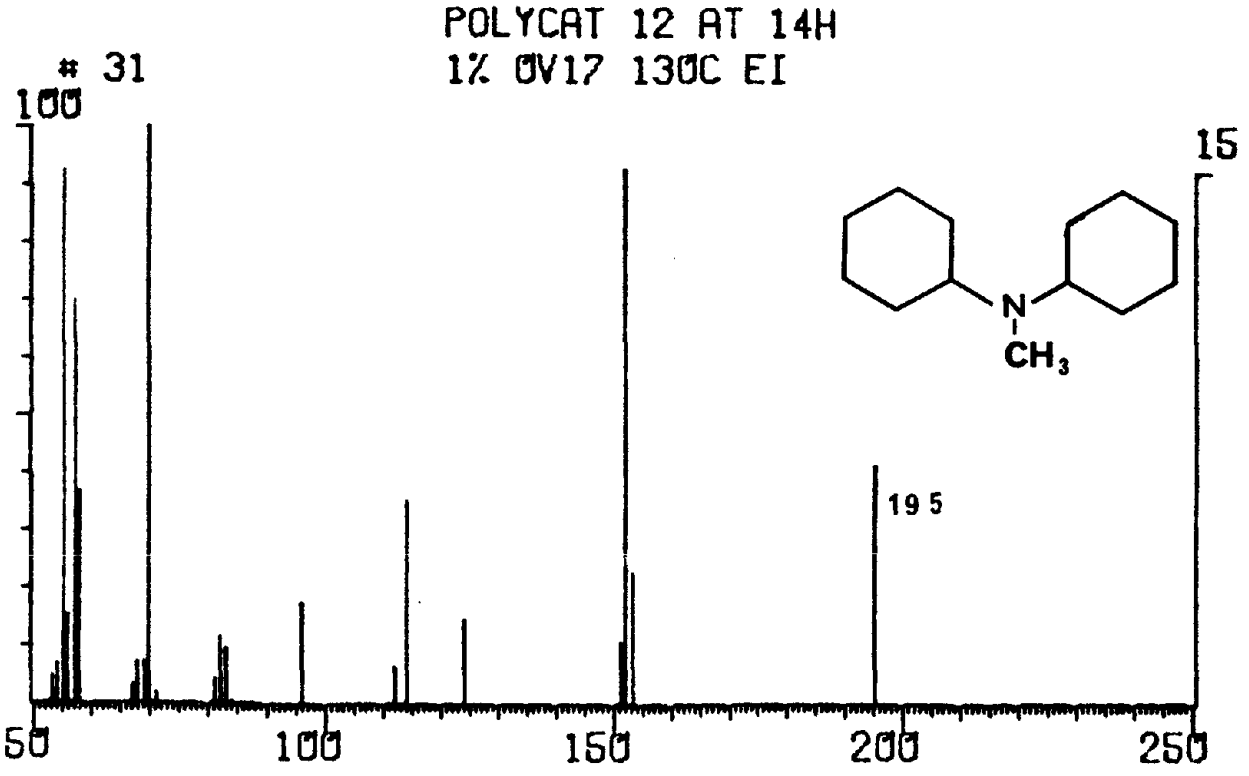


FIGURE 13

N-Methyl dicyclohexylamine
Typical GC-MS of Sample Following Desorption from Charcoal

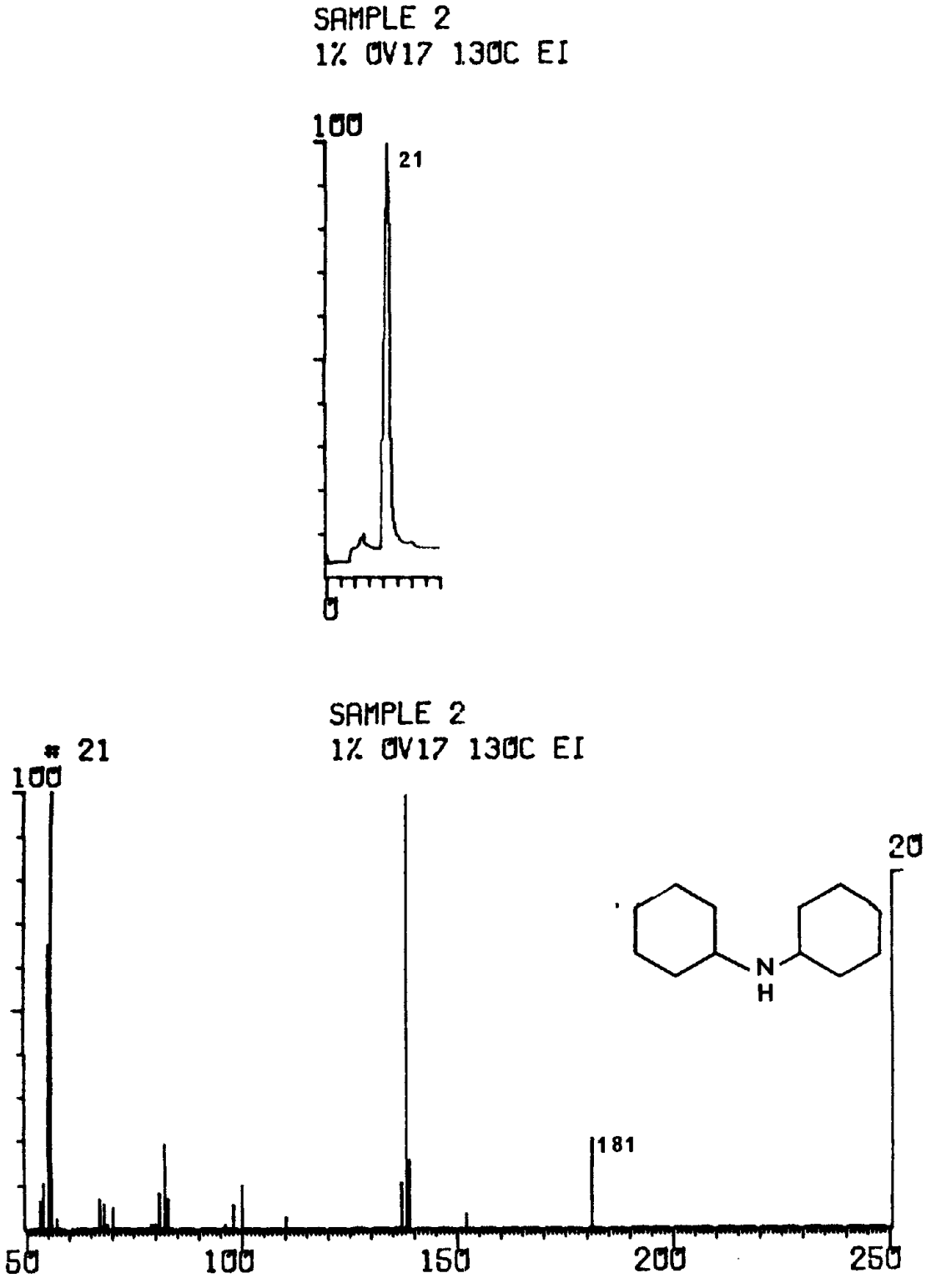


FIGURE 14

N-Methyl dicyclohexylamine
Typical GC-MS of Sample Following Desorption from Charcoal

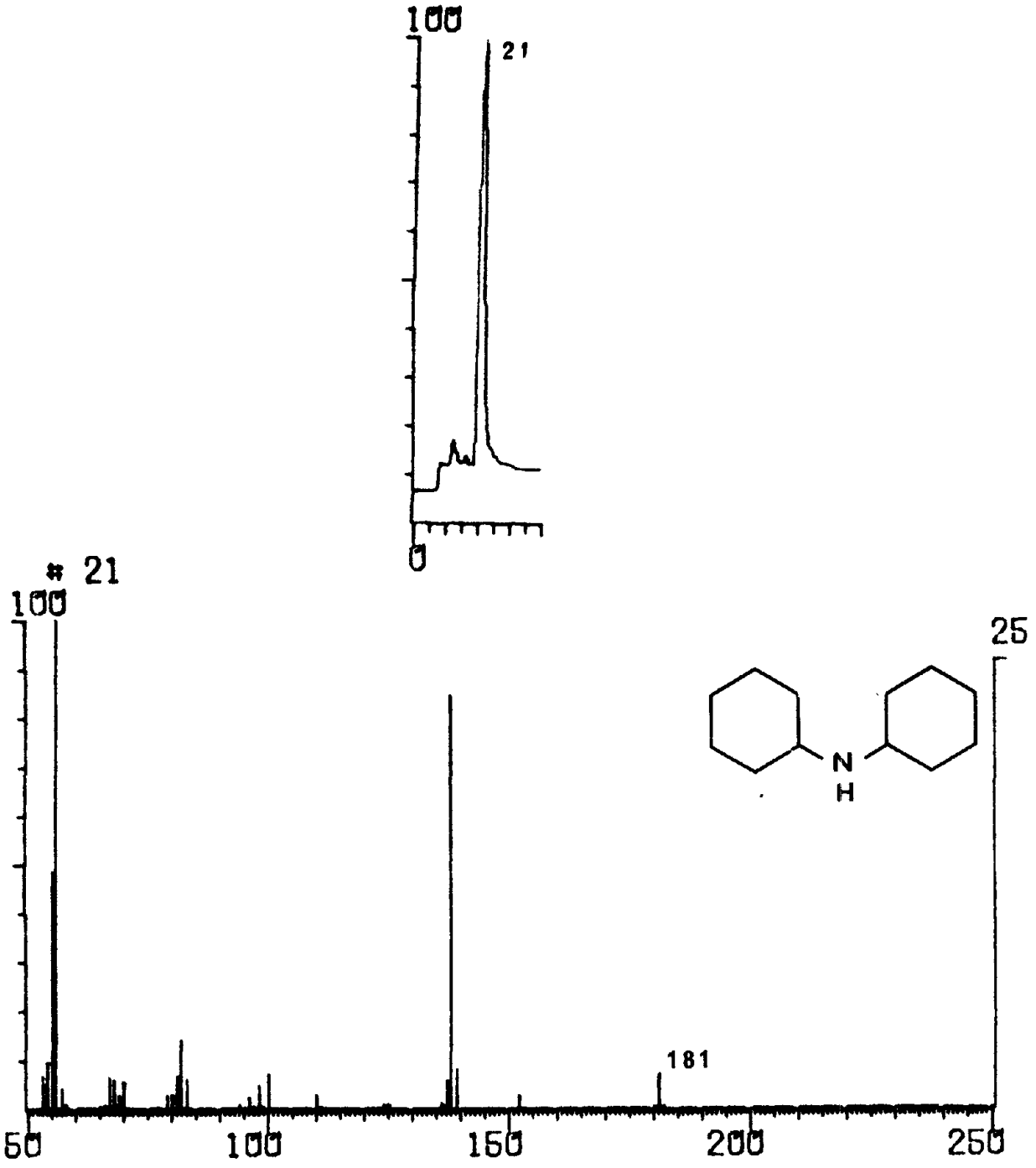
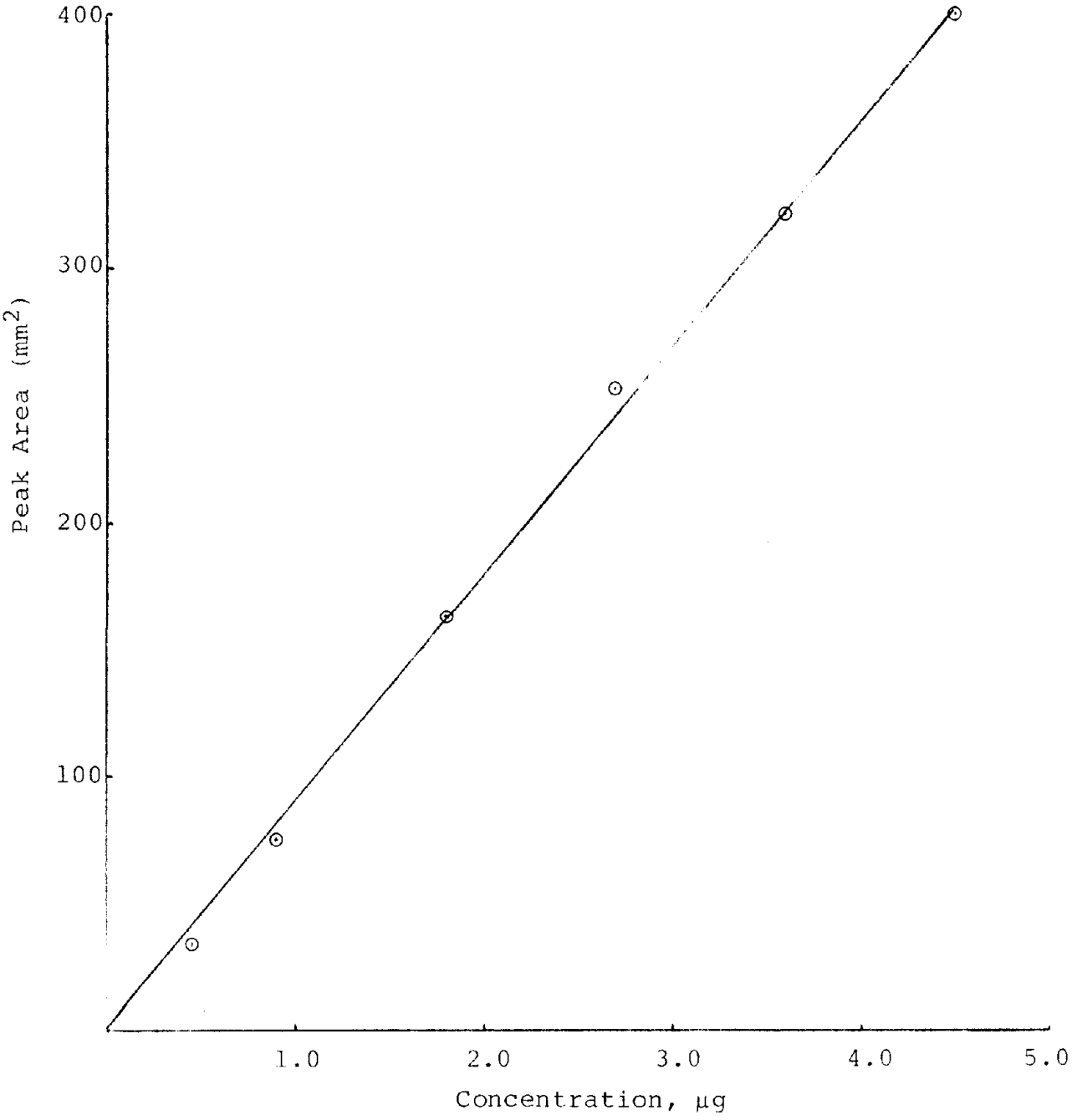


