



HS&E CORPORATE SERVICES

12806



RE: BEHQ-98-14323
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Nov 23, 1998

OPPT Document Control Officer (7407)
US Environmental Protection Agency
401 M Street, SW
Washington, DC 20460
ATTN: Section 8(e) Coordinator

RE: Notifications of Substantial Risk - Lithium Sulfide, Sodium Sulfide, Cerium Sulfide

Dear Sir or Madam:

In accordance with the provisions of Section 8(e) of the Toxic Substances Control Act (TSCA), Rhodia Inc. (Rhodia) is submitting the following notifications of substantial risk:

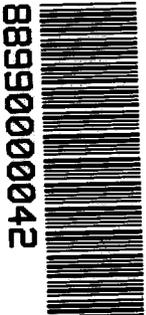
Compound: Lithium Sulfide, CAS# 12136-58-2: The compound was administered to male and female rats at levels of 100, 200 and 500 mg/kg in corn oil. The Oral LD50 in rats was determined to be 274 mg/kg. Symptoms exhibited during the study included piloerection, hunched posture and unsteady gait. Effects were noted in both moribund animals and test survivors. In addition, among rats at the 500 mg/kg dose level, clonic convulsions in were observed in 1 male rat, and tremors in were observed in 1 male and 1 female rat. All effects noted at this dose level were seen in moribund animals.

These types of neurological effects are well-known to occur with overdoses of lithium compounds as a class, but Rhodia is unaware as to whether these effects have been reported in rats exposed orally to lithium sulfide, or at these doses.

Compound: Sodium Sulfide, CAS# 1313-82-2: The chemical was administered to male and female rats at levels of 1000, 1260 and 2000 mg/kg in corn oil. The Oral LD50 in rats was determined to be 1122 mg/kg. Symptoms exhibited during the study included piloerection, hunched posture and unsteady gait. Effects were noted in both moribund animals and study survivors. In addition, several rats at both the 1260 and 2000 mg/kg dose levels exhibited tremors. All effects noted at these two dose levels were observed only among moribund animals.

These types of neurological effects are well-known in occur with overdoses of sodium compounds as a class, but Rhodia is unaware as to whether these effects have been reported in rats exposed orally to sodium sulfide, or at these doses.

Compound: Cerium Sulfide, CAS# 12014-93-6: The chemical was administered to male and female rats at a single dose of 5000 mg/kg in corn oil. The Oral LD50 in rats was determined to be greater than 5000 mg/kg. One animal died within 10 minutes of dosing. Based on an internal review by the laboratory, this death was determined to be



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due to an intubation error, and not attributable to the test compound. All other animals survived to study termination. Clinical signs exhibited during the study included piloerection, hunched posture and unsteady gait. In addition, females only exhibited lethargy, abnormal respiration, walking on toes, pallid extremities and increased sensitivity to touch.

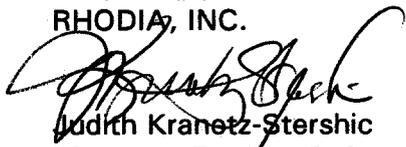
Given this diverse group of signs, it is not clear if these results are indicative of neurological effects due to the test compounds. For example, at such high dose levels, it is quite possible that all of these effects may be attributable to electrolyte imbalances.

These studies were communicated to Rhodia Inc. on November 3, 1998.

Rhodia Inc. asserts that none of this information constitutes confidential business information.

Should you have any questions, or require any further information, please call (732) 821-3324. Thank you.

Very truly yours,
RHODIA, INC.



Judith Kranetz-Stershic
Manager, Product Safety

JKS/
Atts.

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Huntingdon
Life Sciences

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**LITHIUM SULPHIDE
ACUTE ORAL TOXICITY TO THE RAT**

Report

CONFIDENTIAL

RNP 589/984378/AC

LITHIUM SULPHIDE
ACUTE ORAL TOXICITY TO THE RAT

Sponsor

Rhodia
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Report issued 26 October 1998

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COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

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

The UK Good Laboratory Practice Regulations 1997 (Statutory Instrument No 654).

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

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

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

Chemical analysis of formulated test articles for determination of stability, homogeneity and concentration was not undertaken for this study.

Stephen J. Mason

Stephen J. Mason, B.Sc. (Hons.),
Study Director,
Huntingdon Life Sciences Ltd.

26 October 1998

Date

QUALITY ASSURANCE STATEMENT

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

Study Phases Inspected	Date of Inspection	Date of Reporting
Process Based Inspections		
Generic Standard Protocol Review	21 November 1997	24 November 1997
Animal husbandry	14 September 1998	17 September 1998
Housing/environment	14 September 1998	17 September 1998
Weighing of animals	14 September 1998	17 September 1998
Treatment procedures	14 September 1998	17 September 1998
Clinical signs	14 September 1998	17 September 1998
Post mortem	14 September 1998	17 September 1998
Records audit	14 September 1998	17 September 1998
Training records	14 September 1998	17 September 1998
Report	9 October 1998	12 October 1998

Protocol: An audit of the standard protocol generated for this type of study design was conducted and reported to Company Management as indicated above.

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

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

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



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



 Date

RESPONSIBLE PERSONNEL

Stephen J. Mason, B.Sc. (Hons.),
Study Director,
Huntingdon Life Sciences Ltd.

SUMMARY

A study was performed to assess the acute oral toxicity of Lithium sulphide to the rat. The method followed was that described in:

EPA Health Effects Testing Guidelines, Subpart B - General Toxicity Testing § 798.1175 Acute oral toxicity, September 27, 1985 (described in Federal Register Vol. 50, No. 188) and subsequent revisions. Subpart B provides detailed information relating to data requirements of 40 CFR Part 798 and supports the Toxic Substances Control Act (TSCA).

