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Degussa-Hüls Corporation  
2 Turner Place, Piscataway, NJ 08855-0365  
732-560-6800

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April 12, 2000

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Corporation

Document Processing Center (TS-790)  
Office of Toxic Substances  
U.S. Environmental Protection Agency  
401 M Street, SW  
Washington, DC 20460

ATTN: Section 8 (e) Coordinator

RE: Product Name: Allyl Methacrylate  
CAS Registry No. 96-05-9  
CAS Registry Name: 2-Propenoic acid, 2-methyl-, 2-propenyl ester



8EHQ-99-14632

Dear Sir or Madam:

Degussa-Hüls Corporation has received from Röhm GmbH, the enclosed report on "Allyl Methacrylate Acute (Four- Hour) Inhalation Study in Rats," which studies were conducted by Huntingdon Life Sciences. This is a follow up to the TSCA 8(e) submission made December 22, 1999 on the subject product.

Pursuant to Section 8 (e) of the Toxic Substances Control Act, Degussa-Hüls Corporation provides this information to EPA.

Sincerely,  
*Kisha Pippins*  
Kisha Pippins  
Product Safety Specialist

cc: S. Bearman

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ALLYL METHACRYLATE  
ACUTE (FOUR-HOUR) INHALATION STUDY IN RATS

CONTENTS

	Page
COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS .....	3
QUALITY ASSURANCE STATEMENT .....	4
RESPONSIBLE PERSONNEL .....	5
SUMMARY .....	6
INTRODUCTION .....	8
TEST SUBSTANCE .....	9
MATERIALS AND METHODS .....	10
RESULTS .....	15
<b>FIGURES</b>	
1. Vapour generator .....	19
2. Exposure system .....	20
3. Bodyweights - group mean values .....	21
<b>TABLES</b>	
1. Chamber concentration of allyl methacrylate .....	22
2. Clinical signs during exposure .....	23
3. Clinical signs during observation period .....	24
4. Bodyweights - individual and group mean values (g) .....	26
5. Food consumption .....	27
6. Macroscopic pathology - individual findings .....	28
7. Lung weights - individual and group mean values .....	30
<b>APPENDIX</b>	
1. Methods of sample collection and analysis for allyl methacrylate .....	31

CONFIDENTIAL

RGC 028/994951

ALLYL METHACRYLATE  
ACUTE (FOUR-HOUR) INHALATION STUDY IN RATS

**Sponsor**

Röhm GmbH,  
Chemische Fabrik,  
Kirschenallee,  
D-64293 Darmstadt,  
GERMANY.

**Research Laboratory**

Huntingdon Life Sciences Ltd.,  
Woolley Road,  
Alconbury,  
Huntingdon,  
Cambridgeshire,  
PE17 5HS,  
ENGLAND.

## CONTENTS

	Page
COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS .....	3
QUALITY ASSURANCE STATEMENT .....	4
RESPONSIBLE PERSONNEL .....	5
SUMMARY .....	6
INTRODUCTION .....	8
TEST SUBSTANCE .....	9
MATERIALS AND METHODS .....	10
RESULTS .....	15
FIGURES	
1. Vapour generator .....	19
2. Exposure system .....	20
3. Bodyweights - group mean values .....	21
TABLES	
1. Chamber concentration of allyl methacrylate .....	22
2. Clinical signs during exposure .....	23
3. Clinical signs during observation period .....	24
4. Bodyweights - individual and group mean values (g) .....	26
5. Food consumption .....	27
6. Macroscopic pathology - individual findings .....	28
7. Lung weights - individual and group mean values .....	30
APPENDIX	
1. Methods of sample collection and analysis for allyl methacrylate .....	31

**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:

The UK Good Laboratory Practice Regulations 1997 (Statutory Instrument No. 654) and from 14 December 1999, The UK Good Laboratory Practice Regulations 1999 (Statutory Instrument No 3106).

OECD Principles of Good Laboratory Practice (as revised in 1997). ENV/MC/CHEM(98)17.

EC Commission Directive 1999/11/EC of 8 March 1999 (Official Journal No. L 77/8).

The above GLP standards are considered to be equivalent to the following:

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, Joint Directive. (Kanpogyo No. 39 of Environmental Agency, Yakuhatu No. 229 of Ministry of Health and Welfare; 59 Kikyoku No. 85 of Ministry of International Trade and Industry) of 31 March 1984.

Information regarding test substance characterisation, namely the expiry date, was not available to Huntingdon Life Sciences for compliance with the Good Laboratory Practice regulations given above.



.....  
Graham R. Paul, B.Sc. (Hons.), M.Sc., C. Biol., M.I. Biol.,  
Study Director,  
Huntingdon Life Sciences Ltd.

.....  
22 March 2000

Date

QUALITY ASSURANCE STATEMENT

The following have been inspected or audited in relation to this study:

Study Phases Inspected	Date of Inspection	Date of Reporting
Protocol Review	21 October 1999	21 October 1999
Process Based Inspections		
Bodyweight )	7, 8 & 14 October 1999	15 October 1999
Food Consumption )		
Housing and Environment )		
Test Substance Control )		
Exposure Procedures )		
Atmosphere Analysis )		
Clinical Signs recording )		
Records Audit )		
Post Mortem )		
Report	8 March 2000	10 March 2000

Protocol: An audit of the protocol for this study was conducted and reported to the Study Director and Company Management as indicated above.

Process based inspections: At or about the time this study was in progress inspections and audits of routine and repetitive procedures employed on this type of study were carried out. These were conducted and reported to appropriate Company Management as indicated above.

Report Audit: This report has been audited by the Quality Assurance Department. This audit was conducted and reported to the Study Director and Company Management as indicated above.

The methods, procedures and observations were found to be accurately described and the reported results to reflect the raw data.

*Kevin P. de-Salis*  
 Kevin P. de-Salis, B.A. (Hons.), C.Biol., M.I.Biol., Dip.R.Q.A.,  
 Group Manager,  
 Department of Quality Assurance,  
 Huntingdon Life Sciences Ltd.

*J. M. de L...*  
 Date

**RESPONSIBLE PERSONNEL**

Graham R. Paul, B.Sc. (Hons.), M.Sc., C. Biol., M.I. Biol.,  
Study Director.

Anthony M. Bowden, B.Sc. (Hons.),  
Study Supervisor.

Derek W. Coombs, B.Sc.,  
Toxicologist.

Ian S. Gilkison, M.A., Ph.D.,  
Section Head, Aerosol Technology and Analysis.

## SUMMARY

**Test substance**

A clear colourless liquid identified as allyl methacrylate.

**Test animals**

Albino rats (Sprague-Dawley in origin). One control group and 2 test groups, each of 5 male and 5 female rats.

**Route of Administration**

By inhalation of a test atmosphere containing a vapour generated from the test substance.

**Duration of exposure**

Four-hour continuous snout-only exposure.

**Observation period**

Fourteen days post exposure.

**Exposure levels and mortality**

The mean chamber concentration and mortality data are summarised as follows:

Group	Chamber concentration (mg/l)	Mortality		Total
		Males	Females	
2	2.13	5/5	5/5	10/10
3	1.02	0/5	0/5	0/10

All deaths occurred within 1 day of exposure.