FIGURE 15

N-Methyl dicyclohexylamine
Typical Calibration Graph for High Level
5 October 1980



APPENDIX DIET

N-Methyl dicyclohexylamine
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: 068075

Date of Manufacture: 6th August 1980

	Found Analysis
Moisture	8.8 %
Crude Fat	3.9 %
Crude Protein	20.1 %
Ash	5.6 %
Calcium	1.02 %
Phosphorus	0.86 %
Nitrate	12.0 mg/kg
Nitrite	1.0 mg/kg
Selenium	0.26 mg/kg
Lead	1.38 mg/kg
Arsenic	0.74 mg/kg
Cadmium	0.22 mg/kg
Mercury	0.04 mg/kg
Aflatoxins	NONE DETECTED
Total P.C.B.	NONE DETECTED
Total D.D.T.	0.005 mg/kg
Dieldrin	NONE DETECTED
Lindane	0.002 mg/kg
Heptachlor	NONE DETECTED
Malathion	NONE DETECTED
Total Viable Organisms	3.5×10^3/grm
E. Coli Type 1	NONE DETECTED
Salmonella Species	NONE DETECTED
Moulds	375 / grm

Signed .....

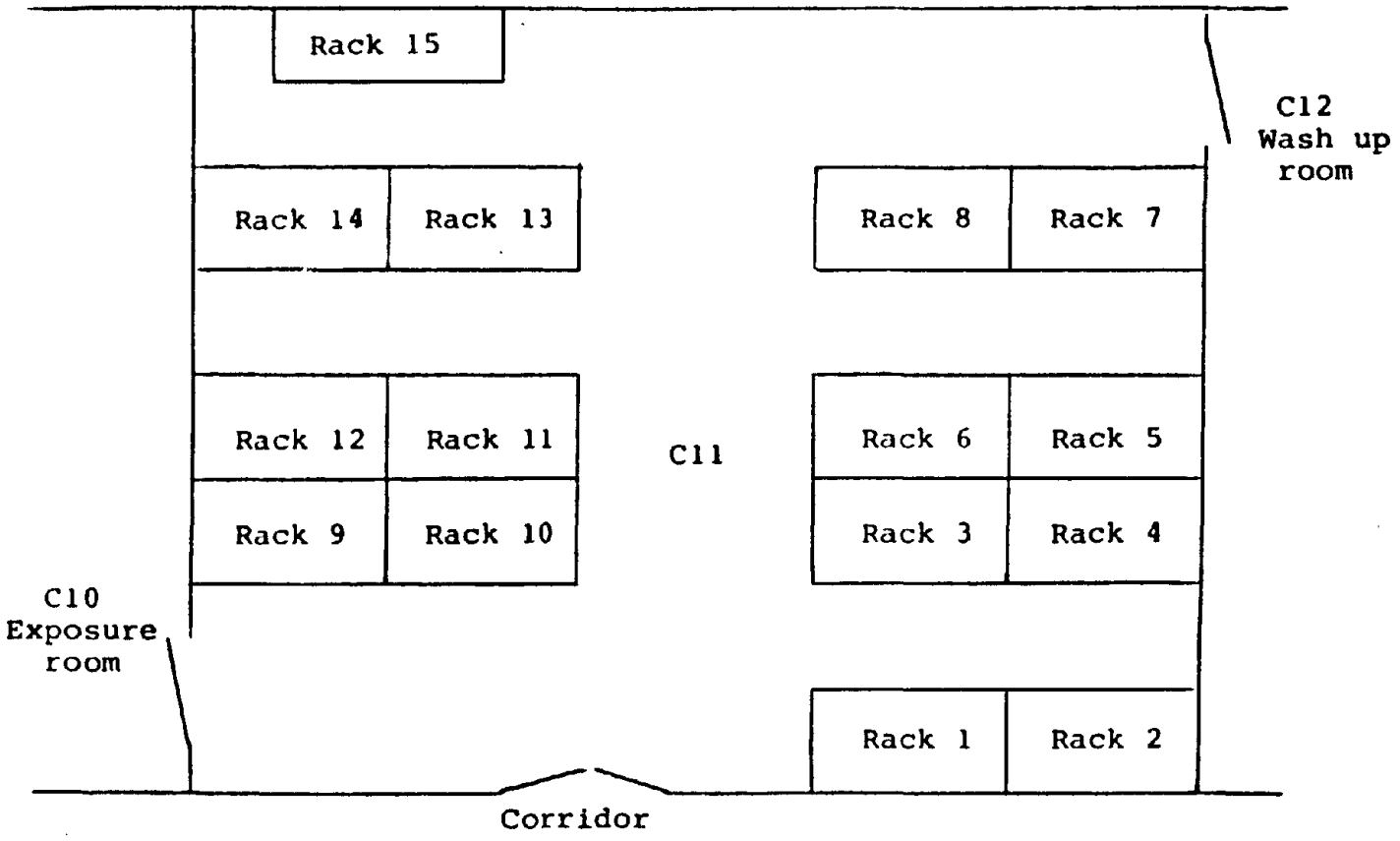
Date ..... 12.16 .....

Registered in England Number 236740
Registered Office: 19 Hanover Square
London, W1R 9DA

A member of the Dalgety Group

APPENDIX Loc-1

N-Methyl dicyclohexylamine
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-Methyl dicyclohexylamine
Examples of Animal Location During Exposure
Exposure Location Sheet

Project No: 409959

Test Compound: Air Control

Exposure Chamber No: 1

Day of Study: 2

Test Concentration: 0

Tier No: 1

Multi-dose Cytogenetic ♂ and ♀

LEFT

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

FRONT REAR

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

RIGHT

SIGNED: _____ DATE: _____

APPENDIX Loc-2 (continued)

N-Methyl dicyclohexylamine
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-Methyl dicyclohexylamine
Exposure Location Sheet

Project No: 409959 Test Concentration: Low

Test Compound: N-Methyl dicyclohexylamine 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-Methyl dicyclohexylamine
Exposure Location Sheet

Project No: 409959 Test Concentration: High

Test Compound: N-Methyl dicyclohexylamine 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-Methyl dicyclohexylamine

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	Bmf	Bw/oF	GAP	Bmf	Bw/oF	EXC.	DIC	DG			
1.												
2.												
3.												
4.												
5.												
6.												
7.												
8.												
9.												
10.												
11.												
12.												
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19.												
20.												
21.												
22.												
23.												
24.												
25.												

APPENDIX FORM-2

N-Methyl dicyclohexylamine

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-Methyl dicyclohexylamine

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-Methyl dicyclohexylamine
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	390	388	392	391	387
	122	409	410	418	421	420
	123	385	380	391	385	386
	124	351	349	352	355	353
	125	370	375	377	379	385
	126	360	360	362	366	366
	127	384	403	405	406	411
	128	384	385	390	392	398
	129	392	392	399	397	403
	130	402	406	411	417	415
	Mean	382.7	384.8	389.7	390.9	392.4
	± S.D.	± 17.9	± 19.7	± 20.9	± 20.9	± 21.4

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	281	227	226	227	228	228
	282	242	240	242	241	245
	283	223	218	217	220	217
	284	240	236	237	240	239
	285	284	278	284	283	282
	286	240	244	240	241	238
	287	246	246	246	245	246
	288	271	270	270	273	266
	289	224	219	225	225	221
	290	271	274	267	274	276
	Mean	246.8	245.1	245.5	247.0	245.8
	± S.D.	± 21.5	± 22.2	± 21.7	± 22.1	± 22.4

APPENDIX TABLE BW-1 (continued)

N-Methyl dicyclohexylamine

Multiple Dosing: 5 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	131	377	380	380	383	383
	132	430	429	431	431	434
	133	375	378	373	371	368
	134	415	416	415	416	416
	135	351	352	347	345	349
	136	350	350	353	352	356
	137	398	397	399	397	399
	138	410	417	416	414	416
	139	364	368	366	368	368
	140	386	390	391	394	396
	Mean	385.6	387.7	387.1	387.1	388.5
	± S.D.	± 27.3	± 27.3	± 28.2	± 28.4	± 28.4

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	291	255	250	246	247	248
	292	232	227	227	228	227
	293	266	264	264	265	265
	294	216	211	212	215	210
	295	281	282	281	281	280
	296	234	234	231	231	232
	297	247	246	247	249	246
	298	234	230	228	231	231
	299	225	220	218	224	221
	300	227	223	223	220	219
	Mean	241.7	238.7	237.7	238.3	237.9
	± S.D.	± 20.3	± 21.8	± 21.7	± 21.5	± 21.9

APPENDIX TABLE BW-1 (continued)

N-Methyl dicyclohexylamine

Multiple Dosing: 25 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	141	379	370	351	347	347
	142	359	347	337	337	331
	143	404	395	377	381	378
	144	357	341	324	322	308
	145	372	360	342	340	344
	146	354	343	330	329	323
	147	325	316	304	302	301
	148	349	327	316	310	306
	149	357	347	336	331	335
	150	366	360	351	348	353
	Mean	362.2	350.6	336.8	334.7	332.6
	± S.D.	± 20.6	± 22.2	± 26.5	± 22.1	± 24.1

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	301	247	240	234	226	225
	302	Dead	Dead	Dead	Dead	Dead
	303	250	242	234	231	228
	304	259	252	250	234	Dead
	305	230	225	219	214	216
	306	238	231	230	228	217
	307	262	257	250	247	241
	308	244	244	236	230	213
	309	239	230	230	222	225
	310	251	240	231	226	224
	Mean	246.7	240.1	234.9	228.7	223.6
	± S.D.	± 10.2	± 10.4	± 9.8	± 9.0	± 8.8

APPENDIX TABLE BW-1 (continued)

N-Methyl dicyclohexylamine

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	151	380	371	364	353	339
	152	396	395	392	387	374
	153	392	385	380	372	370
	154	398	390	385	372	360
	155	375	358	345	331	317
	156	380	362	356	342	331
	157	427	425	418	408	392
	158	416	404	407	393	382
	159	437	432	424	411	408
	160	403	387	366	348	338
	Mean	400.4	390.9	383.7	371.7	361.1
	± S.D.	± 20.7	± 24.5	± 26.7	± 27.9	± 29.3

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Female	311	245	237	233	229	220
	312	254	251	241	237	231
	313	227	223	219	215	212
	314	240	240	240	239	233
	315	271	262	254	242	246
	316	226	223	223	221	216
	317	251	246	242	233	232
	318	251	243	244	239	233
	319	280	275	270	257	255
	320	250	244	240	231	226
	Mean	249.5	244.4	240.6	234.3	230.4
	± S.D.	± 17.0	± 15.9	± 14.5	± 11.6	± 13.1

APPENDIX TABLE BW-2

N-Methyl dicyclohexylamine
Single Exposure Cytogenetics Test
Individual Body Weights (g)

Air Control (0ppm)

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Male	1	362	11	425	21	400
	2	415	12	408	22	411
	3	407	13	416	23	329
	4	380	14	411	24	419
	5	420	15	402	25	379
	6	410	16	430	25	373
	7	440	17	437	27	393
	8	385	18	372	28	383
	9	385	19	376	29	421
	10	399	20	443	30	380
	Mean	400.3		412.0		388.8
	± S.D.	± 22.8		± 23.8		± 27.1

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Female	161	267	171	267	181	238
	162	248	172	235	182	266
	163	223	173	256	183	259
	164	265	174	261	184	230
	165	280	175	245	185	267
	166	300	176	255	186	245
	167	235	177	275	187	269
	168	255	178	229	188	290
	169	265	179	258	189	245
	170	244	180	260	190	250
	Mean	258.2		254.1		255.9
	± S.D.	± 22.3		± 14.1		± 17.7