A group of ten fasted rats (five males and five females) received a single oral gavage dose of the test substance, formulated in corn oil and administered at a dose level of 100 mg/kg bodyweight. This dosage was chosen on the basis of results from a preliminary study. After review of results at 100 mg/kg, further groups of five males and five female were similarly dosed at 200 and 500 mg/kg bodyweight in order to establish the acute median lethal oral dose (LD_{50}).

One male at 100 mg/kg and all rats at 500 mg/kg died during the study. All deaths occurred within approximately 28 minutes of dosing. Macroscopic examination revealed changes in the majority of organs and tissues.

Clinical signs of reaction to treatment included piloerection, hunched posture, waddling/unsteady gait, lethargy, pallid extremities and walking on toes, seen in rats at all dosages. In addition, abnormal respiration, increased salivation, abnormal faeces, increased sensitivity to touch, ungroomed appearance, body tremors, clonic convulsions, prostration (collapsed state), blue/cold extremities and dark colouring to eyes were seen in rats at one or more dose levels. Recovery of surviving rats was complete in all instances by Day 5.

All surviving rats were considered to have achieved satisfactory bodyweight gains throughout the study.

Macroscopic examination of surviving animals killed at study termination on Day 15 revealed inflammation and a white crystalline deposit in the stomach of one male dosed at 200 mg/kg, but otherwise no abnormalities.

The acute median lethal oral doses (LD_{50}) and associated 95% confidence limits to male and female rats of Lithium sulphide were calculated to be:

Males only:	240 (86 to 667) mg/kg bodyweight
Females only:	317 (204 to 494) mg/kg bodyweight
Combined sexes:	274 (104 to 725) mg/kg bodyweight.

INTRODUCTION

The study was designed to assess the toxicity of Lithium sulphide following a single oral dose to the rat. The rats were dosed by oral gavage as the test substance may be ingested accidentally.

The study was conducted in compliance with:

EPA Health Effects Testing Guidelines, Subpart B - General Toxicity Testing § 798.1175 Acute oral toxicity, September 27, 1985 (described in Federal Register Vol. 50, No. 188) and subsequent revisions. Subpart B provides detailed information relating to data requirements of 40 CFR Part 798 and supports the Toxic Substances Control Act (TSCA).

EPA Pesticide Assessment Guidelines, Subdivision F. Hazard Evaluation: Human and Domestic Animals 81-1 (Revised Edition November 1984). Subdivision F provides detailed information relating to data requirements of 40 CFR Part 158 and supports the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA).

Japanese Ministry of Agriculture, Forestry and Fisheries, Requirements for Safety Evaluation of Agricultural Chemicals and Testing Guidelines for Toxicology Studies, Acute Oral Toxicity Study, 59 NohSan No. 4200, Agricultural Production Bureau, January 28, 1985.

The rat was chosen as it has been shown to be a suitable model for this type of study and is one of the animals recommended in the test guideline.

The dose levels for the study were chosen in compliance with the guideline and on the basis of results from a preliminary investigation.

The protocol was approved by Huntingdon Life Sciences Management on 5 June 1998, by the Sponsor on 9 June 1998 and by the Study Director on 18 August 1998.

The experimental phase of the study was undertaken between 18 August and 16 September 1998.

TEST SUBSTANCE

Identity:	Lithium sulphide
Intended use:	Component of pigment
Appearance:	Off white powder
Storage conditions:	4°C in the dark under nitrogen
Lot number:	376515/1
Expiry:	Not advised but assumed stable for six months from date of receipt
Purity:	≥ 98%
Sample received:	29 July 1998

EXPERIMENTAL PROCEDURE

ANIMAL MANAGEMENT

Animals chosen for this study were selected from a stock supply of healthy male and female CD rats of Sprague-Dawley origin (Hsd: Sprague-Dawley(CD) obtained from Harlan U.K. Ltd, Bicester, Oxon, England.

Animals in the main study were in the weight range of 201 to 235 g and approximately eight to ten weeks of age prior to dosing (Day 1). All rats in the main study were acclimatised to the experimental environment for a minimum period of six days prior to dosing.

Rats were allocated without conscious bias to cages within the treatment groups and housed in groups of up to five rats of the same sex in metal cages with wire mesh floors in Building R14 Room 7.

A standard laboratory rodent diet (Special Diet Services RM1 (E) SQC expanded pellet) and drinking water were provided *ad libitum*. Access to food only was prevented overnight prior to and for approximately 4 hours after dosing.

The batch of diet used for the study was analysed for certain nutrients, possible contaminants and micro-organisms.

Results of routine physical and chemical examination of drinking water, as conducted by the supplier, are made available to Huntingdon Life Sciences Ltd. as quarterly summaries.

Animal room temperature was in the range 21 to 23.5°C and relative humidity was in the range 42 - 62%. Permanent daily recordings of these parameters were made and these are archived with other Department raw data. Lighting was controlled by means of a time switch to provide 12 hours of artificial light (0700 - 1900 hours) in each 24-hour period.

Each animal was identified by cage number and ear punching. Each cage was identified by a coloured label displaying the dose level, study schedule number, animal mark and the initials of the Study Director and Home Office licensee.

TEST SUBSTANCE PREPARATION

Lithium sulphide was formulated at various concentrations in corn oil and administered at a volume of 10 ml/kg bodyweight.

The test substance was prepared on the day of dosing.

The absorption of the test substance was not determined.

Characterisation of the homogeneity, stability and purity of the test substance was not undertaken in this study and remains the responsibility of the Sponsor.

TREATMENT PROCEDURE

Preliminary study

A group of two rats (one male and one female) was treated at 2000 mg/kg bodyweight. Following mortalities at this dosage, in order to establish a dosing regime for the main study, two groups of two rats (each comprising one male and one female) were dosed at 200 and 20 mg/kg bodyweight respectively.

