CHAPTER 1

ASSESSMENT OF ACUTE INHALATION TOXICITY OF ACEPHATE FORMULATION IN WISTAR RATS

INTRODUCTION

Acute toxicity is defined as adverse changes occurring immediately or after a short time following a single or short period exposure of a substance (Christopher *et al.*, 1995). It is one of the conventional start points of any toxicological study since it presents the median lethal dose as well as signs of toxicity is a short span of time. Further, it is very important to determine the lethal concentration which may form a part of labelling the compound as per regulatory requirement. Moreover, acute toxicity test also provides a dose concentration range for subsequent long term repeat dose studies.

Apart from the above mentioned experimental significance, the acute toxicity test will also help the clinician in identifying and treating accidental or deliberate pesticide induced human toxicity. Exposure of factory workers during the undisturbed synthesis of pesticides is probably negligible, since the processes are carried out in closed vessels. However, the formulation and dispensing of formulated pesticides may cause accidental contact poisoning. The whole range of workers associated with pesticide treatment of crops or premises are liable to exposure as also workers and segments of the population during disease vector control procedures. Other people coming in direct contact with pesticides are the dealers, the users and other accidental and suicidal cases.

For the above cited reasons it was thought essential to test the selected pesticide for its acute inhalation toxicity. One of the widely used organophsphorous compound the Acephate 97% DF (Trade name *Lancer 97 DF*) was selected for the current study. A review of the literature shows that the selected test article has been screened for its potential toxicity to non-targeted organism. However, even though the compound is largely sprayed in the field by the applicators, and hence chances of inhalation are very high, no comprehensive data was available in literature about the compound's inhalation toxicity in mammals. Therefore, it was thought pertinent to evaluate the inhalation toxicity of Acephate 97% DF in rodents.

As a prelude to the comprehensive screening of the formulation for inhalation toxicity, an acute toxicity test was envisaged herein.

STUDY OBJECTIVE

This study was performed to assess the acute inhalation toxicity of 97% dry flowable formulation of Acephate in rats.

JUSTIFICATION FOR SELECTION OF THE TEST SYSTEM

Wistar rats – Selection of this strain and species was based on a variety of considerations including hardiness, low incidence of respiratory diseases, general acceptability for inhalation toxicity studies and availability of historical control data. The results of the study are expected to be of value in predicting the acute toxicity of the test item in higher mammals including humans.

INSTRUMENTS AND EQUIPMENT USED IN THE EXPERIMENT

Air Compressor, Model T 30/2475

Infusion Pump, 871851/2, PERFUSOR® Secura FT

Inhalation Chamber, Model Head/Nose Only

Diaphragm Vacuum Pump, Model TID-75-P

Thermo-hygrometer, Kusam Meco 918

Oxygen Monitor, Uniphos 310

Seven Stage Cascade Impactor and Impactor Control Unit, Model RC-FB/02-150

Open Face Sampler

$AIMS_{TM}$ Tattoo Machine

Carbon dioxide Monitor, Uniphos 225 (PM)

Sartorius Semi Micro Balance, BP 210 D (Range: 0.01 mg to 210 g)

Electronic Weighing Scale - SMART (Range: 5.0 g to 3.0 kg)

SOLVENTS AND CHEMICALS

Disinfectant	:	Dettol 2.5%
Sodium Hydroxide Pellet	:	Excella R Grade
Distilled Water	:	Milli Pore Elix 10 water purification System

ANIMALS

Five male and five female healthy Wistar rats *(Rattus norvegicus)*, obtained from the Wockhardt Research Centre, Aurangabad, India, were used for the study. Females were nulliparous and non-pregnant. The rats were examined for good health at the time of receipt and acclimatised to the controlled environmental conditions for a period of seven days. At the start of the exposure the male rats weighed between 317.9 g – 360.1 g -, while the female rats weighed between 176.1 g and 204.3 g; the rats were between 12 and 13 weeks old. Before the start of the study, rats were randomised and assigned to the required number of groups using in-house developed validated computer software.

