

A 03

FYI-0199-1348

Berufsgenossenschaft der chemischen Industrie



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MR 15646

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Bearbeiter
Frau Dr. Beth

Ihr Zeichen

Ihre Nachricht vom

22.06.1995

Unser Zeichen
(Bitte stets angeben!)

Dr.Bth/Hs-Tgb.-Nr. 462
630.6/052-1.2.1

Tag:

26.06.1995

No. 108 Methacrolein (78-85-3)



FYI-99-001348

Dear Mr. Sherlock,

thank you for your letter of June 2, 1995 concerning our 90-day inhalation study in rats. I would like to inform you that you may summarize the report's findings in the EPA's public docket, however, it should be mentioned that the study has been performed on behalf of the BG Chemie (Employment Accident Insurance Fund for the Chemical Industry) in Heidelberg/Germany under its "Programme for the Prevention of Health Hazards Caused by Industrial Substances".

→ Scott Sherlock, (MD)

Sincerely yours

Leiter des Bereiches Prävention

Im Auftrag

Dr. Beth

Ø Herrn Dr. C. Auer, EPA

CONTAINS NO CBI



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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

JUN - 2 1995

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Dr. Huber
BG Chemie
Postfach 10 14 80
69004 Heidelberg, Germany

Re: Methacrolein (78-85-3) (B.G. No. 108) Study directed to EPA
January 30, 1995.

Dear Dr. Huber:

You directed the above study to Dr. Auer in January and requested that the Agency "not publish it in a scientific journal." While the Agency has no desire to actually publish the report, we would be interested in placing it in the EPA's public docket and possibly summarizing the report's findings. Would this be possible?

Please telephone me at 202/260-1536 or write to me at:

USEPA, Mail Code 7404
401 M Street, SW,
Washington, DC 20460

Thank you for your attention to this.

Very truly yours,

Scott M. Sherlock
Attorney Advisor
Information Management Division

cc Dr. Charlie Auer



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A 07

Berufsgenossenschaft der chemischen Industrie



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FYI - 95 - 01348

INITIAL

Herrn Director
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Kurfürsten-Anlage 62 461
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Bearbeiter
Herr Dr. Huber

Ihr Zeichen

Ihre Nachricht vom

Unser Zeichen
(Bitte stets angeben!)

Tag

Dr. Hu/Hsch-Tgb.-nr. 66
630.6/052-1.2.1

30.01.1995

No. 108 methacrolein (78-85-3)

Dear Dr. Auer,

please find attached the complete report of the 13-week inhalation toxicity study with methacrolein. This report is for EPA's internal use and should not be published in a scientific journal. If there is interest on the report of a third party we ask you to forward the inquiry to us.

Best regards.

Yours sincerely

Leiter des Bereiches Prävention

Im Auftrag

W. Huber
Dr. Huber

A 08

received
2/6/95 CD

B 01

HRC Report

METHACROLEIN (B.G. No. 108)

13-WEEK INHALATION

TOXICITY STUDY IN RATS

**Huntingdon
Research
Centre**



**METHACROLEIN (B.G. No. 108)
13-WEEK INHALATION TOXICITY STUDY IN RATS**

Sponsor

BG Chemie,
Kurfürsten-Anlage 62,
69115 Heidelberg,
GERMANY.

Sponsor's representative

Dr. Huber

Sponsor's monitoring scientist

Dr. H.J. Klimisch,
BASF AG,
Abteilung Toxikologie,
ZNT-Z 470,
67509 Ludwigshafen,
GERMANY.

Contractor

Huntingdon Research Centre Ltd.,
P.O. Box 2,
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Cambridgeshire,
PE18 6FS,
ENGLAND

Report issued 1 November 1994

CONTENTS

	Page
TITLE PAGE	1
CONTENTS	2
COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS	6
QUALITY ASSURANCE STATEMENTS	7
RESPONSIBLE PERSONNEL	9
SUMMARY	11
INTRODUCTION	14
ANIMALS AND MANAGEMENT	
Animals	15
Accommodation	16
Diet	16
TEST SUBSTANCE AND ADMINISTRATION	
Test substance	18
Administration	19
EXPOSURE SYSTEM AND PROCEDURE	
Test atmosphere generation	20
Exposure chambers	20
Procedure	21
Target concentrations	21

	Page
EXPOSURE CHAMBER CONDITIONS	
Chamber analysed atmosphere concentration methacrolein	22
Nominal concentration	22
Chamber spatial distribution	22
Chamber airflow	22
Chamber pressure	22
Chamber temperature and relative humidity	25
CLINICAL OBSERVATIONS	
Clinical signs	24
Bodyweight	24
Food consumption	24
Ophthalmoscopy	24
LABORATORY INVESTIGATIONS	
Haematology	25
Biochemistry	26
TERMINAL STUDIES	
Sacrifice	28
Macroscopic pathology and organ weight analysis	28
Fixation of tissues	29
Microscopic examination	29
STATISTICAL ANALYSIS	30
REFERENCES	31

	Page
RESULTS	
Chamber atmosphere conditions	
Analysed concentrations of methacrolein	32
Nominal concentrations of methacrolein	32
Chamber temperature and relative humidity	33
Clinical observations	
Mortality	33
Clinical signs	33
Bodyweight	33
Food consumption	34
Ophthalmoscopy	34
Laboratory investigations	
Haematology	34
Biochemistry	35
Terminal studies	
Macroscopic pathology	36
Organ weights	36
Microscopic pathology	37
Treatment-related changes	37
Incidental changes	37
DISCUSSION AND CONCLUSION	39
FIGURES	
1. Vapour generator	40
2. Exposure system	41
3. Location of sample ports on exposure chambers	42
4. Bodyweights - group mean values	43
5. Food consumption - group mean cumulative values	44
TABLES	
1. Exposure mean analysed concentration of methacrolein	45
2. Nominal concentration of methacrolein	47
3. Exposure mean chamber temperature and relative humidity	50
4. Bodyweights - group mean values	53
5. Food consumption - group mean values	54
6. Haematology - group mean values	55
7. Biochemistry - group mean values	56
8(a-b). Macroscopic pathology incidence summary	58
9(a-b). Organ weights - group mean values	62
10(a-c). Microscopic pathology incidence summary	66

	Page
APPENDICES	
1. Composition and quality assurance aspects of diet and drinking water	78
2. Method of analysis for methacrolein	81
3. Purity and stability analysis of methacrolein	84
4. Chamber spatial distribution of methacrolein	106
5. Chamber concentration of methacrolein	107
6. Individual clinical signs	120
7. Bodyweights - individual values	124
8. Food consumption - cage mean values	140
9. Haematology - individual values	142
10. Biochemistry - individual values	146
11(a-b). Organ weights - individual values	150
12. Pathology - individual findings	156
13. Protocol and protocol amendments	295
ANNEX Methacrolein (B.G. No. 108) two-week repeat dose preliminary inhalation toxicity study in rats (HRC Schedule no. BGH 40/920648)	331

COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

The study described in this report was conducted in compliance with the following Good Laboratory Practice standards and I consider the data generated to be valid.

Good Laboratory Practice, The United Kingdom Compliance Programme, Department of Health & Social Security 1986 and subsequent revision, Department of Health 1989.

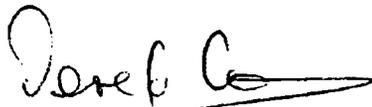
EC Council Directive, 87/18 EEC of 18 December 1986, (No. L 15/29).

Good Laboratory Practice in the testing of Chemicals OECD, ISBN 92-64-12367-9, Paris 1982, subsequently republished OECD Environment Monograph No. 45, 1992.

United States Environmental Protection Agency, (TSCA), Title 40 Code of Federal Regulations Part 792, Federal Register, 29 November 1983 and subsequent amendment Federal Register 17 August 1989.

Japan Ministry of International Trade and Industry, Directive 31 March 1984 (Kanpogyo No. 39 Environmental Agency, Kikyoku No. 85 MITI).

At the time of analysis, Reading Scientific Services Ltd was not part of the UK (DOH) GLP monitoring program.



Derek W. Coombs, B.Sc.,
Study Director,
Huntingdon Research Centre Ltd.

01 November 1994
Date

QUALITY ASSURANCE STATEMENT

This report of study BGX 50, excluding Appendix 3, has been audited by the Huntingdon Research Centre Quality Assurance Department. The methods, practices and procedures reported herein are an accurate description of those employed at HRC during the course of the study. Observations and results presented in this final report form a true and accurate representation of the raw data generated during the conduct of the study at HRC.

Date of reporting audit findings to the
Study Director and HRC Management

14 February 94

G.R. Keeble

G.R. Keeble,
Systems Compliance Auditor,
Department of Quality Assurance,
Huntingdon Research Centre Ltd.

27 October 1994

QUALITY ASSURANCE STATEMENT

Inspections were made by the Quality Assurance Department of various phases of the study BGH 50 as conducted at HRC and described in this report. The dates on which the inspections were made and the dates on which findings were reported to the Study Director and to HRC Management are given below.

Phase of Study	Date of Inspection	Date of Reporting
Protocol Review	-	14 April 93
Pre-experimental Period	28 April 93	30 April 93
Experimental Period	13 - 14 May 93	14 May 93
	19 May 93	20 May 93
	8 July 93	9 July 93
	26 - 30 July 93	30 July 93
	18 August 93	19 August 93
	27 August 93	31 August 93

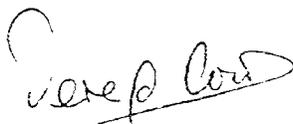
G. R. Keeble

G. R. Keeble,
Systems Compliance Auditor,
Department of Quality Assurance,
Huntingdon Research Centre Ltd.

27 October 1994

RESPONSIBLE PERSONNEL

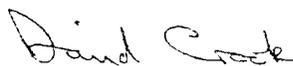
We the undersigned, hereby declare that the work was performed under our supervision according to the procedures herein described, and that this report provides a correct and faithful record of the results obtained.



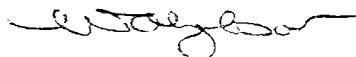
Derek W. Coombs, B.Sc.,
Study Director,
Division of Toxicology.



Terence J. Kenny, B.Sc. (Hons.),
Study Supervisor,
Division of Toxicology.



David Crook, B.Sc. (Hons.), Ph.D.,
Head of Department of Clinical Pathology.



William A. Gibson, B.Sc., (Medical Sciences),
Pathologist - Post Mortem Room,
Department of Pathology.



Richard L. Gregson, M.Phil., Ph.D., C.Biol., M.I.Biol.,
Senior Pathologist,
Department of Pathology.



David J. Lewis, B.Sc., Ph.D., M.I.Biol., M.R.C.Path.,
Deputy Head,
Department of Pathology.

SUMMARY

Test substance: Methacrolein.

Test animals: Albino rats - Cri: CD*(SD)BR Sprague-Dawley strain.

Route of administration: Whole-body exposure to an atmosphere containing the vapour of the test substance.

Duration: Six hours a day, five days a week, for 13 weeks.

Exposure levels.

Group	Designation	Chamber concentration (ppm)		Number of animals	
		Target	Analysed	Males	Females
1	Air control	-		20*	20*
2	Low concentration	1	1.0	10	10
3	Intermediate concentration	5	4.9	10	10
4	High concentration	15	15.3	20*	20*

* 10 animals of each sex were further observed for a 4-week observation period after the end of 13 weeks exposure

Results

Mortality: There were no unscheduled deaths.

Clinical signs: During exposure half-closed eyes were seen in Group 3 (5 ppm) and Group 4 (15 ppm) rats. Licking the inside of the mouth and wet chins, indicating salivation, were also seen occasionally in High concentration (15 ppm) rats.

At other times, no treatment-related signs were seen.

Bodyweight:	<p>Weight gain by Group 4 (15 ppm) rats was decreased in comparison with controls over the 13-week exposure period. The decreased gain was apparent in males from Week 7 of exposure and in females from the commencement of exposures.</p> <p>During the recovery period, weight gain by Group 4 (15 ppm) rats was slightly greater than that of controls.</p>
Food consumption:	<p>During the 13-week exposure period, consumption was decreased in Group 4 (15 ppm) rats compared with controls from Week 7 in males and from commencement of exposures in females.</p> <p>During the recovery period consumption stabilised.</p>
Ophthalmoscopy:	<p>There were no treatment-related findings.</p>
Haematology:	<p>Differences between control and exposed groups were considered not to be of toxicological significance.</p>
Biochemistry:	<p>Differences between control and exposed groups were considered not to be of toxicological significance.</p>
Macroscopic pathology:	<p>There were no treatment-related findings.</p>
Organ weights:	<p>Differences between control and exposed groups were considered not to be toxicological significance.</p>
Microscopic pathology:	<p>Epithelial inflammatory, atrophic and metaplastic changes were seen in the dorsal meatus and dorsal central septum of the nasal passages and, to a lesser degree, in the larynges of animals in the High concentration Group 4 (15 ppm) group only.</p>

B 14

BGH 50/932334

After a period of recovery, the changes seen in the main group animals at termination of exposure showed clear signs of repair and recovery. These included reduced incidence of inflammatory and metaplastic change in the nasal passage with evidence of repair.

Conclusion

The no-adverse-effect concentration in this study was considered to be 4.9 ppm.

INTRODUCTION

This study undertaken at the Huntingdon Research Centre Ltd, Huntingdon, England, was designed to investigate the response of rats to repeated administration by inhalation of the vapour of methacrolein over a period of 13 weeks.

Exposure levels were selected in consultation with the Sponsor, following a 2-week inhalation study (HRC Report No. BGH 40/920648) where rats were exposed to 77, 19 and 5 ppm. Deaths occurred following the first exposure at 77 ppm and exposure of surviving rats was discontinued. Treatment-related effects of exposure at 19 ppm were evident, including reduced bodyweight gain and food consumption together with increased water consumption. Treatment-related changes in the nasal turbinates and larynx were also seen in rats exposed to 19 ppm methacrolein. The no-effect-level in this preliminary study was established at 5 ppm. On the basis of these findings, exposure levels of 0 (Control), 1, 5 and 15 ppm were selected, in order to elicit clear response at the High exposure concentration and with 1 ppm expected to be a no-effect exposure concentration.

On completion of the study, all data pertaining to the study, all specimens and a copy of this report, were retained in the Archive Department of the Huntingdon Research Centre Ltd, Huntingdon, England.

Key dates in the study were:

Approval of protocol by:

Study Director: 3 February 1993

HRC Management: 3 February 1993

Sponsor: 2 March 1993

Arrival of rats: 21 April 1993

Date of first exposure: 30 April 1993

Dates of sacrifice:

Main groups: 30 July 1993

Withdrawal groups: 27 August 1993

This study was designed to comply with the following guidelines:

US EPA (TSCA)

Issued in Federal Register **50** No. 188, Part II of 27 September 1985 and amended Federal Register **52** No. 97 on 20 May 1987 (Guideline 798.2450).

OECD

Guidelines for testing of chemicals No. 413.

ANIMALS AND MANAGEMENT

ANIMALS

One hundred and forty-six (73 male and 73 female) Sprague-Dawley CD rats aged approximately 6 weeks were obtained from Charles River (UK) Ltd, Margate, Kent, England to arrive at Huntingdon Research Centre on 21 April 1993.

On the day of arrival, 5 male and 5 female rats were selected at random, sacrificed and subjected to a detailed macroscopic examination. Nothing abnormal was detected. The remaining rats were arbitrarily selected and identified on a cage basis by a temporary mark on the tail. Ophthalmoscopic examination took place on 22 April 1993. Allocation to 5 groups took place on the following day.

The rats were weighed and the individual bodyweights processed using a computer program which selected 130 rats (65 male and 65 female) for allocation to 5 groups, such that group mean bodyweights were approximately equalised. The rats were uniquely identified by numbers tattooed into the ear pinna. Numbers in excess of 99 were additionally identified by a foot tattoo.

The identification of individual rats in each group, together with the initial allocation group mean bodyweights, were as follows:

Group	Designation	Rat nos.		Group mean bodyweight (g)		
		♂	♀	♂	♀	
1	Air control	- Main	1 - 10	61 - 70	197.3	164.7
		- Recovery	11 - 20	71 - 80		
2	Low concentration		21 - 30	81 - 90	196.8	164.1
3	Intermediate concentration		31 - 40	91 - 100	197.5	164.9
4	High concentration	- Main	41 - 50	101 - 110	197.7	165.3
		- Recovery	51 - 60	111 - 120		
5	Reserve		121 - 125	126 - 130	197.4	165.2

Reserve group rats were retained as potential replacements during the acclimatisation period and were killed when exposures commenced on 30 April 1993.

ACCOMMODATION

The rats were housed 5 of the same sex to a cage, in suspended cages with stainless steel sides and stainless steel mesh floors. Each cage measured 53 cm long, 35 cm wide and 25 cm high. Plastic trays, lined with absorbent paper, were placed below each cage to collect animal excreta. The paper was changed daily and clean cages were introduced at intervals throughout the study.

Each group of rats was kept in a separate ventilated cabinet to prevent any possible cross-contamination between groups once exposures had commenced. The ventilated cabinets drew their air supply from the holding room. Exposure to the test material took place in the same room in which the animal holding cabinets were situated.

The temperature and relative humidity of the room were recorded using a Kent Clearspan M105 chart recorder. The maximum and minimum values over the study period were as follows:

Holding room temperature:	Maximum 25.5°C Minimum 18.0°C
Holding room relative humidity:	Maximum 58% Minimum 31%

The figures quoted above were extremes which were only seen transiently. The temperature and RH were within protocol defined limits for the majority of the time.

Lighting was controlled to give 12 hours light (0800 - 2000 hours) and 12 hours dark per 24 hours.

DIET

While in their cages, all rats had free access to a weighed quantity of standard quality-controlled laboratory rat food (SDS Rat and Mouse No. modified diet, Special Diets Services, Witham, Essex, England).

There was no information available to indicate that any non-nutrient substance likely to influence the effect of the test compound could reasonably be expected to be present in the diet. Analyses were made on all batches of diet used to establish levels of basis nutrients and of specified substances and micro-organisms likely to have been present in the feed components and which, if in excess of specified amounts, might have had an undesirable effect on the test system. All batches of diet used conformed with the acceptable standards agreed by the Study Director and Head, Quality Assurance Department (see Appendix 1). The analytical data have been lodged in HRC Archives.

Tap water was available from moulded polypropylene water bottles at all times while the rats were in the cages. The water bottles were rinsed and refilled daily and thoroughly cleaned at intervals during the study.

There was no information available to indicate that any substance likely to influence the effect of the test substance could reasonably be expected to be present in the drinking water.

The results of the routine physical and chemical analyses of water at source (sampling point, Grafham Final Water) as conducted by the supplier, Anglian Water Services Ltd, have been made available to IIRC. A list of the principal determinands is given in Appendix 1. The analytical data have been lodged in HRC Archives.

TEST SUBSTANCE AND ADMINISTRATION**TEST SUBSTANCE**

Name: Methacrolein (B.G. No. 108)

IUPAC name: 2-Methylpropenal

CAS number: 78-85-3

EINECS number: 201-150-1

Presentation: Liquid

Batch no.: 9200914

Received from: Aldrich Chemical Co Ltd,
Gillingham,
Dorset,
ENGLAND

Receipt date: 9 March 1993 (Manufactured 1 July 1992)

Purity: 90.2% methacrolein
9.4% dimer (3,4-dihydro-2,5-dimethyl-2H-pyran-2-carboxaldehyde)

Stability: Purity analysis by GC/MS was conducted by Reading Scientific Services Ltd on 4 occasions during the study. Dates of analysis and purity details are presented in Appendix 3. There were no essential changes during the study period.

Expiry date: Adequate for the study

Storage: 4°C

ADMINISTRATION

The rats were exposed 6 hours a day, whole-body, to atmospheres containing methacrolein vapour. Exposures commenced on the Friday of the first week and continued for 5 days a week (Monday to Friday) until Week 13 where the rats were exposed for 4 days (Monday to Thursday). This regime allowed for all Main group rats to be killed on the day following the final exposure. The rats received a total of 65 exposures. Recovery groups for the Air control and High concentration were held unexposed for a further 4 weeks.

EXPOSURE SYSTEM AND PROCEDURE

TEST ATMOSPHERE GENERATION (Figure 1, page 40)

The vapour of the test substance was generated by metering the liquid test substance from a glass gas-tight syringe mounted on an infusion pump (Precidor® type 5003) to a sintered glass frit contained in a glass vessel.

Air was fed through each vaporiser at 150 l/min. The air was warmed by passage through a coiled copper tube immersed in a water bath maintained at 80°C.

The vapour/air mixture produced passed from the all glass vaporiser into the base of a glass elutriation column and from there to the chamber inlet duct. After passing through the chamber, the chamber atmosphere was removed via an extract duct using an extract fan.

Different vapour concentrations appropriate to each exposure group were achieved by varying the rate at which the test substance was metered to the glass frit.

EXPOSURE CHAMBERS (Figure 2, page 41)

The exposure chambers were constructed of stainless steel and glass. Each of the 4 chambers used was approximately 0.75 m³ in volume. The chambers were of square plan section fitted with a pyramidal base and top. A square-shaped 3 inch diameter tubular perforated exhaust plenum was fitted in the base of each chamber below exposure cage level. A perforated dispersion plate was fitted at the point of air entry. A 1.5 inch drainage duct fitted with a ball valve was present in the centre base of the chamber and was connected with a drainage system.

Exposure cages, constructed of stainless steel mesh, were suspended on a framework, arranged on four levels. Each level held four cages each capable of individually housing 4 rats. This gave a total potential animal exposure capacity of 64 rats. However, in this investigation a maximum of 8 cages were used, per group.

The chambers were each fitted with eight ports for withdrawal of chamber air samples for analysis. Routinely a mid-centre upper port was used for sample collection.

The chamber airflow was 150 litres per minute. The air entered the chamber through the inlet duct and dispersion plate. Airflows were monitored by tapered tube flow meters, mounted at the front of a purpose-built stainless steel trolley. The generation apparatus was also mounted on the trolley.

A magnehelic pressure gauge (0 - 100 mm water gauge) was connected with each chamber by a nylon tube. This was also mounted on the trolley and was used to monitor chamber internal pressure.

Removal of the test atmosphere from each chamber was accomplished by means of individual air handling units containing coarse and fine filtration media, together with activated charcoal. The flow was adjusted using gate valves, mounted in the exhaust ducting between the chamber and filters, and the internal pressure within each chamber was set to 10 mm water gauge below ambient when operational.

A separate exposure chamber was used for each group of rats. The Air control animals were exposed using a similar system to that used for the test groups receiving methacrolein.

PROCEDURE

The water baths were switched on and set to 70°C.

The animals were removed from their holding cages, and placed within the wire mesh exposure cages in the chamber appropriate to each group. The chamber doors were sealed after fitting of a wet and dry bulb thermohygrometer.

Syringes were filled with the test substance and mounted on Precidor infusion pumps. The initial volume of each syringe was recorded. The airflow to the vaporisers was turned on and the internal pressure of the chambers adjusted to 10 mm water gauge using the gate valves.

Exposure commenced when the infusion pumps were switched on and operating at the determined rate.

Samples for the determination of total chamber concentration were taken during each six-hour exposure. Records of chamber temperature and relative humidity were made, together with the reaction, if any, by exposed rats.

After six hours exposure the infusion pumps were switched off and the volume of methacrolein remaining in the syringes recorded. The vaporiser air supplies were turned off. The rats were unloaded from the chamber into their respective holding cages following a period of chamber clearance of at least 20 - 30 minutes for test group animals.

TARGET CONCENTRATIONS

The target concentrations of methacrolein were as follows:

- Group 2 (Low concentration) - 1 ppm
- Group 3 (Int concentration) - 5 ppm
- Group 4 (High concentration) - 15 ppm

EXPOSURE CHAMBER CONDITIONS

CHAMBER ANALYSED CONCENTRATION OF METHACROLEIN

The concentration of methacrolein present in the exposure chambers used for Groups 2, 3 and 4 was determined on at least 3 occasions during each exposure. Samples were taken at approximately 1, 3 and 5 hours from the start of exposure.

Samples of test atmosphere were withdrawn at 2 litres per minute through charcoal adsorption tubes (Lot 120, NIOSH approved, SKC Inc, PA, USA). The contents of the tubes were eluted into accurately measured 2 ml aliquots of carbon disulphide.

The samples were analysed by flame ionisation chromatography using external standards according to the method detailed in Appendix 2.

NOMINAL CONCENTRATION

The nominal chamber concentration of methacrolein was calculated from the formula:

$$\frac{\text{Volume used (ml)} \times 1000 \times \text{density (0.847 g/ml)}}{\text{Total volume air used over the period of exposure (l)}}$$

CHAMBER SPATIAL DISTRIBUTION (Figure 3)

The spatial distribution of methacrolein in the exposure chambers was determined for each concentration level during preliminary studies. The results are presented in Appendix 4. The spatial distribution of the test vapour was considered satisfactory.

CHAMBER AIRFLOW

Diluent airflow was monitored continuously using tapered tube rotameters and recorded at 30-minute intervals throughout each exposure.

CHAMBER PRESSURE

The chamber internal pressure relative to ambient was monitored continuously by magnehelic pressure gauges and recorded at 30-minute intervals throughout exposure.

CHAMBER TEMPERATURE AND RELATIVE HUMIDITY

The wet and dry bulb temperatures of the thermohygrometer in each chamber were recorded at 30-minute intervals throughout each exposure. The chamber relative humidity was calculated from these data.

CLINICAL OBSERVATIONS**CLINICAL SIGNS****During exposure**

Clinical signs during exposure were recorded as a group response where all visible animals appeared to be responding similarly or a proportion were affected. Group responses seen during exposure were not transferred to the individual clinical sign sheets, and are reported in the text of this report.

At other times

Animals were examined twice each day, usually prior to loading and immediately following unloading from the chambers on exposure days, and in the morning and afternoon of non-exposure days. An entry was made on the individual clinical sign sheets once each week even if abnormalities were not seen.

BODYWEIGHT

Each rat was weighed for allocation to groups, then at weekly intervals commencing 1 week before the start of dosing and continuing throughout the study.

In addition, the weight of each rat at necropsy was recorded.

FOOD CONSUMPTION

The quantity of food consumed by each cage of rats was recorded weekly commencing one week prior to the start of exposures until the end of the study.

OPHTHALMOSCOPY

The eyes of all rats were examined prior to allocation. All rats from the Main groups were examined during the final week of exposure. Examination was carried out using a Keeler Indirect Ophthalmoscope. The pupils of the eyes were dilated with drops of 0.5% tropicamide solution (Mydracil®) prior to examination.

LABORATORY INVESTIGATIONS

Samples of blood were collected from all Main group rats on the morning following the final exposure.

Samples of blood were withdrawn from the orbital sinus while the rats were lightly anaesthetised with ether.

Food was withheld from all animals overnight prior to collection of blood samples.

HAEMATOLOGY

Units

EDTA and sodium citrate (thrombotest) anticoagulants were used. The parameters measured, together with the methods and units used, were as follows:

Packed cell volume (PCV) Ortho ELT-1500	%
Haemoglobin (Hb) Ortho ELT-1500	g/dl
Red cell count (RBC) Ortho ELT-1500	$\times 10^6/\text{mm}^3$
Mean corpuscular haemoglobin concentration (MCHC) by calculation, $\text{Hb (g/dl)} \times 100/\text{PCV (\%)} $	%
Mean corpuscular volume (MCV) - by calculation, $\text{PCV (\%)} \times 10/\text{RBC (}10^6/\text{mm}^3\text{)}$	fl
Total white cell count (WBC)	$\times 10^3/\text{mm}^3$
Platelets (Plts)	$\times 10^3/\text{mm}^3$
Reticulocyte count (Retic - Method of Dacie, J.V. and Lewis, S.M. (Practical Haematology, 1966, 3rd edit. p.28)	% (of red cells)

Differential counts (Diff) - standard microscopy of blood smear, stained with modified Wright's stain, counting 100 cells.

Neutrophils	(N)	}	$\times 10^3/\text{mm}^3$
Lymphocytes	(L)		
Eosinophils	(E)		
Basophils	(B)		
Monocytes	(M)		

Cell morphology: Where abnormal cells were observed when examining any stained slide their presence was recorded

Units

Thrombotest (TT) - Method of Owren, P.A. (Lancet 1959, ii, 754)

s

BIOCHEMISTRY

The blood was placed into proprietary blood collection vials containing lithium heparin anticoagulant. The vials were centrifuged at 3200 'g' for 3 minutes and the plasma analysed for the parameters listed below:

Roche Cobas centrifugal analyser, using appropriate BCL test kit:

Creatine phosphokinase (CPK), also known as creatine kinase - reaction temperature 30°C

mU/ml

Hitachi 737 clinical chemistry analyser:

Glucose - Hexokinase mediated

mg/dl

Glutamic-pyruvic transaminase (GPT), also known as 'alanine aminotransferase'
Reaction temperature 30°C

mU/ml

Glutamic-oxaloacetic transaminase (GOT), also known as 'aspartate aminotransferase'
Reaction temperature 30°C

mU/ml

Gamma glutamyl transferase (γGT)
Reaction temperature 30°C

mU/ml

Total protein

g/dl

Albumin (Alb)

g/dl

Globulin (Glob) - by subtraction,
total protein (g/dl) minus albumin (g/dl)

g/dl

Albumin/Globulin ratio (A/G) by calculation from albumin and total protein concentrations

Urea nitrogen (Urea Nitr)

mg/dl

C 14

BGH 50/932334

	Units
Alkaline phosphatase (AP) Reaction temperature 30°C	mU/ml
Total bilirubin	mg/dl
Creatinine	mg/dl
Sodium (Na)	mEq/l
Potassium (K)	mEq/l
Calcium (Ca)	mEq/l
Inorganic phosphorus (P)	mEq/l
Chloride (Cl)	mEq/l
Cholesterol (Chol)	mg/dl

TERMINAL STUDIES

SACRIFICE

All Main group rats were sacrificed following 13 weeks of exposure. Sacrifice took place on the day following the final exposure.

Recovery group rats were held unexposed for a further 4 weeks and then sacrificed.

The rats were killed by exsanguination from the brachial arteries following anaesthesia induced by intraperitoneal injection of pentobarbitone sodium.

MACROSCOPIC PATHOLOGY AND ORGAN WEIGHT ANALYSIS

The macroscopic appearance of all tissues was noted and the following organs dissected free from each animal and weighed:

adrenals
brain
heart
kidneys

liver
lungs
ovaries
pituitary

prostate
spleen
testes (with epididymides)
thymus

FIXATION OF TISSUES

Samples, or the whole, of the following organs/tissues, together with any macroscopically abnormal entities were preserved in buffered 10% formalin. The eyes were preserved in Davidson's fixative. The lungs were infused with fixative prior to immersion.

^a adrenals	^a heart	^a sciatic nerve
alimentary tract	^a kidneys	seminal vesicle
^a oesophagus	^b larynx (2 levels)	skeletal muscle (thigh)
^a stomach (glandular and non-glandular)	^a liver	skin
^a duodenum	^b lungs (all lobes and mainstem bronchi)	spinal column
^a jejunum,	^a lymph nodes (cervical, mesenteric and tracheobronchial)	^a spinal cord (cervical, thoracic and lumbar)
^a ileum	mammary gland	^a spleen
caecum	^b nasal passages (3 levels)	^a sternum
colon	optic nerve	^a testes (with epididymides)
rectum	^a ovaries	^a thymus
animal identification mark	oviduct	^a thyroid (with parathyroids)
^a aorta	^a pancreas	tongue
^a brain	^a pharynx	^a trachea (including bifurcation)
^a eyes	^a pituitary	ureter
femur with joint (for bone and marrow <i>in situ</i>)	prostate	^a urinary bladder
^a gross abnormalities	^a salivary gland	^a uterus (corpus and cervix)
head (paranasal sinuses, oral cavity, nasopharynx, middle ear, teeth, eyelids, lachrymal gland, Harderian gland and Zymbal's gland)		vagina

MICROSCOPIC EXAMINATION

Light microscopic examination was performed on 4 µm thick sections, stained with haematoxylin and eosin, of these tissues in the table above marked as follows:

- a Rats from Main groups 1 (Air control) and 4 (15 ppm) at termination
- b All Main group rats at termination

Nasal passages and lungs from recovery male and female rats in Groups 1 (Air control) and 4 (15 ppm)

Details of the sectioning of the larynx and nasal passages are included in the Study Protocol presented as Appendix 13 of this report (pages 322 and 323).

STATISTICAL ANALYSIS

All statistical analyses were carried out separately for males and females.

Data relating to food were analysed on a cage basis. For all other parameters the analyses were carried out using the individual animal as the experimental unit. Food consumption data were analysed using cumulative cage weekly average intake per group. Bodyweight data were analysed using weight gains. The following sequence of statistical tests was used for food consumption, bodyweight, organ weight and clinical pathology data:

- (i) If the data consisted predominantly of one particular value (relative frequency of the mode exceeded 75%), the proportion of animals with values different from the mode was analysed by appropriate methods. Otherwise:
- (ii) Bartlett's test (1) was applied to test for heterogeneity of variance between treatments; where significant (at the 1% level) heterogeneity was found, a logarithmic transformation was tried to see if a more stable variance structure could be obtained.
- (iii) If no significant heterogeneity was detected (or if a satisfactory transformation was found), a one-way analysis of variance was carried out. If significant heterogeneity of variance was present, and could not be removed by a transformation, the Kruskal-Wallis analysis of ranks (2) was used.
- (iv) Except for pre-exposure data, analyses of variance were followed by Student's 't' test and Williams' test (3) for a dose-related response, although only Williams' test was reported. The Kruskal-Wallis analyses were followed by Shirley's test (4), the non-parametric equivalent of the 't' and Williams' tests.

Where appropriate, analysis of covariance was used in place of analysis of variance in the above sequence. For organ weight data, the final bodyweight was used as a covariate in an attempt to allow for differences in bodyweight which might affect the organ weights.

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1. Bartlett, M.S., (1937), *Proc. Roy. Soc. Series A*, **160**: 268 - 282.
2. Kruskal, W.H. and Wallis, W.A., (1952/3), *J. Amer. Statist. Ass.*, **47**: 583 - 621 and **48**: 907 - 912.
3. Williams, D.A., (1971/2), *Biometrics*, **27**: 103 - 117 and **28**: 519 - 531.
4. Shirley, E., (1977), *Biometrics*, **33**: 386 - 389.

RESULTS

CHAMBER ATMOSPHERE CONDITIONS

Analysed concentrations of methacrolein

The data are presented as follows:

Table 1 - Exposure mean analysed concentration
Appendix 5 - Chamber concentration of methacrolein

The data are summarised below:

Group	Study mean concentration (ppm)	
	Target	Analysed
2 (1 ppm)	1	1.0
3 (5 ppm)	5	4.9
4 (15 ppm)	15	15.3

Analysed concentrations were in close agreement with targets.

Nominal concentrations of methacrolein

The data are presented in Table 2 and are summarised below:

Group	Study mean	
	(ppm)	A/N (%)
2 (1 ppm)	1.2	88.4
3 (5 ppm)	5.6	89.7
4 (15 ppm)	16.6	92.4

A/N analysed concentration ÷ nominal concentration

Small differences between nominal and analysed concentrations were principally caused by recondensation of the test material within the vaporiser and inlet ducting.

Chamber temperature and relative humidity

The data are presented in Table 3 and are summarised below:

Group	Study mean	
	T(°C)	RH %
1 (Air control)	25.6	38
2 (1 ppm)	25.4	33
3 (5 ppm)	25.0	36
4 (15 ppm)	25.8	47

The differences between groups were small and are considered not to have influenced the outcome of the study.

CLINICAL OBSERVATIONS**Mortality**

There were no unscheduled deaths.

Clinical signs

During exposures half-closed eyes were observed in Group 3 (5 ppm), (Exposures 1 - 6 only) and in Group 4 (15 ppm) (most exposures throughout the study). In addition, occasional observations of licking the inside of the mouth and wet chins, indicating salivation, were made in Group 4 (15 ppm). The observations made are indicative of a non-specific response to exposure to an irritant atmosphere.

Observations made between exposures and at other times are presented for individual animals in Appendix 6. No signs were seen that were considered to be attributable to exposure to methacrolein.

Bodyweight

The data are presented as follows:

- Figure 4 - group mean values
- Table 4 - group mean values
- Appendix 7 - individual values

Recording of bodyweights at Week 13 took place following overnight starvation prior to removal of blood samples, therefore statistical analysis of weight gains following 12 weeks of exposure was also undertaken.

Female rats from Group 4 (15 ppm) gained weight at a reduced rate compared with controls from the commencement to termination of exposures, the reduction was statistically significant ($P < 0.01$ using Williams' test) from Week 3 until Weeks 12 and 13.

Male rats from the Group 4 (15 ppm) gained weight at a similar rate to control rats for the first 7 weeks of exposure. Thereafter, weight gain by Group 4 (15 ppm) males was lower than that of controls. The reduction attained statistical significance ($P < 0.01$) using Williams' test at Week 13 only, however, at Week 12, the degree of significance ($P < 0.05$) was apparent using Student's 't' test.

During the post-exposure observation period a degree of recovery was observed in all Group 4 (15 ppm) rats such that their weight gain over this period was greater than that of controls.

Food consumption

The data are presented as follows:

- Figure 5 - group mean cumulative values
- Table 5 - group mean values
- Appendix 8 - cage mean values

Food consumption measurements at Week 13 were affected by the preceding overnight period of starvation, therefore statistical analysis of cumulative consumption was also undertaken for Week 12 data.

Consumption by female rats from Group 4 (15 ppm) progressively declined relative to control rats from the commencement to the termination of exposures. The reduction attained statistical significance ($P < 0.01$) at Weeks 12 and 13.

Consumption by male rats from Group 4 (15 ppm) was similar to that of controls for the first 7 weeks of exposure, thereafter consumption declined relative to controls. The reduction did not achieve statistical significance.

During the recovery period the progressive decline in consumption by Group 4 (15 ppm) animals halted and consumption stabilised albeit at a lower level than that of control rats. Cumulative consumption by Group 4 (15 ppm) females remained significantly ($P < 0.05$) lower than that of controls.

Ophthalmoscopy

Findings were consistent with the age and strain of the animals examined, there were no treatment-related changes at Week 13.

LABORATORY INVESTIGATIONS

Haematology

The data are presented as follows:

- Table 6 - group mean values
- Appendix 9 - individual values

The following statistically significant differences from control values were seen:

Haemoglobin

Higher concentration in Group 4 (15 ppm) females.

Red cells

Greater numbers in Group 4 (15 ppm) males.

Mean corpuscular haemoglobin concentration

Greater in Group 2 (1 ppm), Group 3 (5 ppm) and Group 4 (15 ppm) females.

Mean corpuscular volume

Lower in Group 3 (5 ppm) and Group 4 (15 ppm) females.

The differences seen were small, inconsistent between the sexes and considered not to be of toxicological significance.

Biochemistry

The data are presented as follows:

- Table 7 - group mean values
- Appendix 10 - individual values

The following statistically significant differences from control values were seen:

Globulin

Lower in Group 4 (15 ppm) females.

Glutamic-pyruvic transaminase

Greater in Group 4 (15 ppm) females.

Electrolytes

Lower sodium concentration in Group 3 (5 ppm) and Group 4 (15 ppm) females.

Lower potassium in Group 4 (15 ppm) females.

Lower inorganic phosphorus in Group 2 (1 ppm), 3 (5 ppm) and 4 (15 ppm) females.

Lower chloride in Group 4 (15 ppm) females.

The differences seen were small, inconsistent between the sexes and considered not to be of toxicological importance.

TERMINAL STUDIES**Macroscopic pathology**

The data are presented as follows:

- Table 8a - Terminal kill incidence summary
- Table 8b - Recovery kill incidence summary
- Appendix 12 - Individual findings

There were no changes revealed in rats killed following 13 weeks of dosing or those killed following 4 weeks of withdrawal that were considered to be attributed to exposure to methacrolein.

Organ weights

The data are presented as follows:

- Table 9a - group mean values (Terminal kill)
- Table 9b - group mean values (Recovery kill)
- Appendix 11a - individual values (Terminal kill)
- Appendix 11b - individual values (Recovery kill)

The following statistically significant differences from control values were seen:

Thymus

Lower weight in Group 4 (15 ppm) Recovery group males.

Spleen

Lower weight in Group 4 (15 ppm) Main group males.

Kidneys

Greater weight when adjusted for bodyweight as covariate in Group 4 (15 ppm) Recovery group males.

Prostate

Lower weight in Groups 2 (1 ppm), 3 (5 ppm) and 4 (15 ppm) Main group males.

The differences seen were small, inconsistent between the sexes and considered not to be of toxicological significance.

Microscopic pathology

The data are presented as follows:

- Table 10a - Terminal kill incidence summary
- Table 10b - Recovery kill incidence summary
- Table 10c - Treatment-related changes incidence summary
- Appendix 12 - Individual findings

Treatment-related changes**Nasal passages**

A range of irritant-related changes characterised by erosion, inflammatory infiltration, atrophy and squamous metaplasia of respiratory and olfactory epithelia of the dorsal meatus and the central septum were seen in the nasal passages of the majority of animals from Group 4 (15 ppm). These changes were not seen in animals in Group 3 (5 ppm) or 2 (1 ppm).

In general (see Table 10c) the atrophic change was more evident in the anterior levels (ie: A had a higher incidence than C) while inflammatory and metaplastic changes predominated in the more posterior B and C levels. Erosive and inflammatory changes, when present, often obscured areas of atrophy and the changes recorded are best considered as a related spectrum of events rather than discrete pathological entities (see Conclusions).

After a period of recovery, clear evidence of attempted repair was seen at the affected sites with a diminution of inflammatory and metaplastic changes.

Larynx

Minor epithelial, primarily hyperplastic, changes were seen in the larynges of Group 4 (15 ppm) animals only. These changes were minor in degree and mostly confined to the anterior aspect of level A and also of the epithelium overlying the arytenoid cartilages as well as some small amounts of squamous metaplastic change of the ventral epithelium at level B.

Incidental changes

The other histological changes that were recorded were all within the normal range of changes seen in animals of this age and strain in this laboratory and were not considered to be of toxicological significance.

