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Attention: TSCA Section 8(e) Coordinator

Dear Sir/Madam:

Enclosed are a summary and relevant studies submitted pursuant to Section 8(e) of the Toxic Substances Control Act (TSCA). Because certain of the information in the enclosed materials is TSCA Confidential Business Information (CBI) under TSCA Section 14 and 40 C.F.R. Part 2 (Attachment 1), both confidential and non-confidential versions of the summary and studies are being submitted.

Please contact me (at 713-229-1114) with any comments or questions that you have about this submittal. Thank you.

Sincerely yours,

Matt Kuryla MH

Matthew L. Kuryla

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Enclosure

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Report Under TSCA Section 8(e) from

operates a process to produce at its facility. The process may, under certain conditions, result in the formation of a residue of a derivative insoluble compound. This derivative insoluble compound residue is encountered during maintenance of some parts of the manufacturing unit. It appears to be principally produced during occasional trip outs and upsets attendant to normal manufacturing operations.

We have received results of toxicity tests performed on this derivative insoluble compound. The Ames screening test for mutagenicity was positive, and it has recently been learned that the follow-up in-vitro mouse lymphoma test also gave a positive result, suggesting the need to consider the derivative insoluble compounds as mutagenic, and a potential carcinogen. In addition to the Ames and in-vitro mouse lymphoma test results, this notice includes a range of reports on toxicological tests carried out on this derivative insoluble compound to date.

The toxicity study results are summarized in Appendix 1. The ecotoxicity study results are summarized in Appendix 2.

Appendix 1

Physico-Chemical Properties

Study reference 599/066
Flammability (solids) - Not flammable
Explosive properties -
BAM fall hammer test, no signs of ignition or explosion
BAM friction test, no signs of ignition or explosion, but a black mark on the porcelain plate might indicate decomposition.
Koenen steel tube test - after heating to a minimum of 285°C for 5 minutes an orange flame was seen from the tube orifice. There was no evidence of explosion.

Toxicological Studies

Acute oral toxicity class (rats). Study reference 599/068 – LD50 greater than 2000mg/kg. (This is outside the "Harmful" classification threshold of 200- 2000mg/kg according to the EU Dangerous Substances Directive.)

Skin sensitization. Study reference 599/070 - no evidence of allergic sensitization.

Acute inhalation toxicity. Study reference 599/069 - The dust can be considered as harmful by inhalation, with a predicted 4 hour LC50 of between 1-5mg dust/litre of air. The effects show the precipitate to be a respiratory irritant. Also, although the effects on the lung (fibrosis, erosion, ulceration, pneumonitis) decreased with time after exposure,

A 05

there was no evidence of the dust being cleared from the lungs up to 21 days after the exposure.

Ames mutagenicity test. Study reference 599/071 – positive result. This may indicate some ability to affect DNA, with possible concerns about mutagenicity risks to humans.

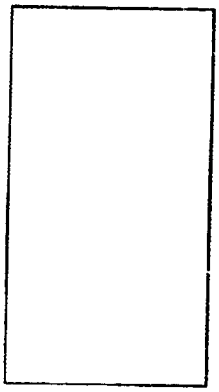
In-vitro mouse lymphoma assay test. Study reference 599/078 – positive result. This is in agreement with the Ames test, and indicates that the precipitate is mutagenic and should be considered a suspect carcinogen.
Note: This study was only completed on November 23, 2000 and the report provided here is a QA-checked draft. The report will be finalized during December 2000.

Appendix 2

During annual plant maintenance shutdowns, vessels are steamed out as part of the cleaning process. As a result, it is possible for derivative insoluble compound residue in the vessels to form aqueous solutions of the solid that would require disposal.

An aqueous solution of the derivative insoluble compound found at the plant was prepared for ecotoxicological testing. A scenario was employed which used a ratio of solid to water that was not necessarily representative of that which would occur during the plant cleaning procedure. The solid was refluxed for six hours in deionized water and then left to stand. Because this resulted in the re-precipitation of a small amount of solid, the solution was filtered to produce the final test material.

This testing program was designed as a simple screen for disposal considerations during scheduled and controlled maintenance events. The original precipitate was analyzed for metal content, and the results of the analysis were as follows:



The reflux solution was analyzed for the most abundant metal, , as a "concentration marker." Ecotoxicological effects may also be influenced by the other

impurities present in the precipitate that may have biological activity (*i.e.* and), so the results cannot necessarily be ascribed solely to the presence of .

On repeating the reflux process (subsequent to the ecotoxicity studies), variability in ruthenium concentrations was observed which appeared to be influenced by length of reflux period as well as precipitate/water ratios.

Ecotoxicity studies

The percentages shown below are volume reflux sample/volume of mixture of water and reflux sample, abbreviated to v/v :

Activated sludge respiration inhibition. Study reference 599/073 - The 3-hour EC50 for inhibition is 10% v/v. Therefore at this concentration the reflux water from the plant could significantly impact a biotreatment works. The No Effect Concentration was 1% v/v.

Algal growth inhibition. Study reference 599/074 – The 72-hr EC50 for total weight of algae is 0.53% v/v. The EC50 for growth rate is 1.3% v/v. The No Effect Concentration was 0.1% v/v.

Acute toxicity to Daphnia. Study reference 599/075 – The 48-hr EC50 is 0.87% v/v. (At this level the test material is classified as toxic to the environment according to the EU Dangerous Substances Directive.) The No Effect Concentration was 0.56% v/v.

Acute toxicity to fish. Study reference 599/076 - 22% v/v. The No Effect Concentration was 10% v/v.

Nitrification Inhibition. Study reference 599/072 - EC50 (4 hours) = 0.37% v/v. No Observed Effect Concentration (NOEC) = 0.010% v/v.

B 01

RS001:

DETERMINATION OF HAZARDOUS PHYSICO-CHEMICAL PROPERTIES

SPL PROJECT NUMBER: 599/066

AUTHOR: S P TREMAIN

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B 02

SPL PROJECT NUMBER: 599/066

QUALITY ASSURANCE REPORT

Inspection of the routine and repetitive procedures that constitute the study is process based (as defined by OECD) and is designed to encompass the major phases at or about the time this study was in progress.

This report has been audited by Safepharm Quality Assurance Unit, and is considered to be an accurate account of the data generated and of the procedures followed.

In each case, the outcome of QA evaluation is reported to the Study Director and Management on the day of evaluation. Audits of study documentation, and process inspections appropriate to the type and schedule of this study were as follows:

§ 07 March 2000	Protocol Compliance Audit
01, 08 March 2000	Flammability (Solids)
05 April 2000	Moisture Content
02, 03, 07, 13, 20 April 2000	Explosive Properties
§ 09 May 2000	Draft Report Audit
§ Date of QA Signature	Final Report Audit

§ Evaluation specific to this study

For Safepharm Quality Assurance Unit* ..... DATE: 17 JUL 2000

*Authorised QA Signatures:

Head of Department: JR Pateman CBiol MIBiol DipRQA
Deputy Head of Department: JM Crowther MISciT
Senior Audit Staff: JV Johnson BSc; C Wren ONC; RJ Gilbert BSc

B 03

SPL PROJECT NUMBER: 599/066

GLP COMPLIANCE STATEMENT

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106)). These Regulations are in accordance with GLP standards published as OECD Principles on Good Laboratory Practice (revised 1997, ENV/MC/CHEM(98)17); and are in accordance with, and implement, the requirements of Directives 87/18/EEC (as amended by Directive 1999/11/EC) and 88/320/EEC (as amended by Directive 1999/12/EC).

These international standards are acceptable to the United States Environmental Protection Agency and Food and Drug Administration, and fulfil the requirements of 40 CFR Part 160, 40 CFR Part 792 and 21 CFR Part 58 (as amended).

This report fully and accurately reflects the procedures used and data generated.

..... Date: 17 JUL 2008

S P Tremain
Study Director

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SUMMARY

STUDY SPONSOR :

STUDY TITLE : DETERMINATION OF HAZARDOUS PHYSICO-CHEMICAL PROPERTIES

TEST MATERIAL : RS001

Two hazardous physico-chemical properties were determined using methods specified in Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548/EEC).

Test	Method	Result
Flammability (Solids)	A10	Not highly flammable
Explosive Properties	A14	Non-explosive

B 06

SPL PROJECT NUMBER: 599/066

RS001:

DETERMINATION OF HAZARDOUS PHYSICO-CHEMICAL PROPERTIES

1. INTRODUCTION

Two hazardous physico-chemical properties of RS001 have been determined at the request of [redacted].

Methods employed were those specified in Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548/EEC).

Testing was conducted between 28 April 2000 and 03 May 2000.

2. TEST MATERIAL

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	RS001
Description	:	red/brown solid
Batch number	:	2 and 3
Date received	:	06 April 2000 and 14 April 2000
Storage conditions	:	room temperature, in the dark

Data relating to the identity, purity and stability of the test material are the responsibility of the Sponsor.

Batch 3 was used for the flammability (solids).
Batch 2 and 3 were used for the explosive properties.

3. ARCHIVES

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Safepharm archives for a period of five years. After this period, the Sponsor's instructions will be sought.

B 07

599/066

5. FLAMMABILITY (SOLIDS)

4.1 Method

The flammability (solids) was determined by measuring the burning rate of t material prepared as a pile of set dimensions. Testing was conducted using Meth A10 of Commission Directive 92/69/EEC (which constitutes Annex V of Coun Directive 67/548/EEC).

4.1.1 Procedures

4.1.1.1 Preliminary screening test

A mould (250 x 20 x 10 mm) was loosely filled with test material (tested received). A non-combustible, non-porous board was placed onto the mould whi was then inverted. The mould was removed and an air-rich Bunsen burner flam applied to one end of the pile for two minutes.