ACCLIMATISATION

Rats were allowed to acclimatise to the experimental room conditions for a period of seven days prior to randomization. During the acclimatisation period, the rats were observed for abnormal behviour or clinical signs, if any. . Rats were also acclimatised to the restrainer tubes for 2 hours on a day before exposure.

ENVIRONMENTAL CONDITIONS

Rats were maintained in an environmentally controlled room. The experimental room temperature and humidity were recorded daily. The temperature range was 20 to 23 °C and the relative humidity level during the experimental period was 65 to 67%. A minimum of 15 air changes per hour was maintained in the experimental room. In the experimental room, 12 hours of artificial fluorescent lighting and 12 hours darkness were maintained, light hours being 0600 hrs to 1800 hrs. The experimental room was cleaned and mopped daily with a disinfectant (Dettol 2.5%).

HUSBANDRY PRACTICES

The rats were housed (five rats/cage as per recommendations made by National Research Council of the National Academies , 2011) in solid floor polypropylene rat cages (size: 410 mm x 282 mm x 180 mm). Each cage was fitted with a stainless steel top grill and a polypropylene water bottle with stainless steel drinking nozzle. The bottom of the cage was layered with clean, sterile rice husk. The cages were kept on six tier racks and their positions were rotated weekly. The cages and bedding material were changed on a daily basis.

FOOD AND WATER

The rats were provided with food and water *ad libitum*. The quality of food and water is regularly monitored at Jai Research Foundation. There were no known contaminants in the food and water at levels that would have interfered with the experimental results obtained.
Food: Teklad Certified Global High Fiber Rat/Mice feed manufactured by Harlan, U.S.A.
Water: UV sterilized water filtered through Kent Reverse Osmosis water filtration system.

ANIMAL IDENTIFICATION

Each rat was uniquely numbered on the tail using a tattoo machine. Appropriate labels were attached to the cages indicating the experiment number, test item name, group number and sex, dose, type of study, cage number and animal number.

This test was undertaken in compliance with the 'Guidelines for Laboratory Animals Facility' issued by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India (CPCSEA, 2010). These guidelines promote the humane care of animals used in research by providing specifications that will enhance animal well being and experimental quality for the advancement of biological knowledge that is relevant to humans and animals.

Project proposal for the experimentation was approved by the Institutional Animal Ethics Committee (IAEC), Jai Research Foundation. The Jai Research Foundation is also accredited with the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) that promotes the humane treatment of animals in science (JRF CPCSEA Approval No. 35/PO/bc/99/CPCSEA, Ethics No. JRF/IAEC/2013/274: JRF Research Number – RES-1-01-7366).

EXPERIMENTAL DESIGN

The rats were exposed using a head/nose-only inhalation exposure system designed to sustain a dynamic air flow rate of 14 to 15 air changes per hour, ensure an adequate oxygen content of at least 19% and CO_2 levels < 1% and a homogeneous, evenly distributed, respirable test aerosol with a Mass Median Aerodynamic Diameter (MMAD) particle size range between 1 and 4 microns. The pressure inside the exposure chamber was maintained slightly negative to the atmospheric pressure of the room to prevent leakage of the test aerosol into the surrounding area (OECD, 2009).

INHALATION EQUIPMENT

The 4 hour acute inhalation exposure was carried out using head/nose-only inhalation exposure equipment supplied by BIO-TOX Instrumentations International, New Delhi, India. This dynamic inhalation exposure system consists of a flow-past head/nose-only inhalation exposure chamber with Perspex rat exposure/restraint tubes, air compressor, flow meter, continuous infusion syringe pump, aerosol generator, cascade impactor, thermohygrometer, oxygen monitor, carbon dioxide monitor and open face gravimetric filter sampler.