Histopathological conclusions

Epithelial inflammatory, atrophic and metaplastic changes were seen in the dorsal meatus and dorsal central septum of the nasal passages and, to a lesser degree, in the larynges of animals from Group 4 (15 ppm) only. These changes are consistent with the inhalation of an irritant substance.

After a period of recovery, the changes seen in nasal passages of the Main group animals at termination of exposure showed clear signs of repair and recovery.

No treatment-related effects were seen in animals from Group 3 (15 ppm) or 2 (1 ppm).

DISCUSSION AND CONCLUSION

In this study, rats were exposed by inhalation to methacrolein vapour for 6 hours a day, 5 days a week (except Week 1 exposed for 1 day and Week 13 for 4 days), for 13 weeks. The mean exposure levels were 1.0, 4.9 and 15.3 ppm.

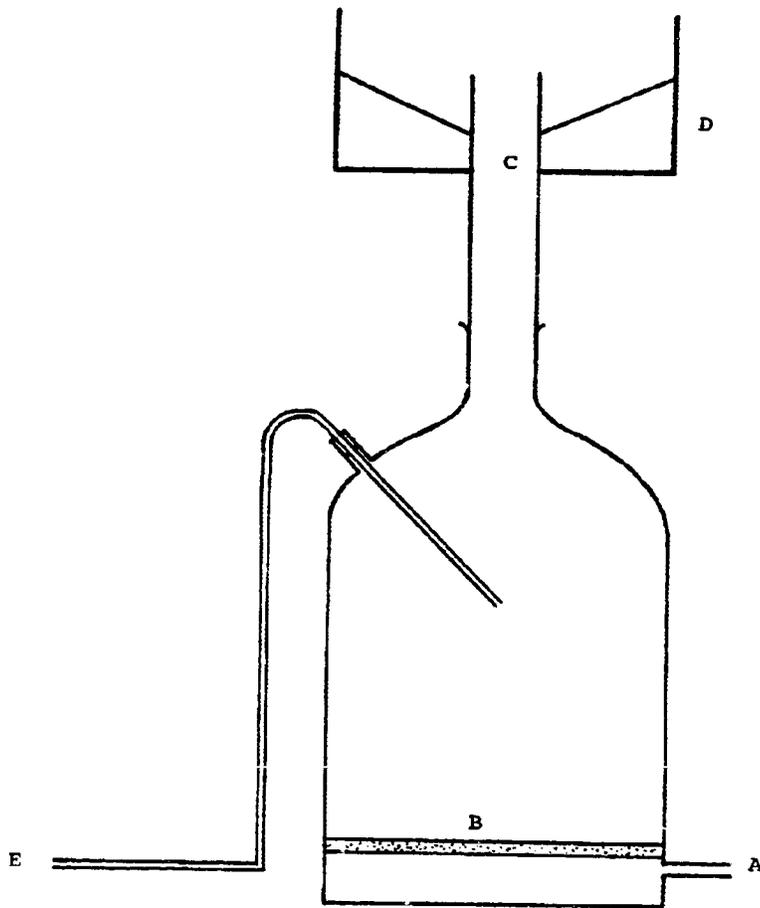
During exposure, clinical signs indicative of exposure to an irritant atmosphere were seen in rats exposed to 4.9 and 15.3 ppm.

Effects on weight gain and food consumption were seen in rats exposed to 15.3 ppm, the effect was more marked in females.

Histopathological changes were confined to the nasal passages and larynges of rats exposed to 15.3 ppm. The changes comprised epithelial inflammatory, atrophic and metaplastic changes seen in the dorsal meatus and dorsal central septum of the nasal passages and, to a lesser degree, in the larynges. These changes are consistent with the inhalation of an irritant substance. Clear signs of repair and recovery of the above changes were seen in rats killed following the 4-week recovery period.

Clinical signs, during exposure, observed in rats exposed to 4.9 ppm were transient (the first 6 exposures only) and of a non-specific nature and in the absence of any other treatment-related effects, the no adverse effect concentration for this study is considered to be 4.9 ppm.

FIGURE 1
Vapour generator

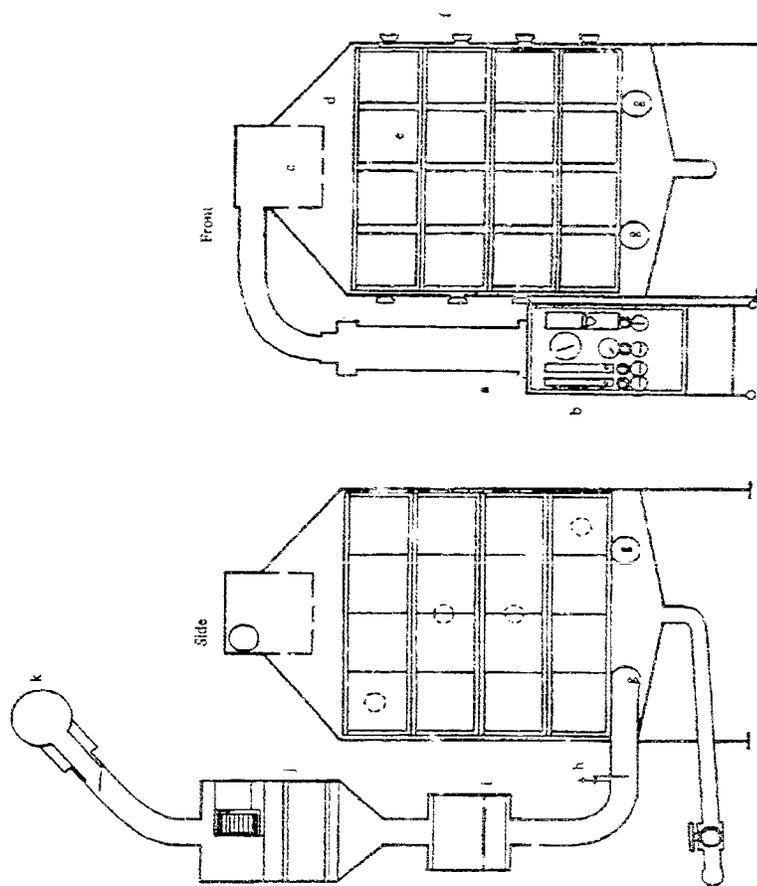


Key

- A. Air line
- B. Fritted glass disc
- C. Vapour outlet
- D. Stainless steel and glass elutriator
- E. Test substance supply line

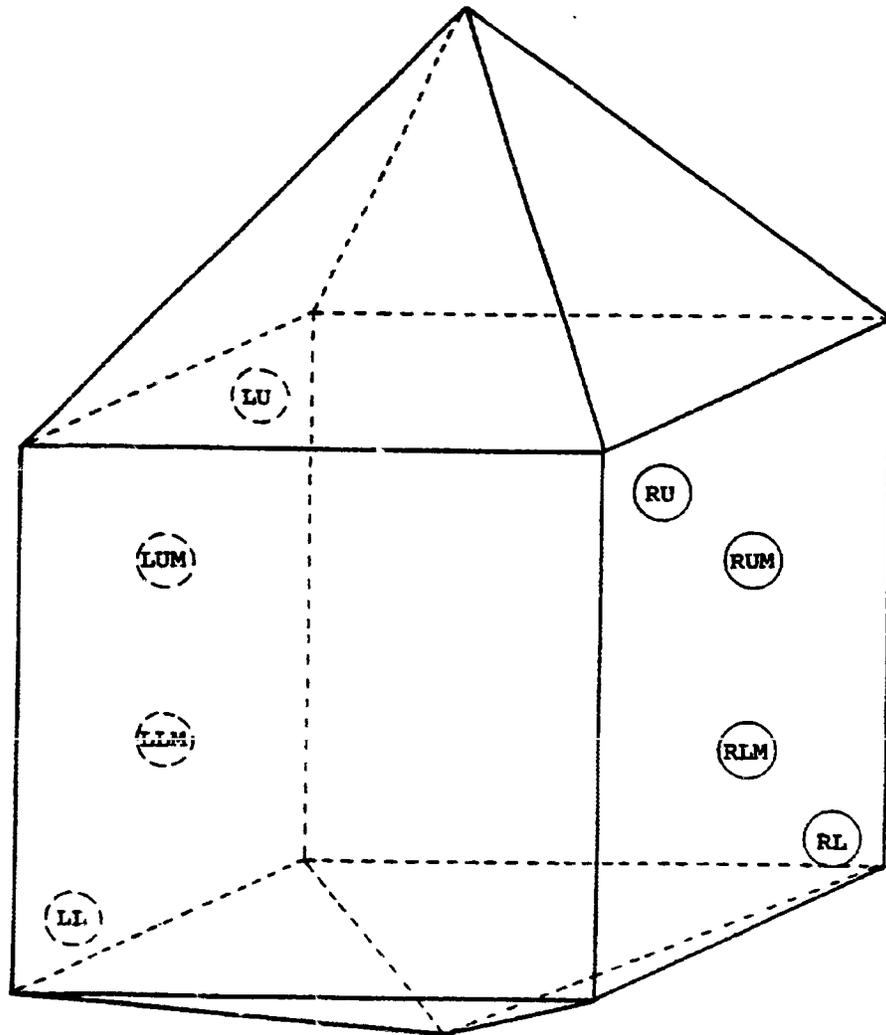
FIGURE 2

Exposure system



- a Glass column
- b Airflow control and chamber monitoring
- c Dispersion device
- d Exposure chamber
- e Animal exposure cages
- f Sampling port
- g Exhaust plenum
- h Gate valve
- i Pre-filter
- j Powered extract filter
- k Main exhaust

FIGURE 3
Location of sample ports on exposure chambers



Key

LU	Left upper	RU	Right upper
LUM	Left upper middle	RUM	Right upper middle
LLM	Left lower middle	RLM	Right lower middle
LL	Left lower	RL	Right lower

FIGURE 4
Bodyweights - group mean values

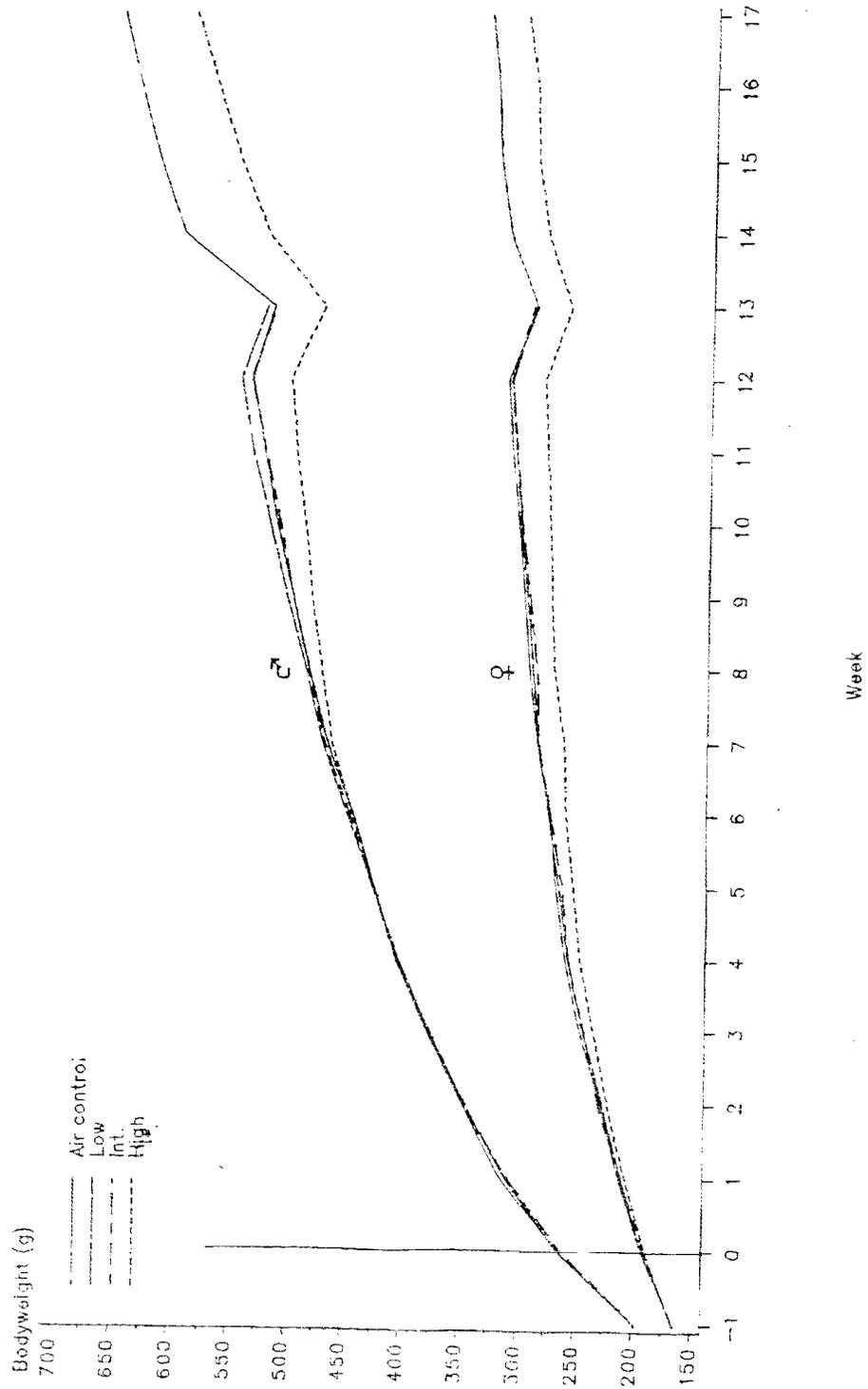


FIGURE 5
Food consumption - group mean cumulative values

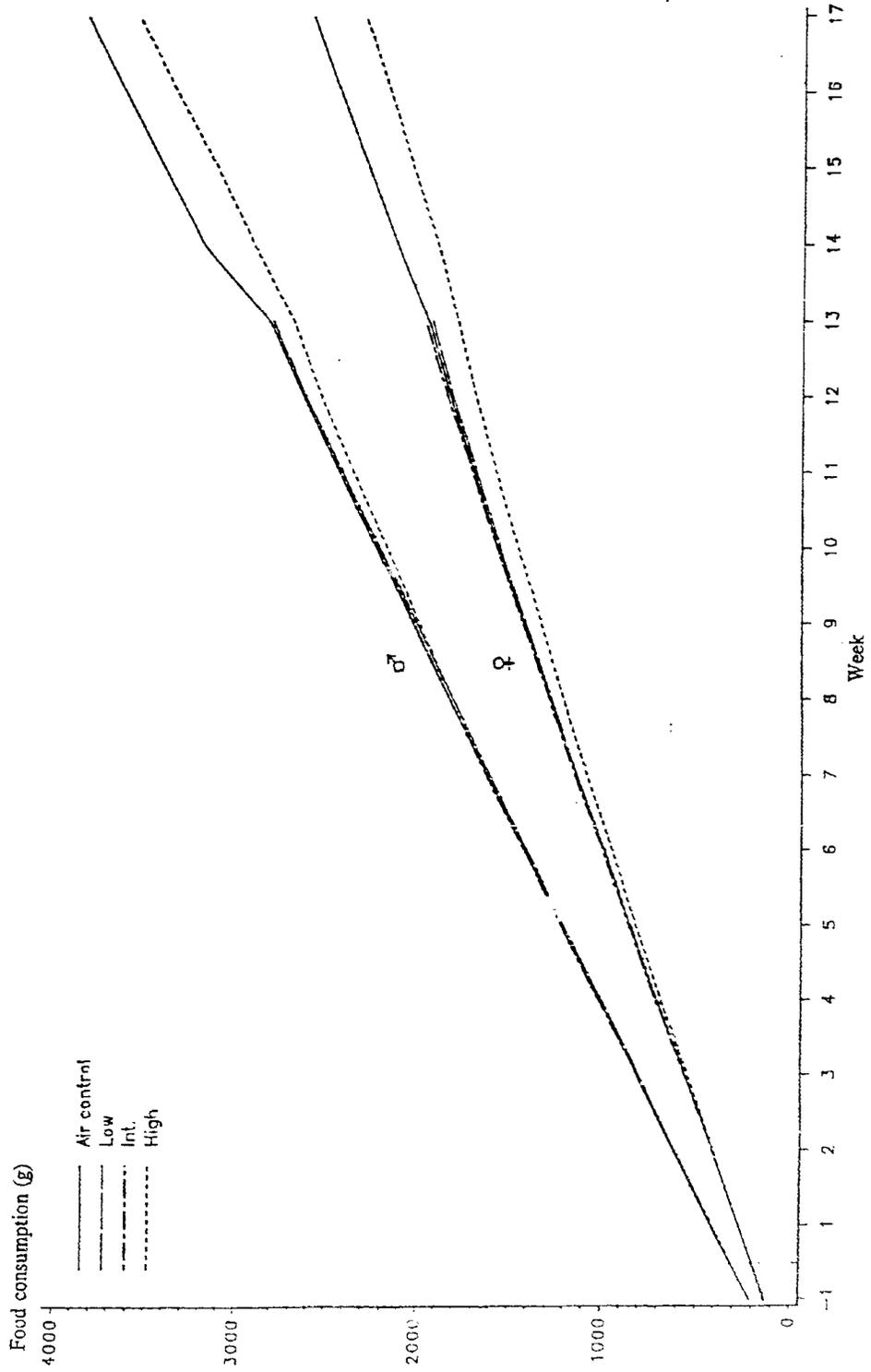


TABLE 1

Exposure mean analysed concentration of methacrolein

Exposure number	Concentrations (ppm)		
	Group 2	Group 3	Group 4
1	0.8	5.4	16.1
2	0.8	5.3	16.6
3	0.8	5.5	17.7
4	1.2	5.0	16.2
5	0.9	4.7	16.0
6	0.9	5.0	16.5
7	1.1	5.5	16.7
8	0.8	4.9	15.1
9	0.8	4.6	13.7
10	0.9	5.1	7.5
11	0.6	5.0	18.1
12	1.3	5.7	16.5
13	0.6	4.1	16.8
14	1.0	5.5	17.9
15	1.2	6.0	18.3
16	1.3	5.2	14.6
17	1.2	5.1	15.3
18	1.2	5.1	15.1
19	1.2	5.0	15.1
20	1.1	5.0	14.8
21	1.1	5.2	15.5
22	1.5	4.7	15.5
23	1.1	5.0	15.9
24	1.3	5.8	17.6
25	1.1	4.9	14.5
26	1.0	4.1	14.7
27	1.1	5.3	12.8
28	1.3	4.3	16.1
29	1.2	4.8	15.0
30	0.9	5.0	14.8
31	0.8	4.6	13.2
32	0.9	4.7	13.0
33	1.4	4.7	14.1
34	0.8	4.1	13.7
35	0.9	4.8	15.2
36	0.9	4.1	13.1
37	0.7	3.5	12.3
38	0.9	3.9	15.5
39	0.9	3.3	14.6
40	0.9	4.2	15.3
41	0.9	4.3	15.2
42	0.9	4.9	15.4
43	0.9	6.1	16.6
44	1.0	4.8	16.5
45	1.1	4.6	16.1

TABLE 1

(Exposure mean analysed concentration of methacrolein - continued)

Exposure number	Concentrations (ppm)		
	Group 2	Group 3	Group 4
46	1.2	5.2	16.2
47	1.1	5.5	18.8
48	1.1	5.6	18.4
49	1.1	5.5	15.5
50	1.1	5.4	17.5
51	1.1	5.2	15.7
52	1.1	5.9	16.3
53	1.3	6.0	17.4
54	1.8	6.8	19.8
55	1.2	5.1	14.4
56	1.1	5.4	14.2
57	1.0	4.2	12.0
58	0.8	4.2	12.1
59	1.0	4.2	12.6
60	1.2	4.4	12.7
61	1.0	4.7	14.1
62	1.2	4.4	13.8
63	0.9	4.5	14.5
64	1.0	5.1	15.0
65	1.0	5.0	14.9
Mean	1.0	4.9	15.2

TABLE 2
Nominal concentration of methacrolein

Exposure number	Group					
	2 (Low conc.)		3 (Inter conc.)		4 (High conc.)	
	Conc. (µg/l)	A/N (%)	Conc. (µg/l)	A/N (%)	Conc. (µg/l)	A/N (%)
1	2.8	81.0	15.7	98.4	47.1	97.8
2	4.4	52.3	15.5	97.4	45.5	104.3
3	2.8	86.9	15.7	99.8	45.5	111.5
4	- ¹	- ¹	14.6	96.9	43.9	105.7
5	3.0	86.7	14.7	91.4	43.9	104.6
6	3.0	81.1	14.9	97.1	43.9	107.4
7	3.1	103.2	14.9	105.8	40.8	117.6
8	3.0	80.0	14.4	98.1	40.8	106.0
9	3.0	73.3	14.9	89.0	42.4	92.8
10	3.1	81.7	14.4	101.6	40.8	53.0
11	3.3	56.6	14.7	98.0	47.1	110.3
12	2.2	160.6	14.9	110.1	47.1	100.4
13	- ²	- ²	- ²	- ²	45.5	105.7
14	3.1	90.3	14.1	111.8	43.9	116.7
15	3.3	100.0	15.1	113.5	47.1	111.6
16	3.3	113.1	15.1	97.7	45.5	91.9
17	3.3	103.0	13.6	107.1	45.5	96.6
18	3.3	104.0	13.5	108.1	43.9	98.8
19	3.0	113.3	14.4	98.6	45.5	94.8
20	3.3	94.9	14.1	102.1	47.1	90.4

¹ Feed line disconnected during exposure to remove an air bubble, therefore total usage could not be determined

² Usages not recorded, in error

A/N Exposure mean analysed concentration × 100 ÷ nominal concentration

TABLE 2
(Nominal concentration of methacrolein - continued)

Exposure number	Group					
	2 (Low conc.)		3 (Inter conc.)		4 (High conc.)	
	Conc. (µg/l)	A/N (%)	Conc. (µg/l)	A/N (%)	Conc. (µg/l)	A/N (%)
21	3.1	105.4	14.7	101.1	47.1	94.4
22	3.3	134.3	14.1	94.8	45.5	98.0
23	3.5	91.4	14.4	98.4	47.1	96.5
24	3.0	121.1	14.1	118.4	47.1	107.4
25	3.0	110.0	14.3	99.3	47.1	88.3
26	3.3	87.9	14.1	83.9	47.1	89.5
27	3.3	92.4	14.1	107.1	47.1	77.7
28	3.3	118.2	14.1	88.2	47.1	97.7
29	(1.7) ³	(209.8) ³	13.2	105.1	40.8	105.3
30	3.3	75.8	14.1	100.7	47.1	90.0
31	3.1	78.5	14.4	91.7	47.1	80.2
32	2.4	106.3	14.7	92.1	47.1	79.1
33	3.5	111.4	14.6	91.8	47.1	85.5
34	3.9	57.7	14.6	81.3	47.1	81.3
35	3.3	78.8	14.3	95.5	47.1	82.4
36	3.3	74.7	14.1	98.8	45.5	82.1
37	3.3	60.6	14.0	72.1	47.1	74.8
38	3.3	73.7	15.7	70.7	51.8	86.2
39	3.8	64.9	14.9	63.8	52.5	79.7
40	3.8	69.3	15.7	76.0	51.8	84.6

³ Error in reading syringe. Not included in mean
A/N Exposure mean analysed concentration × 100 ÷ nominal concentration

TABLE 2
(Nominal concentration of methacrolein - continued)

Exposure number	Group					
	2 (Low conc.)		3 (Inter conc.)		4 (High conc.)	
	Conc. (µg/l)	A/N (%)	Conc. (µg/l)	A/N (%)	Conc. (µg/l)	A/N (%)
41	3.6	74.1	18.0	68.3	51.8	83.9
42	3.8	68.4	18.0	77.6	51.8	85.1
43	3.9	80.8	18.8	93.6	53.3	89.5
44	3.8	79.8	18.8	73.9	50.2	93.9
45	3.8	85.1	18.0	73.7	51.8	89.3
46	3.9	87.8	18.0	83.1	48.6	95.5
47	3.6	88.0	18.8	84.4	51.8	103.9
48	3.6	88.9	18.0	90.2	51.8	101.6
49	3.6	84.3	18.0	86.9	47.1	94.6
50	3.6	84.3	17.3	90.2	47.1	106.6
51	3.6	92.6	17.3	86.1	47.1	95.3
52	3.6	87.0	17.3	97.9	48.6	96.0
53	3.6	101.9	18.0	96.5	47.1	105.9
54	3.6	140.7	18.0	107.6	47.1	120.2
55	3.6	90.7	18.0	82.2	47.1	88.0
56	3.6	82.4	18.0	85.6	47.1	86.8
57	3.6	79.9	18.0	66.3	47.1	73.0
58	3.6	68.5	18.0	67.6	47.1	73.7
59	3.6	78.7	18.0	66.9	48.6	74.0
60	3.5	92.4	18.8	67.9	50.2	72.3
61	3.8	73.7	19.6	69.4	53.3	75.6
62	4.1	84.1	19.6	63.8	53.3	74.4
63	3.8	64.9	20.4	62.6	53.3	77.9
64	3.8	71.9	20.4	70.9	51.8	83.0
65	3.8	73.7	19.6	73.3	50.2	85.1
Study mean	3.4	88.4	16.1	89.7	47.5	92.5
≡ ppm	1.2	-	5.6	-	16.6	-

A/N Exposure mean analysed concentration \times 100 \div nominal concentration

TABLE 3
Exposure mean chamber temperature and relative humidity

Exposure number	Group							
	1 (Air control)		2 (Low conc.)		3 (Inter conc.)		4 (High conc.)	
	T (°C)	RH (%)	T (°C)	RH (%)	T (°C)	RH (%)	T (°C)	RH (%)
1	24.7	56	24.0	37	24.0	30	25.2	46
2	24.8	48	24.0	36	23.6	31	24.9	47
3	24.6	49	23.7	36	23.2	33	24.6	49
4	24.8	49	24.2	37	24.0	32	24.9	51
5	27.5	50	24.6	37	24.1	32	24.7	¹
6	25.6	35	24.8	35	24.2	32	25.1	41
7	25.5	35	24.7	38	24.3	36	25.5	44
8	25.3	38	24.4	41	24.3	36	25.5	46
9	22.3	35	25.0	36	24.5	34	25.5	50
10	25.6	35	24.6	38	24.5	32	25.4	52
11	25.7	33	24.8	38	24.6	32	25.8	45
12	26.0	33	25.4	35	24.9	32	25.8	43
13	25.8	38	26.0	35	24.8	32	26.3	46
14	26.2	40	25.4	40	25.2	49 ²	26.2	46
15	26.0	36	25.0	40	24.5	33	25.5	48
16	25.5	34	24.5	39	24.3	31	25.4	40
17	26.1	37	25.1	41	24.8	36	25.9	49
18	25.8	36	25.1	40	24.7	34	25.5	47
19	25.5	36	24.5	41	24.4	32	25.6	43
20	25.6	35	24.9	37	24.5	31	25.8	42
21	25.3	35	24.3	39	24.2	32	25.4	42
22	26.8	38	26.1	38	24.8	33	26.5	44
23	26.2	36	25.4	41	25.0	36	26.0	42

¹ Wet bulb dried out after 1st recording, further readings unobtainable
² Wet bulb dried out after only 5 readings

TABLE 3

(Exposure mean chamber temperature and relative humidity - continued)

Exposure number	Group							
	1 (Air control)		2 (Low conc.)		3 (Inter conc.)		4 (High conc.)	
	T (°C)	RH (%)	T (°C)	RH (%)	T (°C)	RH (%)	T (°C)	RH (%)
24	26.2	38	25.3	42	25.0	36	26.0	47
25	26.5	35	25.5	41	25.5	34	26.2	45
26	26.5	38	25.7	42	25.7	35	26.8	41
27	26.4	39	25.2	34	25.5	36	26.4	51
28	26.6	41	25.2	33	25.7	37	27.2	46
29	26.7	43	25.8	30	26.0	39	26.9	47
30	26.7	42	25.8	30	25.7	39	26.5	49
31	26.7	39	26.1	32	26.2	38	26.9	49
32	26.4	37	25.5	30	25.2	35	26.6	36
33	25.8	35	25.3	25	24.9	38	26.0	40
34	26.1	38	25.4	26	25.0	38	26.3	41
35	25.8	35	25.1	28	25.2	37	26.0	43
36	26.3	37	25.5	27	25.4	38	26.9	40
37	26.1	36	25.5	27	25.4	37	26.3	42
38	25.4	37	26.4	27	25.3	39	26.5	51
39	25.2	34	26.3	26	25.4	38	25.9	45
40	24.2	39	24.7	32	24.1	36	25.0	48
41	24.1	37	25.0	29	24.2	37	24.8	44
42	25.5	38	26.1	30	25.3	38	26.4	44
43	24.9	40	25.7	33	24.9	39	25.3	51
44	25.1	37	25.7	33	25.1	39	25.6	52
45	24.8	38	25.7	30	24.7	36	25.0	52
46	25.4	37	25.9	32	25.1	38	25.7	50

TABLE 3

(Exposure mean chamber temperature and relative humidity - continued)

Exposure number	Group							
	1 (Air control)		2 (Low conc.)		3 (Inter conc.)		4 (High conc.)	
	T (°C)	RH (%)	T (°C)	RH (%)	T (°C)	RH (%)	T (°C)	RH (%)
47	25.8	38	26.7	30	25.8	34	26.5	50
48	25.7	37	26.0	31	25.2	37	25.8	48
49	24.7	40	25.3	30	24.5	38	25.1	52
50	25.5	38	26.0	32	25.5	39	25.8	50
51	25.3	35	26.2	28	25.2	37	25.8	46
52	25.5	34	26.0	27	25.1	37	25.8	43
53	25.0	39	25.7	30	24.9	40	25.4	50
54	25.2	40	25.8	32	25.2	41	25.9	47
55	25.0	42	25.7	33	25.0	40	25.5	54
56	25.2	42	25.9	34	25.3	42	26.0	52
57	25.3	38	26.3	27	25.4	36	25.9	45
58	25.5	36	26.1	29	25.4	36	25.9	46
59	24.9	38	25.7	29	25.0	37	25.5	50
60	25.8	38	26.3	29	25.5	38	24.9	52
61	25.6	41	26.0	34	25.7	42	25.7	57
62	26.0	34	27.0	26	26.1	39	25.4	53
63	25.0	40	26.4	30	25.2	42	25.3	58
64	25.7	39	26.0	32	25.3	43	25.5	57
65	25.5	43	26.1	35	25.6	44	26.0	63
Study mean	25.6	38	25.4	33	25.0	36	25.8	47

TABLE 4
Bodyweights - group mean values (g)

Week	Group and dosage							
	1♂ Air control	2♂ Low conc.	3♂ Int conc.	4♂ High conc.	1♀ Air control	2♀ Low conc.	3♀ Int conc.	4♀ High conc.
-1	197	197	198	198	165	164	165	165
0	264	265	261	264	193	193	191	190
1	315	310	308	309	214	211	211	208
2	348	345	346	347	230	228	232	224
3	379	378	378	377	248	245	244	236
4	406	404	407	405	261	259	259	248
5	426	427	427	427	270	267	265	255
6	444	449	447	445	275	276	274	261
7	467	471	469	463	287	286	286	264
8	484	486	484	473	294	291	288	273
9	498	503	498	481	299	296	294	275
10	512	518	509	487	304	302	302	278
11	525	533	523	496	311	307	307	281
12	535	545	536	502	315	312	312	284
13	516	522	515	472	291	292	294	261
13 ¹	548			474	294			258
14	595			523	314			282
15	617			548	325			293
16	634			568	327			294
17	650			588	334			304
Gain								
0 - 12	271	280	275	238 ⁺	122	119	122	**
0 - 13	252	257	254	208 ^{**}	NP 98 (98)	100 (107)	103 (100)	** 71 (72)
13 ¹ - 17	102			114	40			46

¹ Recovery animals only

NP Non-parametric analysis employed. Median gain values in parentheses

** P < 0.01 compared with control values using Williams' test

+ P < 0.05 compared with control values using Student's 't' test

TABLE 5
Food consumption - group mean values (g)

Week	Group and dosage							
	1♂ Air control	2♂ Low conc.	3♂ Int conc.	4♂ High conc.	1♀ Air control	2♀ Low conc.	3♀ Int conc.	4♀ High conc.
-1	211	211	207	209	135	133	132	137
1	212	206	207	203	138	138	139	135
2	200	196	205	202	143	141	146	138
3	196	191	198	200	148	149	150	140
4	198	201	207	206	146	149	151	139
5	203	204	204	204	147	144	147	137
6	194	195	194	192	136	139	138	126
7	205	203	208	200	147	145	147	133
8	208	202	199	194	143	138	141	123
9	207	200	195	186	139	138	141	123
10	203	205	199	180	140	136	141	121
11	198	201	199	180	136	132	137	119
12	198	203	203	178	133	132	137	117
13	185	179	177	177	115	112	117	98
14	225			202	147			133
15	218			205	151			135
16	218			224	154			133
17	214			217	152			136
Cumulative								
1 - 12	2422	2407	2417	2325	1696	1683	1714	1556**
1 - 13	2607	2586	2594	2475	1811	1795	1832	1654**
14 - 17	875			848	604			537*

** P < 0.01 compared with control values using Wilcoxon's test

+ P < 0.05 compared with control values using Student's 't' test

TABLE 6

Haematology - group mean values

Week 14

Group	PCV %	Hb g/dl	RBC $\times 10^6$ / mm ³	MCHC %	MCV fl	WBC + Diff $\times 10^3$ /mm ³					Pits $\times 10^3$ / mm ³	TT s	
						Total	N	L	E	B			M
1♂ Air control	57	15.7	8.0	27.5	71	11.8	2.35	9.34	0.09	0.00	0.06	913	24
2♂ Low conc.	57	15.8	8.1	27.5	71	11.0	2.28	8.55	0.13	0.00	0.03	993	25
3♂ Int conc.	58	15.7	8.1	27.3	71	11.2	2.10	8.97	0.09	0.00	0.07	913	25
4♂ High conc.	59	16.0	8.4*	27.2	71	10.8	2.36	8.37	0.06	0.00	0.03	873	24
1♀ Air control	53	14.6	7.3	27.4	73	7.5	1.28	6.12	0.08	0.00	0.04 ^F	907	19
2♀ Low conc.	52	14.7	7.2	28.0*	73	6.4	1.00	5.35	0.08	0.00	0.01	881	20
3♀ Int conc.	52	14.8	7.3	28.4**	71*	6.2	1.21	4.94	0.07	0.00	0.01	917	20
4♀ High conc.	54	15.2*	7.5	28.4**	72*	7.8	1.34	6.38	0.08	0.00	0.02	938	20

* P < 0.05 compared with control values using Williams' test

** P < 0.01 compared with control values using Williams' test

F Frequency analysis applied to the data

APPENDIX 11b
(Organ weights - continued)

Week 18

Group	Animal no.	Body wt g	Brain g	Pituitary mg	Thymus g	Heart g	Lungs g	Liver g	Spleen g	Kidneys g	Adrenals mg	Ovaries mg
1 ♀ Air control	71	327	2.02	12.3	0.208	1.27	1.15	10.3	0.74	1.65	69.2	87.9
	72	328	1.95	17.8	0.474	1.16	1.28	10.4	0.61	1.80	63.9	107.9
	73	387	1.84	14.5	0.288	1.09	1.24	12.0	0.55	1.95	72.0	82.4
	74	330	1.95	17.2	0.259	1.02	1.38	9.9	0.54	1.59	57.0	98.8
	75	336	1.88	12.4	0.203	1.08	1.20	10.5	0.61	1.88	56.5	95.8
	76	285	1.97	13.9	0.244	1.08	1.18	8.9	0.54	1.55	58.6	79.5
	77	295	1.94	12.1	0.134	0.97	1.22	9.2	0.54	1.71	69.4	91.5
	78	321	1.89	14.9	0.184	0.92	1.22	9.9	0.63	1.69	59.4	71.6
	79	310	1.91	13.4	0.246	1.17	1.14	10.2	0.59	1.84	74.9	60.1
	80	385	1.93	17.5	0.293	1.06	1.28	12.0	0.51	1.87	73.4	81.2
4 ♀ High conc.	Mean	331	1.93	14.8	0.253	1.08	1.23	10.3	0.59	1.75	65.4	85.7
	SD	33.5	0.049	2.18	0.0915	0.101	0.073	1.01	0.068	0.134	7.17	12.86
4 ♀ High conc.	111	312	1.83	12.2	0.229	1.13	1.17	9.9	0.53	1.83	63.9	85.3
	112	285	1.74	11.5	0.204	0.98	1.19	9.4	0.48	1.55	80.7	76.8
	113	282	1.88	17.0	0.189	1.05	1.28	9.3	0.60	1.44	75.2	95.7
	114	312	1.99	16.0	0.187	1.17	1.18	10.1	0.58	1.80	78.3	113.1
	115	301	1.86	15.2	0.221	1.07	1.10	9.0	0.53	1.79	74.3	83.3
	116	311	2.01	14.7	0.243	1.00	1.28	10.0	0.60	1.75	63.3	67.3
	117	281	1.85	11.4	0.154	0.86	1.16	8.5	0.46	1.71	56.4	80.1
	118	288	1.96	13.0	0.144	0.98	1.24	10.2	0.53	1.90	57.8	86.9
	119	326	1.98	13.9	0.165	0.93	1.33	9.9	0.56	2.07	65.3	81.3
	120	284	2.00	13.7	0.227	0.82	1.35	9.1	0.63	1.76	71.2	88.6
Standard deviation	Mean	298	1.91	13.9	0.196	1.00	1.23	9.5	0.55	1.76	68.6	85.8
	SD	16.2	0.091	1.89	0.0342	0.111	0.082	0.54	0.056	0.174	8.50	12.18

APPENDIX 12

Rats killed at termination and following a recovery period

Group:	1	2	3	4
Compound:	Control		Methacrolein	
Test substance (ppm):	0	1	5	15

In this appendix the macroscopic and microscopic findings relating to each animal are listed on one page. These findings are presented by an automated data collation system and the following should be noted.

Particular care is taken during removal and processing of all protocol-scheduled tissues. Understandably, omissions or irregularities can occasionally occur, the most vulnerable tissues in this regard being parathyroid, thymus, male mammary gland and autolysed portions of the gastrointestinal tract. For each animal any tissues so affected are listed as missing.

