

CODING FORMS FOR SRC INDEXING

Microfiche No.	OTS0559796		
New Doc ID	88990000268	Old Doc ID	8EHQ-0999-14549
Date Produced	08/03/99	Date Received	09/10/99
		TSCA Section	8E
Submitting Organization	HODOGAYA CHEM (USA) INC		
Contractor	COVANCE LABS LTD		
Document Title	INITIAL SUBMISSION: FINAL REPORT, TP-415 - SINGLE EXPOSURE (INHALATION (HEAD-ONLY) TOXICITY STUDY IN THE RAT, WITH COVER LETTER DATED 9/8/1999		
Chemical Category	1-TETRADECANAMINIUM, N,N-DIMETHYL-N-TETRADECYL-, HEXA-MU.-*		

**INITIAL
SUB-
MISSION**

A 03

8EHQ-0999-14549

HODOGAYA CHEMICAL (U.S.A.), INC.

123 MAIN STREET, 9TH FLOOR

WHITE PLAINS, N.Y. 10601

TEL: (914) 422-0888

FAX: (914) 422-0868

September 8, 1999

MR 26318

Document Control Officer
Office of Pollution Prevention
and Toxic Substances, 7407
U.S. Environmental Protection Agency
401 M Street, SW
Washington, DC 20460

RECEIVED
OPPT/CBIC
1999 SEP 10 AM 11:01

Re: TSCA Health & Safety Study - TP-415

To Whom It May Concern:

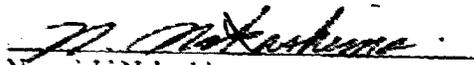
Attached for your review and records, is a copy of a Toxicity Study done by Covance Laboratories Ltd of N. Yorkshire, England in August 1999.

The study was performed on test article, TP-415.

If you have any questions or need further information, please let me know.

Very truly yours,

HODOGAYA CHEMICAL (U.S.A.) INC.


Naomichi Nakashima
Deputy General Manager

Contains No CBI

RECEIVED
OPPT/NCI
1999 SEP 27 AM 9:53

8EHQ-99-14549
06899 0000 2608

A 04

8EHQ-0999-14549

TSCA HEALTH & SAFETY STUDY COVER SHEET - revised 6/25/96

TSCA CBI STATUS:

CHECK IF THIS PAGE CONTAINS CONFIDENTIAL BUSINESS INFORMATION (CBI)

Clearly mark the confidential information with bracketing and check the box in the appropriate section. (Contains CBI. Submit a sanitized cover sheet with CBI deleted. Mark the sanitized copy, "Public Display Copy" in the heading.

1.0 SUBMISSION TYPE <input type="checkbox"/> Contains CBI <input type="checkbox"/> 8(d) <input checked="" type="checkbox"/> 8(e) <input type="checkbox"/> FYI <input type="checkbox"/> 4 <input type="checkbox"/> OTHER: Specify _____ <input type="checkbox"/> Initial Submission <input type="checkbox"/> Follow-up Submission <input type="checkbox"/> Final Report Submission Previous EPA Submission Number or Title if update or follow-up: _____ Docket Number, if any: # _____ <input type="checkbox"/> continuation sheet attached		
2.1 SUMMARY/ABSTRACT ATTACHED (may be required for 8(e); optional for 8(d) & FYI) <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	2.2 SUBMITTER TRACKING NUMBER OR INTERNAL ID	2.3 FOR EPA USE ONLY
3.0 CHEMICAL/TEST SUBSTANCE IDENTITY <input type="checkbox"/> Contains CBI Reported Chemical Name (specify nomenclature if other than CAS name): CAS# <u>117342-25-3</u> 1-Tetradecanaminium, N,N-dimethyl-N-tetradecyl-, hexa-, mu.-oxotetra-, mu.3-oxodi-, mu.5-oxotetradecaooctamolybdate(4-) (4:1) Purity <u>100%</u> <input type="checkbox"/> Single Ingredient <input checked="" type="checkbox"/> Commercial/Tech Grade <input type="checkbox"/> Mixture Trade Name: <u>TP-415</u> Common Name: _____ CAS Number: _____ NAME: _____ % WEIGHT: _____ Other chemical(s) present in tested mixture: _____ <input type="checkbox"/> continuation sheet attached		
4.0 REPORT/STUDY TITLE <input type="checkbox"/> Contains CBI <u>TP-415: Single Exposure Inhalation (Head Only) Toxicity Study in Rat</u> <input type="checkbox"/> continuation sheet attached		
5.1 STUDY/TSCATS INDEXING TERMS (CHECK ONE) HEALTH EFFECTS (HE): <input checked="" type="checkbox"/> ENVIRONMENTAL EFFECTS (EE): _____ ENVIRONMENTAL FATE (EF): _____		
5.2 STUDY/TSCATS INDEXING TERMS (see instructions for 4 digit codes) STUDY TYPE: <u>ATOX</u> SUBJECT ORGANISM (HE, EE only): <u>RATS</u> ROUTES OF EXPOSURE (HE only): <u>LNHL</u> VEHICLE OF EXPOSURE (HE only): _____ Other: _____ Other: _____ Other: _____		
6.0 REPORT/STUDY INFORMATION <input type="checkbox"/> Contains CBI <input type="checkbox"/> Study is GUP Laboratory: <u>Covance Laboratories Ltd</u> Report/Study Date: _____ Source of Data/Study Sponsor (if different than submitter): _____ Number of pages: _____ <input type="checkbox"/> continuation sheet attached		
7.0 SUBMITTER INFORMATION <input type="checkbox"/> Contains CBI Submitter: <u>Toshio Obara</u> Title: <u>Vice President</u> Phone: <u>(914) 922-0888</u> Company Name: <u>Hobogaya Chemical USA</u> Company Address: <u>123 Main St, White Plains, NY 10601</u> Technical Contact: <u>Toshio Obara</u> Submitter Address (if different): _____ Phone: <u>(914) 922-0888</u> <input type="checkbox"/> continuation sheet attached		
8.0 ADDITIONAL/OPTIONAL STUDY COMMENTS <input type="checkbox"/> Contains CBI <p style="text-align: center; font-size: 24pt; font-weight: bold;">Contains No CBI</p> <input type="checkbox"/> continuation sheet attached		

1999 SEP 10 AM 11:01

RECEIVED
DPT CBI/C

BEHQ-99-14549



88990000268



Submitter Signature: Toshio OBARA

Date: 8/29/99

M R 26318

A 05

RECEIVED

Final Report

Study Title TP-415: Single Exposure Inhalation (Head-Only)
Toxicity Study in the Rat

Author N M Shepherd

Sponsor Hodogaya Chemical Co., Ltd.
66-2, Horikawa-cho
Saiwai-ku
Kawasaki-shi 210
Japan

Study Monitor Mr K Kashima

Test Facility Covance Laboratories Ltd
Otley Road, Harrogate
North Yorkshire HG3 1PY
ENGLAND

Covance Report Number 558/9-D6154

Report Issued August 1999

Page Number 1 of 74

Contains No CBI

**STUDY DIRECTOR AUTHENTICATION AND GLP COMPLIANCE
STATEMENT**

TP-415: Single Exposure Inhalation (Head-Only) Toxicity Study in the Rat

I, the undersigned, hereby declare that the work described in this report was performed under my supervision, and that the report provides a true and accurate record of the results obtained.

The study was performed in accordance with the agreed protocol, unless otherwise stated, and the study objectives were achieved. The study was also performed in accordance with Covance Standard Operating Procedures and the principles of the following codes of Good Laboratory Practice:

UK Good Laboratory Practice Regulations 1997

OECD Good Laboratory Practice (revised 1997, issued 1998)

N Shepherd
N Shepherd BSc
Study Director
Covance Laboratories Ltd

3 August 1999
Date

QUALITY ASSURANCE STATEMENT

TP-415: Single Exposure Inhalation (Head-Only) Toxicity Study in the Rat

The study described in this report was subject to audit by the independent Quality Assurance Department as indicated below. The findings of each audit were reported to the Study Director and Management as prescribed by Standard Operating Procedures.

The report audit was designed to confirm that the methods described and results incorporated in the report accurately reflect the raw data produced during the study.

Inspection programme	Inspection date	Report date
Protocol review	12 January 1999	12 January 1999
Dose administration	2 February 1999	2 February 1999
Data review	April 1999	5 May 1999
Draft study report	April 1999	5 May 1999

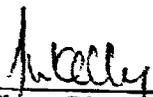
G. Wood
G Wood
Section Head Quality Assurance Unit
Covance Laboratories Ltd

3 August 1999
Date

SCIENTIFIC REVIEWER'S STATEMENT

TP-415: Single Exposure Inhalation (Head-Only) Toxicity Study in the Rat

I, the undersigned, hereby declare that I have reviewed this report in conjunction with the Study Director and that the interpretation and presentation of the data in the report are consistent with the results obtained.



J Kelly Msc Pharm Biochem
Head, Toxicology AH Operations
Covance Laboratories Ltd

3 August 1999
Date

RESPONSIBLE PERSONNEL

TP-415: Single Exposure Inhalation (Head-Only) Toxicity Study in the Rat

In addition the following staff were responsible for key elements of the study:

Deputy Study Director	A C Gibbs
Head of Pathology	J Glaister
Inhalation Chemistry	B Canham
Formulations	B Halliday
Animal house supervisor	J Cunningham
Animal health and welfare	A Basford
Necropsy	I Wilkins
Histology	S Brogden
Data processing	N Darwent
Head of Quality Assurance	S White

ARCHIVE STATEMENT**TP-415: Single Exposure Inhalation (Head-Only) Toxicity Study in the Rat**

All primary data or authenticated copies thereof, specimens and the final report will be retained in the Covance archives for ten years after submission of the final report. At this time the Sponsor will be contacted to determine whether data should be returned, retained or destroyed on their behalf.

Specimens requiring storage deep frozen are specifically excluded from the above. These will be retained for as long as the quality of the material permits evaluation but for no longer than three months after submission of the final report. The Sponsor will be contacted before specimens are destroyed on their behalf.