DESCRIPTION OF INHALATION CHAMBER

(Refer Schematic Layout of Exposure System in Figures 1.1 and 1.2)

The inhalation equipment has 3 main parts namely inlet, exposure and outlet chambers. Each part is 30 cm in height and 30 cm in internal diameter. The total capacity of the chamber is 63.5 litres. The inlet unit (upper) consists of a glass cylinder with facility for the attachment of a spray atomizer. The exposure unit (middle) is made of stainless steel with 20 portholes to accommodate transparent perspex rat exposure tubes. Rat exposure tubes are provided with orifices to eliminate excreta and urine, which are collected in a dropping tray. These exposure tubes are accommodated in the portholes of the inhalation chamber. The adjustable unit of the exposure tube is set in such a way that rats breathe the test item aerosol through the window panel of the exposure tube (nose only) (Cannon *et al*., 1983). Observations during the inhalation experiment were made through the perspex rat exposure tubes. The outlet unit (lower) is made of stainless steel with an outlet provision connected to a suction pump. The outgoing air from the chamber passes through an impinger containing 1.0% sodium hydroxide solution and moisture traps.

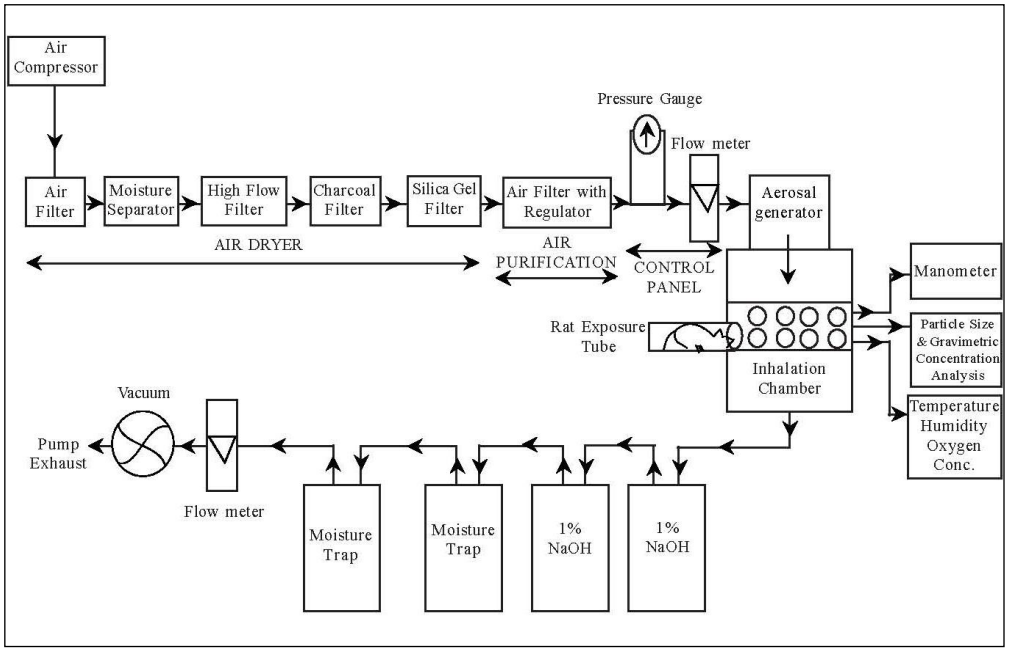


Figure 1.1: Schematic layout of exposure system

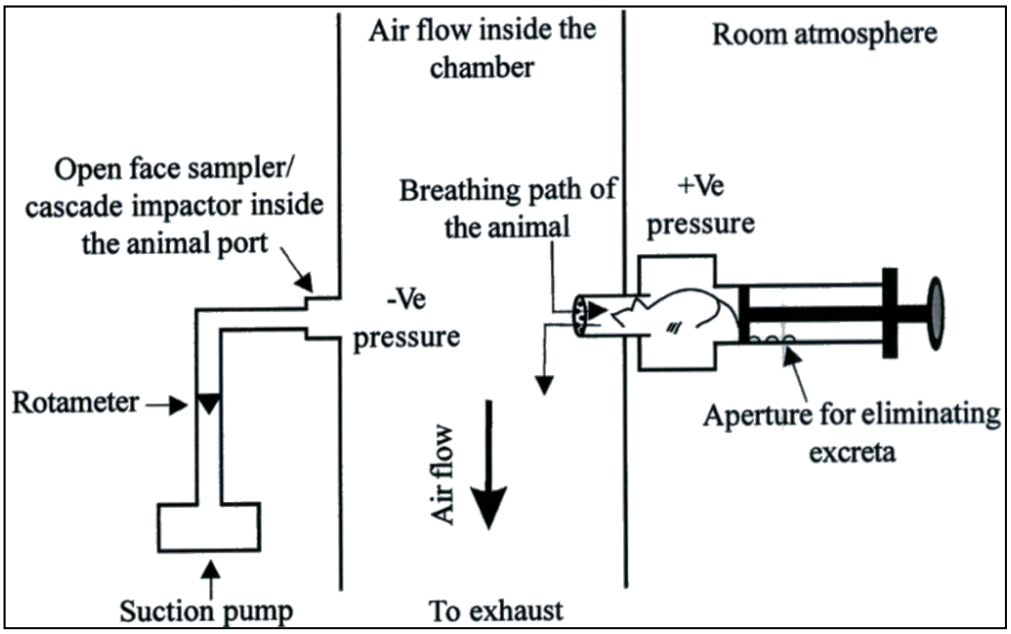


Figure 1.2: Schematic layout of air flow with respect to chamber and exposure tube

PROCEDURE FOR THE GENERATION OF AEROSOLS

Acephate 97% DF is solid and therefore 100 g of test item was diluted with distilled water and final volume was made up to 200 mL (Stock solution concentration of 500 mg/mL). Prepared stock solution was loaded into an infusion syringe, positioned on the continuous infusion syringe pump (manufactured by B. Braun Melsungen AG, West Germany). The test item was infused at the rate of 40 mL/h to generate the aerosols with the help of a spray atomizer. Rats were exposed for 4 hours continuously after the target chamber aerosol concentration was achieved.