4.1.1.2 Moisture content

The moisture content was determined gravimetrically.

An aliquot (approximately 1 g) of test material was weighed (in duplicate; A and B into loss bottles. The samples were dried to constant weight under reduce pressure at approximately 40°C.

4.1.2 Calculation

The moisture content was calculated using Equation 4.1.

$$\text{Moisture content (\%)} = \frac{b - c}{b - a} \times 100$$

Equation 4.

where:

a = mass of loss bottle (g)

b = mass of loss bottle and test material (g)

c = mass of loss bottle and test material after drying (g)

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5. FLAMMABILITY (SOLIDS)

4.2 Results

4.2.1 Preliminary screening test

The pile failed to ignite during the two minutes that the Bunsen flame was applied.

The result of the preliminary screening test obviated the need to perform the main test.

4.2.2 Moisture content

	Determination A	Determination B
a) Mass of loss bottle (g)	11.6652	14.6639
b) Mass of loss bottle and test material (g)	12.6733	15.6742
c) Mass of loss bottle and test material after drying (g)	12.6564	15.6566
Moisture content (% w/w)	1.676	1.742
Mean moisture content (% w/w)	1.709	

4.3 Conclusion

RS001 has been determined to be not highly flammable as it failed to support combustion in the preliminary screening test.

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5. EXPLOSIVE PROPERTIES

5.1 Methods

The explosive properties were determined using Method A14 of Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548/EEC).

The test material was subjected to the following:

1) The BAM fall hammer test which is a test of mechanical sensitivity with respect to shock.
2) The BAM friction test which is a test of mechanical sensitivity with respect to friction.
3) The Koenen steel tube test which is a test of thermal sensitivity.

5.1.1 Procedures

5.1.1.1 BAM fall hammer test

The test material was passed through a 500 μm mesh sieve and dried to constant weight. A steel roller was inserted into a steel collar forming a hollow and an aliquot (40 mm^3) of test material transferred to the hollow. A second steel roller was inserted trapping the sample. The test assembly was placed in the centering ring on the anvil of the apparatus and a weight (10 kg) dropped from a height (40 cm) onto it. Observations were made for any signs of an explosion. This was repeated a further five times.

5.1.1.2 BAM friction test

The test material was passed through a 500 μm mesh sieve and dried to constant weight. An aliquot (10 mm^3) of test material was transferred to a porcelain plate on the apparatus. A porcelain peg was lowered onto the sample and 'Weight 9' hung from 'Position VI' on the arm of the apparatus, this gave a loading of 36 kgf at the peg. The apparatus was switched on and the peg moved back and forth once over 10 mm. Observations were made for any signs of an explosion. This was repeated a further five times.

5.1.1.3 Koenen steel tube test

A steel tube was filled with test material (tested as received) in three increments of 22 g. Each increment was tamped with 80N force. The tube was closed with an

B 10

orifice plate and secured with a nut and threaded collar. The prepared tube was placed into the apparatus and heated by four propane burners over a period of 300 seconds. Three tests were performed with a 2 mm orifice plate and three tests with a 6 mm orifice plate.

5.1.1.4 Moisture content

The moisture content of the test material was determined in duplicate by drying samples to constant weight under reduced pressure at approximately 40 °C details of which are given on pages 7 to 8.

D 01

RS001:

REVERSE MUTATION ASSAY "AMES TEST"

USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

SPL PROJECT NUMBER: 599/071

AUTHORS: A J Bowles
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D 02

SPL PROJECT NUMBER: 599/071

QUALITY ASSURANCE REPORT

This study type is classed as short-term. The standard test method for this study type ("Standardised Study Plan" in OECD terminology) was reviewed for compliance once only on initial production. Inspection of the routine and repetitive procedures that constitute the study is carried out as a continuous process designed to encompass the major phases at or about the time this study was in progress.

This report has been audited by Safepharm Quality Assurance Unit, and is considered to be an accurate account of the data generated and of the procedures followed.

In each case, the outcome of QA evaluation is reported to the Study Director and Management on the day of evaluation. Audits of study documentation, and process inspections appropriate to the type and schedule of this study were as follows:

02 February 2000	Standard Test Method Compliance Audit
05 May 2000	Test Material Preparation
05 May 2000	Test System Preparation
05 May 2000	Exposure to Test Material
05 May 2000	Assessment of Response
§ 28 June 2000	Draft Report Audit
§ Date of QA Signature	Final Report Audit

§ Evaluation specific to this study

G. Wren DATE: 09 AUG 2000

For Safepharm Quality Assurance Unit*

*Authorised QA Signatures:

Head of Department: JR Pateman CBiol MIBiol DipRQA
Deputy Head of Department: JM Crowther MIScT
Senior Audit Staff: JV Johnson BSc; G Wren ONC; RJ Gilbert BSc

D 03

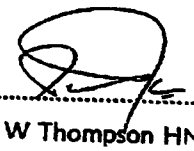
SPL PROJECT NUMBER: 599/071

GLP COMPLIANCE STATEMENT

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106)). These Regulations are in accordance with GLP standards published as OECD Principles on Good Laboratory Practice (revised 1997, ENV/MC/CHEM(98)17); and are in accordance with, and implement, the requirements of Directives 87/18/EEC (as amended by Directive 1999/11/EC) and 88/320/EEC (as amended by Directive 1999/12/EC).

These international standards are acceptable to the United States Environmental Protection Agency and Food and Drug Administration, and fulfil the requirements of 40 CFR Part 160, 40 CFR Part 792 and 21 CFR Part 58 (as amended).

This report fully and accurately reflects the procedures used and data generated.



DATE: 08 AUG 2000

P W Thompson HNC
Study Director
for Safepharm Laboratories

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SPL PROJECT NUMBER: 599/071

SUMMARY

STUDY SPONSOR :

STUDY TITLE : REVERSE MUTATION ASSAY "AMES TEST" USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

TEST MATERIAL : RS001

1. *Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100 and *Escherichia coli* strain WP2uvrA⁻ were treated with suspensions of the test material using the Ames plate incorporation method at up to seven dose levels, in triplicate, both with and without the addition of a rat liver homogenate metabolising system (10% liver S9 in standard co-factors). This method conforms to the guidelines for bacterial mutagenicity testing published by the major japanese Regulatory Authorities including MITI, MHW, MOL and MAFF. It also meets the requirements of the OECD Guidelines for Testing of Chemicals No. 471 "Reverse Mutation Study", Method B14 of Commission Directive 92/69/EEC and the USA, EPA (TSCA) OPPTS harmonised guidelines. The dose range for the first experiment was determined in a preliminary toxicity assay and a non-reported experiment and ranged between 1.5 to 5000 µg/plate. The experiment was repeated on a separate day using a similar dose range of 5 to 1500 µg/plate, fresh cultures of the bacterial strains and fresh test material formulations. Additional dose levels were included to allow for test material induced toxicity and to ensure that a minimum of four non-toxic doses were plated out.

2. The vehicle (dimethyl sulphoxide) control plates gave counts of revertant colonies within the normal range.

3. All of the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies, both with and without metabolic activation. Thus, the sensitivity of the assay and the efficacy of the S9-mix were validated.

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4. The test material caused a visible reduction in the growth of the bacterial lawn at initially 500 µg/plate both with and without S9-mix. The test material was, therefore, tested up to the maximum recommended dose of 5000 µg/plate or its toxic limit depending on strain type and presence or absence of S9. A brown colour was observed at and above 1500 µg/plate with associated dark precipitate observed at 5000 µg/plate, this did not prevent the scoring of revertant colonies.

5. Statistically significant, reproducible and dose-related increases in the frequency of revertant colonies were observed in the upper sub-toxic dose levels of the test material for both TA100 and TA98, both with and without S9-mix. The test material was, therefore, considered to be mutagenic under the conditions of this test.

RS001:

REVERSE MUTATION ASSAY "AMES TEST"

USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

1. INTRODUCTION

This study was conducted according to Safepharm Standard Method 700.07 and was designed to assess the mutagenic potential of the test material using a bacterial/microsome test system. The study was based on the *in vitro* technique described by Ames and his co-workers (1, 2, 3) and Garner *et al* (4) in which mutagenic activity is assessed by exposing histidine auxotrophs of *Salmonella typhimurium* and tryptophan auxotrophs of *Escherichia coli* to various concentrations of the test material. The method conforms to the guidelines for bacterial mutagenicity testing published by the major Japanese Regulatory Authorities including MITI, MHW, MOL and MAFF. It also meets the requirements of the OECD Guidelines for Testing of Chemicals No. 471 "Reverse Mutation Study", Method B14 of Commission Directive 92/69/EEC and the USA, EPA (TSCA) OPPTS harmonised guidelines. A copy of the Certificate of Compliance with GLP, issued by the UK Department of Health, is included as Appendix II.

These mutant strains of *Salmonella* are incapable of synthesising histidine and are, therefore, dependent for growth on an external source of this particular amino acid. When exposed to a mutagenic agent these bacteria may undergo a reverse mutation to histidine independent forms which are detected by their ability to grow on a histidine deficient medium. Using various strains of this organism, revertants produced after exposure to a chemical mutagen may arise as a result of base-pair substitution in the genetic material (miscoding) or frame-shift mutation in which genetic material is either added or deleted. In order to make the bacteria more sensitive to mutation by chemical and physical agents, several additional traits have been introduced. These include a deletion through the excision repair gene ($uvrB^-$ *Salmonella* strains) which renders the organism incapable of DNA excision repair and deep rough mutation (rfa) which increases the permeability of the cell wall. A mutant strain of *Escherichia coli* (WP2$uvrA^-$), which requires tryptophan and which can be reverse mutated by base substitution to tryptophan independence was used to complement the *Salmonella* strains. This strain also has a deletion in an excision repair gene ($uvrA^-$).