CONTENTS

STUDY DIRECTOR AUTHENTICATION AND GLP COMPLIANCE STATEMENT 2

QUALITY ASSURANCE STATEMENT 3

SCIENTIFIC REVIEWER'S STATEMENT 4

RESPONSIBLE PERSONNEL 5

ARCHIVE STATEMENT 6

CONTENTS 7

SUMMARY 9

INTRODUCTION 11

PROTOCOL ADHERENCE 12

TEST AND CONTROL ARTICLES 12

EXPERIMENTAL DESIGN 12

 Regulatory test guidelines 12

 Test article administration 12

 Definitive Study 13

TEST ARTICLE ATMOSPHERE GENERATION 13

 Development of the test atmosphere 13

 Generation of the test atmosphere 13

ATMOSPHERE CONTROL 13

 Exposure chamber temperature and relative humidity 13

 Exposure chamber air flow 14

 Exposure chamber oxygen concentration 14

 Nominal concentration 14

 Exposure concentration 14

 Particle size 14

TEST SYSTEM 15

 Species, strain and supplier 15

 Specification 15

 Environment and husbandry 15

 Housing 15

 Diet and water 15

PRE-EXPERIMENTAL OBSERVATIONS 16

 Acclimatisation and health procedures 16

 Allocation to treatment group 16

 Identification of the test system 16

EXPERIMENTAL PROCEDURES 17

 Clinical signs 17

 Morbidity and mortality 17

 Body weight 17

TERMINAL PROCEDURES	17
Necropsy.....	17
Lung weights.....	17
Histopathology.....	18
DATA EVALUATION	18
RESULTS	19
Exposure chamber temperature and relative humidity.....	19
Exposure chamber air flow.....	19
Exposure chamber oxygen concentration.....	19
Measured exposure chamber atmosphere concentrations.....	20
Exposure chamber particle size analysis.....	20
Mortality.....	20
Clinical observations.....	21
Body weight.....	21
Lung weight.....	22
Necropsy.....	22
Acute median lethal concentration.....	23
Toxicity assessment classification.....	23
CONCLUSION	24
FIGURES	25
Figure 1 Group mean body weight as a percentage of pre-exposure value - males.....	26
Figure 2 Group mean body weight as a percentage of pre-exposure value - females.....	27
TABLES	28
Table 1 Individual and group mean inhalation data.....	29
Table 2 Group summary of clinical signs.....	31
Table 3 Group mean body weight as a percentage of pre-exposure value.....	35
Table 4 Group mean lung weights and lung/body weight ratios.....	36
APPENDICES	37
Appendix 1 Inhalation procedures.....	38
Appendix 2 Individual and group mean body weights.....	42
Appendix 3 Individual and group mean lung weights and lung/body weight ratios.....	44
Appendix 4 Individual necropsy data.....	46
Appendix 5 Sponsor's Certificate of Test Article Analysis.....	50
Appendix 6 Certificates of diet analysis.....	51
Appendix 7 Study protocol.....	53

SUMMARY

The objective of the study was to estimate the toxicity of the test article, TP-415, in the rat following a 4-hour exposure (head-only). Groups of ten rats (five males and five females) of the CrI:CD(SD)IGSBR strain were exposed to the following chamber concentrations by head-only inhalation over a period of four hours:

Group number	Group description	Nominal concentration (mg/L)	Chamber concentration (mg/L)	Median value	
				MMAD (μ m)	GSD
1	Control	0	0	0	0
2	Low	0.7	0.22	1.83	2.15
3	Intermediate	4.1	1.27	2.77	2.33
4	High	175	5.34	4.45	2.33

MMAD = mass median aerodynamic diameter

Exposure was followed by an observation period of 14 days. As deaths occurred in the high exposure group additional animals were obtained, and the intermediate and low exposure groups were exposed 21 and 28 days after the control exposure. The animals were approximately 7 to 9 weeks at the start of exposure.

The test article was used as supplied.

The exposure chamber temperature recorded for the control and treated groups were in the ranges 21 to 22 °C and 22 to 29 °C, respectively, with values for relative humidity in the ranges 58 to 72 % and 21 to 40 %. The number of air exchanges per hour for the control, low, intermediate and high exposure chambers were approximately 15, 113, 36 and 36. The oxygen concentration was above protocol minimum of 19 % on all occasions with an actual range of 19.8 to 20.7 %. The median mass median aerodynamic diameter of the particles indicated the mean diameter of the aerosol was within the respirable range of the rat (up to 5 μ m).

All animals in the control and low exposure groups survived to the scheduled termination date. At the end of Day 3 all animals in the intermediate and high exposure groups had died or had been removed from study due to their clinical condition. Clinical signs, attributable to exposure to the test article atmosphere, were observed at all exposure concentrations. Treatment related signs, which may be summarised as loss of condition, a reduction in activity, disruption of the breathing pattern and reduced consumption of diet and water, were observed in all treated animals following exposure. Loss of body weight during exposure was as expected, but in treated groups continued at Day 2. Between Days 3 and 15, low exposure

animals gained body weight, and for the period Days 8 to 15 body weight gain was similar to controls. When normalised to pre-exposure body weight, the amount of weight gained in low exposure male and female groups was 18 and 9 %, respectively, less than the weight gained in controls.

At the low exposure, relative lung weight and necropsy findings did not suggest any effect of the test article. Macropathological findings in the animals which died or were removed from study may be attributed to irritation of the pulmonary tissues.

In conclusion, a single exposure to the test article, TP-415, at atmospheric concentrations of 1.27 and 5.34 mg/L for 4 hours resulted in marked adverse reactions and deaths (100% mortality: 1/10 at 1.27 mg/L and 2/10 at 5.34 mg/L found dead, all others killed in extremis). All animals survived exposure at 0.22 mg/L with transient toxicological observations. Therefore it can be concluded that the acute median lethal concentration (LC₅₀) is between 0.22 and 1.27 mg/L. With regard to acute inhalation toxicity under EU Directive 93/21/EEC Annex IV (May 1993), the classification of the test article is "T" (toxic).

INTRODUCTION

The objective of the study was to estimate the toxicity of the test article, TP-415, in the rat following a 4-hour inhalation exposure (head-only).

The test article was assigned a toxicity category according to published toxicity assessment criteria (see Appendix 1 of the report). This determination of toxicity class minimises animal usage.

The inhalation route was chosen as it is a possible route of human exposure.

The rat was selected as it is a readily available rodent species acceptable to the regulatory authorities with documented susceptibility to a wide range of toxic substances.

The study protocol was signed by the Study Director on 8 January 1999. The animals were received by Covance on 26 January 1999 (control and high exposure groups) and 16 February 1999 (low and intermediate exposure groups). The first exposure was conducted on 2 February 1999 (high exposure group), 3 February 1999 (control group), 24 February 1999 (intermediate group) and 3 March 1999 (low exposure group). Terminal necropsies were started on 17 February 1999 (control group) and completed on 17 March 1999 (low exposure group). The study completion date will be the date the final report is signed by the Study Director.

PROTOCOL ADHERENCE

The study was conducted in accordance with the agreed definitive protocol and two amendments. There were no major deviations from the protocol. Minor deviations, which did not affect the integrity or outcome of the study, are presented in the report.

TEST AND CONTROL ARTICLES

The test article, a white powder, identified as TP-415, was received at Covance as follows:

Test Article	Covance Lot Number	Batch Number	Quantity supplied (g)	Expiry Date	Date of receipt at Covance
TP-415	1	1412	1700	31 October 1999	26 October 1998

The purity of the test article was stated to be 100.0%. When not in use the test article was stored cool (1 to 10°C), under desiccant in the dark. The control article was filtered air.

EXPERIMENTAL DESIGN

Regulatory test guidelines

This study was designed to meet the known requirements of Annex to Commission Directive 92/69/EEC, Method B2, and OECD Guidelines for the Testing of Chemicals Method 403, adopted 12 May 1981.

Test article administration

The test article was administered by head-only inhalation for a period of four hours. The chamber was of appropriate size and internal volume for the exposure.

Definitive Study

The following groups were exposed:

Group number	Group description	Nominal concentration (mg/L)	Chamber concentration (mg/L)	Animals	
				Male	Female
1	Control	0	0	5	5
2	Low	0.7	0.22	5	5
3	Intermediate	4.1	1.27	5	5
4	High	17.5	5.34	5	5

The day of exposure was followed by a 14 day observation period. Group 3 and 4 animals were removed from the study during the observation period due to deaths and their poor condition. As deaths occurred in Group 4 additional animals were obtained, and Groups 3 and 2 were exposed 21 and 28 days after the Group 1 exposure. The animals' acclimatisation periods were seven days for Group 4, eight days for Groups 1 and 3 and fifteen days for Group 2. As the exposures were conducted on different days, the necropsy days were staggered accordingly.

TEST ARTICLE ATMOSPHERE GENERATION

Development of the test atmosphere

Atmosphere development was conducted using the test article as supplied. Data collected during atmosphere development are retained in the study records but not reported.

Generation of the test atmosphere

A Wright dust feed generator located immediately above the chamber was used to produce the atmosphere of test article. A schematic diagram of the continuous flow system used is shown in Appendix 1.

ATMOSPHERE CONTROL

Exposure chamber temperature and relative humidity

The temperature and relative humidity in the chamber were monitored continuously and recorded twice hourly. The temperature and relative humidity ranges were

slightly outside the target ranges of 20 to 24°C and 40 to 60%, respectively. The actual ranges recorded were 21 to 29°C and 21 to 72%, respectively.

Exposure chamber air flow

The air flow through the exposure chamber was monitored continuously and recorded twice hourly.

Exposure chamber oxygen concentration

The oxygen concentration in the exposure chamber was monitored continuously and recorded twice hourly. The oxygen level did not fall below the protocol minimum target value of 19% v/v.

Nominal concentration

The test article was weighed before and after exposure to determine the amount utilised during the exposure period.

Exposure concentration

The actual concentration in the exposure chamber was measured gravimetrically approximately twice hourly during the exposure period.

Particle size

The particle size of the atmospheres was measured prior to exposures, and then approximately one measurement/hour for each test group using gravimetric methodology. The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were calculated for each occasion.

TEST SYSTEM

Species, strain and supplier

A sufficient number of rats of the Cri:CD(SD)IGSBR strain were obtained from Charles River (UK) Ltd, Margate, to provide 20 healthy animals of each sex. Animals were obtained on two occasions, 10 animals of each sex per occasion.

Specification

The animals were ordered in the age range of about 6 to 7 weeks old on arrival and each sex within a 15g weight range. They were approximately 7 to 9 weeks old at the start of exposure.

Environment and husbandry

The animals were housed in a single room air-conditioned to provide a minimum of 15 air changes/hour and routinely maintained at a temperature of 19 to 25°C and a relative humidity of 40 to 70%. Although on some occasions values were recorded outside these ranges, these occasions were of short duration and are considered not to have affected the integrity or outcome of the study. The actual ranges recorded for temperature and humidity were between 16 to 28°C and 36 to 59% respectively. Fluorescent lighting was controlled automatically to give a cycle of 12 hours light (0600 to 1800 h) and 12 hours darkness.

Housing

The animals were housed in groups of five in stainless steel wire mesh cages (size 55 x 34 x 20 cm, floor area 1870 cm²) suspended over cardboard-lined trays.

Diet and water

Throughout the study, except during exposure, the animals had access *ad libitum* to SQC Rat and Mouse Maintenance Diet No 1, Expanded, (Special Diets Services Ltd. Witham). Each batch of diet was analysed for specific constituents and contaminants.

A certificate of analysis for each batch of diet used on this study is presented in Appendix 6.

Mains drinking water was available *ad libitum*, except during the exposure period, from water bottles attached to the cages.

The diet and water were considered not to have contained any contaminant at a level which might have affected the integrity or outcome of the study.

PRE-EXPERIMENTAL OBSERVATIONS

Acclimatisation and health procedures

All animals were given a clinical inspection for ill health on arrival. They were acclimatised for a minimum of 7 days and a veterinary inspection was performed before the start of exposure to ensure their suitability for the study.

Allocation to treatment group

Animals were assigned, by sex, as they came to hand on arrival, one to each cage for the available cages, until each cage contained the required number. Cages were arbitrarily allocated to study.

Identification of the test system

After allocation to treatment group each animal was permanently numbered by indelible ink on the tail as follows:

Group number	Colour code	Animal identification numbers	
		Male	Female
1	Buff	1-5	6-10
2	Blue	31-35	36-40
3	Green	21-25	26-30
4	Pink	11-15	16-20

Cages were appropriately identified with study information including study number and animal numbers.

EXPERIMENTAL PROCEDURES**Clinical signs**

Animals were observed for signs of ill health or overt toxicity. The animals were observed at hourly intervals during the exposure period, for the remainder of the working day and once daily thereafter for 14 days. An individual record was maintained of the clinical condition of each animal.