The nominal concentration of aerosolized Acephate 97% DF was calculated to be 22.222 mg/L air based on the mass of test item used per hour and the total volumetric flow through the exposure chamber per hour (900 L/hour) (Table 1.1). The air inflow and outflow rates were maintained at 15 and 20 litres per minute (Appendix 1.1), respectively and were monitored throughout the exposure period. The temperature, humidity, oxygen and carbon dioxide concentrations were monitored and recorded at every 60 minutes.

The chamber temperature and the relative humidity recorded using the thermo-hygrometer was 20.1 to 22.5 °C and 68.1 to 68.4%, respectively (Appendix 1.1). The oxygen concentration inside the chamber was recorded using the oxygen monitor; Uniphos 310 was 20.5 to 21.7% (Appendix 1.1). The carbon dioxide concentration inside the chamber recorded using the carbon dioxide monitor; Uniphos 225 (PM) was 0.03 to 0.04% (Appendix 1.1).

Each rat was restrained in a single transparent perspex exposure (nose only) tube with adjustable unit. The exposure tubes were accommodated in the portholes of the inhalation chamber. The adjustable unit of the exposure tube was set so that animals breathed Acephate 97% DF aerosols through the window panel of the exposure tube. Observations during inhalation exposure were made through the transparent perspex exposure tubes.

GRAVIMETRIC CONCENTRATION ANALYSIS

Gravimetric samples were collected and analyzed to determine the chamber aerosol concentration (Willeke and Baron, 1993). The concentration of aerosol present in the chamber was determined gravimetrically two times during the four-hour exposure period. Each sample was taken by drawing chamber atmosphere from the animal breathing zone at a set rate using a constant flow air sampling pump (1.77 LPM). The aerosol particles were

collected on pre-weighed glass micro fibre filters (Whatman Glass Microfibre Filters, GF/A, 25 mm diameter, manufactured by Whatman International Ltd., Maidstone, England). On the day of the acute 4-hour inhalation exposure, the breathing zone concentration was calculated (5.133 mg/L air) from the gravimetric measurements (Table 1.2).