APPENDIX TABLE BW-2 (continued)

N-Methyl dicyclohexylamine

Single Dosing: 5 ppm

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Male	31	382	41	385	51	397
	32	362	42	405	52	394
	33	406	43	406	53	453
	34	424	44	404	54	424
	35	401	45	386	55	404
	36	400	46	399	56	402
	37	374	47	451	57	434
	38	428	48	479	58	418
	39	363	49	438	59	388
	40	411	50	404	60	416
	Mean	395.1		415.7		413.0
	± S.D.	± 23.8		± 30.4		± 20.1

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Female	191	215	201	261	211	255
	192	242	202	254	212	288
	193	219	203	268	213	232
	194	264	204	232	214	255
	195	236	205	271	215	288
	196	250	206	291	216	266
	197	265	207	277	217	269
	198	240	208	255	218	246
	199	298	209	233	219	275
	200	256	210	260	220	268
	Mean	248.5		260.2		264.2
	± S.D.	± 24.2		± 18.3		± 17.7

APPENDIX TABLE BW-2 (continued)

N-Methyl dicyclohexylamine

Single Dosing: 20 ppm

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Male	61	350	71	410	81	427
	62	420	72	404	82	386
	63	412	73	391	83	396
	64	407	74	422	84	397
	65	449	75	413	85	386
	66	412	76	418	86	378
	67	398	77	405	87	363
	68	426	78	417	88	375
	69	370	79	390	89	408
	70	363	80	Dead	90	381
	Mean ± S.D.	400.7 ± 30.9		407.8 ± 11.4		389.7 ± 18.2

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Female	221	272	231	287	241	231
	222	232	232	263	242	245
	223	267	233	266	243	234
	224	228	234	225	244	234
	225	270	235	287	245	260
	226	276	236	217	246	296
	227	236	237	266	247	266
	228	246	238	245	248	255
	229	Dead	239	300	249	288
	230	262	240	268	250	269
	Mean ± S.D.	254.3 ± 18.9		262.4 ± 26.8		257.8 ± 22.6

APPENDIX TABLE BW-2 (continued)

N-Methyl dicyclohexylamine

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	420	101	411	111	392
	92	390	102	425	112	436
	93	450	103	415	113	375
	94	430	104	411	114	450
	95	395	105	411	115	401
	96	395	106	340	116	395
	97	410	107	409	117	395
	98	435	108	425	118	347
	99	420	109	497	119	385
	100	365	110	443	120	441
	Mean ± S.D.	411.0 ± 25.1		418.7 ± 38.4		404.7 ± 37.9

Sex	Animal Number	6 h Sample Weight	Animal Number	24 h Sample Weight	Animal Number	48 h Sample Weight
Female	251	302	261	280	271	270
	252	265	262	272	272	286
	253	262	263	275	273	230
	254	263	264	255	274	246
	255	267	265	280	275	243
	256	280	266	256	276	263
	257	256	267	256	277	271
	258	280	268	219	278	266
	259	265	269	266	279	274
	260	255	270	252	280	260
	Mean ± S.D.	269.5 ± 14.2		261.6 ± 18.2		260.9 ± 16.7

APPENDIX TABLE BW-3

N-Methyl dicyclohexylamine
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	384	384	386	382	384
	362	431	434	436	440	442
	363	413	414	414	410	410
	364	409	409	413	410	413
	365	394	402	405	405	407
	366	386	389	389	392	401
	367	347	352	351	356	351
	368	381	382	382	388	385
	369	343	339	344	348	341
	370	374	385	375	386	384
	Mean	386.2	389.0	389.5	391.7	391.8
	± S.D.	± 27.7	± 28.2	± 28.6	± 26.9	± 29.9

APPENDIX TABLE BW-3 (continued)

N-Methyl dicyclohexylamine

Multiple Dosing: 5 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	371	302	367	364	361	362
	372	450	460	463	462	466
	373	395	405	404	401	403
	374	359	357	356	352	355
	375	416	411	412	414	417
	376	365	367	365	366	371
	377	385	391	388	390	390
	378	319	320	317	322	325
	379	387	382	381	381	387
	380	366	366	370	373	376
	Mean	380.4	382.6	382.0	382.2	385.4
	± S.D.	± 35.6	± 37.6	± 38.9	± 38.2	± 38.4

APPENDIX TABLE BW-3 (continued)

N-Methyl dicyclohexylamine

Multiple Dosing: 25 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	381	361	339	328	305	300
	382	339	320	310	309	309
	383	345	330	318	310	294
	384	364	347	337	332	326
	385	367	345	344	326	330
	386	388	354	344	336	336
	387	348	330	325	322	328
	388	369	349	342	339	334
	389	352	335	327	325	320
	390	343	335	322	312	316
	Mean	357.6	338.4	329.7	321.6	319.3
	± S.D.	± 15.0	± 10.4	± 11.7	± 12.1	± 14.4

APPENDIX TABLE BW-3 (continued)

N-Methyl dicyclohexylamine

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	391	377	366	395	352	335
	392	387	377	371	357	347
	393	381	376	368	355	342
	394	409	404	396	388	380
	395	442	435	427	410	400
	396	354	347	336	331	327
	397	407	395	387	378	371
	389	405	392	381	367	357
	399	360	349	347	334	330
	400	385	380	369	360	354
	Mean	390.7	382.1	374.6	363.2	354.3
	± S.D.	± 25.9	± 26.2	± 25.8	± 24.0	± 22.4

APPENDIX TABLE BW-4

N-Methyl dicyclohexylamine
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	28	28	27	27	28
	322	26	26	26	27	27
	323	27	28	27	27	27
	324	27	27	28	27	27
	325	27	27	27	27	27
	326	27	27	28	28	28
	327	24	25	26	25	25
	328	26	25	26	25	26
	329	26	26	27	27	27
	330	25	25	26	26	26
	Mean ± S.D.	26.3 ± 1.2	26.4 ± 1.2	26.8 ± 0.8	26.6 ± 1.0	26.8 ± 0.9

APPENDIX TABLE BW-4 (continued)

N-Methyl dicyclohexylamine

Multiple Dosing: 5 ppm

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	331	26	26	27	28	28
	332	25	24	24	26	25
	333	23	23	25	25	25
	334	23	22	23	22	24
	335	24	24	26	25	27
	336	23	24	25	24	25
	337	22	22	23	23	24
	338	22	22	23	24	24
	339	20	21	22	22	22
	340	23	23	24	24	25
	Mean ± S.D.	23.1 ± 1.7	23.1 ± 1.4	24.2 ± 1.5	24.3 ± 1.8	24.9 ± 1.7

APPENDIX TABLE BW-4 (continued)

N-Methyl dicyclohexylamine

Multiple Dosing: 25 ppm

Sex	Animal Number	Day of Dosing	Animal Number	Day of Dosing	Animal Number	Day of Dosing	
		1		1		1	2
Male	341	All Mice Dead	401	All New Mice Dead	421	23	23
	342		402		422	26	Dead
	343		403		423	26	Dead
	344		404		424	26	Dead
	345		405		425	25	24
	346		406		426	27	Dead
	347		407		427	Dead	-
	348		408		428	26	23
	349		409		429	Dead	-
	350		410		430	Dead	-
	Mean ± S.D.					24.7 ± 1.5	23.3 ± 0.6

APPENDIX TABLE BW-4 (continued)

N-Methyl dicyclohexylamine

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Sex	Animal Number	Day of Dosing				
		1	2	3	4	5
Male	351	23	24	25	24	22
	352	25	25	26	24	25
	353	26	26	26	25	23
	354	28	28	27	26	25
	355	25	24	25	23	22
	356	25	25	26	24	23
	357	27	26	27	26	25
	358	25	25	25	24	23
	359	25	25	25	25	23
	360	26	26	26	25	24
	Mean ± S.D.	25.5 ± 1.4	25.4 ± 1.2	25.8 ± 0.8	24.6 ± 1.0	23.5 ± 1.2

APPENDIX TABLE CA-MD-M

N-Methyl dicyclohexylamine
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	19/3	50	25	24							1 Chromatid Fragment	13.6 x 119.1
	19/1		25	25								
129	99/5	50	25	23	1							15.9 x 116.4
					1							12.1 x 113.9
	99/1		25	25								
124	108/4	50	25	25								
	108/5		25	25								
125	86/2	50	25	25								
	86/3		25	25								
128	15/1	50	25	23		1						12.5 x 119.4
					1	1						15.1 x 118.9
	15/3		25	24		1						18.4 x 119.1
127	34/1	50	25	25								
	34/4		25	25								
122	22/2	50	25	24	1							32.9 x 117.6
	22/5		25	25								
121	78/2	50	25	25								
	78/4		25	24	1							12.8 x 118.3
123	58/3	50	25	21	1							21.7 x 118.6
											1 Chromatid Fragment	25.5 x 119.0
					1							28.7 x 119.2
					1							29.5 x 118.9
	58/2		25	24	1							15.8 x 113.9
126	77/3	50	25	25								
	77/1		25	25								

APPENDIX TABLE CA-MD-M

N-Methyl dicyclohexylamine
Males

Multiple Dosing: 5 ppm

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
137	16/2	50	25	24		1						14.7 x 119.2
	16/3		25	24	1							23.1 x 118.3
133	125/1	50	25	25								
	125/5		25	25								
134	128/2	50	25	25								
	128/5		25	22	2							38.0 x 120.3
											1 Chromatid Fragment	33.3 x 116.8
						1						26.0 x 114.5
140	60/3	50	25	25								
	60/5		25	25								
132	152/4	50	25	25								
	152/5		25	25								
139	109/2	50	25	25								
	109/1		25	25								
138	153/1	50	25	24	1							42.3 x 113.6
	153/5		25	25								
135	140/1	50	25	24							1 Ring Chromosome	41.5 x 115.6
	140/3		25	24	1							40.4 x 116.5
136	48/2	50	25	25								
	48/4		25	23	1							16.5 x 115.9
					1							31.2 x 115.4
131	7/2	50	25	24	1							15.2 x 118.0
	7/3		25	24	1							31.6 x 114.2

APPENDIX TABLE CA-MD-M (continued)

N-Methyl dicyclohexylamine
Males

Multiple Dosing: 25 ppm

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
144	113/1	50	25	24	1							31.5 x 115.1
	113/3		25	24	1							14.9 x 117.9
147	134/1	50	25	24		1						11.3 x 120.9
	134/3		25	25								
142	74/2	50	25	24	1						1 Dicentric	16.1 x 119.5
	74/1		25	25								
149	151/3	50	25	25								
	151/5		25	25								
145	30/1	50	25	25								
	30/3		25	25								
143	71/2	50	25	25								
	71/5		25	25								
146	154/1	50	25	24							1 Dicentric	24.1 x 119.3
	154/3		25	25								
148	51/1	50	25	24	1							29.9 x 119.0
	51/3		25	25								
150	83/2	50	25	24	1							42.2 x 114.1
	83/4		25	24	1							33.8.x 119.9
141	44/3	50	25	24	1							13.8 x 115.0
	44/4		25	23	1							19.6 x 118.3
					1							11.2 x 115.7

APPENDIX TABLE CA-MD-M (continued)

N-Methyl dicyclohexylamine
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	65/3	50	25	22	2	4						14.8 x 120.1
					1							21.4 x 119.8
					1	2						24.8 x 120.4
	65/5		25	21	1							28.7 x 119.1
					1							30.7 x 119.3
					1	1						36.0 x 117.2
					1							34.5 x 117.2
155	5/2	50	25	23		1						42.7 x 115.6
					1							22.8 x 115.1
	5/3		25	24							1 Chromatid Fragment	34.2 x 120.4
156	90/4	50	25	23	1							16.0 x 115.9
					1							11.3 x 115.3
	90/3		25	24							1 Chromatid Fragment	21.9 x 115.9
158	157/5	50	25	24	1							40.5 x 115.5
	157/3		25	25								
152	50/1	50	25	21	1	1		1				11.1 x 119.5
											1 Exchange	13.5 x 119.4
					1							19.1 x 119.3
					1	1						35.9 x 116.9
	50/5		25	21	1							15.3 x 119.6
					1							16.5 x 119.6
					1							21.3 x 119.6
					1							21.9 x 119.2

APPENDIX TABLE CA-MD-M (continued)

N-Methyl dicyclohexylamine

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	9/1	50	25	21							1 Chromatid Fragment	13.1 x 117.6
											1 Chromatid Fragment	14.2 x 117.7
						1						21.5 x 117.9
								1				25.5 x 117.0
	9/4		25	24							1 Chromatid Fragment	36.5 x 115.7
153	73/4	50	25	21	1							9.6 x 119.0
						2		1				20.7 x 119.6
					1							16.4 x 114.1
					2	4						39.5 x 113.0
	73/3		25	22	1							5.3 x 117.5
					1	2						13.5 x 118.1
							4					35.0 x 118.5
151	11/1	50	25	23		2					2 Chromatid Fragments	36.4 x 119.0
					1							35.0 x 114.7
	11/2		25	24	2	1						35.2 x 119.4
157	84/1	50	25	24		1						16.0 x 118.3
	84/5		25	24	1							34.7 x 113.2
154	139/1	50	25	25								
	139/3		25	21	1						1 Chromatid Fragment	13.0 x 119.7
											1 Chromatid Fragment	19.0 x 118.8
					1							39.1 x 114.8
						1						35.8 x 114.7