**Clinical signs**

**During exposure** - Exaggerated breathing was first noted in test rats from 15 and 30 minutes into exposure for Groups 2 and 3 respectively. A decreased breathing rate was evident in all Group 3 test rats from 2 hours into exposure.

**Observation period** - Gasping, noisy and exaggerated breathing was evident in test rats following exposure, persisting until death for Group 2. Gasping was no longer evident for Group 3 on the day following exposure (Day 1) and noisy and exaggerated breathing persisted up to Days 2 and 3 of the observation period respectively. A slow breathing rate was evident in all Group 2 rats following exposure, persisting until death.

In addition, eyes partially closed, lethargy and whole body cold to touch (Group 2 only) and wet fur (snout/jaws) and peripheral vaso-dilation (characterised by 'red feet') were noted in test rats post exposure. Brown staining on head, whole body and around snout/jaws was noted for Group 3 rats from Day 1, persisting in females to Day 6. Poor grooming was also noted for Group 3 rats from Day 1, persisting for a Group 3 female to Day 12 of the observation period.

A large area of fur in the urino-genital region of a Group 3 female was soiled with excreta and wet from Day 4, persisting up to Day 12 of the observation period. Walking on toes was also noted for this Group 3 female from Day 4, persisting up to Day 7. Matted fur (urino-genital region) was evident for this Group 3 female from Day 8, persisting for the remainder of the observation period.

All Group 3 males were of normal appearance and behaviour from Day 4 of the observation period.

#### Bodyweight

Bodyweight losses were recorded for all Group 2 decedents prior to necropsy.

A mean bodyweight loss was evident for Group 3 rats during the 4-day period following exposure. Thereafter, a general bodyweight gain was evident for Group 3 rats for Days 4 to 7 of the observation period. The mean bodyweight gain of Group 3 rats during the second week of the observation period was similar to or greater than control values for males and females respectively.

#### Food consumption

A reduction in the food consumption of Group 3 rats was evident during the first week following exposure.

#### Water consumption

There were no treatment-related effects.

#### Macroscopic pathology

**Decedents (Group 2)** - The lungs of a proportion of decedents were minimally/moderately congested. A clear or white frothy discharge from the trachea was evident in a proportion of decedents.

Gas-filled stomachs and intestines were noted in all decedents and a decedent male respectively.

External findings noted prior to necropsy included crusty brown staining around snout and jaws, clear discharge from snout, wet fur (snout/jaws) and fur soiled with excreta.

**Rats surviving the 14-day observation period** - There were no treatment-related findings.

#### Lung weights

**Decedents (Group 2)** - The lung weights of female decedents were higher than similarly treated rats surviving the 14-day observation period.

**Rats surviving the 14-day observation period** - There were no treatment-related effects.

External findings of fur soiled with excreta and matted fur in the urino-genital region were noted for a Group 3 female prior to necropsy.

#### Conclusion

The 'mortality curve' (mortality plotted against chamber concentration) in this investigation was relatively steep, with no unscheduled deaths following exposure at 1.02 mg/l and 100% mortality at 2.13 mg/l. Exposure of further groups was therefore considered unnecessary. The LC<sub>50</sub> (4-hour) for allyl methacrylate is between 1.02 and 2.13 mg/l in air and may be considered equivalent to 1.47 mg/l, the geometric mean of these two concentrations.

## INTRODUCTION

The acute inhalation toxicity of allyl methacrylate was assessed by exposing 2 groups of rats, for a period of 4 hours, to a vapour generated from the test substance at target concentrations of 1 mg/l and 2 mg/l. A further group, acting as a control was exposed to clean air only.

The study was conducted by the Inhalation Studies Group, Huntingdon Research Centre, Huntingdon Life Sciences Ltd., during the period 13 October 1999 to 18 November 1999. The protocol for the study was approved by the Study Director and Huntingdon Management on 6 October 1999 and approved by the Sponsor on 7 October 1999.

This study was designed in compliance with EEC (Annex, point 5.2.3), OECD (403), US EPA (Health Effects Test Guidelines, OPPTS 870.1300, Acute Inhalation Toxicity, 5 August 1998) and J-MAFF test guidelines for acute inhalation studies.

On completion of the study all data relating to the study, including all specimens and a copy of the final report, were lodged in the Huntingdon Life Sciences Archives. The data will be retained in Archives for a period of 5 years from the completion date for the study. After such time, the Sponsor will be contacted and advice sought on the return, disposal or further retention of the study data.

## TEST SUBSTANCE

Identity:	Allyl methacrylate
CAS number:	96-05-9
Assay:	99.6%
Intended use:	Industrial
Appearance:	Clear colourless liquid
Amount received:	1 kg
Batch number:	1296710258
Date received:	16 September 1999
Expiry date:	Nominally March 2000 (6 months from receipt)
Storage conditions:	In the dark at ambient room temperature and in the original container
Supplier:	Sponsor

The complete description of the chemical and physical properties of the test substance, including stability, is the responsibility of the Sponsor

A small sample (1 ml) was sealed in a suitable container and stored in archives at an appropriate temperature.

## MATERIALS AND METHODS

### ANIMALS AND MAINTENANCE

Ten male and 10 female albino rats (Sprague-Dawley in origin), approximately 7 and 8 weeks old respectively, were selected from a consignment of rats obtained from Charles River UK Limited, Manston Road, Margate, Kent, England on 13 October 1999. Five male and 5 female rats (same supplier and age) were selected from a second consignment on 27 October 1999.

On arrival the rats were allocated to 1 of 3 groups, each of 5 males and 5 females, and were identified individually by a number tattooed on the ear pinnae. The rats were housed by sex in groups of 5 and acclimatised to laboratory conditions for at least 5 days before the day of exposure.

The holding cages (size 35cm x 53 cm x 25 cm height) were made of stainless steel sheet and wire mesh and were suspended on a movable rack. While in their cages all rats had free access to a measured excess amount of food, SDS rat and mouse diet (RM1) and tap water supplied by Anglian Water. Water bottles were emptied and refilled daily. Food and water were analysed routinely to determine the levels of chemical or microbiological contaminants.

The rats remained in a holding room except for the 4-hour exposure and an overnight post exposure period when the rats in the test group were kept in a ventilated cabinet to allow dispersal of any residual test substance.

The temperature and relative humidity of the holding room air was recorded continuously using a Kent Clearspan thermohydrograph. Air extraction was *via* a balanced system providing at least 15 air changes per hour. The animal holding room conditions remained within the environmental control settings of 22°C ± 3°C and 50% ± 20% respectively. The actual recorded environmental limits were as follows:

Temperature	- maximum	22.5°C
	- minimum	20.5°C
Relative humidity	- maximum	64%
	- minimum	36%

Room lighting was by artificial light between 07:30 and 19:30 daily and controlled automatically.

### INHALATION EXPOSURES

Two preliminary exposures, each with 1 male and 1 female rat, were conducted for a period of 4 hours in order to assess the likely response of rats to the test vapour and hence enable selection of an appropriate target concentration for the first test group (Group 2). The mean chamber concentrations were 0.24 mg/l and 1.00 mg/l.

Two groups, each of 5 male and 5 female rats, were exposed continuously for 4 hours to a vapour generated from allyl methacrylate at target concentrations of 1 mg/l and 2 mg/l.

A further group acting as a control received clean air only for 4 hours.