DESCRIPTION OF SEVEN STAGE CASCADE IMPACTOR (MODEL N°: 02-150)

The unit (manufactured by In-Tox Products, USA) consisted of an inlet cone, seven stages, seven collection plates and a final (backup) filter (Whatman Glass Microfibre Filters). The stages were numbered from 1 to 7 and F. Stage F contained the final filter. All these impactor stages contained multiple precision-drilled orifices. The orifices of the stages were progressively smaller from top to bottom. When air was drawn through the sampler, multiple jets of air in each stage directed any airborne particles towards the surface of the collection plate for the respective stage. The base stage had an outlet nipple connected to an exhaust pump. Aerosols from the chamber were drawn into the cascade impactor (7 stages) with pre-weighed stainless steel collection plates using a vacuum pump. The sampling flow rate was maintained at 1.77 litres per minute. At the end of the sampling period (1 minute), the collection plates with test item were disassembled and weighed. The increase in the weight of each collection plate was the mass of particles in the size range of that impact stage. The total mass of particles and the percent mass of particles in each size range was calculated. The mean cumulative percent particle size was calculated for a required particle size by adding the mean particle size distribution from 0 to that required particle size. Mass Median Aerodynamic Diameter (MMAD) was calculated directly from percent particle size distribution (Phalen, 2009) (Table 1.3; Figure 1.3).

OBSERVATIONS

SIGNS OF TOXICITY

All rats were observed for any signs of toxicity as per the guideline (OECD, 2000) and mortality at hourly intervals during the 4 h exposure period and at 1 and 2 h after the exposure on the day of exposure and once a day for morbidity and mortality for a period of 14 days following exposure (Appendix 1.2).

BODY WEIGHT

Body weights were recorded prior to exposure on day 0 and on post-exposure days 1, 3, 7 and 14 (Table 1.4).

NECROPSY

At the end of observation period (on day 14) all the rats were sacrificed by intraperitoneal administration of thiopentone sodium 150 mg/kg body weight and were subjected to gross pathological examination. This consisted of an external examination and the opening of the nasal passage, abdominal and thoracic cavities. The appearance of any macroscopic abnormalities was recorded (Appendix 1.3).

STATISTICAL ANALYSIS OF RESULTS

As this study was conducted as a limit test (Technical Committee of the Inhalation Specialty Section, SOT, 1992) at the maximum achievable breathing zone concentration of 5.133 mg/L air, no statistical analysis was required to calculate the LC_{50}.

RESULTS

CONCENTRATION DETAILS OF TEST ITEM IN THE INHALATION CHAMBER

Breathing zone concentration of Acephate 97% DF in the exposure chamber was 5.113 mg/L air (Table 1.2). The average Mass Median Aerodynamic Diameter (MMAD) of aerosolized Acephate 97% DF was determined to be 3.24 µm with an average geometric standard deviation (GSD) of 2.81 (Table 1.3, Figure 1.3). The chamber aerosol concentration of Acephate 97% DF was determined from the entire mass collected on the glass fibre filter x 0.5 (dilution factor).

MORTALITIES

No mortality was observed in rats nose-only exposed for 4 hours to the breathing zone concentration of 5.133mg Acephate 97% DF /L air (Table 1.5).

CLINICAL OBSERVATIONS

No treatment-related clinical signs were observed in any of the rats exposed to the breathing zone concentration of 5.133mg Acephate 97% DF/L air in distilled water (Appendix 1.2).

BODY WEIGHT

Normal gain in body weight was observed throughout the experiment (Table 1.4).

NECROPSY (MACROSCOPIC FINDINGS)

External

External examination of terminally sacrificed animals did not reveal any abnormality of pathological significance (Appendix 1.3).

Internal

Visceral examination of terminally sacrificed male and female rats did not reveal any abnormality of pathological significance (Appendix 1.3).

INTERPRETATION OF RESULTS AND CONCLUSION

No mortality was observed following nose-only inhalation exposure to the breathing zone concentration of 5.113mg Acephate 97% DF/L air. Under the conditions of this study, the 4h acute inhalation median lethal concentration (LC_{50}) of Acephate 97% DF in male and female Wistar rats was found to be greater than the breathing zone concentration of 5.113 mg/L air.