APPENDIX TABLE CA-MD-F

N-Methyl dicyclohexylamine
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		
282	182/1	50	25	23	1							13.2 x 119.0
						1						14.5 x 119.0
	182/2		25	24	1							23.7 x 114.1
286	237/1	50	25	25								
	237/4		25	25								
283	218/5	50	25	25								
	218/1		25	25								
284	268/4	50	25	24	1							15.7 x 118.6
	268/1		25	23	1							19.2 x 119.4
					1							37.9 x 120.3
289	259/1	50	25	25								
	259/5		25	25								
290	179/4	50	25	25								
	179/5		25	24	1							30.8 x 113.9
287	194/4	50	25	24	1							40.9 x 115.5
	194/5		25	25								
281	238/1	50	25	23	2	1						25.1 x 116.4
											1 Chromatid Fragment	19.2 x 115.2
	238/4		25	24	2							14.7 x 110.6
285	246/2	50	25	24		1						40.2 x 118.8
	246/3		25	24	1							34.7 x 118.3
288	175/5	50	25	25								
	175/3		25	25								

APPENDIX TABLE CA-MD-F (continued)

N-Methyl dicyclohexylamine

Females

Multiple Dosing: 5 ppm

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
300	220/4	50	25	25								
	220/2		25	23	1							35.6 x 119.1
					1							18.5 x 114.7
296	208/2	50	25	22	2							10.0 x 119.3
											1 Chromatid Fragment	42.3 x 115.9
											1 Dicentric	35.0 x 115.4
	208/4		25	24	1							34.2 x 120.1
294	288/1	50	25	24	1	1						17.9 x 117.0
	288/2		25	24	1							34.7 x 115.9
291	167/1	50	25	25								
	167/2		25	25								
293	285/5	50	25	23		1						28.7 x 119.1
					1							22.5 x 113.2
	285/2		25	22	1							36.3 x 121.3
					2							17.2 x 116.6
						1						20.2 x 113.7
295	300/3	50	25	25								
	300/1		25	25								
299	269/1	50	25	25								
	269/3		25	25								
298	313/2	50	25	25								
	313/3		25	25								
292	312/2	50	25	25								
	312/4		25	25								

APPENDIX TABLE CA-MD-F (continued)

N-Methyl dicyclohexylamine
Females

Multiple Dosing: 5 ppm

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
297	176/4	50	25	23	2							13.3 x 118.7
					1							13.8 x 112.4
	176/3		25	23	1							15.1 x 118.7
					1							19.4 x 118.9

APPENDIX TABLE CA-MD-F (continued)

N-Methyl dicyclohexylamine
Females

Multiple Dosing: 25 ppm

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
309	311/1	50	25	22	1							46.6 x 114.3
					1							43.5 x 114.2
					1							12.9 x 111.3
	311/5		25	22	1							38.6 x 120.8
											1 Chromatid Fragment	47.0 x 116.6
					1						1 Chromatid Fragment	10.9 x 111.9
307	294/4	50	25	24		1						43.2 x 113.0
	294/2		25	23	1							37.2 x 119.9
					1							20.5 x 116.0
303	231/2	50	25	24	1							40.1 x 116.2
	231/5		25	23	2							36.9 x 119.5
					1							26.7 x 112.1
301	204/3	50	25	22							1 Chromatid Fragment	35.5 x 115.5
					2							34.6 x 110.9
					1							19.4 x 110.5
	204/5		25	24	1							11.3 x 110.7
310	243/1	50	25	22							1 Chromatid Fragment	14.1 x 118.2
					3	1						18.1 x 119.1
					1							23.0 x 119.9
	243/3		25	23	3							10.9 x 119.6
					1							28.3 x 119.8
306	314/2	50	25	24	1							12.0 x 111.9
	314/4		25	24	1							35.6 x 116.7
305	190/1	50	25	24	1							14.0 x 119.8
	190/2		25	25								

APPENDIX TABLE CA-MD-F (continued)

N-Methyl dicyclohexylamine

Females

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
315	165/4	50	25	23	1							12.5 x 119.1
						1						32.5 x 115.9
	165/3		25	20		1						15.7 x 119.5
					1							17.3 x 119.2
											1 Chromatid Fragment	17.6 x 119.8
					1							33.8 x 116.3
					1							30.7 x 115.7
313	233/2	50	25	24	1							30.8 x 109.8
	233/5		25	19	1							22.6 x 119.0
					1							36.6 x 116.4
					1							6.8 x 113.2
					2	1						9.0 x 113.0
					1	1	1				1 Chromatid Fragment	11.4 x 112.9
					1							21.9 x 112.7
311	171/3	50	25	24	1	5						11.5 x 114.9
	171/1		25	24	1							31.0 x 115.0
319	225/2	50	25	18							1 Multi Aberration	8.1 x 119.4
						1						10.5 x 119.1
					2							10.6 x 119.1
					1	1						42.0 x 117.4
					1							20.7 x 117.1
											1 Multi Aberration	16.3 x 117.1
					1							13.9 x 117.5

APPENDIX TABLE CA-MD-F (continued)

N-Methyl dicyclohexylamine

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		
319	225/3		25	21	3	1						15.6 x 119.7
					1							16.5 x 119.6
						1						17.7 x 119.6
								2				39.5 x 115.3
320	169/3	50	25	25								
	169/4		25	21	1	4						13.4 x 119.1
						1						16.5 x 119.3
					1							38.4 x 118.6
											1 Chromatid Fragment	20.3 x 116.6
316	250/3	50	25	23		2						40.3 x 113.6
					1							22.1 x 113.4
	250/1		25	22		1						19.3 x 120.4
					1							28.5 x 120.9
								1				37.4 x 120.2
318	317/1	50	25	21	1							12.5 x 119.2
					1							43.8 x 115.8
					1	1						34.0 x 115.0
					1							31.4 x 115.5
	317/3		25	24	1							16.9 x 118.3
312	210/5	50	25	24	1							25.0 x 115.8
	210/1		25	23		1						39.9 x 121.1
					1							21.1 x 113.1

APPENDIX TABLE CA-MD-F (continued)

N-Methyl dicyclohexylamine
Females

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
314	299/4	50	25	24	1							48.6 x 119.0
	299/3		25	22	1							35.4 x 115.5
					1			1				19.9 x 114.8
						1						37.2 x 115.0
317	244/5	50	25	22	1							33.4 x 116.1
					1	1						41.0 x 116.4
					1							43.1 x 113.2
	244/4		25	22	1							20.6 x 117.4
					1							25.0 x 117.5
					1			1				43.3 x 114.4

APPENDIX TABLE CA-M6

N-Methyl dicyclohexylamine
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Males

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
1	156/4	50	25	24	1							117.2 x 19.2
	156/5		25	24	1							84.0 x 13.4
9	85/1	50	25	24	1							114.0 x 20.0
	85/4		25	25								
7	2/4	50	25	25								
	2/1		25	25								
3	111/2	50	25	19	1							87.0 x 11.2
					1							109.0 x 15.0
					3			1				117.0 x 10.4
								1				83.8 x 8.2
					2							115.0 x 8.0
					1							110.0 x 5.5
	111/3		25	23	2							106.6 x 15.0
					2							108.8 x 13.4
10	147/3	50	25	24	1							91.0 x 23.0
	147/4		25	25								
6	80/2	50	25	25								
	80/3		25	24	1							95.0 x 12.0
2	136/1	50	25	24	1							94.8 x 22.5
	136/3		25	25								

APPENDIX TABLE CA-M6 (continued)

N-Methyl dicyclohexylamine

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		
4	43/2	50	19	17	1							90.9 x 18.0
					1							104.0 x 15.9
	43/3		25	25								
	43/5		6	6								
5	105/2	50	25	25								
	105/3		25	23							1 Robertsonian Translocation	107.5 x 17.4
					1							116.6 x 16.9
8	36/2	50	25	22				1				87.0 x 23.0
					2							96.0 x 17.0
					1							111.1 x 15.8
	36/4		25	24	1							113.0 x 12.9

APPENDIX TABLE CA-M6 (continued)

N-Methyl dicyclohexylamine
Males

Single Dosing: 5 ppm

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
36	33/1	50	25	22	1							109.5 x 16.9
						1						116.8 x 14.2
											1 Robertsonian Translocation	103.1 x 13.1
	33/2		25	24					1			40.9 x 22.0
39	68/3	50	25	23	1							105.5 x 19.0
					1							96.8 x 15.0
	68/4		25	23	1							97.0 x 23.0
					1							111.1 x 20.5
33	124/3	50	25	25								
	124/2		25	21	1							114.8 x 19.1
					1							81.0 x 18.5
					2							80.8 x 18.5
					1							97.0 x 18.0
37	69/2	50	25	25								
	69/5		25	25								
38	155/1	50	25	21	1							79.0 x 21.2
					1							79.9 x 19.9
					1							108.9 x 16.0
					1							82.0 x 15.8
	155/2		25	25								
31	52/1	50	25	24	1							107.0 x 20.4
	52/2		25	24	1			1				105.0 x 20.9

APPENDIX TABLE CA-M6 (continued)

N-Methyl dicyclohexylamine

Males

Single Dosing: 5 ppm

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
34	131/2	50	25	24	1							101.0 x 21.0
	131/4		25	25								
35	110/2	50	25	24	1							85.0 x 20.6
	110/3		25	25								
40	120/5	50	25	25								
	120/3		25	25								
32	150/1	5	2	2								
	150/2		1	1								
	150/4		2	2								
	150/3,5		0	0								

APPENDIX TABLE CA-M6 (continued)

N-Methyl dicyclohexylamine

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
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
69	148/4	50	25	25								
	148/5		25	25								
61	49/3	50	25	25								
	49/4		25	24				1				103.0 x 13.0
66	54/1	50	25	25								
	54/2		25	25								
64	23/3	50	25	25								
	23/4		25	25								
67	40/1	50	25	25								
	40/2		25	25								
63	119/1	50	25	24	1							114.1 x 20.9
	119/4		25	24	1							105.1 x 22.0
65	12/4	50	25	23	1							115.9 x 16.6
					1							86.0 x 16.5
	12/3		25	24	1							96.2 x 21.1
70	142/2	50	25	25								
	142/5		25	23	1							88.6 x 23.0
								1				85.5 x 17.8
68	107/1	50	25	25								
	107/5		25	24	1							88.1 x 18.2
62	57/2	50	25	25								
	57/4		25	25								

APPENDIX TABLE CA-M6 (continued)

N-Methyl dicyclohexylamine
Males

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
94	76/3		20	14	2							115.8 x 21.9
					3							108.2 x 21.0
					3							94.0 x 21.0
					3							108.2 x 21.1
					2							83.1 x 10.9
											1 Chromosomal Fragment	107.0 x 10.1
	76/4		13	7	1							80.2 x 21.4
					2							104.1 x 19.2
					1							105.2 x 18.9
					1							119.2 x 16.5
					1							91.4 x 15.1
					1							93.4 x 11.0
	76/5		1	1								
99	29/4	50	25	25								
	29/5		25	22	1							100.0 x 16.0
					1			1				72.0 x 8.9
					1							109.0 x 7.0
95	95/2	50	25	19	1							105.0 x 21.8
					1							103.5 x 17.5
					1							109.9 x 16.2
					1							92.8 x 11.8
					2							87.0 x 11.4
					2							109.4 x 11.8

APPENDIX TABLE CA-M6 (continued)

N-Methyl dicyclohexylamine
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		
97	126/1	50	25	20	1							82.0 x 24.0
					1							85.0 x 23.0
					1							111.0 x 14.5
											1 Chromosomal Fragment	95.6 x 14.2
					1					1	1 Chromosomal Fragment	120.8 x 13.2
	126/3		25	24	1							114.0 x 21.0
98	102/3	50	25	23	1							103.0 x 20.0
					1							109.9 x 19.9
	102/4		25	24	2							120.0 x 18.0
100	10/2	50	25	23		1		1				102.8 x 19.9
					1							118.9 x 17.1
	10/3		25	20							1 Chromatid Fragment	79.0 x 19.1
					1							108.2 x 14.0
					1							101.5 x 10.0
					1							102.1 x 18.0
								1				120.1 x 17.9
94	76/1	50	5	4	3							115.0 x 12.5
	76/2		11	5	3							88.8 x 20.0
					1			1				116.1 x 18.0
					1							99.0 x 15.1
					1							108.1 x 13.1
					1							114.0 x 6.2
					1							86.1 x 6.1

APPENDIX TABLE CA-M6 (continued)

N-Methyl dicyclohexylamine
Males

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
95	95/5		25	22	1							110.6 x 17.5
					2							77.9 x 16.9
					1							82.0 x 16.9
92	39/3	50	25	25								
	39/5		25	25								
91	81/1	50	25	25								
	81/5		25	24		1						91.1 x 15.9
93	20/4	50	25	23					1			94.8 x 20.9
					1							97.1 x 18.9
	20/5		25	23	1							90.5 x 21.0
								1				96.0 x 21.0
96	13/4	1	1	1								
	13/1,2, 3 & 5		0	0								