Morbidity and mortality

All animals were examined twice daily to detect any which were dead or moribund. Moribund animals were killed and necropsied to prevent autolysis.

Body weight

Individual body weights were recorded immediately before and after exposure on Day 1, and on Days 2, 3 (Group 2 only) 8 and 15 of the study, and before necropsy.

TERMINAL PROCEDURES

The following procedures were applied to all animals killed at the end of the study, those killed in extremis, and where possible, to those found dead:

Necropsy

At the terminal and unscheduled kills the animals were given an intraperitoneal injection of sodium pentobarbitone. Following exsanguination a full internal and external examination was made under the general supervision of a pathologist and all lesions were recorded. The nasal cavity and respiratory tract were examined and any irritation assessed.

Lung weights

The animals were weighed before necropsy. The lungs and trachea were dissected free from fat and other contiguous tissue and weighed prior to fixation.

Histopathology

Samples of all gross lesions were fixed in the appropriate fixative and retained without further processing.

DATA EVALUATION

Any signs of toxicity and mode of death were assessed. Data were processed, where appropriate, to give group mean values and standard deviations.

No further statistical analyses were performed.

RESULTS**Exposure chamber temperature and relative humidity (Table 1)**

The temperature recorded for the control and treated groups were in the ranges 21 to 22 °C and 22 to 29 °C, respectively. The majority of values recorded during the exposures of the low and intermediate groups were above the protocol range of 20 to 24 °C. Values recorded for relative humidity during the control and treated groups exposures were in the ranges 58 to 72 % and 21 to 40 %, respectively, slightly outside the target protocol range of 40 to 60 %. For treated groups the relative humidity range below the protocol range may be attributable to the test article being a dry powder. The temperature and relative humidity ranges observed are considered not to have affected the outcome or interpretation of the study.

Exposure chamber air flow (Table 1)

Exposure chamber air flow was 10, 75, 24 and 24 L/min for the control, low, intermediate and high exposure groups respectively. The corresponding number of air exchanges per hour were approximately 15, 113, 36 and 36.

Exposure chamber oxygen concentration (Table 1)

The oxygen concentration was above the protocol minimum of 19 % on all occasions with an actual range of 19.8 to 20.7 %. These values were considered to be satisfactory.

Measured exposure chamber atmosphere concentrations (Table 1)

For the treated groups the atmosphere concentrations were as follows:

Low exposure -	mean 0.22 mg/L, SD 0.05 mg/L individual values 0.15 to 0.27 mg/L nominal concentration 0.7 mg/L efficiency of generation 31 %
Intermediate exposure -	mean 1.27 mg/L, SD 0.22 mg/L individual values 0.91 to 1.60 mg/L nominal concentration 4.1 mg/L efficiency of generation 31 %
High exposure -	mean 5.34 mg/L, SD 0.77 mg/L individual values 3.54 to 6.11 mg/L nominal concentration 17.5 mg/L efficiency of generation 31 %

Variation between individual concentration values within an exposure was acceptable, although occasionally values extended the ranges recorded. The efficiency of generation for all the treated groups was comparable, with values of 31 % for the three exposures. These values are towards the upper end of the efficiency range that may be expected for a powder test article.

Exposure chamber particle size analysis (Table 1)

The median value for the mass median aerodynamic diameter (MMAD) for the low intermediate and high exposure groups was 1.83, 2.77 and 4.43 μm , with corresponding geometric standard deviation values of 2.18, 2.33 and 2.33. These geometric standard deviation values indicate the atmospheres were of an aerosol that was poly-dispersed. The increase in particle size with increased atmosphere concentration may be attributable to the individual particles agglomerating into larger ones. Overall these values indicate that the mean diameter of the aerosol was within the respirable range of the rat (up to 5 μm).

Mortality (Table 2)

All animals in the control and low exposure groups survived to the scheduled termination date. At the end of Day 3, 1/10 and 2/10 animals in the intermediate and high exposure groups respectively had been found dead and 9/10 and 8/10 respectively had been killed in extremis due to their poor clinical condition.

All intermediate and high exposure animals survived exposure, but one high exposure animal was removed from the study after 3 hours of the observation period. At Day 2 one intermediate female animal and one male and one female high exposure animal were found dead. At Day 3 all surviving animals from the intermediate (5 males 4 females) and high (3 males, 4 females) exposure animals were removed from the study due to their poor condition.

Clinical observations (Table 2)

Clinical signs, attributable to exposure of the test article atmosphere were observed at all exposure concentrations. Treatment related signs were observed in all animals in the intermediate and high exposure groups until their removal from the study and in animals of the low exposure group up to Day 6.

In the intermediate and high exposure groups the clinical signs attributed to the test article during the observation period included persistent staining of the eyes and fur, squinting, piloerection, hunched posture, lethargy, ataxia, effects on breathing and nasal discharge. Other clinical signs observed on Day 3 included swollen abdomen, rough fur, staining around the nose and mouth. Following exposure, the clinical signs observed in the low exposure group were similar to those in other groups on the day of exposure and on Day 2. However, the frequency and severity of the clinical signs observed decreased at Day 3 and all animals were normal at Day 6. It was also observed that the amount of diet and water consumed by the treated animals was less than expected at Days 2 and 3 for the intermediate and high exposure groups and for the low exposure group at Day 2.

Clinical signs observed in the control animals on the day of exposure (nasal discharge, stained eyes / fur and wet fur) are considered to be attributable to the restraint procedure. The clinical observations of rough fur, (one control animal) and stained head, (one low exposure animal) observed during the observation period, are considered to reflect the routine occasional observations seen in animals of this strain at this laboratory.

Body weight (Figures 1 & 2, Table 3, Appendix 2)

Following exposure, percentage body weight loss was similar in all groups. At Day 2, the body weight of control animals was similar to or greater than their pre-exposure body weight but all surviving treated animals continued to lose body weight. The loss of body weight in the treated groups at Day 2 and individual animals in the low

exposure group at Day 3 may be attributable to their poor condition after exposure and their reduced diet and water consumption.

At Day 3, the majority of low exposure animals had gained body weight when compared with Day 2, and at Day 8 all animals were similar to or greater than their pre-exposure body weight. Percentage body weight gain was similar for control and the low exposure groups between Days 8 and 15. When normalised to pre-exposure body weight, at Day 15 the amount of weight gained in low exposure male and female groups was 18 and 9 % less than the weight gained in controls. The animals from the low exposure group were heavier than those in the other groups at the start of the exposure period but the animals were still in the rapid growth phase and the variation in starting body weight is considered not to be the direct cause of the variation in body weight gain.

The EC Guidelines recommends that inter-animal variation in body weight at the start of exposure should not exceed $\pm 20\%$ of the overall mean weight. For males, two animals in the low exposure group exceeded this recommendation by up to 8 %. However, this variation is considered not to have affected the validity of the study.

Lung weight (Table 4, Appendix 3)

At the scheduled necropsy, group mean absolute lung weight for the low exposure group was increased when compared with controls (males 24 %, females 28 %). However, lung weight relative to body weight was only slightly increased, (males 10 % and females 11 %). These differences in absolute lung weight may be attributable to the difference in terminal body weights of control and low exposure animals.

When removed from study the absolute and relative lung weights of the animals in the intermediate and high exposure groups were all increased above the range of the terminal control animals. Although there were no concurrent controls, it is considered that these increases may be attributed to exposure to the test article.

Necropsy (Appendix 4)

At the terminal kill the findings in the low exposure animals did not suggest an effect of treatment. Findings in the control animals are similar to those that could be expected for animals of this age and strain at this laboratory.

Necropsy findings for the lung in the intermediate and the high exposure groups may be attributed to irritation of the pulmonary tissues. The findings of distension in tissues of the gastrointestinal track may be considered to be secondary effects attributable to the difficulties encountered by the animal in breathing due to the condition of the lungs. Other finding may be attributed to the overall condition of the animal and the onset of agonal changes.

Acute median lethal concentration

The acute median lethal concentration (LC_{50}) is between 0.22 and 1.27 mg/L for both sexes and the sexes combined. As the level of mortality at 1.27 mg/L was 10/10, the acute median lethal concentration (LC_{50}) and its fiducial limits could not be calculated.

Toxicity assessment classification

Under the EU toxicity assessment classifications the test article is allocated the toxicity classification - "T" (toxic).

CONCLUSION

In conclusion, a single exposure to the test article, TP-415, at atmospheric concentrations of 1.27 and 5.34 mg/L for 4 hours resulted in marked adverse reactions and deaths (100% mortality: 1/10 at 1.27 mg/L and 2/10 at 5.34 mg/L found dead, all others killed in extremis). All animals survived exposure at 0.22 mg/L with transient toxicological observations. Therefore it can be concluded that the acute median lethal concentration (LC_{50}) is between 0.22 and 1.27 mg/L. With regard to acute inhalation toxicity under EU Directive 93/21/EEC Annex IV (May 1993), the classification of the test article is "T" (toxic).

FIGURES

Figure 1
Group mean body weight as a percentage of pre-exposure value -- males

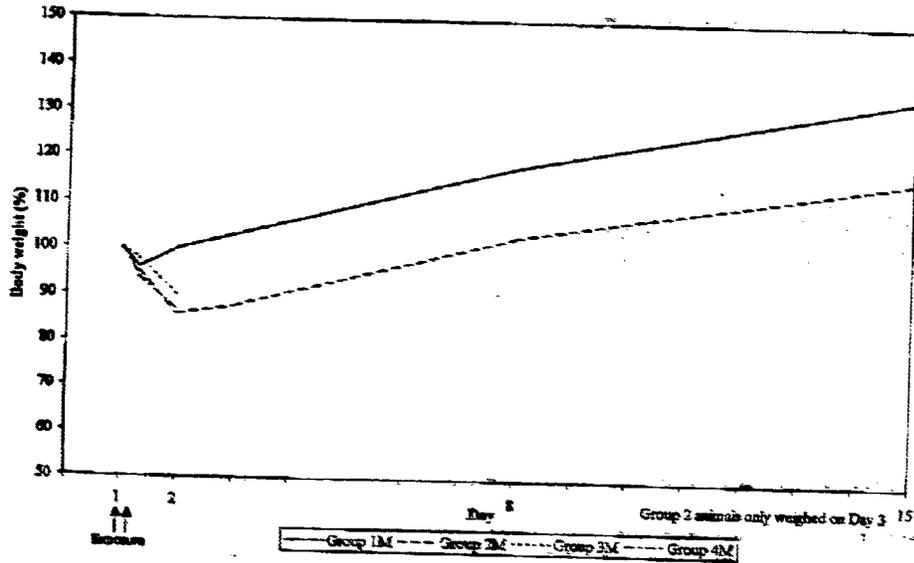
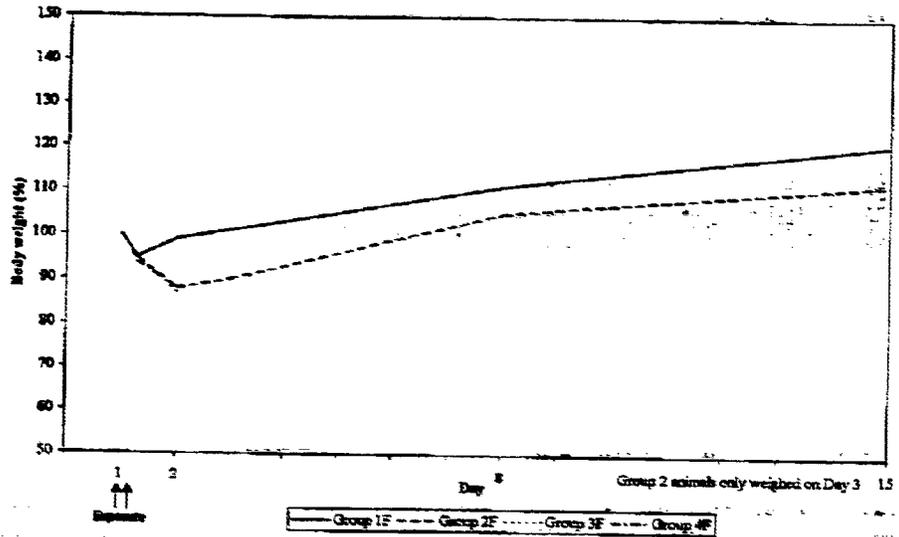