The group identification and date of exposure for the groups were:

Group 1 (Control):	19 October 1999
Group 2 (2.13 mg/l):	19 October 1999
Group 3 (1.02 mg/l):	4 November 1999

The mean chamber concentration of the vapour for each test group is presented in the **RESULTS** section of this report.

## EXPOSURE SYSTEM

### Vapour generator

The vapour generator, illustrated in Figure 1, was designed to produce and maintain an atmosphere containing vapour by evaporation of the test substance from a fritted glass disc with a countercurrent of air. The air supply to the vaporiser was warmed by passage through a stainless steel coil immersed in water maintained at 35 - 40°C, in a water bath, as an aid to evaporation. All parts of the generator in contact with the test substance were made of glass, except the syringe (polypropylene) and feed line (Teflon®).

The test substance was supplied to the generator from a syringe driven at a constant rate by a syringe pump (Precidor® Type 5003). The compressed air supply to the generator was dried, filtered and oil free.

### Conditioning of the test atmosphere

The resultant test vapour was passed through a glass column containing glass wool in order to remove any condensate.

### Exposure chambers

The snout-only exposure chambers<sup>1</sup> used for the exposures were of cylindrical form (30 cm internal diameter, 45 cm height) and made of aluminium alloy. The internal surfaces of the chamber have a conformal chemically resistant coating. The chambers have an enclosed volume of approximately 30 litres. The rats were held for exposure in moulded polycarbonate restraining tubes, which were attached at evenly spaced ports in the cylindrical section of the chamber, and were designed to allow only the snout to project into the chamber. Each rat was restrained in a forward position by an adjustable foamed plastic stopper, which also provided a seal for the tube.

The conditioned test atmosphere entered through a port at the top centre of the chamber and passed out through a port at the base section below the level of the rats. Each chamber was positioned in a large cabinet equipped with an extract fan exhausting through an absolute filter.

The configuration of the exposure system is shown in Figure 2.

ADG Developments Ltd., Hitchin, Hertfordshire, England

### PROCEDURE

A pre-heated supply of clean dried air was connected to the vapour generator and the supply pressure was adjusted to give a flow rate of 12 litres/minute measured at the generator outlet tube. An in-line flow meter was used to monitor generator airflow during the exposure. The chamber exhaust was calibrated at the point of attachment to the exposure chamber and was adjusted to produce a slightly negative chamber pressure.

A syringe filled with the test substance was fitted to the syringe pump and connected to the generator via Teflon® tubing. Feed rates of 0.038 and 0.018 ml/minute were selected for Groups 2 and 3 respectively, as a result of preliminary generation trials. These feed rates were expected to generate chamber concentrations close to the targets of 2 and 1 mg/l for Groups 2 and 3 respectively.

The rats to be exposed were placed into separate restraining tubes and were then attached to the exposure chamber.

The syringe pump was switched on and the exposure timed for 4 hours, following a 6-minute<sup>2</sup> equilibration period, from the appearance of the test substance on the fritted glass disc. No adjustment of the exposure system was necessary during exposure of test groups.

After 4 hours, the syringe pump was switched off and the exposure chamber allowed to clear before the rats were removed for examination.

Following exposure, the rats were returned to the holding cages and food and water supplies were restored. The test rats were kept in a ventilated cabinet overnight and then returned to the holding room for the remainder of the observation period.

The control group was treated similarly but exposed to air only for 4 hours. The control rats were returned to the holding room at the end of the exposure procedure.

### CHAMBER ATMOSPHERE ANALYSIS

At least five air samples were taken from the chamber during each exposure in order to determine the concentration of the test substance in air. Samples were obtained following equilibration and then approximately at hourly intervals. An additional sample was taken during the exposure of Group 2 (Sample 2) in order to ensure satisfactory generation of the test atmosphere. The times of sampling are presented in Table 1.

<sup>2</sup> 6 minutes is the theoretical time required for the concentration of vapour to reach 90% of its final value under the conditions of exposure employed. The equilibration time ( $t_{eq}$ ) is calculated as follows:

$$t_{eq} \text{ (min)} = \ln \left[ \frac{100}{100 - \% \text{ final conc.}} \right] \times \frac{\text{Chamber volume (litres)}}{\text{Chamber airflow (litres / min)}}$$

Air samples were withdrawn at 2 litres/minute through a gas absorption trap (bubbler) using hexane as a 'trapping medium'. The 'bubbler' stood in 'ice-cold' water during sampling to minimise evaporation of the solvent. The volume of air sampled was measured with a wet-type gas meter.<sup>3</sup> The contents of the 'bubbler' were transferred to a glass vial, together with the 'washings', and retained for chemical analysis.

During the preliminary exposure of rats at 1.00 mg/l, a sample of the test atmosphere was drawn through 2 'bubblers' connected in series. There was no breakthrough of test substance evident in the second 'bubbler'.

The method of sampling and chemical analysis is described in Appendix 1.

#### NOMINAL CONCENTRATION

The nominal concentration of the test substance was calculated from the total mass of allyl methacrylate delivered to the vaporiser and the total volume of air flowing through the exposure system during the period of generation.

#### CHAMBER AIR TEMPERATURE AND RELATIVE HUMIDITY

The air temperature in the exposure chamber was measured using an alcohol-in-glass thermometer and the relative humidity was determined using an Analytical Development Company Ltd infra red water vapour analyser, Model 225. The temperature and humidity were recorded at the start of exposure and then at 30-minute intervals during the 4-hour exposure.

#### OBSERVATIONS

##### Mortality

Throughout the study, all cages were checked at least twice daily, once in the morning and again towards the end of the working day, for dead or moribund animals.

Any animals found dead were subjected to a detailed macroscopic examination (see **TERMINAL STUDIES**).

##### Clinical signs

The rats were observed intermittently for signs of reaction to the test substance during exposure and at least twice daily throughout the observation period.

The clinical signs were recorded at the end of the chamber equilibration period, at 0.25, 0.5 and 1.0 hours then at hourly intervals during the exposure. Clinical signs were recorded immediately following completion of the exposure and then at 1.0 and 2.0 hours post-exposure.

During the observation period, the clinical signs were recorded once in the morning and then as necessary following a later check for survival.

<sup>3</sup> Model DM3D, G.H. Zeal Ltd., London, England (formally Alexander Wright and Co., Sutton, Surrey, England)

**Bodyweight**

All rats were weighed twice during the week prior to exposure, immediately before exposure (Day 0) and weekly during the observation period. Daily measurement of bodyweights for Group 3 rats was instigated from Day 4 of the observation period to monitor the condition of these rats. Weekly measurement of bodyweights was resumed for Group 3 rats from Day 7 of the observation period.

**Food consumption**

The amount of food consumed by each cage of rats was measured from weighday to weighday throughout the study. The daily mean intakes of food for each cage were calculated from the recorded data.

**Water consumption**

A visual inspection of water bottles was conducted daily.

**TERMINAL STUDIES**

At the end of the 14-day observation period, the surviving rats were killed by intraperitoneal injection of pentobarbitone sodium and exsanguinated when clinically dead.

All rats were subjected to a detailed macroscopic examination. The lungs (including the larynx and trachea) were removed, dissected clear of surrounding tissue, weighed and the weights recorded.

The lungs were discarded following necropsy.