APPENDIX TABLE CA-M24

N-Methyl dicyclohexylamine
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		
19	121/1	50	25	25								
	121/2		25	25								
11	137/3	50	25	24				1				6.3 x 92.8
	137/5		25	25								
16	118/5	50	25	25								
	118/4		25	24	1							10.2 x 125.7
14	61/1	50	25	25								
	61/5		18	16	1							12.9 x 116.1
					1							9.4 x 110.5
	61/4		7	7								
20	94/3	50	25	25								
	94/2		25	25								
15	112/4	50	25	25								
	112/5		25	25								
12	53/5	50	25	25								
	53/4		25	25								
17	25/3	50	25	24	1			1				38.0 x 110.2
	25/5		25	22	1							7.6 x 87.7
								1				32.0 x 85.5
								1				2.1 x 113.0
13	135/5	50	25	25								
	135/4		25	24	2			1				7.9 x 113.9

APPENDIX TABLE CA-M24 (continued)

N-Methyl dicyclohexylamine

Males

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
18	46/3	7	2	2								
	46/5		3	2	1							11.9 x 128.7
	46/1		1	1								
	46/4		1	1								
	46/2		0	0								

APPENDIX TABLE CA-M24 (continued)

N-Methyl dicyclohexylamine

Males

Single Dosing: 5 ppm

Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
49	91/4	50	25	25								
	91/5		25	25								
46	104/3	50	25	25								
	104/2		25	25								
50	3/3	50	25	25								
	3/5		25	25								
42	31/5	50	25	25								
	31/2		25	24							1 Chromosomal Fragment	10.0 x 124.9
47	97/3	50	25	25								
	97/5		25	24	1							13.0 x 123.0
44	64/3	50	25	25								
	64/4		25	25								
45	100/2	50	25	25								
	100/1		25	25								
48	160/4	50	25	25								
	160/5		25	24	1							10.0 x 125.0
43	138/1	50	25	24	1							8.8 x 116.9
	138/2		25	25								
41	106/4	50	25	25								
	106/3		25	25								

APPENDIX TABLE CA-M24 (continued)

N-Methyl dicyclohexylamine

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
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
77	6/5	50	25	23	1							10.0 x 126.0
											1 Exchange	8.8 x 123.2
	6/4		25	25								
73	103/2	50	25	25								
	103/3		25	25								
72	127/5	50	25	24	1							9.0 x 120.7
	127/3		25	25								
74	146/3	50	25	24	1							9.1 x 111.0
	146/2		25	25								
79	42/3	50	25	25								
	42/2		25	25								
71	62/1	50	25	25								
	62/4		25	25								
75	56/5	50	25	25								
	56/3		25	25								
78	133/2	50	25	25								
	133/1		25	24				1				13.7 x 104.0
76	18/2	50	25	24	1							13.7 x 120.1
	18/1		25	25								

APPENDIX TABLE CA-M24 (continued)

N-Methyl dicyclohexylamine

Males

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
107	8/1	50	25	19		2					2 Exchanges	14.7 x 121.1
					3	1					1 Exchange 1 Pair of Minutes	14.9 x 114.4
					1		1				1 Exchange	13.0 x 120.4
					1						1 Minute	13.8 x 121.1
					1	1		1				13.8 x 121.8
					1							13.7 x 121.8
	8/5		25	22							1 Exchange	8.0 x 118.2
						1	2				1 Minute	6.4 x 132.0
					1							5.1 x 121.1
104	55/5	50	25	24	1							12.1 x 115.5
	55/4		25	24							1 Exchange	9.0 x 127.9
101	116/2	50	25	24							1 Multi Aberration	6.2 x 121.1
	116/3		25	23		2					1 Exchange	11.9 x 111.8
					1							11.2 x 124.6
102	32/5	50	25	22							1 Multi Aberration	9.9 x 124.2
					1						1 Exchange	5.0 x 117.2
											1 Multi Aberration	3.5 x 131.1
	32/3		25	19							1 Multi Aberration	12.9 x 122.0
					1							12.8 x 112.0
					1	1						11.1 x 117.6
					2	1						11.8 x 123.0
						1					1 Minute	10.0 x 115.6
					2			1				10.1 x 115.6

APPENDIX TABLE CA-M24 (continued)

N-Methyl dicyclohexylamine

Males

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
109	93/3	50	25	22	1	2						12.9 x 119.1
					1							11.3 x 124.9
					1							11.0 x 125.0
	93/2		25	22							1 Multi Aberration	13.7 x 119.0
					1	2						13.8 x 115.0
						1						14.0 x 115.0
103	92/1	50	25	20	1							6.3 x 110.5
					1						1 Exchange	6.8 x 104.9
								1				6.3 x 95.2
					1							6.0 x 94.0
					1							6.3 x 86.1
	92/5		25	21		1					2 Exchanges	9.0 x 117.1
					1							7.2 x 121.1
											1 Chromatid Fragment	7.5 x 119.0
					1	2						7.0 x 111.5
108	143/4	50	25	20	1							12.5 x 121.2
					2	1					1 Exchange	13.5 x 124.0
					1							13.6 x 124.1
					1							13.1 x 126.1
											1 Chromatid Fragment	12.4 x 127.5
					1							12.1 x 124.2

APPENDIX TABLE CA-M24 (continued)

N-Methyl dicyclohexylamine

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		
	143/2		25	21	1							10.0 x 121.2
					1						2 Exchanges	10.0 x 123.7
											1 Exchange	9.6 x 111.9
					1							8.8 x 114.1
105	67/4	50	25	24	1	3					1 Exchange	13.2 x 113.4
	67/1		25	21	1							15.0 x 128.0
											1 Multi Aberration	14.5 x 124.8
											1 Exchange	14.5 x 123.0
						1					2 Exchanges	13.9 x 116.0
110	89/1	50	25	24				1				10.0 x 110.1
	89/5		25	25								
106	72/1	50	25	24	1	1						12.9 x 99.9
	72/2		25	24	1							13.3 x 124.8

APPENDIX TABLE CA-M48 (continued)

N-Methyl dicyclohexylamine
Males

Single Dosing: 5 ppm

Sampling Time: 48 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
57	158/2	50	25	25								
	158/3		25	23	1							64.9 x 109.8
					1							55.4 x 109.0
58	88/1	50	25	25								
	88/3		25	23	2	1						60.1 x 107.2
					1							32.4 x 105.5
51	145/2	50	25	25								
	145/3		25	25								
56	132/1	50	25	25								
	132/2		25	25								
52	159/2	50	25	25								
	159/3		25	24	1							56.4 x 104.5
53	37/1	50	25	25								
	37/4		25	25								
54	24/1	50	25	25								
	24/2		25	25								
60	66/2	50	25	25								
	66/3		25	25								
59	27/1	50	25	25								
	27/2		25	25								
55	101/1	50	25	23	1							63.0 x 109.0
					1							60.2 x 108.0
	101/2		25	24	1							55.0 x 103.3

APPENDIX TABLE CA-M48 (continued)

N-Methyl dicyclohexylamine

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		
90	26/1	50	25	25								
	26/2		25	25								
88	38/1	50	25	25								
	38/2		25	24	1							65.6 x 107.8
87	14/4	50	25	25								
	14/5		25	25								
82	114/1	50	25	25								
	114/2		25	25								
84	96/1	50	25	25								
	96/2		25	25								
86	59/1	50	25	25								
	59/2		25	25								
81	123/2	50	25	25								
	123/3		25	25								
83	21/1	50	25	25								
	21/5		25	25								
89	130/1	50	25	22	1						1 Chromatid Fragment	66.7 x 110.7
					1							58.6 x 111.3
					1							54.4 x 109.9
	130/2		25	22	1							60.9 x 108.4
					1							59.9 x 110.0
					1							54.4 x 107.5
85	122/2	50	25	24	1							64.9 x 112.2
	122/4		25	25								

APPENDIX TABLE CA-M48 (continued)

N-Methyl dicyclohexylamine

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		
112	79/4	50	25	21		1						71.2 x 109.4
						1						68.1 x 110.0
					1							61.4 x 108.9
					1							60.4 x 108.4
	79/5		25	19	1							65.0 x 108.9
					4							63.5 x 108.9
											1 Multi Aberration	56.4 x 108.0
					1							54.2 x 108.0
					2							54.1 x 108.5
					1							51.4 x 107.7
111	75/1	50	25	23	1							57.2 x 104.1
					1							50.6 x 102.3
	75/2		25	24							1 Multi Aberration	56.5 x 105.0
113	4/1	50	25	25								
	4/2		25	25								
114	47/1	50	25	25								
	47/2		25	23		1					1 Chromatid Fragment	57.1 x 106.9
					1	1					1 Exchange	54.1 x 106.1
119	129/1	50	25	25								
	129/2		25	23	1							67.5 x 108.0
											1 Exchange	67.4 x 107.5
						1						67.2 x 107.1
118	87/1	50	25	25								
	87/3		25	25								

APPENDIX TABLE CA-M48 (continued)

N-Methyl dicyclohexylamine
Males

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sampling Time: 48 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
115	149/1	50	25	25								
	149/4		25	25								
116	144/1	50	25	25								
	144/2		25	25								
117	1/1	50	25	25								
	1/2		25	25								
120	41/2	50	25	25								
	41/3		25	25								

APPENDIX TABLE CA-M48

N-Methyl dicyclohexylamine
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		
25	63/2	50	25	25								
	63/3		25	25								
24	45/5	50	25	24	1							42.0 x 105.5
	45/4		25	25								
21	98/1	50	25	24	1							60.3 x 106.9
	98/2		25	24							1 Pair of Minutes	55.2 x 109.7
26	28/1	50	25	25								
	28/2		25	25								
23	82/1	17	15	15								
	82/2		2	2								
	82/3,4,5		0	0								
30	115/5	50	25	25								
	115/1		25	25								
27	17/1	50	25	25								
	17/2		25	25								
28	141/2	50	25	25								
	141/3		25	24	1							48.7 x 105.5
29	70/2	50	25	25								
	70/4		25	25								
22	35/3	50	25	25								
	35/4		25	25								

APPENDIX TABLE CA-F6

N-Methyl dicyclohexylamine
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		
164	203/2	50	25	25								
	203/3		25	24	2							109.1 x 21.1
167	162/4	50	25	24	1							118.8 x 15.9
	162/5		25	25								
165	265/2	50	25	24				1				82.5 x 16.2
	265/4		25	22	1							80.0 x 20.9
					1							108.2 x 18.1
					1							107.6 x 17.1
162	296/5	50	25	21	1							82.0 x 16.0
					1							113.0 x 12.9
					1							89.0 x 8.5
								1				75.0 x 8.8
	296/4		25	24	1							116.8 x 12.9
168	196/4	50	25	25								
	196/2		25	25								
161	316/3	50	25	25								
	316/5		25	25								
166	240/2	50	25	25								
	240/5		25	25								
169	245/2	50	25	22	1							114.0 x 21.0
						1						82.9 x 21.0
					1							79.0 x 15.1
	245/5		25	25								

APPENDIX TABLE CA-F6 (continued)

N-Methyl dicyclohexylamine
Females

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
163	271/5	50	25	25								
	271/2		25	24	1							80.4 x 15.0
170	307/1	50	25	25								
	307/3		25	23	1							109.9 x 24.1
					1							99.1 x 20.4

APPENDIX TABLE CA-F6 (continued)

N-Methyl dicyclohexylamine

Females

Single Dosing: 5 ppm

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
197	229/2	50	25	25								
	229/3		25	24				1				84.9 x 16.5
192	310/4	50	25	25								
	310/5		16	15	1							106.0 x 17.8
	310/2		9	9								
194	291/3	50	25	25								
	291/5		25	25								
195	270/1	50	25	23	1							112.2 x 22.0
					1							90.2 x 18.9
	270/2		25	24	1							100.2 x 19.8
191	212/1	50	25	25								
	212/4		25	23	1							87.0 x 16.0
					1							106.8 x 23.4
200	280/1	50	25	25								
	280/2		25	25								
193	284/2	50	25	25								
	284/4		25	25								
196	193/3	50	25	25								
	193/5		25	25								
199	228/2	50	25	25								
	228/3		25	25								

APPENDIX TABLE CA-F6 (continued)

N-Methyl dicyclohexylamine
Females

Single Dosing: 5 ppm

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
198	315/1	12	2	2								
	315/2		3	3								
	315/3		4	4								
	315/4		3	3								
	315/5		0	0								

APPENDIX TABLE CA-F6 (continued)