Figure 2
Group mean body weight as a percentage of pre-exposure value – females



C 04

Covance Study 558/9
Final Report

TABLES

Table 1
Individual and group mean inhalation data

Group 1					
Time point (min into exposure)	Temperature (°C)	Chamber conditions			Air flow (L/min)
		Humidity (%)	Oxygen (%)		
0	22	58	20.6		10
30	21	72	19.9		10
60	22	68	19.9		10
90	22	65	19.8		10
120	22	65	19.8		10
150	22	64	19.8		10
180	22	62	19.9		10
210	22	62	19.9		10
240	21	52	19.9		10
Mean	22	64	19.9		10
SD	0	4	0		0

Group 2							
Time point (min into exposure)	Temperature (°C)	Chamber conditions			Chamber concentration (mg/L)	Particle size	
		Humidity (%)	Oxygen (%)	Air flow (L/min)		MPPD (µm)	GSD
0	24	33	20.6	75			
15					0.24		
19							
30	26	24	20.6	75		1.68	2.21
44							
48					0.15†		
59					0.15†		
60	26	24	20.7	75	0.25		
75							
81					0.19		
90	27	23	20.6	75		1.78	2.01
105							
120	26	22	20.6	75	0.25		
139							
144					0.27		
150	25	22	20.6	75		2.21	2.15
160					0.20		
180	25	22	20.6	75			
199							
202						1.77	2.51
210	25	22	20.6	75	0.23		
222							
240	26	22	20.6	75	0.27		
Mean	26	24	20.6	75	0.22	1.63#	2.18#
SD	1	4	0.0	0	0.05		

Normal concentration (µg/L) = 0.7

median value

† low atmosphere concentration suggested the development of a technical difficulty within the generator. Difficulty resolved and exposure continued.

Table 1
Individual and group mean inhalation data

Group 3								
Time point (min into exposure)	Temperature (°C)	Chamber conditions			Air flow (L/min)	Chamber concentration (mg/L)	Particle size	
		Humidity (%)	Oxygen (%)	MMAD (µm)			GSD	
0	26	32	20.4	24				
20					1.47			
30	27	23	20.6	24				
33						2.74	2.41	
52					1.34			
60	27	21	20.7	24				
66					1.37	2.75	2.12	
77								
90	28	21	20.6	24				
100					1.22			
120	28	26	20.6	24				
122						2.79	2.26	
137					1.04			
150	29	34	20.6	24				
157					0.91			
180	25	35	20.5	24				
183						2.78	2.39	
197					1.60			
210	24	29	20.5	24				
220					1.24			
240	24	24	20.5	24				
Mean	26	27	20.6	24	1.27	2.77#	2.33#	
SD	2	5	0.1	0	0.22			

Nominal concentration (mg/L) = 4.1
median value

Group 4								
Time point (min into exposure)	Temperature (°C)	Chamber conditions			Air flow (L/min)	Chamber concentration (mg/L)	Particle size	
		Humidity (%)	Oxygen (%)	MMAD (µm)			GSD	
0	22	40	19.8	24				
12					3.54			
27					5.60			
30	22	40	19.8	24				
32						4.96	3.12	
55					5.90			
60	23	39	19.9	24				
75					5.10			
90	23	39	19.9	24				
95						3.58	2.42	
106					5.03			
120	24	38	20.0	24				
127					5.53			
132						4.44	2.24	
150	23	40	19.8	24				
160					5.86			
180	22	38	19.9	24				
194					6.11			
196						4.42	2.04	
210	22	36	19.9	24				
227					5.43			
240	23	40	19.9	24				
Mean	23	39	19.9	24	5.34	4.43#	2.33#	
SD	1	1	0.1	0	0.77			

Nominal concentration (mg/L) = 17.3
median value

Table 2
Group summary of clinical signs

Group and sex	Signs of reaction	Number showing sign during day of exposure	Number showing sign on day of observation:							
			2#	3	4	5	6	7	8	
1M	Normal		5	5	5	5	5	5	4	
	Nasal discharge	3								
	Eyes - stained	3								
	Fur - stained	5								
	Fur - wet	5								
	Rough fur - back								1	
2M	Normal				4	5	5	4		
	Breathing - laboured		2							
	Breathing - noisy		4	2	2	1				
	Breathing - rapid	1								
	Body - stained	5	5							
	Eyes - semi-closed	5	2							
	Eyes - stained	1								
	Fur - wet	5								
	Head - stained									
	Hunched	5	5						1	
	Lethargic	5	5							
	Nasal discharge									
	Piloerection	5	5	2	2					
	Thin			2	2					

Group 2 animals consumed less diet and water than expected

Group and sex	Signs of reaction	Number showing sign on day of observation:							
		9	10	11	12	13	14	15	
1M	Normal	4	4	4	4	4	4	5	
	Nasal discharge								
	Eyes - stained								
	Fur - stained								
	Fur - wet								
	Rough fur - back	1	1	1	1	1	1		
2M	Normal	4	5	5	5	5	5	5	
	Breathing - laboured								
	Breathing - noisy								
	Breathing - rapid								
	Body - stained								
	Eyes - semi-closed								
	Eyes - stained								
	Fur - wet								
	Head - stained	1							
	Hunched								
	Lethargic								
	Nasal discharge								
	Piloerection								
	Thin								

Table 2
Group summary of clinical signs

Group and sex	Signs of reaction	Number showing sign during day of exposure	Number showing sign on day of observation:	
			2#	3#
3M	Normal			
	Body - stained	5	5	5
	Eyes - stained	1	5	5
	Eyes - semi-closed	5	5	5
	Fur - wet	5		
	Hunched	5	5	5
	Laboured breathing	5	5	5
	Lethargic	5	5	5
	Nasal discharge	5	5	
	Noisy breathing	2	5	5
	Piloerection	5	5	
	Rough fur			5
	Staining - Nose and mouth			5
	Killed in extremis			5
4M	Normal			
	Abdomen - swollen			1
	Ataxia	1		
	Eyes - stained	4		2
	Fur - stained	5	2	3
	Fur - wet	5	3	
	Gasping	3	3	3
	Hunched	5	3	3
	Laboured respiration	5	3	3
	Lethargic	5	3	3
	Nasal discharge	3		
	Noisy respiration	5	3	3
	Squinting	5	3	3
	Thin			3
	Dead		1	
	Killed in extremis	1		3

Groups 3 and 4 animals consumed less diet and water than expected

Table 2
Group summary of clinical signs

Group and sex	Signs of reaction	Number showing sign during day of exposure	Number showing sign on day of observation:							
			2#	3	4	5	6	7	8	
1F	Normal		5	5	5	5	5	5	5	
	Nasal discharge	2								
	Eyes - stained	2								
	Fur - stained	5								
	Fur - wet	5								
2F	Normal		3	4	4	5	5	5		
	Breathing - laboured		3							
	Breathing - noisy		2	1	1	1				
	Breathing - rapid	1								
	Body - stained	5								
	Eyes - semi-closed	5								
	Eyes - stained	1								
	Fur - wet	5								
	Hunched	5								
	Lethargic	5								
	Nasal discharge			2	1					
	Piloerection	5	5							

Group 2 animals consumed less diet and water than expected

Group and sex	Signs of reaction	9	Number showing sign on day of observation:						
			10	11	12	13	14	15	
1F	Normal	5	5	5	5	5	5	5	
	Nasal discharge								
	Eyes - stained								
	Fur - stained								
	Fur - wet								
2F	Normal	5	5	5	5	5	5	5	
	Breathing - laboured								
	Breathing - noisy								
	Breathing - rapid								
	Body - stained								
	Eyes - semi-closed								
	Eyes - stained								
	Fur - wet								
	Hunched								
	Lethargic								
	Nasal discharge								
	Piloerection								

Table 2
Group summary of clinical signs

Group and sex	Signs of reaction	Number showing sign during day of exposure	Number showing sign on day of observation:	
			2	3
3F	Normal			
	Body - stained	5	4	4
	Breathing - laboured	5	4	4
	Breathing - noisy	3	4	4
	Eyes - stained		4	4
	Eyes - semi-closed	5	4	4
	Fur - wet	5		4
	Hunched	5	4	4
	Lethargic	5	4	4
	Nasal discharge	5	4	
	Piloerection	5	4	
	Rough fur			4
	Staining: nose and mouth			4
	Dead		1	
	Killed in extremis			4
4F	Normal			
	Abdomen - swollen			2
	Ataxia	1		
	Eyes - stained	2		2
	Fur - stained	5	4	4
	Fur - wet	5	4	
	Gasping		4	4
	Hunched	5	4	4
	Lethargic	5	4	4
	Respiration - Laboured	5	4	3
	Respiration - noisy	5	4	4
	Squinting	5	4	4
	Thin			4
	Dead		1	
	Killed in extremis			4

* Groups 3 and 4 animals consumed less diet and water than expected

Table 3
Group mean body weight as a percentage of pre-exposure value

Group and sex		1		% Pre-exposure on Day:				
		Pre-exposure	Post-exposure	2	3	8	15	
1M	Mean	100	96	100			118	134
	SD	0	2	2			2	5
2M	Mean	100	95	86	88	103	116	
	SD	0	2	3	5	2	4	
3M	Mean	100	96	90				
	SD	0	2	2				
4M	Mean	100	94	87				
	SD	0	2	1				

Group 2 animals only weighed on Day 3

Group and sex		1		% Pre-exposure on Day:				
		Pre-exposure	Post-exposure	2	3	8	15	
1F	Mean	100	95	99			111	121
	SD	0	1	0			3	4
2F	Mean	100	95	88	90	105	112	
	SD	0	2	2	3	4	5	
3F	Mean	100	95	87				
	SD	0	1	1				
2F	Mean	100	94	86				
	SD	0	1	0				

Group 2 animals only weighed on Day 3

Table 4
Group mean lung weights and lung/body weight ratios

Group and sex		Body weight (g)	Lung weight (g)	Ratio (%)
1M	Mean	309.3	1.624	0.5261
	SD	10.2	0.102	0.0447
2M	Mean	347.1	2.015	0.5802
	SD	16.8	0.163	0.0329
Group and sex		Body weight (g)	Lung weight (g)	Ratio (%)
1F	Mean	211.5	1.319	0.6251
	SD	20.6	0.107	0.0408
2F	Mean	244.0	1.698	0.6914
	SD	10.6	0.129	0.0363

APPENDICES

Appendix 1
Inhalation procedures

The following methods were used:

Generation of Test Atmosphere

A Wright dust feed generator located immediately above the chamber and supplied with compressed air was used to produce the atmosphere of test article.