Since many compounds do not exert a mutagenic effect until they have been metabolised by enzyme systems not available in the bacterial cell, the test material and the bacteria are also incubated in the presence of a liver microsomal preparation (S9-mix) prepared from rats pre-treated with a compound known to induce an elevated level of these enzymes.

The study was performed between 28 March 2000 and 08 June 2000.

2. TEST MATERIAL

Sponsor's identification	:	RS001
Batch number	:	1
Description	:	red/brown solid
Date received	:	15 March 2000
Storage conditions	:	room temperature in the dark

Data relating to the identity, purity and stability of the test material are the responsibility of the Sponsor.

3. METHODS

3.1 Tester Strains

Salmonella typhimurium TA1535, TA1537, TA98 and TA100

Escherichia coli WP2uvrA$^-$

The *Salmonella* strains were obtained from the University of California at Berkeley on culture discs on 4 August 1995 whilst *Escherichia coli* strain WP2uvrA- was obtained from the British Industrial Biological Research Association on 17 August 1987. All of the strains were stored at -196°C in a Statebourne liquid nitrogen freezer, model SXR 34. Prior to the master strains being used, characterisation checks were carried out to confirm the amino-acid requirement, presence of rfa, R factors, uvrB or uvrA mutation and the spontaneous reversion rate.

In this assay, overnight sub-cultures of the appropriate coded stock cultures were prepared in nutrient broth (Oxoid Limited; lot number 220940 06/04) and incubated at 37°C for approximately 10 hours. Each culture was monitored spectrophotometrically for turbidity with titres determined by viable count analysis on nutrient agar plates.

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3.2 Preparation of Test and Control Materials

The test material was accurately weighed and approximate half-log dilutions prepared in dimethyl sulphoxide by mixing on a vortex mixer and sonication for 90 minutes at 40°C on the day of each experiment. As an aid to both dosing and suspending all of the test material formulations were prepared at half the concentration required on Vogel-Bonner plates. To compensate, the formulations were dosed using 200 µl aliquots (6). Also to ensure that the test material did not adhere to the inside of the glass containers above the vehicle, small volume universal containers were used for all of the test material formulations. Analysis for concentration, homogeneity and stability of the test material formulations is not a requirement of the test guidelines and was, therefore, not determined. Prior to use, the solvent was dried using molecular sieves (sodium alumino-silicate) ie 2 mm pellets with a nominal pore diameter of 4×10^{-4} microns.

Vehicle and positive controls were used in parallel with the test material. A solvent treatment group was used as the vehicle control and the positive control materials were as follows:

N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG) : 3 µg/plate for TA100, 5 µg/plate for TA1535 and 2 µg/plate for WP2uvrA$^-$

9-Aminoacridine (9AA) : 80 µg/plate for TA1537

4-Nitroquinoline-1-oxide (4NQO) : 0.2 µg/plate for TA98

In addition, 2-Aminoanthracene (2AA) and Benzo(a)pyrene (BP) which are non-mutagenic in the absence of metabolising enzymes were used in the series of plates with S9-mix at the following concentrations:

2AA at 1 µg/plate for TA100

2AA at 2 µg/plate for TA1535 and TA1537

BP at 5 µg/plate for TA98

2AA at 10 µg/plate for WP2uvrA$^-$

3.3 Microsomal Enzyme Fraction

S9 was prepared in-house on 08 January 2000 and 08 April 2000 from the livers of male Sprague-Dawley rats weighing – 250g. These had each orally received three consecutive daily doses of phenobarbitone/β-naphthoflavone (80/100 mg per kg per day) prior to S9 preparation. Before use, each batch of S9 was assayed for its ability to metabolize the indirect mutagens 2-Aminoanthracene and Benzo(a)pyrene. The S9 was stored at -196°C.

3.4 S9-Mix and Agar

The S9-mix was prepared immediately before use using sterilised co-factors and maintained on ice for the duration of the test.

S9	5.0 ml
1.65 M KCl/0.4 M $MgCl_2$	1.0 ml
0.1 M Glucose-6-phosphate	2.5 ml
0.1 M NADPH	2.0 ml
0.1 M NADH	2.0 ml
0.2 M Sodium phosphate buffer (pH 7.4)	25.0 ml
Sterile distilled water	12.5 ml

A 0.5 ml aliquot of S9-mix and 2 ml of molten, trace histidine or tryptophan supplemented, top agar was overlaid onto a sterile Vogel-Bonner Minimal agar plate in order to assess the sterility of the S9-mix. This procedure was repeated, in triplicate, on the day of each experiment.

Top agar was prepared using 0.6% Difco Bacto agar (lot number 142311 01/02) and 0.5% sodium chloride with 5 ml of 1.0 mM histidine and 1.0 mM biotin or 1.0 mM tryptophan solution added to each 100 ml of top agar. Vogel-Bonner Minimal agar plates were purchased from Oxoid Ltd. They were prepared using 1.6% Oxoid Agar Technical No.3 (lot numbers 810616-2 01/05 and 808388-2 08/04) with Vogel-Bonner Medium E and 20 mg/ml D-glucose.

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3.5 Test Procedure

3.5.1 Preliminary Toxicity Study

In order to select appropriate dose levels for use in the main study, a preliminary test was carried out to determine the toxicity of the test material. The dose range of the test material was 0, 0.15, 0.5, 1.5, 5, 15, 50, 150, 500, 1500 and 5000 µg/plate. The study was performed by mixing 0.1 ml of bacterial culture (TA100 or WP2uvrA$^-$), 2 ml of molten, trace histidine or tryptophan supplemented, top agar, 0.2 ml of test material formulation and 0.5 ml of S9-mix or phosphate buffer and overlaying onto sterile plates of Vogel-Bonner Minimal agar (30 ml/plate). Ten concentrations of the test material formulation and a vehicle control (dimethyl sulphoxide) were tested. In addition, 0.2 ml of the maximum concentration of the test material and 2 ml of molten, trace histidine or tryptophan supplemented, top agar was overlaid onto sterile Vogel-Bonner Minimal agar plates in order to assess the sterility of the test material. After approximately 48 hours incubation at 37°C the plates were assessed for numbers of revertant colonies using a Domino colony counter and examined for effects on the growth of the bacterial background lawn.

3.5.2 Mutation Study - Experiment 1

Up to seven concentrations of the test material (1.5, 5, 15, 50, 150, 500, 1500 and 5000 µg/plate) were assayed in triplicate against each tester strain, using the direct plate incorporation method.

The dose ranges for each tester strain with (+) and without (-) S9-mix were as follows:

TA100 (+/-S9), TA1535 (+/-S9), WP2uvrA$^-$ (+/-S9), TA98 (+S9) and TA1537 (+S9):	5 to 1500 µg/plate
TA1537 (-S9):	15 to 5000 µg/plate
TA98 (-S9):	1.5 to 1500 µg/plate

Additional dose levels were included to allow for test material induced toxicity and to ensure a minimum of four non-toxic dose levels were plated out.

D 12

Measured aliquots (0.1 ml) of one of the bacterial cultures were dispensed into sets of test tubes followed by 2.0 ml of molten, trace histidine or tryptophan supplemented, top agar, 0.2 ml of the test material formulation, vehicle or 0.1 ml of the positive control and either 0.5 ml of S9-mix or phosphate buffer. The contents of each test tube were mixed and equally distributed onto the surface of Vogel-Bonner Minimal agar plates (one tube per plate). This procedure was repeated, in triplicate, for each bacterial strain and for each concentration of test material both with and without S9-mix.

All of the plates were incubated at 37°C for approximately 48 hours and the frequency of revertant colonies assessed using a Domino colony counter.

3.5.3 Mutation Study - Experiment 2

The second experiment was performed using methodology as described for Experiment 1, using fresh bacterial cultures, test material and control solutions. The test material dose range was similar to Experiment 1 and was 5 to 1500 µg/plate.

3.6 Acceptance Criteria

The reverse mutation assay may be considered valid if the following criteria are met:

All tester strain cultures exhibit a characteristic number of spontaneous revertants per plate in the vehicle and untreated controls. Acceptable ranges are presented in the standard test method section 3.2 with historical control ranges for 1998 and 1999 presented in Appendix I.

The appropriate characteristics for each tester strain have been confirmed, eg rfa cell-wall mutation and pkM101 plasmid R-factor etc.

All tester strain cultures should be in the approximate range of 1 to 9.9 x 10^9 bacteria per ml.

F 01

RS001:

ACUTE ORAL TOXICITY STUDY

IN THE RAT - ACUTE TOXIC CLASS METHOD

SPL PROJECT NUMBER: 599/068

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F 02

SPL PROJECT NUMBER: 599/068

QUALITY ASSURANCE REPORT

This study type is classed as short-term. The standard test method for this study type ("Standardised Study Plan" in OECD terminology) was reviewed for compliance once only on initial production. Inspection of the routine and repetitive procedures that constitute the study is carried out as a continuous process designed to encompass the major phases at or about the time this study was in progress.

This report has been audited by Safepharm Quality Assurance Unit, and is considered to be an accurate account of the data generated and of the procedures followed.