N-Methyl dicyclohexylamine
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
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
227	200/3	50	25	24	1							101.9 x 20.9
	200/4		25	25								
223	279/1	50	25	24	1			1				84.0 x 14.4
	279/2		25	23	1							103.0 x 18.0
					1							111.1 x 114.8
225	172/2	50	25	24	1							113.0 x 21.1
	172/3		25	24	1							111.0 x 16.9
221	209/4	50	25	24							1 Chromatid Fragment	97.0 x 24.0
	209/5		25	24	1							121.8 x 19.5
226	214/4	50	25	25								
	214/3		25	24	1							106.9 x 23.0
228	267/2	50	25	25								
	267/3		25	25								
224	183/1	50	25	25								
	183/2		25	24	1							80.9 x 14.9
222	217/3	50	25	25								
	217/5		25	24				1				116.0 x 21.5
230	302/1	14	5	5								
	302/2		2	2								
	302/3		1	1								
	302/5		6	5							1 Chromatid Fragment	113.1 x 12.2
	302/4		0	0								

APPENDIX TABLE CA-F6 (continued)

N-Methyl dicyclohexylamine
Females

Single Dosing: Ethyl methanesulphonate, 250 mg/kg

Sampling Time: 6 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
257	286/3	50	25	24	2							75.9 x 15.1
	286/4		25	23	1							88.2 x 20.9
					2							88.9 x 12.0
258	262/3	50	25	23				1				83.9 x 19.0
					1							106.1 x 9.9
	262/5		25	20	1							113.8 x 24.8
					1							99.0 x 21.1
					1							107.1 x 21.0
					1							119.2 x 19.1
					1							118.8 x 15.0
260	170/4	50	25	23		1						105.0 x 19.9
					1			1				103.8 x 19.8
	170/5		25	23		1						114.0 x 23.8
					1							93.1 x 22.0
251	241/1	50	25	25								
	241/2		25	25								
259	189/3	50	25	19		1					1 Chromatid Fragment	102.5 x 21.0
					4							105.8 x 19.5
					1							97.0 x 18.9
					1							106.2 x 18.8
					2							119.9 x 18.0
					1							76.0 x 15.3

APPENDIX TABLE CA-F6 (continued)

N-Methyl dicyclohexylamine
Females

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
259	189/4		25	21	1			1				106.0 x 22.9
					1							84.8 x 18.1
					1							83.0 x 16.9
					1							101.0 x 17.0
256	173/1	50	25	25								
	173/3		25	24	1							81.4 x 18.9
254	236/1	50	25	25								
	236/5		25	24					2			113.0 x 14.9
255	255/4	50	25	25								
	255/5		25	25								
253	180/2	50	25	24	1							112.6 x 14.9
	180/5		25	21	1							109.0 x 25.5
					1							107.0 x 21.9
					1							94.2 x 20.6
						1						85.2 x 20.1
252	199/5	50	25	24	1							56.0 x 19.1
	199/2		25	22	1							77.0 x 22.8
					1							81.0 x 22.0
					1							114.9 x 18.8

APPENDIX TABLE CA-F24

N-Methyl dicyclohexylamine
Cytogenetic Analysis of Rat Bone Marrow Cells
Chromatid/Chromosomal Aberrations Scored
Females

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
180	254/2	50	25	25								
	254/4		25	25								
179	281/5	50	25	25								
	281/4		25	25								
176	278/4	50	25	24	1							11.8 x 121.0
	278/5		25	25								
173	295/5	50	25	25								
	295/4		25	23	1							13.5 x 117.2
					1							13.0 x 91.9
174	221/2	50	25	25								
	221/4		25	24	1							6.9 x 111.1
171	297/1	50	25	25								
	297/4		25	24	1							11.5 x 88.2
175	272/1	50	25	24	1							8.1 x 118.4
	272/2		25	25								
172	213/4	50	25	25								
	213/2		25	24				1				13.9 x 119.1
178	206/3	50	25	25								
	206/1		25	25								
177	185/2	50	25	25								
	185/1		25	24	1							11.3 x 120.0

APPENDIX TABLE CA-F24 (continued)

N-Methyl dicyclohexylamine
Females

Single Dosing: 5 ppm

Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
203	298/5	50	4	4								
	298/1		25	24	1							8.2 x 108.5
	298/2		21	21								
204	224/5	50	25	24	1							9.3 x 105.5
	224/4		25	24	1							2.2 x 111.7
206	264/3	50	25	25								
	264/1		25	25								
201	266/4	50	25	25								
	266/3		25	25								
205	260/3	50	25	22		1						15.0 x 117.0
					1							11.9 x 112.0
					1							11.4 x 101.5
	260/2		25	22	1							10.2 x 117.1
					1							7.8 x 125.0
					1							2.7 x 119.0
208	320/1	50	25	23	1							9.1 x 102.8
					1							8.9 x 102.0
	320/3		25	25								
207	257/2	50	25	23	1							7.8 x 115.0
					1							8.6 x 117.1
	257/1		25	25								
210	163/2	50	25	24				1				11.2 x 110.5
	163/3		25	25								

APPENDIX TABLE CA-F24 (continued)

N-Methyl dicyclohexylamine
Females

Single Dosing: 5 ppm

Sampling Time: 24 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
202	191/1	50	25	25								
	191/5		25	25								
209	251/2	50	25	25								
	251/4		25	24	1							10.8 x 121.1

APPENDIX TABLE CA-F24 (continued)

N-Methyl dicyclohexylamine
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		
232	287/1	50	25	25								
	287/4		25	25								
236	178/1	49	11	8	1							10.8 x 111.4
								1				8.3 x 112.1
					1							7.0 x 116.9
	178/3		15	14	1							1.9 x 93.4
	178/4		8	8								
	178/5		7	6	1							0.8 x 100.8
	178/2		8	8								
233	263/4	50	25	25								
	263/5		25	23	1							14.0 x 117.9
					1							13.2 x 120.0
234	306/3	50	25	25								
	306/4		25	25								
238	293/1	50	25	25								
	293/2		25	25								
235	216/5	50	25	24	1							10.8 x 121.1
	216/1		25	25								
237	166/4	50	25	24	1							0.9 x 109.0
	166/3		25	23	1							9.0 x 131.1
								1				4.0 x 121.2

APPENDIX TABLE CA-F24 (continued)

N-Methy dicyclohexylamine

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		
240	277/2	50	25	25								
	277/4		25	25								
239	202/4	50	25	24	1							13.0 x 114.5
	202/5		25	24				1				10.0 x 112.8
231	222/5	50	25	25								
	222/3		25	25								

APPENDIX TABLE CA-F24 (continued)

N-Methyl dicyclohexylamine
Females

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
267	168/1	50	25	23							2 Exchanges	8.8 x 121.2
					1							7.5 x 103.2
	168/4		25	19	1							13.0 x 117.0
								1				10.8 x 111.0
					1	1						9.7 x 113.3
						1						8.9 x 113.0
											1 Multi Aberration	6.3 x 125.4
					1							32.0 x 120.0
269	253/5	50	25	22							1 Multi Aberration	12.9 x 119.8
						1						11.2 x 116.1
											1 Exchange	
					1	2		1			1 Minute	10.2 x 123.2
	253/1		25	21		1						10.1 x 118.0
											1 Exchange	10.0 x 130.5
					2			1				6.6 x 111.4
					2							6.4 x 111.4
											2 Exchanges	
264	215/4	50	25	20	2						1 Minute	14.0 x 125.9
						1						12.4 x 127.3
						1						12.3 x 123.1
											1 Exchange	
					1	2					1 Minute	11.8 x 118.6
											1 Exchange	
											1 Minute	11.9 x 118.4

APPENDIX TABLE CA-F24 (continued)

N-Methyl dicyclohexylamine
Females

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
264	215/3		25	18	1							11.2 x 127.2
											1 Exchange	
						2	1				1 Minute	12.1 x 117.1
					1	2						12.2 x 112.2
					1							11.1 x 115.9
					4	1						11.0 x 116.5
											1 Multi Aberration	11.2 x 121.1
					2	2						9.6 x 128.2
263	252/5	50	25	21	1							13.2 x 125.2
											1 Exchange	14.1 x 122.2
											1 Exchange	13.6 x 117.4
					1							13.2 x 116.4
	252/4		25	20		3					1 Exchange	11.7 x 124.1
					1							11.2 x 120.0
											1 Multi Aberration	9.5 x 127.0
						3					1 Exchange	9.1 x 129.0
											1 Multi Aberration	8.0 x 130.4
262	192/3	50	25	19	1	3						11.0 x 121.4
					1							8.3 x 125.4
					1							8.1 x 117.8
											1 Exchange	
					1						1 Minute	7.0 x 104.9
					1	2						4.6 x 124.2
					1							3.0 x 113.3

APPENDIX TABLE CA-F24 (continued)

N-Methyl dicyclohexylamine
Females

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

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
					Chromatid			Chromosome			Miscellaneous	
		Per Animal	Per Slide		Gap	B w F	B w/o F	Gap	B w F	B w/o F		
262	192/4		25	21	1							10.9 x 112.9
					1							7.7 x 117.2
											1 Multi Aberration	7.2 x 109.8
265	227/1	50	25	25								
	227/5		25	23	3	1						7.5 x 95.6
					3							6.3 x 100.6
270	249/1	50	25	24	1							11.9 x 110.0
	249/4		25	20	1	1					1 Exchange	11.2 x 121.2
					3							9.4 x 119.9
											1 Exchange	8.8 x 128.0
					2						2 Exchanges	9.1 x 129.0
						1						8.2 x 120.2
268	363/3	50	25	18	1							13.5 x 122.1
					3	1					1 Exchange	12.0 x 114.1
					1							11.7 x 113.0
					1	1					1 Exchange	11.9 x 112.9
					1							10.4 x 118.7
											1 Multi Aberration	4.1 x 129.0
								1				3.9 x 124.2
	363/2		25	20							1 Chromatid Fragment	11.4 x 118.0
						1						8.1 x 122.0
					1							4.8 x 119.9
											1 Multi Aberration	3.8 x 109.8
					1							0.5 x 121.0

APPENDIX TABLE CA-F24 (continued)

N-Methyl dicyclohexylamine

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		
261	276/3	50	25	25								
	276/4		25	24							1 Chromatid Fragment	11.6 x 123.8
266	232/4	50	25	24	2						1 Exchange	14.8 x 114.2
	232/5		25	25								

APPENDIX TABLE CA-F48

N-Methyl dicyclohexylamine
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
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
185	223/2	50	25	24	1							58.7 x 102.1
	223/3		25	25								
186	188/1	50	25	24	1							59.5 x 111.2
	188/2		25	25								
190	275/1	50	25	25								
	275/2		25	25								
183	242/2	50	25	25								
	242/3		25	25								
181	258/2	50	25	24							1 Pair of Minutes	66.4 x 105.4
	258/3		25	25								
184	205/2	50	25	25								
	205/4		25	24	1							63.9 x 107.5
188	301/4	50	25	24	1							66.3 x 102.2
	301/5		25	25								
182	195/2	50	25	25								
	195/3		25	25								
187	177/2	50	25	25								
	177/4		25	25								
189	230/1-5	0	0	0								

APPENDIX TABLE CA-F48 (continued)

N-Methyl dicyclohexylamine

Females

Single Dosing: 5 ppm

Sampling Time: 48 h

Animal Number	Slide Number	Spreads Examined		Number of Spreads Without Aberrations	Observed Aberrations per Spread							Vernier Key
		Per Animal	Per Slide		Chromatid			Chromosome			Miscellaneous	
					Gap	B w F	B w/o F	Gap	B w F	B w/o F		
213	197/2	50	25	24	1							27.9 x 97.9
	197/3		25	25								
217	318/1	50	25	25								
	318/2		25	25								
219	187/1	50	25	25								
	187/4		25	25								
218	248/1	50	25	25								
	248/2		25	25								
215	261/2	50	25	24							1 Exchange	65.6 x 102.8
	261/3		25	25								
216	292/3	50	25	25								
	292/4		25	25								
211	305/1	50	25	24	1							61.4 x 104.7
	305/2		25	24	1							63.4 x 103.0
212	319/1	50	25	25								
	319/2		25	25								
220	226/1	50	25	25								
	226/2		25	25								
214	184/1	50	25	25								
	184/3		25	25								

APPENDIX TABLE CA-F48 (continued)

N-Methyl dicyclohexylamine
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		
244	256/1	50	25	24	1							54.0 x 101.5
	256/2		25	25								
241	283/1-5	0	0	0								
243	181/1	50	25	25								
	181/3		25	24	1							60.2 x 106.6
248	198/1	50	25	25								
	198/2		25	25								
250	186/4	15	8	8								
	186/5		7	7								
	186/1,2,3		0	0								
242	274/1	50	25	25								
	274/2		25	25								
246	219/1	50	25	24	1							60.9 x 106.7
	219/2		25	24	1							59.6 x 104.9
249	290/1	50	25	25								
	290/2		25	25								
247	174/2	50	25	25								
	174/3		25	24	1							64.1 x 108.1
245	282/3	50	25	25								
	282/4		25	25								

APPENDIX TABLE CA-F48 (continued)

N-Methyl dicyclohexylamine

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		
271	235/3	15	8	8								
	235/4		3	3								
	235/5		4	4								
	235/1,2		0	0								
273	164/2	5	5	5								
	164/1,3,4,5		0	0								
275	309/1	50	25	25								
	309/2		25	25								
272	239/1	50	25	25								
	239/4		25	22	1							66.1 x 109.4
					1	1						64.9 x 109.8
					1							60.9 x 110.8
279	289/2	50	25	25								
	289/5		25	25								
278	247/1	50	25	24							1 Exchange	67.0 x 106.0
	247/2		25	25								
276	304/1	50	25	25								
	304/2		25	25								
277	161/1	50	25	22	1							56.5 x 104.8
											1 Multi Aberration	55.8 x 104.2
											1 Multi Aberration	55.7 x 104.0
	161/2		25	22							1 Exchange	69.7 x 104.0
					1							67.4 x 104.5
					1							65.1 x 105.0