The atmosphere was introduced into the top of a cylindrical chamber constructed of aluminium and glass (approximately 40 L internal volume).

The test atmospheres were scrubbed/filtered using a Vokes cartridge particulate filter exhausted to the outside of the building and vented.

ATMOSPHERE CONTROL**Exposure chamber temperature and relative humidity**

The temperature and relative humidity inside the exposure chamber were monitored using a digital thermometer with a remote probe located inside the chamber and a hair hygrometer located in the exhaust duct.

Chamber air flow

Chamber air flow was monitored continuously using flow meters throughout the exposure period.

Exposure chamber oxygen concentration

The oxygen concentration inside the chamber was monitored using an oxygen analyser incorporating a remote sensor.

Nominal concentration

Nominal concentration is defined as the amount of test article released per minute from the generator divided by the airflow per minute passing through the exposure system. The nominal concentration of the test article in the exposure chamber was calculated as shown:

$$\text{Nominal concentration (mg/L)} = \frac{\text{weight of test article used (mg)}}{\text{air flow (L/min)} \times \text{duration (min)}}$$

Exposure concentration

Samples were obtained during the exposure period. The concentration of the test article was determined gravimetrically. The atmosphere was sampled by drawing a known volume through a glass fibre open face filter positioned at a site representative of that occupied by the external nares of the experimental animals. The filter was weighed before and after sampling and the measured concentration of the test atmosphere was calculated as follows:

$$\text{Gravimetric concentration (mg/L)} = \frac{\text{total weight gain (mg)}}{\text{volume of sample (L)}}$$

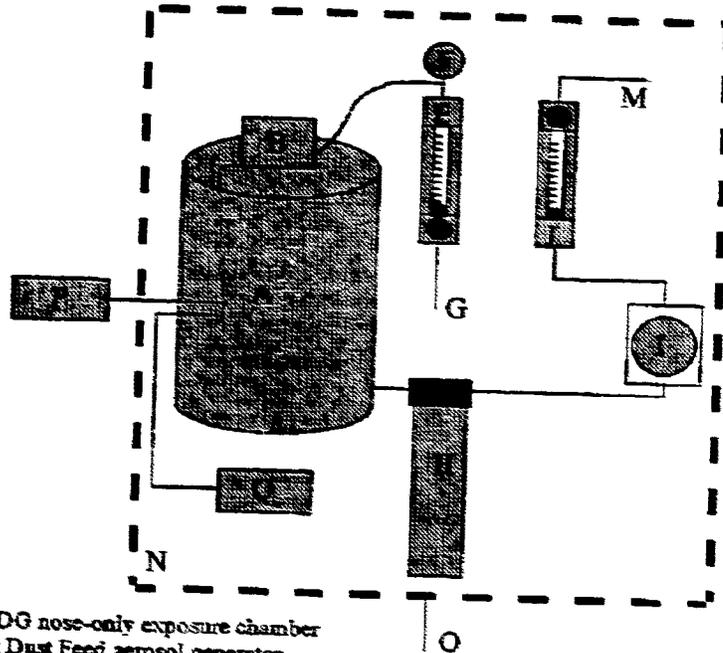
Exposure chamber particle size analysis

The particle size was determined using an Andersen 298 Marple Cascade Impactor, with six separation stages corresponding to maximum mass median aerodynamic diameters of 0.52, 0.93, 1.55, 3.50, 6.00 and 9.80 μm . The samples were obtained hourly over a period of up to 2 minutes at each exposure period.

The cumulative percentage by weight of test article collected at each successive stage was plotted by computer as a probability value against the logarithmic value of the upper class limit of that stage. The point at which the cumulative distribution line crossed the 50 percentile was the estimate of the mass median aerodynamic diameter (MMAD). The Geometric Standard Deviation was calculated.

Ref: The Operating Manual for Andersen 2000 Inc. 1 ACFM Ambient Particle Sizing Samplers January 1976. Revised September 9 1977.

Figure 1
Schematic representation of exposure system



KEY

- A - 40L ADG nose-only exposure chamber
- B - Wright Dust Feed aerosol generator
- E - Wright Dust Feed air supply flowmeter
- F - Pressure gauge
- G - Breathing quality compressed air supply
- H - 10" 3.0µm Vokes filter
- I - Humidity probe housed in polycarbonate cylinder
- J - Chamber extraction flowmeter
- M - Vacuum source
- N - Wooden chronic exposure cabinet
- O - Cabinet extract connected to Nilfisk extract system
- P - Temperature probe and readout box
- Q - Oxygen probe and readout box

Toxicity assessment criteria

The data obtained in the single dose inhalation study can be compared with the proposed toxicity classification below.

Commission Directive 93/21/EEC Annex IV (May 1993)

Toxicity category by inhalation	LC ₅₀ (mg/L)
very toxic	LC ₅₀ ≤ 0.25 (aerosols or particulates)
	LC ₅₀ ≤ 0.50 (gases and vapours)
toxic	0.25 < LC ₅₀ ≤ 1 (aerosols or particulates)
	0.50 < LC ₅₀ ≤ 2 (gases and vapours)
harmful	1 < LC ₅₀ ≤ 5 (aerosols or particulates)
	2 < LC ₅₀ ≤ 20 (gases and vapours)

Appendix 2
Individual and group mean body weights

Group and sex	Animal number	Body weight (g) on Day:				
		1 - Pre-exposure	1 - Post-exposure	2	8	15
1M	1	235	227	239	282	322
	2	228	220	232	272	311
	3	245	239	246	289	327
	4	227	213	224	266	310
	5	241	227	236	274	303
	Mean	235	225	235	277	315
SD	8	13	8	9	10	

Group and sex	Animal number	Body weight (g) on Day:					
		1 - Pre-exposure	1 - Post-exposure	2	3	8	15
2M	31	331	303	294	311	349	374
	32	293	277	247	243	305	349
	33	307	293	263	251	306	355
	34	302	286	248	271	314	361
	35	297	287	260	267	306	330
	Mean	306	290	262	269	316	354
SD	15	9	15	26	19	16	

Group and sex	Animal number	Body weight (g) on Day:				
		1 - Pre-exposure	1 - Post-exposure	2	8	15
3M	21	236	234	215	-	-
	22	242	242	223	-	-
	23	263	252	230	-	-
	24	252	248	228	-	-
	25	241	238	219	-	-
	Mean	247	243	223	-	-
SD	10	7	6	-	-	

Group and sex	Animal number	Body weight (g) on Day:				
		1 - Pre-exposure	1 - Post-exposure	2	8	15
4M	11	232	216	-	-	-
	12	232	215	201	-	-
	13	223	214	196	-	-
	14	230	213	-	-	-
	15	238	227	204	-	-
	Mean	231	217	200	-	-
SD	6	6	4	-	-	

Appendix 2
Individual and group mean body weights

Group and sex	Animal number	Body weight (g) on Day:				
		1 - Pre-exposure	1 - Post-exposure	2	8	15
1F	6	189	181	186	216	240
	7	187	177	185	204	230
	8	177	169	176	194	208
	9	160	153	159	179	193
	10	177	166	175	191	211
	Mean	178	169	176	197	216
	SD	11	11	11	14	19

Group and sex	Animal number	Body weight (g) on Day:					
		1 - Pre-exposure	1 - Post-exposure	2	3	8	15
2F	36	224	214	202	208	233	241
	37	230	217	205	208	250	262
	38	222	206	194	193	218	235
	39	220	213	187	194	239	259
	40	225	215	204	211	239	253
	Mean	224	213	198	203	236	250
	SD	4	4	8	9	12	12

Group and sex	Animal number	Body weight (g) on Day:				
		1 - Pre-exposure	1 - Post-exposure	2	8	15
3F	26	187	186	171	-	-
	27	196	195	-	-	-
	28	203	196	176	-	-
	29	205	197	181	-	-
	30	195	187	168	-	-
	Mean	199	190	174	-	-
	SD	4	6	6	-	-

Group and sex	Animal number	Body weight (g) on Day:				
		1 - Pre-exposure	1 - Post-exposure	2	8	15
4F	16	173	167	157	-	-
	17	171	159	149	-	-
	18	163	157	-	-	-
	19	170	160	149	-	-
	20	164	154	143	-	-
	Mean	170	158	150	-	-
	SD	6	5	6	-	-

Appendix 3
Individual and group mean lung weights and lung/body weight ratios

Terminal Kill				
Group and sex	Animal number	Body weight (g)	Lung weight (g)	Ratio (%)
1M	1	314.0	1.639	0.5220
	2	309.2	1.732	0.5620
	3	323.9	1.485	0.4585
	4	302.0	1.562	0.5172
	5	298.2	1.703	0.5711
	Mean	309.3	1.624	0.5261
	SD	10.2	0.102	0.0447
2M	31	370.4	2.100	0.5670
	32	342.9	2.079	0.6063
	33	347.8	2.157	0.6202
	34	350.9	1.998	0.5694
	35	323.5	1.741	0.5382
	Mean	347.1	2.015	0.5802
	SD	16.8	0.163	0.0329
1F	6	239.9	1.392	0.5802
	7	222.6	1.434	0.6442
	8	201.3	1.355	0.6731
	9	185.9	1.196	0.6434
	10	208.0	1.216	0.5846
	Mean	211.5	1.319	0.6251
	SD	20.6	0.107	0.0408
2F	36	234.9	1.619	0.6892
	37	257.3	1.705	0.6627
	38	231.8	1.505	0.6493
	39	250.0	1.824	0.7296
	40	246.1	1.797	0.7261
	Mean	244.0	1.688	0.6914
	SD	10.6	0.129	0.0363

Appendix 3
Individual and group mean lung weights and lung/body weight ratios
Sporadic deaths

Group and sex	Animal number	Body weight (g)	Lung weight (g)	Ratio (%)
3M	21	208.6	1.961	0.9113
	22	197.2	2.262	1.1471
	23	204.4	2.190	1.0714
	24	203.7	2.201	1.0805
	25	191.6	1.966	1.0261
4M	11	204.8	2.684	1.3105
	12	181.9	1.829	1.0055
	13	170.8	2.233	1.3074
	14	209.6	2.061	0.9833
	15	182.7	2.498	1.3673
3F	26	155.3	1.586	1.0212
	27	169.2	2.532	1.4964
	28	156.4	2.010	1.2852
	29	162.0	2.256	1.3926
	30	149.2	2.061	1.3814
4F	16	142.7	1.931	1.3532
	17	135.8	2.020	1.4875
	18	146.6	3.702	2.5252
	19	136.3	1.758	1.2898
	20	130.9	1.786	1.3644

Appendix 4
Individual necropsy data
Terminal kill

Group 1

Animal number and sex	1M	2M	3M	4M	5M	6F	7F	8F	9F	10F
NECROPSY										
Animal not remarkable (NR)		NR	NR		NR					NR
Lung - dark focus: left lobe	P									
- depressed area: dark, left lobe 4mm diameter								P		
pale: slight, all lobes									P	
Kidney - depressed area: few, bilateral 2mm diameter				P						
Tail - sore: near base				P						
Thymus - red: moderate, left						P				
- red focus: several right						P				
Uterus - distension: minimal, bilateral							P			

P - present

Group 2

Animal number and sex	31M	32M	33M	34M	35M	36F	37F	38F	39F	40F
NECROPSY										
Animal not remarkable (NR)	NR									
Lung - pale area: left lobe 2mm diameter										P