**CALCULATIONS**

In order to minimise the cumulative errors, which result from repeated rounding of numbers, some of the data in this report have been calculated using unrounded data and only rounded for reporting. Consequently any further calculation using the data as presented will include rounding errors in the last significant figure, possibly leading to small apparent discrepancies with other data in this report.

## RESULTS

## CHAMBER ATMOSPHERE CONDITIONS

## Chamber concentration of Allyl Methacrylate

The data are presented in Table 1.

The mean chamber concentration data are summarised as follows:

Group	Chamber concentration (mg/l)	
	Mean	sd
2	2.13	0.165
3	1.02	0.066

sd Standard deviation

The mean chamber concentrations were in agreement with target (2 mg/l and 1 mg/l for Groups 2 and 3 respectively).

## Nominal concentration

The nominal concentrations for Groups 2 and 3 were 3.18 and 1.49 mg/l. The mean chamber concentrations of allyl methacrylate were approximately 67 % and 68 % of the nominal concentrations for Groups 2 and 3 respectively. Such differences are considered likely to be associated with condensation of the test substance in the exposure system.

## Chamber air temperature and relative humidity

The mean chamber air temperature, the relative humidity and the standard deviation (sd) of the means during exposure of the groups were:

Group	Temperature (°C)		Relative Humidity (%)	
	Mean	sd	Mean	sd
1 (Control)	20.4	0.17	38	1.5
2 (2.13 mg/l)	20.1	0.33	35	3.5
3 (1.02 mg/l)	20.8	0.44	33	3.0

The mean relative humidity of test atmospheres was marginally lower than the control value and was considered not to have affected the outcome of the study<sup>4</sup>. The mean chamber air temperature was similar for the control and test atmospheres.

<sup>4</sup> PAULUHN, J. and MOHR, U. (1999) Repeated 4-week inhalation exposure of rats: effect of low, intermediate and high-humidity chamber atmospheres. *Exp. Toxic. Pathol.*, **51**, 178-187

## CLINICAL OBSERVATIONS

### Mortality

The mortality data are summarised as follows:

Group	Number of deaths		
	Males	Females	Total
2 (2.13 mg/l)	5/5	5/5	10/10
3 (1.02 mg/l)	0/5	0/5	0/10

All Group 2 rats were found dead at the early check on Day 1 of the observation period.

### Clinical signs

**During the exposure** (Table 2) - Exaggerated breathing was first noted in test rats from 15 and 30 minutes into exposure for Groups 2 and 3 respectively. A decreased breathing rate was evident in all Group 3 test rats from 2 hours into exposure.

Soiling of the fur with excreta was observed in control and test rats from 15 and 30 minutes into exposure respectively and was considered to be associated with the method of restraint.

**During the observation period** (Table 3) - Gasping, noisy and exaggerated breathing was evident in test rats following exposure, persisting until death for Group 2. Gasping was no longer evident for Group 3 on the day following exposure (Day 1) and noisy and exaggerated breathing persisted up to Days 2 and 3 of the observation period respectively. A slow breathing rate was evident in all Group 2 rats following exposure, persisting until death.

In addition, eyes partially closed, lethargy and whole body cold to touch (Group 2 only) and wet fur (snout/jaws) and peripheral vaso-dilation (characterised by 'red feet') were noted in test rats post exposure. Brown staining on head, whole body and around snout/jaws was noted for Group 3 rats from Day 1, persisting in females to Day 6. Poor grooming was also noted for Group 3 rats from Day 1, persisting for a Group 3 female to Day 12 of the observation period.

A large area of fur in the urino-genital region of a Group 3 female was soiled with excreta and wet from Day 4, persisting to Day 12 of the observation period. Walking on toes was also noted for this Group 3 female from Days 4 to 7 and matted fur (urino-genital region) was evident for this Group 3 female from Day 8, persisting for the remainder of the observation period.

Brown staining on head was also noted for one control male post exposure. Soiling of the fur with excreta was observed in both test and control rats immediately after exposure. These signs were considered to be associated with the method of restraint.

Hair loss from the body was noted on one control female from Day 2, persisting for the remainder of the observation period.

All Group 3 males and most Group 3 females were of normal appearance and behaviour from Days 4 and 7 of the observation period respectively.

**Bodyweight**

The data are presented in Figure 3 and Table 4.

Group 3 rats were weighed daily from Days 4 to 7 of the observation period in order to aid assessment of the condition of surviving test rats.

Bodyweight losses were recorded for all Group 2 decedents prior to necropsy.

A mean bodyweight loss was evident for Group 3 rats during the 4-day period following exposure. Thereafter, a general bodyweight gain was evident for Group 3 rats for Days 4 to 7 of the observation period. The mean bodyweight gain of Group 3 rats during the second week of the observation period was similar to or greater than control values for males and females respectively.

**Food consumption**

The data are presented in Table 5.

A reduction in the food consumption of Group 3 rats was evident during the first week following exposure. Thereafter, the food consumption of Group 3 rats was similar to control values.

**Water consumption**

There were no treatment-related effects.

A visual appraisal of the water bottles indicated that the amount of water consumed by test rats was similar to control rats.

**TERMINAL STUDIES****Macroscopic pathology**

The data are presented in Table 6.

**Decedents (Group 2)** - The lungs of a proportion of decedents were minimally/moderately congested. A clear or white frothy discharge from the trachea was evident in a proportion of decedents.

Gas-filled stomachs and intestines were noted in all decedents and a decedent male respectively.

External findings noted prior to necropsy included crusty brown staining around snout and jaws, clear discharge from snout, wet fur (snout/jaws) and fur soiled with excreta.

**Rats surviving the 14-day observation period** - There were no treatment-related findings.

The external findings of fur soiled with excreta and matted fur in the urino-genital region were noted for a Group 3 female prior to necropsy.

**Lung weights**

The data are presented in Table 7.

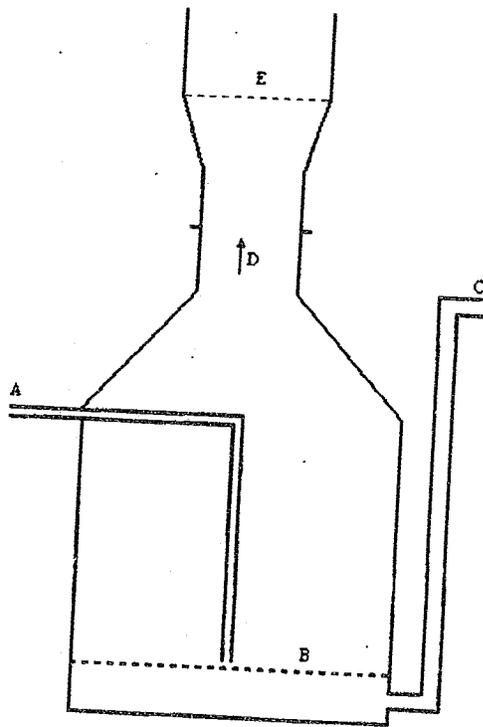
**Decedents (Group 2)** - The mean lung weight of female decedents was higher than that of control rats surviving the 14-day observation period. This finding was not apparent for decedent males.