APPENDIX TABLE CA-F48 (continued)

N-Methyl dicyclohexylamine
Females

Single Dosing: Ethyl méthanesulphonate, 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		
280	201/1	50	25	25								
	201/2		25	25								
274	207/3	50	25	25								
	207/4		25	25								

APPENDIX TABLE DL

N-Methyl dicyclohexylamine
Dominant Lethal Assessment

Multiple Dosing: Air Control (0 ppm)

Week No.	Male No.	361		362		363		364		365		366		367		368		369		370		Total
	Female	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
1	Corpora lutea	15	11	14	11	13	9	13	14	11	0	13	17	11	13	15	0	10	14	12	0	216
	Total Implants	14	9	14	14	16	13	14	15	12	0	12	17	11	14	16	0	10	14	13	0	228
	Live Implants	14	8	12	13	16	13	13	14	12	0	12	17	9	13	14	0	9	14	12	0	215
	Early Deaths	0	1	2	1	0	0	1	1	0	0	0	0	2	1	2	0	0	0	1	0	12
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
2	Corpora lutea	13	10	15	12	9	13	14	10	0	11	15	13	10	16	12	0	11	12	14	15	225
	Total Implants	13	14	14	11	9	15	13	11	0	15	14	14	11	19	13	0	11	13	12	12	234
	Live Implants	13	14	13	11	9	15	12	11	0	14	14	14	6	18	12	0	10	12	12	12	222
	Early Deaths	0	0	1	0	0	0	1	0	0	1	0	0	5	1	1	0	1	1	0	0	12
	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	15	15	14	9	14	12	8	14	13	16	12	14	11	14	12	11	12	11	10	11	248
	Total Implants	16	16	15	10	14	13	12	14	12	14	14	17	12	15	2	10	5	12	12	13	248
	Live Implants	15	16	14	8	14	13	10	13	12	13	13	16	10	15	2	9	5	12	12	11	233
	Early Deaths	1	0	1	2	0	0	2	1	0	1	1	1	2	0	0	1	0	0	0	2	15
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	Corpora lutea	15	14	12	17	16	14	3	14	10	15	13	0	13	18	15	12	14	15	10	13	253
	Total Implants	15	14	11	13	13	14	1	14	13	12	12	0	14	13	16	12	13	16	10	14	240
	Live Implants	15	14	10	13	13	12	0	14	12	10	12	0	14	12	16	11	13	16	10	13	230
	Early Deaths	0	0	0	0	0	2	1	0	1	2	0	0	0	1	0	0	0	0	0	1	8
	Late Deaths	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2
5	Corpora lutea	0	13	11	0	9	12	13	13	13	14	13	12	16	12	16	10	16	14	16	0	223
	Total Implants	0	13	12	0	2	13	14	13	13	10	13	13	16	11	15	10	16	15	13	0	212
	Live Implants	0	13	12	0	2	13	14	12	12	9	13	13	14	11	13	10	15	14	9	0	199
	Early Deaths	0	0	0	0	0	0	0	1	1	1	0	0	2	0	2	0	1	1	4	0	13
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

APPENDIX TABLE DL (continued)

N-Methyl dicyclohexylamine

Multipl Dosing: Air Control (0 ppm)

Week No.	Male No.	361		362		363		364		365		366		367		368		369		370		Total
	Female	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
6	Corpora lutea	15	10	13	14	11	14	15	11	12	10	11	12	14	19	10	0	10	14	14	12	241
	Total Implants	8	10	11	17	12	14	14	12	12	13	11	8	13	16	11	0	14	12	13	12	233
	Live Implants	7	10	11	17	11	14	14	12	12	12	11	8	13	16	11	0	14	12	12	12	229
	Early Deaths	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	4
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	Corpora lutea	15	15	9	8	16	11	7	12	15	14	14	14	22	14	16	11	15	13	13	14	268
	Total Implants	15	17	16	4	16	13	4	14	13	14	15	11	14	12	16	10	14	13	10	14	255
	Live Implants	15	16	16	3	13	13	4	13	12	14	14	11	14	12	14	10	13	13	10	14	244
	Early Deaths	0	0	0	1	3	0	0	1	1	0	1	0	0	0	2	0	1	0	0	0	10
	Late Deaths	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
8	Corpora lutea	15	15	15	12	10	13	14	15	13	11	13	11	18	14	13	14	*	11	13	13	253
	Total Implants	13	14	14	13	12	14	14	14	14	13	14	14	14	16	14	13	*	14	16	13	263
	Live Implants	13	14	12	12	12	12	13	13	14	11	11	13	14	14	12	13	*	14	14	12	243
	Early Deaths	0	0	2	1	0	2	1	1	0	2	3	1	0	2	1	0	*	0	2	1	19
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	*	0	0	0	1
9	Corpora lutea	0	15	15	14	12	17	14	14	16	12	14	13	14	15	13	14	12	15	11	13	263
	Total Implants	0	16	11	15	12	15	15	14	17	12	14	14	14	15	11	14	13	15	11	14	262
	Live Implants	0	14	10	14	12	15	15	14	17	12	13	13	14	15	11	13	9	15	11	13	250
	Early Deaths	0	2	1	1	0	0	0	0	0	0	1	0	0	0	0	1	4	0	0	1	11
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
10	Corpora lutea	0	11	8	18	14	12	15	11	15	9	13	14	12	10	19	0	12	16	14	12	235
	Total Implants	0	13	12	15	14	10	15	10	14	11	11	13	12	13	16	0	13	14	2	15	223
	Live Implants	0	10	11	14	14	10	15	10	13	11	11	13	11	13	14	0	11	14	2	15	212
	Early Deaths	0	3	1	1	0	0	0	0	1	0	0	0	1	0	2	0	2	0	0	0	11
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

* - Ambiguous result

APPENDIX TABLE DL (continued)

N-Methyl dicyclohexylamine

Multiple Dosing: 5 ppm

Week No.	Male No.	371		372		373		374		375		376		377		378		379		380		Total
	Female	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
1	Corpora lutea	13	14	12	13	13	10	13	17	11	11	14	13	12	12	13	13	12	10	6	12	244
	Total Implants	16	13	12	14	11	13	14	16	5	12	15	13	13	14	14	13	12	14	5	14	253
	Live Implants	16	11	12	13	10	13	14	15	3	12	15	11	12	14	14	13	11	13	5	14	241
	Early Deaths	0	2	0	1	1	0	0	1	2	0	0	2	1	0	0	0	1	1	0	0	12
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	Corpora lutea	14	12	13	21	11	14	13	12	12	15	16	12	12	15	18	19	13	13	0	11	266
	Total Implants	13	12	10	13	13	14	13	16	10	14	12	15	13	14	8	13	9	7	0	13	232
	Live Implants	12	9	10	13	12	10	10	16	9	12	11	14	13	12	8	13	8	6	0	13	211
	Early Deaths	1	3	0	0	1	4	3	0	1	1	0	1	0	1	0	0	1	1	0	0	18
	Late Deaths	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	3
3	Corpora lutea	13	11	14	14	15	11	13	15	12	16	10	17	13	13	12	15	11	14	11	12	262
	Total Implants	13	11	15	14	15	14	13	15	14	16	11	12	14	14	17	13	11	13	11	14	270
	Live Implants	13	11	15	13	13	13	13	15	13	14	11	12	14	14	16	11	11	12	11	12	257
	Early Deaths	0	0	0	1	2	1	0	0	1	2	0	0	0	0	1	1	0	1	0	2	12
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
4	Corpora lutea	13	16	14	22	17	10	13	16	12	13	16	14	16	15	12	14	17	11	14	12	287
	Total Implants	12	13	13	14	18	13	14	13	13	12	12	13	14	14	12	15	13	13	13	12	266
	Live Implants	11	13	13	14	18	13	13	13	12	12	12	12	14	12	12	14	13	13	11	12	257
	Early Deaths	1	0	0	0	0	0	1	0	0	0	0	1	0	2	0	1	0	0	2	0	8
	Late Deaths	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
5	Corpora lutea	9	11	9	13	10	11	15	14	14	16	14	18	13	13	13	16	11	14	12	12	258
	Total Implants	10	11	9	12	11	12	15	12	12	15	15	16	13	13	12	16	12	11	13	12	255
	Live Implants	10	11	8	12	11	12	15	12	12	13	14	15	13	12	11	16	12	14	12	12	247
	Early Deaths	0	0	1	0	0	0	0	0	0	2	1	1	0	1	1	0	0	0	1	0	8
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

APPENDIX TABLE DL (continued)

N-Methyl dicyclohexylamine

Multiple Dosing: 5 ppm

Week No.	Male No.	371		372		373		374		375		376		377		378		379		380		Total
	Female	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
6	Corpora lutea	12	15	13	13	14	12	13	12	12	15	13	10	13	15	12	14	13	14	10	10	255
	Total Implants	10	17	14	11	14	14	14	15	13	15	13	13	14	16	13	13	14	15	13	8	269
	Live Implants	10	17	13	11	14	14	14	15	13	13	13	8	14	16	12	13	13	15	12	7	257
	Early Deaths	0	0	1	0	0	0	0	0	0	2	0	5	0	0	1	0	1	0	0	1	11
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
7	Corpora lutea	15	14	13	15	14	17	13	11	17	11	15	12	16	14	16	15	16	14	17	13	288
	Total Implants	15	14	13	16	14	17	15	15	15	11	15	11	18	13	16	15	15	16	10	13	287
	Live Implants	15	14	12	16	14	17	15	15	14	11	15	9	14	11	16	15	15	14	10	12	274
	Early Deaths	0	0	1	0	0	0	0	0	1	0	0	2	4	2	0	0	0	2	0	1	13
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	Corpora lutea	9	13	9	11	10	12	14	12	13	22	10	19	16	17	14	14	14	13	15	14	271
	Total Implants	10	14	7	10	11	14	15	13	13	15	15	14	13	15	13	15	13	12	15	13	260
	Live Implants	9	14	8	10	11	13	13	13	11	15	14	13	12	15	12	14	11	12	13	13	246
	Early Deaths	1	0	1	0	0	0	0	0	2	0	1	0	1	0	1	1	2	0	2	0	12
	Late Deaths	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	2
9	Corpora lutea	11	16	13	15	4	13	13	15	14	14	12	15	13	12	15	14	18	11	14	13	265
	Total Implants	11	15	11	14	1	13	13	15	14	13	14	13	16	11	12	14	15	13	8	10	246
	Live Implants	8	15	11	14	1	13	12	15	13	12	13	13	16	10	11	14	14	13	7	8	233
	Early Deaths	3	0	0	0	0	0	1	0	1	1	1	0	0	0	1	0	1	0	1	2	12
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
10	Corpora lutea	11	12	11	13	12	14	14	12	15	12	11	10	15	15	11	14	11	16	17	16	262
	Total Implants	13	15	12	14	13	14	12	14	13	12	11	10	12	15	11	13	11	12	14	14	255
	Live Implants	11	15	12	14	13	13	11	14	13	11	11	10	11	15	10	13	11	12	14	13	247
	Early Deaths	2	0	0	0	0	1	1	0	0	1	0	0	1	0	1	0	0	0	0	0	7
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1

APPENDIX TABLE DL (continued)

N-Methyl dicyclohexylamine

Multiple Dosing: 25 ppm

Week No.	Male No.	381		382		383		384		385		386		387		388		389		390		Total
	Female	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
1	Corpora lutea			14	17	0	0	13	12	0	14	12	11	13	12	15	11	12	16	12	15	199
	Total Implants			14	18	0	0	13	12	0	14	13	10	11	13	15	12	13	14	15	14	201
	Live Implants			14	17	0	0	12	11	0	14	12	10	11	12	15	12	13	13	15	14	195
	Early Deaths			0	1	0	0	1	1	0	0	1	0	0	1	0	0	0	1	0	0	6
	Late Deaths			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	Corpora lutea			13	15	13	11	14	0	13	11	14	16	15	13	12	15	13	11	13	12	224
	Total Implants			12	15	14	11	14	0	11	12	16	15	16	11	13	15	13	12	15	14	229
	Live Implants			12	15	13	9	11	0	10	12	16	15	15	11	13	15	12	12	15	14	220
	Early Deaths			0	0	1	2	3	0	1	0	0	0	1	0	0	0	1	0	0	0	9
	Late Deaths			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	Corpora lutea	MALE		12	12	10	5	10	11	11	10	11	15	15	12	13	14	14	13	14	12	214
	Total Implants			16	11	12	1	10	12	11	13	13	13	16	14	10	14	16	14	14	11	221
	Live Implants			15	11	11	1	10	12	11	13	11	13	16	13	10	14	15	14	14	10	214
	Early Deaths			1	0	1	0	0	0	0	0	2	0	0	1	0	0	1	0	0	1	7
	Late Deaths			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	Corpora lutea			4	13	9	16	11	16	16	12	15	0	8	14	12	14	13	13	12	10	208
	Total Implants			0	14	11	14	12	11	15	10	15	0	9	14	14	14	13	13	13	9	201
	Live Implants			0	14	10	13	11	11	15	10	14	0	8	12	14	14	12	12	13	9	192
	Early Deaths			0	0	1	1	0	0	0	0	1	0	1	2	0	0	1	1	0	0	8
	Late Deaths			0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
5	Corpora lutea			16	15	2	13	12	13	19	16	14	15	16	13	0	12	14	15	14	14	233
	Total Implants			15	14	0	13	10	13	16	14	13	15	16	14	0	13	13	15	13	14	221
	Live Implants			15	14	0	13	10	13	16	14	12	15	16	13	0	12	13	14	12	14	216
	Early Deaths			0	0	0	0	0	0	0	0	1	0	0	1	0	1	0	1	1	0	5
	Late Deaths			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