P - present

Appendix 4
Individual necropsy data
Sporadic deaths

Group 3										
Animal number and sex	21M	22M	23M	24M	25M	26F	27F	28F	29F	30F
NECROPSY										
Animal not remarkable (NR)										
Lung - dark areas: few, all lobes 1-2mm diameter		P								
- dark areas: few, all lobes 2-4mm diameter							P			
- dark focus: few, all lobes				P					P	
- dark focus: numerous, all lobes	P							P		
- dark focus: several, all lobes			P		P					P
- dark: slight, all lobes							P			
- pale: minimal, all lobes									P	
- pale: moderate, all lobes						P				
- pale: slight, all lobes				P						
Caecum - abnormal contents: dark substance, adhering to mucosal surface						P				
- distension: marked, gaseous			P							
- distension: moderate					P					
- distension: moderate, gaseous		P				P		P		
- distension: slight, gaseous	P			P						P
Colon - distension: moderate					P					
- distension: moderate, gaseous		P				P		P		
- distension: slight, gaseous	P			P						P
Duodenum - distension: slight, gaseous	P									P
Ileum - distension: moderate					P					
- distension: moderate, gaseous		P				P		P		
- distension: slight, gaseous										P
Jejunum - distension: moderate					P					
- distension: moderate, gaseous		P	P	P		P		P		
- distension: slight							P			
- distension: slight, gaseous	P								P	P
Liver - dark: slight, all lobes	P									
- mottled: slight, all lobes	P	P				P				P
- pale: slight, all lobes						P				P
Mandibular lymph node - red: slight		P								
Nasal cavity - abnormal contents: thick, cream substance		P								
Stomach - distension: marked, gaseous							P			
- distension: slight, gaseous	P									P
Day of death	3	3	3	3	3	3	2#	3	3	3

P - present
animal found dead
all other animals killed in extremis

Appendix 4
Individual necropsy data
Sporadic deaths

Group 4

Animal number and sex	11M	12M	13M	14M	15M	16F	17F	18F	19F	20F
NECROPSY										
Animal not remarkable (NR)										
Lung - dark areas: few, all lobes 2-6mm diameter							P			
- dark areas: numerous, all lobes 1-4mm diameter						P				
- dark areas: several, all lobes 2-4mm diameter		F								
- dark areas: several, all lobes 1-3mm diameter					P					
- dark areas: several, all lobes 1-8mm diameter										P
- dark focus: numerous, all lobes										P
- dark: marked, all lobes										P
- inflated, all lobes	U							P		
- inflated, marked, all lobes	U			U	U	U		U		
- mottled: moderate, all lobes				U				U		
- pale areas: few, all lobes 2-5mm diameter							U			
- pale focus: few, all lobes										P
- pale: moderate, all lobes		P								
- red areas: few, all lobes 2-8mm diameter			P							
Heart - large: slight, all chambers										U
Caecum - dark contents								P		U
- distension: slight, gaseous										U
- distension: moderate, gaseous		P			P				P	
Colon - distension: slight, gaseous									P	
- distension: moderate, gaseous		U			U					
Duodenum - distension: slight, gaseous									P	
- distension: moderate, gaseous		P						P		
- thick: slight, both surfaces										U
Ileum - distension: moderate, gaseous		P			P		U			
- distension: slight, gaseous									P	
Jejunum - distension: moderate, gaseous		U			P		P			
- distension: slight			P							
- distension: slight, gaseous									P	P
Kidney - cyst: clear, right										
- pelvic dilation: marked, right						P		U		
Liver - mottled: slight, all lobes						P				
- mottled: moderate, all lobes										P
- pale areas: few, all lobes, 3-5mm diameter					F					

P - present
F animal found dead
all other animals killed in extremis

Appendix 4
Individual necropsy data
Sporadic deaths

Group 4 (continued)	11M	12M	13M	14M	15M	16F	17F	18F	19F	20F
Animal number and sex										
Mandibular lymph node - large: slight			P							
Pituitary - dark: marked		P								
Spleen - small: moderate							P			
Stomach - dark: marked, mucosal surface, around oesophageal sphincter										P
- dark: slight, mucosal surface, fundus region										P
- distension: slight, gaseous									P	
- distension: moderate, gaseous		P	P		P		P			P
- distension: marked, gaseous						P				
Uterus - distension: slight, bilateral										P
Thin		P	P		P		P		P	P
Day of death	2#	3	3	1	3	3	3	2#	3	3

P - present
animal found dead
all other animals killed in extremis

Appendix 5
Sponsor's Certificate of Test Article Analysis



October 12, 1998

Certificate of Analysis

We hereby certify that following commodity have been duly inspected by us and found to be in full conformity with our standard of quality.

Commodity Name TP-415
Lot No. 1412
Quantity 1.7Kg

ITEM	TEST RESULTS	
Appearance	Slightly Yellowish White Powder	
Average Particle Size	(μ m)	5.6
Apparent Specific Gravity	(g/ml)	0.170
Volatile Matter	(%)	0.08
pH	(-)	4.6
Purity	(%)	100.0


 保土谷化学工業株式会社
 HODOGAYA CHEMICAL CO., LTD.
 〒210-8585 川崎市幸区堀川町66-2
 66-2, HODOGAYA-CHO, SAIWAI-KU, KANSAI-KU, SHU 210-8585, JAPAN

NANYOU PLANT
Norio Yamamoto
 Manager of Quality Assurance Group

Appendix 6
Certificates of diet analysis

SDS

Special Quality Control
Certificate of Analysis

PRODUCT: HIR (10 SQ)
BATCH NO: 5155
DATE OF MANUFACTURE: 16th September 1998
PREFIX BATCH NO: 391

Nutrient	Found Analysis		Contaminant	Found Analysis	Limit of Detection
Moisture	10.0	%	Fluoride	5 mg/kg	1.0 mg/kg
Gross Fat	2.6	%	Nitrate as NaNO_3	17 mg/kg	1.0 mg/kg
Gross Protein	15.2	%	Nitrite as NaNO_2	3.0 mg/kg	1.0 mg/kg
Gross Fibre	4.1	%	Lead	Non detected	0.25 mg/kg
Ash	4.9	%	Arsenic	Non detected	0.2 mg/kg
Calcium	0.71	%	Cadmium	Non detected	0.05 mg/kg
Phosphorus	0.49	%	Mercury	Non detected	0.01 mg/kg
Sodium	0.22	%	Selenium	Non detected	0.05 mg/kg
Chloride	0.49	%			
Potassium	0.70	%			
Magnesium	0.16	%	Total Aflatoxins	Non detected	1 mcg/kg each of B1, B2, G1, G2
Iron	131	mg/kg	Total P.C.B	Non detected	10.0 mcg/kg
Copper	22	mg/kg	Total D.D.T	Non detected	10.0 mcg/kg
Manganese	64	mg/kg	Dieldrin	Non detected	10.0 mcg/kg
Zinc	46	mg/kg	Lindane	Non detected	10.0 mcg/kg
			Heptachlor	Non detected	10.0 mcg/kg
			Malathion	Non detected	20.0 mcg/kg
Vitamin A	3.6	iu/g	Total Viable Organisms x 1000	Non detected	1000/g
Vitamin E	41	mg/kg			
Vitamin C		mg/kg	Neophilic Spores x 100	5.0	per gram 100/g
			Salmonella Species	Non detected	per gram Absent in 20 gram
			Enterobacteriaceae	Non detected	per gram Absent in 20 gram
			Escherichia Coli	Non detected	per gram Absent in 20 gram
			Fungal Units	Non detected	per gram Absent in 20 gram
			Antibiotic Activity	Non detected	

Signed R.S.F.W.
Dated 21/10/98





Special Quality Control
Certificate of Analysis

PRODUCT: ENI (E) SQC

BATCH NO: 5325

PREP BATCH NO: 472

DATE OF MANUFACTURE: 23-NOV-98

Nutrient	Found Analysis		Contaminant	Found Analysis	Limit of Detection
Moisture	18.6	%	Fluoride	5 mg/kg	1.0 mg/kg
Crude Fat	3.0	%	Nitrate as KNO_3	21 mg/kg	1.0 mg/kg
Crude Protein	15.0	%	Nitrite as KNO_2	1.5 mg/kg	1.0 mg/kg
Crude Fibre	5.1	%	Lead	Non Detected	0.25 mg/kg
Ash	6.0	%	Arsenic	Non Detected	0.2 mg/kg
Calcium	0.78	%	Calcium	0.07 mg/kg	0.05 mg/kg
Phosphorus	0.53	%	Mercury	Non Detected	0.01 mg/kg
Sodium	0.22	%	Selenium	0.07 mg/kg	0.05 mg/kg
Chloride	0.49	%			
Potassium	0.68	%			
Magnesium	0.18	%	Total Aflatoxins	Non Detected	1 mcg/kg each of B1, B2, G1, G2
Iron	164	mg/kg			
Copper	7	mg/kg	Total P.C.B	Non Detected	10.0 mcg/kg
Sulphur	60	mg/kg	Total D.B.I	Non Detected	10.0 mcg/kg
Zinc	33	mg/kg	Dieldrin	Non Detected	10.0 mcg/kg
			Lindane	Non Detected	10.0 mcg/kg
			Heptachlor	Non Detected	10.0 mcg/kg
			Malathion	Non Detected	20.0 mcg/kg
Vitamin A	4.3	iu/g	Total Viable Organisms x 1000	Non Detected	per gram 1000/g
Vitamin E	48	mg/kg			
Vitamin C		mg/kg	Mesophilic Spores x 100	Non Detected	per gram 100/g
			Salmonellae Species	Non Detected	Absent in 20 gram
			Shigera Bacteriaceae	Non Detected	Absent in 20 gram
			Escherichia Coli	Non Detected	Absent in 20 gram
			Fungal Units	Non Detected	Absent in 20 gram
			Antibiotic Activity	Non Detected	

Signed RSF
Date 9/2/99



Appendix 7
Study protocol**COVANCE****DEFINITIVE PROTOCOL****TP-415: Single Exposure Inhalation (Head-Only) Toxicity Study
in the Rat**Test Facility: Covance Laboratories Ltd., Otley Road, Harrogate,
North Yorkshire, HG5 1PY, England.

Protocol status: Definitive

Covance Study Number: 558/9

CLE Study Director Signature *N.M. Shepard* Date 8 January 1999

Printed name: N.M. Shepard

CLE Management Signature *J.L. Kelly* Date 8 January 1999

Printed name and title: J.L. Kelly, Section Manager - General Toxicology

Sponsor* Signature *Akin Yamamoto* Date 18 January 1999Printed name and title: Mr. A. Yamamoto, Deputy General Manager Environmental Health
& Safety Quality Assurance Dept.Sponsor's Monitor:
Sponsor:Signature *K. Kashima* Mr. K. Kashima
Hodogaya Chemical Co., Ltd.
66-2, Honkawa-cho
Saiwai-ku
Kawasaki-shi 210
Japan

Page Number: 1 of 15

* Please sign on back approval pages; return one to the Study Director and retain one for
your records.

INTRODUCTION

The objective of the study is to estimate the toxicity of the test article, TP-415, in the rat following a 4-hour inhalation exposure (head-only).

The rat has been selected, because it is a rodent species recommended by various regulatory authorities. Background data are available.

An inhalation exposure has been chosen because it is a possible route of human exposure.

TEST ARTICLE

The test article is identified as TP-415. The minimum normally required for materials of low toxicity is 1500 g (nose-only). When not in use the test article will be stored in a sealed container at 1 to 10°C, over a desiccant in the dark.