In each case, the outcome of QA evaluation is reported to the Study Director and Management on the day of evaluation. Audits of study documentation, and process inspections appropriate to the type and schedule of this study were as follows:

23 December 1999	Standard Test Method Compliance Audit
04 April 2000	Test Material Preparation
04 April 2000	Animal Preparation
04 April 2000	Dosing
05 April 2000	Assessment of Response
14 April 2000	Necropsy
§ 01 June 2000	Draft Report Audit
§ Date of QA Signature	Final Report Audit

§ Evaluation specific to this study

G. Wren ..... DATE: 17 JUL 2000

For Safepharm Quality Assurance Unit*

---

*Authorised QA Signatures:

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Deputy Head of Department: JM Crowther MIScT
Senior Audit Staff: JV Johnson BSc; G Wren ONC; RJ Gilbert BSc

F 03

SPL PROJECT NUMBER: 599/068

GLP COMPLIANCE STATEMENT

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106)). These Regulations are in accordance with GLP standards published as OECD Principles on Good Laboratory Practice (revised 1997, ENV/MC/CHEM(98)17); and are in accordance with, and implement, the requirements of Directives 87/18/EEC (as amended by Directive 1999/11/EC) and 88/320/EEC (as amended by Directive 1999/12/EC).

These international standards are acceptable to the United States Environmental Protection Agency and Food and Drug Administration, and fulfil the requirements of 40 CFR Part 160, 40 CFR Part 792 and 21 CFR Part 58 (as amended).

This report fully and accurately reflects t[illegible] procedures used and data generated.

R Driscoll DATE: 14 JUL 2000

R Driscoll BTech (Hons)
Study Director
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F 04

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SUMMARY

STUDY SPONSOR	:	
STUDY TITLE	:	ACUTE ORAL TOXICITY STUDY IN THE RAT - ACUTE TOXIC CLASS METHOD
TEST MATERIAL	:	RS001

1. A study was performed to assess the acute oral toxicity of the test material following a single oral administration to the Sprague-Dawley CD strain rat. The method followed that in the OECD Guidelines for Testing of Chemicals No. 423 "Acute Oral Toxicity - Acute Toxic Class Method" (adopted 22 March 1996) and Method B1 tris of Commission Directive 96/54/EC (which constitutes Annex V of Council Directive 67/548/EEC).

The results may be used as a basis for classification and labelling under Annex VI of Council Directive 67/548/EEC (as adapted to technical progress by Commission Directive 93/21/EEC) relating to the classification, packaging and labelling of dangerous substances.

2. Using all available information, 2000 mg/kg bodyweight was selected as the starting dose.

A group of three fasted females was treated with the starting dose. This was followed by a group of three fasted animals of the other sex at the same dose level.

The test material was administered orally as a dispersion in arachis oil BP. The animals were observed ½, 1, 2 and 4 hours after dosing and then once daily for fourteen days. Bodyweights were recorded on Day 0 (day of dosing) and on Days 7 and 14. At the end of the observation period all animals were killed by cervical dislocation and subjected to gross necropsy.

3. There were no deaths.

4. Clinical signs of toxicity noted in males were ataxia, hunched posture, lethargy, decreased respiratory rate and laboured respiration. All males recovered two days after dosing. There were no clinical signs of toxicity noted in females.

5. All animals showed expected gains in bodyweight over the study period.

6. No abnormalities were noted at necropsy.

7. The acute oral median lethal dose, (LD_{50}) of the test material, in the Sprague-Dawley CD strain rat, was estimated as being greater than 2000 mg/kg bodyweight. No mortalities were noted in animals treated with 2000 mg/kg bodyweight.

No symbol and risk phrase are required according to EU labelling regulations.

RS001:

ACUTE ORAL TOXICITY STUDY

IN THE RAT - ACUTE TOXIC CLASS METHOD

1. INTRODUCTION

The purpose of the study was to assess the acute oral toxicity of the test material following a single oral administration to the Sprague-Dawley CD strain rat. The method followed the OECD Guidelines for Testing of Chemicals No. 423 "Acute Oral Toxicity - Acute Toxic Class Method" (adopted 22 March 1996) and Method B1 tris of Commission Directive 96/54/EC (which constitutes Annex V of Council Directive 67/548/EEC).

The results may be used as a basis for classification and labelling under Annex VI of Council Directive 67/548/EEC (as adapted to technical progress by Commission Directive 93/21/EEC) relating to the classification, packaging and labelling of dangerous substances.

The rat was the species of choice for this study as it is widely used for safety evaluation studies and was recommended in the test method.

The study was performed between 29 March 2000 and 17 April 2000.

2. TEST MATERIAL AND EXPERIMENTAL PREPARATION

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	RS001
Batch number	:	1
Date received	:	15 March 2000
Description	:	red/brown solid
Storage conditions	:	room temperature in the dark

Data relating to the identity, purity and stability of the test material are the responsibility of the Sponsor.

2.2 Experimental Preparation

For the purpose of the study the test material was freshly prepared, as required, as a dispersion at the appropriate concentration in arachis oil BP.

Homogeneity was assured by the use of a Silverson Homogeniser and vortex mixer.

Determination by analysis of the concentration, homogeneity and stability of the test material preparations was not appropriate because it was not specified in the Study Plan and is not a requirement of the Test Guideline.

3. METHODS

3.1 Animals and Animal Husbandry

Male and female Sprague-Dawley CD (Crl : CD ® (SD) IGS BR) strain rats supplied by Charles River (UK) Ltd, Margate, Kent, UK were used. At the start of the study the males weighed 200 to 211g, and the females 205 to 225g, and were eight to twelve weeks old. After an acclimatisation period of at least five days the animals were selected at random and given a number unique within the cage by tail marking.

The animals were housed in groups of three by sex in solid-floor polypropylene cages furnished with woodflakes. With the exception of an overnight fast immediately before dosing and for approximately three to four hours after dosing, free access to mains drinking water and food (Rat and Mouse SQC Expanded Diet No.1, Special Diets Services Limited, Witham, Essex, UK) was allowed throughout the study.

The temperature and relative humidity were set to achieve limits of 19 to 25°C and 30 to 70% respectively. Any occasional deviations from these targets were considered not to have affected the purpose or integrity of the study. The rate of air exchange was approximately fifteen changes per hour and the lighting was controlled by a time switch to give twelve hours continuous light and twelve hours darkness.

3.2 Study Design

The information available suggested a starting dose of 2000 mg/kg bodyweight. The testing sequence followed the flow chart described in Appendix Ic.

Groups of fasted animals were therefore treated as follows:

DOSE LEVEL mg/kg	CONCENTRATION mg/ml	DOSE VOLUME ml/kg	NUMBER OF RATS	
			MALE	FEMALE
2000	200	10	-	3
2000	200	10	3	-

All animals were dosed once only by gavage, using a metal cannula attached to a graduated syringe. The volume administered to each animal was calculated according to the fasted bodyweight at the time of dosing. Treatment of animals was sequential. Sufficient time was allowed between each sex to confirm the survival of the previously dosed animals.

The animals were observed for deaths or overt signs of toxicity ½, 1, 2 and 4 hours after dosing and subsequently once daily for fourteen days.

Individual bodyweights were recorded prior to dosing and seven and fourteen days after treatment.

At the end of the observation period the animals were killed by cervical dislocation and subjected to gross pathological examination. This consisted of an external examination and opening of the abdominal and thoracic cavities for examination of major organs. The appearance of any macroscopic abnormalities was recorded. No tissues were retained.

3.3 Evaluation of Data

The number of animals dying during the study, or killed for humane reasons, was determined. The nature, severity, time of onset and duration of toxic effects were also determined. Effects on bodyweights and abnormalities noted at necropsy were identified.

Using the mortality data obtained, an estimate of the acute oral median lethal dose (LD_{50}) of the test material was made as shown in the schematic diagram in Appendix Ic.

3.3.1 **Interpretation According to Annex VI Section 3.2**

The results were interpreted according to the Commission Directive 93/21/EEC which adapts Council Directive 67/548/EEC on the regulations relating to the classification, packaging and labelling of dangerous substances.

The test material will be classified and assigned the appropriate symbol and risk phrase as follows:

CATEGORY	ACUTE ORAL LD_{50} mg/kg	SYMBOL	RISK PHRASE
VERY TOXIC	≤25	T+	R 28 "VERY TOXIC IF SWALLOWED"
TOXIC	>25 to 200	T	R 25 "TOXIC IF SWALLOWED"
HARMFUL	>200 to 2000	Xn	R 22 "HARMFUL IF SWALLOWED"

Test materials with acute oral LD_{50} values greater than 2000 mg/kg require no symbol and risk phrase.

4. **ARCHIVES**

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Safepharm archives for a period of five years. After this period, the Sponsor's instructions will be sought.

A 01

RS001:

BUEHLER DELAYED CONTACT HYPERSENSITIVITY

STUDY IN THE GUINEA PIG

SPL PROJECT NUMBER: 599/070

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A 02

SPL PROJECT NUMBER: 599/070

QUALITY ASSURANCE REPORT

This study type is classed as short-term. The standard test method for this study type ("Standardised Study Plan" in OECD terminology) was reviewed for compliance once only on initial production. Inspection of the routine and repetitive procedures that constitute the study is carried out as a continuous process designed to encompass the major phases at or about the time this study was in progress.

This report has been audited by Safepharm Quality Assurance Unit, and is considered to be an accurate account of the data generated and of the procedures followed.