MALE DEAD

APPENDIX TABLE DL (continued)

N-Methyl dicyclohexylamine

Multiple Dosing: 25 ppm

Week No.	Male No.	381		382		383		384		385		386		387		388		389		390		Total
	Female	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
6	Corpora lutea			14	9	10	12	10	15	15	15	14	11	11	13	11	12	10	13	14	15	224
	Total Implants			12	13	12	12	11	19	14	17	14	12	13	14	14	13	12	13	13	14	242
	Live Implants			12	12	10	12	11	18	13	16	14	12	12	14	11	11	12	12	12	12	226
	Early Deaths			0	1	2	0	0	1	1	1	0	0	1	0	3	2	0	1	1	2	16
	Late Deaths			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	Corpora lutea			11	0	0	16	9	11	16	15	14	14	11	17	16	12	14	14	12	14	216
	Total Implants			12	0	0	14	12	12	17	16	14	14	11	16	15	13	14	13	12	14	219
	Live Implants			11	0	0	12	11	11	17	16	13	12	10	15	14	12	14	13	12	14	207
	Early Deaths			1	0	0	2	1	0	0	0	1	2	1	1	0	1	0	0	0	0	10
	Late Deaths			0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	2
8	Corpora lutea	MALE	DEAD	14	13	12	18	12	11	13	12	13	19	17	9	12	11	13	10	14	12	235
	Total Implants			13	13	13	14	14	12	13	13	14	16	13	11	13	13	16	9	12	13	235
	Live Implants			13	13	11	13	12	12	12	12	11	16	13	11	12	12	14	9	12	13	221
	Early Deaths			0	0	2	1	2	0	1	1	2	0	0	0	1	1	2	0	0	0	13
	Late Deaths			0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1
9	Corpora lutea			12	12	14	17	0	12	16	11	13	15	15	17	11	11	11	15	12	14	228
	Total Implants			12	13	14	15	0	15	16	9	13	15	13	11	11	12	12	16	12	11	220
	Live Implants			12	13	12	13	0	13	14	9	13	14	13	11	11	12	9	15	11	10	205
	Early Deaths			0	0	1	2	0	1	2	0	0	1	0	0	0	0	3	1	1	0	12
	Late Deaths			0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	3
10	Corpora lutea			11	12	14	15	10	12	0	12	15	13	7	8	12	14	13	11	13	12	204
	Total Implants			13	15	13	15	4	13	0	15	16	14	5	15	12	16	12	13	14	14	219
	Live Implants			13	15	11	15	4	13	0	15	16	14	5	15	12	15	12	11	14	14	214
	Early Deaths			0	0	2	0	0	0	0	0	0	0	0	0	0	1	0	2	0	0	5
	Late Deaths			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

APPENDIX TABLE DL (continued)

N-Methyl dicyclohexylamine

Multiple Dosing: Ethyl methanesulphonate, 100 mg/kg

Week No.	Male No.	391		392		393		394		395		396		397		398		399		400		Total
	Female	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
1	Corpora lutea	11	6	10	7	10	0	7	9	0	0	6	9	13	10	0	10	1	0	7	8	124
	Total Implants	10	6	9	10	13	0	7	12	0	0	1	14	13	9	0	6	0	0	14	12	136
	Live Implants	4	0	3	2	1	0	0	12	0	0	1	4	5	4	0	1	0	0	1	1	39
	Early Deaths	6	6	6	8	12	0	7	0	0	0	0	10	8	5	0	5	0	0	13	11	97
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	Corpora lutea	0	2	3	7	0	3	4	1	0	1	4	2	1	0	0	0	0	0	7	0	35
	Total Implants	0	0	2	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	5
	Live Implants	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Early Deaths	0	0	2	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	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	1	1	2	0	1	7	3	2	5	3	2	0	0	2	4	5	0	0	4	2	44
	Total Implants	0	0	2	0	0	1	0	0	1	0	0	0	0	1	2	1	0	0	1	2	11
	Live Implants	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Early Deaths	0	0	2	0	0	1	0	0	1	0	0	0	0	1	2	1	0	0	1	2	11
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	Corpora lutea	12	2	8	16	18	12	5	14	13	15	12	20	12	14	13	0	0	0	10	7	203
	Total Implants	11	0	8	16	11	12	1	14	12	11	14	11	14	11	14	0	0	0	7	0	167
	Live Implants	1	0	1	9	11	9	0	8	10	8	12	7	11	11	11	0	0	0	5	0	114
	Early Deaths	10	0	7	7	0	3	1	6	2	3	2	4	3	0	3	0	0	0	2	0	53
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	Corpora lutea	13	15	13	14	18	15	16	11	11	10	16	14	12	13	11	11	0	0	11	14	238
	Total Implants	5	15	13	14	14	14	15	11	11	12	14	14	11	13	12	12	0	0	8	14	222
	Live Implants	4	13	13	12	13	12	15	11	11	12	14	13	10	12	12	11	0	0	6	14	208
	Early Deaths	1	2	0	2	1	2	0	0	0	0	0	1	1	1	0	1	0	0	2	0	14
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

APPENDIX TABLE DL (continued)

N-Methyl dicyclohexylamine

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	14	14	14	13	15	17	12	10	11	19	13	17	13	10	10	16	2	3	12	15	250
	Total Implants	17	13	14	13	12	16	14	10	11	14	13	14	11	13	13	16	0	0	13	10	237
	Live Implants	16	12	13	13	10	16	12	9	11	12	13	14	11	11	11	15	0	0	12	9	221
	Early Deaths	1	1	1	0	2	0	2	1	0	1	0	0	0	2	2	0	0	0	1	1	15
	Late Deaths	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
7	Corpora lutea	13	14	12	16	13	16	13	13	16	14	14	14	0	11	15	15	0	0	18	13	240
	Total Implants	16	13	12	13	13	17	13	13	16	15	12	13	0	9	15	16	0	0	13	12	231
	Live Implants	15	13	10	13	6	15	13	12	16	13	12	13	0	9	15	15	0	0	13	8	211
	Early Deaths	1	0	2	0	7	1	0	1	0	2	0	0	0	0	0	1	0	0	0	4	19
	Late Deaths	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
8	Corpora lutea	13	15	10	16	12	0	12	11	15	13	15	9	13	11	19	12	13	0	15	12	236
	Total Implants	13	14	12	15	14	0	12	12	13	15	16	11	10	9	14	12	14	0	16	14	236
	Live Implants	13	14	12	13	13	0	12	12	13	13	16	11	10	9	11	11	14	0	13	14	224
	Early Deaths	0	0	0	2	0	0	0	0	0	2	0	0	0	0	3	1	0	0	2	0	10
	Late Deaths	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2
9	Corpora lutea	0	15	17	15	20	11	10	11	12	15	13	11	13	13	14	12	1	14	14	12	243
	Total Implants	0	14	16	14	18	3	9	9	12	15	13	11	13	14	13	11	0	14	15	13	227
	Live Implants	0	12	16	14	18	3	9	9	12	15	13	10	11	14	11	10	0	14	14	12	21
	Early Deaths	0	2	0	0	0	0	0	0	0	0	0	1	2	0	2	1	0	0	1	1	10
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	Corpora lutea	14	14	13	11	16	10	12	13	16	11	12	16	14	14	11	13	12	15	13	0	250
	Total Implants	14	14	13	12	15	13	10	13	16	14	13	11	13	14	12	12	13	15	13	0	250
	Live Implants	12	14	12	12	15	13	10	12	15	14	12	11	13	14	11	11	13	15	12	0	241
	Early Deaths	2	0	1	0	0	0	0	1	1	0	1	0	0	0	1	1	0	0	1	0	9
	Late Deaths	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

APPENDIX TABLE SA

N-Methyl dicyclohexylamine
Sperm Abnormality Assessment

Multiple Dosing: Air Control (0 ppm)
Low, 5 ppm
High, 25 ppm
Positive, Ethyl methanesulphonate 200 mg/kg

Slide No.	Normal	Abnormality					Total Abnormal	Total Examined	De-coded Information	
		A	B	C	D	E			Animal No.	Group
355	967	1	3	11	10	8	33	1000	321	Air
324	967	0	1	8	9	15	33	1000	322	Air
349	950	0	5	26	9	10	50	1000	323	Air
333	975	0	0	13	4	8	25	1000	324	Air
356	973	0	2	12	1	12	27	1000	325	Air
323	943	1	10	18	7	21	57	1000	326	Air
345	933	1	5	31	10	20	67	1000	327	Air
325	962	2	3	16	7	10	38	1000	328	Air
344	962	0	2	17	2	17	39	1000	329	Air
322	970	2	0	10	9	9	30	1000	330	Air
335	895*	0	1	19	78*	7	105*	1000	331*	Low
346	964	0	3	12	9	12	36	1000	332	Low
337	958	0	1	29	5	7	42	1000	333	Low
329	960	1	1	16	12	10	40	1000	334	Low
350	954	0	1	13	26	6	46	1000	335	Low
343	958	1	0	20	9	12	42	1000	336	Low
326	947	2	4	24	4	19	53	1000	337	Low
341	951	0	4	26	16	13	49	1000	338	Low
357	972	0	1	9	12	6	28	1000	339	Low
334	968	1	1	14	3	13	32	1000	340	Low

* Animal No. 331: abnormal recording for Category E

APPENDIX TABLE SA (continued)

N-Methyl dicyclohexylamine

Multiple Dosing: Air Control (0 ppm)
Low, 5 ppm
High, 25 ppm
Positive, Ethyl methanesulphonate, 200 mg/kg

Slide No.	Normal	Abnormality					Total Abnormal	Total Examined	De-coded Information	
		A	B	C	D	E			Animal No.	Group
-	-	-	-	-	-	-	-	-	341	High**
-	-	-	-	-	-	-	-	-	342	High**
-	-	-	-	-	-	-	-	-	343	High**
-	-	-	-	-	-	-	-	-	344	High**
-	-	-	-	-	-	-	-	-	345	High**
-	-	-	-	-	-	-	-	-	346	High**
-	-	-	-	-	-	-	-	-	347	High**
-	-	-	-	-	-	-	-	-	348	High**
-	-	-	-	-	-	-	-	-	349	High**
-	-	-	-	-	-	-	-	-	350	High**
351	937	0	4	34	8	17	63	1000	351	+
339	926	1	5	34	10	24	74	1000	352	+
352	939	3	4	24	8	22	61	1000	353	+
330	922	0	5	38	20	15	78	1000	354	+
354	821	4	17	139	5	14	179	1000	355	+
347	922	2	7	38	8	23	78	1000	356	+
359	942	0	1	29	8	20	58	1000	357	+
360	934	2	3	32	6	23	66	1000	358	+
340	925	1	4	34	12	24	75	1000	359	+
348	916	0	2	41	21	20	84	1000	360	+

** High Dose Group: all animals dead as a result of dosing

APPENDIX TABLE 5A (Repeat)

N-Methyl dicyclohexylamine
Sperm Abnormality Assessment

Multiple Dosing: Air Control (0 ppm)
Low, 5 ppm
High, 25 ppm
Positive, Ethyl methanesulphonate, 200 mg/kg

Slide No.	Normal	Abnormality					Total Abnormal	Total Examined	De-coded Information	
		A	B	C	D	E			Animal No.	Group
411	964	0	1	19	12	4	36	1000	411	Air*
412	957	0	3	22	8	10	43	1000	412	Air*
413	955	1	0	23	10	11	45	1000	413	Air*
421	967	0	0	18	10	5	33	1000	421	High**
422	***	-	-	-	-	-	***	***	422	High**
423	***	-	-	-	-	-	***	***	423	High**
424	***	-	-	-	-	-	***	***	424	High**
425	964	0	1	19	12	4	36	1000	425	High**
426	***	-	-	-	-	-	***	***	426	High**
427	***	-	-	-	-	-	***	***	427	High**
428	962	0	3	13	7	15	38	1000	428	High**
429	***	-	-	-	-	-	***	***	429	High**
430	***	-	-	-	-	-	***	***	430	High**

* Control animals not exposed in inhalation chambers

** First replacement batch of high dose group animals (Nos. 401-410) all dead as a result of dosing. Second replacement batch (Nos. 421-430) exposed for 2 days

*** Animal dead as a result of dosing