The following abbreviation is used:

Adj - Adjacent

APPENDIX 12

(Pathology - continued)

Compound: Air
Dosage Level: Control
Rat No/Sex: 1♂ (Terminal)

MACROSCOPIC FINDINGS

No abnormalities were seen in the animal

MICROSCOPIC FINDINGS

The following observations were noted:

Heart

Myocarditis: (Minimal , Focus)

The following tissues were considered normal:

Nasal Passages - Levels A/b/c; Pharynx; Larynx - Levels A/b; Trachea; Lungs; Aorta; Thymus;
Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Spleen; Liver; Pancreas; Kidneys; Urinary
Bladder; Epididymides; Testes; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands;
Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Colon; Eyes; Spinal Cord; Sciatic Nerve;
Brain; Bone Marrow/sternum

Tissues not available for examination were:

Lymph Nodes - Tracheobronchial : (Not seen)

Pathologist: R.L.Gregson

APPENDIX 12

(Pathology - continued)

Compound: Air
Dosage Level: Control
Rat No/Sex: 2♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm

Liver
Pale subcapsular area/s - median cleft: (One) 1mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Plasmacytosis
Prominent distended venules containing small lymphocytes

The following tissues were considered normal:

Nasal Passages - Levels A/b/c; Pharynx; Larynx - Levels A/b; Trachea; Lungs; Aorta; Heart; Thymus; Lymph Nodes - Mesenteric; Spleen; Liver : (W.N.L.); Pancreas; Kidneys; Urinary Bladder; Epididymides; Testes; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Colon; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Tissues not available for examination were:

Lymph Nodes - Tracheobronchial : (Not seen)

Pathologist: R.L.Gregson

APPENDIX 12

(Pathology - continued)

Compound: Air
Dosage Level: Control
Rat No/Sex: 3♂ (Terminal)

MACROSCOPIC FINDINGS

Skin Alopecia
Side/s of face

Lymph Nodes - Cervical
Enlarged: 11mm

Liver
Pale subcapsular area/s - median cleft: (One) 2mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Plasmacytosis
Prominent distended venules containing small lymphocytes

Liver
Area of foamy hepatocytes near the median cleft

The following tissues were considered normal:

Nasal Passages - Levels A/b/c; Pharynx; Larynx - Levels A/b. Trachea; Lungs; Aorta; Heart; Thymus; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Spleen; Pancreas; Kidneys; Urinary Bladder; Epididymides; Testes; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Colon; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 12
(Pathology - continued)

Compound: Air
Dosage Level: Control
Rat No/Sex: 4♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm

Lungs
Pale subpleural foci: (Multiple) 1mm

Seminal Vesicles
Distended: (Minimal)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lungs
Subpleural aggregation of alveolar macrophages: (Trace)

Lymph Nodes - Cervical
Plasmacytosis
Prominent paracortex

Seminal Vesicles
Dilated with normal seminal colloid

Thyroids
Ectopic thymic tissue present

APPENDIX 12

(Pathology - continued)

Rat No/Sex: 4♂ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Nasal Passages - Levels A/b/c; Pharynx; Larynx - Levels A/b; Trachea; Aorta; Heart; Thymus;
Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Spleen; Liver; Pancreas; Kidneys;
Urinary Bladder; Epididymides; Testes; Parathyroids; Adrenals; Pituitary; Salivary Glands;
Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Colon; Eyes; Spinal Cord; Sciatic Nerve;
Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 12

(Pathology - continued)

Compound: Air
Dosage Level: Control
Rat No/Sex: 5♂ (Terminal)

MACROSCOPIC FINDINGS

Skin Alopecia

Side/s of face: (Minimal , Diffuse)

Lymph Nodes - Cervical

Enlarged: 10mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical

Plasmacytosis

The following tissues were considered normal:

Nasal Passages - Levels A/b/c; Pharynx; Larynx - Levels A/b; Trachea; Lungs; Aorta; Heart; Thymus; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Spleen; Liver; Pancreas; Kidneys; Urinary Bladder; Epididymides; Testes; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Colon; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 12

(Pathology - continued)

Compound: Air
Dosage Level: Control
Rat No/Sex: 6♂ (Terminal)

MACROSCOPIC FINDINGS

No abnormalities were seen in the animal

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Nasal Passages - Levels A/b/c; Pharynx; Larynx - Levels A/b; Trachea; Lungs; Aorta; Heart; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Spleen; Liver; Pancreas; Kidneys; Urinary Bladder; Epididymides; Testes; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Colon; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 12

(Pathology - continued)

Compound: Air
Dosage Level: Control
Rat No/Sex: 7♂ (Terminal)

MACROSCOPIC FINDINGS

Stomach Antrum Mucosa

White nodule, near to limiting ridge: 1mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Pituitary

Cyst(s) in pars anterior: (Minimal)

The following tissues were considered normal:

Nasal Passages - Levels A/b/c; Pharynx; Larynx - Levels A/b; Trachea; Lungs; Aorta; Heart; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Spleen; Liver; Pancreas; Kidneys; Urinary Bladder; Epididymides; Testes; Thyroids; Parathyroids; Adrenals; Salivary Glands; Oesophagus; Stomach : (W.N.L.); Duodenum; Jejunum; Ileum; Colon; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 12

(Pathology - continued)

Compound: Air
Dosage Level: Control
Rat No/Sex: 8♂ (Terminal)

MACROSCOPIC FINDINGS

No abnormalities were seen in the animal

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Nasal Passages - Levels A/b/c; Pharynx; Larynx - Levels A/b; Trachea; Lungs; Aorta; Heart; Thymus; Lymph Nodes - Cervical; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Spleen; Liver; Pancreas; Kidneys; Urinary Bladder; Epididymides; Testes; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Colon; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 12**(Pathology - continued)**

Compound: Air
Dosage Level: Control
Rat No/Sex: 9♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged

Liver
Pale subcapsular area/s - median cleft: (One) 3mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Plasmacytosis

The following tissues were considered normal:

Nasal Passages - Levels A/b/c, Pharynx; Larynx - Levels A/b; Trachea; Lungs; Aorta; Heart;
Thymus; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Spleen; Liver :
(W.N.L.); Pancreas; Kidneys; Urinary Bladder; Epididymides; Testes; Thyroids; Adrenals;
Pituitary; Salivary Glands; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Colon; Eyes;
Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Tissues not available for examination were:

Parathyroids : (Not seen)

Pathologist: R.L.Gregson

APPENDIX 12

(Pathology - continued)

Compound: Air
Dosage Level: Control
Rat No/Sex: 10♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Plasmacytosis

The following tissues were considered normal:

Nasal Passages - Levels A/b/c; Pharynx; Larynx - Levels A/b; Trachea; Lungs; Aorta; Heart; Thymus; Lymph Nodes - Mesenteric; Lymph Nodes - Tracheobronchial; Spleen; Liver; Pancreas; Kidneys; Urinary Bladder; Epididymides; Testes; Thyroids; Parathyroids; Adrenals; Pituitary; Salivary Glands; Oesophagus; Stomach; Duodenum; Jejunum; Ileum; Colon; Eyes; Spinal Cord; Sciatic Nerve; Brain; Bone Marrow/sternum

Pathologist: R.L.Gregson

APPENDIX 12
(Pathology - continued)

Compound: Air
Dosage Level: Control
Rat No/Sex: 11♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 11mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Nasal Passages - Levels A/b/c; Lungs

Pathologist: R.L.Gregson

APPENDIX 12

(Pathology - continued)

Compound: Air
Dosage Level: Control
Rat No/Sex: 12♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 9mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Nasal Passages - Levels A/b/c; Lungs

Pathologist: R.L.Gregson

APPENDIX 12

(Pathology - continued)

Compound: Air
Dosage Level: Control
Rat No/Sex: 13♂ (Recovered)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical

Enlarged: 12mm

Liver

Pale subcapsular area/s - median cleft: (One) 2mm

Stomach Antrum Mucosa

White nodule, near to limiting ridge: 1mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Nasal Passages - Levels A/b/c; Lungs

Pathologist: R.L.Gregson

APPENDIX 12
(Pathology - continued)

Compound: Air
Dosage Level: Control
Rat No/Sex: 14♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Nasal Passages - Levels A/b/c; Lungs

Pathologist: R.L Gregson

BGH 50/932334

APPENDIX 12

(Pathology - continued)

Compound: Air
Dosage Level: Control
Rat No/Sex: 15♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Nasal Passages - Levels A/b/c; Lungs

Pathologist: R.L.Gregson

APPENDIX 12

(Pathology - continued)

Compound: Air
Dosage Level: Control
Rat No/Sex: 16♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 13mm

Liver
Pale subcapsular area/s - median cleft: (One) 2mm

Stomach Antrum Mucosa
White nodules, near to limiting ridge: (Two) 1mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Nasal Passages - Levels A/b/c; Lungs

Pathologist: R.L.Gregson

APPENDIX 12

(Pathology - continued)

Compound: Air
Dosage Level: Control
Rat No/Sex: 17♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 10mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Nasal Passages - Levels A/b/c; Lungs

Pathologist: R.L.Gregson

APPENDIX 12
(Pathology - continued)

Compound: Air
Dosage Level: Control
Rat No/Sex: 18♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 3mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Nasal Passages - Levels A/b/c; Lungs

Pathologist: R.L.Gregson

APPENDIX 12

(Pathology - continued)

Compound: Air
Dosage Level: Control
Rat No/Sex: 19♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical

Enlarged: 9mm

Liver

Pale subcapsular area/s - median cleft: (One) 1mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Nasal Passages - Levels A/b/c; Lungs

Pathologist: R.L.Gregson

APPENDIX 12

(Pathology - continued)

Compound: Air
Dosage Level: Control
Rat No/Sex: 20♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 11mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Nasal Passages - Levels A/b/c; Lungs

Pathologist: R.L.Gregson

APPENDIX 12

(Pathology - continued)

Compound: Methacrolein
Dosage Level: Low
Rat No/Sex: 21♂ (Terminal)

MACROSCOPIC FINDINGS

Head

Pinna/e - torn: (Right)

Lymph Nodes - Cervical

Enlarged: 7mm

Liver

Pale subcapsular area/s - median cleft: (One) 1mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Nasal Passages - Levels A/b/c; Larynx - Levels A/b; Lungs

Pathologist: R.L.Gregson

APPENDIX 12
(Pathology - continued)

Compound: Methacrolein
Dosage Level: Low
Rat No/Sex: 22♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 12mm

Liver
Pale subcapsular area/s - median cleft: (One) 2mm

Forestomach
Limiting ridge - cyst: 2mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Nasal Passages - Levels A/b/c; Larynx - Levels A/b; Lungs

Pathologist: R.L.Gregson

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METHACROLEIN (B.G. No. 108)
TWO-WEEK REPEAT-DOSE PRELIMINARY
INHALATION TOXICITY STUDY IN RATS

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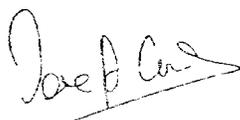
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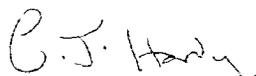
We the undersigned, hereby declare that the work was performed under our supervision according to the procedures herein described, and that this report provides a correct and faithful record of the results obtained.



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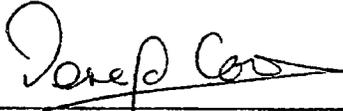
COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

To the best of my knowledge and belief the study described in this report was conducted in compliance with the following Good Laboratory Practice Standards.

Good Laboratory Practice, The United Kingdom Compliance Programme, Department of Health & Social Security 1986 and subsequent revision, Department of Health, 1989.

United States Environmental Protection Agency, (TSCA), Title 40 Code of Federal Regulations Part 792, Federal Register, 29 November 1983 and subsequent amendment Federal Register 17 August 1989.

Organisation for Economic Co-operation and Development, ISBN 92-64-12367-9, Paris 1982.



Derek W. Coombs, B.Sc.,
Study Director,
Huntingdon Research Centre Ltd.

01/09/92
Date

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QUALITY ASSURANCE STATEMENT

This report has been audited by the Quality Assurance Department. It is considered to be an accurate description of the procedures and practices employed during the course of the study and an accurate presentation of the findings.

Date of reporting audit findings
to the Study Director and HRC Management

17.08.92



K.P. de-Salis, B.A.,
Systems Compliance Auditor,
Department of Quality Assurance,
Huntingdon Research Centre Ltd.

28 August 92

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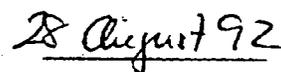
QUALITY ASSURANCE STATEMENT**DATES OF STUDY INSPECTIONS**

Inspections were made by the Quality Assurance Department of the various phases of the study described in this report. The dates on which the inspections were made and the dates on which the findings were reported to the Study Director and to HRC Management are given below.

Phase of Study	Date of Inspection	Date of Reporting
Protocol Review	29.04.91	29.04.91
Pre-experimental Period	08.05.91	08.05.91
Experimental Period	15.05.91	16.05.91
	23.05.91	23.05.91
	30.05.91	30.05.91



K.P. de-Salis, B.A.,
Systems Compliance Auditor,
Department of Quality Assurance,
Huntingdon Research Centre Ltd.



28 August 92

CONTENTS

	Page
SUMMARY	i - v
INTRODUCTION	1
ANIMALS AND MANAGEMENT	
Animals	2
Accommodation	2 - 3
Diet	3
TEST SUBSTANCE AND ADMINISTRATION	
Test substance	4
Administration	4
EXPOSURE SYSTEM AND PROCEDURE	
Atmosphere generation	5
Exposure chambers	5
Procedure	5 - 6
Target concentrations	6
Exposure chamber conditions	
Chamber atmosphere concentration of methacrolein	6
Nominal concentration	6
Chamber air flow	6
Chamber pressure	7
Chamber temperature and relative humidity	7
CLINICAL OBSERVATIONS	
Clinical signs	8
Bodyweight	8
Food consumption	8
Water consumption	8
LABORATORY INVESTIGATIONS	
Haematology	9 - 10
Blood biochemistry	10 - 11

SACRIFICE AND POST MORTEM PROCEDURE		Page
Sacrifice		12
Macroscopic pathology and organ weight analysis		12
Tissue preservation		12
Microscopic examination		13
STATISTICAL ANALYSIS		14
ARCHIVING		15
REFERENCES		16
RESULTS		
Chamber atmosphere conditions		17
Analysed concentration of methacrolein		17
Nominal concentration of methacrolein		17
Chamber temperature and relative humidity		18
Clinical observations		
Mortality		18
Clinical signs	18	- 19
Bodyweights		19
Food consumption		19
Water consumption		20
Laboratory investigations		
Haematology		20
Biochemistry		21
Terminal studies		
Macroscopic pathology	22	- 23
Organ weights		23
Microscopic pathology	23	- 24
DISCUSSION		25
FIGURES		
1. Vapour generator		26
2. Exposure system		27
3. Bodyweights - group mean values		28
4. Food consumption - group mean cumulative values		29
5.(a-b) Water consumption - group mean cumulative values	30	- 31

TABLES

	Page
1. Chamber concentration of methacrolein	32 - 33
2. Nominal concentration of methacrolein	34
3. Temperature and relative humidity during exposures (exposure mean values)	35
4. Clinical signs during exposure	36
5. Bodyweights - group mean values	37
6. Food consumption - group mean cumulative values	38
7. Water consumption - group mean cumulative values	39
8. Haematology - group mean values	40
9. Biochemistry - group mean values	41 - 42
10. Macroscopic pathology incidence summary	43 - 46
11. Organ weights - group mean values	47 - 48
12. Microscopic pathology - incidence summary	49 - 56

APPENDICES

1.(a-b) Composition and quality assurance aspects of diet and water	57 - 59
2a. Method of analysis for methacrolein	60 - 61
2b. Purity analysis of methacrolein	62 - 76
3. Individual clinical signs	77 - 78
4. Bodyweights - individual values	79 - 86
5. Food consumption - cage mean values	87 - 88
6. Water consumption - cage mean values	89 - 90
7. Haematology - individual values	91 - 93
8. Biochemistry - individual values	94 - 97
9. Organ weights - individual values	98 - 99
10. Pathology - individual findings	100 - 153
11. Sites of histological examination for larynx and nasal passages	154 - 155

BGH 50/932334

BGH 40/920648

SUMMARY

Test substance: Methacrolein (B.G. No. 108).

Test animals: Albino Crl: CD® (SD) BR Sprague-Dawley strain.

Route of administration: Whole-body exposure to the vapour of the test substance.

Duration of exposures: Six hours a day, 5 days a week (Monday to Friday) for 2 weeks and on the Monday of the following week. A total of 11 exposures. Only 1 exposure was completed at the highest exposure level due to the severe response.

Exposure levels:

Group	Designation	Concentration of methacrolein			
		Target		Analysed	
		(ppm)	(mg/l)	(ppm)	(mg/l)
1	Air control			Air only	
2	Low concentration MTHCRLN	5	0.014	5	0.013
3	Int. concentration MTHCRLN	20	0.057	19	0.054
4	High concentration MTHCRLN	80	0.230	(77)	(0.221)

() Single exposure

Results

Mortality: Unscheduled deaths occurred in the High concentration group as follows:

One male and 1 female found dead on the morning following the first exposure.

Two females died later the same day (Day 2).

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BGH 50/932334

BGH 40/920648

The 4 surviving males were moribund and were sacrificed *in extremis* on Day 2.

One female was found dead on Day 3.

Clinical signs:

During exposure signs indicative of exposure to an irritant substance were observed in all exposed groups typified by closed or half-closed eyes. The Intermediate and High concentration groups also adopted a hunched posture. A prone posture, irregular shallow breathing and pronounced (diaphragmatic) breathing movements were observed during the single exposure at the high concentration level.

At other times red/brown staining around the head was seen in a proportion of rats exposed to the Inter. concentration level from Week 2.

Following Exposure 1, all rats exposed at the high concentration were gasping or had pronounced breathing movements and red/brown staining around the snout. Signs persisted until the animals died or were killed.

Râles was occasionally observed in the surviving High concentration group female throughout the period until scheduled sacrifice.

Bodyweights:

Intermediate concentration rats gained slightly less weight than controls.

Food consumption:

Intermediate concentration rats consumed less than control rats.

Water consumption:

Intermediate concentration rats consumed more than control rats. The effect was more marked in females.

Haematology:

Differences between control and exposed groups were considered not to be of toxicological significance.

BGH 50/932334

BGH 40/920648

Biochemistry:

Differences between control and exposed groups were considered not to be of toxicological significance.

Macroscopic pathology:

Higher incidence of the following, compared with controls, seen in decedent rats from Group 4 (High concentration MTHCRLN) were considered to be related to exposure to methacrolein

Lungs - congested
- not fully collapsed on opening thoracic cavity

Adipose tissue - reduced

Liver - pale areas

Pancreas - pallor

Gastro-intestinal tract - gaseous distension

Skeletal muscle - congestion close to the larynx.

Organ weights:

Differences between control and groups exposed at 5 or 19 ppm were considered not to be of toxicological significance. The single surviving rat exposed for 1 day at 77 ppm had a high lung weight.

Microscopic pathology:

The following treatment-related changes were seen in decedent rats from Group 4 (High concentration MTHCRLN):

Nasal turbinates -erosion/ulceration/necrosis of the respiratory epithelium and erosion of the olfactory epithelium in all rats. Purulent exudate in the nasal cavity and evidence of epithelial regeneration in a majority of rats. Erosion and ulceration of the olfactory epithelium in a single female.

BGH 50/932334

BGH 40/920648

Larynx - areas/extensive epithelial ulceration in all rats. Moderate subepithelial inflammation and fibrino-purulent exudate in the lumen with bacterial colonisation in the majority of rats. Minimal epithelial hyperplasia with squamous metaplasia in a single female.

Trachea - epithelial erosion, necrosis or ulceration in a proportion of rats. Inflammatory cells in the lamina propria in a single female. Fibropurulent exudate in the lumen of a single female.

Lungs - necrosis of the bronchiolar epithelium in all rats, with peribronchiolar inflammatory cells in a single male. Vascular congestion in one male and all females. Focal alveolar oedema in a single male.

Liver - minimal centrilobular hepatocyte vacuolation in a proportion of males. Generalised hepatocyte vacuolation in a proportion of females. Subcapsular hepatocyte necrosis in two males, centrilobular hepatocyte necrosis in one male.

Adrenals - reduced cortical vacuolation in all males. Apparent hypertrophy of the zona fasciculata in two females.

The following treatment-related changes were seen in rats killed after the exposure period:

Nasal turbinates - pseudoglandular goblet cell hyperplasia and erosion and/or disorganisation of the olfactory epithelium in a majority of Intermediate concentration group rats and the High concentration rat. Minimal hyperplasia of the respiratory epithelium in a majority of Intermediate concentration rats. Hypertrophy of the respiratory epithelium in 2 Intermediate concentration males.

Larynx - minimal epithelia hyperplasia in a proportion of Intermediate concentration rats with necrosis of the ventral cartilage in the High concentration rat.

BGH 50/932334

BGH 40/920648

Trachea - epithelial hypertrophy in a proportion of Intermediate concentration males and the High concentration female. Epithelial hyperplasia in the majority of intermediate concentration rats.

Lungs - hypertrophy of bronchiolar epithelium with prominent goblet cells in the High concentration female.

Adrenals - apparent hypertrophy of the zona fasciculata in 3 Intermediate concentration females.

The no-adverse effect concentration of Methacrolein was 0.013 mg/l (5 ppm).

BGH 50/932334

BGH 40/920648

INTRODUCTION

This study undertaken at the Huntingdon Research Centre Ltd., Huntingdon, England, was designed to investigate the effect on rats of inhalation of a test atmosphere containing methacrolein vapour, administered for 6 hours a day, 5 days a week for 2 weeks and 1 day of the following week.

The data from this investigation was intended to assist the selection of exposure levels for a subsequent 90-day inhalation study.

Key dates during this study were:

Protocol approval:

by Study Director: 24 April 1991.

by HRC Management: 24 April 1991.

by Sponsor: 15 July 1991.

Animals arrived: 1 May 1991.

First exposure: 13 May 1991.

Last exposure: 27 May 1991.

Terminal kill: 28 May 1991.

This report contains all the relevant data collected during the study. All specimens, raw data and other documents generated at HRC during the study, together with a copy of this final report, have been lodged in the HRC Archives, Huntingdon, Cambridgeshire, England.

BGH 50/932334

BGH 40/920648

ANIMALS AND MANAGEMENT

Animals

Fifty rats (25 male and 25 female) Sprague-Dawley CD rats, aged approximately 6 weeks, were ordered from Charles River (JK) Ltd., Manston Road, Margate, Kent, England, to arrive at Huntingdon Research Centre on 1 May 1991.

All rats were arbitrarily selected and identified on a cage basis by a temporary mark on the tail. Veterinary examination took place on 3 May 1991. Allocation took place on 3 May 1991.

The rats were weighed and the individual bodyweights processed using a computer program which selected 50 rats (25 male and 25 female) for allocation to 5 groups, such that the group mean bodyweights were approximately equalised. The rats were uniquely identified by numbers tattooed into the ear pinna.

The identification of individual rats in each group, together with the initial allocation group mean bodyweights, were as follows:

Group	Designation	Rat numbers		Group mean bodyweights (g)	
		Male	Female	Male	Female
1	Air control	51 -	21 - 25	173.0	143.8
2	Low concentration MTHCRLN	6 - 10	26 - 30	173.8	144.8
3	Int. concentration MTHCRLN	11 - 15	31 - 35	173.4	143.0
4	High concentration MTHCRLN	16 - 20	36 - 40	172.6	144.0
R	Reserve	41 - 45	46 - 50	173.6	145.4

The reserve group rats were retained as potential replacements during the acclimatisation period and were killed when exposures commenced (13 May 1991).

Accommodation

The rats were housed 5 of the same sex to a cage, in suspended cages with stainless steel sides and stainless steel mesh floors. Each cage measured 53 cm long, 35 cm wide and 25 cm high. Plastic trays, lined with absorbent paper, were placed below each cage to collect animal excreta. The paper was changed daily and clean cages were introduced at intervals throughout the study.

Each group of rats was kept in a separate ventilated cabinet to prevent any possible cross-contamination between groups once exposure had commenced. The ventilated cabinets drew their air supply from the holding room. Exposure to the material took place in the same room.

BGH 50/932334

BGH 40/920648

The temperature and relative humidity of the room was recorded using a Kent Clearspan M105 chart recorder. The maximum and minimum values over the study period were as follows:

Holding room temperature:	Maximum 23.0°C
	Minimum 19.0°C
Holding room relative humidity:	Maximum 62%
	Minimum 40%

Lighting was controlled to give 12 hours light (08.00 - 20.00 hours) and 12 hours dark per 24 hours.

Diet

While in their cages all rats had free access to a weighed quantity of standard quality controlled laboratory rat food (SDS Rat and Mouse No. 1 modified diet, Special Diets Services, Witham, Essex, England).

There was no information available to indicate that any non-nutrient substance likely to influence the effect of the test compound could reasonably be expected to be present in the diet. Analyses were made on all batches of diet used to establish levels of basic nutrients and of specified substances and micro-organisms likely to have been present in the feed components and which, if in excess of specified amounts, might have had an undesirable effect on the test system. All batches of diet used conformed with the acceptable standards agreed by the Study Director and Head, Quality Assurance Department. The data have been lodged in HRC Archives.

Tap water was available from moulded polypropylene water bottles at all times while the rats were in the cages. The water bottles were rinsed and refilled daily and thoroughly cleaned at intervals during the study.

There was no information available to indicate that any substance likely to influence the effect of the test system could reasonably be expected to be present in the drinking water.

The results of the routine physical and chemical analyses of water at source (sampling point, Grafham Final Water) as conducted by the supplier, Anglian Water Services Ltd., have been made available to HRC. Additionally, levels of specified substances known to be present from time to time in local water and which, if in excess of the maxima recommended for (human) drinking water, might have had an undesirable effect on the test system, are determined in the tap water at approximately six-monthly intervals. The analytical data have been lodged in HRC Archives.

BGH 50/932334

BGH 40/920648

TEST SUBSTANCE AND ADMINISTRATION

Test substance

Name: Methacrolein (B.G. No. 108).
IUPAC name: 2-methylpropenal.
Batch no.: 02505PX.
Presentation: Liquid.
Received from: Aldrich Chemical Co. Ltd.,
The Old Brickyard,
New Road,
Gillingham,
Dorset,
England.
Receipt date: 25 April 1991.
Purity: 97.02% (G.C.). The major impurity is the dimer
of methacrolein.
Stability: Adequate for study. Result of re-analysis after
end of study: 93.94% (G.C.).
Storage conditions: Refrigerated (4°C).

Administration

The animals were exposed whole-body to the vapour of methacrolein for 6 hours a day, 5 days a week (Monday to Friday) for 2 weeks and on the Monday of the following week. A total of 11 exposures.

Control rats were similarly treated but no test substance was introduced into the exposure chamber.

EXPOSURE SYSTEM AND PROCEDURE

Atmosphere generation (Figure 1)

The vapour was produced by metering the liquid from an all polypropylene syringe, mounted on a Precidor® type 5003 syringe pump, to a sintered glass disc contained in a glass vessel, through which air was passed at a rate of 150 litres per minute.

The air was pre-warmed having been passed through a tube immersed in a water bath maintained at 70°C.

The vapour-laden air passed into the chamber *via* the inlet duct.

The control group received 150 l/minute of air only.

Exposure chambers (Figure 2)

The exposure chambers were constructed from stainless steel and glass and were of approximately 750 l internal volume. The chambers were of square cross-section fitted with a pyramidal base and top. An extraction plenum was fitted in the base.

The chamber atmosphere was extracted by means of individual air handling units, each fitted with filters. A gate valve fitted in each extract line was used to adjust the pressure within each chamber to approximately 10 mm of water below that of the room.

Each chamber was fitted with ports for withdrawal of chamber air samples for analytical purposes. The atmosphere from the test group chambers was sampled from an upper middle port on the chamber side wall.

The rats were held within individual compartments of stainless steel wire mesh cages during exposure.

Procedure

The water bath was switched on and set to operate at 70°C.

The rats were placed within the exposure cages, the cages loaded into the exposure chambers and the chamber doors sealed.

Syringes appropriate to each group were filled with test substance, mounted on the syringe pumps and connected to the vaporisers with a PTFE tube. The volume of test substance in each syringe was recorded.

The air supply to the vaporisers was switched on. Chamber pressure was checked and adjusted if necessary to 10 mm of water below ambient.

BGH 50/932334

BGH 40/920648

Exposure commenced when the syringe pumps were switched on. The concentration of methacrolein within each chamber was controlled by the rate at which the test material was fed to the vaporiser by the syringe pump. The feed rate required to achieve the target concentration was determined during preliminary investigations.

Following 6 hours of exposure the syringe pumps were switched off. The volume of test substance remaining in each syringe was recorded.

The air supply was switched off and a sample bung removed from the side wall of the test chamber to facilitate clearance of the chamber atmosphere. After approximately 20 minutes the rats were unloaded from the chambers and returned to their holding cages.

Target concentrations

The target concentrations of methacrolein were as follows:

Group	Designation	Target concentration	
		(ppm)	(mg/l)
1	Air control	Air	
2	Low concentration MTHCRLN	5	0.014
3	Int. concentration MTHCRLN	20	0.057
4	High concentration MTHCRLN	80	0.230

Exposure chamber conditions

Chamber atmosphere concentration of methacrolein

The concentration of methacrolein present in each exposure chamber was measured at approximately hourly intervals during exposure.

The method of sampling and analysis is detailed in Appendix 2.

Nominal concentration

Nominal concentration for each exposure level was calculated for each exposure from the volume of liquid delivered from each syringe.

Chamber air flow

Air flow to the vaporiser was monitored continuously using tapered tube rotameters and recorded at 30-minute intervals throughout exposure.

BGH 50/932334

BGH 40/920648

Chamber pressure

The chamber internal pressure relative to ambient was monitored continuously by magnehelic pressure gauge and recorded at 30-minute intervals throughout exposure.

Chamber temperature and relative humidity

The temperature and relative humidity in each chamber were monitored continuously with a wet and dry bulb thermohygrometer and recorded at 30-minute intervals. Relative humidity was calculated from these data.

CLINICAL OBSERVATIONS

Clinical signs

During exposure

Clinical signs during exposure were recorded as a group response where all visible animals appeared to be responding similarly or a proportion were affected. Group responses seen during exposure were not transferred to the individual clinical signs sheets, and are reported in the text of this report.

At other times

Animals were examined twice each day, usually prior to loading and immediately following unloading from the chambers on exposure days, and in the morning and afternoon of non-exposure days. An entry was made in the individual clinical signs sheets twice daily, even if abnormalities were not seen.

Bodyweight

Each rat was weighed for allocation to groups, then on each day commencing 1 week before the start of exposures and continuing throughout the study.

In addition, the weight of each rat at necropsy was recorded.

Food consumption

The quantity of food consumed by each cage of rats was recorded daily commencing 1 week prior to the start of exposures until the end of the study.

Water consumption

The quantity of water consumed by each cage of rats was recorded daily commencing 1 week prior to the start of exposures until the end of the study.

BGH 50/932334

BGH 40/920648

LABORATORY INVESTIGATIONS

Samples of blood were collected from all surviving rats during Week 2 of the study (23 May 1991).

All rats were deprived of food overnight. Food was available to all rats for a period of approximately one hour immediately after removal of blood samples and before exposure.

Blood was removed from the orbital sinus of each rat while under light ether anaesthesia.

Haematology

EDTA and sodium citrate (for thrombotest) anticoagulants were used. The parameters measured together with the methods and units were as follows:

The following estimations were performed with an Ortho ELT-1500 haematology analyser using standard Ortho methodology:

	Units
Packed cell volume (PCV)	%
Haemoglobin (Hb)	g/dl
Red cell count (RBC)	$\times 10^6 / \text{mm}^3$
Mean corpuscular haemoglobin concentration (MCHC) - calculated: $\text{Hb (g/dl)} \times 100 / \text{PCV (\%)} $	%
Mean corpuscular volume (MCV) - calculated: $\text{PCV (\%)} \times 10 / \text{RBC } (\times 10^6 / \text{mm}^3)$	fl
Mean corpuscular haemoglobin (MCH) - calculated: $\text{Hb (g/dl)} \times 10 / \text{RBC } (\times 10^6 / \text{mm}^3)$	pg
Total white cell count (WBC total)	$\times 10^3 / \text{mm}^3$
Platelet count (Plts)	$\times 10^3 / \text{mm}^3$

The following were performed using the methodology described below:

Differential count (Diff) - standard microscopy of blood smear, stained with modified Wright's stain, counting 100 cells:

Neutrophils (N)	}	$\times 10^3 / \text{mm}^3$
Lymphocytes (L)		
Eosinophils (E)		
Basophils (B)		
Monocytes (M)		

: 9 :

: 352 :

BGH 50/932334

BGH 40/920648

Thrombotest (TT) - Method of Owren, P.A.
(Lancet 1959, ii, 754)

Units

s

Reticulocyte count (Retic) - Method of Dacie, J.V.
and Lewis, S.M. (Practical Haematology, 1966,
3rd edit. p. 28)

% (of
red cells)

A smear for reticulocyte count was prepared. In the absence of changes in red cell parameters the smear was not examined.

Blood biochemistry

The blood was placed into proprietary blood collection vials containing lithium heparin anticoagulant. The blood was centrifuged at 3200 'g' for 3 minutes and the plasma analysed as below:

The following parameters were analysed with a Roche Cobas centrifugal analyser, using the appropriate BCL test kit:

	Units
Glucose - hexokinase mediated	mg/dl
Glutamic-pyruvic transaminase (GPT), also known as 'alanine aminotransferase' - reaction temperature 30°C	mU/ml
Glutamic-oxaloacetic transaminase (GOT), also known as 'aspartate aminotransferase' - reaction temperature 30°C	mU/ml
Creatine phosphokinase (CPK) also known as 'creatin kinase' - reaction temperature 30°C	mU/ml
Gamma-glutamyl transferase (γGT) - reaction temperature 30°C	mU/ml

The following parameters were analysed with a Hitachi 737 Clinical chemistry analyser, using standard Hitachi 737 methodology:

Total protein	g/dl
Albumin (Alb)	g/dl
Globuline (Glob) - by subtraction, total protein (g/dl) minus albumin (g/dl)	g/dl
Albumin/Globulin ratio (A/G) - by calculation from albumin and total protein concentrations	-

: 10 :

: 353 :

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BGH 50/932334

BGH 40/920648

	Units
Urea nitrogen (Urea Nitr)	mg/dl
Alkaline phosphatase (AP) - reaction temperature 30°C	mU/ml
Total bilirubin	mg/dl
Creatinine	mg/dl
Sodium (Na)	mEq/l
Potassium (K)	mEq/l
Calcium (Ca)	mEq/l
Inorganic phosphorus (P)	mEq/l
Chloride (Cl)	mEq/l
Cholesterol (Chol)	mg/dl

SACRIFICE AND POST MORTEM PROCEDURE

Sacrifice

Following two weeks and one day of exposure all surviving rats were killed on 28 May 1991.

The rats were killed by exsanguination from the brachial arteries following anaesthesia induced by intraperitoneal injection of pentobarbitone sodium.

Macroscopic pathology and organ weight analysis

The macroscopic appearance of all tissues was noted. The following organs were dissected free from each animal and weighed:

- | | |
|----------|-----------------------------|
| adrenals | ovaries |
| brain | pituitary |
| heart | spleen |
| kidneys | testes (with epididymides) |
| liver | thyroid (with parathyroids) |
| lungs | |

Tissue preservation

Samples, or the whole, of the following tissues together with any other macroscopically abnormal entities were preserved in 10% neutral buffered formalin (except eyes which were preserved in Davidson's fixative). The lungs were infused via the trachea with fixative prior to immersion:

- | | | |
|--|---|---|
| ^b adrenals | ^b lungs | spinal column |
| aorta | lymph nodes (cervical, mesenteric and tracheobronchial) | spinal cord (cervical, mid-thoracic and lumbar) |
| brain (sections of medulla/pons, cerebellum and cerebral cortex) | mammary glands | ^a spleen |
| caecum | ^b nasal passages (3 levels) | sternum/ribs (for bone and marrow) |
| colon | oesophagus | stomach |
| duodenum | optic nerve | testes (with epididymides) |
| eyes | ovaries | thymus |
| eyelids | oviduct | thyroid (with parathyroids) |
| exorbital lachrymal gland | pancreas | tongue |
| femur (with joint) | pharynx | ^b trachea (including bifurcation) |
| harderian gland | pituitary | ureter |
| ^a heart | prostate | urinary bladder |
| ileum | rectum | uterus |
| jejunum | salivary gland | vagina |
| ^a kidneys | sciatic nerve | |
| ^b larynx (2 levels) | seminal vesicles | |
| ^b liver | skeletal muscle (thigh) | |
| | skin | |

: 12 :

: 355 :

BGH 50/932334

BGH 40/920648

Microscopic examination

Light microscopic examination was performed of 4 μ m thick sections, stained with haematoxylin and eosin, of those tissues in the table above marked as follows:

- ^a Air control and High concentration rats only
- ^b All rats

STATISTICAL ANALYSIS

All statistical analyses were carried out separately for males and females.

The analyses were carried out using the individual animal as the basic experimental unit.

Bodyweight data were analysed using weight gain.

The following sequence of statistical tests was used for bodyweight and organ weight data:

- (i) If the data consisted predominantly of one particular value (relative frequency of the mode exceeded 75%), the proportion of animals with values different from the mode was analysed by appropriate methods. Otherwise:
- (ii) Bartlett's test (1) was applied to test for heterogeneity of variance between treatments. Where significant (at the 1% level) heterogeneity was found, a logarithmic transformation was tried to see if a more stable variance structure could be obtained.
- (iii) If no significant heterogeneity was detected (or if a satisfactory transformation was found), a one-way analysis of variance was carried out. If significant heterogeneity of variance was present, and could not be removed by a transformation, the Kruskal-Wallis analysis of ranks (2) was used.
- (iv) Analyses of variance were followed by Student's 't' test and Williams' test (3) for a dose related response. Williams' test was reported for exposed groups, when and where considered appropriate. The Kruskal-Wallis analyses were followed by the non-parametric equivalents of the 't' test and Williams' test (Shirley's test (4)).

Where appropriate, analysis of covariance was used in place of analysis of variance in the above sequence. For organ weight data, bodyweight was used as covariate in an attempt to allow for differences in bodyweight which might influence the organ weights.

F 12

BGH 50/932334

BGH 40/920648

ARCHIVING

All specimens, raw data and other documents generated at HRC during the course of the study, together with a copy of the final report, have been lodged in the Huntingdon Research Centre Archives, Huntingdon, England.

: 15 :

: 358 :

BGH 50/932334

BGH 40/920648

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2. Kruskal, W.H. and Wallis, W.A., (1952/3), J. Amer. Statist. Ass., **47**: 583 - 621 and **48**: 907 - 912.
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4. Shirley, E., (1977), Biometrics, **33**: 386 - 389.

RESULTS

CHAMBER ATMOSPHERE CONDITIONS

Group 4 (High concentration MTHCRLN) exposures were terminated following deaths after Exposure 1. Details are presented in the appropriate section. Data relating to the High concentration group presented below refer to Exposure 1 only.

Analysed concentration of methacrolein

The data are presented in Table 1 and are summarised below:

Group	Study mean analysed concentration of methacrolein	
	(mg/l)	(ppm)
1 (Air control)		Air only
2 (Low concentration MTHCRLN)	0.013	5
3 (Int. concentration MTHCRLN)	0.054	19
4 (High concentration MTHCRLN)	(0.221)	(77)

$$\text{ppm} = \frac{24450 \times (\text{mg/l})}{\text{molecular weight of methacrolein (70.09)}} \text{ at } 760 \text{ mmHg and } 25^\circ\text{C}$$

Nominal concentration of methacrolein

The data are presented in Table 2 and are summarised below:

Group	Study mean nominal concentration of methacrolein (mg/l)	Analysed nominal (%)
1 (Air control)	-	-
2 (Low concentration MTHCRLN)	0.014	91.4
3 (Int. concentration MTHCRLN)	0.055	97.7
4 (High concentration MTHCRLN)	(0.220)	(100.5)

Chamber temperature and relative humidity

The data are presented in Table 3 and are summarised below:

Group	T(°C)	RH(%)
1 (Air control)	22.5	37
2 (Low concentration MTHCRLN)	23.6	29
3 (Int. concentration MTHCRLN)	23.3	32
4 (High concentration MTHCRLN)	(21.0)	(45)

The small differences between groups were considered not to have influenced the outcome of the study.

CLINICAL OBSERVATIONS**Mortality**

There were a number of unscheduled deaths in Group 4 (High concentration MTHCRLN) following Exposure 1. Following this Group 4 rats received no further exposures. Details of deaths were as follows:

One male (no. 20) and 1 female (no. 38) died overnight following Exposure 1.

No. 37♀ died in the morning of Day 2.

Nos. 16♂, 17♂, 18♂ and 19♂ were sacrificed in the afternoon of Day 2 following a review of their clinical condition.

No. 39♀ died in the afternoon of Day 2.

No. 40♀ was found dead in the morning of Day 3.

Clinical signs

The data are presented as follows:

Table 4 - clinical signs during exposure
Appendix 3 - individual clinical signs.

During exposure all groups exposed to methacrolein were observed to close or half-close their eyes in response to irritation. Groups 3 (Int. concentration MTHCRLN) and 4 (High concentration MTHCRLN) adopted a hunched and occasionally a prone posture, wet chins indicating salivation were also seen. During the single exposure the High concentration group received, irregular, shallow breathing was observed followed by pronounced breathing movements.

Between exposures and at other times there were no exposure-related signs seen in Groups 1 (Air control) and 2 (Low concentration MTHCRLN).

: 18 :

: 361 :

Red/brown staining around the snout and head was seen from Week 2 of exposure in a proportion of male and female rats from Group 3 (Int. concentration MTHCRLN).

Following Exposure 1, all Group 4 (High concentration MTHCRLN) rats were seen to be gasping or have pronounced breathing movements (diaphragmatic breathing) with red/brown staining around the snout. Râles was observed in 5/5 males and 2/5 females.

Of the 4/5 males and 4/5 females surviving until the morning of Day 2, all of the males had pronounced breathing movements and red/brown staining around the snout, these animals were subsequently seen to be moribund and were sacrificed. All surviving females had red/brown staining around the snout and pronounced breathing movements or hunched posture. Three of the females subsequently died. The one surviving female showed some evidence of recovery, however, occasional episodes of râles were observed up to and including the day of scheduled sacrifice.

Bodyweights

The data are presented as follows:

- Figure 3 - group mean values
- Table 5 - group mean values
- Appendix 4 - individual values

All decedent rats from Group 4 (High concentration MTHCRLN) lost weight following Exposure 1.

The surviving female rat from the High concentration group did not regain its pre-dose bodyweight until Day 5. Subsequently, this animal's rate of weight gain was similar to that of the control group.

The rate of gain by Group 3 (Int. concentration MTHCRLN) rats was slightly lower than controls, however, this was not statistically significant.

Food consumption

The data are presented as follows:

- Figure 4 - group mean cumulative consumption
- Table 6 - group mean cumulative consumption
- Appendix 5 - cage mean values

Consumption by the surviving High concentration female after Day 2 and by rats from Group 2 (Low concentration MTHCRLN) was similar to controls.

Consumption by males and females from Group 3 (Int. concentration MTHCRLN) was slightly lower than controls.

Water consumption

The data are presented as follows:

Figure 5 - group mean cumulative values
Table 7 - group mean cumulative values
Appendix 6 - cage mean values.

Consumption by the surviving High concentration female after Day 2 and rats from Group 2 (Low concentration MTHCRLN) was similar to controls.

Consumption by rats from the Int. concentration group was slightly greater than controls. The difference was more marked in females.

LABORATORY INVESTIGATIONS**Haematology**

The data are presented as follows:

Table 8 - group mean values
Appendix 7 - individual values.

Data from the surviving High concentration female were not included in statistical analyses.

The following statistically significant differences ($P < 0.05$) from control values were seen:

Red cell count

Greater in males from Group 3 (Int. concentration MTHCRLN).

Mean corpuscular haemoglobin concentration

Lower in males from Group 2 (Low concentration MTHCRLN) and in males and females from Group 3 (Int. concentration MTHCRLN).

Mean corpuscular haemoglobin

Lower in males from Group 2 (Low concentration MTHCRLN) and 3 (Int. concentration MTHCRLN).

Thrombotest

Slower clotting time in males from Group 3 (Int. concentration MTHCRLN).

The above differences were small with one exception inconsistent between the sexes. They are considered not to be of toxicological significance.

Biochemistry

The data are presented as follows:

Table 9 - group mean values
Appendix 8 - individual values.

Data from the surviving High concentration female were not included in statistical analyses.

The following statistically significant differences ($P < 0.05$) from control values were seen:

Albumin concentration

Lower in males and females from Group 3 (Int. concentration MTHCRLN).

Albumin/globulin ratio

Lower in males from Group 3 (Int. concentration MTHCRLN).

Urea nitrogen concentration

Greater in males from Group 2 (Low concentration MTHCRLN) and 3 (Int. concentration MTHRLN).

Gamma-glutamyl transferase activity

Lower in males from Group 3 (Int. concentration MTHCRLN).

Creatine phosphokinase activity

Greater in males from Group 3 (Int. concentration MTHCRLN).

Calcium concentration

Greater in males from Group 3 (Int. concentration MTHCRLN).

The above differences were small and with one exception inconsistent between the sexes. They are considered not to be of toxicological significance.

TERMINAL STUDIES

Macroscopic pathology

The data are presented as follows:

Table 10 - incidence summary
Appendix 10 - individual findings.

Macroscopic examination revealed the following treatment-related changes:

Lungs

Congestion was observed in 2/5 male decedent rats and 3/4 female decedent rats treated with the high concentration of methacrolein compared with 0/5 male and female terminal control rats.

The lungs were seen to be not fully collapsed on opening the thoracic cavity in 3/5 male decedent rats and 1/1 female terminal rat treated with the high concentration of methacrolein compared with 0/5 male and female terminal control rats.