In each case, the outcome of QA evaluation is reported to the Study Director and Management on the day of evaluation. Audits of study documentation, and process inspections appropriate to the type and schedule of this study were as follows:

17 January 2000	Standard Test Method Compliance Audit
08 May 2000	Test Material Preparation
04 May 2000	Animal Preparation
11 May 2000	Dosing
23 May 2000	Assessment of Response
§ 01 June 2000	Draft Report Audit
§ Date of QA Signature	Final Report Audit

§ Evaluation specific to this study

..... G. Wren ..... DATE: 17 JUL 2000

For Safepharm Quality Assurance Unit*

*Authorised QA Signatures:

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Senior Audit Staff: JV Johnson BSc; G Wren ONC; RJ Gilbert BSc

A 03

SPL PROJECT NUMBER: 599/070

GLP COMPLIANCE STATEMENT

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106)). These Regulations are in accordance with GLP standards published as OECD Principles on Good Laboratory Practice (revised 1997, ENV/MC/CHEM(98)17); and are in accordance with, and implement, the requirements of Directives 87/18/EEC (as amended by Directive 1999/11/EC) and 88/320/EEC (as amended by Directive 1999/12/EC).

These international standards are acceptable to the United States Environmental Protection Agency and Food and Drug Administration, and fulfil the requirements of CFR Part 160, 40 CFR Part 792 and 21 CFR Part 58 (as amended).

This report fully and accurately reflects the procedures used and data generated.

R Driscoll ..... DATE: 14 JUL 2000

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A 04

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A 05

SPL PROJECT NUMBER: 599/070

SUMMARY

STUDY SPONSOR	:	
STUDY TITLE	:	BUEHLER DELAYED CONTACT HYPERSENSITIVITY STUDY IN THE GUINEA PIG
TEST MATERIAL	:	RS001

1. A study was performed to assess the contact sensitisation potential of the test material in the albino guinea pig. The study was performed in compliance with the OECD Guidelines for Testing of Chemicals No. 406 "Skin Sensitisation" (adopted 17 July 1992) and Method B6 of Commission Directive 96/54/EC (which constitutes Annex V of Council Directive 67/548/EEC).

   The results may be used as a basis for classification and labelling under Annex VI of Council Directive 67/548/EEC (as adapted to technical progress by Commission Directive 93/21/EEC).

2. Twenty test and ten control animals were used for the main study.

   Based on the results of sighting tests, the concentrations of test material for the induction and challenge phases were selected as follows:

Topical Induction	:	50% w/w in petroleum jelly BP
Topical Challenge	:	50% and 25% w/w in petroleum jelly BP

3. The test material produced a 0% (0/20) sensitisation rate and was considered to be a non-sensitiser to guinea pig skin. The test material did not meet the criteria for classification as a sensitiser according to EU labelling regulations. No risk phrase is required.

RS001:
BUEHLER DELAYED CONTACT HYPERSENSITIVITY STUDY IN THE GUINEA PIG

1. INTRODUCTION

The study was performed to assess the contact sensitisation potential of the test material. The study was performed in compliance with the OECD Guidelines for the Testing of Chemicals No. 406 "Skin Sensitisation" (adopted 17 July 1992) and Method B6 of Commission Directive 96/54/EC (which constitutes Annex V of Council Directive 67/548/EEC).

The results may be used as a basis for classification and labelling under Annex VI of Council Directive 67/548/EEC (as adapted to technical progress by Commission Directive 93/21/EEC).

The test system was chosen because the guinea pig has been shown to be a suitable species for this type of study and is recommended in the test method. The strain used in these laboratories has been shown to produce satisfactory sensitisation responses using known positive sensitisers (see Appendix VIII). The results of the study are believed to be of value in predicting the likely contact sensitisation potential of the test material to man.

The study was performed between 03 April 2000 and 19 May 2000.

2. TEST MATERIAL AND EXPERIMENTAL PREPARATION

2.1 Description, Identification and Storage Condit`.

Sponsor's identification	:	RS001
Batch number	:	1
Date received	:	15 March 2000
Description	:	red/brown solid
Storage conditions	:	room temperature in the dark

Data relating to the identity, purity and stability of the test material are the responsibility of the Sponsor.

2.2 **Experimental Preparation**

For the purpose of this study the test material was freshly prepared as follows:

Topical Induction	:	50% w/w in petroleum jelly BP
Topical Challenge	:	50% and 25% w/w in petroleum jelly BP

Determination by analysis of the concentration, homogeneity and stability of the test material preparations was not appropriate because it was not specified in the Study Plan and is not a requirement of the Test Guideline.

3. METHODS

3.1 Animals and Animal Husbandry

Thirty-four male albino Dunkin Hartley guinea pigs supplied by David Hall Limited, Burton-on-Trent, Staffordshire, UK were used. At the start of the main study the animals weighed 363 to 437g, and were approximately eight to twelve weeks old. After a minimum acclimatisation period of five days, each animal was selected at random and given a number unique within the study which was written on a small area of clipped rump using a black indelible marker-pen.

The animals were housed singly or in pairs in solid-floor polypropylene cages furnished with woodflakes. Free access to mains tap water and food (Guinea Pig FD1 Diet, Special Diets Services Limited, Witham, Essex, UK) was allowed throughout the study.

The temperature and relative humidity were set to achieve limits of 17 to 23°C and 30 to 70% respectively. Any occasional deviations from these targets were considered not to have affected the purpose or integrity of the study. The rate of air exchange was approximately fifteen changes per hour and the lighting was controlled by a time switch to give twelve hours continuous light and twelve hours darkness.

3.2 Procedure

The sensitising properties of the test material were assessed using the procedure of Buehler E V, Toxicol. Appl. Pharmacol (1964) 6: 341 and Arch. Dermatol (1965) 91: 171-177.

3.2.1 Selection of Concentrations for Main Study (Sighting Tests)

The concentrations of test material to be used at each stage of the main study were determined by 'sighting tests' in which groups of guinea pigs were treated with various concentrations of test material. The procedures were as follows:

3.2.1.1 Selection of Concentration for Topical Induction

Two previously untreated guinea pigs were treated with four concentrations of the test material (50%, 25%, 10%, and 5% w/w in petroleum jelly BP). Applications were made to the clipped flanks under occlusive dressings for an exposure period of 6 hours. The degree of erythema and oedema was evaluated 24 and 48 hours after dressing removal. The highest concentration of the test material producing only mild dermal irritation was selected for the topical induction stage of the main study.

3.2.1.2 Selection of Concentration for Topical Challenge

Two guinea pigs were treated with two concentrations of the test material (50% and 25% w/w in petroleum jelly BP). These animals had been treated identically to the control animals of the main study on Days 0, 7 and 14. Applications were made to the clipped flanks under occlusive dressings for an exposure period of six hours. The degree of erythema and oedema was evaluated 24 and 48 hours after dressing removal. The highest concentration of the test material which produced no evidence of dermal irritation, and one lower concentration were selected for the topical challenge stage of the main study.

3.2.2 Main Study

A group of thirty guinea pigs was used for the main study, twenty test and ten control. The bodyweight of each animal was recorded at the start and end of the study.

Two main phases were involved in the main study; (a) an induction of a response and (b) a challenge of that response.

3.2.2.1 Induction

Induction of the Test Animals: The hair was removed from an area on the left flank of each animal with veterinary clippers and treated with a topical application of the test material formulation. An absorbent cotton lint patch (approximate size 20 mm x 20 mm) loaded with the test material formulation (50% w/w in petroleum jelly BP) as a thick, even layer was applied to the prepared skin and held in place under a strip of surgical adhesive tape and covered with an overlapping length of aluminium foil. The patch and foil were further secured by a strip of elastic adhesive bandage wound in a double layer around the torso of each animal. This occlusive dressing was kept in place for 6 hours. After 6 hours the dressing was carefully cut using scissors, removed and discarded. The treatment sites were decontaminated using diethyl ether and the position of the treatment sites was identified using a black indelible marker-pen. The induction procedure was repeated on the same site on Days 7 and 14 for a total of three 6-hour exposures.

Approximately 24 hours after each induction application (Days 1, 8 and 15) the degree of erythema and oedema was quantified using the scale shown in Appendix VII.

Induction of the Control Animals: The topical applications followed the same procedure as for the test animals except that the vehicle alone was applied.

3.2.2.2 Challenge

Shortly before treatment on Day 28 the right flank of each animal was clipped free of hair with veterinary clippers.

An absorbent cotton lint patch (approximate size 20 mm x 20 mm) loaded with a thick, even layer of test material at the maximum non-irritant concentration (50% w/w in petroleum jelly BP) was applied to the shorn right flank of each animal and was held in place with a strip of surgical adhesive tape. To ensure that the maximum non-

irritant concentration was used at challenge, the test material at a concentration of 25% w/w in petroleum jelly BP was similarly applied to a separate skin site on the right shorn flank.

The patches were covered with an overlapping length of aluminium foil and further secured by a strip of elastic adhesive bandage wound in a double layer around the torso.

After six hours, the dressing was carefully cut using scissors, removed and discarded. The treatment sites were decontaminated using diethyl ether and the position of the treatment sites was identified by using a black indelible marker-pen. On Day 29 the flanks were clipped free of hair using veterinary clippers.

3.2.2.3 **Evaluation of Skin Reactions**

Approximately 24 and 48 hours after dressing removal, the degree of erythema and oedema was quantified using the scale shown in Appendix VII.

Any other reactions were also recorded.

3.3 **Interpretation of Results**

A comparison of the intensities and durations of reactions at the test material challenge sites in the test and control animals permits identification of sensitisation reactions.

If the test material at the maximum non-irritant concentration produces reactions in test animals at the 24 or 48-hour reading, these reactions are attributed to contact sensitisation. This pre-supposes that no similar reactions are observed in the test material challenge sites of the control animals. If irritation is observed in the control animals, only reactions in the test animals that exceed the most severe reaction seen in the control animals are attributed to contact sensitisation. The results are expressed in terms of incidence and severity of responses, ie.

Incidence Score: The number of test group animals showing skin reactions greater than the most severe reaction observed in the control group animals, expressed as a fraction of the total number of test group animals.