Main study

A group of ten rats (five males and five females) was treated at 100 mg/kg bodyweight. Further groups of five males and five females were dosed at 200 and 500 mg/kg bodyweight in order to establish the acute median lethal oral dose (LD₅₀).

The treatment regime and constitution of the treatment groups are shown below:

Dates dosed	Dose (mg/kg)	Dose concentration (% w/v)	Dose volume (ml/kg)	No. of rats	
				M	F
18.08.98	2000	20	10	1	1
24.08.98	200	2	10	1	1
25.08.98	20	0.2	10	1	1
27.08.98	100	1	10	5	5
02.09.98	200	2	10	5	5
09.09.98	500	5	10	5	5

In the above and following Tables, M: denotes male rats and F: denotes female rats.

Control animals

No control animals were included in this study.

ADMINISTRATION OF TEST SUBSTANCE

The appropriate dose volume of the test substance was administered to each rat by oral gavage by using a syringe and plastic catheter (8 choke).

The day of dosing was designated Day 1.

OBSERVATIONS

Mortality

Cages of rats were checked at least twice daily for mortalities.

Clinical signs

Animals were observed soon after dosing and at frequent intervals for the remainder of Day 1. On subsequent days, surviving animals were observed once in the morning and again at the end of the experimental day (with the exception of Day 15 - morning only). The nature and severity of the clinical signs and time were recorded at each observation. Animals surviving treatment in the main study were observed for 14 days after dosing.

Bodyweight

The bodyweight of each rat in the main study was recorded on Days 1 (prior to dosing), 8 and 15, or at death. Individual weekly bodyweight changes and group mean bodyweights were calculated.

TERMINAL STUDIES

Termination

All animals surviving treatment in the main study were killed on Day 15 by carbon dioxide asphyxiation.

Macroscopic pathology

All animals were subjected to a macroscopic examination which consisted of opening the cranial, abdominal and thoracic cavities. The macroscopic appearance of all tissues was recorded.

STATISTICAL ANALYSIS

The acute median lethal oral dose (LD_{50}) to rats was calculated using the method of Finney [FINNEY, D.J. (1971) *Probit Analysis*, 3rd ed., Cambridge University Press, Cambridge].

ARCHIVES

All raw data and study related documents generated during the course of the study at Huntingdon, together with a copy of the final report will be lodged in the Huntingdon Life Sciences Ltd. Archive, Huntingdon.

Such records will be retained for a minimum period of five years from the date of issue of the final report. At the end of the five year retention period the client will be contacted and advice sought on the future requirements. Under no circumstances will any item be discarded without the client's knowledge.

DEVIATIONS FROM PROTOCOL

As a result of a failure in the automatic chart recorder, no readings were taken for temperature and humidity on 14 August. In addition, on Day 7 of the study only one observation was made for animals dosed at 100 mg/kg bodyweight. However, animals were not exhibiting any signs of reaction to treatment at this time this deviation was not considered to have affected the integrity or validity of the study.

RESULTS

PRELIMINARY STUDY (Tables 1 and 3)

A group of two rats (one male and one female) was dosed at 2000 mg/kg bodyweight. Both animals died within approximately 13 minutes of dosing. Clinical signs prior to death included piloerection, hunched posture and increased salivation, seen in both rats. In addition, waddling/unsteady gait, abnormal respiration, pallid extremities and prostration were also seen in the male only. Macroscopic examination of decedents revealed congestive changes in the majority of organs and tissues.

On the basis of the above results, an additional group of two rats was dosed at 200 mg/kg bodyweight. Again, both animals died within 13 minutes of dosing. Clinical signs among these animals included piloerection, hunched posture, waddling/unsteady gait, abnormal respiration and pallid extremities, seen in both rats with lethargy seen in the male only. Macroscopic examination of decedents revealed congestive changes in the majority of organs and tissues.

To provide information on a dosing regime for the main study, a final group of two rats was dosed at 20 mg/kg. There were no deaths and clinical signs among these animals were confined to piloerection only. Bodyweight gain was considered satisfactory for studies of this nature and duration and no abnormalities were revealed at the terminal necropsy on Day 8.

After review of the results from all preliminary study dosages, it was decided that 100 mg/kg bodyweight was a suitable initial dose level for the main study.

MAIN STUDY

A group of ten rats (five males and five females) was dosed at 100 mg/kg bodyweight. On the basis of results at this dosage, further groups of five males and five females were dosed at 200 and 500 mg/kg bodyweight in order to establish the acute median lethal oral dose (LD₅₀).

MORTALITY (Table 2)

One male at 100 mg/kg and all rats at 500 mg/kg died during the study. All deaths occurred within approximately 28 minutes of dosing. Macroscopic examination of these animals revealed:

Male - 100 mg/kg

Congestion (characterised by dark colouration / injected blood vessels) in the brain, liver and spleen, with clear liquid contents in the stomach.

Males and females - 500 mg/kg

Congestion (characterised by dark colouration / injected blood vessels / inflammation) in subcutaneous tissue, brain, heart, liver, spleen and kidneys, with congestion and clear liquid contents in the lungs and thoracic cavity. Congestion with clear/yellow/red fluid contents was also observed in the stomach and along the alimentary tract.

CLINICAL SIGNS (Table 3)

Clinical signs were observed in animals immediately following dosing and comprised:

Piloerection in four males and all females at 100 mg/kg, in all rats at 200 mg/kg and in one male and one female at 500 mg/kg;

hunched posture, waddling/unsteady gait and walking on toes, each in four males and all females at 100 mg/kg, in all rats at 200 mg/kg and in one male at 500 mg/kg;

lethargy in all females at 100 mg/kg, in all rats at 200 mg/kg and in two males and one female at 500 mg/kg;

abnormal respiration in one male at 200 mg/kg and in one male and one female at 500 mg/kg;

pallid extremities in four males and all females at 100 mg/kg, in all rats at 200 mg/kg and in two males and one female at 500 mg/kg;

increased salivation in one female at both 100 and 200 mg/kg bodyweight;

abnormal faeces in and ungroomed appearance, each in four males and all females at 100 mg/kg, and in all rats at 200 mg/kg;

increased sensitivity to touch in two females at 100 mg/kg and in one female at 200 mg/kg;

body tremors in one male and one female at 500 mg/kg;

clonic convulsions in one female at 500 mg/kg;

prostration (collapsed state) in two males and one female at 500 mg/kg;

blue/cold extremities in one female at 200 mg/kg;

dark colouring to eyes in all females at 100 mg/kg.