**Rats surviving the 14-day observation period** - There were no treatment-related effects.

**CONCLUSION**

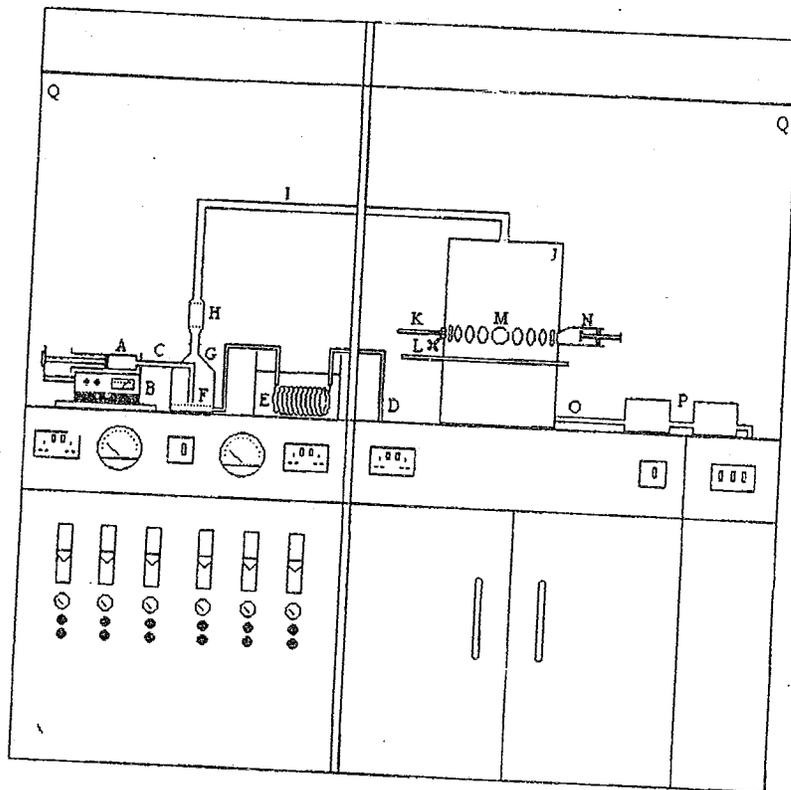
The 'mortality curve' (mortality plotted against chamber concentration) in this investigation was relatively steep, with no unscheduled deaths following exposure at 1.02 mg/l and 100% mortality at 2.13 mg/l. Exposure of further groups was therefore considered unnecessary. The  $LC_{50}$  (4-hour) for allyl methacrylate may be considered equivalent to 1.47 mg/l, the geometric mean of these two concentrations.

FIGURE 1  
Vapour generator



- A Test substance feed line
- B Fritted glass disc
- C Air supply
- D Vapour outlet
- E Glass wool trap (to remove any condensate)

FIGURE 2  
Exposure system



- |  |                                     |
|--|-------------------------------------|
| A Test substance in syringe                    | I Glass tubing                      |
| B Syringe pump                                 | J Exposure chamber (30 litres)      |
| C Teflon® feed line                            | K Thermometer                       |
| D Vaporiser air supply                         | L Water vapour analyser sample line |
| E Water bath and coiled stainless steel tubing | M Animal exposure/sample ports      |
| F Fritted glass disc                           | N Rat holding tube                  |
| G Large vaporiser                              | O Extract from exposure chamber     |
| H Glass wool trap (to remove any condensate)   | P Vapour absorbers <sup>a</sup>     |
|  | Q Air extraction chamber            |

<sup>a</sup> Fluosorber (shirley Aldred & Co. Ltd., Sheffield, England)

FIGURE 3

Bodyweights - group mean values

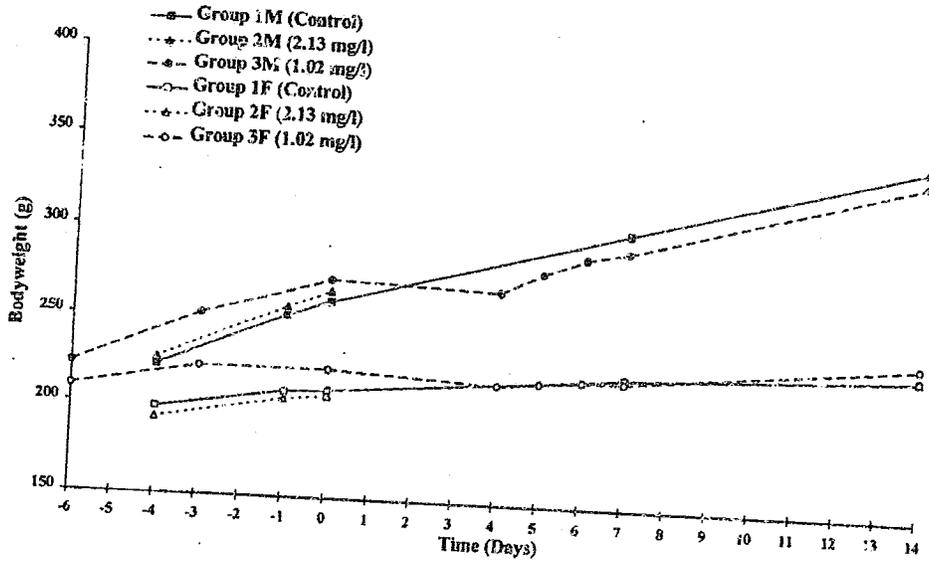


TABLE I

## Chamber concentration of allyl methacrylate

Group	Sample	Time taken (h:min)	Chamber concentration (mg/l)	Nominal concentration <sup>a</sup> (mg/l)
2 (2.13 mg/l)	1	0:12	1.88	
	2	0:44	1.97	
	3	1:00	2.24	
	4	2:00	2.25	
	5	3:00	2.21	
	6	3:44	2.25	
		Mean		2.13
	sd		0.165	
3 (1.02 mg/l)	1	0:11	0.97	
	2	1:01	1.06	
	3	2:00	1.12	
	4	3:00	0.98	
	5	3:47	0.97	
		Mean		1.02
	sd		0.056	0.056

sd Standard deviation

Calculated from the total mass of the test substance dispersed by the generator (9.4 g and 4.4 g for Groups 2 and 3 respectively) and the total volume of air (2932 litres) supplied to the exposure system during the generation periods as follows:

$$\text{Nominal concentration (mg/l)} = \frac{\text{Usage (g)} \times 10^3}{\text{Air flow (l/min)} \times \text{Duration (mins)}}$$

TABLE 2  
Clinical signs during exposure

Group	Signs	Number showing signs						
		Time in hours						
		0*	0.25	0.5	1.0	2.0	3.0	4.0
1M (Control)	Normal appearance	5	4	1	1			
	Fur soiled with excreta		1	4	4	5	5	5
2M (2.13 mg/l)	Normal appearance	5	3					
	Exaggerated breathing		2	3	4	5	5	5
	Fur soiled with excreta			5	5	5	5	5
3M (1.02 mg/l)	Normal appearance	5	5					
	Exaggerated breathing			3	5	5	5	5
	Decreased breathing rate					5	5	5
	Fur soiled with excreta				5	5	5	5
1F (Control)	Normal appearance	5	5					
	Fur soiled with excreta			5	5	5	5	5
2F (2.13 mg/l)	Normal appearance	5	3					
	Exaggerated breathing		2	3	3	4	5	5
	Fur soiled with excreta			5	5	5	5	5
3F (1.02 mg/l)	Normal appearance	5	5					
	Exaggerated breathing			5	5	5	5	5
	Decreased breathing rate					5	5	5
	Fur soiled with excreta					5	5	5