Adipose tissue

A reduction in adipose tissue was observed in 2/5 male decedent rats and 3/4 female decedent rats treated with the high concentration of methacrolein compared with 0/5 male and female terminal control rats.

Liver

Pale area(s) were noted in 2/5 male decedent rats treated with the high concentration of methacrolein compared with 0/5 male terminal control rats.

Pancreas

Pallor was observed in 2/5 male decedent rats and 2/4 female decedent rats treated with the high concentration of methacrolein compared with 0/5 male and female terminal control rats.

Gastro-intestinal tract

Gaseous distension was observed in 1/5 male decedent rats and 2/4 female decedent rats treated with the high concentration of methacrolein compared with 0/5 male and female terminal control rats.

Small intestine

Gaseous distension was noted in 3/5 male decedent rats and 2/4 female decedent rats treated with the high concentration of methacrolein compared with 0/5 male and female terminal control rats.

Fur

Staining was seen in all male and female decedent rats treated with the high concentration of methacrolein compared with none in the male and female terminal control rats.

Skeletal muscle

Congestion of the muscle close to the larynx was observed in 1/5 male decedent rats and 1/4 female decedent rats treated with the high concentration of methacrolein compared with 0/5 male and female terminal control rats.

The incidence and distribution of all other findings were considered to fall within the expected background range of macroscopic changes.

Organ weights

The data are presented as follows:

Table 11 - group mean values
Appendix 9 - individual values.

Data from the surviving High concentration female were not included in statistical analyses.

There were no differences between control and Groups 2 and 3 that were considered to be of toxicological significance. The lung weight for the single female survivor in Group 4 (High concentration MTHCRLN) was higher than expected.

Microscopic pathology

The data are presented as follows:

Table 12 - incidence summary
Appendix 10 - individual findings.

The following comments are made in summary:

Treatment-related findings;

Decedent rats

Nasal turbinates - erosion/ulceration/necrosis of the respiratory epithelium in all rats. Degeneration of the respiratory epithelium in three male rats. Erosion of the olfactory epithelium in all rats, with ulceration of the olfactory epithelium in a single female rat. Purulent exudate in the nasal cavity in the majority of rats. Evidence of epithelial regeneration in the majority of rats. These findings were seen at all 3 levels (Appendix 11), but were generally less extensive at level C.

Larynx - areas/extensive epithelial ulceration in all rats. Moderate subepithelial inflammation in the majority of rats. Fibrino-purulent exudate in the lumen with bacterial colonisation in the majority of rats. Minimal epithelial hyperplasia with squamous metaplasia in a single female rat. These findings were seen at both levels examined (Appendix 11).

Trachea (with bifurcation) - epithelial erosion, necrosis or ulceration in a proportion of rats. Inflammatory cells in the lamina propria in a single female rat. Fibropurulent exudate in the lumen of a single female rat.

BGH 50/932334

BGH 40/920648

Lungs - necrosis of the bronchiolar epithelium in all rats, with peribronchiolar inflammatory cells in a single male rat. Vascular congestion in one male and all female rats. Focal alveolar oedema in a single male rat.

Liver - subcapsular hepatocyte necrosis in two male rats. Centrilobular hepatocyte necrosis in a single male rat. Minimal centrilobular hepatocyte vacuolation in a proportion of male rats. Generalised hepatocyte vacuolation in a proportion of female rats.

Adrenals - apparent hypertrophy of the zona fasciculata in two female rats. Reduced cortical vacuolation in Group 4 male rats.

In all decedent rats, the lesions in the respiratory tract were considered to be contributory to death.

Terminal rats

Nasal turbinates - hypertrophy of the respiratory epithelium in two Group 3 male rats. Minimal hyperplasia of the respiratory epithelium in the majority of Group 3 rats. Pseudoglandular goblet cell hyperplasia in the majority of Group 3 rats and in a single Group 4 rat. Erosion and/or disorganisation of the olfactory epithelium in the majority of Group 3 rats and in a single Group 4 rat. These findings were seen at all 3 levels examined (Appendix 11) but were generally less extensive at level C.

Larynx - minimal epithelial hyperplasia in a proportion of Group 3 rats and in the Group 4 rat (this was generally seen at level A). Necrosis of the ventral cartilage in the Group 4 rat (level B).

Trachea (with bifurcation) - epithelial hypertrophy in a proportion of Group 3 male rats and in the Group 4 female rat. Epithelial hyperplasia in the majority of Group 3 rats.

Lungs - hypertrophy of bronchiolar epithelium with prominent goblet cells in the Group 4 rat.

Liver - subcapsular hepatocyte necrosis in a single Group 3 female rat.

Adrenals - apparent hypertrophy of the zona fasciculata in three Group 3 female rats.

Additional comments

The significance of the centrilobular hepatocyte necrosis with prominent inflammatory cells found in a single liver lobe in a single Group 2 rat is uncertain, but unlikely to be related to treatment.

All other findings were considered to be spontaneous in origin and of no toxicological importance.

DISCUSSION

In this study rats were exposed by inhalation to the vapour of methacrolein for 6 hours a day, 5 days a week for 2 weeks and on 1 day of the following week. The mean analysed exposure levels were 0.013, 0.054 and 0.221 mg/l (5, 19 and 77 ppm).

Following the first exposure all male rats and 4 out of 5 female rats exposed to 0.221 mg/l (77 ppm) died or were sacrificed *in extremis*. The surviving female rat was held unexposed until the scheduled day of sacrifice.

Signs indicative of exposure to an irritant substance were seen including closed or half-closed eyelids in all exposed groups, a hunched posture in groups exposed to 0.054 (19 ppm) and 0.221 mg/l (77 ppm) and irregular shallow and/or pronounced breathing movements observed during the single exposure at 0.221 mg/l (77 ppm). At other times red/brown staining of the head was observed in rats exposed to 0.054 (19 ppm) and 0.221 mg/l (77 ppm). Gasping or pronounced breathing movements were observed post exposure in all rats exposed to 0.221 mg/l (77 ppm).

A slightly reduced weight gain (not statistically significant) with lower food consumption and increased water consumption, compared with controls, was recorded for rats exposed to 0.054 mg/l (19 ppm).

There were no effects on haematology or blood biochemistry as a result of exposure.

There were no effects on organ weights as a result of exposure at 0.013 (5 ppm) or 0.054 mg/l (19 ppm). The single rat that survived 1 exposure of 0.221 mg/l (77 ppm) had a high lung weight.

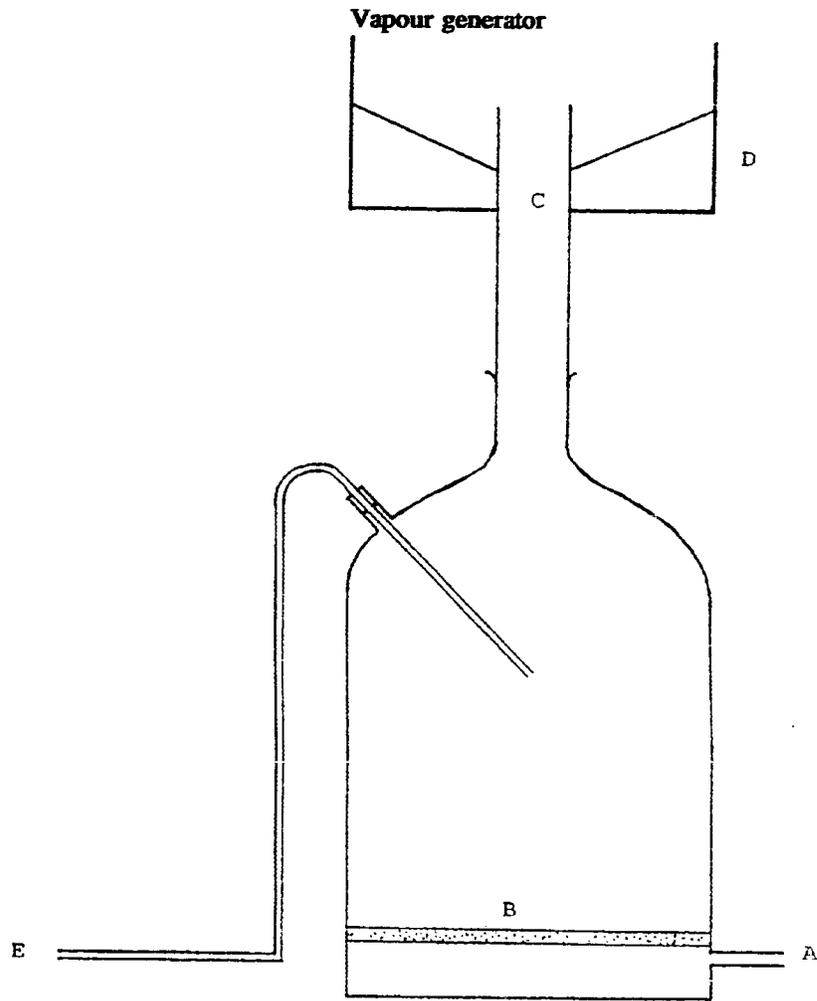
Macroscopic pathological changes were confined to rats exposed at 0.221 mg/l (77 ppm). These included; congested lungs, lungs not collapsed on opening thoracic cavity, congestion of muscle close to the larynx, reduced adipose tissue and pale areas in the liver.

Histopathological changes were seen in all decedent rats and included; epithelial erosion and/or ulceration of the epithelium in the nasal turbinates, larynx and trachea, necrosis of the bronchiolar epithelium, hepatocyte vacuolation and vacuolation in the adrenal cortex.

Histopathological changes seen in rats exposed to 0.054 mg/l (19 ppm) included; hyperplasia of the olfactory, respiratory, laryngeal and tracheal epithelia, hypertrophy of the respiratory, tracheal and bronchiolar epithelia, subcapsular hepatocyte necrosis and apparent hypertrophy of the adrenal zona fasciculata.

No treatment-related histopathological changes were observed in rats exposed to 0.013 mg/l (5 ppm), this is therefore considered to be the no-adverse effect level for this study.

FIGURE 1



Key

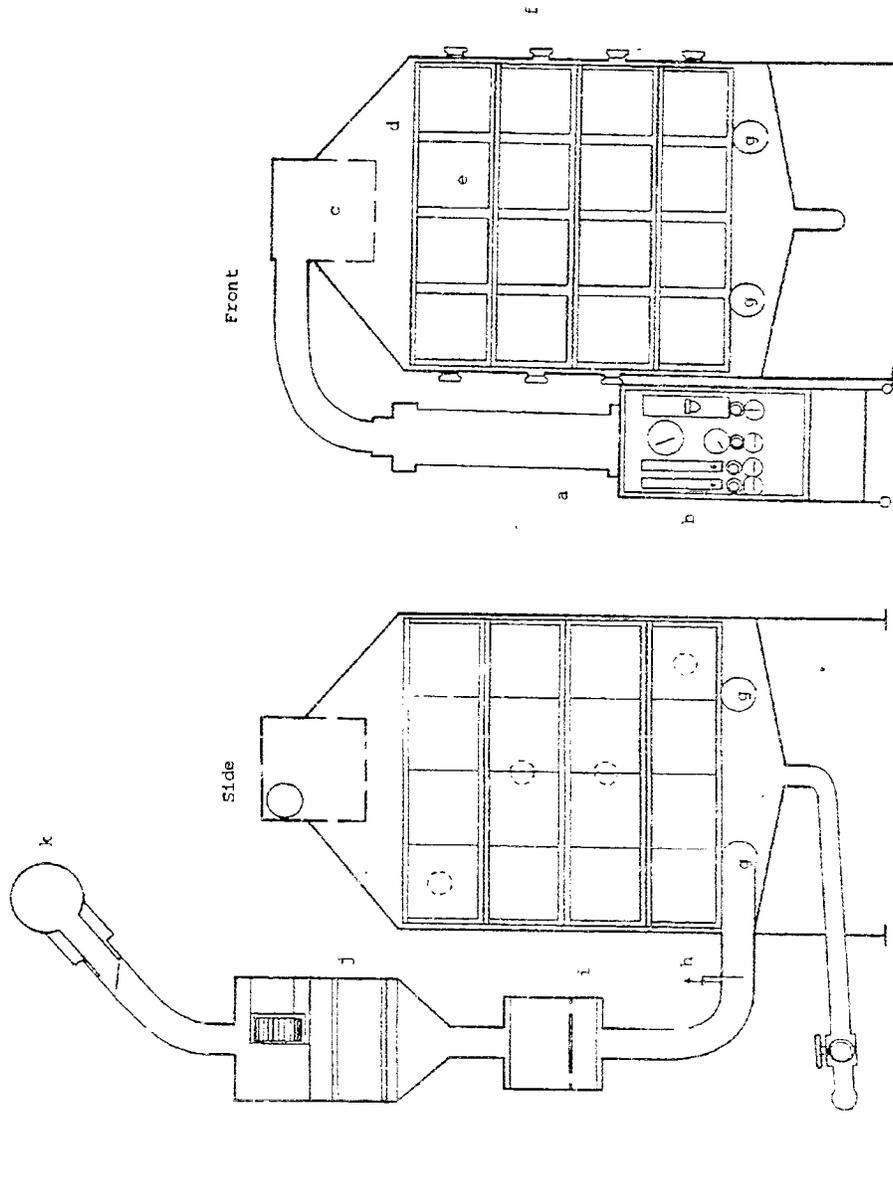
- A. Air line
- B. Fritted glass disc
- C. Vapour inlet
- D. Stainless steel and glass elutriator
- E. Test substance supply line

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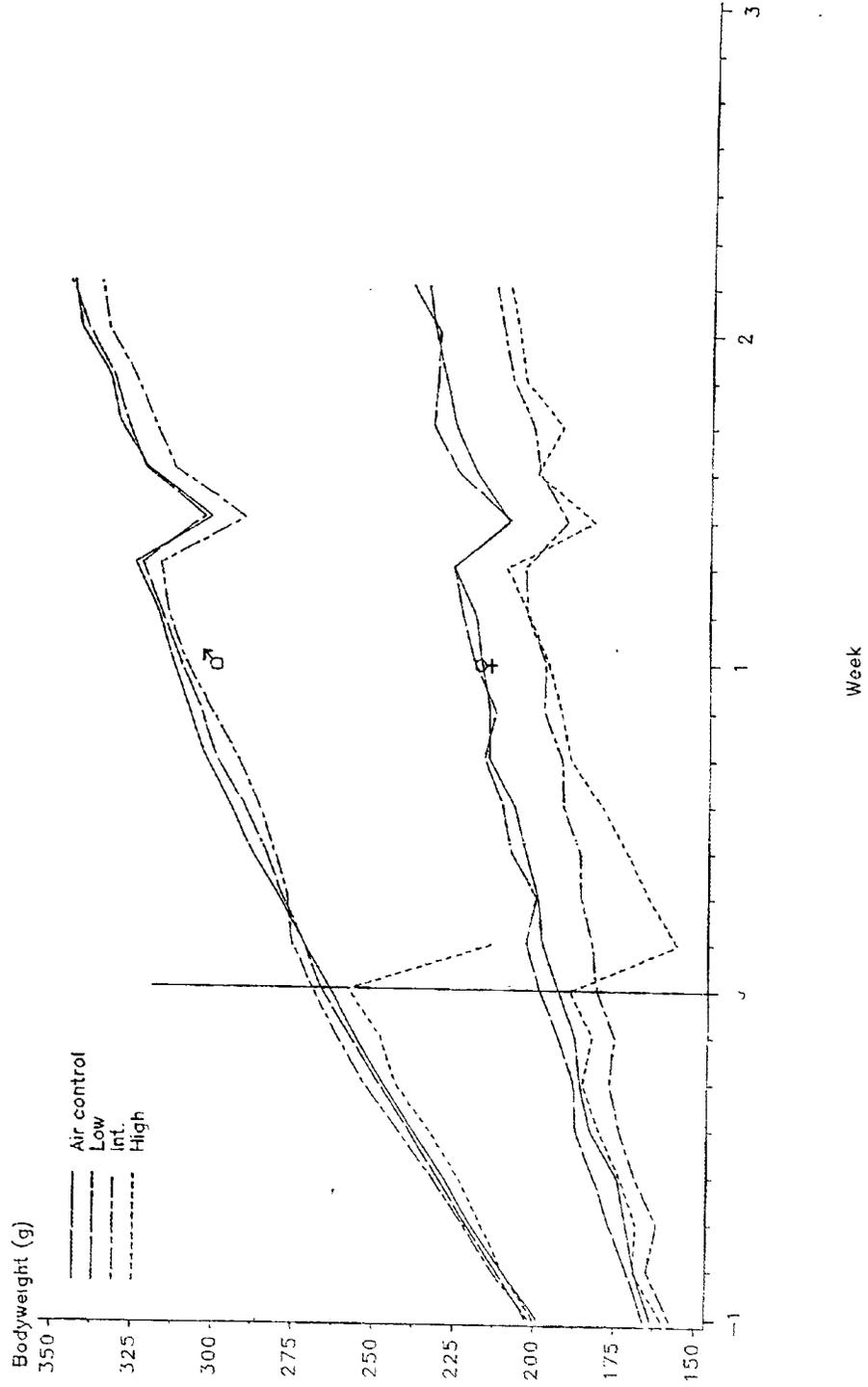
FIGURE 2

Exposure system



- a Glass elutriation column.
- b Airflow control and chamber pressure monitoring.
- c Dispersion device.
- d Exposure chamber.
- e Animal exposure cages.
- f Sampling port.
- g Exhaust plenum.
- h Gate valve.
- i Pre-filter.
- j Powered extract filter.
- k Main exhaust.

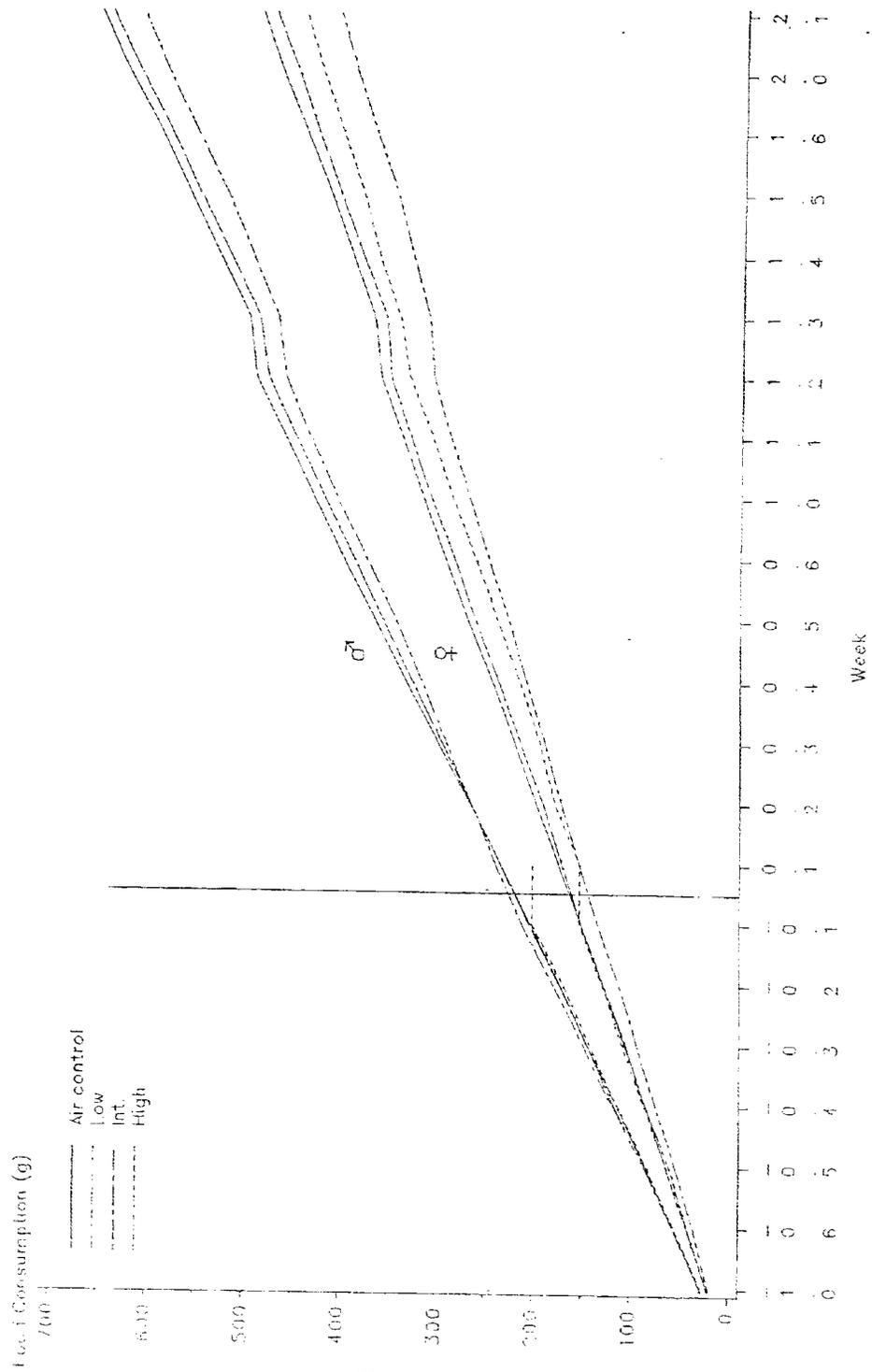
FIGURE 3
Bodyweights - group mean values



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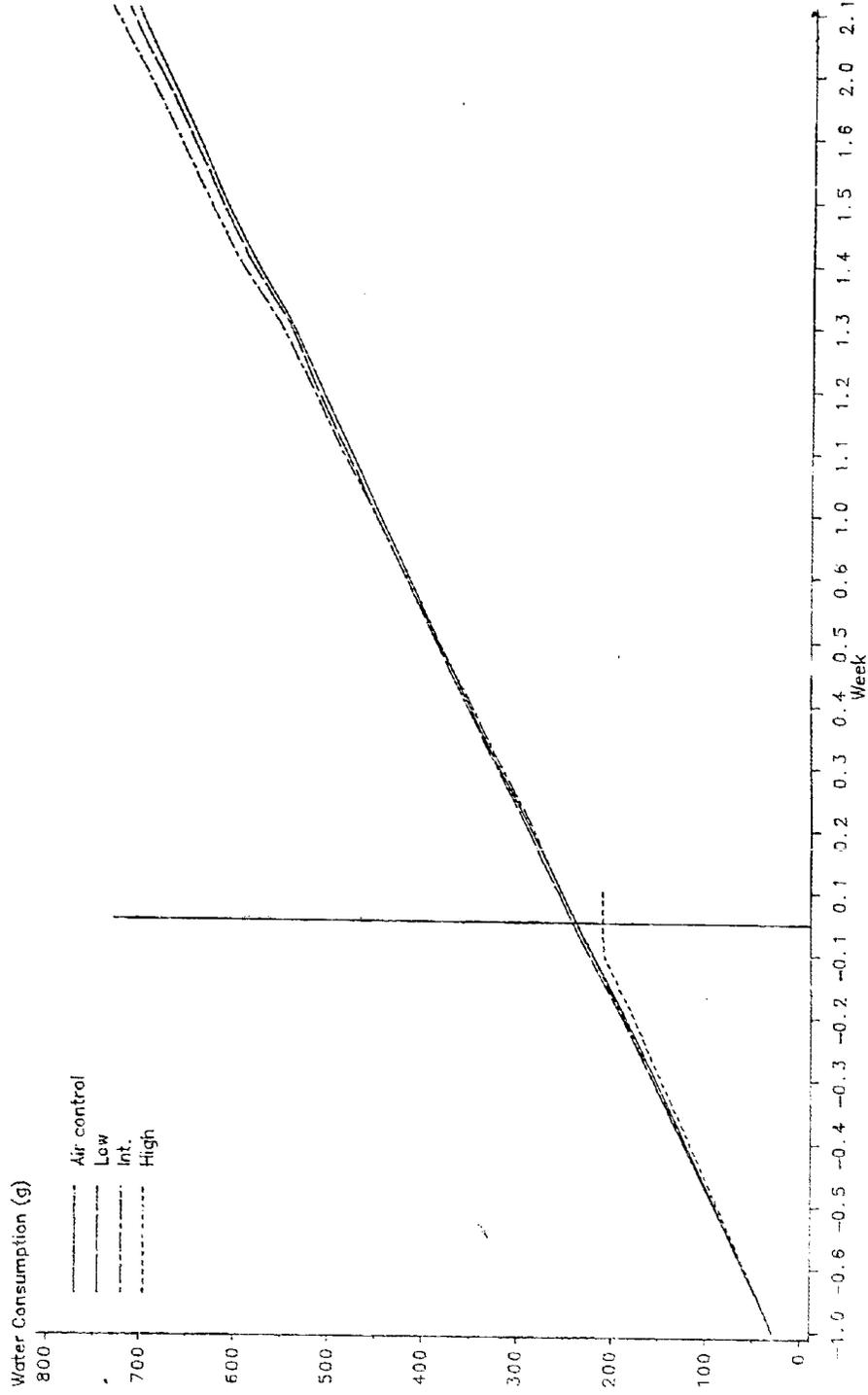
FIGURE 4
Food consumption - group mean cumulative values



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FIGURE 5a
Water consumption - group mean cumulative values - males



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FIGURE 5b
Water consumption .. group mean cumulative values - females

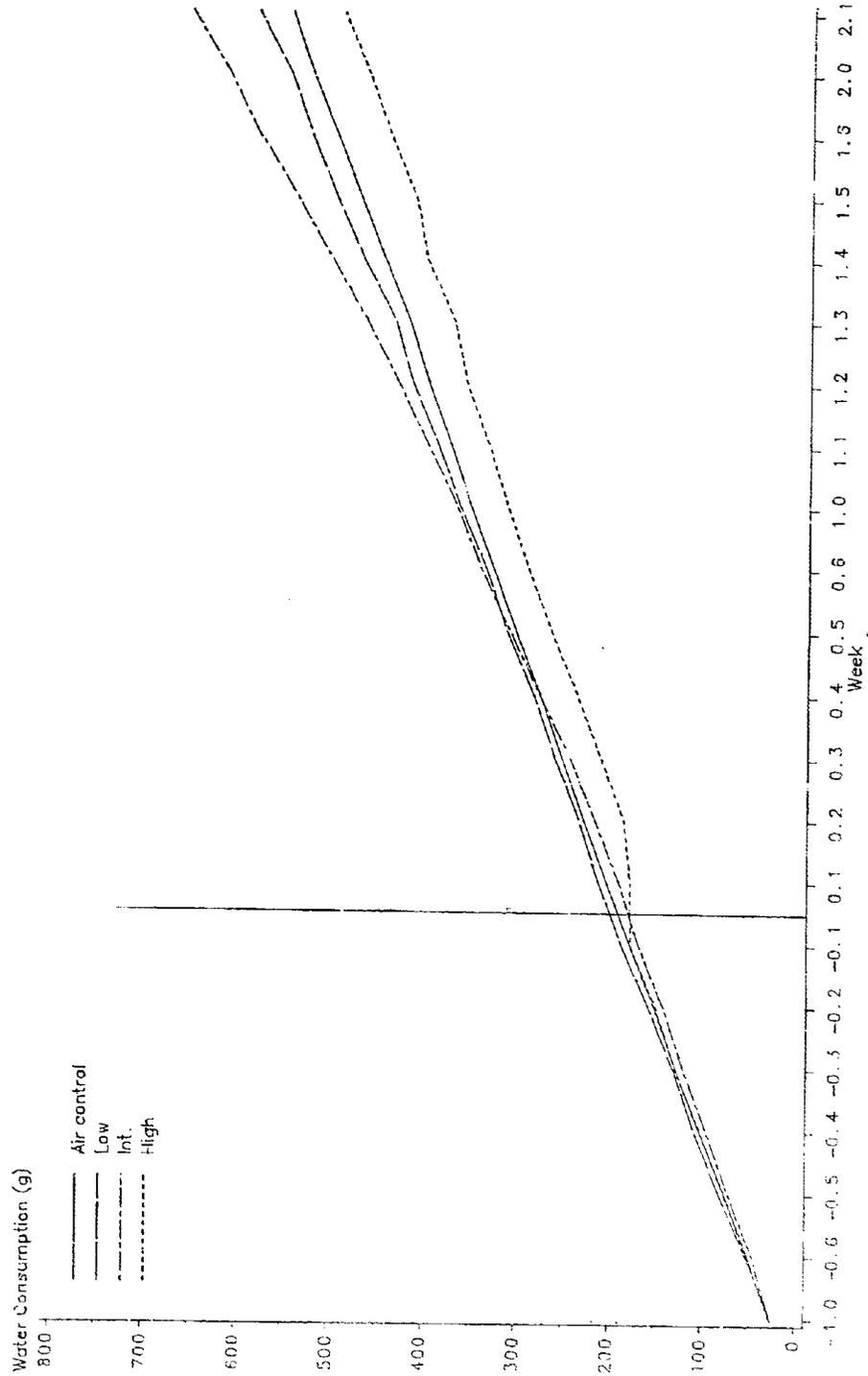


TABLE 1
Chamber concentration of methacrolein (mg/l)

Exposure	Sample	Group		
		2 (Low conc. MTHCRLN)	3 (Int. conc. MTHCRLN)	4 (High conc. (MTHCRLN))
1	1	0.013	0.062	0.308
	2	0.011	0.049	0.217
	3	0.010	0.060	0.218
	4	0.015	0.063	0.223
	5	0.014	0.063	0.192
	6	0.008	0.053	0.165
	Mean	0.012	0.058	0.221
2	7	0.013	0.059	*
	8	0.013	0.059	
	9	0.012	0.060	
	10	0.012	0.048	
	11	0.013	0.055	
	12	0.010	0.049	
	Mean	0.012	0.055	
3	13	0.013	0.053	
	14	0.011	0.050	
	15	0.013	0.050	
	16	0.012	0.058	
	17	0.013	0.051	
	18	0.012	0.046	
	Mean	0.012	0.051	
4	19	0.014	0.058	
	20	0.015	0.058	
	21	0.014	0.059	
	22	0.013	0.056	
	23	0.014	0.056	
	24	0.013	0.053	
	Mean	0.014	0.057	
5	25	0.016	0.056	
	26	0.016	0.052	
	27	0.014	0.053	
	28	0.013	0.050	
	29	0.014	0.053	
	30	0.014	0.052	
	Mean	0.015	0.053	
6	31	0.013	0.049	
	32	0.011	0.049	
	33	0.010	0.050	
	34	0.011	0.046	
	35	0.012	0.046	
	36	0.011	0.048	
	Mean	0.011	0.048	

* High dose exposure terminated due to deaths

TABLE 1
(Chamber concentration of methacrolein - continued)

Exposure	Sample	Group		
		2 (Low conc. MTHCRLN)	3 (Int. conc. MTHCRLN)	4 (High conc. (MTHCRLN))
7	37	0.014	0.054	
	38	0.014	0.052	
	39	0.015	0.052	
	40	0.012	0.052	
	41	0.013	0.054	
	42	0.012	0.053	
	Mean	0.013	0.053	
8	43	0.012	0.052	
	44	0.013	0.054	
	45	0.013	0.053	
	46	0.012	0.055	
	47	0.012	0.053	
	48	0.012	0.055	
	Mean	0.012	0.054	
9	49	0.014	0.068	
	50	0.014	0.059	
	51	0.014	0.028	
	52	0.014	0.064	
	53	0.014	0.062	
	54	0.011	0.053	
	Mean	0.014	0.056	
10	55	a	0.056	
	56	0.017	0.050	
	57	0.010	0.049	
	58	0.010	0.051	
	59	0.012	0.051	
	50	0.012	0.050	
	Mean	0.012	0.051	
11	61	0.012	0.061	
	62	0.012	0.054	
	63	0.012	0.049	
	64	0.012	0.057	
	65	0.012	0.057	
	66	0.012	0.051	
	Mean	0.012	0.055	
	Study mean	0.013	0.054	(0.221)
	SD	0.0016	0.0058	(0.048)
	ppm+	4.5	18.8	(77.1)

SD Standard deviation
a Test substance not generating when sample removed. Liquid not in contact with glass frit
() Single exposure
ppm+ Calculated using ppm = 24450 x mg/l / M.W. (70.09)

TABLE 2
Nominal concentration of methacrolein (mg/l)

Exposure	Group					
	2 (Low conc. MTHCRLN)		3 (Int. conc. (MTHCRLN)		4 (High conc. MTHCRLN)	
	Conc. (mg/l)	A/N* (%)	Conc. (mg/l)	A/N (%)	Conc. (mg/l)	A/N (%)
1	0.014	85.7	0.056	103.6	0.220 #	100.5
2	0.014	85.7	0.056	98.2		
3	0.014	85.7	0.056	91.1		
4	0.014	100.0	0.056	101.8		
5	0.014	107.1	0.056	94.6		
6	0.014	78.6	0.056	85.7		
7	0.014	92.9	0.053	100.0		
8	0.014	85.7	0.056	96.4		
9	0.014	100.0	0.050	112.0		
10	0.011	109.1	0.055	92.7		
11	0.016	75.0	0.056	98.2		
Study mean	0.014	91.4	0.053	97.7		

$$A/N = \frac{\text{Analysed concentration}}{\text{Nominal concentration}} \times 100$$

High concentration exposure terminated due to deaths

TABLE 3
Temperature and relative humidity
during exposures
(exposure mean values)

Exposure	Group							
	1 (Air control)		2 (Low conc. MTHCRLN)		3 (Int conc. MTHCRLN)		4 (High conc. MTHCRLN)	
	T (°C)	RH (%)	T (°C)	RH (%)	T (°C)	RH (%)	T (°C)	RH (%)
1	21.2	40	23.2	29	23.0	31	21.0	45
2	21.8	38	23.7	29	23.5	31	#	
3	22.7	35	23.4	29	22.9	31		
4	22.4	34	23.1	28	22.7	32		
5	22.4	35	22.8	31	22.8	31		
6	22.7	35	23.2	30	22.8	33		
7	23.1	38	23.5	30	23.3	33		
8	23.8	35	24.3	29	24.0	32		
9	22.4	38	24.0	29	23.8	31		
10	22.3	39	24.5	27	23.7	31		
11	22.2	37	23.8	28	23.4	32		
Mean	22.5	37	23.6	29	23.3	32		

High concentration exposure terminated due to deaths

TABLE 4
Clinical signs during exposure

Group	Observation	Exposure										
		1	2	3	4	5	6	7	8	9	10	11
1 Air control	Nothing abnormal detected	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
2 Low conc. MTHCRLN	Eyes closed/half-closed	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
3 Int. conc. MTHCRLN	Eyes closed/half-closed	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Hunched posture	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Prone posture			✓							✓	✓
	Wet chin									✓	✓	✓
4 High conc. MTHCRLN	Eyes closed/half-closed	✓										
	Prone posture	✓										
	Wet chin	✓										
	Irregular shallow breathing	✓										
	Diaphragmatic breathing	✓										

TABLE 5
Bodyweights - group mean values (g)

Day	Group and dosage							
	1♂ Air control	2♂ Low conc. MTHCRLN	3♂ Int. conc. MTHCRLN	4♂ High conc. MTHCRLN	1♀ Air control	2♀ Low conc. MTHCRLN	3♀ Int. conc. MTHCRLN	4♀ High conc. MTHCRLN
Pre-dose								
-7	199	202	201	200	164	166	158	160
-6	210	212	213	210	169	171	165	169
-5	220	222	223	217	172	177	162	168
-4	229	231	232	224	175	182	169	174
-3	238	240	243	233	183	188	174	180
-2	248	249	254	244	186	198	177	185
-1	257	258	262	249	188	193	176	182
Dosing								
0	264	267	269	259	193	199	181	190
1	273	272	276	213	198	203	183	156
2	281	279	278		200	200	186	165
3	289	285	282		204	208	187	172
4	296	292	287		207	211	192	180
5	304	301	294		215	217	193	190
6	309	305	302		215	213	198	193
7	314	312	310		218	220	198	197
8	318	317	315		220	224	204	203
9	326	323	318		227	227	204	210
10	302	304	291		209	205	191	183
11	322	323	314		220	226	200	201
12	331	329	320		227	233	202	193
13	334	333	326		230	232	209	205
14	343	341	335		233	232	212	207
15	346	347	337		235	240	214	210

A 08

BGH 50/932334

BGH 40/920648

TABLE 6

Food consumption - group mean cumulative values (g)

Day	Group and dosage							
	1♂ Air control	2♂ Low conc. MTHCRLN	3♂ Int. conc. MTHCRLN	4♂ High conc. MTHCRLN	1♀ Air control	2♀ Low conc. MTHCRLN	3♀ Int. conc. MTHCRLN	4♀ High conc. MTHCRLN
Pre-dose								
-7	28	27	28	26	21	21	20	21
-6	56	56	57	55	41	43	36	42
-5	84	85	87	83	61	64	55	64
-4	114	116	118	113	84	85	75	87
-3	144	145	149	142	107	105	94	109
-2	173	175	181	170	128	128	112	130
-1	204	206	213	202	152	151	134	153
Dosing								
1	235	236	240	202	175	172	153	153
2	266	265	265	202	198	191	172	179
3	299	295	291		220	215	190	195
4	332	326	316		243	237	208	215
5	364	357	343		268	261	226	238
6	397	386	373		292	282	248	261
7	429	418	405		316	307	268	285
8	458	447	433		337	329	287	306
9	489	477	459		361	350	305	331
10	498	487	467		368	356	311	342
11	528	518	495		391	380	329	364
12	560	549	522		415	405	346	381
13	591	580	553		440	428	368	403
14	625	613	585		466	450	389	425
15	655	644	612		489	475	408	445

TABLE 7
Water consumption - group mean cumulative values (g)

Day	Group and dosage							
	1♂ Air control	2♂ Low conc. MTHCRLN	3♂ Int. conc. MTHCRLN	4♂ High conc. MTHCRLN	1♀ Air control	2♀ Low conc. MTHCRLN	3♀ Int. conc. MTHCRLN	4♀ High conc. MTHCRLN
Pre-dose								
-7	30	30	29	30	25	24	25	26
-6	60	61	59	59	49	52	45	50
-5	91	93	92	89	74	80	70	75
-4	123	125	125	118	102	108	95	101
-3	154	157	156	148	128	131	120	128
-2	187	190	188	178	151	158	142	150
-1	220	225	222	210	179	188	169	178
Dosing								
1	254	258	254	211	204	213	191	179
2	287	291	285	211	229	233	217	186
3	321	323	318		253	260	244	211
4	353	356	351		276	283	277	236
5	386	389	387		303	312	305	265
6	417	421	421		327	334	340	290
7	449	453	454		352	363	367	312
8	479	485	489		374	388	399	333
9	511	516	521		399	415	431	358
10	542	545	555		419	435	467	372
11	582	587	599		447	470	503	402
12	616	621	634		474	499	542	414
13	645	653	666		499	526	583	442
14	678	686	700		526	550	616	467
15	710	721	739		550	585	657	494

TABLE 8
Haematology - group mean values

Week 2 (23 May 1991)

Group	PCV %	Hb g/dl	RBC $\times 10^6/\text{mm}^3$	MCHC %	MCV fl	MCH pg	WBC + Diff $\times 10^3/\text{mm}^3$					Plts $\times 10^3/\text{mm}^3$	TT s	
							Total	N	L	E	B			M
♂ Air control	51	14.9	6.1	29.5	83	24.6	12.8	2.17	10.58	0.08	0.00	0.00	1210	F
♂ Low conc. MTHCRLN	51	14.8	6.3	28.9*	81	23.5*	15.2	3.34	11.69	0.10	0.00	0.04	1272	20
♂ Int. conc. MTHCRLN	52	15.2	6.4*	29.1*	83	23.9*	12.9	3.29	9.70	0.02	0.00	0.00	1143	21*
♀ Air control	52	15.2	6.2	29.2	84	24.4	11.4	1.96	9.35	0.00	0.00	0.09	1220	19
♀ Low conc. MTHCRLN	50	14.6	5.9	29.2	84	24.7	10.0	2.04	7.84	0.06	0.00	0.06	1197	18
♀ Int. conc. MTHCRLN	53	15.4	6.5	28.8*	82	23.6	9.0	1.40	7.59	0.02	0.00	0.00	1160	19
a 4♀ High conc. MTHCRLN	58	(16.5)	(7.0)	(28.4)	(83)	(23.6)	(11.5)	(1.61)	(9.78)	(0.00)	(0.00)	(0.12)	(1319)	(18)

* P < 0.05 compared with control data using Williams' test
a Data from single survivor - not included in statistical analysis
F Frequency analysis applied to data

TABLE 9
Biochemistry - group mean values

Week 2 (23 May 1991)

Group	Glu- cose mg/dl	Protein g/dl			A/G	Urea Nitr mg/dl	Creat- inine mg/dl	AP mU/ ml	GIT mU/ ml	GOT mU/ ml
		Total	Alb	Glob						
1♂ Air control	113	5.9	2.9	3.0	0.99	11	0.5	424	24	52
2♂ Low conc. MTHCRLN	99	6.0	2.9	3.1	0.92	16*	0.5	410	25	53
3♂ Int. conc. MTHCRLN	111	6.1	2.9*	3.3	0.87*	13*	0.5	389	26	56
1♀ Air control	94	6.1	3.0	3.1	0.96	15	0.5	245	17	59
2♀ Low conc. MTHCRLN	110	6.0	3.0	3.0	0.99	19	0.5	276	18	52
3♀ Int. conc. MTHCRLN	92	5.9	2.9*	3.1	0.94	16	0.6	300	22	65
a 4♀ High conc. MTHCRLN	(112)	(5.5)	(2.8)	(2.8)	(1.00)	(19)	(0.5)	(323)	(19)	(53)

* P < 0.05 compared with control data using Williams' test

a Data from single survivor - not included in statistical analysis

TABLE 9
(Biochemistry - continued)

Week 2 (23 May 1991)