There were no other signs of reaction to treatment and recovery of surviving rats was complete by either Day 4 (females 100 mg/kg, males and females 200 mg/kg), or Day 5 (males 100 mg/kg).

BODYWEIGHT (Tables 4 and 5)

All surviving rats were considered to have achieved satisfactory bodyweight gains throughout the study.

MACROSCOPIC EXAMINATION

Macroscopic examination of surviving animals killed at study termination on Day 15 revealed inflammation and a white crystalline deposit in the stomach of one male dosed at 200 mg/kg, but otherwise no abnormalities.

ESTIMATION OF LD₅₀ VALUES

The acute median lethal oral doses (LD₅₀) and associated 95% confidence limits to male and female rats of Lithium sulphide were calculated to be:

Males only:	240 (86 to 667) mg/kg bodyweight
Females only:	317 (204 to 494) mg/kg bodyweight
Combined sexes:	274 (104 to 725) mg/kg bodyweight.

The slope of the parallel probit lines was 5.56 with a standard error of 1.58 using log transformation of dose. The heterogeneity factor was not significant.

CONCLUSION

The acute median lethal oral doses (LD_{50}) and associated 95% confidence limits to male and female rats of Lithium sulphide were calculated to be:

Males only:	240 (86 to 667) mg/kg bodyweight
Females only:	317 (204 to 494) mg/kg bodyweight
Combined sexes:	274 (104 to 725) mg/kg bodyweight.

TABLE 1

Mortality data - preliminary study

Sex	Dose (mg/kg)	Number of deaths	Day
			1
			Time after dosing
Male	20	0/1	-
Female		0/1	-
Male	200	1/1	~ 13 minutes
Female		1/1	~ 13 minutes
Male	2000	1/1	~ 13 minutes
Female		1/1	~ 13 minutes

Preliminary study investigations comprised one male and one female

TABLE 2

Mortality data - main study

Sex	Dose (mg/kg)	Number of deaths	Day					
			1					
			Time after dosing (min)					
			<5	5	8	10	15	28
Male	100	1/5	1					
Female		0/5						
Male	200	0/5						
Female		0/5						
Male	500	5/5	3			1		1
Female		5/5		4		1		

TABLE 3

Signs of reaction to treatment

Signs	No. of rats showing signs											
	Dose (mg/kg)											
	20 [#]		200 [#]		2000 [#]		100		200		500	
	M	F	M	F	M	F	M	F	M	F	M	F
Piloerection	1	1	1	1	1	1	4	5	5	5	1	1
Hunched posture	0	0	1	1	1	1	4	5	5	5	1	0
Waddling/unsteady gait	0	0	1	1	1	0	4	5	5	5	1	0
Lethargy	0	0	1	0	0	0	0	5	5	5	2	1
Abnormal respiration*	0	0	1	1	1	0	0	0	1	0	1	1
Pallid extremities	0	0	1	1	1	0	4	5	5	5	2	1
Walking on toes	0	0	0	0	0	0	4	5	5	5	1	0
Increased salivation	0	0	0	0	1	1	0	1	0	1	0	0
Abnormal faeces**	0	0	0	0	0	0	4	5	5	5	0	0
Increased sensitivity to touch	0	0	0	0	0	0	0	2	0	1	0	0
Ungroomed appearance***	0	0	0	0	0	0	4	5	5	5	0	0
Body tremors	0	0	0	0	0	0	0	0	0	0	1	1
Clonic convulsions	0	0	0	0	0	0	0	0	0	0	0	1
Prostration (collapsed state)	0	0	1	1	1	0	0	0	0	0	2	1
Blue/cold extremities	0	0	0	0	0	0	0	0	0	1	0	0
Dark colouring to eyes	0	0	0	0	0	0	0	5	0	0	0	0

Preliminary study comprised one male and one female

* Characterised by increased, decreased or gasping/noisy respiration

** Characterised by mucoïd, soft to liquid or discoloured (yellow/green/brown) faeces

*** Characterised by soiled stained fur around face/muzzle or ano/genital region or all or most of the body

TABLE 4

Individual and group mean bodyweights (g)

Dose (mg/kg)	Animal No. & Sex	Bodyweight (g) at			
		Day 1	Day 8	Day 15	Death
100	11 M	224	276	320	-
	12 M	215	-	-	215
	13 M	219	279	319	-
	14 M	219	272	316	-
	15 M	213	258	290	-
	Mean	218	271	311	
200	21 M	226	300	369	-
	22 M	218	272	323	-
	23 M	235	305	375	-
	24 M	214	279	326	-
	25 M	214	265	308	-
	Mean	221	284	340	
500	31 M	221	-	-	221
	32 M	231	-	-	231
	33 M	233	-	-	233
	34 M	227	-	-	227
	35 M	230	-	-	230
	Mean	228			
100	16 F	207	231	258	-
	17 F	216	247	266	-
	18 F	207	236	248	-
	19 F	209	244	265	-
	20 F	201	224	223	-
	Mean	208	236	252	
200	26 F	212	252	268	-
	27 F	207	229	245	-
	28 F	210	243	254	-
	29 F	203	226	239	-
	30 F	218	240	254	-
	Mean	210	238	252	
500	36 F	210	-	-	210
	37 F	210	-	-	210
	38 F	213	-	-	212
	39 F	210	-	-	210
	40 F	207	-	-	207
	Mean	210			