\* Clinical signs recorded during the 6-minute equilibration period

TABLE 3  
Clinical signs during observation period

Group	Signs	Post-exposure*		Number showing signs														
		0 hr	1 hr	2 hr	Day of observation													
					1	2	3	4	5	6	7	8	9	10	11	12	13	14
1M (Control)	Normal appearance and behaviour	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Fur/skin soiled with excreta	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Brown staining on head	1																
2M (2.13 mg/l)	Exaggerated breathing	5	5	5														
	Slow breathing rate	5	5	5														
	Gasping	5	5	5														
	Noisy breathing	5	5	5														
	Lethargic	5	5	5														
	Whole body cold to touch	5	5	5														
	Eyes partially closed	5	5	5														
	Peripheral vaso-dilation ('red feet')	5	5	5														
	Fur/skin soiled with excreta	2																
	Wet fur (snout and jaws)	5	5	5														
3M (1.02 mg/l)	Dead (total)	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Normal in appearance at: !				5	5	5	5	5	5	5	5	5	5	5	5	5	5
	behaviour																	
	Exaggerated breathing				3	5	5	5	5	5	5	5	5	5	5	5	5	5
	Gasping	5	5	5	2													
	Noisy breathing	5	5	5														
	Peripheral vaso-dilation ('red feet')	2	5	5														
	Fur/skin soiled with excreta	5	5	5														
	Wet fur (snout and jaws)	5	4	4														
	Poorly groomed	5	5	5														
Brown staining around snout/jaws				3														
Brown staining on head				4														
				5	2													

\* Clinical signs recorded after exposure on the day of exposure

TABLE 3  
(Clinical signs during observation period - continued)

Group	Signs	Post-exposure*		Number showing signs														
		0 hr	1 hr	2 hr	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1F (Control)	Normal appearance and behaviour	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Fur/skin soiled with excreta																	
	Hair loss from body																	
2F (2.13 mg/l)	Exaggerated breathing	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Slow breathing rate	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Gasping	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Noisy breathing	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Leithargic	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Whole body cold to touch	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Eyes partially closed	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Peripheral vaso-dilation ('red feet')	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Fur/skin soiled with excreta	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Wet fur (snout and jaws)	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
3F (1.02 mg/l)	Dead (total)	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Normal in appearance and behaviour	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Exaggerated breathing	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Gasping	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Noisy breathing	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Peripheral vaso-dilation ('red feet')	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Fur/skin soiled with excreta	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Wet fur (snout and jaws)	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Poorly groomed	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Brown staining around snout/jaws	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Brown staining on head	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	
Brown staining on whole body	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	
Large area of fur in urino-genital region soiled with excreta and wet	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	
Walking on toes	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	
Matted fur (urino-genital region)	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	

\* Clinical signs recorded after exposure on the day of exposure

TABLE 4

Bodyweights - individual and group mean values (g)

Group	Rat	Day of observation										
		-6	-4	-3	-1	0	4	5	6	7	14	Terminal
1M (Control)	61		216		251	260				295	346	
	62		227		262	271				328	385	
	63		228		256	263				302	337	
	64		220		243	253				288	313	
	65		221		247	258				307	349	
	Mean		222		252	261				304	346	
2M (2.13 mg/l)	71		225		252	262						243
	72		233		266	279						257
	73		222		251	260						230
	74		220		255	265						248
	75		229		260	270						242
	Mean		226		257	267						
3M (1.02 mg/l)	81	224	261		276	247	266	270	274	324		
	82	219	253		270	273	279	292	292	336		
	83	222	250		276	270	281	291	298	337		
	84	219	242		269	280	291	297	302	348		
	85	224	253		276	281	289	300	303	346		
	Mean	222	252		273	270	281	290	294	338		
1F (Control)	66		205		218	221				232	236	
	67		202		211	215				217	217	
	68		198		211	211				233	249	
	69		190		199	196				212	226	
	70		193		204	208				219	218	
	Mean		198		209	210				223	229	
2F (2.13 mg/l)	76		195		207	214						195
	77		194		209	203						193
	78		186		197	203						192
	79		192		208	210						192
	80		191		202	206						193
	Mean		192		205	207						
3F (1.02 mg/l)	86	205	221		227	222	223	220	220	229		
	87	205	220		213	217	218	223	221	232		
	88	216	231		231	201	208	210	212	233		
	89	201	206		213	213	214	214	216	225		
	90	217	231		227	231	232	238	237	259		
	Mean	209	222		222	217	219	221	221	236		

0 Immediately prior to exposure on the day

TABLE 5

## Food consumption

Period of Consumption (Day) ‡	Food consumption (g/rat/day)					
	1M (Control)	2M (2.13 mg/l)	3M (1.02 mg/l)	1F (Control)	2F (2.13 mg/l)	3F (1.02 mg/l)
Pre-exposure						
A	30	32	29	22	23	24
B	31	32	31	21	19	21
Post-exposure						
C	30	1	23	21	2	16
8 to 14	31	-	30	20	-	22
Cumulative (g/rat) 1 to 14	422	-	371	285	-	264

‡ Periods of food consumption are summarised as follows:

Group	Period of consumption		
	A	B	C
1 (Control)	-4 to -2	-1	1 to 7
2 (2.13 mg/l)	-4 to -2	-1	1
3 (1.02 mg/l)	-6 to -4	-3 to -1	1 to 7

TABLE 6

## Macroscopic pathology

Group	Rat	Region/organ affected	Observation
1M (Control)	61		No abnormalities detected
	62		No abnormalities detected
	63		No abnormalities detected
	64		No abnormalities detected
	65		No abnormalities detected
2M (2.13 mg/l)	71*	Stomach	Gas-filled
		External appearance	Fur soiled with excreta, wet fur (snout/jaws), clear discharge from snout, crusty brown staining around snout/jaws
	72*	Lungs	Moderate congestion right posterior lobe, minimal congestion right anterior lobe
	73*	Stomach	Gas-filled
		External appearance	Fur soiled with excreta
	74*	Stomach	Gas-filled
		External appearance	Fur soiled with excreta
	75*	Stomach	Gas-filled
		Intestines	Gas-filled
		External appearance	Fur soiled with excreta, clear discharge from snout
3M (1.02 mg/l)	81	Lungs	Small area of congestion top and bottom sections of left lung, clear discharge from trachea
		Stomach	Gas-filled
	82	External appearance	Fur soiled with excreta
83		No abnormalities detected	
84		No abnormalities detected	
85		No abnormalities detected	

\* Decedent

TABLE 6

(Macroscopic pathology - continued)