Group	γ GT mU/ ml	CPK mU/ ml	Bili- rubin mg/dl	Na mEq/ l	K mEq/ l	Ca mEq/ l	P mEq/ l	Cl mEq/ l	Chol mg/dl
1 σ Air control	2	104	0.1	141	3.6	5.3	6.1	97	89
2 σ Low conc. MTHCRLN	2	114	0.1	141	3.6	5.3	5.9	97	83
3 σ Int. conc. MTHCRLN	* <1	* 143	0.1	142	3.4	* 5.5	5.6	97	82
1 φ Air control	<1	143	F 0.1	141	3.4	5.3	4.7	98	# 91 (81)
2 φ Low conc. MTHCRLN	<1	119	0.1	140	3.6	5.2	4.4	99	90 (92)
3 φ Int. conc. MTHCRLN	<2	160	0.1	140	3.6	5.2	4.5	99	82 (81)
a 4 φ High conc. MTHCRLN	(1)	(137)	(0.1)	(141)	(4.3)	(5.3)	(5.9)	(100)	(105)

* P < 0.05 compared with control data using Williams' test

a Data from single survivor - not included in statistical analysis

F Frequency analysis applied to data

Distribution free method of analysis applied to data. Median values in parentheses

TABLE 10
Macroscopic pathology incidence summary (Terminal)

Removal reason: Terminal	Males				Female			
	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
Animals on study	5	5	5	5	5	5	5	5
Animals completed	5	5	5	0	5	5	5	1
Fur Stained	0	0	0	0	0	1	0	0
Eyes Congested	0	0	0	0	0	0	0	0
Harderian Glands Haemorrhagic	0	0	0	0	0	1	0	0
Incisors Pale	0	0	1	0	1	0	0	0
Lymph Nodes - Cervical Enlarged	4	5	4	0	5	5	4	0
Lungs Not collapsed Pale	0	0	0	0	0	0	0	1
Lymph Nodes - Tracheobronchial Enlarged	0	0	0	0	0	3	2	1
Stomach Antrum Mucosa White nodule/s	0	1	1	0	0	0	1	0
Kidneys Hydronephrotic	0	2	2	0	0	0	0	0

TABLE 10
(Macroscopic pathology incidence summary (Terminal) - continued)

	Males				Females			
	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
Removal reason: Terminal	5	5	5	5	5	5	5	5
Animals on study	5	5	5	5	5	5	5	5
Animals completed	0	0	0	0	1	0	0	1
Uterus Fluid distension	0	0	0	0	1	0	0	1

TABLE 10
(Macroscopic pathology incidence summary (Decedent) - continued)

Removal reason: Intercurrent	Group	
	Males	Females
Animals on study Animals completed	5 5	5 4
Fur Stained	5	4
Oral Cavity Contained gelatinous material	1	1
Lymph Nodes - Cervical Enlarged	2	1
Thymus Congested	0	1
Lungs Not collapsed Congested	3 2	0 3
Lymph Nodes - Tracheobronchial Enlarged Congested	0 0	1 1
Adipose Tissue Minimal	2	3
Liver Pale subcapsular area/s - median cleft Pale subcapsular area/s	0 2	1 0

TABLE 10
(Macroscopic pathology incidence summary (Decedent) - continued)

Removal reason: Intereurrent	Group	
	Males	Females
Animals on study Animals completed	5 5	5 4
Pancreas Pale	(Continued) 2	2
Gastro-intestinal Tract Gaseous distension Contents minimal	1 1	2 0
Stomach Gaseous distension	3	1
Small Intestine Gaseous distension	3	2
Large Intestine Gaseous distension	1	0
Adrenal Congested Enlarged	0 0	1 1
Skeletal Muscle Congested - proximal to liver	1	1

TABLE 11

Organ weights - group mean values

Week 3

Group	Body wt. g	Brain g	Pituitary mg	Thyroids mg	Heart g	Lungs g	Liver g	Spleen g	Kidneys g	Adrenals mg	Testes+ Epidids g
1♂ Air control	344	1.87	10.5	15.5	A 1.18 (1.169)	A 1.25 (1.221)	A 14.6 (14.28)	A 0.78 (0.749)	A 2.50 (2.450)	46.9	3.69
2♂ Low conc. MTHCRLN	337	1.86	9.6	15.3	1.19 (1.196)	1.27 (1.273)	13.3 (13.32)	0.79 (0.797)	2.28 (2.287)	48.3	3.91
3♂ Int. conc. MTHCRLN	332	1.91	9.4	15.6	1.10 (1.116)	1.27 (1.287)	13.8 (14.16)	0.78 (0.801)	2.32 (2.367)	51.4	3.92

A Statistical analysis performed with values adjusted using bodyweight as covariate. Adjusted mean values in parentheses

(Organ weights continued)

Week 3

Group	Body wt. g	Brain g	Pituitary mg	Thyroids mg	Heart g	Liver g	Spleen g	Kidneys g	Adrenals mg	Ovaries mg
1♀ Air control	231	1.79	11.1	13.3	A 0.86 (0.845)	3 9.7 (9.95)	A 0.60 (0.587)	A 1.55 (1.67)	63.2	83.6
2♀ Low conc. MTHCRLN	234	1.82	11.2	11.6	0.57 (0.349)	1 0.5 (0.95)	0.62 (0.504)	1.01 (1.057)	56.2	86.2
3♀ Int. conc. MTHCRLN	208	1.76	9.7	10.5	0.80 (0.851)	1 8.5 (9.36)	0.49 (0.73)	1.5 (1.616)	63.0	75.0
a 4♀ High conc. MTHCRLN	(206)	(1.79)	(10.7)	(11.9)	(0.56)	(7.5)	(0.48)	(1.36)	(63.7)	(81.3)

A Statistical analysis performed with values adjusted using bodyweight as covariate. Adjusted mean values in parentheses

a Single survivor - not included in statistical analysis

TABLE 12
Microscopic pathology incidence summary

Males on study	Group 1		Group 2		Group 3		Group 4	
	Decedent	Terminal	Decedent	Terminal	Decedent	Terminal	Decedent	Terminal
Animals completed	5	5	5	5	5	5	5	5
Nasal turbinates	0	0	0	0	0	0	0	0
Examined	5	5	5	5	5	5	5	5
No abnormalities detected	3	4	4	4	4	4	4	4
Erosion of respiratory epithelium	1	0	0	0	0	0	0	0
Ulceration of respiratory epithelium	0	0	0	0	0	0	1	0
Degeneration of respiratory epithelium	0	0	0	0	0	0	3	0
Necrosis of respiratory epithelium	0	0	0	0	0	0	4	0
Hypertrophy of respiratory epithelium	0	0	0	0	0	2	0	0
Hyperplasia of respiratory epithelium (Total)	0	0	0	0	0	4	0	0
Minimal	0	0	0	0	0	4	0	0
Pseudoglandular goblet cell hyperplasia	0	0	0	0	0	4	0	0
Erosion of olfactory epithelium	0	0	0	0	0	5	0	0
Disorganisation of olfactory epithelium	0	0	0	0	0	3	5	0
Rosette formation in olfactory epithelium	0	0	0	0	0	5	0	0
Evidence of epithelial regeneration	1	1	1	1	1	0	0	0
Purulent exudate in nasal cavity	0	0	0	0	0	0	0	0
Larynx	0	0	0	0	0	0	0	0
Examined	5	5	5	5	5	5	5	5
No abnormalities detected	3	4	4	4	4	4	4	4
Epithelial ulceration	0	0	0	0	0	2	0	0
Epithelial hyperplasia (Total)	0	0	0	0	0	3	0	0
Minimal	0	0	0	0	0	3	0	0
Evidence of epithelial regeneration	0	0	0	0	0	0	0	0
Subepithelial inflammation (Total)	0	0	0	0	0	0	3	0
Moderate	0	0	0	0	0	0	4	0
Fibrinopurulent exudate in lumen	0	0	0	0	0	0	4	0

TABLE 12
(Microscopic pathology incidence summary - continued)

Males on study	Group 1		Group 2		Group 3		Group 4	
	Terminal	Decedent	Terminal	Decedent	Terminal	Decedent	Terminal	Decedent
Animals completed	5	0	5	0	5	0	5	0
(continued)								
Larynx	0	0	0	0	0	0	1	0
Haemorrhage in lumen	0	0	0	0	0	0	4	0
Bacterial colonies present	0	0	0	0	0	0	0	0
Trachea (With Bifurcation)	0	0	0	0	0	0	0	0
Examined	5	0	5	0	5	0	5	0
No abnormalities detected	0	0	0	0	0	0	0	0
Epithelial erosion	0	0	0	0	0	0	1	0
Epithelial ulceration	0	0	0	0	0	0	1	0
Epithelial necrosis	0	0	0	0	0	0	4	0
Epithelial hypertrophy	0	0	0	0	0	0	0	0
Epithelial hyperplasia (Total)	0	0	0	0	0	0	0	0
Minimal	0	0	0	0	0	0	0	0
Lungs	0	0	0	0	0	0	0	0
Examined	5	0	5	0	5	0	5	0
No abnormalities detected	0	0	0	0	0	0	0	0
Necrosis of bronchiolar epithelium	0	0	0	0	0	0	0	0
Peribronchiolar inflammatory cells (Total)	0	0	0	0	0	0	0	0
Minimal	0	0	0	0	0	0	0	0
Vascular congestion	0	0	0	0	0	0	1	0
Subpleural aggregation of alveolar macrophages	0	0	0	0	0	0	1	0
Alveolar oedema	0	0	0	0	0	0	0	0
Thrombus in pulmonary blood vessel	0	0	0	0	0	0	0	0
Alveolar mineralisation	0	0	0	0	0	0	0	0
Arterial medial hypertrophy	0	0	0	0	0	0	0	0
Alveolar haemorrhage (Total)	0	0	0	0	0	0	1	0
Minimal	0	0	0	0	0	0	0	0
Heart	0	0	0	0	0	0	0	0
Examined	5	0	0	0	0	0	5	0
No abnormalities detected	0	0	0	0	0	0	5	0
Lymph Nodes - Cervical	0	0	0	0	0	0	0	0
Examined	4	0	0	0	0	0	2	0

TABLE 12
(Microscopic pathology incidence summary - continued)

Males on study	Group 1		Group 2		Group 3		Group 4	
	5	5	5	5	5	5	5	5
Animals completed	Decedent 0	Terminal 5	Decedent 0	Terminal 5	Decedent 0	Terminal 5	Decedent 0	Terminal 5
Lymph Nodes - Cervical	(continued)							
Plasmacytosis	1	0	0	0	0	0	0	0
Prominent germinal centres	4	0	0	0	0	0	2	0
Lymphocytolysis	0	0	0	0	0	0	1	0
Spleen								
Examined	5	0	0	0	0	0	5	0
No abnormalities detected	5	0	0	0	0	0	4	0
Extramedullary haemopoiesis (Total)	0	0	0	0	0	0	1	0
Minimal	0	0	0	0	0	0	1	0
Liver								
Examined	5	0	5	0	5	0	5	0
No abnormalities detected	5	0	4	0	5	0	2	0
Subcapsular hepatocyte necrosis (Total)	0	0	0	0	0	0	2	0
Moderate	0	0	0	0	0	0	1	0
Centrilobular hepatocyte necrosis (Total)	0	0	0	0	0	0	1	0
Moderate	0	0	0	0	0	0	1	0
Centrilobular hepatocyte necrosis with portal to portal fibrous bridging, bile duct proliferation and giant cells	0	0	0	0	0	0	1	0
Centrilobular hepatocyte vacuolation (Total)	0	0	0	0	0	0	3	0
Minimal	0	0	0	0	0	0	3	0
Centrilobular hepatocyte necrosis with prominent inflammatory cells (Total)	0	0	1	1	0	0	0	0
Minimal	0	0	0	0	0	0	0	0
Pancreas								
Examined	0	0	0	0	0	0	2	0

TABLE 12
(Microscopic pathology incidence summary - continued)

Males on study	Group 1		Group 2		Group 3		Group 4	
	5	5	5	5	5	5	5	5
Animals completed	Decedent 0	Terminal 5	Decedent 0	Terminal 5	Decedent 0	Terminal 5	Decedent 5	Terminal 0
Pancreas	(continued)							
Exocrine degranulation (Total)	0	0	0	0	0	0	2	0
Minimal	0	0	0	0	0	0	2	0
Kidneys	0	5	0	0	0	0	5	0
Examined	0	5	0	0	0	0	4	0
No abnormalities detected	0	0	0	0	0	0	1	0
Medullary cyst	0	0	0	0	0	0	1	0
Lined papillary tubule	0	0	0	0	0	0	1	0
Livers	0	5	0	5	0	5	5	0
Examined	0	5	0	5	0	5	5	0
Cirrhotic vacuolation (Total)	0	0	0	1	0	0	4	0
Minimal	0	0	0	1	0	0	4	0
Moderate	0	0	0	4	0	0	1	0
Factors Contributory To Death	0	0	0	0	0	0	5	0
Examined	0	0	0	0	0	0	5	0
Lesions in respiratory tract								

TABLE 12
(Microscopic pathology incidence summary - continued)

Females on study	Group 1		Group 2		Group 3		Group 4	
	Decedent	Terminal	Decedent	Terminal	Decedent	Terminal	Decedent	Terminal
Nasal Turbinates	0	5	0	5	0	5	4	1
Examined	0	5	0	5	0	5	4	1
No abnormalities detected	0	5	0	5	0	5	4	1
Erosion of respiratory epithelium	0	0	0	4	0	0	0	0
Ulceration of respiratory epithelium	0	0	0	0	0	0	1	0
Necrosis of respiratory epithelium	0	0	0	0	0	0	4	0
Hyperplasia of respiratory epithelium (Total)	0	0	0	0	0	0	0	0
Minimal	0	0	0	0	0	2	0	0
Pseudoepithelioid goblet cell hyperplasia	0	0	0	0	0	2	0	0
Erosion of olfactory epithelium	0	0	0	0	0	3	0	1
Ulceration of olfactory epithelium	0	0	0	0	0	5	4	0
Disorganisation of olfactory epithelium	0	0	0	0	0	0	1	0
Kosette formation in olfactory epithelium	0	0	0	0	0	5	0	1
Evidence of epithelial regeneration	0	0	0	1	0	1	0	0
Regenerative hyperplasia of olfactory epithelium	0	0	0	0	0	0	2	0
Purulent exudate in nasal cavity	0	0	0	0	0	1	0	0
Larynx	0	0	0	0	0	0	4	0
Examined	0	5	0	5	0	5	4	1
No abnormalities detected	0	5	0	5	0	5	4	1
Epithelial ulceration	0	0	0	0	0	3	0	0
Epithelial hyperplasia (Total)	0	0	0	0	0	2	1	1
Minimal	0	0	0	0	0	2	1	1
Epithelial squamous metaplasia	0	0	0	0	0	0	1	0
Evidence of epithelial regeneration	0	0	0	0	0	0	2	0
Subepithelial inflammation (Total)	0	0	0	0	0	0	4	0
Moderate	0	0	0	0	0	0	4	0

TABLE 12
(Microscopic pathology incidence summary - continued)

Females on study	Group 1		Group 2		Group 3		Group 4	
	Decedent	Terminal	Decedent	Terminal	Decedent	Terminal	Decedent	Terminal
Animals completed	5	5	5	5	5	5	5	5
Larynx	0	0	0	0	0	0	0	1
Necrosis of ventral cartilage	0	0	0	0	0	0	0	0
Fibrinopurulent exudate in lumen	0	0	0	0	0	0	4	0
Bacterial colonies present	0	0	0	0	0	0	3	0
Trachea (With Bifurcation)	5	5	5	5	5	5	4	1
Examined	5	5	5	5	5	5	4	0
No abnormalities detected	0	0	0	0	0	0	0	0
Epithelial erosion	0	0	0	0	0	0	2	0
Epithelial ulceration	0	0	0	0	0	0	1	0
Epithelial necrosis	0	0	0	0	0	0	2	0
Epithelial hypertrophy	0	0	0	0	0	0	0	1
Epithelial hyperplasia (Total)	0	0	0	0	0	3	0	0
Minimal	0	0	0	0	0	3	0	0
Inflammatory cells in the lamina propria	0	0	0	0	0	0	1	0
Fibrinopurulent exudate in lumen	0	0	0	0	0	0	1	0
Necrotic debris in lumen	0	0	0	0	0	0	1	0
Lungs	5	5	5	5	5	5	4	1
Examined	4	4	4	4	4	4	0	0
No abnormalities detected	0	0	0	0	0	0	4	0
Necrosis of bronchiolar epithelium	0	0	0	0	0	0	0	0
Hypertrophy of bronchiolar epithelium with prominent goblet cells	0	0	0	0	0	0	0	1
Vascular congestion	0	0	0	0	0	0	4	0
Alveolar mineralisation	0	0	0	0	0	0	0	0
Microgranuloma	0	0	0	0	0	0	0	1
Heart	5	5	5	5	5	5	4	1
Examined	5	5	5	5	5	5	4	1
No abnormalities detected	0	0	0	0	0	0	4	1
Thymus	0	0	0	0	0	0	1	0
Examined	0	0	0	0	0	0	1	0

TABLE 12
(Microscopic pathology incidence summary - continued)

Females on study	Group 1		Group 2		Group 3		Group 4	
	Terminal	Decedent	Terminal	Decedent	Terminal	Decedent	Terminal	Decedent
Animals completed	5	5	5	5	5	5	5	5
Thymus	0	0	0	0	0	0	0	0
Haemorrhage	0	0	0	0	0	0	1	0
Lymph Nodes - Cervical	5	0	0	0	0	0	1	0
Examined	1	0	0	0	0	0	0	0
Plasmacytosis	5	0	0	0	0	0	1	0
Prominent germinal centres	0	0	0	0	0	0	0	0
Lymph Nodes - Tracheobronchial	0	0	0	0	0	0	2	1
Examined	0	0	0	0	0	0	0	1
Prominent germinal centres	0	0	0	0	0	0	0	2
Congestion	0	0	0	0	0	0	0	0
Spleen	5	0	1	0	1	0	4	1
Examined	5	0	1	0	1	0	4	1
No abnormalities detected	0	0	0	0	0	0	0	0
Liver	5	0	4	0	4	0	4	1
Examined	4	0	3	0	3	0	2	1
No abnormalities detected	0	0	0	0	0	0	0	0
Subcapsular hepatocyte necrosis	0	0	0	0	1	0	0	0
(Total)	0	0	0	0	1	0	0	0
Minimal	0	0	0	0	0	0	0	0
Generalised hepatocyte vacuolation	0	0	0	0	0	0	2	0
(Total)	0	0	0	0	0	0	2	0
Minimal	0	0	0	0	0	0	0	0
Parenchymal inflammatory cells	1	0	1	0	0	0	0	0
(Total)	1	0	1	0	0	0	0	0
Minimal	0	0	0	0	0	0	0	0
Prominent sinusoidal cells	0	0	0	0	0	0	1	0
Pancreas	0	0	0	0	0	0	2	0
Examined	0	0	0	0	0	0	0	0

: 55 :

: 398 :

TABLE 12
(Microscopic pathology incidence summary - continued)

Females on study	Group 1		Group 2		Group 3		Group 4	
	Decedent	Terminal	Decedent	Terminal	Decedent	Terminal	Decedent	Terminal
Animals completed	5	5	5	5	5	5	5	5
Pancreas	0	5	0	5	0	5	0	5
Exocrine degranulation (Total)	0	0	0	0	0	0	0	0
Minimal	0	0	0	0	0	0	1	0
Vacuolation in exocrine acini	0	0	0	0	0	0	1	0
Kidneys	0	5	0	0	0	0	4	1
Examined	0	5	0	0	0	0	4	1
No abnormalities detected	0	0	0	0	0	0	0	0
Adrenals	0	5	0	5	0	5	5	1
Examined	0	3	0	3	0	0	0	1
No abnormalities detected	0	0	0	0	0	0	0	0
Apparent hypertrophy of zona fasciculata	0	0	0	0	0	3	2	0
Cortical vacuolation (Total)	0	2	0	2	0	5	4	0
Minimal	0	0	0	0	0	2	4	0
Moderate	0	2	0	0	0	3	0	0
Sinusoidal congestion	0	0	0	0	0	0	1	0
Uterus	0	1	0	0	0	0	0	1
Examined	0	1	0	0	0	0	0	1
Luminal dilatation (Total)	0	1	0	0	0	0	0	1
Moderate	0	0	0	0	0	0	0	0
Factors Contributory To Death	0	0	0	0	0	0	4	0
Examined	0	0	0	0	0	0	4	0
Lesions in respiratory tract	0	0	0	0	0	0	4	0

APPENDIX 3

Individual clinical signs

Group	Designation	Rat no. /sex	Observation
1	Air control	1♂ 2♂ 3♂ 4♂ 5♂	Nothing abnormal detected Nothing abnormal detected Nothing abnormal detected Nothing abnormal detected Nothing abnormal detected
2	Low conc. MTHCRLN	6♂ 7♂ 8♂ 9♂ 10♂	Nothing abnormal detected Nothing abnormal detected Nothing abnormal detected Nothing abnormal detected Nothing abnormal detected
3	Int. conc. MTHCRLN	11♂ 12♂ 13♂ 14♂ 15♂	Nothing abnormal detected Red/brown staining under the chin post exposure; Days 9 and 15 Red/brown staining around the snout post exposure; Day 12 Nothing abnormal detected Red/brown staining under the chin post exposure; Days 8 and 9 Red/brown staining around the snout post exposure; Day 12
4	High conc. MTHCRLN	16♂ 17♂ 18♂ 19♂ 20♂	Gasping, râles and red/brown staining around the snout post exposure; Day 1 Red/brown staining around the snout and forepaws, gasping, loud squeak when inhaling and pronounced breathing movements (diaphragmatic breathing); Day 2 Moribund; sacrificed 14 May 1991; Day 2 Gasping, râles and red/brown staining around the snout post exposure; Day 1 Red/brown staining around the snout and forepaws, pronounced breathing movements and a loud squeak when inhaling; Day 2 Moribund; sacrificed 14 May 1991; Day 2 Gasping, râles and red/brown staining around the snout post exposure; Day 1 Red/brown staining around the snout and forepaws, pronounced breathing movements and a hunched posture; Day 2 Moribund; sacrificed 14 May 1991; Day 2 Gasping, râles and red/brown staining around the snout post exposure; Day 1 Red/brown staining around the snout and forepaws, gasping, loud squeak when inhaling and pronounced breathing movements (diaphragmatic breathing); Day 2 Moribund; sacrificed 14 May 1991; Day 2 Gasping, râles and red/brown staining around the snout post exposure; Day 1. Found dead 14 May 1991; Day 2

APPENDIX 3

(Individual clinical signs - continued)

Group	Designation	Rat no. /sex	Observation
1	Air control	21 ♀	Left upper top lip swoller; Day 15
		22 ♀	Nothing abnormal detected
		23 ♀	Nothing abnormal detected
		24 ♀	Scabs on tail; Days 11 - 15
		25 ♀	Nothing abnormal detected
2	Low conc. MTHCRLN	26 ♀	Nothing abnormal detected
		27 ♀	Nothing abnormal detected
		28 ♀	Nothing abnormal detected
		29 ♀	Nothing abnormal detected
		30 ♀	Nothing abnormal detected
3	Int. conc. MTHCRLN	31 ♀	Red/brown staining under the chin; Day 9, and around the snout; Day 12
		32 ♀	Red/brown staining around the snout; Days 12 and 15, and both forepaws; Day 15
		33 ♀	Red/brown staining under the chin; Days 8 to 10, around the left eye; Days 8 and 10, snout; Days 11 and 15 and forepaws; Days 8 and 15
		34 ♀	Red/brown staining around the left eye; Day 10
		35 ♀	Nothing abnormal detected
4	High conc. MTHCRLN	36 ♀	Pronounced breathing movements (diaphragmatic breathing) and red/brown staining around the snout post exposure; Day 1 Red/brown staining around the snout and forepaws; hunched posture; Day 2. Râles; Days 3 to 4 and 10 to 15
		37 ♀	Râles, pronounced breathing movements (diaphragmatic breathing) and red/brown staining around the snout post exposure; Day 1 Pronounced breathing movements (diaphragmatic breathing), red/brown staining around the snout and forepaws; Day 2 Spasm followed by death, 14 May 1991; Day 2
		38 ♀	Gasping and red/brown staining around the snout; Day 1 Found dead in cage, 14 May 1991; Day 2
		39 ♀	Pronounced breathing movements (diaphragmatic breathing) and red/brown staining around the snout post exposure; Day 1 Gasping, pronounced breathing movements (diaphragmatic breathing), red/brown staining around the snout and forepaws and hunched posture; Day 2 Found dead in cage, 14 May 1991; Day 2
		40 ♀	Râles, pronounced breathing movements (diaphragmatic breathing) and red/brown staining around the snout post exposure; Day 1 Red/brown staining around the snout and forepaws, hunched posture and half-closed eyes; Day 2 Found dead in cage, 15 May 1991; Day 2

APPENDIX 4

Bodyweights - individual values (g)

Group: 1♂ Air control

Cage number	Animal number	Day											
		-7	-6	-5	-4	-3	-2	-1	0	1	2	3	4
1	1	201	208	216	224	238	248	259	263	274	280	291	301
	2	196	212	220	226	236	245	253	260	271	278	283	291
	3	201	210	221	233	239	250	258	266	275	285	292	300
	4	194	209	221	227	237	244	256	265	269	275	288	294
	5	202	212	224	233	242	252	257	265	274	285	292	294

Group 1♂ Air control

Cage number	Animal number	Day										
		5	6	7	8	9	10	11	12	13	14	15
1	1	312	320	332	335	346	315	340	351	353	364	373
	2	297	302	308	311	322	303	318	327	332	339	343
	3	303	307	311	315	319	293	318	322	327	332	337
	4	306	310	311	316	324	305	322	334	338	347	339
	5	302	306	309	314	317	293	312	321	321	334	336

BGH 50/932334

BGH 40/920648

APPENDIX 4

(Bodyweights - continued)

Group 2♂ Low conc. MTHCRLN

Cage number	Animal number	Day											
		-7	-6	-5	-4	-3	-2	-1	0	1	2	3	4
2	6	205	214	219	231	238	248	254	263	266	274	278	284
	7	197	202	213	225	232	244	252	253	263	267	274	279
	8	199	212	218	226	231	240	246	256	259	266	271	279
	9	205	217	227	234	247	255	266	277	285	295	300	308
	10	205	215	231	237	250	259	273	284	289	295	300	309

Group 2♂ Low conc. MTHCRLN

Cage number	Animal number	Day										
		5	6	7	8	9	10	11	12	13	14	15
2	6	294	297	301	306	311	304	312	316	321	328	337
	7	285	287	295	300	304	289	306	305	313	317	325
	8	284	288	295	300	301	284	303	307	308	319	322
	9	319	330	336	341	352	321	350	358	363	372	374
	10	321	325	332	340	349	322	343	357	360	369	375

BGH 50/932334

BGH 40/920648

APPENDIX 4

(Bodyweights - continued)

Group 3♂ Int.conc. MTHCRLN

Cage number	Animal number	Day											
		-7	-6	-5	-4	-3	-2	-1	0	1	2	3	4
3	11	207	219	231	239	248	258	264	273	277	278	286	289
	12	199	212	221	233	243	253	262	267	274	275	276	279
	13	197	208	216	224	233	242	248	255	259	264	263	268
	14	206	220	226	237	247	261	269	276	287	285	291	294
	15	198	208	219	229	244	254	267	276	284	286	294	304

Group 3♂ Int.conc. MTHCRLN

Cage number	Animal number	Day										
		5	6	7	8	9	10	11	12	13	14	15
3	11	296	306	311	317	317	292	314	322	326	334	332
	12	289	300	312	316	314	290	306	313	324	331	329
	13	273	278	283	289	289	268	286	291	299	305	310
	14	299	307	315	318	323	292	321	326	325	339	339
	15	312	321	330	337	345	314	341	349	354	365	376

BGH 50/932334

BGH 40/920648

APPENDIX 4
(Bodyweights - continued)

Group 4♂ High conc. MTHCRLN

Cage number	Animal number	Day											
		-7	-6	-5	-4	-3	-2	-1	0	1	2	3	4
4	16	199	206	215	220	228	238	244	254	207			
	17	199	208	215	223	231	244	248	259	218			
	18	191	204	208	211	219	227	229	238	202			
	19	205	216	224	234	245	257	263	269	226			
	20	207	216	222	233	243	253	260	273				

Group 4♂ High conc. MTHCRLN

Cage number	Animal number	Day											
		5	6	7	8	9	10	11	12	13	14	15	
4	16												
	17												
	18												
	19												
	20												

BGH 50/932334

BGH 40/920648

APPENDIX 4

(Bodyweights - continued)

Group 1♀ Air control

Cage number	Animal number	Day											
		-7	-6	-5	-4	-3	-2	-1	0	1	2	3	4
5	21	162	173	173	166	177	183	184	179	188	194	199	196
	22	161	169	168	174	183	189	187	199	207	208	206	215
	23	162	167	166	173	180	185	182	192	196	198	193	204
	24	151	159	162	169	175	181	178	181	182	191	191	186
	25	182	178	190	191	201	194	209	216	219	209	230	236

Group 1♀ Air control

Cage number	Animal number	Day										
		5	6	7	8	9	10	11	12	13	14	15
5	21	206	209	210	206	214	200	207	207	211	218	220
	22	226	230	223	238	245	220	229	245	251	257	249
	23	210	210	209	214	223	205	209	222	226	232	225
	24	194	195	200	193	199	188	196	199	196	203	206
	25	240	231	246	247	253	234	258	261	255	256	277

BGH 50/932334

BGH 40/920648

APPENDIX 4

(Bodyweights - continued)

Group 2♀ Low conc. MTHCRLN

Cage number	Animal number	Day											
		-7	-6	-5	-4	-3	-2	-1	0	1	2	3	4
6	26	167	167	176	181	186	184	190	198	201	201	208	213
	27	166	172	176	182	180	182	190	193	195	189	199	199
	28	162	171	175	182	188	188	194	198	203	198	205	209
	29	171	177	181	184	195	198	196	205	213	209	214	215
	30	163	170	177	180	189	189	196	201	204	204	215	218

Group 2♀ Low conc. MTHCRLN

Cage number	Animal number	Day										
		5	6	7	8	9	10	11	12	13	14	15
6	26	220	218	224	229	234	214	234	240	243	237	249
	27	203	197	210	210	213	197	210	220	221	216	226
	28	212	210	220	222	224	205	231	233	230	233	239
	29	223	222	217	227	227	213	221	230	232	235	232
	30	225	218	228	231	235	215	232	244	236	238	254

D 13

BGH 50/922334

BGH 40/920648

APPENDIX 4

(Bodyweights - continued)

Group 3♀ Int.conc. MTHCRLN

Cage number	Animal number	Day											
		-7	-6	-5	-4	-3	-2	-1	0	1	2	3	4
7	31	160	165	161	171	178	183	174	181	185	186	181	191
	32	160	160	149	157	166	163	151	166	172	171	168	177
	33	161	170	169	175	179	186	185	191	196	198	196	198
	34	154	166	164	169	173	178	184	185	183	191	198	200
	35	153	164	166	173	174	175	184	183	177	185	191	195

Group 3♀ Int.conc. MTHCRLN

Cage number	Animal number	Day											
		5	6	7	8	9	10	11	12	13	14	15	
7	31	196	196	187	199	201	186	193	197	204	195	209	
	32	177	180	174	181	189	172	182	185	192	198	193	
	33	203	210	211	211	217	200	210	210	216	223	222	
	34	196	206	209	216	208	202	212	212	217	228	231	
	35	191	200	208	211	207	197	204	207	215	214	216	

BGH 50/932334

BGH 40/920648

APPENDIX 4

(Bodyweights - continued)

Group 4♀ High conc. MTHCRLN

Cage number	Animal number	Day											
		-7	-6	-5	-4	-3	-2	-1	0	1	2	3	4
8	36	153	163	166	171	169	177	181	181	154	165	172	180
	37	156	164	165	165	173	180	174	184	151			
	38	166	174	171	181	189	194	188	197				
	39	164	174	173	180	190	189	184	194	160			
	40	162	172	167	172	181	187	185	192	160			

Group 4♀ High conc. MTHCRLN

Cage number	Animal number	Day										
		5	6	7	8	9	10	11	12	13	14	15
8	36	190	193	197	203	210	183	201	193	205	207	210
	37											
	38											
	39											
	40											

APPENDIX 9

Organ weights - individual values

Week 3

Group	Rat no.	Body wt. g	Brain g	Pituitary mg	Thyroids mg	Heart g	Lungs g	Liver g	Spleen g	Kidneys g	Adrenals mg	Testes+ Epidids g
1♂ Air control	1	366	1.80	11.1	18.0	1.32	1.20	16.8	0.82	2.89	30.1	3.26
	2	341	1.83	9.8	13.5	1.20	1.17	13.8	0.76	2.53	48.3	3.98
	3	334	1.82	10.1	16.3	1.15	1.26	13.7	0.64	2.50	56.9	3.58
	4	346	1.88	10.6	14.7	1.21	1.34	15.8	0.89	2.32	54.4	3.83
	5	332	2.01	10.8	14.8	1.04	1.26	13.1	0.77	2.27	44.9	3.81
	Mean SD	344 13.6	1.87 0.085	10.5 0.53	15.5 1.73	1.18 0.102	1.25 0.065	14.6 1.58	0.78 0.092	2.50 0.244	46.9 10.54	3.69 0.282
2♂ Low conc. MTHCRLN	6	324	1.91	10.9	13.9	1.38	1.25	13.7	0.78	2.42	51.1	4.07
	7	315	1.85	9.2	19.3	1.06	1.11	12.1	0.57	2.18	47.2	3.66
	8	314	1.71	8.4	13.4	1.15	1.19	11.9	0.75	1.84	46.9	4.09
	9	367	1.91	8.3	12.2	1.12	1.41	14.3	0.96	2.50	42.1	3.83
	10	366	1.89	11.1	17.8	1.26	1.39	14.4	0.91	2.47	54.3	3.88
	Mean SD	337 27.0	1.85 0.084	9.6 1.34	15.3 3.06	1.19 0.127	1.27 0.129	13.3 1.20	0.79 0.153	2.28 0.277	48.3 4.62	3.91 0.179
3♂ Int. conc. MTHCRLN	11	328	1.96	7.5	15.5	1.04	1.21	12.4	0.75	2.28	57.2	4.16
	12	324	1.92	9.4	16.0	1.03	1.13	13.8	0.64	2.15	50.5	3.74
	13	308	1.90	8.7	13.2	1.02	1.17	12.6	0.80	2.06	45.6	3.34
	14	336	1.88	9.8	16.6	1.18	1.35	13.7	0.69	2.63	50.2	4.14
	15	366	1.92	11.8	16.8	1.24	1.46	16.7	1.00	2.48	53.5	4.20
	Mean SD	332 21.4	1.92 0.030	9.4 1.58	15.6 1.45	1.10 0.101	1.26 0.137	13.8 1.72	0.78 0.139	2.32 0.234	51.4 4.30	3.92 0.371

SD Standard deviation

APPENDIX 9

(Organ weights - continued)

Week 3

Group	Rat no.	Body wt. g	Brain g	Pituitary mg	Thyroids mg	Heart g	Lungs g	Liver g	Spleen g	Kidneys g	Adrenals mg	Ovaries mg
1♀ Air control	21	217	1.74	10.8	12.3	0.76	1.05	8.1	0.62	1.46	71.9	81.2
	22	246	1.81	10.2	16.0	1.05	1.18	9.9	0.70	1.83	65.8	68.1
	23	221	1.90	13.8	13.2	0.74	0.96	8.8	0.54	1.65	44.0	93.0
	24	202	1.73	8.0	11.8	0.81	0.83	8.0	0.53	1.53	58.9	68.8
	25	271	1.78	12.8	13.2	0.96	1.11	11.9	0.63	1.81	75.5	106.8
	Mean SD	231 27.2	1.79 0.068	11.1 2.27	13.3 1.62	0.86 0.135	1.05 0.136	9.3 1.62	0.60 0.070	1.66 0.165	63.2 12.46	83.6 16.52
2♀ Low conc. MTHCRLN	26	243	1.77	14.2	7.8	0.99	1.16	10.3	0.66	1.63	69.7	85.5
	27	221	1.76	9.7	12.3	0.80	1.13	8.2	0.50	1.58	60.6	79.5
	28	222	1.82	9.9	15.4	0.83	#2.23	9.1	0.75	1.73	75.6	85.1
	29	230	1.89	10.0	12.1	0.94	1.18	9.1	0.55	1.71	71.9	91.8
	30	242	1.84	12.1	10.5	0.81	1.09	10.6	0.67	1.75	53.1	89.3
	Mean SD	234 9.1	1.82 0.053	11.2 1.95	11.6 2.77	0.87 0.086	1.14 0.039	9.5 0.98	0.63 0.100	1.68 0.068	66.2 9.17	86.2 4.63
3♀ Int. conc. MTHCRLN	31	205	1.78	9.5	8.5	0.82	1.22	9.6	0.50	1.62	61.7	77.2
	32	188	1.79	11.0	12.1	0.85	1.03	7.6	0.45	1.40	63.2	79.5
	33	217	1.74	9.8	10.5	0.89	0.97	7.5	0.40	1.41	55.9	65.0
	34	223	1.69	8.9	3.9	0.74	0.94	9.1	0.64	1.65	62.6	75.2
	35	208	1.79	9.4	12.3	0.73	1.09	8.5	0.47	1.61	72.8	77.9
	Mean SD	208 13.4	1.76 0.043	9.7 0.79	9.5 3.46	0.81 0.069	1.05 0.111	8.5 0.92	0.49 0.090	1.54 0.122	63.2 6.08	75.0 5.73
4♀ High dose MTHCRLN	36	206	1.7	10.7	11.9	0.88	1.38	7.8	0.48	1.36	63.7	91.4
	Mean SD	206	1.79	10.7	11.9	0.88	1.38	7.8	0.48	1.36	63.7	91.4

SD Standard deviation

Value excluded from calculation of mean and statistical analysis. Suspected weighing error

COMPLETED STUDIES

- Acute Oral LD₅₀ in Rats (No MRID) - study enclosed
- 21-Day Acute Avian Oral LD₅₀ Mallard Duck (MRID 42183301)
- Acute Toxicity to Eastern Oyster Under Flow-Through Conditions with Acrolein (MRID 43164302)
- Acute Toxicity to Mysid Shrimp Under Flow-Through Conditions with Acrolein (MRID 43164301)
- Acute Toxicity to Sheepshead Minnow Under Flow-Through Conditions with Acrolein (MRID 43225202)
- 14-Day Oral Toxicity in Mice (No MRID) - study enclosed
- *Salmonella*/Mammalian-Microsome Preincubation Mutagenicity Assay (Ames Test) and *Escherichia coli* WP2uvr A Reverse Mutation Assay with a Confirmatory Assay (No MRID) - study enclosed
- Teratology Study in Mice (MRID 145214)
- Pathology Working Group Report on the Lifetime Experimental Study of Acrolein in Female F344 Rats (No MRID) - study enclosed
- Determination of the Anaerobic Aquatic Metabolism of ¹⁴C-Acrolein (MRID 42332901/42949201)
- Determination of the Aerobic Aquatic Metabolism of ¹⁴C-Acrolein (MRID 42288901/42837601/43227101)
- Validation and Stability of an Assay for the Determination of Acrolein and 3-Hydroxypropanol in Water (MRID 41970501)
- Solubility of Acrolein: Representative Polar and Non-Polar Solvents (MRID 42032501)
- Storage Stability (MRID 42117801)
- Acrolein: Manufacturing Process and Discussion of Impurities (MRID 41014801/42552701)



STUDIES IN PROGRESS

- **Nature of Residue in Livestock (Lactating Goats and Laying Hens)**
Radio-labeled material was given by oral administration to livestock. Residue and metabolite identification are presently being determined. In the meanwhile, interim reports have been submitted to update EPA: MRID 42157901 (Goat, 1st Interim), MRID 42158101 (Goat, 2nd Interim), MRID 42933301 (Hen, 2nd Interim), MRID 42933601 (Goat, 2nd Interim).
- **Nature of Residue in Plants (Leaf Lettuce)**
A concentration of 15 ppm was applied (spray irrigated) directly to lettuce plants on a recurring basis. No plant damage was associated with applications, levels of radioactivity are extremely low and investigations are underway to determine metabolites. In the meanwhile, interim reports have been submitted to update EPA: MRID 42295101 (1st Interim) and MRID 43225203 (2nd Interim).



Acrolein: Analysis and Certification of Product Ingredients (MRID 41896901)

Acrolein: Product Identity and Disclosure of Ingredients (MRID 41894101)

Henry's Constant for Acrolein (No MRID) - study enclosed

Physical Properties of Acrolein: Color, Physical State, Odor, Boiling Point, Density, Specific Gravity, pH, Stability, Explodability, and Corrosion Characteristics (MRID 40840601)

Toxicity of Acrolein to *Lemna gibba* G3 (Duckweed) Tier 2 Growth and Reproduction of Aquatic Plants (MRID 42620904)

Toxicity of Acrolein to *Selenastrum capricornutum* (green algae) Tier 2 Growth and Reproduction of Aquatic Plants (MRID 42620905)

Toxicity of Acrolein to *Navicula pelliculosa* (freshwater diatom) Tier 2 Growth and Reproduction of Aquatic Plants (MRID 42620902)

Toxicity of Acrolein to *Anabaena flos-aquae* (blue-green algae) Tier 2 Growth and Reproduction of Aquatic Plants (MRID 42620901)

Toxicity of Acrolein to *Skeletonema costatum* (marine diatom) Tier 2 Growth and Reproduction of Aquatic Plants (MRID 42620903)

Acrolein (Magnacide® H): Nature and Magnitude of Residues Study Using Freshwater Fish and Shellfish (MRID 43225201)

Acrolein Metabolism (42031001 - Preliminary and Definitive, 43177101 - Supplement 1, 43275901 - Supplement 2)

Primary Dermal Irritation in Albino Rabbits with Acrolein (No MRID) - study enclosed

Insecticide Efficacy Study in Fleas (No MRID) - study enclosed

Message

In discussing
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this communi
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C.05



Facsimile Transmittal

Date: September 21, 1998

Addresses: Scott Sheriack
EPA

Addressee Fax: 202-260-9555

of Pages: 3 (including this page)

Sender: Pamela Kreis

Sender Fax: 410-480-0956

Sender Phone: 410-480-0955

Message: Attached is a letter in follow-up to our telephone conversation last week. In discussing the issues further with the company, we have determined that keeping the information reported confidential from the reporting facility is not really an issue. This was the subject of my conversation with you. However, we still want clear guidance on how to handle this information. If you want to discuss this further by phone, please feel free to call me. I will be traveling Wed. - Mon. returning Tues. in the afternoon. If you call me at your telephone number and I will return your call. Thanks.