TABLE 5

Individual bodyweight changes (g)

Dose (mg/kg)	Animal No. & Sex	Bodyweight gains (g) at	
		Day 8	Day 15
100	11 M	52	44
	12 M	-	-
	13 M	60	40
	14 M	53	44
	15 M	45	32
200	21 M	74	69
	22 M	54	51
	23 M	70	70
	24 M	65	47
	25 M	51	43
500	31 M	-	-
	32 M	-	-
	33 M	-	-
	34 M	-	-
	35 M	-	-
100	16 F	24	27
	17 F	31	19
	18 F	29	12
	19 F	35	21
	20 F	23	(1)
200	26 F	40	16
	27 F	22	16
	28 F	33	11
	29 F	23	13
	30 F	22	14
500	36 F	-	-
	37 F	-	-
	38 F	-	-
	39 F	-	-
	40 F	-	-

() Bodyweight loss

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SODIUM SULPHIDE

ACUTE ORAL TOXICITY TO THE RAT

Report

SODIUM SULPHIDE
ACUTE ORAL TOXICITY TO THE RAT

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Research Laboratory

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Report issued 26 October 1998

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Stephen J. Mason

26 October 1998

Stephen J. Mason, B.Sc. (Hons.),
Study Director,
Huntingdon Life Sciences Ltd.

Date

QUALITY ASSURANCE STATEMENT

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

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Process Based Inspections		
Generic Standard Protocol Review	21 November 1997	24 November 1997
Husbandry	14 September 1998	17 September 1998
Housing/Environment	14 September 1998	17 September 1998
Weighing of animals	14 September 1998	17 September 1998
Treatment procedure	14 September 1998	17 September 1998
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Post mortem	14 September 1998	17 September 1998
Records audit	14 September 1998	17 September 1998
Training records	14 September 1998	17 September 1998
Report	8 October 1998	12 October 1998

Protocol: An audit of the standard protocol generated for this type of study design was conducted and reported to Company Management as indicated above.

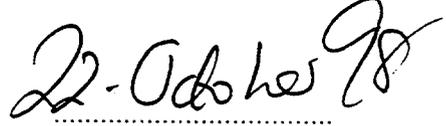
Process based inspections: At or about the time this study was in progress inspections and audits of routine and repetitive procedures employed on this type of study were carried out. These were conducted and reported to appropriate Company Management as indicated above.

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

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



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



 Date

RESPONSIBLE PERSONNEL

Stephen J. Mason, B.Sc. (Hons.),
Study Director ,
Huntingdon Life Sciences Ltd.

SUMMARY

A study was performed to assess the acute oral toxicity of Sodium sulphide to the rat. The method followed was that described in:

EPA Health Effects Testing Guidelines, Subpart B - General Toxicity Testing § 798.1175 Acute oral toxicity, September 27, 1985 (described in Federal Register Vol. 50, No. 188) and subsequent revisions. Subpart B provides detailed information relating to data requirements of 40 CFR Part 798 and supports the Toxic Substances Control Act (TSCA).

EPA Pesticide Assessment Guidelines, Subdivision F. Hazard Evaluation: Human and Domestic Animals 81-1 (Revised Edition November 1984). Subdivision F provides detailed information relating to data requirements of 40 CFR Part 158 and supports the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA).

Japanese Ministry of Agriculture, Forestry and Fisheries, Requirements for Safety Evaluation of Agricultural Chemicals and Testing Guidelines for Toxicology Studies, Acute Oral Toxicity Study, 59 NohSan No. 4200, Agricultural Production Bureau, January 28, 1985.

A group of ten fasted rats (five males and five females) received a single oral gavage dose of the test substance, formulated in corn oil and administered at a dose level of 1000 mg/kg bodyweight. This dosage was chosen on the basis of results from a preliminary study. As a result of evident toxicity at 1000 mg/kg, further groups of five males and five female were dosed at 1260 and 2000 mg/kg bodyweight in order to establish the acute median lethal oral dose (LD₅₀).

All rats at 1260 and 2000 mg/kg died during the study. All deaths occurred within approximately 20 minutes of dosing. Macroscopic examination revealed changes in the majority of organs and tissues.

Clinical signs of reaction to treatment included piloerection and pallid extremities, seen in rats at all dosages. In addition, hunched posture, waddling/unsteady gait, lethargy, abnormal respiration, partially closed eyelids, walking on toes, increased salivation, abnormal faeces, ungroomed appearance, protruding eyes, body tremors, prostration (collapsed state) and dark colouring to eyes were seen in rats at one or more dose levels. Recovery of surviving rats was complete in all instances by Day 4.

All surviving rats were considered to have achieved satisfactory bodyweight gains throughout the study.

Macroscopic examination of surviving animals killed at study termination on Day 15 revealed no abnormalities.

The acute median lethal oral doses (LD₅₀) and associated 95% confidence limits to male and female rats of Sodium sulphide were calculated to be:

Males only:	1122 (938 to 1343) mg/kg bodyweight
Females only:	1122 (938 to 1343) mg/kg bodyweight
Combined sexes:	1122 (939 to 1342) mg/kg bodyweight.

INTRODUCTION

The study was designed to assess the toxicity of Sodium sulphide following a single oral dose to the rat. The rats were dosed by oral gavage as the test substance may be ingested accidentally.

The study was conducted in compliance with:

EPA Health Effects Testing Guidelines, Subpart B - General Toxicity Testing § 798.1175 Acute oral toxicity, September 27, 1985 (described in Federal Register Vol. 50, No. 188) and subsequent revisions. Subpart B provides detailed information relating to data requirements of 40 CFR Part 798 and supports the Toxic Substances Control Act (TSCA).