Group	Rat	Region/organ affected	Observation
1F (Control)	66		No abnormalities detected
	67		No abnormalities detected
	68	External appearance	Hair loss from body
	69		No abnormalities detected
2F (2.13 mg/l)	70		No abnormalities detected
	76*	Lungs	Small area of moderate congestion right anterior lobe, small area of minimal congestion right posterior lobe, white frothy discharge from trachea
		Stomach	Gas-filled
	77*	External appearance	Fur soiled with excreta, clear discharge from snout, crusty brown staining around snout/jaws
		Lungs	Minimal congestion left lung and right anterior lobe, clear frothy discharge from trachea
	78*	Stomach	Gas-filled
		External appearance	Fur soiled with excreta, clear discharge from snout/jaws, crusty brown staining around snout/jaws
	79*	Lungs	clear frothy discharge from trachea
		Stomach	Gas-filled
	80*	External appearance	Fur soiled with excreta, clear discharge from snout/jaws, crusty brown staining around snout/jaws
		Lungs	Small area of minimal congestion left lung, white frothy discharge from trachea
	81*	Stomach	Gas-filled
External appearance		Fur soiled with excreta, clear discharge from snout/jaws, crusty brown staining around snout/jaws	
82*	Lungs	Moderate congestion right middle lobe, minimal congestion left lung, white frothy discharge from trachea	
	Stomach	Gas-filled	
83*	External appearance	Fur soiled with excreta, clear discharge from snout/jaws, crusty brown staining around snout/jaws	
	84*		No abnormalities detected
3F (1.02 mg/l)	85*		No abnormalities detected
	86*	External appearance	Fur soiled with excreta, matted fur urino-genital region
	87*		No abnormalities detected
	88*		No abnormalities detected
89*		No abnormalities detected	
90*		No abnormalities detected	

\* Decedent

TABLE 7

Lung weights - individual and group mean values

Group	Rat	Lung weight (g)	
		Decedent	Survivor
1M (Control)	61		1.56
	62		1.90
	63		1.50
	64		1.43
	65		1.64
	Mean sd		1.61 0.182
2M (2.13mg/l)	71	1.77	
	72	1.67	
	73	1.55	
	74	1.79	
	75	1.64	
	Mean sd	1.68 0.098	- -
3M (1.02 mg/l)	81		1.66
	82		1.51
	83		1.49
	84		1.48
	85		1.62
	Mean sd		1.55 0.082
1F (Control)	66		1.44
	67		1.37
	68		1.42
	69		1.31
	70		1.33
	Mean sd		1.37 0.056
2F (2.13mg/l)	76	1.53	
	77	1.71	
	78	1.44	
	79	1.80	
	80	1.54	
	Mean sd	1.60 0.147	- -
3F (1.02 mg/l)	86		1.34
	87		1.34
	88		1.17
	89		1.24
	90		1.37
	Mean sd		1.29 0.084

sd Standard deviation

## APPENDIX 1

## Methods of sample collection and analysis for allyl methacrylate

## SAMPLE COLLECTION

## Chamber concentration

A sample (5 or 10 litres according to group) of the chamber atmosphere was drawn through a solvent trap containing hexane. The sample volume was measured using a wet-type gas meter placed in-line with the pump. The sampling rate (2 litres/minute) was set, daily before use, using a tapered tube rotameter.

During the preliminary exposure with rats at 1.00 mg/l, a sample (10 litres) of the chamber atmosphere was drawn through 2 solvent traps, connected in series and containing hexane, in order to determine whether breakthrough of the test substance occurred.

## SAMPLE PREPARATION AND METHOD OF ANALYSIS

The resulting solutions were analysed by GC.

The methods of sample preparation and sample analysis are detailed together with a summary of the method validation in the Inhalation Analytical Procedure at the end of this appendix.

## CALCULATIONS

## GC

The method for calculating the concentration of allyl methacrylate in the chamber ( $\mu\text{g/l}$ ) is given below in equation 1.

$$\frac{C (\mu\text{g} / \text{ml}) \times V_s (\text{ml})}{V_a (\text{l})} \quad (1)$$

where C = concentration of analysed sample solution  
 $V_s$  = volume of sample solution  
 $V_a$  = volume of atmosphere sampled

In order to minimise the cumulative errors which result from repeated rounding of numbers, much of the data in this report has been calculated continuously using unrounded numbers and only rounded for printing. Consequently, any further calculation using these rounded numbers will include rounding errors in the last significant figure, possibly leading to small apparent discrepancies with other data in the report.

APPENDIX 1

(Methods of sample collection and analysis for allyl methacrylate - continued)

COMPOUND SPECIFIC INHALATION ANALYTICAL PROCEDURE FOR ALLYL METHACRYLATE

The analysis of Allyl methacrylate in air sample substrate

This document details the basic procedures for the analysis of allyl methacrylate collected using solvent traps (bubblers). Hexane is used to trap the analyte. The resulting solutions of approximate concentration 50 to 1000 µg/ml are quantified by GC.

Reference to Sponsor's methodology: Adapted from a method supplied by the sponsor.

Title: Method provided by e-mail.

Dated: 13 August 1999

Authorisation

The method outlined in this document has been validated and is considered fit for the purpose of monitoring conditions in an Inhalation Toxicology study.

Prepared by: N C Lloyd  
Senior Study Analyst

Approved by: Ian S Gilkison  
Head of Section

*This document contains the core method of analysis. Study specific amendments and additions will be detailed within a supplementary document.*

**NOTE** Throughout this document, the symbol § indicates that the relevant information is not available at present, but will be included in a Study specific supplement.

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## APPENDIX 1

(Methods of sample collection and analysis for allyl methacrylate - continued)

**Test substance**Allyl methacrylate, has the following formula:  $C_7H_{10}O_2$ 

Appearance Clear liquid

Storage 4°C

**Reagents**

Hexane Glass Distilled Grade (Distol) Rathburn

**Equipment**

Balance and data printer Sartorius R160P with YDP-01

General laboratory glassware

**Consumables**

Scintillation vials ca 20 ml capacity Packard Instruments BV

Autosampler vials, septa and caps Glass vials, silicone/teflon septa, polypropylene caps Fisher Scientific Ltd.

**Method of sample extraction**

The solution from a bubbler sample was transferred to a volumetric flask. The bubbler was further rinsed with hexane and this was added to the volumetric flask. The volumetric flask is made up to volume with further hexane. The flask is stoppered and the contents mixed thoroughly and transferred to an appropriately labelled scintillation vial.

The volumes of extraction solvent added to each sample are as detailed in the study specific supplement. All samples not already contained within appropriately labelled scintillation vials will be transferred to appropriately labelled scintillation vials prior to storage.

**Preparation of standard solutions**

Weigh approximately 100 mg of Allyl methacrylate (to 0.01 mg) into a volumetric flask (100 ml), dissolve in hexane, mix thoroughly and make up to volume with hexane to provide standard S1. Using glass volumetric pipettes, transfer aliquots of standard S1 into volumetric flasks and dilute to volume using hexane to provide standards at the required concentrations as detailed in the study specific supplement.

## APPENDIX 1

(Methods of sample collection and analysis for allyl methacrylate - continued)

**Preparation of solutions for analysis**

Allow the solutions to equilibrate to room temperature prior to preparation.

**Storage of standards and samples**

Type	Storage conditions	Maximum storage period
Samples solutions	ca 4°C	7 Days
Standard solutions	ca 4°C	7 Days

**Calibration and quantification**

Calibration of the instrument is performed using a standard of nominal concentration 500µg/ml. Calibrate by injecting at least 6 replicates at the beginning of each analytical sequence. Measure the peak area response for these 6 injections to derive a single point calibration line passing through the origin.