Severity Score: The sum of the values assigned to the erythematous skin responses at the test material challenge sites of the test and control group animals, at each evaluation, divided by the number of animals in that group.

The data obtained may be used to classify the test material according to Commission Directive 93/21/EEC adapting Council Directive 67/548/EEC on the classification, packaging and labelling of dangerous substances.

The test material will be classified as sensitising and assigned the symbol "Xi", the indication of danger 'irritant' and the risk phrase R 43 "MAY CAUSE SENSITISATION BY SKIN CONTACT" if 15% of the test animals show a sensitisation response.

4. ARCHIVES

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Safepharm archives for a period of five years. After this period, the Sponsor's instructions will be sought.

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5. **RESULTS**

5.1 Topical Sighting Tests

The individual skin reactions observed in the topical sighting tests are given in Appendices I and II.

Based on these results, the following concentrations were selected for the main study:

Topical Induction	:	50% w/w in petroleum jelly BP
Topical Challenge	:	50% and 25% w/w in petroleum jelly BP

5.2 Main Study

5.2.1 Skin Reactions Observed After Topical Induction

Individual skin reactions observed at the topical induction sites of test and control group animals are given in Appendices III and IV.

No signs of skin irritation were elicited by the test material.

5.2.2 Skin Reactions Observed After Topical Challenge

Individual skin reactions at the challenge sites of test and control group animals are given in Tables 1 and 2.

50% w/w in Petroleum Jelly BP

No skin reactions were noted at the challenge sites of the test or control group animals at the 24 or 48-hour observations.

25% w/w in Petroleum Jelly BP

No skin reactions were noted at the challenge sites of the test or control group animals at the 24 or 48-hour observations.

5.3 Bodyweight

Individual bodyweights and bodyweight gains of test and control group animals at Day 0 and Day 30 are given in Appendices V and VI.

Bodyweight gains of guinea pigs in the test group, between Day 0 and Day 30, were comparable to those observed in the control group animals over the same period.

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SafePharm Laboratories

RS001:

FIVE DAY REPEATED EXPOSURE WITH SINGLE EXPOSURE INHALATION (NOSE ONLY) TOXICITY STUDY IN THE RAT

SPL PROJECT NUMBER: 599/069

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GLP COMPLIANCE STATEMENT

The work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106)). These regulations are in accordance with GLP standards published as OECD Principles on Good Laboratory Practice (revised 1997, ENV/MC/CHEM(98)17); and are in accordance with, and implement, the requirements of Directives 87/18/EEC (as amended by Directive 1999/11/EC) and 88/320/EEC (as amended by Directive 1999/12/EC).

These international standards are acceptable to the United States Environmental Protection Agency and Food and Dug Administration, and fulfil the requirements of 40 CFR Part 160, 40 CFR Part 792 and 21 CFR Part 58 (as amended).

This report fully and accurately reflects the procedures used and the data generated.

..... DATE: 04 OCT 2000

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QUALITY ASSURANCE STATEMENT

This study type is classed as short-term. Inspection of the routine and repetitive procedures that constitute the study is carried out as a continuous process designed to encompass the major phases at or about the time this study was in progress.

This report has been audited by Safepharm Quality Assurance Unit, and is considered to be an accurate account of the data generated and of the procedures followed.

In each case, the outcome of QA evaluation is reported to the Study Director and Management on the day of evaluation. Audits of study documentation, and process inspections appropriate to the type and schedule of this study were as follows:

◊	07 March 2000	Study Plan Compliance Audit
	20 April 2000	Test Material Preparation
	26 April 2000	Animal Preparation
	20 April 2000	Test System Preparation
	19 April 2000	Assessment of Response
	11April 2000	Necropsy
◊	21 July 2000	Draft Report Audit
◊	Date of QA Signature	Final Report Audit

◊ Evaluation specific to this study

..... DATE: - 9 OCT 2000

For Safepharm Quality Assurance Unit*

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RS001:

Five Day Repeated Exposure with Single Exposure

Inhalation (Nose Only) Toxicity Study in The Rat

SUMMARY

Method. A study was performed to assess the inhalation toxicity of the test material by single and repeated exposures. The test material was administered as a dust atmosphere to five groups of animals, each comprising of two male animals. All groups were initially exposed to a mean achieved atmosphere concentration of 1.98mg/L for four hours on Day 1 and then four of the groups were exposed to three further exposures, of six hour duration, at mean achieved concentrations of 0.19 or 0.20mg/L on Days 3, 4 and 5. Groups of animals were then sacrificed after various recovery/observation periods following completion of the exposures (Days 6, 12, 19 and 26). The single exposure group was sacrificed on Day 15. Clinical signs and bodyweight development were monitored during the study and all animals were subjected to a gross necropsy examination with histopathological evaluation of selected tissues of all animals.

Results. *Atmosphere Characteristics.*

Exposure Number	Atmosphere Concentration			Particle Size Distribution		
	Mean Achieved (mg/L)	Standard Deviation	Nominal (mg/L)	Mean Mass Aerodynamic Diameter (µm)	Inhalable Fraction (% < 4 µm)	Geometric Standard Deviation
1	1.98	0.22	22.9	3.70	53.8	2.29
2	0.20	0.02	0.78	3.18	59.8	2.52
3	0.19	0.01	0.87	2.87	64.7	2.42
4	0.20	0.01	0.82	3.12	60.9	2.43

Mortality. Two animals died following the initial exposure, one was found dead at the beginning of Day 2 and the other was found dead at the beginning of Day 3.

Clinical Observations. Following the initial exposure, all except one female animal showed substantial respiratory effects such as laboured and/or noisy respiration and increased respiratory rate. This was especially apparent the following day. Surviving animals, including those subjected to repeated exposures, recovered well and although respiratory abnormalities persisted for several days, one animal appeared asymptomatic from Day 5 and all animals were asymptomatic from Day 10.

Bodyweight. The majority of animals showed bodyweight loss three days into the study. On Day 6, only one animal showed a loss and by Day 10 normal development was noted for all surviving animals.

Necropsy. Amongst animals that died during the study, the following macroscopic abnormalities were recorded:

Lungs – enlarged, abnormally red, fluid filled, abnormally dark;
Liver – dark, patchy pallor;
Small intestine - gaseous distension, mucoid contents.

At terminal kill, four animals showed no macroscopic abnormalities. Amongst the remaining animals, the following abnormalities were detected:

Lungs – dark or pale patches, abnormally red, dark foci;
Kidneys – hydronephrosis.

Histopathology. The single and repeated inhalation exposure of RS001 to male rats followed by recovery periods of up to 21 days resulted in treatment-related morphological changes in the lungs and nasal cavities. Such changes were indicative of damage caused by an irritant substance. In general terms the severity of treatment related conditions lessened with increasing recovery period, but pulmonary accumulations of the test material remained unchanged.

Conclusion. Given the nature of the clinical and histopathological changes produced by exposure to the test material, it is concluded that RS001 is a respiratory irritant. Exposure to the test material, at the concentrations and particle sizes achieved during this study, has been shown to cause a range of effects to the respiratory system including focal pneumonitis, focal alveolar fibrosis and hyperplasia, disruption, erosion and ulceration of respiratory epithelium. In general terms, the severity of observations decreased with increasing recovery period but there was no evidence of clearance of the test material from the lungs within twenty-one days.

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RS001:

FIVE DAY REPEATED EXPOSURE

WITH SINGLE EXPOSURE

INHALATION (NOSE ONLY) TOXICITY STUDY

IN THE RAT

C 09

1. INTRODUCTION

The study was performed according to the protocol presented in Appendix 25 and was designed to assess the inhalation toxicity of the test material in the Sprague-Dawley Crl:CD® (SD) IGS BR strain rat by single and repeated nose-only exposures to dust atmospheres of the test material

The test system was chosen because the rat has been shown to be a suitable model for this type of study and has been historically used for safety evaluation studies of this type. The results of the study are believed to be of value in predicting the likely toxicity of the test material to man.

The study was performed between 27 March 2000 and 30 June 2000.

2. TEST MATERIAL

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	RS001
Description	:	Red/brown solid
Batch number	:	1 (characterisation and sightings) and 2 (main exposures)
Date received	:	15 March 2000 and 6 April 2000
Storage conditions	:	Room temperature, in the dark

Data relating to the identity, purity and stability of the test material are the responsibility of the Sponsor. In order to generate a suitable atmosphere, the test material was ground, prior to use, using a Moulinex electric grinder.

The absorption of the test material was not determined.

3. METHODS

3.1 Animals and Animal Husbandry

10 male Sprague-Dawley Crl:CD® (SD) IGS BR strain rats were supplied by Charles River (UK) Ltd, Margate, Kent. On receipt the animals were randomly allocated to cages. After an acclimatisation period of at least five days the animals were given a number unique within the study by ear punching and a number written on a colour coded cage card. At the start of the study the animals were approximately eight to ten weeks old and weighed from 277g to 304g.

The animals were housed in groups of two in solid-floor polypropylene cages with stainless steel lids, furnished with softwood flakes (Datesand Ltd., Cheshire, UK) and provided with environmental enrichment items: wooden chew blocks (B & K Universal Ltd, Grimston, Hull, UK) and cardboard "fun tunnels" (Datesand Ltd., Cheshire, UK). With the exception of the exposure period, free access to mains drinking water and food (Rat and Mouse Expanded Diet No. 1, Special Diets Services Limited, Witham, Essex, UK) was allowed throughout the study. The diet, drinking water, bedding and enrichment items are routinely analysed and are considered not to contain any contaminants that could reasonably be expected to affect the purpose or integrity of the study.