EPA Pesticide Assessment Guidelines, Subdivision F. Hazard Evaluation: Human and Domestic Animals 81-1 (Revised Edition November 1984). Subdivision F provides detailed information relating to data requirements of 40 CFR Part 158 and supports the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA).

Japanese Ministry of Agriculture, Forestry and Fisheries, Requirements for Safety Evaluation of Agricultural Chemicals and Testing Guidelines for Toxicology Studies, Acute Oral Toxicity Study, 59 NohSan No. 4200, Agricultural Production Bureau, January 28, 1985.

The rat was chosen as it has been shown to be a suitable model for this type of study and is one of the animals recommended in the test guideline.

The dose levels for the study were chosen in compliance with the guideline and on the basis of results from a preliminary investigation.

The protocol was approved by Huntingdon Life Sciences Management on 5 June 1998, by the Sponsor on 9 June 1998 and by the Study Director on 18 August 1998.

The experimental phase of the study was undertaken between 18 August and 9 September 1998.

TEST SUBSTANCE

Identity:	Sodium sulphide
Intended use:	Component of pigment
Appearance:	Clear colourless solid
Storage conditions:	4°C in the dark under nitrogen
Lot number:	369673/1
Expiry:	Not advised but assumed stable for six months from date of receipt
Purity:	≥ 98%
Sample received:	29 July 1998

EXPERIMENTAL PROCEDURE

ANIMAL MANAGEMENT

Animals chosen for this study were selected from a stock supply of healthy male and female CD rats of Sprague-Dawley origin (Hsd: Sprague-Dawley(CD) obtained from Harlan U.K. Ltd, Bicester, Oxon, England.

Animals in the main study were in the weight range of 200 to 277 g and approximately eight to ten weeks of age prior to dosing (Day 1). All rats in the main study were acclimatised to the experimental environment for a minimum period of six days prior to dosing.

Rats were allocated without conscious bias to cages within the treatment groups and housed in groups of up to five rats of the same sex in metal cages with wire mesh floors in Building R14 Room 7.

A standard laboratory rodent diet (Special Diet Services RM1 (E) SQC expanded pellet) and drinking water were provided *ad libitum*. Access to food only was prevented overnight prior to and for approximately 4 hours after dosing.

The batch of diet used for the study was analysed for certain nutrients, possible contaminants and micro-organisms.

Results of routine physical and chemical examination of drinking water, as conducted by the supplier, are made available to Huntingdon Life Sciences Ltd. as quarterly summaries.

Animal room temperature was in the range 20 to 23.5°C and relative humidity was in the range 41 - 62%. Permanent daily recordings of these parameters were made and these are archived with other Department raw data. Lighting was controlled by means of a time switch to provide 12 hours of artificial light (0700 - 1900 hours) in each 24-hour period.

Each animal was identified by cage number and ear punching. Each cage was identified by a coloured label displaying the dose level, study schedule number, animal mark and the initials of the Study Director and Home Office licensee.

TEST SUBSTANCE PREPARATION

Sodium sulphide was formulated at various concentrations in corn oil and administered at a volume of 10 ml/kg bodyweight.

The test substance was prepared on the day of dosing.

The absorption of the test substance was not determined.

Characterisation of the homogeneity, stability and purity of the test substance was not undertaken in this study and remains the responsibility of the Sponsor.

TREATMENT PROCEDURE

Preliminary study

A group of two rats (one male and one female) was treated at 2000 mg/kg bodyweight. Following mortalities at this dosage, a further one male and female was dosed at 200 mg/kg to establish a dosing regime for the main study.

Main study

A group of ten rats (five males and five females) was treated at 1000 mg/kg bodyweight. Further groups of five males and five females were dosed at 1260 and 2000 mg/kg bodyweight in order to establish the acute median lethal oral dose (LD₅₀).

The treatment regime and constitution of the treatment groups are shown below:

Dates dosed	Dose (mg/kg)	Dose concentration (% w/v)	Dose volume (ml/kg)	No. of rats	
				M	F
18.08.98	2000	20	10	1	1
24.08.98	200	2	10	1	1
27.08.98	1000	10	10	5	5
02.09.98	2000	20	10	5	5
09.09.98	1260	12.6	10	5	5

In the above and following Tables, M: denotes male rats and F: denotes female rats.

Control animals

No control animals were included in this study.

ADMINISTRATION OF TEST SUBSTANCE

The appropriate dose volume of the test substance was administered to each rat by oral gavage by using a syringe and plastic catheter (8 choke).

The day of dosing was designated Day 1.

OBSERVATIONS

Mortality

Cages of rats were checked at least twice daily for mortalities.

Clinical signs

Animals were observed soon after dosing and at frequent intervals for the remainder of Day 1. On subsequent days, surviving animals were observed once in the morning and again at the end of the experimental day (with the exception of Day 15 - morning only). The nature and severity of the clinical signs and time were recorded at each observation.

Animals surviving treatment in the main study were observed for 14 days after dosing.

Bodyweight

The bodyweight of each rat in the main study was recorded on Days 1 (prior to dosing), 8 and 15, or at death. Individual weekly bodyweight changes and group mean bodyweights were calculated.

TERMINAL STUDIES

Termination

All animals surviving treatment in the main study were killed on Day 15 by carbon dioxide asphyxiation.

Macroscopic pathology

All animals were subjected to a macroscopic examination which consisted of opening the cranial, abdominal and thoracic cavities. The macroscopic appearance of all tissues was recorded.

STATISTICAL ANALYSIS

The acute median lethal oral dose (LD_{50}) to rats was calculated using the method of Finney [FINNEY, D.J. (1971) *Probit Analysis*, 3rd ed., Cambridge University Press, Cambridge].

ARCHIVES

All raw data and study related documents generated during the course of the study at Huntingdon, together with a copy of the final report will be lodged in the Huntingdon Life Sciences Ltd. Archive, Huntingdon.