Table 1.1

NOMINAL CONCENTRATION (mg/L air) DETAILS OF ACEPHATE 97% DF AND T_{95} CALCULATION IN INHALATION CHAMBER

Calculation of Stock Solution Concentration

A quantity of 100 g of test item was diluted with distilled water and final volume was made upto 200 mL to generate the aerosols (Stock solution concentration of 500 mg/mL).

Calculation of Nominal Concentration

$$\text{Nominal Concentration (mg/L air)} = \frac{\text{Total Test Item Consumed per Hour [Infusion Rate (mL/hour)} \times \text{Stock Solution Concentration]}}{\text{Air Flow Rate/Hour (LPH)}}$$

Stock Solution Concentration	Infusion Rate (mL/hour)	Air Flow Rate (LPM)	Air Flow Rate (LPH)	Nominal Concentration (mg/L air)
500	40	15	900	22.222

Key: LPM = Litres per minute, LPH = Litres per hour

Calculation of T_{95} (Equilibration period calculation)

T_{95} (minutes)

=3 × (Chamber volume/Chamber airflow)

=3 × (63.5/15)

=12.7 minutes

Table 1.2

CONCENTRATION MEASUREMENTS OF ACEPHATE 97% DF IN THE INHALATION CHAMBER

Breathing zone concentration was analysed gravimetrically with an open face sampler using glass microfibre filters (GF/A).

Sampling speed was 1.77 litres per minute, i.e., for 1 minute, 1.77 litres sample was drawn from the chamber.

Breathing Zone Concentration Calculation after Animals Loaded to the Inhalation Chamber

Group: I

S. N^{o}	Time (Minutes)	Pre weight (mg)	Post weight (mg)	Difference in Weight (mg)	Breathing Zone Concentration (mg/L air)	Mean ± SD (mg/L air)
1	30	25.33	43.54	18.21	5.144	5.133 ± 0.016
2	150	25.17	43.30	18.13	5.121	

Key: SD = Standard deviation

Note: Animals were loaded to the inhalation chamber after 15 minutes from the start of the equilibration period, post verification of the target aerosol concentration in the chamber; the gravimetric concentration analysis was initiated after 30 minutes from the start of exposure.

Table 1.3

PERCENT AND MEAN PERCENT PARTICLE SIZE DISTRIBUTION IN THE INHALATION CHAMBER

Group: I **Breathing Zone Concentration (mg/L air): 5.113**

Particle Size Range (µm)	ECD (µm)	Particle Size Distribution During Exposure				Mean Percent Particle Size	Cumulative Percent Particle Size
		1 Hour		3 Hour			
		Mass (mg)	%	Mass (mg)	%		
> 6.35	6.35	0.27	25.23	0.25	22.73	23.98 (1.77)	100.00
4.25 – 6.35	4.25	0.21	19.63	0.20	18.18	18.90 (1.02)	76.02
2.85 – 4.25	2.85	0.12	11.21	0.14	12.73	11.97 (1.07)	57.12
1.95 – 2.85	1.95	0.12	11.21	0.12	10.91	11.06 (0.22)	45.15
1.45 – 1.95	1.45	0.18	16.82	0.17	15.45	16.14 (0.97)	34.09
0.97 – 1.45	0.97	0.09	8.41	0.11	10.00	9.21 (1.12)	17.95
0.64 – 0.97	0.64	0.07	6.54	0.09	8.18	7.36 (1.16)	8.74
0 – 0.64	-	0.01	0.93	0.02	1.82	1.38 (0.62)	1.38
Total mass in 1.77 litres		1.07	100.00	1.10	100.00	-	-

Key: ECD = Effective cut off diameter, - = Not applicable

Values shown in parentheses are standard deviations.