Temperature and humidity in the animal holding room were generally maintained within target ranges of 21 ± 2°C and 55 ± 15% respectively. Although there were occasional deviations from the humidity target values, these were considered not to have affected the purpose or integrity of the study. The rate of air exchange was at least fifteen changes per hour and the lighting was controlled to give twelve hours continuous light and twelve hours darkness. The animals were retained in this accommodation at all times except during the exposure period.

3.2 Inhalation Exposure

3.2.1 Atmosphere Generation

A dust atmosphere was produced from the test material using a 'Wright's Dust Feed' mechanism (L Adams Ltd, London, UK) located at the top of the exposure chamber and driven by a variable speed motor. The dust feed was connected to a metered compressed air supply.

Compressed air was supplied by means of an oil free compressor and passed through a water trap and respiratory quality filters before it was introduced to the dust feed.

The cylindrical exposure chamber (ADG Developments Ltd, Hitchin, Herts, UK) had a volume of approximately 30 litres (dimensions: 28 cm diameter x 50 cm high). The concentration within the exposure chamber was controlled by adjusting the rate of the variable speed motor. The extract from the exposure chamber passed through a 'scrubber' trap and was connected with a high efficiency filter to a metered exhaust system. The chamber was maintained under negative pressure. A schematic diagram of the dynamic (continuous flow) system employed is shown in Figure 1.

Homogeneity of the test atmosphere within the chamber was not specifically determined during this study. Chambers of the same design have been fully validated and shown to produce evenly

distributed atmospheres in the animals' breathing zone with a wide variety of test materials (Green J D *et al*, 1984).

Prior to the start of the study, test material atmospheres were generated within the exposure chamber. During this characterisation period test material input rates were varied to achieve the required atmospheric concentrations.

3.2.2 Pre-Study Sighting

During characterisation, groups of two rats were exposed to various concentrations of the test material, for up to six hours, to enable dose levels to be selected for the main study.

3.2.3 Exposure Procedure

Animals were allocated to treatment groups as follows:

Group Number	1 x 4-hour exposure (Day 1) Target = 2.0mg/L	3 x 6-hour exposure (Days 3-5) Target = 0.2mg/L	Necropsy Day
1	Yes	No	15
2	Yes	Yes	6
3	Yes	Yes	12
4	Yes	Yes	19
5	Yes	Yes	26

Each rat was individually held in a tapered, polycarbonate restraining tube fitted onto a single tier of the exposure chamber and sealed by means of a rubber 'O' ring. Only the nose of each animal was exposed to the test atmosphere.

On Day 1 of the study, following an appropriate equilibration period, five groups, each of two male rats, were subjected to a single exposure to the test material for a period of four hours. Based on the results of the pre-study sightings, a target concentration of 2.0mg/L was used for this exposure.

On Days 3, 4 and 5 following an appropriate equilibration period, Groups 2 to 5 were again exposed to an atmosphere of the test material, at a target concentration of 0.2mg/L, for a six hour period.

3.2.4 Exposure Chamber Temperature and Relative Humidity

The temperature and relative humidity inside the exposure chamber were measured by an electronic thermometer/humidity meter (Comark Ltd, Welwyn Garden City, Hertfordshire, UK)

located in a vacant port in the animals' breathing zone of the chamber and recorded every thirty minutes throughout each exposure period. Individual values are given in Appendix 21 and 22.

3.2.5 Exposure Chamber Oxygen Concentration

Oxygen levels within the exposure chamber were measured by an electronic oxygen analyser (Servomex (UK) Ltd, Crowborough, East Sussex) located in a sampling port in the animals breathing zone during each exposure period. The test atmosphere was generated to contain at least 19% oxygen. Individual values are given in Appendix 23.

3.2.6 Exposure Chamber Atmosphere Concentration

The actual chamber concentration was measured at regular intervals during each exposure period. The gravimetric method used, employed glass fibre filters (Gelman type A/E 25 mm) placed in a filter holder. The holder was temporarily sealed in a vacant port in the exposure chamber in the animals' breathing zone and a suitable, known volume of exposure chamber air was drawn through the filter using a vacuum pump.

Each filter was weighed before and after sampling in order to calculate the weight of collected test material. The difference in the two weights, divided by the volume of atmosphere sampled, gave the actual chamber concentration.

The nominal chamber concentrations were calculated by dividing the mass of test material used by the total volume of air passed through the chamber.

3.2.7 Particle Size Distribution

The particle size of the generated atmosphere inside the exposure chamber was determined three times during each exposure period using a Marple Personal Cascade Impactor (Schaefer Instruments Ltd, Oxon, UK). This device consisted of six impactor stages (9.8, 6.0, 3.5, 1.55, 0.93 and 0.52 µm cut points) with stainless steel collection substrates and a back up glass fibre filter, housed in an aluminium sampler. The sampler was temporarily sealed in a sampling port in the animals' breathing zone and a suitable, known volume of exposure chamber air was drawn through it using a vacuum pump.

The collection substrates and backup filter were weighed before and after sampling and the weight of test material, collected at each stage, calculated by difference.

The mean amount for each stage was used to determine the cumulative amount below each cut-off point size. In this way, the proportion (%) of aerosol less than 9.8, 6.0, 3.5, 1.55 0.93 and 0.52 µm was calculated.

The resulting values were converted to probits and plotted against Log_{10} cut-point size. From this plot, the Mass Median Aerodynamic Diameter (MMAD) was determined (as the 50% point) and the geometric standard deviation was calculated. In addition, the proportion (%) of aerosol less than 4 µm (considered to be the respirable portion) was determined.

All particle size distribution calculations were performed using a purpose designed computer programme (Chrom Series Data Server and Reg 2000 Graph Plotter).

3.3 Observations

3.3.1 Clinical Signs

During the exposure phase of the study, animals were observed for clinical signs prior to the repeat exposures, at hourly intervals during exposure, immediately on removal from the restraining tubes at the end of exposure and one hour after completion of the exposure. During the recovery period, observations were carried out at least once daily. Any deaths or evidence of overt toxicity were recorded at each observation.

3.3.2 Bodyweight

Individual bodyweights were recorded just prior to the first exposure and on Days 3, 6, 10, 12, 19 and 26 (where applicable).

3.3.3 Pathology

Gross necropsies were performed on all animals that died during the study and on all survivors killed by intravenous injection of sodium pentobarbitone at the end of the relevant observation period. All animals, including those that died, were subjected to a full external and internal examination, and any macroscopic abnormalities were recorded. The respiratory tract was subjected to a detailed macroscopic examination for signs of irritancy or local toxicity.

3.3.4 Histopathology

Samples of the lungs (inflated to approximately normal inspiratory volume with buffered 10% formalin, prior to immersion in fixative), nasal cavity and larynx were taken from all animals and preserved in buffered 10% formalin. These tissues were despatched to Propath UK Ltd, Willow

Court, Netherwood Road, Rotherwas, Hereford, UK for processing. The preserved tissues were prepared as paraffin blocks, sectioned at a nominal thickness of 5µm and stained with haematoxylin and eosin for subsequent microscopic examination by the Study Pathologist. All findings were entered into the ROELEE 84 pathology computerisation system for tabulation and report production.

In addition, for animals from Groups 3, 4, and 5, samples of the following tissues were taken and preserved in buffered 10% formalin:

Adrenals;
Aorta (thoracic),
Bone and bone marrow (femur including stifle joint);
Bone and bone marrow (sternum);
Brain;
Caecum;
Colon;
Duodenum;
Kidneys;
Eyes;
Gross Lesions;
Heart;
Ileum,
Jejunum;
Liver;
Lymph nodes (cervical and mesentric);
Muscle (skeletal);
Oesophagus;
Pancreas;
Pituitary;
Prostate;
Rectum;
Salivary Glands (submaxillary);
Sciatic nerve;
Seminal vesicles;
Skin (hind limb);
Spleen;
Stomach;
Testes (with epididymides);
Thymus;
Thyroid/parathyroid;
Trachea;
Urinary bladder.

3.4 Evaluation of Data

Data evaluations included the relationship, if any, between the animals' exposure to the test material and the incidence and severity of all abnormalities including behavioural and clinical observations, necropsy findings, histopathology, bodyweight changes, mortality and any other toxicological effects.

4. ARCHIVES

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Safepharm archives for five years, after which instructions will be sought as to further retention or disposal.

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RESULTS

5. Results

5.1 Pre-Study Sighting.

Three groups of two animals were exposed to various atmospheres of the test material:

5.1.1 Sighting 1

One male and one female animal were exposed to a mean achieved atmosphere concentration of 4.99mg/L (standard deviation = 0.36) for 122 minutes. Differing sexes were chosen to demonstrate that there were no significant sex differences. On removal from the chamber, the only respiratory effects noted were decreased respiratory rate for both animals. This observation persisted until the third day post-exposure and was accompanied on Day 1 by noisy respiration. Both animals were asymptomatic on Days 3 and 4 and the sighting was terminated.

5.1.2 Sighting 2

One male and one female animal were exposed to a mean achieved atmosphere concentration of 4.94mg/L (standard deviation = 0.71) for 240 minutes. Differing sexes were chosen to demonstrate that there were no significant sex differences. On removal from the chamber, observations included increased respiratory rate and noisy respiration and one hour after exposure this had changed to decreased respiratory rate and noisy respiration. No respiratory effects were noted in the male animal on Day 1, though observations of splayed and tiptoe gait were recorded. The female showed laboured and noisy respiration and lethargy. Both animals were found dead on the second day post-exposure.