Such records will be retained for a minimum period of five years from the date of issue of the final report. At the end of the five year retention period the client will be contacted and advice sought on the future requirements. Under no circumstances will any item be discarded without the client's knowledge.

DEVIATIONS FROM PROTOCOL

There were no deviations from the protocol.

RESULTS

PRELIMINARY STUDY (Tables 1 and 3)

A group of two rats (one male and one female) was dosed at 2000 mg/kg bodyweight. Both animals died within approximately 38 minutes of dosing. Clinical signs prior to death included piloerection, lethargy, abnormal respiration, pallid extremities and prostration, seen in both rats. In addition, hunched posture, waddling/unsteady gait and walking on toes were seen in the female only with partially closed eyelids and blue/cold extremities seen in the male. Macroscopic examination of decedents revealed congestive changes in the majority of organs and tissues.

On the basis of the above results, a further group of two rats (one male and one female) was dosed at 200 mg/kg bodyweight. There were no deaths. Clinical signs among these animals were confined to piloerection, hunched posture, waddling/unsteady gait and pallid extremities, seen in both rats with walking on toes, abnormal faeces and ungroomed appearance seen in the female only. Bodyweight gain was considered satisfactory for studies of this nature and duration and no macroscopic abnormalities were observed at the terminal necropsy on Day 8.

After review of the results from both preliminary study dosages, it was decided that 1000 mg/kg bodyweight was a suitable initial dose level for the main study.

MAIN STUDY

A group of ten rats (five males and five females) was dosed at 1000 mg/kg bodyweight. There were no deaths and on the basis of results at this dosage, further groups of five males and five females were dosed at 1260 and 2000 mg/kg bodyweight in order to establish the acute median lethal oral dose (LD₅₀).

MORTALITY (Table 2)

All rats at 1260 and 2000 mg/kg died during the study. All deaths occurred within approximately 20 minutes of dosing. Macroscopic examination of these animals revealed:

Males and females - 1260 mg/kg

Congestion (characterised by dark colouration / injected blood vessels / inflammation) in subcutaneous tissue, brain, heart, liver, spleen and kidneys. Congestion with clear fluid contents was noted in the lungs and thoracic cavity and congestion with yellow/green and red fluid contents was observed in the stomach and along the alimentary tract.

Males and females - 2000 mg/kg

Congestion (characterised by dark colouration / injected blood vessels / inflammation) in subcutaneous tissue, brain, heart, lungs, liver, spleen and kidneys, with liver and spleen tissue adhering to the abdominal wall. Congestion with yellow/green and red fluid contents was observed in the stomach and along the alimentary tract.

CLINICAL SIGNS (Table 3)

Clinical signs were observed in animals immediately following dosing and comprised:

Piloerection in all rats at 1000 mg/kg, one female at 1260 mg/kg and in all females at 2000 mg/kg;

hunched posture in all rats at 1000 mg/kg and in one female at 1260 mg/kg;

waddling/unsteady gait in all females at 1000 mg/kg and in one female at 1260 mg/kg;

lethargy, abnormal respiration and prostration (collapsed state), each in one male and two females at 1260 mg/kg and in four males and all females at 2000 mg/kg;

partially closed eyelids in one male at 1260 mg/kg and in one female at 2000 mg/kg;

pallid extremities in all males at 1000 mg/kg, one male and three females at 1260 mg/kg and in four males and all females at 2000 mg/kg;

walking on toes and protruding eyes, each in one female at 1260 mg/kg;

increased salivation in two males at 2000 mg/kg;

abnormal faeces in all rats at 1000 mg/kg;

ungroomed appearance in four males and one female at 1000 mg/kg;

body tremors in one male and one female at 1260 mg/kg and in two males at 2000 mg/kg;

dark colouring to eyes in three females at 1260 mg/kg and in four males and all females at 2000 mg/kg.

There were no other signs of reaction to treatment and recovery of surviving rats was complete by Day 3.

BODYWEIGHT (Tables 4 and 5)

All surviving rats were considered to have achieved satisfactory bodyweight gains throughout the study.

MACROSCOPIC EXAMINATION

Macroscopic examination of surviving animals killed at study termination on Day 15 revealed no abnormalities.

ESTIMATION OF LD₅₀ VALUES

The acute median lethal oral doses (LD₅₀) and associated 95% confidence limits to male and female rats of Sodium sulphide were calculated to be:

Males only:	1122 (938 to 1343) mg/kg bodyweight
Females only:	1122 (938 to 1343) mg/kg bodyweight
Combined sexes:	1122 (939 to 1342) mg/kg bodyweight.

The slope of the probit line was 57.3 with a standard error of 44.4 using log transformation of dose. The heterogeneity factor was not significant.

CONCLUSION

The acute median lethal oral doses (LD_{50}) and associated 95% confidence limits to male and female rats of Sodium sulphide were calculated to be:

Males only:	1122 (938 to 1343) mg/kg bodyweight
Females only:	1122 (938 to 1343) mg/kg bodyweight
Combined sexes:	1122 (939 to 1342) mg/kg bodyweight.