The Sponsor has provided information on the physical appearance, hazardous properties, stability and a date of expiry. The Sponsor will provide information on the purity of the batch of test article supplied (Batch 1412). A sample for archive will be the responsibility of the Sponsor.

The control article and vehicle for the test article will be filtered air.

EXPERIMENTAL DESIGN

Regulatory test guidelines

This protocol is designed to meet the known requirements of Annex to Commission Directive 92/69/EEC, Method B2, and OECD Guidelines for the Testing of Chemicals Method 403, adopted 12 May 1981.

Exposure to the test article

The test article will be administered as a single 4-hour exposure via inhalation (nose-only), in a chamber having a volume of approximately 0.040 M³ and being constructed of aluminium and glass. A schematic diagram of the exposure system is in Appendix 3.

The exposure concentration will be approximately 5 mg/L or the maximum achievable concentration for 5 males and 5 females exposed simultaneously for 4 hours. Animals will be observed for 14 days after the exposure for signs of clinical toxicity and morbidity.

TEST ATMOSPHERE

Generation of the test atmosphere

An appropriate method of generation of the test atmosphere will be chosen. If the test article is to be generated as a dust, efforts will be made to generate a "respirable" size distribution of particles. The test atmosphere will be generated from the bulk test article as supplied by the Sponsor.

Exposure chamber temperature and relative humidity

The temperature and relative humidity in the chamber will be monitored continuously and recorded twice hourly. The temperature and relative humidity ranges will be 20 to 24°C and 40 to 60%, respectively. Values outside these ranges can occur; however, they are generally brief in duration and do not affect the integrity of the study. Also, any deviations will be recorded in the study notes.

Exposure chamber air flow

The air flow through the exposure chamber will be monitored continuously and recorded twice hourly. There will not be less than 12 air changes/hour.

Exposure chamber oxygen concentration

The oxygen concentration in the exposure chamber will be monitored and recorded twice hourly. The oxygen level will not fall below a minimum value of 19% v/v.

Nominal concentration

The test article will be weighed before and after exposures to determine the amount utilized during the exposure period.

Exposure concentration

The actual concentration in the exposure chamber will be measured approximately twice hourly during the exposure period.

Particle size

The particle size of the aerospheres will be measured prior to exposures, and then hourly for each exposure group using gravimetric methodology. The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) will be calculated for each occasion.

TEST SYSTEM**Species, strain and supplier**

Sufficient rats of the CRJ:CD(SD)MGSBR strain will be obtained to provide enough healthy animals of each sex.

Specification

The animals will be obtained as young adults, each sex ordered in an age range of 35 days \pm 1 day, each sex within a 15 g weight range. At randomisation their body weight will be within \pm 20% of the overall mean for each sex.

Environment

The animals will be housed in a single room, air-conditioned to provide a minimum of 15 air changes/hour. Routinely, the temperature and relative humidity ranges will be 19 to 25°C and 40 to 70%, respectively. Fluorescent lighting will be controlled automatically to give a cycle of 12 hours light (0600 to 1800) and 12 hours dark.

The animals will be housed in groups of five in stainless steel mesh cages of size 55 x 34 x 20 cm, floor area 1870 cm².

Diet and water

Throughout the study, except during exposure, the animals will have access *ad libitum* to SQC Rat and Mouse Maintenance Diet No 1, Expanded (Special Diets Services Ltd. Wiltam). Each batch of diet is analysed for specific constituents. Typical values are presented in Appendix 1.

Drinking water will be provided *ad libitum* via an automatic watering system or bottles. The water is periodically analysed for specific contaminants. Typical values are presented in Appendix 2.

No contaminants are expected to be present in diet or water at levels which might interfere with achieving the objective of the study.

ANIMAL HEALTH AND WELFARE

All procedures to be carried out on live animals as part of this study will be subject to the provisions of United Kingdom National Law, in particular the Animals (Scientific Procedures) Act, 1986.

In order to monitor the welfare of an individual or group of animals, additional observations to those already detailed may be instigated at the discretion of the Study Director. In certain instances this may include treatment as advised by a CLE animal welfare veterinary surgeon.

PRE-EXPERIMENTAL PROCEDURES**Acclimatisation and health procedures**

All animals will be given a clinical inspection for ill health on arrival. They will be acclimatised for a minimum of 5 days and a veterinary inspection will be performed before the start of exposures to ensure their suitability for the study.

Allocation of animals to the exposure groups

The animals will be assigned arbitrarily to the exposure groups. Group mean body weights will be calculated and inspected to ensure there are no unacceptable differences between groups.

Identification of the animals

The animals will be individually identified by tail marking with indelible ink.

Group number	Group color	Males	Females
1	buff	1-5	6-10
2	pink	11-15	16-20

Cages will be appropriately identified with study information including study number and animal numbers.

EXPERIMENTAL OBSERVATIONS

Clinical signs

Animals will be observed for signs of ill health or overt toxicity. Animals will be observed approximately hourly during the exposure period and for the remainder of the exposure day. They will be observed daily during the remainder of the study. Additional observations may be undertaken at the discretion of the Study Director or the observer. The study will be extended if significant abnormalities remain after 14 days. An individual record will be maintained on the clinical condition of each animal.

Morbidity and mortality

All animals will be examined at the beginning and the end of the working day to ensure the animals are in good health. Any animal which shows marked signs of ill health may be isolated. Moribund animals will be killed and necropsied.

Body weights

Individual body weights will be recorded before and after exposure on Day 1, on Days 2, 8 and 15, and before necropsy.

TERMINAL PROCEDURES

Necropsy

All animals including decedents will be subject to necropsy.

At the scheduled necropsies an intraperitoneal sodium pentobarbitone overdose will be given prior to exsanguination and a full macroscopic examination will be performed under the general supervision of a pathologist and all lesions will be recorded. The nasal cavity and respiratory tract will be examined and any irritation assessed.

Organ weights

Animals will be weighed before necropsy. The lungs and trachea will be dissected free from fat and other contiguous tissue and weighed prior to any fixation.

Histopathology

Samples of all gross lesions will be preserved in the appropriate fixative. No histopathological assessment of tissues will be undertaken in the first instance. If exposure-related lesions are identified macroscopically, the Sponsor will be consulted to see if histopathology is required. If histopathology is required, a protocol amendment and a cost estimate will be prepared by the Study Director. The nasal cavity will not normally be examined histologically as the macroscopic examination precludes preservation.

DATA EVALUATION

Any signs of toxicity and mode of death will be assessed. The data will be processed where appropriate to give group mean values and standard deviations.

GOOD LABORATORY PRACTICE COMPLIANCE

This study will be conducted in accordance with the United Kingdom Good Laboratory Practice Regulations 1997, Statutory Instrument No. 654 and the OECD Principles on Good Laboratory Practice (revised 1997, issued January 1998) ENV/MC/CHEM(98)17. All procedures will be performed in accordance with detailed Standard Operating Procedures. The records to be kept for this study are indicated in the appendices. Wherever appropriate, any change to this protocol will be made by an amendment issued in agreement with the Sponsor.

Protocol review, in-life and report audits will be performed in accordance with Standard Operating Procedures laid down by the Covance Quality Assurance department.

In the event of inspection by an outside authority, the Sponsor will be consulted before the inspectors are permitted access to any of the study records (unless required by law or regulation).

REPORTS

The Sponsor will be informed promptly of any significant findings and issued progress reports at appropriate intervals. The final report will contain all procedures and results. An unaudited draft report will be issued for discussion with the Sponsor (see Appendices).

ARCHIVE

All primary data or authenticated copies thereof, specimens and the final report will be retained in the CLE archives for ten years after submission of the final report. At this time the Sponsor will be contacted to determine whether data should be returned, retained or destroyed on their behalf.

Specimens requiring storage deep frozen are specifically excluded from the above. These will be retained for as long as the quality of the material permits evaluation but for no longer than three months after submission of the final report. The Sponsor will be contacted before specimens are destroyed on their behalf.

APPENDIX I
Diet summary
Year ending 31 December 1997

Nutrients		Mean	SD	Contaminants		Mean	SD	
Moisture	%	9.7	1.2	Fluoride	mg/kg	8	2	
Crude Fat	%	3.1	0.4	Nitrate as NaCO ₃	mg/kg	19	5	
Crude protein	%	14.7	0.7	Nitrite as NaNO ₂	mg/kg	2.6	0.5	
Crude Fiber	%	4.1	0.6	Lead	mg/kg	0.42	0.26	
Ash	%	4.6	0.3	Acetic	mg/kg	ND	0	
Calcium	%	0.66	0.06	Cadmium	mg/kg	0.05	0.03	
Phosphorus	%	0.30	0.06	Mercury	mg/kg	0	0.01	
Sodium	%	0.22	0.01	Selenium	mg/kg	0.07	0.01	
Chloride	%	0.39	0.05	Total Aflatoxins	mg/kg	ND	0	
Potassium	%	0.30	0.08	Total PCB	mg/kg	ND	0	
Magnesium	%	0.17	0.02	Total DDT	mg/kg	ND	0	
Iron	mg/kg	141	24	Dieldrin	mg/kg	ND	0	
Copper	mg/kg	11	1	Lindane	mg/kg	ND	0	
Manganese	mg/kg	57	7	Heptachlor	mg/kg	ND	0	
Zinc	mg/kg	44	5	Melathion	mg/kg	8	11	
Vitamin A	log	4.5	1.5	Total viable organisms	x 10 ³	per g	0.7	1.11
Vitamin E	mg/kg	46	10	Mouldy species	x 10 ²	per g	5.94	7.79
Vitamin C	mg/kg			Salt-tolerant species		per g	ND	0
				Presumptive E.coli		per g	ND	0
				E.coli type 1		per g	ND	0
				Fungal units		per g	46	22
				Antibiotic activity		per g	ND	0

ND = Not detected

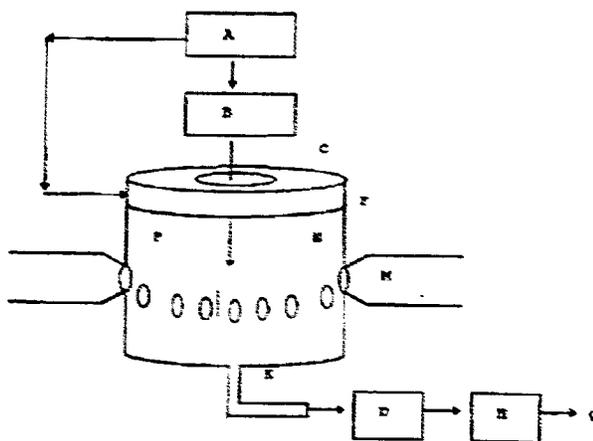
APPENDIX 2
Summary of water analysis - 1997

Covance water supply sampled at point of use (ground water)

Parameter	Unit	Range	Mean
Conductivity 20c	uS/cm	7.5 - 4.5	7.0
pH		7.5 - 8.0	7.8
Turbidity	NTU	0.0 - 0.15	0.05
Strontium	mg/L	0.0 - 0.15	0.05
Nitrate	mg/L	0.0 - 0.15	0.05
Nitrogen ammoniacal	mg/L	0.0 - 0.15	0.05
Iron	mg/L	0.0 - 0.15	0.05
Aluminium	mg/L	0.0 - 0.15	0.05
Fluoride	mg/L	0.0 - 0.15	0.05
Colour True	PCU	0.0 - 0.15	0.05
Aldrin	ug/L	0.0 - 0.15	0.05
DDE (ppb)	ug/L	0.0 - 0.15	0.05
Dieldrin	ug/L	0.0 - 0.15	0.05
Endrin	ug/L	0.0 - 0.15	0.05
Heptachlor	ug/L	0.0 - 0.15	0.05
Chlordane	ug/L	0.0 - 0.15	0.05
Copper	ug/L	0.0 - 0.15	0.05
Lead	ug/L	0.0 - 0.15	0.05
Zinc	ug/L	0.0 - 0.15	0.05
Chloride	mg/L	0.0 - 0.15	0.05
Sulphate	mg/L	0.0 - 0.15	0.05
Calcium	mg/L	0.0 - 0.15	0.05
Magnesium	mg/L	0.0 - 0.15	0.05
Sodium	mg/L	0.0 - 0.15	0.05
Potassium	mg/L	0.0 - 0.15	0.05
Phosphate	mg/L	0.0 - 0.15	0.05
Hardness total	mg/L	0.0 - 0.15	0.05
Alkalinity total	mg/L	0.0 - 0.15	0.05
Faecal coliforms	MPN/100ml	0.0 - 0.15	0.05
Total coliforms	MPN/100ml	0.0 - 0.15	0.05
Chlorine free	mg/L	0.0 - 0.15	0.05
Chlorine total	mg/L	0.0 - 0.15	0.05
Chlorine 1 day 10c	mg/L	0.0 - 0.15	0.05
Chlorine 3 day 20c	mg/L	0.0 - 0.15	0.05

APPENDIX 3.