Table 1.4

INDIVIDUAL AND MEAN BODY WEIGHT (G) AND BODY WEIGHT CHANGE (%)

Breathing Zone Concentration (mg/L air)	Sex	Rat N°	Body Weights (g) on Day					Percent Body Weight Change on Day			
			0	1	3	7	14	1	3	7	14
5.113		1	360.1	361.4	363.6	371.4	383.3	0.4	1.0	3.1	6.4
	Male	2	332.5	333.1	334.2	342.7	353.7	0.2	0.5	3.1	6.4
		3	317.9	319.0	320.1	330.9	347.5	0.3	0.7	4.1	9.3
		4	340.8	341.2	343.1	351.8	360.1	0.1	0.7	3.2	5.7
		5	341.6	341.9	342.4	350.9	361.8	0.1	0.2	2.7	5.9
		Mean	**338.6**	**339.3**	**340.7**	**349.5**	**361.3**	**0.2**	**0.6**	**3.2**	**6.7**
		Standard Deviation	**±15.3**	**±15.4**	**±15.8**	**±14.8**	**±13.5**	**±0.1**	**±0.3**	**±0.5**	**±1.5**
	Female	6	204.3	204.8	205.3	208.1	212.3	0.2	0.5	1.9	3.9
		7	176.1	177.0	178.4	179.8	181.4	0.5	1.3	2.1	3.0
		8	190.3	191.3	192.6	198.6	199.9	0.5	1.2	4.4	5.0
		9	202.5	202.8	204.6	206.4	208.2	0.1	1.0	1.9	2.8
		10	201.3	202.6	204.3	206.1	208.9	0.6	1.5	2.4	3.8
		Mean	**194.9**	**195.7**	**197.0**	**199.8**	**202.1**	**0.4**	**1.1**	**2.5**	**3.7**
		Standard Deviation	**±11.8**	**±11.7**	**±11.7**	**±11.8**	**±12.5**	**±0.2**	**±0.4**	**±1.1**	**±0.9**