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C.05.

Acute Oral Toxicity (LD₅₀) of
Acrolein (Lot No. SFSL-5893) in
Rats (FIFRA Guidelines)

BSC Project Number: 10258

PREPARED FOR:

Magna Corporation
2434 Holmes Road
Houston, TX 77051
Project Officer: Mr. Charles MacDonald

PREPARED BY:


Indu A. Muni, Ph.D.

Bioassay Systems Corporation
225 Wildwood Avenue
Woburn, Massachusetts 01801

May 6, 1981

TABLE OF CONTENTS

	Page
SUMMARY	1
I. Introduction	2
II. Materials and Methods	3
A. Chemical	
1. General Information	
2. Dosage Preparation	
3. Dosage Analyses	
B. Animals	
1. Rats	
2. Observation and Examination During Quarantine	4
3. Randomization Method	
4. Animal Maintenance	
C. Experimental Design	
III. Results	
A. Experimental Results	
1. Survival	
2. Pharmacotoxic Signs	
3. Observations at Necropsy	6
4. Discussion and Conclusions	
B. Tables	
Table 1 - The Acute Oral Toxicity of Acrolein in the rat	7
C. Figures	
Figure 1 - Dose Response Curves for Acrolein	8
D. Appendix	
Appendix A - ESC Personnel Assigned to the Study	9

IV. Storage Information	10
V. Signatures	11
VI. Quality Assurance Report	12

SUMMARY

An acute oral LD₅₀ determination of acrolein administered by gavage was performed in male and female Sprague-Dawley rats obtained from Charles River Breeding Laboratory, Wilmington, MA. The dosages were 20, 25, 30 and 40 mg/kg of acrolein in distilled water and the total volume administered was 5 ml/kg.

A total of 20 males and 11 females died within 4 days after dosing. All animals were observed for 14 days following compound administration. Terminally sacrificed animals showed minimal signs of toxicity.

The method of Litchfield and Wilcoxon¹ was used to calculate the LD₅₀ values. Based on the results of this experiment, the oral LD₅₀'s were found to be:

	LD ₅₀ mg/kg (95% Confidence Limit)	
Male Rats:	25.0	(22.9-27.5)
Female Rats:	33.3	(30.0-36.9)
Combined Population:	29.0	(26.0-31.6)

¹Litchfield, J.T. JR., and Wilcoxon, F., A Simplified Method of Evaluating Dose-Effect Experiments, J. Pharm. Exp. Therap., 96, 99-115, 1949.

I. INTRODUCTION

The objective of this study was to assess the acute oral toxicity of acrolein (Lot No. SFSL-5893) in rats by the methodology described in FIFRA guidelines (43FR, Part II, p.37355, August 22, 1978). Doses were chosen following consideration of the results of a previous range-finding study² with acrolein which was conducted at Bioassay Systems Corporation from December, 1979 to February, 1981. This study was initiated on April 1, 1981 and was completed on April 19, 1981.

¹FIFRA requires that laboratory rats should be used as a test system for acute oral LD₅₀ studies.

²A preliminary report of range-finding study was submitted to the Sponsor.

II. MATERIALS AND METHODS

A. Chemical

1. General Information

Chemical Name: Acrolein (6/4/80)

Source: Magna Corporation, Sante Fe Springs, CA

Lot No: SFSL-5893

Physical State: Liquid

Solubility: About 16-18% w/v in distilled water

Purity: 95%

Density: 0.839 at 20°C

2. Dosage Preparation

Separate stock solutions were prepared, fresh for each dose level. An appropriate volume of acrolein was placed in a 100 ml-volumetric flask and the volume was adjusted to 100 ml with distilled water. The dose solutions contained 4, 5, 6 and 8 mg of acrolein/ml. All animals (treatment groups) received 5 ml/kg of the respective dose solutions. These dose solutions containing various concentrations of acrolein are stable for at least 5 days, when stored at 5°C.

3. Dosage Analyses:

After serial dilutions (with distilled water), the acrolein concentrations were monitored at 210 nm. A standard curve was obtained prior to dose analyses (0.67 to 2.67 µg/ml range).

B. Animals

1. Rats

Species: Rattus norvegicus albinus

Strain: Sprague-Dawley

Source: Charles River Breeding Laboratory,
Wilmington, MA

C.11.

Age at Start of Study 5-6 weeks

Weight at Start of Study: 100-150 grams

Number and Sex of Animals: 50 males, 50 females

Identification: Unique number code ear punched on each animal

2. Observation and Examination during Quarantine

Upon receipt, animals were inspected for signs of disease and deformity. The animals were quarantined for 12 days. All animals were examined daily and appeared healthy throughout the quarantine period. The room temperature and relative humidity were $72^{\circ} \pm 2^{\circ} \text{F.}$ and $50 \pm 10\%$, respectively, throughout the quarantine period.

3. Randomization Method

Animals were randomly assigned to treatment groups in order to control bias.

4. Animal Maintenance

Housing: Polycarbonate cages, five animals (same sex) per cage, Hazelton System

Food: Charles River Pelleted Diet, ad libitum, 4 hours after dosing. The animals were fasted overnight prior to dosing.

Water: Ad libitum from water bottles, Untreated city water.

Light: Fluorescent, automatically controlled, 12-hour light/dark cycle (lights on 7 AM to 7 PM)

Therapeutic agents: None

Room Temperature: $72^{\circ} \pm 2^{\circ} \text{F.}$

Room Humidity: $50\% \pm 10\%$

Air Flow: 10-15 complete changes of 100% fresh air every hour filtered through roughing Varicel, HEPA and charcoal filters prior to introduction into the animal room.

Cage-changing Schedule: Twice weekly

¹Steel, R.G.D., and Torrie, J.H., Principles and Procedures of Statistics. McGraw-Hill Book Company, Inc., New York, 1960.

C 12

C. Experimental Design

Dosages: 20, 25, 30, 40 mg/kg (Levels I to IV).

No. of Animals/dose concentration: 10 males, 10 females.

Control Animals: Untreated controls, 10 males, 10 females.

Dose Volumes: 5 ml/kg.

Route of Administration: Oral administration by gavage.

Vehicle: Distilled water.

Frequency of Administration: Once on day 1.

Duration of Study: 14 days.

III. RESULTS

A. Experimental Results

1. Survival: Table I represents the acute oral toxicity of acrolein in rats. After acrolein administration, a total of 31 rats died during the 14-day study period. Ten males in Level IV, 8 males in Level III and 2 male rats in Level I died during the first 15 hours after dosing. All other males survived to terminal sacrifice. Among the females, 7 females in Level IV and 3 females in Level III group died during the first 15 hours while 1 female in Level III died on the fourth day after dosing. All other females survived to terminal sacrifice.
2. Pharmacotoxic signs: Immediately following dose administration, Level III and Level IV animals showed signs of lethargy, respiratory distress and squinted eyes until death occurred. The surviving animals exhibited similar signs for varying lengths of time through the observation period. Levels II and I showed similar signs but in most cases the conditions were less severe and did not persist as long in the survivors. Control animals remained healthy throughout the observation period.

3. Observations at Necropsy: Animals which died 1.5-4 hours after dosing showed overall reddening of lungs, hemorrhagic stomach and intestines (blood-filled) and dilation of blood vessels on the brain's surface. Darkening of the medulla of the kidneys was evident in most animals which died greater than five hours after dosing. All levels of animals sacrificed at the termination of the study showed few significant lesions. Varying degrees of darkening of the lung, kidneys (medulla) and stomach were noted. No obvious differences between levels of treated animals existed. Control animals had few, non-specific lesions (several pinpoint black spots on lungs).

4. Discussion and Conclusions: Results of the acute or 1 toxicity study with acrolein are summarized in Table 1 and Figure 1. Table 1 lists the mortality rate of the animals at the following concentrations: 20, 25, 30 and 40 mg/kg. The dose response curves are illustrated in Figure 1. The slope values were found to be 1.10, 1.22 and 1.16 for males, females and the combined population, respectively. The acute LD₅₀ values (mg/kg) with 95% confidence limit at 20% intervals were calculated to be 25.0 (22.9-27.5), 33.3 (30.0-36.9) and 29.0 (26.6-31.6) for male rats, female rats and combined rat population, respectively.

TABLE 1
THE ACUTE ORAL TOXICITY OF ACROLEIN IN THE RAT

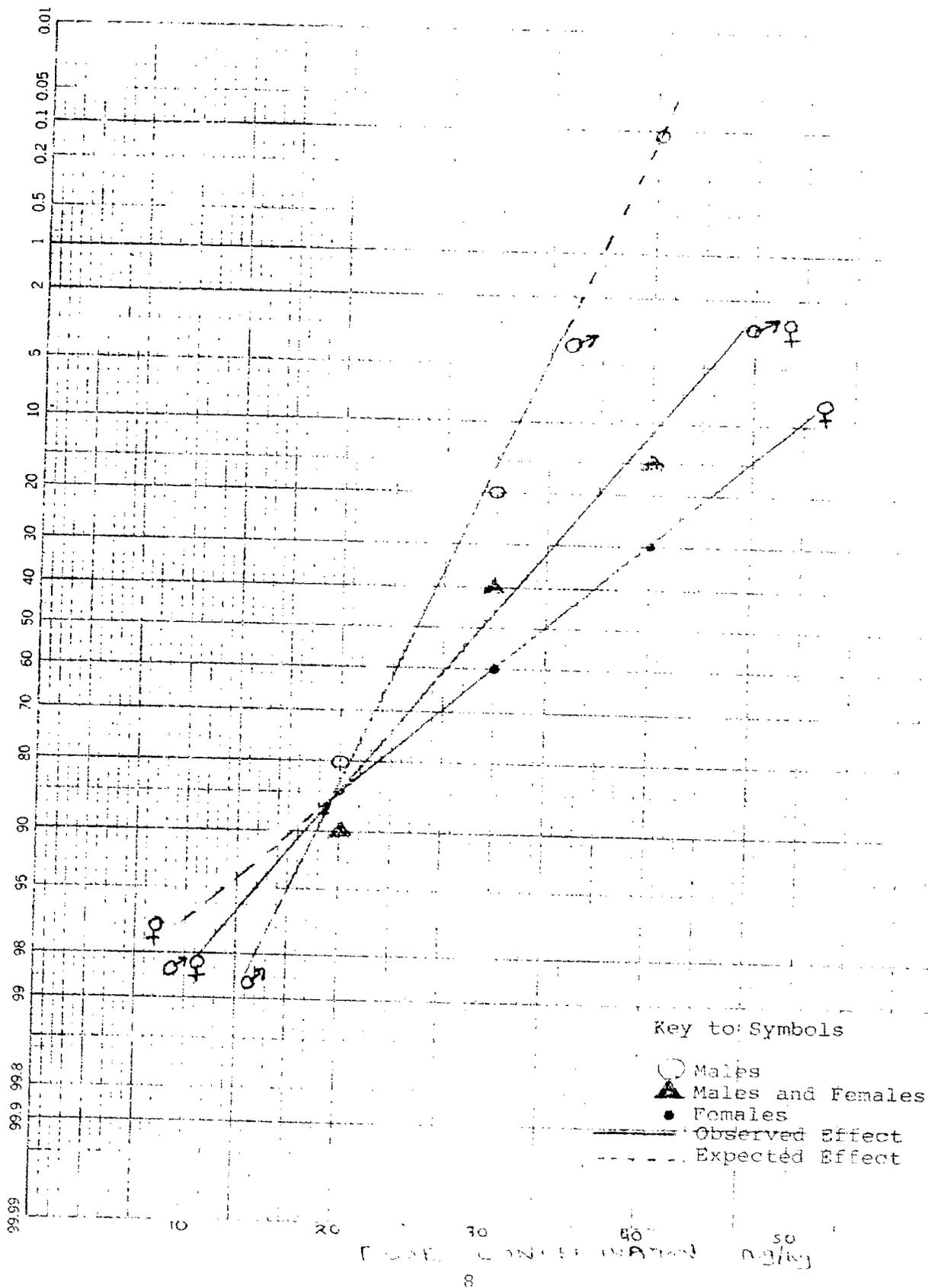
Dose (mg/kg) ^a	MALES		FEMALES		Time to Death
	Mortality	% Mortality	Mortality	% Mortality	
20	2/10	20	0/10	0	
25 ^c	0/10	0	0/10	0	
30	8/10	80	4/10	40	5-15 hours(1) 4th day (1)
40	10/10	100	7/10	70	1-4 hours(5) 5-15 hours(2)

^a Distilled water solution of acrolein
^b Following administration of the test material
^c Number of animals died during the time interval specified
doses solution (25 mg/kg) was not analyzed for acrolein concentration

Figure 1

46 8003

K·Σ PROBABILITY X % DIVISIONS
KEUPTEL & ESSER CO. 4102 N. 224



APPENDIX A

BSC PERSONNEL ASSIGNED TO THE STUDY

Indu A. Muni, Ph.D.	Study Director, Toxicologist
Jack A. Reynolds, D.V.M.	Animal Care Veterinarian
Matthew King	Technical Supervisor, Toxicology Necropsy Room Supervisor
Druanne Twombly	Technician, Toxicology
Theodore A. Olsson	Technical Supervisor, Chemistry
Peter J. Mione	Manager, Quality Assurance

% DEATH

D.03.

IV. STORAGE INFORMATION

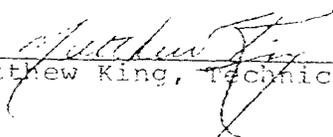
The unused acrolein (Lot # SFSL-5893) is stored in the Chronic Chemistry repository (cabinet #1) at Bioassay Systems Corporation. A copy of the protocol, all the raw data generated during this study and a copy of the final report are stored in the archives at BSC.

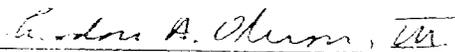
D.04.

V. Signatures

Study Title: Acute oral toxicity (LD₅₀) of acrolein
in rats (EPA Guidelines).


Druanne Twombly, Technician, Toxicology


Matthew King, Technical Supervisor,
Toxicology


Theodore A. Olsson, Technical
Supervisor, Chemistry


Indu A. Munn, Study Director

D.05.

Bioassay Systems Corporation

225 Wildwood Avenue Woburn, Massachusetts 01801
617-933-9229

QUALITY ASSURANCE REPORT

SPONSOR: Magna Corporation

TEST SUBSTANCES: Acrolein (SFSL-5893)

FINDINGS:

The acute 14-day oral toxicity test was conducted according to the approved protocol. All reported results were inspected and found to accurately reflect the original data. The environmental records, however, were not maintained during the entire study.

DATE: 5-20-81

QUALITY ASSURANCE OFFICER: Gregory A. Roscoe

D 06

14-Day Oral Toxicity Test in Mice

BSC Project Number 11496

11496

14-Day Oral Toxicity Test in Mice

BSC Project Number 11496

Prepared For:

Magna Corporaton
11808 South Bloomfield Avenue
Santa Fe Springs, CA. 90670

Project Officer: Ms. Bonnie M. Baldrige

Study Director:

Carole A. Mansur 6-20-83
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TABLE OF CONTENTS

	<u>Page</u>
SUMMARY	1
I. INTRODUCTION	2
II. MATERIALS AND METHODS	3
A. Chemical	
1. General Information	
2. Dose Preparation	
3. Dose Analyses	
B. Animals	4
1. Mice	
2. Observation and Examination During Quarantine	
3. Randomization Method	
4. Animal Maintenance	
C. Experimental Design	5
III. RESULTS	6
A. Survival	
B. Pharmacotoxic Signs	
C. Body Weights	
D. Food Consumption	
E. Observation at Necropsy	
F. Discussion and Conclusions	
IV. STORAGE INFORMATION	7
TABLES	
Table 1 Mortality Among CD-1 Mice Administered Various Levels of Acrolein	8
Table 2 Mean and Standard Deviation of Body Weights In Acrolein-Treated Male Mice	9
Table 3 Mean and Standard Deviation of Body Weights In Acrolein-Treated Female Mice	10
Table 4 Individual Body Weights of Male Mice Administered Acrolein	11
Table 5 Individual Body Weights of Female Mice Administered Acrolein	13

TABLE OF CONTENTS (Continued)

	<u>Page</u>
Table 6 Incidence of Clinical Observations Among CD-1 Mice Administered Various Levels of Acrolein	15
Table 7 Clinical Observations Seen in CD-1 Mice Administered Acrolein	16
Table 8 Food Consumption of CD-1 Mice Administered Various Levels of Acrolein	17
Table 9 Incidence of Gross Lesions Seen in CD-1 Mice Administered Various Levels of Acrolein	18
Table 10 Gross Lesions Seen in Male Mice Administered Acrolein	19
Table 11 Gross Lesions Seen in Female Mice Administered Acrolein	21
V. SIGNATURES	22
VI. BSC SUPERVISORY PERSONNEL ASSIGNED TO STUDY	23
VII. PROTOCOL AMENDMENTS	24
Quality Assurance Report	

SUMMARY

A 14 day toxicity test of acrolein administered by gavage was conducted using 100 CD-1 mice obtained from Charles River Breeding Laboratory, Wilmington, Massachusetts. The doses were 0.0, 4.6, 5.8, 7.2 and 9.0 mg/kg of acrolein in deionized water administered in a dose volume of 10 ml/kg.

A total of 3 male mice died within four days. One female (level II) died on Day 6. All animals were administered the appropriate dose volume and observed for 14 days. Terminally sacrificed animals showed minimal signs of toxicity.

I. INTRODUCTION

The objective of this study was to assess the oral toxicity of acrolein (Bioassay Systems Corporation Lot No. 12/22/82) in CD-1 mice. This information will be used in assessing dose levels to be used in subsequent chronic studies. The study was initiated on December 21, 1982 (animal receipt) and completed on January 18, 1983. Doses were chosen following consideration of the results of a previous LD50 in female CD-1 mice with acrolein which was conducted at Bioassay Systems Corporation (BSC) in December 1981 and an LD50 in male mice which was conducted in December 1982. The doses used in this study were 0.0, 4.6, 5.8, 7.2, and 9.0 mg/kg of acrolein in deionized water administered in a dose volume of 10 ml/kg.

II. MATERIALS AND METHODS

A. Chemical

1. <u>General Information:</u>	<u>Test Substance</u>	<u>Control</u>
Identification:	Acrolein	Deionized Water
Source:	Magna Corporation Santa Fe Springs, CA	N/A
BSC Lot #:	12/22/82	N/A
Manufacturer's Lot #:	6288	N/A
Physical State:	Liquid	Liquid
Solubility:	16% w/v in water	N/A
Purity:	Greater than 96%	Resistance greater than 10 megaohms

2. Dose Preparation

Separate stock solutions were prepared fresh for each dose level. An appropriate volume of acrolein was placed in a volumetric flask and the volume was adjusted to the volume mark with deionized water. The dose solutions contained 0, 0.46, 0.58, 0.72 and 0.90 mg of acrolein per ml. All animals received 10 ml/kg of the respective dose solutions. These dose solutions containing various concentrations of acrolein were stable for at least 5 days, when stored at room temperature.

3. Dose Analyses

The concentrations of acrolein in the dose formulations were analyzed spectrophotometrically at 210 nm.

The formulations containing acrolein were diluted with deionized water to yield absorbance measurements at 210 nm between 0.2 and 0.8 absorbance units. These absorbance values were then converted to acrolein concentrations by comparison to a standard absorbance curve of known concentrations of acrolein. After correcting for dilution, the analyzed concentrations of acrolein in the dose formulations were found to be within 4% of the target dose levels.

B. Animals1. Mice

Species: Mus musculus

Strain: CD-1

Source: Charles River Breeding Laboratory, Wilmington,
Massachusetts

Age at Start of Study: 7 weeks

Weight at Start of Study: 22.3-37.0 grams

Number and Sex of Animals: 50 males; 50 females (10
animals/sex/dose level)

Identification: Unique number code ear punched on each animal

2. Observation and Examination During Quarantine

After receipt, animals were inspected for signs of disease and deformity. The animals were quarantined for 14 days. All animals were examined daily and appeared healthy throughout the quarantine period.

3. Randomization Method

Animals were randomly assigned to treatment groups in order to control bias using an electronic calculator capable of generating random numbers.

4. Animal Maintenance

Housing: Polycarbonate cages, five animals per cage, Hazelton System.

Food: Zeigler NIH-07 Pelleted Diet, ad libitum.

Water: Ad libitum from an automatic watering system, untreated city water.

Light: Fluorescent, automatically controlled, 12-hour light/dark cycle (lights on 7AM to 7PM).

Therapeutic agents: none

Room Temperature: 68-72°F; the temperature was not documented on December 21, 1982.

Room Humidity: 45-70%; the humidity reached 7% on one occasion for one day; the humidity was not documented on December 21, 1982.

Air Flow: 12-16 complete changes of 100% fresh air every hour filtered through roughing Varicel, HEPA and charcoal filters prior to introduction into the animal room. The air flow system is checked twice yearly by Air Balance Corp., Burlington, MA.

Cage-changing Schedule: Twice weekly; once weekly during quarantine

Food-hopper Changing Schedule: Once weekly

Rack Changing Schedule: Once every two weeks; the rack was changed during the third week

C. Experimental Design

Upon receipt, the animals were inspected and placed in quarantine. All animals were ear punched with a unique number and randomized into test groups. Food consumption and body weights were measured weekly beginning on day 1 and clinical observations were noted twice daily with the exception of number 50 on January 9, 1983. Morbidity and mortality checks were done twice daily with the exception of the quarantine period and January 16, 1983, when death checks were done once daily.

All animals were dosed orally by gavage using a syringe and stainless steel ball tip gavage tube. The dose volume was 10 ml/kg based on the most recent body weight. The dose levels used were 4.6, 5.8, 7.2 and 9.0 mg/kg. Each animal was dosed once daily for 14 consecutive days (January 4-17, 1983). All animals were subjected to a gross necropsy. All animals which survived to terminal sacrifice were euthanized by CO₂ and necropsied on January 18, 1983 at the age of nine weeks.

III. RESULTS

- A. Survival: Table 1 represents the mortality of CD-1 mice dosed at various levels of acrolein. A total of 4 mice died during the study. Two males in Level III died on day 3 and day 4 and one male in Level IV died on day 4. One female mouse in Level II died on day 6. All other animals survived to terminal sacrifice.
- B. Clinical Observations: Table 6 shows the incidence of clinical signs and Table 7 represents individual clinical signs of the CD-1 mice dosed at various levels of acrolein. All ten males in Level IV showed rough coats. Other signs in some Level IV males were pilo erection, reddening of the tip of the tail, bite wounds, closed eyes, and the presence of exudate around the eyes. Some Level III males showed hunching, lethargy, rough coats, and squinted eyes. One Level III male showed blood around the mouth after dosing which may be an artifact of gavage. One Level II female displayed lethargy, one Level III and two Level IV females showed reddened tail tips. All other animals appeared healthy.
- C. Body Weights: Tables 2 and 3 represent the body weight changes of mice administered acrolein and Tables 4 and 5 represent the individual weights. All animals were weighed on the first day of dosing, after one week and at terminal sacrifice. There are no evident signs of toxicity based on body weights.
- D. Food Consumption: Table 8 shows the food consumption of mice administered various levels of acrolein. There are no evident signs of toxicity based on food consumption.
- E. Observation at Necropsy: The most common findings at necropsy were in the squamous portion of the gastric mucosa. One Level II male, two Level III males, nine Level IV males and six Level IV females showed white and thickened gastric mucosa in the squamous portion of the stomach. Five Level III males and two Level IV females had pinpoint raised foci or nodules in the squamous portion of the stomach. Other lesions seen in the stomach include ulcers, black flecks in the gastric contents, black pinpoint foci, and red foci or reddened appearance in the squamous portion of the stomach. Other observations include hemorrhagic lungs and tails darkened near the tip. Table 9 represents the incidence of gross lesions seen and Tables 10 and 11 show the individual lesions seen.
- F. Discussion and Conclusions: Based on the mortality and necropsy observations seen in the 14 day toxicity study, the levels recommended for the chronic study are 5.8, 4.6 and 2.3 mg/kg in male mice and 7.2, 5.8 and 4.6 mg/kg in female mice.

IV. STORAGE INFORMATION

The unused acrolein (BSC Lot # 12/22/82) is stored in the Chronic Chemistry Repository (Cabinet #1) at Bioassay Systems Corporation. A copy of the protocol, all raw data generated during this study and a copy of the final report are stored in the archives at BSC.

TABLE 1
MORTALITY AMONG CD-1 MICE
ADMINISTERED VARIOUS LEVELS OF ACROLEIN^a

Week 1:	Dose mg/kg	Mortality	Animal Number	Manner of Death	Day of Death	Age at Death (Weeks)	
Males:	0.0	0/10	-	-	-	-	
	4.6	0/10	-	-	-	-	
	5.8	0/10	-	-	-	-	
	7.2	2/10	21	found dead	4	8	
			30	found dead	3	8	
	9.0	1/10	3	found dead	4	8	
	Females:	0.0	0/10	-	-	-	-
		4.6	0/10	-	-	-	-
		5.8	1/10	70	found dead	6	8
		7.2	0/10	-	-	-	-
9.0	0/10	-	-	-	-		

Week 2: No animals were found dead in week 2.

^adeionized water solution of acrolein

TABLE 2
 MEAN AND STANDARD DEVIATION OF
 BODY WEIGHT IN
 ACROCELIN-TREATED MALE MICE^a

Group	Initial Mean Body Weight	Interim Mean Body Weight	Mean Body Weight of Surviving Animals
Control	32.4 ± 1.9	33.4 ± 2.2	34.1 ± 2.7
Level I	32.9 ± 2.7	33.3 ± 3.2	34.5 ± 3.1
Level II	32.2 ± 1.7	33.3 ± 1.7	34.0 ± 1.4
Level III	31.4 ± 2.1	32.1 ± 1.5 (8)	32.8 ± 1.6 (8)
Level IV	31.9 ± 1.4	32.7 ± 1.7 (9)	33.8 ± 2.2 (9)

^aMean body weight (g) ± standard deviations; n = 10 except where noted in parentheses.

TABLE C
MEAN AND STANDARD DEVIATION OF
BODY WEIGHT IN
ACROLEIN-TREATED FEMALE MICE^a

Group	Initial Mean Body Weight	Interim Mean Body Weight	Mean Body Weight of Surviving Animals
Control	25.5 ± 1.1	26.2 ± 1.3	27.1 ± 1.0
Level I	25.4 ± 1.6	25.6 ± 1.3	27.1 ± 1.5
Level II	24.9 ± 0.9	25.7 ± 1.1 (9)	26.6 ± 1.2 (9)
Level III	24.1 ± 0.8	25.2 ± 1.6	25.4 ± 2.3
Level IV	25.8 ± 1.8	26.4 ± 1.7	27.1 ± 1.3

^aMean body weight (g) ± standard deviations; n = 10 except where noted in parentheses

BIOASSAY SYSTEMS CORPORATION

QUALITY ASSURANCE REPORTBSC PROJECT No.: 11496SPONSOR: Magna CorporationTEST SUBSTANCE: AcroleinFINDINGS:

The 14-Day Oral Toxicity in Mice Test was conducted according to the methods described in the protocol. Any and all changes in the protocol have been appropriately documented. The final report has been reviewed by BSC Quality Assurance Unit personnel and has been found to accurately represent the original data. This study was performed in compliance with FDA Good Laboratory Practice Regulations (21 CFR 58.1-58.219, 1979).

Inspections:

<u>Phase</u>	<u>QA Inspection</u>	<u>Findings Reported to Study Director</u>	<u>Findings Reported to BSC Management</u>
Protocol Review	12/20/82	12/20/82	1/10/83
Dose Formulation	1/3/83	1/4/83	1/10/83
Dosage Analysis	1/3/83	1/4/83	1/10/83
Test/Control Article Administration	1/5/83	1/5/83	1/10/83
Body Weights	1/11/83	1/12/83	1/21/83
Necropsy	1/18/83	1/19/83	1/21/83
Final Report Review	5/20/83	5/24/83	6/20/83

QUALITY ASSURANCE OFFICER:

Peter A. Loyka

DATE:

6/20/83

TABLE 4
INDIVIDUAL BODY WEIGHTS OF MALE MICE ADMINISTERED ACROLEIN

Group	Animal #	Day 1 (g)	Day 8 (g)	Day 15 (g)
0.0 mg/kg	23	34.1	36.1	37.6
	24	29.9	30.8	30.9
	25	32.2	33.5	35.2
	26	36.0	37.0	38.2
	27	32.8	34.3	35.6
	28	30.7	30.2	30.6
	31	33.7	33.8	33.2
	32	33.2	32.9	34.9
	35	31.4	34.0	33.5
	48	30.1	31.7	31.5
4.6 mg/kg	2	33.0	30.3	32.1
	4	29.3	29.7	30.0
	5	28.2	29.2	31.1
	9	37.0	38.5	39.3
	11	32.6	32.4	33.7
	25	35.0	36.2	37.1
	36	35.2	35.6	37.2
	41	34.3	35.8	37.2
	42	31.1	31.5	32.3
	46	32.8	33.8	34.8
5.8 mg/kg	6	33.7	34.8	36.0
	12	30.4	31.6	32.2
	13	33.0	34.9	34.5
	14	33.2	34.7	34.7
	18	32.7	33.6	33.6
	20	32.9	34.7	35.5
	29	32.9	33.5	34.2
	33	32.1	32.5	34.1
	37	32.7	33.5	33.9
	47	28.2	29.5	31.3
7.2 mg/kg	1	34.4	34.9	36.3
	7	28.9	30.8	32.2
	15	31.0	32.1	32.6
	21	29.1	a	a
	24	28.8	31.4	32.9
	30	33.2	a	a
	34	31.0	30.0	31.6
	39	34.1	32.6	31.7
	43	30.8	32.2	31.5
	50	32.3	32.7	33.4

^aAnimal died on test.

TABLE 4 (continued)

Group	Animal #	Day 1 (g)	Day 8 (g)	Day 15 (g)
9.0 mg/kg	3	31.7	a	a
	10	31.8	33.4	35.1
	16	30.9	32.0	34.1
	19	32.4	35.9	37.1
	23	33.9	33.8	36.0
	38	34.4	32.7	34.3
	40	29.8	29.8	29.8
	44	30.3	31.9	31.8
	45	31.7	33.2	33.8
	49	31.6	31.4	32.3

^aAnimal died on test.

TABLE 5
INDIVIDUAL BODY WEIGHTS OF FEMALE MICE ADMINISTERED ACROLEIN

Group	Animal #	Day 1 (g)	Day 8 (g)	Day 15 (g)
0.0 mg/kg	58	27.6	27.7	27.5
	67	25.5	24.9	27.3
	72	24.8	25.0	27.1
	76	26.0	25.6	26.8
	77	25.5	26.4	27.6
	78	26.6	27.9	26.8
	81	25.3	26.8	26.7
	82	23.5	24.1	25.0
	85	24.4	26.4	26.8
	98	25.5	27.0	29.2
4.6 mg/kg	52	24.5	25.2	26.1
	54	27.3	26.1	27.6
	55	25.9	24.1	26.1
	59	24.2	25.5	25.0
	61	26.6	27.8	29.1
	75	26.6	27.4	28.4
	86	22.7	23.6	28.4
	91	24.8	25.1	28.1
	92	27.6	26.2	25.0
	96	23.8	25.2	27.1
5.8 mg/kg	56	26.6	26.6	28.6
	62	23.8	23.6	25.3
	63	24.0	25.3	26.4
	64	24.4	25.4	25.5
	68	25.3	25.7	27.7
	70	25.9	a	a
	79	24.7	25.8	26.4
	83	25.1	26.5	25.5
	87	24.0	24.7	26.0
	97	25.4	27.3	27.7
7.2 mg/kg	51	24.5	23.2	29.0
	57	25.0	28.2	28.2
	65	24.5	24.9	24.2
	71	23.9	23.8	23.4
	74	22.3	26.1	22.7
	80	24.0	25.5	24.7
	84	23.3	22.9	22.4
	89	24.2	26.2	26.5
	93	24.2	25.9	26.5
	100	24.9	25.5	26.1

^aAnimal died on test.

TABLE 5 (continued)

Group	Animal #	Day 1 (g)	Day 8 (g)	Day 15 (g)
9.0 mg/kg	53	28.9	26.5	26.8
	60	26.5	26.6	25.7
	66	25.3	25.2	26.0
	69	26.1	26.5	28.4
	73	25.8	29.1	29.0
	88	28.0	26.9	28.0
	90	25.1	26.4	25.8
	94	24.0	24.7	26.3
	95	25.7	28.7	28.8
	99	22.5	23.5	25.9

Table 6

Incidence of Clinical Observations Among CD-1 Mice Administered Various Levels of Acrolein

Clinical Observation	Vehicle Control	Male				Vehicle Control	Level I	Level II	Level III	Level IV	Female				
		Level I	Level II	Level III	Level IV						Level I	Level II	Level III	Level IV	
Hunched	0/10	0/10	0/10	2/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Lethargy	0/10	0/10	0/10	3/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	0/10	0/10	0/10	0/10
Rough coat	0/10	0/10	0/10	3/10	10/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Pilo Erection	0/10	0/10	0/10	0/10	1/9	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Squinted Eyes	0/10	0/10	0/10	3/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Tip of tail red	0/10	0/10	0/10	0/10	3/9	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	0/10	2/10
Bite wounds	0/10	0/10	0/10	0/10	3/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Eye closed	0/10	0/10	0/10	0/10	1/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Exudate around eye (s)	0/10	0/10	0/10	0/10	2/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Blood around mouth after dosing	0/10	0/10	0/10	1/8	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10

TABLE 7
 Clinical Observations Seen in CD-1 Mice
 Administered Acrolein

Group	Animal #	Sex	Day of Test	Condition(s)
5.8 mg/kg	70	F	6	lethargy and extreme, very shallow breathing found dead in pm
7.2 mg/kg	21	M	2,3	hunched; lethargic; rough coat; squinted eyes
	30	M	4	found dead in am
			2	hunched; lethargic; rough coat; squinted eyes
	43	M	3 14	found dead in am blood around mouth after dosing
	50	M	3	lethargy; rough coat; squinted eyes
9.0 mg/kg	3	M	10-12	end of tail reddened
			2,3	rough coat
			3	exudate around eyes
	10	M	4	found dead in pm
			2,3	rough coat
	16	M	11-14	bite wounds on back
			2,3	rough coat
	19	M	11-14	bite wounds on back
			2-4,6-8	rough coat
			4-8	orange stain around left eye
	23	M	4-8,10-14	left eye closed or partially closed
			2,3	rough coat
			10	end of tail reddened
			11-14	bite wounds on back
			38	M
40	M	2,3	rough coat	
		10-14	end of tail reddened	
44	M	2,3	rough coat	
45	M	2,3,12	rough coat	
		8	pilo erection	
49	M	2,3	rough coat	
		7,8,10-14	1 cm of tail reddened	
60	F	11-13	end of tail reddened	
99	F	10-14	end of tail reddened	

Table 8
 Mean Food Consumption of CD-1 Mice Administered
 Various Levels of Acrolein^a

<u>Males:</u>			<u>Females:</u>		
<u>Dose Level</u>	<u>Mean and Standard Deviation</u>		<u>Dose Level</u>	<u>Mean and Standard Deviation</u>	
	<u>Week 1</u>	<u>Week 2</u>		<u>Week 1</u>	<u>Week 2</u>
Vehicle Control	78.9 ± 15.4	78.5 ± 12.6	Vehicle Control	70.1 ± 12.8	75.5 ± 13.6
Level I	80.6 ± 12.6	79.8 ± 13.5	Level I	65.5 ± 10.9	73.3 ± 12.4
Level II	80.9 ± 16.0	81.6 ± 9.6	Level II	68.0 ± 11.0 (9)	64.2 ± 15.5 (9)
Level III	64.8 ± 12.5 (8)	66.2 ± 11.0 (8)	Level III	72.0 ± 20.7	72.8 ± 10.4
Level IV	76.3 ± 19.7 (9)	81.5 ± 10.6 (9)	Level IV	73.7 ± 15.3	76.1 ± 11.2

^aValues are for 10 animals unless otherwise indicated in ().

Table 9

Incidence of Gross Lesions Seen in CD-1 Mice Administered
Various Levels of Acrolein

Gross Lesion	Male				Female					
	Vehicle Control	Level I	Level II	Level III	Level IV	Vehicle Control	Level I	Level II	Level III	Level IV
White thickened gastric mucosa (squamous portion)	0/10	0/10	1/10	2/10	9/10	0/10	0/10	0/10	0/10	6/10
Ulcers of stomach	0/10	0/10	0/10	0/10	2/10	0/10	0/10	0/10	0/10	0/10
Black flecks (like blood) in gastric contents	0/10	0/10	0/10	0/10	2/10	0/10	0/10	0/10	0/10	1/10
Black pinpoint foci over mucosal surface of stomach	0/10	0/10	0/10	0/10	1/10	0/10	0/10	0/10	0/10	0/10
Pinpoint raised foci or nodules on squamous portion of stomach	0/10	0/10	0/10	5/10	0/10	0/10	0/10	0/10	1/10	2/10
Cardia portion of stomach reddened or red foci	0/10	0/10	0/10	2/10	1/10	0/10	0/10	0/10	0/10	0/10
Tail darkened near tip	0/10	0/10	0/10	1/10	0/10	0/10	0/10	0/10	0/10	0/10
Lungs hemorrhagic	0/10	0/10	0/10	0/10	1/10	0/10	0/10	0/10	0/10	0/10

TABLE 10

Gross Lesions Seen in Male Mice Administered Acrolein

Group	Animal Number	Date of Death	Gross Lesions Observed
5.8 mg/kg	13	1/18/83	<u>Stomach</u> - thickened, white nodular, corrugated, gastric mucosa in squamous portion
7.2 mg/kg	15	1/18/83	<u>Stomach</u> - thickened, white ridge .1 cm wide, .5 cm long on squamous portion
	24	1/18/83	<u>Stomach</u> - three .1 cm diameter, raised white
	1	1/18/83	<u>Stomach</u> - multiple raised white foci in dorso-lateral section of squamous portion
	7	1/18/83	<u>Stomach</u> - multiple raised white foci in dorso-lateral section of squamous portion
	50	1/18/83	<u>Stomach</u> - several thick white 0.1 cm nodules on squamous mucosa
	43	1/18/83	<u>Stomach</u> - craterous rim of thickened white squamous mucosa
	39	1/18/83	<u>Stomach</u> - several thick white nodules on squamous mucosa
	30	1/6/83	<u>Stomach</u> - pinpoint red foci on cardiac portion
	21	1/7/83	<u>Stomach</u> - pinpoint red foci over surface of cardiac portion <u>Tail</u> - darkened near tip (2cm)
9.0 mg/kg	3	1/7/83	<u>Stomach</u> - cardiac portion reddened <u>Lungs</u> - hemorrhagic
	44	1/18/83	<u>Stomach</u> - many black pinpoint spots over mucosal surface; forestomach is thickened, mucosa is white and thickened

TABLE 10
Gross Lesions Seen in Male Mice Administered Acrolein

Group	Animal Number	Date of Death	Gross Lesions Observed
9.0 mg/kg	45	1/18/83	<u>Stomach</u> - forestomach mucosa is white and thickened
	40	1/18/83	<u>Stomach</u> - forestomach is white and slightly thickened
	49	1/18/83	<u>Stomach</u> - forestomach is white and slightly thickened
	38	1/18/83	<u>Stomach</u> - forestomach is white and slightly thickened
	23	1/18/83	<u>Stomach</u> - Black flecks of material (like blood) throughout gastric contents, one ulcer in squamous portion; one ulcer in fundic portion; severe white hyperkeratosis and acanthosis of squamous portion especially in a knurled corrugated fold along the squamous-fundic border
	19	1/18/83	<u>Stomach</u> - old ulcer (0.3 cm) in squamous portion with surrounding white marked hyperkeratosis and acanthosis forming the edges of a crater
	10	1/18/83	<u>Stomach</u> - Moderate disseminated white irregular thickening of squamous portion of gastric mucosa
	16	1/18/83	<u>Stomach</u> - Black flecks (like blood) throughout gastric contents, very thick white corrugated border between fundic and squamous portion; thickened white irregular corrugated squamous portion of gastric mucosa

TABLE 11
Gross Lesions Seen in Female Mice Administered Acrolein

Group	Animal Number	Date of Death	Gross Observations Seen
7.2 g/kg	57	1/18/83	<u>Stomach</u> - multiple pinpoint raised foci in most dorso-lateral area of squamous portion
9.0 g/kg	73	1/18/83	<u>Stomach</u> - 0.5 cm diameter thickened white area
	66	1/18/83	<u>Stomach</u> - slight thickening of stomach lining
	60	1/18/83	<u>Stomach</u> - thickened white area (1 cm x 0.2 cm) on stomach lining
	53	1/18/83	<u>Stomach</u> - thickened white area (1 cm x 0.5 cm) on stomach lining
	94	1/18/83	<u>Stomach</u> - mildly thickened, white diffuse corrugated squamous gastric mucosa, a few black flecks (like dried blood) in gastric contents)
	95	1/18/83	<u>Stomach</u> - slightly thickened ridge about 0.7 cm long in squamous portion of gastric mucosa
	90	1/18/83	<u>Stomach</u> - four white thickened nodules 0.1 cm in squamous portion of mucosa
	88	1/18/83	<u>Stomach</u> - multiple white nodules in squamous portion (0.1 cm diameter)

V. SIGNATURES

Study Title: 14-Day Oral Toxicity Test in Mice

Theodore A. Olsson 6-22-83
Theodore A. Olsson, Technical Supervisor,
Analytical Chemistry

Carole A. Mansur 6-20-83
Carole A. Mansur, Study Director

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VI. BSC SUPERVISORY PERSONNEL ASSIGNED TO THE STUDY

Carole A. Mansur	Study Director
Kirby N. Smith, D.V.M.	Animal Care Veterinarian
Theodore A. Olsson	Technical Supervisor, Chemistry
Peter J. Mione	Manager, Quality Assurance

A.01.