SYSTEM FOR HEAD-ONLY EXPOSURE



- | | |
|-------------------------------|--|
| A - Air supply | H - Relative humidity hygrometer |
| B - Atmosphere generator | M - Animal holding tube |
| C - Diluent air cyclone inlet | N - Atmosphere sampling port |
| D - Atmosphere analyzer | P - G ₂ and temperature sensor port |
| F - Animal exposure cage | Q - Exhaust |
| K - Atmosphere outlet | |

APPENDIX 4.
Study records

The following study records will be routinely maintained and will include but may not be limited to the following:

Protocol	Animal house experimental procedures, observations and recording as listed in the protocol
Protocol amendment(s)	
File notes(s)	
Study schedule	
Study correspondence	The following data will be maintained if the analyses are required by protocol and performed by CLE:
Any despatch/receipt of samples from CLE	
Dispensary information pertaining to:	Initiation chemistry
Test article receipt	Macro/micro pathological observations and recording as specified in the protocol
Test article description	
Test article usage	
Test article disposal	
Preparation of dosing formulations (if appropriate)	
Animal house information pertaining to:	
Diet batches used	
Diet and water analysis	
Supplier's animal data	
Acceptability and randomisation of animals	
Details of experimental environment	
• - central records maintained	

APPENDIX 3

Reports

Draft report

An unaudited draft report will be issued for the study Sponsor's comments. The report will be prepared to contain the following information:

The name and address of the testing facility and the location of all raw data and final report.

The objectives and procedures stated in the approved protocol including any amendments made to the original protocol and any unforeseen circumstances which may have affected the quality or integrity of the study.

The identity of the test and control substances, by name or code number, their quality or purity and accuracy of formulation will be presented if appropriate. The experimental design including study start and completion dates, exposure concentrations, route, frequency and duration of the exposures.

The supplier, species, strain and sex of the animals and the method of identification.

The reports of the individual scientists involved in the study, e.g. pathologist

Data collected and pertaining to the experimental observations and pathology sections of this protocol will be presented in the report unless considered not appropriate by the Study Director. In these circumstances a textual comment will describe any effects, relationship or absence thereof to exposure.

Other data not specified in the above sections (e.g. data supplied by the Sponsor or suppliers) may be reported where appropriate.

Final report

The final report will be produced on paper following quality assurance evaluation of the complete draft report. In addition to all the details described for the draft report it will contain the Study Director's signature, other scientists involved in the study as authentication of the report and a statement that the study and the report have been subject to quality assurance evaluation.

Electronic reports

An electronic copy of the draft and final reports above may be made available on request from the Sponsor. The study reports will be produced in PDF format unless agreed otherwise by Covance before the start of the study. Reports requiring specialised Sponsor formats/alternative

Sponsor formats/alternative computer software packages may be possible on request from the Sponsor but may involve extra time and cost.

It should be noted that an electronic final report will be despatched only at the request of the Sponsor and contains, to the best of our knowledge, a full copy of the available information presented in the original and verified hard copy of the final report. However, the electronic copy will not be subject to quality assurance approval. Covance will accept no responsibility for subsequent operations carried out on this electronic information, or copies thereof, after despatched to the Sponsor.

**APPENDIX 6.
Responsible personnel**

Study Director	N M Shepherd
Deputy Study Director	A C Gibbs
Head of Pathology	J Glister
Formulations	B Hildley
Inhalation chemistry	B Cashen
Animal house supervisor	J Cunningham
Animal health and welfare	A Basford
Necropsy	I Wilkins
Histology	S Brogden
Data processing	N Darwent
Head of Quality Assurance	S White

Proposed Dates	
Animals on site	January 1999
First treatment	January 1999
Study termination	February 1999
Draft report	March 1999

A change to the Study Director will be documented by protocol amendment, other changes to personnel or dates will be documented in study records.

Distribution

Personnel above. Animal House Supervisor (2 copies). Manager AH5.3, Production planning. Contracts administration via Production planning. C Springall.

Protocol Amendment

Study Title	TP-415: Single Exposure Inhalation (Head-Only) Toxicity Study in the Rat
Study Director	N M Shepherd
Sponsor	Hodogaya Chemical Co., Ltd. 66-2, Horikawa-cho Sairei-ku Kawasaki-shi 210 Japan
Study Monitor	Mr K Yuhla
Performing Laboratory	Covance Laboratories Ltd. Osley Road, Harrogate North Yorkshire, HG3 1PY England
Covance Study Number	5539
Amendment Number	1
Page Number	1 of 3

The following amendment documents revisions to the protocol sections of

Study Monitor
Test system supplier

Study monitor
At the request of the Sponsor Mr K Kashima replaces Mr K Yuhta as the Study Monitor.

Test system supplier
To provide additional information on the animal supplier, the supplier is identified as Charles River (UK) Limited.

Amendment approval

Please print or type your name and company status below your signature on both approval pages; return one to the Study Director and retain one for your records.

Akira Yamamoto 10 February 1999
Name: Mr A Yamamoto Date:
General Manager, Environmental Health & Safety Quality Assurance Dept.
Hodogaya Chemical Co., Ltd.

N M Shepherd 3 February 1999
Name: N M Shepherd Date:
Study Director
Covance Laboratories Ltd.

J L Kelly 3 February 1999
Name: J L Kelly Date:
Section Manager - General Toxicology
Covance Laboratories Ltd.

Protocol Amendment

Study Title	TP-415: Single Exposure Inhalation (Head-Only) Toxicity Study in the Rat
Study Director	N M Shepherd
Sponsor	Hodogaya Chemical Co., Ltd. 66-2, Honikawa-cho Saiwai-ku Kawasaki-shi 210 Japan
Study Monitor	M K Kashima
Performing Laboratory	Covance Laboratories Ltd. Otley Road, Harrogate North Yorkshire, HG3 1PY England
Covance Study Number	5589
Amendment Number	2
Page Number	1 of 4

The following amendment documents revisions to the protocol sections of:

Test system - Specification
Additional exposure groups
Experimental design
Identification of the animals
Data evaluation
Reports
Toxicity assessment criteria
Appendix 6 - Proposed dates

Test system - Specification

When ordering animals to meet the study schedule the animal supplier was unable to supply animals in the protocol age range of 35 days \pm 1 day but was able to supply animals that were 42 to 49 days old. As animals 42 to 49 days old were still young adults and would be supplied within a weight range of 15 g, to maintain the study schedule the Study Director agreed to accept these animals. For the follow on exposures, animals of similar age and weight specifications to those used earlier have been ordered. So that when exposed the age of each group will be similar and inter group comparisons can be made. The deviation in the age of the animals ordered is considered not to have affected the integrity of the study or the data obtained.

Additional exposure groups

At the request of the Sponsor additional exposures are to be conducted to estimate the toxicity of the test article and classify it according to the EU packaging and labelling requirements.

Experimental design

An additional exposure will be conducted at a concentration of approximately 1 mg/L to expose 5 males and 5 females simultaneously for 4 hours. The animals will be observed for 14 days after the exposure for signs of clinical toxicity and morbidity.

If 5 or more animals die then an additional group of 5 males and 5 females will be exposed at a concentration of approximately 0.25 mg/L. The animals will be observed for 14 days after the exposure for signs of clinical toxicity and morbidity.

Identification of the animals

The animals will be identified by tail marking with indelible ink as follows:

Group number	Group color	Male	Female
to be allocated and documented in directly readable response of exposure to be known	Buff Green Blue Pink	1-5 21-25 31-35 41-45	6-10 26-30 36-40 46-50

Data evaluation

From the atmosphere concentration data the range within which the median lethal concentration is expected to occur will be provided.

Reports

Two copies of the unedited draft report will be issued as directed by the Sponsor.

Toxicity assessment criteria

The data obtained in the single exposure inhalation study will be compared with the toxicity classification below:

Commission Directive 93/21/EEC Annex IV (May 1993)

Exposure category by inhalation	LC ₅₀ (mg/L)
very toxic	LC ₅₀ ≤ 0.25 (gases and particulates) LC ₅₀ ≤ 0.10 (gases and vapours)
toxic	0.25 < LC ₅₀ ≤ 1 (gases and particulates) 0.50 < LC ₅₀ ≤ 2 (gases and vapours)
harmful	1 < LC ₅₀ ≤ 5 (gases and particulates) 1 < LC ₅₀ ≤ 20 (gases and vapours)

Appendix 5 - Proposed dates

Draft report April 1999

Amendment approval

Please print or type your name and company status below your signature on both approval pages; return one to the Study Director and retain one for your records.

A Yamamoto 29 March 1999
Name: Mr A Yamamoto Date
General Manager: Environmental Health & Safety Quality Assurance Dept.
Hodogaya Chemical Co., Ltd.

N M Shepherd 26 February 1999
Name: N M Shepherd Date
Study Director
Covance Laboratories Ltd.

J L Kelly 26 February 1999
Name: J L Kelly Date
Section Manager - General Toxicology
Covance Laboratories Ltd.

Covance is an independent, publicly held company operating in over 15 countries and over 30 offices worldwide, with headquarters in Princeton, New Jersey, USA.

THE AMERICAS

Princeton
+1 (609) 609 452 9550

EUROPE

Brussels
+32 (0) 2 773 29 10

ASIA/PACIFIC

Singapore
+65 (0) 7747233

COVANCE LABORATORIES

Madison, WI, USA
+1 (608) 241 4471

Vienna, VA, USA
+1 (703) 593 5400

Harrrogate, UK
+44 (0) 1423 500011

Münster, Germany
+49 (0) 261 9796-0

COVANCE
THE DEVELOPMENT SERVICES COMPANY

Shaping Solutions

CERTIFICATE OF AUTHENTICITY

THIS IS TO CERTIFY that the microimages appearing on this microfiche are accurate and complete reproductions of the records of U.S. Environmental Protection Agency documents as delivered in the regular course of business for microfilming.

Data produced 08 - 04 - 2000 Susan Rivera
(Month) (Day) (Year) Camera Operator

Place Syracuse New York
(City) (State)



END