5.1.3 Sighting 3

Two male animals were exposed to a mean achieved atmosphere concentration of 1.96mg/L (standard deviation = 0.23) for 240 minutes. On removal from the chamber, the only respiratory effects noted were increased respiratory rate for both animals. No deterioration in their condition was noted on Day 1 and so the animals were exposed to a repeat exposure, on Day 2, at a mean achieved atmosphere concentration of 0.25mg/L (standard deviation = 0.12) for 304 minutes. Respiratory observations, on removal from the chamber, now included increased respiratory rate and laboured and/or noisy respiration. In addition, one of the animals showed pallor of the extremities. Increased respiratory rate and/or noisy respiration persisted for several days in both animals until the sighting was terminated on Day 4.

5.2 Atmosphere Characteristics

5.2.1 Achieved Atmosphere Concentration

The actual concentration of the test material was measured at approximately fifteen minute intervals during each exposure period. The mean values obtained were:

	Atmosphere Concentration		
Exposure Number	Mean Achieved (mg/L)	Standard Deviation	Nominal (mg/L)
1	1.98	0.22	22.9
2	0.20	0.02	0.78
3	0.19	0.01	0.87
4	0.20	0.01	0.82

The exposure chamber concentrations and chamber flow rates are presented graphically in Figures 2 to 5 and are given in Appendix 1 to 4.

The chamber air flow rate was maintained at 20 L/min, providing 40 air changes per hour.

The theoretical chamber equilibration time (T_{99}) was 7 minutes (Silver, 1946).

5.2.2 Particle Size Distribution

The particle size analyses of the atmosphere drawn from the animals' breathing zone, were as follows:

Group Number	Mean Achieved Atmosphere Concentration (mg/L)	Mean Mass Median Aerodynamic Diameter (µm)	Inhalable Fraction (% <4 µm)	Geometric Standard Deviation
1	1.98	3.70	53.8	2.29
2	0.20	3.18	59.8	2.52
3	0.19	2.87	64.7	2.42
4	0.20	3.12	60.9	2.43

The particle size distribution data are presented graphically in Figures 6 to 13 and are given in Appendix 5 to 8.

5.3 Mortality

Two animals died following the initial exposure at a mean achieved concentration of 1.98mg/l. One was found dead at the beginning of Day 2 and the other was found dead at the beginning of Day 3.

5.4 Clinical Observations

Individual clinical observations are given in Appendix 9 to 13.

Exposure 1 (Day 1)

During this exposure, wet fur was seen in all animals and increased or decreased respiratory rate and fur staining by test material were common. On removal, increased respiratory rate or decreased respiratory rate, hunched posture, pilo-erection and wet fur were noted for all animals and noisy respiration and fur staining by test material were present in the majority. One hour post exposure, hunched posture and pilo-erection remained and all animals now exhibited decreased respiratory rate with noisy respiration frequently recorded.

One animal was found dead the following morning and the condition of the majority of the remaining animals had deteriorated, with laboured respiration and red/brown staining around the snout or eyes now apparent in all except one animal and sneezing present in several. There were isolated instances of pallor of the extremities and ptosis.

On Day 3, another animal was found dead. However, the condition of the remaining animals had markedly improved, with only three instances of laboured respiration.

Exposures 2 to 4 (Days 3 to 5)

During these exposures, common observations recorded included increased respiratory rate and wet fur. There were instances of laboured respiration during Exposure 2, but this observation did not reappear later during the study.

On removal from the chamber and one-hour post exposure common observations were limited to increased respiratory rate, hunched posture, pilo-erection and wet fur. Noisy respiration and red/brown staining around the eyes and/or snout became more frequent during this three day period and there was an isolated instance of sneezing on Day 5.

Hunched posture, increased respiratory rate and/or noisy respiration were recorded intermittently for all animals prior to exposure on Days 4 and 5 and during Day 6, but for Days 7 to 9, observations were limited to increased respiratory rate only. Asymptomatic animals were noted from Day 5, prior to the final exposure and from Day 10 all animals appeared normal.

Instances of wet fur, hunched posture and pilo-erection on removal from the exposure chambers are considered not to be toxicologically significant as data from historic, negative control groups indicate that such observations are associated with the restraint procedure rather than exposure to a test material.

5.5 Bodyweight

Individual bodyweights are given in Appendix 14.

Most animals showed bodyweight loss three days into the study. On Day 6, only one animal showed a loss and by Day 10 normal development was noted for all surviving animals.

5.6 Necropsy

Individual necropsy findings are given in Appendix 15.

Amongst animals that died during the study, the following macroscopic abnormalities were recorded:

Lungs – enlarged, abnormally red, fluid filled, abnormally dark;
Liver – dark, patchy pallor;
Small intestine - gaseous distension, mucoid contents.

Though gaseous distension is often associated with extreme breathing difficulty, in particular gasping respiration, it is considered that the other non-lung associated abnormalities detected are probably post-mortem effects.

At terminal kill, four animals showed no macroscopic abnormalities. Amongst the remaining animals, the following abnormalities were detected:

Lungs – dark or pale patches, abnormally red, dark foci;
Kidneys – hydronephrosis.

Hydronephrosis is considered to be a spontaneous abnormality and not to be caused by exposure to the test material.

6. HISTOPATHOLOGY

Individual histopathological findings are given in Appendix 16 to 20.

There were two unscheduled deaths during the course of the study. Rats 1 (group 1) and 7 (group 4) died prior to scheduled termination. In general, the contribution made by interim death animals to a group assessment of treatment-related pathology should be regarded with caution. Animals dying before termination of the dosing period will not have received their full exposure to the test material, and those recovery group animals dying before completion of the recovery period may continue to illustrate the morphological consequences of treatment.

6.1 Trachea:

No changes of toxicological significance were observed.

6.2 Lungs:

The lungs of all animals regardless of exposure or recovery period had minimal to slight bronchus associated lymphoid tissue, which was unrelated in presence or severity to treatment. Accumulations of granular pigment were also seen in the lung parenchyma of all animals regardless of treatment group. This pigment probably represented accumulations of the inhaled test material and remained undiminished in extent after the full 21-day recovery period. Other treatment-related changes included focal pneumonitis, accumulations of alveolar macrophages, focal alveolar fibrosis, and hyperplasia of the airways epithelium. Pulmonary congestion was seen in both interim death animals, and in one interim death rat pulmonary oedema was also present. There was an absence of pneumonitis in the lungs of repeated exposure animals necropsied from Day 12 onwards although the significance of this is dubious given that the surviving group 1 animal had moderate pneumonitis after necropsy on Day 15 of the study. The severity of alveolar macrophage accumulations also showed some indication of amelioration with increasing recovery period. A clear group distribution for severity of any of the remaining conditions was not present.

6.3 Nasal Turbinates:

Pigment accumulations, probably representing deposition of the test material, were seen overlying the turbinates epithelium for the majority of animals in the study regardless of treatment group or recovery period. In addition, occasional instances of inflammatory exudate, epithelial disruption,

and epithelial erosion were also observed with a possible generalised reduction in severity and incidence with increasing recovery period. The more severe grades of inflammatory exudate and epithelial disruption, together with focal epithelial ulceration were seen for both interim death animals and such irritation to the nasal surfaces may have been a factor contributing towards moribundity in these rats.

7. DISCUSSION

Both single and repeated exposures of the test material RS001 to rats produced toxicologically significant findings in the clinical, macroscopic and microscopic condition of the animals.

Exposure to the test material at a concentration of 1.98mg/L for four hours resulted in a severe deterioration in condition amongst the animals, manifest as clearly observable signs such as laboured and noisy respiration and increased respiratory rate. This effect on animal health was also observed as a weight loss amongst the majority of animals. The histopathological findings for the two animals that died following this exposure complement these observations, with significant damage to the nasal epithelium and lung tissue reported.

Repeated exposures of the test material at approximate concentrations of 0.20mg/L appeared to produce little additional effect upon the lungs of the animals and the surviving single-exposure animal showed similar histopathological changes as the repeated exposure animals. Detrimental effects on the nasal cavity, however, were noted amongst animals that were subjected to repeated exposures and this may be associated with the increasing incidence of noisy respiration during the repeat exposures.

There would appear to be a treatment related increase in alveolar macrophages and a corresponding decrease with increasing recovery period, though such conclusions are tentative given the small group size. Such an increase in alveolar macrophages is usually a response to particulate matter in the alveoli. However, deposition of the test material in the lungs, in the form of accumulation of granular pigment, showed little variation between single and repeated exposures and was unaffected by the length of recovery period. There is, therefore, no indication of any substantial clearance of the test material. It is probable that the lungs were simply overloaded with particulate matter and, in such cases, stasis or significant slowing of alveolar macrophage-mediated clearance of insoluble particles is typical. (Morrow, 1988: Morrow, 1992)

Attention should be drawn to the differing severity of the response to an atmosphere of approximately 2mg/L in the sighting phase and the main phase. With a mean achieved atmosphere concentration of 1.96mg/L for 240 minutes for Sighting 3, the only respiratory effects noted were increased respiratory rate in the two animals exposed. The first main phase exposure was completed at a mean achieved concentration of 1.98mg/L and yet the effect on the animals was substantially different with two deaths and the following exposure postponed due to the severity of the observations. It cannot be determined, from the data available, whether such a marked

difference is due to the differing test material batches used for the sighting and main exposures or whether this is simply due to natural variability of response in a population.

Conclusion:

Given the nature of the clinical and histopathological changes produced by exposure to the test material, it is concluded that RS001 is a respiratory irritant. Exposure to the test material, at the concentrations and particle sizes achieved during this study, has been shown to cause a range of effects to the respiratory system including focal pneumonitis, focal alveolar fibrosis and hyperplasia, disruption, erosion and ulceration of respiratory epithelium. In general terms, the severity of observations decreased with increasing recovery period but there was no evidence of clearance of the test material from the lungs within twenty-one days.

8. REFERENCES

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