Key: 0 = Before exposure

Table 1.5

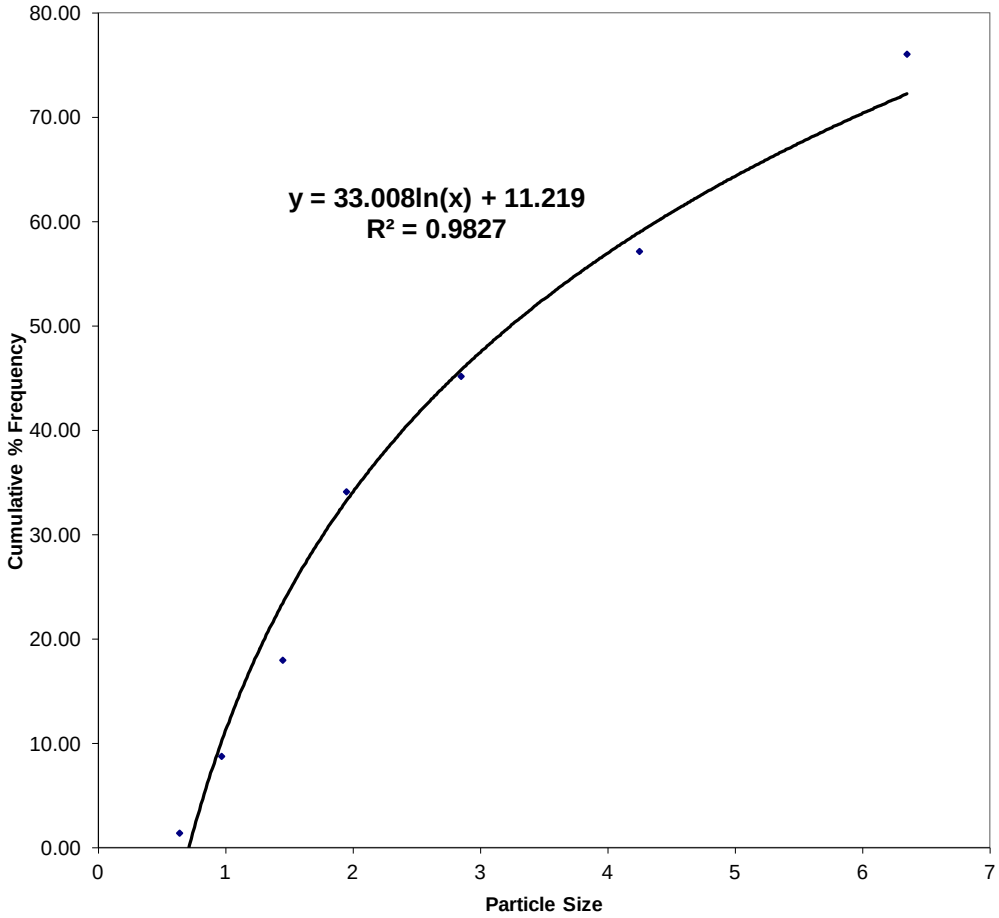
MORTALITY

Group N°	Breathing Zone Concentration (mg/L air)	Number and Sex of Rats Used	Mortality										
			During Exposure	After Exposure						Number		Percent	
			1-4 h	1h	2h	24 h	48 h	72 h	4-7 Day	8-14 Day	M	F	M	F
I	5.113	5 M + 5 F	0	0	0	0	0	0	0	0	0	0	0	0

Key: M = Male, F = Female, h = Hour

Figure 1.3

PERCENT PARTICLE SIZE DISTRIBUTION CURVE



Mass Median Aerodynamic Diameter (50% MMAD) = 2.96 µm

Mass Median Aerodynamic Diameter (84.1% MMAD) = 8.60 µm

$$\text{Geometric Standard Deviation (GSD)} = \frac{\text{Size at 84.1\% mass}}{\text{Size at 50\% mass}} = \frac{8.60}{2.96} = 2.91$$

Appendix 1.1

EXPOSURE DETAILS

Group: I **Breathing Zone Concentration (mg/L air): 5.113**

Time (Minutes)	Exposure Chamber					
	Air In-Flow Rate (LPM)	Air Out-Flow Rate (LPM)	Temperature (°C)	Relative Humidity (% RH)	Oxygen Concentration (%)	Carbon Dioxide Concentration (%)
0	15	20	22.0	68.1	20.6	0.03
60	15	20	22.5	68.3	20.7	0.03
120	15	20	22.1	68.0	20.5	0.04
180	15	20	22.0	68.2	20.6	0.03
240	15	20	20.1	68.4	20.6	0.04

Key: LPM = Litres per minute

Note: The ‘0’ minute reading is when animals were loaded to the exposure chamber.

Appendix 1.2

INDIVIDUAL CLINICAL OBSERVATIONS

Group: I **Breathing Zone Concentration (mg/L air): 5.113**

Rat N°	Sex	Clinical Signs Observed																			
		At Hour (Day 0)						On Day													
		1	2	3	4	*	**	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	Male	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	Male	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3	Male	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
4	Male	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	Male	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	Female	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	Female	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	Female	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	Female	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	Female	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Key: 1 = Normal, * = 1st hour post-exposure, ** = 2nd hour post-exposure

Note: At hour (Day 0), 1 to 4 hours observations were during the exposure period.

Appendix 1.3

INDIVIDUAL NECROPSY FINDINGS

Group: I **Breathing Zone Concentration (mg/L air): 5.113**

Rat N°	Sex	Mode of Death	External	Internal
1	Male	Terminal sacrifice	No abnormality detected	No abnormality detected
2	Male	Terminal sacrifice	No abnormality detected	No abnormality detected
3	Male	Terminal sacrifice	No abnormality detected	No abnormality detected
4	Male	Terminal sacrifice	No abnormality detected	No abnormality detected
5	Male	Terminal sacrifice	No abnormality detected	No abnormality detected
6	Female	Terminal sacrifice	No abnormality detected	No abnormality detected
7	Female	Terminal sacrifice	No abnormality detected	No abnormality detected
8	Female	Terminal sacrifice	No abnormality detected	No abnormality detected
9	Female	Terminal sacrifice	No abnormality detected	No abnormality detected
10	Female	Terminal sacrifice	No abnormality detected	No abnormality detected