For each injection of the sample, measure the peak area response and determine the concentration of the sample using the equation below:

$$\text{Amount}(\mu\text{g}) = \frac{A}{S} \times V$$

Where

- A = Peak area response in the sample chromatogram
- S = Slope of calibration line derived from calibration data
- V = Dilution volume of sample (mL); = extraction volume x dilution factor

## APPENDIX 1

(Methods of sample collection and analysis for allyl methacrylate - continued)

**Chromatographic conditions**

Analytical column	HP-5 30m x 0.32mm id; df=0.25µm
Carrier gas	Helium (1.5 ml/min)
Split vent	Helium (75 ml/min)
Septum purge	Helium (1 ml/min)
Split ratio	1:50
Oxidant	Air (430 ml/min)
Fuel	Hydrogen (43 ml/min)
Injection volume	2 µl
Injector temperature	150°C
Detector temperature	250°C
Detector range	0
Column temperature	100°C
Retention time	Allyl methacrylate approximately 3.9 minutes

**Quality assurance measures**

When the method is established on a chromatographic system six injections of a standard will be used to verify performance of the system. The parameters and acceptance criteria are set out below:

Parameter	Typical value	Acceptance criteria
Plate count (USP)	34885	>2000
Tailing factor (USP)	0.70	$1.5 > x > 0.5$
Measurement repeatability (n=6) expressed as coefficient of variation	1.3%	<5%
QC Tolerance at LOQ	0.3%	<10%
Resolution	>1000	>1

The highest calibration standard will be compared against a standard of similar concentration prepared independently. The ratio of response factors will be acceptable if within the range 0.95 to 1.05.

A quality check standard must follow every 6 concentration samples for the analysis to be regarded as valid. The results of the quality check standards must lie within 5% of the nominal value except at concentrations approaching the limit of quantification (LOQ), where 10% is acceptable.

## APPENDIX I

(Methods of sample collection and analysis for allyl methacrylate - continued)

Samples must lie within the range of acceptable quality check standards for the result to be regarded as valid. The LOQ for the run will be regarded as the concentration of the lowest acceptable quality check standard.

The population relative standard deviation of duplicate injections must be less than 5 % except at concentrations approaching the limit of quantification, where 10 % is acceptable.

**Summary of method validation**

The raw data for the method validation is located in study RGC/028.

Comparison of test blanks, standards and test samples showed that the analyte was well resolved from any potential interfering peak.

Precision data showed coefficients of variation for allyl methacrylate of less than 1.3% with solutions in the range of 1114.75 to 111.48  $\mu\text{g/ml}$ , and 0.3% at 44.59  $\mu\text{g/ml}$ .

A single point calibration of the peak area response against concentration of standard (557.38  $\mu\text{g/ml}$ ) produced a correlation coefficient of 1.0 and relative errors less than 0.4% in the range 1114.57 to 111.48  $\mu\text{g/ml}$  and 0% at 44.59  $\mu\text{g/ml}$ . The Limit of Quantification (LOQ) for allyl methacrylate will be set by the lowest acceptable check standard, however, the LOQ and Limit of Detection (LOD) are potentially as low as 1.23 and 0.37  $\mu\text{g/ml}$  respectively (calculated statistically using the standard deviation obtained for a solution of concentration 44.59  $\mu\text{g/ml}$ ).

Standards of allyl methacrylate in hexane in the nominal range 50 to 1000  $\mu\text{g/ml}$  stored at approximately 4°C for 7 days and subsequently analysed against fresh standards showed concentrations within 5% of their nominal concentrations except at concentrations approaching 50  $\mu\text{g/ml}$ , the Limit of Quantification, where concentrations within 10% of their nominal were observed.

No breakthrough of allyl methacrylate through the bubbler was observed by placing a second trap in series. The concentration of trap two was less than 5% than that of trap one.

## APPENDIX 1

(Methods of sample collection and analysis for allyl methacrylate - continued)

## Chromatographs

System No.	Components of gas chromatography system		
1	Hewlett Packard	5890A	Chromatograph with capillary inlets, heated gas sampling valve, ECD and FID. } 7673 Autosampler } A/D interface } Integration software
	Hewlett Packard	18593B	
	Hewlett Packard	18596CX	
	Hewlett Packard	G1512AX	
	TSP*	SP4500	
	TSP	PC1000	
2	Pye Unicam	PU4550	Chromatograph with gas valve and FID. Autosampler A/D interface Integration software
	Pye Unicam	PU4700	
	TSP	SP4500	
	TSP	PC1000	
3	Shimadzu	GC-14A	Chromatograph with FID. Autosampler Autoinjector A/D interface Integration software
	Shimadzu	AOC-1400	
	Shimadzu	AOC-14	
	TSP	SP4500	
	TSP	PC1000	
6	Shimadzu	GC-14A	Chromatograph with FID. Automated gas valve Integrator
	Shimadzu	MGS-4	
	Shimadzu	CR4-A	
7	Shimadzu	GC-14A	Chromatograph with FID. Automated gas valve Integrator
	Shimadzu	MGS-4	
	Shimadzu	CR4-A	
8	Hewlett Packard	5890A	Chromatograph with capillary inlets, heated automatic gas sampling valve and FID. } 6890 Series Autosampler } A/D interface } Integration software
	Hewlett Packard	G1513A	
	Hewlett Packard	18596CX	
	Hewlett Packard	G1512AX	
	TSP	SP4500	
	TSP	PC1000	
9	Perkin Elmer	Autosystem XL	Automatic Chromatograph with programmable split/less capillary injector, heated automatic gas sampling valve and FID.

\* TSP Thermo Separation Products (formerly Spectra-Physics)

APPENDIX 1

(Methods of sample collection and analysis for allyl methacrylate - continued)

**RGC/028 - STUDY SPECIFIC SUPPLEMENT TO THE INHALATION ANALYTICAL PROCEDURE FOR ALLYL METHACRYLATE**

This method details the procedure to be used for the GC assay of allyl methacrylate in air at concentrations in the range of 50 to 1000 µg/ml.

Details given in this supplement supersede those in the compound specific IAP.

Copy	Distribution List	Signature	Date Issued	Signature	Date Withdrawn
Master	ATAS:- IAP File		13 October 1999		
<b>CONTROLLED COPIES:</b>					
1	Study Box file		13 October 1999		
2					
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Archive	Study Data				

**Analytical standard**

Name: Allyl methacrylate  
 Batch number: Not Provided  
 Purity: 98%  
 Expiry date: 16 March 2000  
 Supplier: Röhm GmbH Chemische Fabrik

**Method of sample extraction**

Bubbler samples were collected and made up to a volume of 25 ml

**Preparation of standard solutions**

Prepare standard solutions in the nominal range 50 to 1000 µg/ml.

**Calibration and Quantification**

Calibration of the instrument is performed using a standard solution of approximately 500 µg/ml.

**APPENDIX 1**

**(Methods of sample collection and analysis for allyl methacrylate - continued)**

**Chromatographs**

The analysis is performed using chromatograph 8.

**Authorisation**

The method outlined in this document and the compound specific Inhalation Analytical Procedure has been validated and is considered fit for the purpose of monitoring conditions in an Inhalation Toxicology study.

**Prepared by:**

▲  
N C Lloyd  
Senior Study Analyst

**Approved by:**

▲  
Ian S Gilkison  
Head of Section

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▲ Signed on original.