FINAL REPORT

Study Title

SALMONELLA/MAMMALIAN-MICROSOME
PREINCUBATION MUTAGENICITY ASSAY
(AMES TEST)

AND

ESCHERICHIA COLI WP2uvr A
REVERSE MUTATION ASSAY
WITH A CONFIRMATORY ASSAY

Test Article

Acrolein, Inhibited

Authors

Richard H.C. San, Ph.D.
Karen A. Springfield, B.S.

Study Report Date

10/26/89

Performing Laboratory

Microbiological Associates, Inc.
9900 Blackwell Road
Rockville, MD 20850

Laboratory Study Number

T8799.502071

Submitted To

Baker Performance Chemicals Inc.
3920 Essex Lane
Houston, TX 77027

STATEMENT OF COMPLIANCE

To the best of my knowledge, the Salmonella/Mammalian-Microsome Preincubation Mutagenicity Assay (Ames Test) And Escherichia Coli WP2uvr A Reverse Mutation Assay With A Confirmatory Assay of test article Acrolein, Inhibited was conducted in compliance with the U.S. FDA Good Laboratory Practice Regulations (21CFR58), the U.S. EPA GLP's (40CFR792 and 40CFR160) and the OECD guidelines in all material aspects with the following reservations:

The identity, strength, purity and composition or other characteristics to define the test or control articles have not been determined by the testing facility (Section 105 (a)).

The stability of the test or control articles under the test conditions has not been determined by the testing facility and is not included in the final report (Sections 105 (a) and (b) and 185 (a) (5)).

Analyses to determine the uniformity, concentration, or stability of the test or control mixtures were not performed by the testing facility (Section 113 (a)).

R. H. C. San

10/20/99

Richard H.C. San, Ph.D.
Study Director

Date

A 03

QUALITY ASSURANCE STATEMENT

Study Title: SALMONELLA/MAMMALIAN-MICROSOME PREINCUBATION
MUTAGENICITY ASSAY (AMES TEST) AND ESCHERICHIA
COLI WP2uvr A REVERSE MUTATION ASSAY WITH
A CONFIRMATORY ASSAY

Study Number: T8799.502071

Study Director: Richard H.C. San, Ph.D.

Initiation Date: 89/07/06

Review Completed Date: 89/10/27

This study has been divided into a series of phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice regulations (21CFR58), the U.S. EPA GLPs (40CFR792 and 40CFR160), and the OECD guidelines and to assure that the study is conducted according to the protocol.

The following are the inspection dates, phases inspected, and report dates of QA inspections of the study.

INSPECT ON 89/07/07 - 89/07/07, TO STUDY DIR 89/07/07, TO MGMT 89/07/07
PHASES: PROTOCOL REVIEW

INSPECT ON 89/08/01 - 89/08/01, TO STUDY DIR 89/08/01, TO MGMT 89/08/01
PHASES: STRAIN CHARACTERIZATION

INSPECT ON 89/10/21 - 89/10/24, TO STUDY DIR 89/10/24, TO MGMT 89/10/27
PHASES: FINAL REPORT

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Janette A. John
Quality Assurance
RA/QA Department

10/27/89
Date

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FINAL REPORT
SALMONELLA/MAMMALIAN-MICROSOME
PREINCUBATION MUTAGENICITY ASSAY
(AMES TEST)
AND
ESCHERICHIA COLI WP2uvr A
REVERSE MUTATION ASSAY
WITH A CONFIRMATORY ASSAY

Sponsor: Baker Performance Chemicals Inc.
3920 Essex Lane
Houston, TX 77027

Testing Facility: Microbiological Associate Inc. (MBA)
9900 Blackwell Road
Rockville, Maryland 20850

MBA Study No.: T8799.502071

Test Article I.D.: Acrolein, Inhibited

Test Article Lot No.: UTLX 89446

Test Article Description: Clear liquid

Test Article Purity (Determined by sponsor): 96.58%

Storage Conditions: 4°C

Date Received: 06/29/89

Date Study Started: 07/06/89

Date Study Completed: 10/26/89

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TABLE OF CONTENTS

Introduction page 7
Conclusions page 7
Materials and Methods. page 9
Discussion of the Dose Range-Finding Study page 22
Dose Range-Finding Study Results page 24
Discussion of the Mutagenicity Assay page 27
Mutagenicity Assay Results page 30
Appendix (Study Protocol). page 65

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INTRODUCTION

AND

CONCLUSIONS

Introduction

Baker Performance Chemicals Inc. submitted

Test Article ID: Acrolein, Inhibited

Lot No.: UTLX 89446

Receipt Date: 06/29/89

MBA Test Article ID: T8799

for testing in the Salmonella/Mammalian-Microsome Preincubation Mutagenicity Assay (Ames Test) and Escherichia Coli WP2uvr A Reverse Mutation Assay With A Confirmatory Assay. This assay evaluates the mutagenic potential of the test article (or its metabolites) for its ability to induce back mutations at selected loci of several strains of Salmonella typhimurium in the presence and absence of microsomal enzymes derived from Aroclor 1254 induced rat liver. The tester strains used in this study were TA98, TA100, TA1535, TA1537 TA1538, TA102, TA104 and WP2uvr A.

Conclusions

The results of the Salmonella/Mammalian-Microsome Preincubation Mutagenicity Assay (Ames Test) and Escherichia Coli WP2uvr A Reverse Mutation Assay With A Confirmatory Assay indicate that under the conditions of this study, Baker Performance Chemicals Inc.'s test article Acrolein, Inhibited (MBA# T8799) did cause positive responses with tester strains TA98 and TA100 in the presence and absence of microsomal enzymes and with tester strain WP2uvr A in the presence of microsomal enzymes.

Should also include tests that had negative findings

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MATERIALS AND METHODS

The experimental materials, methods and procedures are based on those described by Ames et al. (1975), and Maron and Ames (1983).

1.0 MEDIA AND REAGENT PREPARATION

Note: All references to water imply sterile, deionized distilled grade water produced by the water processing system at MBA.

1.1 Top Agar for Selection of Histidine Revertants: Minimal top agar was prepared containing 0.8% agar (W/V) and 0.5% NaCl (W/V). After autoclaving, the molten agar was distributed into sterile bottles and stored at room temperature. On the day of its use, the top agar was melted and each 100 ml was supplemented with 10 ml of sterile 0.5 mM L-histidine/0.5 mM D-biotin solution.

1.2 Top Agar for Selection of Tryptophan Revertants: Minimal top agar was prepared with 0.8% agar (w/v) and 0.5% NaCl (w/v). After autoclaving, the molten agar was distributed into sterile bottles and stored at room temperature. On the day of its use, the top agar was melted and each 100 ml was supplemented with 10 ml per 100 ml agar of a sterile solution which contained 0.11 mg/ml L-tryptophan.

1.2 Top Agar for Salmonella Viable Count Determination and Genotype Characterization: Minimal top agar, as described above, was supplemented with 10 ml of a sterile 0.5 mM L-histidine/0.5 mM D-biotin solution along with 25 ml of sterile water per 100 ml of top agar.

1.2 Top Agar for E. coli Viable Count Determinations: Minimal top agar, as described above, was supplemented with 10 ml of a sterile 0.11 mg/ml tryptophan solution along with 25 ml of sterile water per 100 ml of top agar.

1.3 Minimal Bottom Agar: Bottom agar was Vogel-Bonner minimal medium E (Vogel and Bonner, 1956) containing 1.5% (W/V) agar.

1.4 Nutrient Broth: Nutrient Broth used for growing overnight cultures of the tester strains was Vogel-Bonner salt solution supplemented with 2.5% (W/V) Oxoid Nutrient Broth No. 2 (dry powder).

1.5 Nutrient Bottom Agar: Nutrient bottom agar was Vogel-Bonner minimal medium E containing 1.5% (W/V) agar and supplemented with 2.5% (W/V) Oxoid Nutrient Broth No. 2 (dry powder).

1.6 Tester Strain Diluent: The diluent used for tester strain titering was Vogel-Bonner salt solution supplemented with 2.5% (W/V) Oxoid Nutrient Broth No. 2 (dry powder).

1.7 Test Article Vehicle: The vehicle used to deliver Acrolein, Inhibited to the test system was dimethylsulfoxide (DMSO), (CAS# 67-68-5), certified A.C.S., purchased from Fisher Scientific Company, Silver Spring, MD.

1.8 Exogenous Metabolic Activation

1.8.1 Liver Microsomal Enzymes - S-9 Homogenate

1.8.1.1 Species, Strain, Sex, Inducer: Liver microsomal enzymes were prepared from male, Sprague-Dawley rats that had been injected with Aroclor 1254 at 500 mg/kg. The Aroclor was diluted in corn oil to a concentration of 200 mg/ml. Five days after i.p. injection with the Aroclor, the rats were sacrificed by decapitation, and their livers were excised.

1.8.1.2 Homogenate Preparation: The preparation of the microsomal enzyme fraction was carried out with sterile glassware and solutions at 0-4°C. Excised livers were placed in 0.15M KCl contained in a pre-weighed beaker. After the liver was weighed, it was transferred to another beaker containing 3 volumes of 0.15M KCl (3 ml/g of wet liver) where it was minced with sterile scissors. The minced liver was homogenized and centrifuged at 9000 x g for 10 minutes. The supernatant (referred to by Ames as the S-9 fraction) was removed, and small aliquots were distributed into freezing ampules that were stored at $\leq -70^{\circ}\text{C}$.

1.8.1.3 S-9 Characterization: The S-9 homogenate was characterized for its ability to metabolize the promutagens 7,12-dimethylbenzanthracene and 2-aminoanthracene to mutagens, as described by deSerres and Shelby (1979).

1.8.2 S-9 Mix: The S-9 mix was prepared immediately before its use. The microsomal enzyme mixture (S-9 mix) contained the following components:

H ₂ O	0.56 ml
1.00M NaH ₂ PO ₄ /K ₂ HPO ₄ , pH 7.4	0.10 ml
0.05M Glucose-6-phosphate	0.10 ml
0.04M NADP	0.10 ml
0.2M MgCl ₂ /0.825M KCl	0.04 ml
S-9	<u>0.10 ml</u>
	1.00 ml

Where appropriate, 0.5 ml of the S-9 mix was added to the preincubation mixture for each plate.

1.8.3 Sham S-9 Mix: The Sham S-9 mix was prepared immediately before its use. The sham microsomal enzyme mixture (Sham S-9 mix) contained the following components:

H ₂ O	0.90 ml
1.00M NaH ₂ PO ₄ /K ₂ HPO ₄ , pH 7.4	<u>0.10 ml</u>
	1.00 ml

Where appropriate, 0.5 ml of the Sham S-9 mix was added to the preincubation mixture for each plate.

2.0 TEST SYSTEM

The Ames Test has been shown to be a sensitive, rapid, accurate indicator of the mutagenic activity of a wide range of chemical classes.

The tester strains to be used were the Salmonella typhimurium histidine auxotrophs TA98, TA100, TA1535, TA1537 and TA1538 as described by Ames et al. (1975), TA102 as described by Levin et al. (1982), TA104 as described by Marnett et al. (1985), and the Escherichia coli tryptophan derivative WP2uvr A as described by Bridges (1972).

2.1 Salmonella

GENOTYPE OF THE SALMONELLA STRAINS USED FOR MUTAGEN TESTING

Figure 1

HISTIDINE MUTATION				ADDITIONAL MUTATIONS		
<u>hisG46</u>	<u>hisC3076</u>	<u>hisD3052</u>	<u>hisG428</u>	LPS	Repair	R-factor
TA1535	TA1537	TA1538		<u>rfa</u>	<u>uvrB</u>	-
TA100		TA98		<u>rfa</u>	<u>uvrB</u>	+R
			TA102	<u>rfa</u>	-	+R
			TA104	<u>rfa</u>	<u>uvrB</u>	+R

The tester strains contain, in addition to a mutation in the histidine operon, additional mutations that enhance their sensitivities to some mutagenic compounds. The rfa wall mutation causes a loss of one of the enzymes responsible for the synthesis of part of the lipopolysaccharide layer of the cell wall. The resulting cell wall deficiency increases the permeability of the cell to certain classes of chemicals, such as those containing large ring systems that would otherwise be excluded by a normal cell wall.

The mutation in the uvrB gene results in a deficient DNA excision-repair system. This deficiency results in greatly enhanced sensitivity to some mutagens. Since the uvrB deletion extends through the bio gene, tester strains containing this deletion also require the vitamin biotin for growth.

Finally, tester strains TA98, TA100, TA102 and TA104 also contain the pKM101 plasmid (carrying the R factor) that further increases the sensitivities of these four strains to some mutagens. It has been suggested that this plasmid increases

sensitivity to mutagens by modifying an existing bacterial DNA repair polymerase complex involved with the mismatch-repair process.

Tester strains TA98, TA1537 and TA1538 are reverted from histidine dependence (auxotrophy) to histidine independence (prototrophy) by frameshift mutagens. Tester strain TA1535 is reverted by mutagens that cause basepair substitutions. Tester strain TA100 is reverted by mutagens that cause both frameshift and basepair substitution mutations.

Strain TA102 is a relatively new tester strain which possesses A-T base pairs at the site of the mutation unlike the earlier tester strains which possess G-C base pairs at the mutation sites. Additionally, this strain is unique in that the hisG428 ochre mutation has been introduced into a plasmid (pAQ1) so that under the appropriate experimental conditions, approximately 30 copies of the mutant gene are available for back mutation. The uvrB mutation has not been introduced into this strain so it can detect cross-linking agents such as mitomycin C. This strain has been shown to be useful for detecting oxidative mutagens such as bleomycin that are not detected by other tester strains.

TA104 exhibits a sensitivity spectrum similar to that of TA102 but it does not detect cross-linking agents.

2.2 E. coli: The WP2uvr A reverse mutation assay system measures reversion from trp⁻ to trp⁺ at a site blocking a step of tryptophan biosynthesis prior to the formation of anthranilic acid (Brusick et al., 1980). Tryptophan revertants can arise due to a base change at the originally mutated site or by a base change elsewhere in the chromosome causing the original mutation to be suppressed. Thus, the specificity of the reversion mechanism is sensitive to base-pair substitution mutations, rather than frameshift mutations (Green and Muriel, 1976).

2.3 Source

2.3.1 Salmonella: Tester strains in use at Microbiological Associates Inc. (MBA) were received directly from Dr. Bruce Ames, Department of Biochemistry, University of California, Berkeley.

2.3.2 E. coli: Tester strain WP2uvr A in use at Microbiological Associates, Inc. was received from the National Collection of Industrial Bacteria, Torrey Research Station, P.O. Box 31, 135 Abbey Road, Aberdeen, AB9 8DG, Scotland (United Kingdom).

2.4 Storage

2.4.1 Frozen Permanent Stocks: Frozen Permanent Stocks were prepared by growing fresh overnight cultures, adding DMSO

(0.09 ml/ml of culture) and freezing away approximately 1.5 ml aliquots in glass vials. Frozen Permanent Stocks are stored at $\leq -70^{\circ}\text{C}$.

2.4.2 Master Plates

2.4.2.1 Salmonella: Master plates were prepared by streaking each tester strain from a frozen permanent stock onto minimal medium supplemented with histidine (260 μM), biotin (3 μM) and, for strains containing the R-factor, ampicillin (25 $\mu\text{g/ml}$). Master plates are stored at $4 \pm 2^{\circ}\text{C}$.

2.4.2.2 E. coli: Master plates of the tester strain will be stored at 4°C . Master plates are prepared by streaking the strain from a frozen permanent stock onto Vogel-Bonner minimal medium E supplemented with 2.5% (W/V) Oxoid Nutrient Broth No. 2 (dry powder).

2.5 Overnight Culture Preparation: Overnight cultures were prepared by removing a colony of the appropriate tester strain from the appropriate master plate and transferring it to a vessel containing ~50 ml of culture medium. To assure that cultures were harvested in late log phase, the length of incubation was controlled and monitored. Following inoculation, each flask was placed in a resting shaker/incubator at room temperature. The shaker/incubator was programmed to begin shaking at approximately 125 rpm at $37 \pm 2^{\circ}\text{C}$ approximately 12 hours before the anticipated time of harvest. Each culture was monitored spectrophotometrically for turbidity and was harvested at a percent transmittance yielding a titer of approximately $1-2 \times 10^9$ cells per milliliter.

2.5.1 Tester Strain Culture Titters: Tester strain titers were determined by viable count assays on nutrient agar plates. The number of cells seeded per plate is reported on the strain data forms.

2.6 Genotype Characterization: On the day of their use in the mutagenicity assay, tester strain cultures were checked for the following genetic markers:

2.6.1 rfa Wall Mutation: The presence of the rfa wall mutation was confirmed for the Salmonella by demonstration of sensitivity to crystal violet. An aliquot of an overnight culture of each strain was overlaid onto plates containing selective media. An antibiotic sensitivity disk and 10 μl of a 1.0 mg/ml crystal violet solution were added. Sensitivity was demonstrated by inhibition of bacterial growth in a zone immediately surrounding the disk.

2.6.2 pKM101 Plasmid R-factor: The presence of the pKM101 plasmid was confirmed for tester strains TA98, TA100, TA102 and TA104 by demonstration of resistance to ampicillin. An aliquot of the overnight culture of each strain was overlaid onto plates containing selective media and an antibiotic sensitivity

disk containing 10 ug of ampicillin was added. Resistance was demonstrated by bacterial growth in the zone immediately surrounding the disk.

2.6.3 Characteristic Number of Spontaneous Revertants: Spontaneous reversion frequencies in the vehicle controls that are characteristic of the respective strains were demonstrated by plating 100 ul aliquots of the culture along with the appropriate vehicle on selective media.

3.0 EXPERIMENTAL DESIGN

The assay was performed in two phases. The first phase, the dose-range finding study, was used to establish the dose-range over which the test article would be assayed. The second phase, the mutagenicity assay, was used to evaluate the mutagenicity of the test article under the conditions of the assay.

3.1 Dose Range-Finding Study: Ten dose levels of the test article were plated per tester strain, one plate per dose, with an overnight culture of TA100 and WP2uvr A on selective minimal agar in both the presence and absence of microsomal enzymes.

3.2 Mutagenicity Assay: The test article was tested at five dose levels along with appropriate vehicle and positive controls on tester strains TA98, TA100, TA1535, TA1537, TA1538, TA102, TA104 and WP2uvr A in the presence and absence of S-9 mix. In the mutagenicity assay, all dose levels of the test article, vehicle controls and positive controls were plated in triplicate.

3.3 Frequency and Route of Administration: The test system was exposed to the test article via the preincubation modification of the Ames test originally described by Yahagi et al. (1977). This methodology has been shown to detect a wide range of classes of chemical mutagens.

4.0 CONTROLS

4.1 Positive Controls: The positive controls plated concurrently with the assay are listed in Figure 2.

POSITIVE CONTROL AND TESTER STRAIN COMBINATIONS

Figure 2

<u>Strain</u>	<u>Activation</u>	<u>Positive Controls</u>	<u>Conc. per Plate</u>
TA98	+	2-aminoanthracene	0.25 ug
TA98	-	2-nitrofluorene	1.0 ug
TA100	+	2-aminoanthracene	0.25 ug
TA100	-	sodium azide	0.5 ug
TA1535	+	2-aminoanthracene	0.25 ug
TA1535	-	sodium azide	0.5 ug
TA1537	+	2-aminoanthracene	0.25 ug
TA1537	-	ICR-191	0.25 ug
TA1538	+	2-aminoanthracene	0.25 ug
TA1538	-	2-nitrofluorene	1.0 ug
TA102	+	sterigmatocystin	10.0 ug
TA102	-	cumene hydroperoxide	100.0 ug
TA104	+	2-aminoanthracene	4.0 ug
TA104	-	cumene hydroperoxide	75.0 ug
WP2uvr A	+	2-aminoanthracene	10000.0 ug
WP2uvr A	-	methyl methanesulfonate	1000.0 ug

Source and Grade

2-aminoanthracene (CAS #613-13-8), Sigma Chemical Co., practical grade

ICR-191 (CAS #1707-45-0), Polysciences Inc., 95% pure

2-nitrofluorene (CAS #607-57-8), Aldrich Chemical Co., 98% pure

sodium azide (CAS #26628-22-8), Sigma Chemical Co., practical grade

Cumene hydroperoxide (CAS #80-15-9), Aldrich Chemical Company., 80 % pure

methyl methanesulfonate (CAS #: 66-27-3), Aldrich Chemical Company., 99 % pure

Sterigmatocystin (CAS #10048-13-2), Aldrich Chemical Company.

4.2 Vehicle Controls: Vehicle controls (dimethylsulfoxide (DMSO)) were plated for all tester strains with and without microsomal enzymes.

4.3 Sterility Controls

4.3.1 Test Article Sterility Determination: To determine the sterility of the test article, the highest test article dose level used in the mutagenicity assay was checked for sterility by plating on selective agar an aliquot volume equal to that used in the assay.

4.3.2 S-9 Mix and Sham S-9 Mix Sterility Determination: To determine the sterility of the S-9 mix and the Sham S-9 mix, a 0.5 ml aliquot of each was plated on selective agar.

5.0 PLATING PROCEDURES

5.1 Test System Identification: Each plate was labeled with a code system that identified the test article, test phase, dose level, tester strain, and activation, as described in detail in Microbiological Associates, Inc.'s Microbial Mutagenesis Standard Operating Procedures.

5.2 Plating Procedure: The test article was serially diluted immediately before its use. When S-9 mix was required, a 500 ul aliquot of S-9 mix was added to 13 X 100 mm glass culture tubes pre-heated to $37\pm 2^{\circ}\text{C}$. To these tubes were added 100 ul of appropriate tester strain and either 50 ul of vehicle or test article, or 50 ul of positive control. In the absence of S-9 mix, a 500 ul aliquot of Sham S-9 mix was added to 13 X 100 mm glass culture tubes pre-heated to $37\pm 2^{\circ}\text{C}$. To these tubes were added 100 ul of appropriate tester strain and either 50 ul of vehicle or test article, or 50 ul of positive control. After vortexing, the mixture was incubated without shaking for 20 ± 2 minutes at $37\pm 2^{\circ}\text{C}$. Following the preincubation, 2.0 ml of selective top agar was added to each tube and the mixture was vortexed and overlaid onto the surface of 25 ml of minimal bottom agar. After the overlay had solidified, the plates were inverted and incubated for approximately 48 hours at $37\pm 2^{\circ}\text{C}$. Plates that were not counted immediately following the 48 hour incubation period were stored at $4\pm 2^{\circ}\text{C}$ until colony counting could be conducted.

6.0 SCORING THE MUTAGENICITY ASSAY

6.1 Colony Counting: Revertant colonies for a given tester strain and activation condition, except for the positive controls, were counted either entirely by automated colony counter or entirely by hand. Plates with sufficient test article precipitate to interfere with automated colony counting were counted manually.

6.2 Evaluation of the Bacterial Background Lawn: The condition of the bacterial background lawn was evaluated for evidence of test article toxicity by using a dissecting microscope. This toxicity was scored relative to the vehicle control plate, and it was evaluated using the criteria and codes that appear in Figure 3.

6.3 Analysis of the Data: For all replicate platings, the mean number of revertants per plate was calculated and the standard deviation around the mean was also calculated. The

results of these calculations are presented on the individual strain data forms.

7.0 CRITERIA FOR DETERMINATION OF A VALID TEST

The following criteria must be met for the mutagenicity assay to be considered valid:

7.1 Tester Strain Integrity

7.1.1 rfa Wall Mutation: To demonstrate the presence of the deep rough mutation (rfa), all Salmonella tester strain cultures must exhibit sensitivity to crystal violet.

7.1.2 pKM101 Plasmid R-factor: To demonstrate the presence of the pKM101 plasmid R-factor, cultures of tester strains TA98, TA100, TA102 and TA104 must exhibit resistance to ampicillin.

7.1.3 Characteristic Number of Spontaneous Revertants: Each tester strain culture must exhibit a characteristic mean number of spontaneous revertants in the vehicle control. The acceptable ranges are as follows:

TA98	10 - 50
TA100	80 - 240
TA1535	5 - 45
TA1537	3 - 21
TA1538	5 - 35
TA102	200 - 380
TA104	200 - 380
WP2uvr A	10 - 60

7.1.4 Tester Strain Titters: To ensure that appropriate numbers of bacteria are plated, tester strain culture titters must be greater than or equal to 0.6×10^9 cells/ml.

7.1.5 Positive Control Values: The mean of each positive control must exhibit at least a three-fold increase in the number of revertants over the mean value of the respective vehicle control.

7.2 Toxicity

7.2.1 Minimal Number of Dose Levels: A minimum of three non-toxic dose levels are required to evaluate assay data.

8.0 CRITERIA FOR EVALUATION OF TEST RESULTS

For a test article to be evaluated as positive, it must cause at least a doubling in the mean revertants per plate of at least one tester strain. This increase in the mean number of revertants per plate must be accompanied by a dose response to

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increasing concentrations of the test article. If the study is to be judged positive solely on the basis of a dose-responsive, less than 3-fold increase in TA1537 or TA1538, the response must be confirmed in a repeat experiment.

9.0 ARCHIVES

All experimental records (raw data and appropriate reports) of the study are maintained in the Microbiological Associates, Inc.'s archives located at 9900 Blackwell Road, Rockville, Maryland 20850.

The Director of the Quality Assurance Unit is responsible for maintaining the archives.

10.0 STABILITY OF THE TEST ARTICLE

The stability of the test article under the actual experimental conditions used in this study was not determined by Microbiological Associates, Inc.

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BACTERIAL BACKGROUND LAWN EVALUATION CODE

Figure 3

The condition of the background bacterial lawn is evaluated, first macroscopically and then microscopically (using a dissecting microscope). The evaluation is recorded using the following code:

<u>CODE</u>	<u>DEFINITION</u>	<u>CHARACTERISTICS</u>
1	Normal	Distinguished by a healthy microcolony lawn.
2	Slightly Reduced	Distinguished by a noticeable thinning of the microcolony lawn and an increase in the size of the microcolonies compared to the vehicle control plate.
3	Moderately Reduced	Distinguished by a marked thinning of the microcolony lawn and an increase in the size of the microcolonies compared to the vehicle control plate.
4	Extremely Reduced	Distinguished by an extreme thinning of the microcolony lawn and an increase in the size of the microcolonies compared to the vehicle control plate.
5	Absent	Distinguished by a complete lack of any microcolony lawn.
6	Obscured by Precipitate	The background bacterial lawn cannot be accurately evaluated due to microscopic test article precipitate.

Evidence of macroscopic test article precipitate on the plates is recorded by addition of the following precipitate code to the code number used to evaluate the condition of the background bacterial lawn.

SP	Slight Precipitate	Distinguished by noticeable precipitate on the plate, however, the precipitate does not influence automated counting of the plate.
MP	Moderate Precipitate	Distinguished by a marked amount of precipitate on the plate, requiring the plate to be hand counted.
HP	Heavy Precipitate	Distinguished by a large amount of precipitate on the plate, making the required hand count difficult.

Thus, 3MP would indicate a plate observed to have a moderately reduced background lawn with a marked amount of precipitate that required the plate to be counted manually.

B.07

DISCUSSION

OF THE

DOSE RANGE-FINDING STUDY

The Salmonella/Mammalian-Microsome Preincubation Mutagenicity Assay (Ames Test) And Escherichia Coli WP2uvr A Reverse Mutation Assay With A Confirmatory Assay was performed in two phases. The first phase, the dose range-finding study, was used to establish the dose range over which the test article would be assayed. The second phase, the mutagenicity assay, was used to evaluate the mutagenicity of the test article.

The results of the dose range-finding study of

Test Article ID: Acrolein, Inhibited
Lot No.: UTLX 89446
Sponsor: Baker Performance Chemicals Inc.
MBA Test Article ID: T8799

are presented in Table 1.

The maximum dose tested in the dose range-finding study was 10000 ug per plate. This dose was delivered to the test system as a solution in dimethylsulfoxide (DMSO) using a plating aliquot of 50 ul.

The results of the dose range-finding study of MBA #T8799 conducted in the presence and ~~absence~~ of microsomal enzymes indicate that because of toxicity and mutagenicity to the test system, the appropriate maximum dose to be plated in the mutagenicity assay in the presence of microsomal enzymes would be 75 ug per plate for Salmonella and 67 ug per plate for WP2uvr A and 33 ug per plate in the absence of microsomal enzymes for both Salmonella and WP2uvr A.

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DOSE RANGE-FINDING

STUDY RESULTS

Salmonella Mutagenicity Assay

Dose Range-Finding Study

Table 1

Test Article Id : Acrolein, Inhibited
 Study Number : T8799.502071
 Experiment No. : A1
 Date Plated : 07/07/89
 Counted by : machine
 Vehicle : dimethyl sulfoxide (DMSO)
 Plating Aliquot : 50ul

Test Article Concentration ug per plate	TA100 With S-9 Activation		TA100 Without Activation	
	Revertants per plate	Background Lawn *	Revertants per plate	Background Lawn *
Vehicle	147	1	93	1
10	109	1	249	1
33	125	1	464	1
67	246	1	0	5
100	194	1	0	5
333	0	5	0	5
667	0	5	0	5
1000	0	5	0	5
3333	0	5	0	5
6667	0	5	0	5
10000	0	5	0	5

*Background bacterial evaluation code

1=Normal 2=Slightly reduced 3=Moderately reduced
 4=Extremely reduced 5=Absent 6=Obscured by precipitate
 SP=Slight precipitate MP=Moderate precipitate HP=heavy precipitate

Escherichia coli Mutagenicity Assay

Dose Range-Finding Study

Table 1 Continued

Test Article Id : Acrolein, Inhibited
 Study Number : T8799.502071
 Experiment No. : A1
 Date Plated : 07/07/89
 Counted by : machine
 Vehicle : dimethylsulfoxide (DMSO)
 Plating Aliquot : 50ul

Test Article Concentration ug per plate	WP2uvr A With S-9 Activation		WP2uvr A Without Activation	
	Revertants per plate	Background Lawn *	Revertants per plate	Background Lawn *
Vehicle	81	1	81	1
10	51	1	16	1
33	40	1	36	1
67	26	1	0	3
100	0	2	0	4
333	0	5	0	5
667	0	5	0	5
1000	0	5	0	5
3333	0	5	0	5
6667	0	5	0	5
10000	0	5	0	5

*Background bacterial evaluation code

1=Normal 2=Slightly reduced 3=Moderately reduced
 4=Extremely reduced 5=Absent 6=Obscured by precipitate
 SP=Slight precipitate MP=Moderate precipitate HP=Heavy precipitate

B 12

DISCUSSION
OF THE
MUTAGENICITY ASSAY

The results of the Salmonella/Mammalian-Microsome
Preincubation Mutagenicity Assay (Ames Test) And Escherichia Coli
WP2uvr A Reverse Mutation Assay With A Confirmatory Assay of

Test Article ID: Acrolein, Inhibited
Lot No.: UTLX 89446
Sponsor: Baker Performance Chemicals Inc.
MBA Test Article ID: T8799

are presented in Tables 2 through 35. These data were generated in Experiment T8799-B2, T8799-B3, T8799-B4, T8799-B5 and T8799-B6.

In Experiment T8799-B1, no positive responses or no cytotoxicity were observed with any of the tester strains either in the presence or absence of microsomal enzymes. Based on the absence of cytotoxicity, the test system was not adequately exposed to the test article. Therefore, the results were not reported and the test was repeated in Experiment T8799-B2 using screw cap dilution tubes.

In Experiment T8799-B2, positive responses were observed with tester strains TA98 (2.1-fold, maximum increase) and TA100 (3.9-fold, maximum increase) in the absence of microsomal enzymes. No positive responses were observed with tester strains TA98, TA1537, TA1538, TA102, TA104 and WP2uvr A in the presence of microsomal enzymes and with tester strains TA1535, TA1537, TA1538 and WP2uvr A in the absence of microsomal enzymes. The data for tester strains and TA102 and TA104 in the absence of microsomal enzymes and tester strains TA100 and TA1535 in the presence of microsomal enzymes were not reported due to unacceptable positive control values. In Experiment T8799-B3, tester strains TA100 and TA1535 in the presence of microsomal enzymes and TA102 and TA104 in the absence of microsomal enzymes were retested.

In Experiment T8799-B3, no positive response was observed with tester strain TA1535 in the presence of microsomal enzymes. Tester strains TA102 and TA104 in the absence of microsomal enzymes and tester strain TA100 in the presence of microsomal enzymes were not reported due to unacceptable positive control values. In Experiment T8799-B4, tester strain TA100 in the presence of microsomal enzymes and TA102 and TA104 in the absence of microsomal enzymes were retested. Due to the unavailability of Difco bacto agar the laboratory was forced to switch to BBL select agar in May 1989. Since this switch, these three positive control values have repeatedly fallen short of the minimum 3-fold increase criteria. Therefore, additional dose levels of positive control were included. Due to the toxicity observed with tester strain TA104 using cumene hydroperoxide at 75 ug/plate, several lower dose levels were used. The positive control concentrations were as follows:

<u>Tester Strain</u>	<u>Positive Control</u>	<u>Dose Levels/Plate</u>
TA102 without S-9	cumene hydroperoxide	100, 150, 200 ug
TA104 without S-9	cumene hydroperoxide	10, 12, 15 ug
TA100 with S-9	2-aminoanthracene	0.25, 0.5, 1.0 ug

In Experiment T8799-B4, a positive response was observed with tester strain TA100 (2.2-fold, maximum increase) in the presence of microsomal enzymes. No positive responses were observed with tester strains TA102 and TA104 in the absence of microsomal enzymes. The confirmatory assay was conducted in Experiment T8799-B5.

In Experiment T8799-B5, positive responses were observed with tester strains TA98 (2.1-fold, maximum increase), TA100 (3.4-fold, maximum increase) and WP2uvr A (2.2-fold, maximum increase) in the presence of microsomal enzymes and with tester strains TA98 (2.4-fold, maximum increase) and TA100 (5.7-fold, maximum increase) in the absence of microsomal enzymes. The results with tester strains TA1538 and TA104 in the presence and absence of microsomal enzymes were not reported due to unacceptable vehicle control values; therefore, these tester strains were retested in Experiment T8799-B6. No other positive responses were observed with any of the remaining experimental conditions.

In Experiment T8799-B6, no positive response were observed with tester strains TA1538 and TA104 in the presence or absence of microsomal enzymes.

All criteria for a valid study were met as described in the protocol with the exception of the positive control values for tester strains TA100 with microsomal enzymes and TA102 and TA104 without microsomal enzymes. However, the inclusion of additional dose levels indicate that these tester strains were capable of detecting various dose levels of a mutagen or promutagen. Therefore, the study director has accepted the data generated with these tester strains.

C 01

MUTAGENICITY ASSAY RESULTS

Salmonella Mutagenicity Assay

Table 2

Test Article Id : Acrolein, Inhibited
 Study Number : T8799.502071
 Strain : TA98
 Liver Microsomes : None
 Vehicle : dimethylsulfoxide (DMSO)
 Plating Aliquot : 50ul
 Experiment No : B2
 Cells Seeded : 0.7 X 10⁸
 Date Plated : 08/01/89
 Counted by : hand

Concentration ug per plate	Plate Number	Revertants per plate	Background Code *	Average Revertants	Standard Deviation
Vehicle	01	16	1	14	4
	02	9	1		
	03	17	1		
0.3	01	20	1	19	2
	02	20	1		
	03	16	1		
1.0	01	13	1	15	3
	02	18	1		
	03	14	1		
3.3	01	23	1	24	8
	02	16	1		
	03	32	1		
10	01	31	2	29	9
	02	19	2		
	03	37	2		
33	01	0	4	0	0
	02	0	5		
	03	0	4		
**Positive Control 2-nitrofluorene 1.0 ug per plate					
	01	886	1	909	31
	02	898	1		
	03	944	1		

*Background bacterial evaluation code
 1=Normal 2=Slightly reduced 3=Moderately reduced
 4=Extremely reduced 5=Absent 6=Obscured by precipitate
 SP=Slight precipitate MP=Moderate precipitate HP=Heavy precipitate

** Positive control plates were machine counted

Salmonella Mutagenicity Assay

Table 3

Test Article Id : Acrolein, Inhibited
 Study Number : T8799.502071
 Strain : TA98
 Liver Microsomes : Rat liver S-9
 Vehicle : dimethylsulfoxide (DMSO)
 Plating Aliquot : 50ul
 Experiment No : B2
 Cells Seeded : 0.7 X 10⁸
 Date Plated : 08/01/89
 Counted by : hand

Concentration ug per plate	Plate Number	Revertants per plate	Background Code *	Average Revertants	Standard Deviation
Vehicle	01	21	1	19	2
	02	18	1		
	03	19	1		
6.7	01	22	1	19	4
	02	19	1		
	03	15	1		
10	01	23	1	26	3
	02	25	1		
	03	29	1		
33	01	19	1	22	7
	02	30	1		
	03	18	1		
67	01	6	3	12	6
	02	17	3		
	03	12	3		
75	01	0	5	0	0
	02	0	5		
	03	0	5		
**Positive Control 2-aminoanthracene 0.25 ug per plate					
	01	205	1	268	82
	02	361	1		
	03	237	1		

*Background bacterial evaluation code
 1=Normal 2=Slightly reduced 3=Moderately reduced
 4=Extremely reduced 5=Absent 6=Obscured by precipitate
 SP=Slight precipitate MP=Moderate precipitate HP=Heavy precipitate
 ** Positive control plates were machine counted

Salmonella Mutagenicity Assay

Table 4

Test Article Id : Acrolein, Inhibited
 Study Number : T8799.502071
 Strain : TA100
 Liver Microsomes : None
 Vehicle : dimethylsulfoxide (DMSO)
 Plating Aliquot : 50ul
 Experiment No : B2
 Cells Seeded : 4.7 X 10⁸
 Date Plated : 05/01/89
 Counted by : machine

Concentration ug per plate	Plate Number	Revertants per plate	Background Code *	Average Revertants	Standard Deviation
Vehicle	01	108	1	97	10
	02	89	1		
	03	94	1		
0.3	01	99	1	115	20
	02	138	1		
	03	108	1		
1.0	01	122	1	121	2
	02	119	1		
	03	121	1		
3.3	01	175	1	170	8
	02	173	1		
	03	161	1		
10	01	398	1	376	22
	02	376	1		
	03	355	1		
33	01	0	3	11	17
	02	3	3		
	03	31	3		
**Positive Control sodium azide 0.5 ug per plate					
	01	686	1	714	25
	02	728	1		
	03	729	1		

*Background bacterial evaluation code

1=Normal 2=Slightly reduced 3=Moderately reduced
 4=Extremely reduced 5=Absent 6=Obscured by precipitate
 SP=Slight precipitate MP=Moderate precipitate HP=Heavy precipitate

** Positive control plates were machine counted

Salmonella Mutagenicity Assay

Table 5

Test Article Id : Acrolein, Inhibited
 Study Number : T8799.502071
 Strain : TA1535
 Liver Microsomes : None
 Vehicle : dimethylsulfoxide (DMSO)
 Plating Aliquot : 50ul
 Experiment No : B2
 Cells Seeded : 3.5 X 10⁸
 Date Plated : 08/01/89
 Counted by : hand

Concentration ug per plate	Plate Number	Revertants per plate	Background Code *	Average Revertants	Standard Deviation
Vehicle	01	7	1	6	1
	02	5	1		
	03	6	1		
0.3	01	14	1	8	5
	02	4	1		
	03	7	1		
1.0	01	14	1	10	5
	02	5	1		
	03	10	1		
3.3	01	13	1	10	4
	02	6	1		
	03	12	1		
10	01	6	1	9	3
	02	9	1		
	03	12	1		
33	01	1	3	0	1
	02	0	3		
	03	0	3		
**Positive Control sodium azide 0.5 ug per plate					
	01	399	1	378	21
	02	379	1		
	03	357	1		

*Background bacterial evaluation code

1=Normal 2=Slightly reduced 3=Moderately reduced
 4=Extremely reduced 5=Absent 6=Obscured by precipitate
 SP=Slight precipitate MP=Moderate precipitate HP=Heavy precipitate

** Positive control plates were machine counted

Salmonella Mutagenicity Assay

Table 6

Test Article Id : Acrolein, Inhibited
 Study Number : T8799.502071
 Strain : TA1537
 Liver Microsomes : None
 Vehicle : dimethylsulfoxide (DMSO)
 Plating Aliquot : 50ul
 Experiment No : B2
 Cells Seeded : 0.8 X 10⁸
 Date Plated : 08/01/89
 Counted by : hand

Concentration ug per plate	Plate Number	Revertants per plate	Background Code *	Average Revertants	Standard Deviation
Vehicle	01	4	1		
	02	4	1		
	03	6	1	5	1
0.3	01	10	1		
	02	5	1		
	03	6	1	7	3
1.0	01	2	1		
	02	7	1		
	03	5	1	5	3
3.3	01	4	1		
	02	4	1		
	03	5	1	4	1
10	01	10	1		
	02	6	1		
	03	6	1	7	2
33	01	1	4		
	02	2	4		
	03	1	3	1	1
**Positive Control ICR-191 0.25 ug per plate					
	01	176	1		
	02	171	1		
	03	162	1	170	7

*Background bacterial evaluation code
 1=Normal 2=Slightly reduced 3=Moderately reduced
 4=Extremely reduced 5=Absent 6=Obscured by precipitate
 SP=Slight precipitate HP=Moderate precipitate HP=Heavy precipitate
 ** Positive control plates were machine counted

Salmonella Mutagenicity Assay

Table 7

Test Article Id : Acrolein, Inhibited
 Study Number : T8799.502071
 Strain : TA1537
 Liver Microsomes : Rat liver S-9
 Vehicle : dimethylsulfoxide (DMSO)
 Plating Aliquot : 50ul
 Experiment No : B2
 Cells Seeded : 0.8 X 10⁸
 Date Plated : 08/01/89
 Counted by : hand

Concentration ug per plate	Plate Number	Revertants per plate	Background Code *	Average Revertants	Standard Deviation
Vehicle	01	5	1	5	1
	02	6	1		
	03	5	1		
6.7	01	2	1	3	1
	02	3	1		
	03	3	1		
10	01	6	1	6	1
	02	7	1		
	03	5	1		
33	01	4	1	5	2
	02	7	1		
	03	4	1		
67	01	2	3	1	1
	02	0	3		
	03	1	3		
75	01	0	5	0	0
	02	0	5		
	03	0	5		
**Positive Control 2-aminoanthracene 0.25 ug per plate					
	01	35	1	24	12
	02	27	1		
	03	11	1		

*Background bacterial evaluation code
 1=Normal 2=Slightly reduced 3=Moderately reduced
 4=Extremely reduced 5=Absent 6=Obscured by precipitate
 SP=Slight precipitate MP=Moderate precipitate HP=Heavy precipitate
 ** Positive control plates were machine counted



Salmonella Mutagenicity Assay

Table 8

Test Article Id : Acrolein, Inhibited
 Study Number : T8799.502071
 Strain : TA1538
 Liver Microsomes : None
 Vehicle : dimethylsulfoxide (DMSO)
 Plating Aliquot : 50ul
 Experiment No : B2
 Cells Seeded : 1.0 X 10⁸
 Date Plated : 08/01/89
 Counted by : hand

Concentration ug per plate	Plate Number	Revertants per plate	Background Code *	Average Revertants	Standard Deviation
Vehicle	01	10	1	11	1
	02	10	1		
	03	12	1		
0.3	01	11	1	12	2
	02	14	1		
	03	12	1		
1.0	01	10	1	13	3
	02	16	1		
	03	14	1		
3.3	01	5	1	8	4
	02	13	1		
	03	6	1		
10	01	10	1	10	2
	02	12	1		
	03	9	1		
33	01	0	3	0	0
	02	0	3		
	03	0	3		
**Positive Control 2-nitrofluorene 1.0 ug per plate					
	01	1547	1	1634	80
	02	1650	1		
	03	1705	1		

*Background bacterial evaluation code

1=Normal

2=Slightly reduced

3=Moderately reduced

4=Extremely reduced

5=Absent

6=Obscured by precipitate

SP=Slight precipitate MP=Moderate precipitate

HP=heavy precipitate

** Positive control plates were machine counted

Salmonella Mutagenicity Assay

Table 9

Test Article Id : Acrolein, Inhibited
 Study Number : T8799.502071
 Strain : TA1538
 Liver Microsomes : Rat liver S-9
 Vehicle : dimethylsulfoxide (DMSO)
 Plating Aliquot : 50ul
 Experiment No : B2
 Cells Seeded : 1.0 X 10⁸
 Date Plated : 08/01/89
 Counted by : hand

Concentration ug per plate	Plate Number	Revertants per plate	Background Code *	Average Revertants	Standard Deviation
Vehicle	01	11	1	14	8
	02	9	1		
	03	23	1		
6.7	01	13	1	13	4
	02	17	1		
	03	10	1		
10	01	13	1	12	1
	02	13	1		
	03	11	1		
33	01	6	1	6	2
	02	5	1		
	03	8	1		
67	01	3	3	2	1
	02	1	3		
	03	2	3		
75	01	0	5	0	0
	02	0	5		
	03	0	5		
**Positive Control 2-aminoanthracene 0.25 ug per plate					
	01	299	1	278	32
	02	241	1		
	03	295	1		

*Background bacterial evaluation code

1=Normal 2=Slightly reduced 3=Moderately reduced
 4=Extremely reduced 5=Absent 6=Obscured by precipitate
 SP=Slight precipitate MP=Moderate precipitate HP=Heavy precipitate

** Positive control plates were machine counted

Salmonella Mutagenicity Assay

Table 10

Test Article Id : Acrolein, Inhibited
 Study Number : T8799.502071
 Strain : TA102
 Liver Microsomes : Rat liver S-9
 Vehicle : dimethylsulfoxide (DMSO)
 Plating Aliquot : 50ul
 Experiment No : B2
 Cells Seeded : 1.0 X 10⁸
 Date Plated : 03/01/89
 Counted by : machine

Concentration ug per plate	Plate Number	Revertants per plate	Background Code *	Average Revertants	Standard Deviation
Vehicle	01	386	1	380	27
	02	404	1		
	03	350	1		
6.7	01	374	1	374	3
	02	377	1		
	03	371	1		
10	01	336	1	352	14
	02	364	1		
	03	356	1		
33	01	370	1	364	11
	02	351	1		
	03	370	1		
67	01	85	2	90	8
	02	99	2		
	03	86	2		
71	01	17	3	23	12
	02	37	3		
	03	15	3		
**Positive Control sterigmatocystin 10.0 ug per plate					
	01	1438	1	1449	108
	02	1562	1		
	03	1346	1		

*Background bacterial evaluation code

1=Normal 2=Slightly reduced 3=Moderately reduced
 4=Extremely reduced 5=Absent 6=Obscured by precipitate
 SP=Slight precipitate MP=Moderate precipitate HP=Heavy precipitate

** Positive control plates were machine counted

Salmonella Mutagenicity Assay

Table 11

Test Article Id	: Acrolein, Inhibited		Experiment No	: B2	
Study Number	: T8799.502071		Cells Seeded	: 0.9 X 10 ⁸	
Strain	: TA104		Date Plated	: 08/01/89	
Liver Microsomes	: Rat liver S-9		Counted by	: machine	
Vehicle	: dimethylsulfoxide (DMSO)				
Plating Aliquot	: 50ul				

Concentration ug per plate	Plate Number	Revertants per plate	Background Code *	Average Revertants	Standard Deviation
Vehicle	01	253	1	249	3
	02	248	1		
	03	247	1		
6.7	01	243	1	251	7
	02	256	1		
	03	253	1		
10	01	293	1	284	13
	02	269	1		
	03	289	1		
33	01	355	1	349	5
	02	346	1		
	03	347	1		
67	01	4	3	5	2
	02	3	3		
	03	7	3		
75	01	C		0	0
	02	C	5		
	03	0	5		
**Positive Control 2-aminoanthracene 3.8 ug per plate					
	01	787	1	826	47
	02	841	1		
	03	880	1		

*Background bacterial evaluation code

- 1=Normal
- 2=Slightly reduced
- 3=Moderately reduced
- 4=Extremely reduced
- 5=Absent
- 6=Obscured by precipitate
- SP=Slight precipitate
- MP=Moderate precipitate
- HP=Heavy precipitate

** Positive control plates were machine counted
C=Contaminated

Escherichia coli Mutagenicity Assay

Table 12

Test Article Id : Acrolein, Inhibited
 Study Number : T8799.502071
 Strain : WP2uvrA
 Liver Microsomes : None
 Vehicle : dimethylsulfoxide (DMSO)
 Plating Aliquot : 50ul
 Experiment No : B2
 Cells Seeded : 1.9 X 10⁸
 Date Plated : 08/01/89
 Counted by : hand

Concentration ug per plate	Plate Number	Revertants per plate	Background Code *	Average Revertants	Standard Deviation
Vehicle	01	12	1	14	2
	02	16	1		
	03	15	1		
0.3	01	14	1	13	3
	02	15	1		
	03	10	1		
1.0	01	15	1	17	3
	02	15	1		
	03	20	1		
3.3	01	21	1	18	4
	02	14	1		
	03	18	1		
10	01	17	1	18	3
	02	21	1		
	03	15	1		
33	01	14	2	18	11
	02	30	2		
	03	9	2		
**Positive Control methyl methanesulfonate 1000 ug per plate					
	01	189	1	212	27
	02	206	1		
	03	242	-		

*Background bacterial evaluation code

1=Normal 2=Slightly reduced 3=Moderately reduced
 4=Extremely reduced 5=Absent 6=Obscured by precipitate
 SP=Slight precipitate MP=Moderate precipitate HP=Heavy precipitate

** Positive control plates were machine counted

Escherichia coli Mutagenicity Assay

Table 13

Test Article Id : Acrolein, Inhibited
 Study Number : T8799.502071
 Strain : WP2uvrA
 Liver Microsomes : Rat liver S-9
 Vehicle : dimethylsulfoxide (DMSO)
 Plating Aliquot : 50ul
 Experiment No : B2
 Cells Seeded : 1.9 X 10⁸
 Date Plated : 08/01/89
 Counted by : hand

Concentration ug per plate	Plate Number	Revertants per plate	Background Code *	Average Revertants	Standard Deviation
Vehicle	01	21	1	21	3
	02	24	1		
	03	18	1		
1.0	01	21	1	17	6
	02	10	1		
	03	21	1		
3.3	01	25	1	24	3
	02	21	1		
	03	26	1		
10	01	20	1	22	4
	02	27	1		
	03	19	1		
33	01	33	1	35	7
	02	29	1		
	03	43	1		
67	01	34	2	36	9
	02	45	2		
	03	28	2		
**Positive Control 2-aminoanthracene 10000 ug per plate					
	01	140	1	110	25
	02	108	1		
	03	90	1		

*Background bacterial evaluation code

1=Normal 2=Slightly reduced 3=Moderately reduced
 4=Extremely reduced 5=Absent 6=Obscured by precipitate
 SP=Slight precipitate MP=Moderate precipitate HP=Heavy precipitate

** Positive control plates were machine counted

Salmonella Mutagenicity Assay

Table 14

Test Article Id : Acrolein, Inhibited
 Study Number : T8799.502071
 Strain : TA1535
 Liver Microsomes : Rat liver S-9
 Vehicle : dimethylsulfoxide (DMSO)
 Plating Aliquot : 50ul
 Experiment No : B3
 Cells Seeded : 1.7 X 10⁸
 Date Plated : 08/11/89
 Counted by : hand

Concentration ug per plate	Plate Number	Revertants per plate	Background Code *	Average Revertants	Standard Deviation
Vehicle	01	11	1	9	2
	02	9	1		
	03	7	1		
3.3	01	8	1	8	2
	02	7	1		
	03	10	1		
6.7	01	11	1	12	1
	02	13	1		
	03	11	1		
10	01	9	1	8	1
	02	7	1		
	03	7	1		
33	01	17	1	10	5
	02	7	1		
	03	6	1		
67	01	11	1	12	2
	02	10	1		
	03	14	1		
**Positive Control 2-aminoanthracene 0.25 ug per plate					
	01	27	1	29	3
	02	32	1		
	03	27	1		

*Background bacterial evaluation code
 1=Normal 2=Slightly reduced 3=Moderately reduced
 4=Extremely reduced 5=Absent 6=Obscured by precipitate
 SP=Slight precipitate NP=Moderate precipitate HP=Heavy precipitate
 ** Positive control plates were machine counted

Salmonella Mutagenicity Assay

Table 15

Test Article Id : Acrolein, Inhibited
 Study Number : T8799.502071
 Strain : TA100
 Liver Microsomes : Rat liver S-9
 Vehicle : dimethylsulfoxide (DMSO)
 Plating Aliquot : 50ul
 Experiment No : B4
 Cells Seeded : 1.0 X 10⁸
 Date Plated : 08/30/89
 Counted by : machine

Concentration ug per plate	Plate Number	Revertants per plate	Background Code *	Average Revertants	Standard Deviation
Vehicle	01	144	1	142	12
	02	153	1		
	03	130	1		
3.3	01	173	1	168	6
	02	161	1		
	03	169	1		
6.7	01	129	1	130	21
	02	110	1		
	03	152	1		
10	01	136	1	130	7
	02	130	1		
	03	123	1		
33	01	363	1	315	48
	02	268	1		
	03	315	1		
67	01	C		312	54
	02	350	2		
	03	273	2		
**Positive Control 2-aminoanthracene 0.5 ug per plate					
	01	690	1	679	19
	02	690	1		
	03	657	1		

*Background bacterial evaluation code
 1=Normal 2=Slightly reduced 3=Moderately reduced
 4=Extremely reduced 5=Absent 6=Obscured by precipitate
 SP=Slight precipitate MP=Moderate precipitate HP=Heavy precipitate
 ** Positive control plates were machine counted
 C=Contaminated

Salmonella Mutagenicity Assay

Table 16

Test Article Id : Acrolein, Inhibited
 Study Number : T8799.502071
 Strain : TA102
 Liver Microsomes : None
 Vehicle : dimethylsulfoxide (DMSO)
 Plating Aliquot : 50ul
 Experiment No : B4
 Cells Seeded : 1.6 X 10⁸
 Date Plated : 08/30/89
 Counted by : machine

Concentration ug per plate	Plate Number	Revertants per plate	Background Code *	Average Revertants	Standard Deviation
Vehicle	01	245	1	252	8
	02	252	1		
	03	260	1		
0.3	01	262	1	251	10
	02	248	1		
	03	243	1		
1.0	01	204	1	235	29
	02	241	1		
	03	261	1		
3.3	01	209	1	200	10
	02	190	1		
	03	201	1		
10	01	144	1	162	34
	02	201	1		
	03	142	1		
33	01	24	3	25	1
	02	26	3		
	03	24	3		
**Positive Control cumene hydroperoxide 100 ug per plate					
	01	974	1	1008	30
	02	1017	1		
	03	1032	1		

*Background bacterial evaluation code

1=Normal 2=Slightly reduced 3=Moderately reduced
 4=Extremely reduced 5=Absent 6=Obscured by precipitate
 SP=Slight precipitate MP=Moderate precipitate HP=Heavy precipitate

** Positive control plates were machine counted

Salmonella Mutagenicity Assay

Table 17

Test Article Id : Acrolein, Inhibited
 Study Number : T8799.502071
 Strain : TA104
 Liver Microsomes : None
 Vehicle : dimethylsulfoxide (DMSO)
 Plating Aliquot : 50ul
 Experiment No : B4
 Cells Seeded : 0.7 X 10⁸
 Date Plated : 08/30/89
 Counted by : machine

Concentration ug per plate	Plate Number	Revertants per plate	Background Code *	Average Revertants	Standard Deviation
Vehicle	01	284	1	238	48
	02	241	1		
	03	188	1		
0.3	01	289	1	264	29
	02	233	1		
	03	271	1		
1.0	01	288	1	259	47
	02	284	1		
	03	205	1		
3.3	01	296	1	286	15
	02	294	1		
	03	269	1		
10	01	335	1	323	15
	02	328	1		
	03	307	1		
33	01	0	4	1	1
	02	2	4		
	03	0	4		
**Positive Control cumene hydroperoxide 10 ug per plate					
	01	1082	1	1104	24
	02	1101	1		
	03	1129	1		

*Background bacterial evaluation code

- 1=Normal
- 2=Slightly reduced
- 3=Moderately reduced
- 4=Extremely reduced
- 5=Absent
- 6=Obscured by precipitate
- SP=Slight precipitate
- MP=Moderate precipitate
- HP=Heavy precipitate

** Positive control plates were machine counted

Salmonella Mutagenicity Assay

Table 18

Test Article Id : Acrolein, Inhibited
 Study Number : T8799.502071
 Strain : TA98
 Liver Microsomes : None
 Vehicle : dimethylsulfoxide (DMSO)
 Plating Aliquot : 50ul
 Experiment No : B5
 Cells Seeded : 2.2 X 10⁸
 Date Plated : 09/20/89
 Counted by : hand

Concentration ug per plate	Plate Number	Revertants per plate	Background Code *	Average Revertants	Standard Deviation
Vehicle	01	14	1	12	4
	02	14	1		
	03	7	1		
1.0	01	10	1	12	7
	02	19	1		
	03	6	1		
3.3	01	17	1	14	4
	02	15	1		
	03	10	1		
6.7	01	22	1	25	4
	02	23	1		
	03	30	1		
10	01	31	1	29	2
	02	28	1		
	03	28	1		
20	01	3	3	2	1
	02	2	3		
	03	2	3		
**Positive Control 2-nitrofluorene 1.0 ug per plate					
	01	640	1	627	13
	02	614	1		
	03	628	1		

*Background bacterial evaluation code

1=Normal 2=Slightly reduced 3=Moderately reduced
 4=Extremely reduced 5=Absent 6=Obscured by precipitate
 SP=Slight precipitate MP=Moderate precipitate HP=Heavy precipitate

** Positive control plates were machine counted

Salmonella Mutagenicity Assay

Table 19

Test Article Id : Acrolein, Inhibited
 Study Number : T8799.502071
 Strain : TA98
 Liver Microsomes : Rat liver S-9
 Vehicle : dimethylsulfoxide (DMSO)
 Plating Aliquot : 50ul
 Experiment No : B5
 Cells Seeded : 2.2 X 10⁸
 Date Plated : 09/20/89
 Counted by : hand

Concentration ug per plate	Plate Number	Revertants per plate	Background Code *	Average Revertants	Standard Deviation
Vehicle	01	12	1	15	6
	02	22	1		
	03	12	1		
6.7	01	15	1	17	3
	02	16	1		
	03	21	1		
10	01	18	1	19	4
	02	23	1		
	03	16	1		
33	01	20	1	31	10
	02	33	1		
	03	39	1		
50	01	32	2	31	1
	02	32	2		
	03	30	2		
67	01	16	3	18	5
	02	14	3		
	03	24	3		
**Positive Control 2-aminoanthracene 0.25 ug per plate					
	01	182	1	172	18
	02	183	1		
	03	152	1		

*Background bacterial evaluation code
 1=Normal 2=Slightly reduced 3=Moderately reduced
 4=Extremely reduced 5=Absent 6=Obscured by precipitate
 SP=Slight precipitate MP=Moderate precipitate HP=Heavy precipitate
 ** Positive control plates were machine counted

Salmonella Mutagenicity Assay

Table 20

Test Article Id : Acrolein, Inhibited
 Study Number : T8799.502071
 Strain : TA100
 Liver Microsomes : None
 Vehicle : dimethylsulfoxide (DMSO)
 Plating Aliquot : 50ul
 Experiment No : B5
 Cells Seeded : 2.3 X 10⁸
 Date Plated : 09/20/89
 Counted by : machine

Concentration ug per plate	Plate Number	Revertants per plate	Background Code *	Average Revertants	Standard Deviation
Vehicle	01	176	1	127	43
	02	95	1		
	03	110	1		
3.3	01	314	1	238	66
	02	204	1		
	03	196	1		
6.7	01	108	1	236	113
	02	323	1		
	03	276	1		
10	01	310	1	335	33
	02	323	1		
	03	373	1		
20	01	740	1	730	12
	02	733	1		
	03	717	1		
33	01	0	3	0	0
	02	0	3		
	03	0	3		
**Positive Control sodium azide 0.5 ug per plate					
	01	386	1	409	40
	02	386	1		
	03	455	1		

*Background bacterial evaluation code

1=Normal 2=Slightly reduced 3=Moderately reduced
 4=Extremely reduced 5=Absent 6=Obscured by precipitate
 SP=Slight precipitate MP=Moderate precipitate HP=Heavy precipitate

** Positive control plates were machine counted

FINAL REPORT

Study Title

SALMONELLA/MAMMALIAN-MICROSOME
PREINCUBATION MUTAGENICITY ASSAY
(AMES TEST)
AND
ESCHERICHIA COLI WP2uvr A
REVERSE MUTATION ASSAY
WITH A CONFIRMATORY ASSAY

Test Article

Acrolein, Inhibited

Authors

Richard H.C. San, Ph.D.
Keren A. Springfield, B.S.

Study Report Date

10/26/89

Performing Laboratory

Microbiological Associates, Inc.
9900 Blackwell Road
Rockville, MD 20850

Laboratory Study Number

T8799.502071

Submitted To

Baker Performance Chemicals Inc.
3920 Essex Lane
Houston, TX 77027

THIS PAGE REVISED	Study No. 78799.502071	Date
	<i>Revised</i>	11/9/90
Signature		Date

FINAL REPORT
SALMONELLA/MAMMALIAN-MICROSOME
PREINCUBATION MUTAGENICITY ASSAY
 (AMES TEST)
 AND
ESCHERICHIA COLI WP2uvr A
REVERSE MUTATION ASSAY
 WITH A CONFIRMATORY ASSAY

Sponsor: Baker Performance Chemicals Inc.
 3920 Essex Lane
 Houston, TX 77027

Testing Facility: Microbiological Associates, Inc. (MBA)
 9900 Blackwell Road
 Rockville, Maryland 20850

MBA Study No.: T8799.502071

Test Article I.D.: Acrolein, Inhibited

Test Article Lot No.: UTLX 89446

Test Article Description: Clear liquid

Test Article Purity (Determined by sponsor): 96.58%

Storage Conditions: 4°C

Date Received: 06/29/89

Date Study Started: 07/06/89

Date Study Completed: 10/26/89

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Study No. <u>T8799.502071</u>	
<u>Richard H.C. San</u>	<u>11/9/90</u>
Signature	Date

Sponsor's Representative: Richard A. Parent, Ph.D., D.A.B.T.
 Consultox, Ltd.

Study Director: Richard H.C. San, Ph.D.
 Microbiological Associates, Inc.

<u>Richard H.C. San</u>	<u>11/9/90</u>	<u>Valentine O. Wagner, III</u>	<u>11/9/90</u>
Richard H.C. San, Ph.D.	Date	Valentine O. Wagner, III, M.S.	Date
Study Director		Department Head	
<u>Mark B. Schadly</u>	<u>11/9/90</u>	<u>Christine Krueel</u>	<u>11/9/90</u>
Mark B. Schadly, B.S.	Date	Christine Krueel, B.S.	Date
Biologist II		Biologist	
<u>Karen A. Springfield</u>	<u>11/9/90</u>	<u>J. Blair Shelton</u>	<u>11/9/90</u>
Karen A. Springfield, B.S.	Date	J. Blair Shelton, R.S.	Date
Biologist		Biologist	
<u>Dale E. Ozarowski</u>	<u>11/9/90</u>		
Dale E. Ozarowski, B.S.	Date		
Biologist			

TABLE OF CONTENTS

Introduction page 7
Conclusions. page 7
Materials and Methods. page 9
Discussion of the Dose Range-Finding Study page 22
Dose Range-Finding Study Results page 24
Discussion of the Mutagenicity Assay page 27
Mutagenicity Assay Results page 30
Appendix A (Study Protocol). page 65
Appendix B (List of materials and supplies). page 82

MBA Study No. T8799.502071 5

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Introduction

Baker Performance Chemicals Inc. submitted

Test Article ID: Acrolein, Inhibited
Lot No.: UTLX 89446
Receipt Date: 06/29/89
MBA Test Article ID: T8799

for testing in the Salmonella/Mammalian-Microsome Preincubation Mutagenicity Assay (Ames Test) and Escherichia Coli WP2uvr A Reverse Mutation Assay With A Confirmatory Assay. This assay evaluates the mutagenic potential of the test article (or its metabolites) for its ability to induce back mutations at selected loci of several strains of Salmonella typhimurium in the presence and absence of microsomal enzymes derived from Aroclor 1254 induced rat liver. The tester strains used in this study were TA98, TA100, TA1535, TA1537, TA1538, TA102, TA104 and WP2uvr A.

Conclusions

The results of the Salmonella/Mammalian-Microsome Preincubation Mutagenicity Assay (Ames Test) and Escherichia Coli WP2uvr A Reverse Mutation Assay With A Confirmatory Assay indicate that under the conditions of this study, Baker Performance Chemicals Inc.'s test article Acrolein, Inhibited (MBA# T8799) did cause positive responses with tester strains TA98 and TA100 in the presence and absence of microsomal enzymes and with tester strain WP2uvr A in the presence of microsomal enzymes. No mutagenic responses were observed with tester strains TA1535, TA1537, TA1538, TA102 and TA104 in the presence and absence of microsomal enzymes and with tester strain WP2uvr A in the absence of microsomal enzymes.

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disk containing 10 ug of ampicillin was added. Resistance was demonstrated by bacterial growth in the zone immediately surrounding the disk.

2.6.3 Characteristic Number of Spontaneous Revertants: Spontaneous reversion frequencies in the vehicle controls that are characteristic of the respective strains were demonstrated by plating 100 ul aliquots of the culture along with the appropriate vehicle on selective media.

3.0 EXPERIMENTAL DESIGN

The assay was performed in two phases. The first phase, the dose-range finding study, was used to establish the dose-range over which the test article would be assayed. The second phase, the mutagenicity assay, was used to evaluate the mutagenicity of the test article under the conditions of the assay.

3.1 Dose Range-Finding Study: Ten dose levels of the test article were plated per tester strain, one plate per dose, with an overnight culture of TA100 and WP2uvr A on selective minimal agar in both the presence and absence of microsomal enzymes.

3.2 Mutagenicity Assay: The test article was tested at five dose levels along with appropriate vehicle and positive controls on tester strains TA98, TA100, TA1535, TA1537, TA1538, TA102, TA104 and WP2uvr A in the presence and absence of S-9 mix. In the mutagenicity assay, all dose levels of the test article, vehicle controls and positive controls were plated in triplicate. A confirmatory mutagenicity assay was conducted with all of the above tester strains in the presence and absence of microsomal enzymes.

3.3 Frequency and Route of Administration: The test system was exposed to the test article via the preincubation modification of the Ames test originally described by Yahagi et al. (1977). This methodology has been shown to detect a wide range of classes of chemical mutagens.

4.0 CONTROLS

4.1 Positive Controls: The positive controls plated concurrently with the assay are listed in Figure 2.

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APPENDIX A
STUDY PROTOCOL

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A 01



PATHOLOGY WORKING GROUP REPORT
ON THE LIFETIME EXPERIMENTAL
STUDY OF ACROLEIN
IN FEMALE F344 RATS

PATHCO No. 90-49

Submitted To:

Consuitox Ltd.
P.O. Box 14082
Baton Rouge, LA 70898

Prepared by:

Dawn G. Goodman, V.M.D.
Pathology Working Group Chairperson
Diplomate, American College of
Veterinary Pathologists

PATHCO, Inc.
10075 Tyler Place, Suite 16
Ijamsville, MD 21754

September 6, 1990

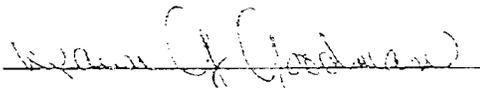


Acrolein

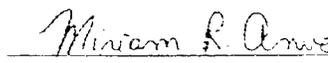
PATHOLOGY WORKING GROUP (PWG)

SPECIES : F344 Rats
TYPE OF STUDY : Life-time Experimental Study
LABORATORY : NCI-Frederick Cancer Research Center
PATHOLOGIST : Melvin D. Reuber, M.D.
DATE OF PWG : August 22, 1990
SITE OF PWG : PATHCO, Inc.
10075 Tyler Place #16
Ijamsville, MD 21754

PWG PARTICIPANTS :



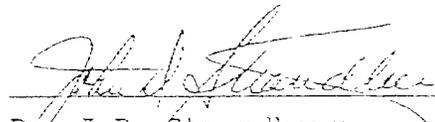
Dr. D.G. Goodman
PATHCO, Inc. (Chairperson)



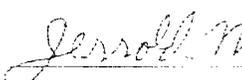
Dr. M.R. Anver
Clement Associates



Dr. W.R. Brown
Research Pathology Services, Inc.



Dr. J.D. Strandberg
Johns Hopkins University



Dr. J.M. Ward
Consultant



TABLE OF CONTENTS

PATHOLOGY WORKING GROUP SUMMARY SHEET

PATHOLOGY WORKING GROUP REPORT

- I. SUMMARY AND CONCLUSIONS OF THE PATHOLOGY WORKING GROUP (PWG) REVIEW
- II. INTRODUCTION
- III. STUDY DESIGN
- IV. AUDIT OF SLIDES AND BLOCKS
- V. CONDUCT OF THE PWG REVIEW
 - A. Chairperson's Review
 - B. PWG Review
- VI. FINDINGS AND INTERPRETATIONS
 - A. Adrenal Neoplasms
 - B. Nonneoplastic Lesions of the Adrenal Gland
 - C. Interpretation and Conclusions

REFERENCES

APPENDICES

- APPENDIX A Chairperson's Worksheets
- APPENDIX B Inventory of Slides Received from FCRC (July 23, 1990)
- APPENDIX C Recut Requests by PATHCO, Inc.
- APPENDIX D Inventory of Slides Received from FCRC (August 10, 1990)
- APPENDIX E Other Data Supplied by FCRC
- APPENDIX F C.V.s
 - D.G. Goodman, Chairperson
 - M.R. Anver, Participant
 - W.R. Brown, Participant
 - J.D. Strandberg, Participant
 - J.M. Ward, Participant
- APPENDIX G Literature

PATHCO REPORT NO. 31-B
ON THE LIFE-SPAN OF F344 RATS
ACROLEIN IN DRINKING WATER
PATHCO NO. 31-B

I. SUMMARY AND CONCLUSIONS OF THE PATHOLOGY WORKING GROUP
(PWG) REVIEW

The PWG examination of the adrenal glands from the female F344 rats given 625 ppm of acrolein in the drinking water in a lifetime study found no compound-related morphologic changes. No cortical tumors were diagnosed, and the incidence of pheochromocytomas was within the range of the historical database. Therefore, there is no evidence in this study to indicate that acrolein has any carcinogenic effect on the adrenal glands of female F344 rats.

II. INTRODUCTION

PATHCO, Inc., was requested to conduct an independent panel review of the slides from the lifetime experimental study of acrolein in F344 rats. The study was conducted at the NCI-Frederick Cancer Research Facility (FCRC) by Dr. W. Lijinsky and Dr. M. D. Reuber. The results were subsequently published (Lijinsky and Reuber, 1987 [Appendix G]). In this study, 25% (5/20) of the high dose (625 ppm) female rats were reported as having adenomas of the adrenal cortex and hyperplastic nodules of the adrenal cortex were reported in 10% (2/20) of the high dose animals. Adrenal tumors were not found in male rats. This is the only study reported which might indicate a possible carcinogenic effect of acrolein. It was subsequently found that this study did not have adequate controls (see Section IV).

III. STUDY DESIGN

Acrolein was administered in the drinking water to male F344 rats at dosage levels of 100, 250, and 625 ppm for 5 days a week for up to 132 weeks. Female rats received 625 ppm only. There were 20 animals per sex per dose group at the start of the study. The control group arrangement is discussed in Section IV.



IV. AUDIT OF SLIDES AND BLOCKS

Hematoxylin and eosin stained microscope slides were received from FCRC by PATHCO. Upon receipt of the slides, it was noted that the number of slides received did not match the number of blocks listed on the inventory (Appendix B), i.e. multiple slides per animal were missing. It was determined that for the female rats, only 2 adrenal slides (one control and one treated) had been received. Male rats were not reviewed as no compound-related effect was reported by Lijinsky and Reuber (1987).

Because of the missing slides, a slide/block match-up was performed by an FCRC technician and Dr. Dawn G. Goodman of PATHCO on all female rats from the acrolein study (Group MP - 625 ppm, high dose, acrolein and Group OE - controls). At this time, the FCRC laboratory provided the original protocol for trimming the tissues for the acrolein study (Appendix E). This protocol indicated that in both experimental and control groups only adrenals with gross lesions had been processed. At a later time, apparently upon request of the study pathologist, additional tissues were processed (designated as retrim blocks).

Results of the slide/block match-up were as follows. One adrenal slide was present for a single female rat in the acrolein group (Group MP). Blocks for adrenals were found for all animals in the acrolein-treated group; most of these were retrim blocks. In the designated control group (Group OE), one adrenal slide and its corresponding block were available. No retrim blocks of adrenal were available for Group OE. It was unclear whether adrenals had been processed for histology in Group OE. In addition, the wet tissues for this group (OE) had been discarded (Appendix E).

To assure that all adrenals reported by the original investigators were available, all blocks with no corresponding slide (except the block containing the aorta which was readily identifiable) were recut for both the treated and control groups (Appendix C). Even though the original slides were available for the adrenals from two animals (one Group MP rat and one Group OE rat), these blocks were also recut. All recut slides were made available to PATHCO (Appendix D).

Based on the above information, it is apparent that there is no appropriate control group for the acrolein study in female F344 rats. The data reported in the paper by



Lijinsky and Reuber (1987) is apparently based upon microscopic evaluation of the adrenals in the treated animals and gross examination of the adrenals in the controls. It is not known whether additional control adrenal glands were examined microscopically in the initial study or if the results reported were based solely on gross findings. Most lesions of the adrenal gland are found upon microscopic examination rather than by gross observation.

Because only one adrenal slide/block and no wet tissue was available from the original designated control group (Group OE), an additional control group (Group QN) with 40 animals was identified. This group appeared to have been on study at approximately the same time as the acrolein study. The start dates for the experiments involving the three groups (Groups MP, OE, and QN) were provided by the FCRC laboratory (Appendix E). The acrolein-treated group was started in September, 1978, the designated control (Group OE) in July, 1979, and the additional control (Group QN) in April, 1980.

Only one adrenal slide was available from the designated control group (Group OE) for the acrolein study. In addition, the designated control group (Group OE) is inappropriate to use as a control for the acrolein study since apparently the adrenal glands were not examined microscopically for all animals. It should be noted that the designated control group (Group OE) was started approximately 10 months later than the acrolein-treated animals. This group is not a concurrent control group. It was determined that slides/blocks for the adrenals of 34 of the 40 animals were available for the additional control group (Group QN). The slides for the adrenals from this group were obtained by PATHCO to provide reference data (Appendix D). This additional control group (Group QN) was started approximately 19 months after the acrolein-treated animals. Thus, both control groups (OE and QN) can be considered laboratory controls rather than study controls since neither was started concurrently with the acrolein-treated group.

V. CONDUCT OF THE PWG

The Pathology Working Group was chaired by Dr. Dawn S. Goodman who organized and presented the material to a panel of four additional pathologists. The other members of the PWG were Dr. M.R. Anver, Dr. W.R. Brown, Dr. J.D. Strandberg, and Dr. J.M. Ward. Dr. R.A. Parent of Consultox was present



as an observer. C.V.s for the Chairperson and the PWG participants are presented in Appendix F.

A. Chairperson's Review

Only data from female rats were considered in this review. The data available included:

- 1) summary data presented in the published report (Lijinsky and Reuber, 1987 [Appendix G]);
- 2) necropsy records for control (Group OE) and acrolein-treated animals (Group MP);
- 3) all microscope slides for control (Group OE) and acrolein-treated animals (Group MP), including recut slides for those originally missing;
- 4) all microscope slides for adrenals from the additional control group (Group QN).

The individual animal diagnoses made by the study pathologist (Dr. M.D. Reuber) were not available. The original microscope slides for adrenals from the acrolein-treated animals were not available. However, recuts from the original blocks were made. These slides were used in this review.

The Chairperson evaluated all the adrenal slides available from the acrolein-treated animals (Group MP), the designated control group (Group OE), and the additional control group (Group QN). The results of her review are presented in Appendix A.

B. PWG Review

The PWG examined coded slides without knowledge of treatment or dose group. All adrenal slides supplied by FCRC for female rats were reviewed including those from the acrolein-treated animals (Group MP), the designated control group (Group OE), and the additional control group (Group QN).

The participants each recorded their diagnoses and/or comments on their worksheets. The worksheets are on file at PATHCO, Inc. Each lesion was discussed by the group, re-examined, if necessary, and the final opinions were recorded on the Chairperson's worksheets (Appendix A). In determining the PWG diagnosis, the diagnoses of the Chairperson and the 4 PWG members were considered. The PWG



diagnosis for a lesion was determined when at least 3 out of the 5 pathologists agreed.

After the PWG completed the slide review and diagnoses were recorded, the slides were decoded by treatment group and the lesion incidences were tabulated. The results were then discussed and conclusions drawn by the PWG.

VI. INTERPRETATIONS AND CONCLUSIONS

Prior to examination of the slides by the PWG, there was a PWG discussion on the diagnostic criteria for adrenal cortical neoplasms, adrenal cortical hyperplasia, and hypertrophy. The criteria used by the PWG for the diagnosis of adrenal cortical lesions are currently accepted criteria (Strandberg, 1983, a,b,c [Appendix C]) and are described in detail below.

A. Adrenal Neoplasms

Adrenal cortical adenomas usually involve the zona fasciculata and/or zona reticularis. They are generally well-demarcated from surrounding parenchyma by compression; however, they are not encapsulated. There is distortion and loss of the normal architectural pattern of the affected cortex. The cords are irregular and branching and may be more than one cell layer thick. The cells are often enlarged with abundant eosinophilic cytoplasm and large round centrally placed nuclei with prominent nucleoli. In some neoplasms, the cells may be smaller than normal with amphophilic or slightly basophilic cytoplasm. Cellular atypia is minimal and the presence of mitotic figures are variable. Fatty change, angiectasis, hemorrhage or thrombosis may be present. When present, they are often seen in the portion of the tumor closest to the adrenal medulla.

Adrenal cortical carcinomas are large irregularly-shaped masses involving large areas of the cortex. They often compress the medulla and distort or invade the adrenal capsule. Invasion of adjacent structures is common. Within the tumor there is complete loss of the normal cortical architecture with the cells arranged in irregular cords, trabeculae, solid nests, or combinations thereof. The cells are frequently large, with eosinophilic cytoplasm and round nuclei. However, cellular pleomorphism and necrosis are common. Cellular atypia and the mitotic index are quite variable. As with the adenomas, fatty change, angiectasis, and thrombosis may occur within the tumor.



Pheochromocytomas are neoplasms of the adrenal medulla. They are usually easily distinguished from adrenal cortical neoplasms. Pheochromocytomas are composed of polyhedral cells with amphophilic or basophilic cytoplasm and centrally located nuclei. The cells are arranged in trabeculae or nests separated by dilated vascular spaces. There is compression of the adjacent tissue. It is difficult to distinguish benign from malignant pheochromocytomas on the basis of cytology alone. By convention, tumors which have invaded the adrenal capsule or have spread beyond the adrenal gland are diagnosed as malignant. Those still within the adrenal gland, even if replacing most of the adrenal cortex, are considered benign.

B. Nonneoplastic Lesions of the Adrenal Gland

Focal adrenal cortical hyperplasia consists of a poor to moderately well-demarcated focus within the zona fasciculata and/or zona reticularis. There may be some compression of the adjacent parenchyma but this is not a prominent feature. Within the focus, the cells, although increased in number, maintain normal architectural relationships. There is often crowding of the cells which are generally smaller than usual, and the cords may be more tortuous than usual. The cells usually have small amounts of slightly basophilic cytoplasm, and the round vesicular nuclei are often smaller than normal as well. In some lesions the cells may be enlarged with abundant eosinophilic cytoplasm or a combination of small and large cells may be present. Fatty change may be present, most commonly affecting the portion of the lesion adjacent to the medulla.

Focal adrenal cortical hypertrophy consists of a focus of cortical cells which are markedly enlarged with abundant eosinophilic cytoplasm and large round vesicular nuclei. The normal cord architecture is maintained. Fatty change may also be present.

Focal vacuolation (fatty change) consists of a focus of cells with one or more clear cytoplasmic vacuoles. When this becomes severe, there may be cell loss and formation of cystic spaces. When this occurs, it is termed cystic degeneration.

C. Interpretation and Conclusions

The results of the PWG examination are summarized in the Table I. There was general agreement on the diagnoses by all



pathologists participating in the PWG. While the original slides were not available for examination, with the exception of 2 animals, the fact that most recuts contained both cortical and medullary tissue indicated that neoplasms would be unlikely to be missed or to have been cut through. Microscopically, there were lesions observed, predominantly in the adrenal cortex, that were typical of those that occur spontaneously in aged female F344 rats. The most common lesions were foci of hyperplasia, hypertrophy, and/or vacuolation of cells mostly in the zona fasciculata. For purposes of analysis, hyperplastic and hypertrophic lesions were considered equivalent and tabulated together. The incidence of these lesions was higher in the laboratory control female rats (Group QN) than in acrolein-treated group (MP) (Table I). Although adrenal slides from the laboratory control group (QN) only were available for examination, in the experience of the PWG members, the incidences of these changes are within the expected range for aged female rats of this strain.

One cortical adenoma was diagnosed in a laboratory control group (QN) rat and none in the acrolein treatment group (MP) or the designated control group (OE). The PWG results are in contrast to the original published results in which five cortical adenomas were diagnosed in the acrolein treatment group and one in the control group.

Medullary tumors (pheochromocytomas) were diagnosed by the PWG in three female rats in each of the acrolein group (MP) and laboratory control (QN) group and in the single control group OE rat. Although the incidence of pheochromocytoma was slightly higher in the acrolein-treated rats (15%) than in the laboratory control rats (9%), this difference was not considered to be of any biological relevance. A 15% incidence of pheochromocytoma has been reported in control female F344 rats from lifetime studies (Solleveld, et.al. 1984 [Appendix G]).

Based on findings of the PWG, there were no compound-related toxic or proliferative lesions of the adrenal cortex. The PWG did not identify cortical neoplasms in the acrolein group (MP); the only proliferative cortical lesions diagnosed were focal hyperplasia and hypertrophy. These nonneoplastic lesions occurred at a lower incidence than in the laboratory control group QN (Table I). The disparity in cortical adenoma incidences between the PWG review and the published report (Lijinsky and Reuber, 1987 [Appendix G]) are likely a result of the more clearly defined diagnostic criteria for

A 11



proliferative lesions used by the PWG. It is possible that the original study pathologist diagnosed the focal proliferative lesions of cortical hypertrophy/hyperplasia as benign tumors. Based on the current and accepted criteria for proliferative/ neoplastic lesions of the adrenal cortex it is the opinion of the PWG that these lesions are nonneoplastic changes.

In conclusion, it is the opinion of the PWG that there is no evidence of any carcinogenic effect of acrolein on the adrenal glands of female rats in this study.



TABLE 1
 Incidences of Adrenal Lesions
 in Female F344 Rats

Group Dose	MP (acrolein - 625ppm)	OE (designated control)	QN (laboratory control)
# Adrenals Examined Microscopically	20	1	34
Cortical Adenoma	--	--	1 (3%)
Cortical Hyperplasia/ hypertrophy	7 (35%)	--	16 (48%)
Cortical Vacuolation (Focal)	8 (40%)	1	9 (50%)
Cortical Diffuse Vacuolation	--	--	2 (6%)
Cortical Cystic Degeneration	--	--	1 (3%)
Pheochromocytoma	3 (15%)	1	3 (9%)



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A 14

APPENDIX A
Chairperson's Worksheets