TABLE 1

Mortality data - preliminary study

Sex	Dose (mg/kg)	Number of deaths	Day
			1
			Time after dosing
Male	200	0/1	-
Female		0/1	-
Male	2000	1/1	~ 11 minutes
Female		1/1	~ 38 minutes

Preliminary study investigations comprised one male and one female

TABLE 2

Mortality data - main study

Sex	Dose (mg/kg)	Number of deaths	Day				
			1				
			Time after dosing (min. approx)				
			<5	5	10	15	20
Male	1000	0/5					
Female		0/5					
Male	1260	5/5	4			1	
Female		5/5	2			1	2
Male	2000	5/5	1		4		
Female		5/5			3		2

TABLE 3

Signs of reaction to treatment

Signs	No. of rats showing signs									
	Dose (mg/kg)									
	200 [#]		2000 [#]		1000		1260		2000	
	M	F	M	F	M	F	M	F	M	F
Piloerection	1	1	1	1	5	5	0	1	0	5
Hunched posture	1	1	0	1	5	5	0	1	0	0
Waddling/unsteady gait	1	1	0	1	0	5	0	1	0	0
Lethargy	0	0	1	1	0	0	1	2	4	5
Abnormal respiration*	0	0	1	1	0	0	1	2	4	5
Partially closed eyelids	0	0	1	0	0	0	1	0	0	1
Pallid extremities	1	1	1	1	5	0	1	3	4	5
Walking on toes	0	1	0	1	0	0	0	1	0	0
Increased salivation	0	0	0	0	0	0	0	0	2	0
Abnormal faeces**	0	1	0	0	5	5	0	0	0	0
Ungroomed appearance***	0	1	0	0	4	1	0	0	0	0
Protruding eyes	0	0	0	0	0	0	0	1	0	0
Body tremors	0	0	0	0	0	0	1	1	2	0
Prostration (collapsed state)	0	0	1	1	0	0	1	2	4	5
Blue/cold extremities	0	0	1	0	0	0	0	0	0	0
Dark colouring to eyes	0	0	0	0	0	0	0	3	4	5

Preliminary study comprised one male and one female

* Characterised by increased, decreased or gasping/noisy respiration

** Characterised by mucoid, soft to liquid or discoloured (yellow/brown) faeces

*** Characterised by soiled stained fur around the ano/genital region

TABLE 4

Individual and group mean bodyweights (g)

Dose (mg/kg)	Animal No. & Sex	Bodyweight (g) at			
		Day 1	Day 8	Day 15	Death
1000	11 M	221	284	330	-
	12 M	209	246	295	-
	13 M	215	276	330	-
	14 M	224	288	336	-
	15 M	205	247	285	-
	Mean	215	268	315	
1260	31 M	264	-	-	264
	32 M	263	-	-	264
	33 M	259	-	-	257
	34 M	277	-	-	277
	35 M	232	-	-	232
	Mean	259			
2000	21 M	210	-	-	210
	22 M	223	-	-	222
	23 M	220	-	-	220
	24 M	222	-	-	222
	25 M	224	-	-	224
	Mean	220			
1000	16 F	203	234	240	-
	17 F	203	227	246	-
	18 F	202	220	237	-
	19 F	205	237	248	-
	20 F	212	233	257	-
	Mean	205	230	246	
1260	36 F	221	-	-	221
	37 F	223	-	-	223
	38 F	209	-	-	209
	39 F	223	-	-	223
	40 F	226	-	-	226
	Mean	220			
2000	26 F	207	-	-	207
	27 F	205	-	-	205
	28 F	209	-	-	210
	29 F	205	-	-	206
	30 F	200	-	-	200
	Mean	205			

TABLE 5

Individual bodyweight changes (g)

Dose (mg/kg)	Animal No. & Sex	Bodyweight gains (g) at	
		Day 8	Day 15
1000	11 M	63	46
	12 M	37	49
	13 M	61	54
	14 M	64	48
	15 M	42	38
1260	31 M	-	-
	32 M	-	-
	33 M	-	-
	34 M	-	-
	35 M	-	-
2000	21 M	-	-
	22 M	-	-
	23 M	-	-
	24 M	-	-
	25 M	-	-
1000	16 F	31	6
	17 F	24	19
	18 F	18	17
	19 F	32	11
	20 F	21	24
1260	36 F	-	-
	37 F	-	-
	38 F	-	-
	39 F	-	-
	40 F	-	-
2000	26 F	-	-
	26 F	-	-
	27 F	-	-
	28 F	-	-
	30 F	-	-

CERIUM SULPHIDE
ACUTE ORAL TOXICITY TO THE RAT

Report

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COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

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

The UK Good Laboratory Practice Regulations 1997 (Statutory Instrument No 654).

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

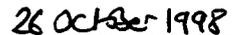
EC Council Directive 87/18/EEC of 18 December 1986 (Official Journal No L 15/29).

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

Chemical analysis of formulated test articles for determination of stability, homogeneity and concentration was not undertaken for this study.



Stephen J. Mason, B.Sc. (Hons.),
Study Director,
Huntingdon Life Sciences Ltd.



Date

QUALITY ASSURANCE STATEMENT

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

Study Phases Inspected	Date of Inspection	Date of Reporting
Process Based Inspections		
Generic Standard Protocol Review	21 November 1997	24 November 1997
Husbandry	14 September 1998	17 September 1998
Housing/Environment	14 September 1998	17 September 1998
Weighing of animals	14 September 1998	17 September 1998
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Treatment procedure	14 September 1998	17 September 1998
Post mortem	14 September 1998	17 September 1998
Report	8 October 1998	12 October 1998

Protocol: An audit of the standard protocol generated for this type of study design was conducted and reported to Company Management as indicated above.

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

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

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



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



.....
 Date

RNP 590/984377/AC

RESPONSIBLE PERSONNEL

Stephen J. Mason, B.Sc. (Hons.),
Study Director,
Huntingdon Life Sciences Ltd.

SUMMARY

This study was performed to assess the acute oral toxicity of Cerium sulphide to the rat. The method followed was that described in:

EPA Health Effects Testing Guidelines, Subpart B - General Toxicity Testing § 798.1175 Acute oral toxicity, September 27, 1985 (described in Federal Register Vol. 50, No. 188) and subsequent revisions. Subpart B provides detailed information relating to data requirements of 40 CFR Part 798 and supports the Toxic Substances Control Act (TSCA).

EPA Pesticide Assessment Guidelines, Subdivision F. Hazard Evaluation: Human and Domestic Animals 81-1 (Revised Edition November 1984). Subdivision F provides detailed information relating to data requirements of 40 CFR Part 158 and supports the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA).

Japanese Ministry of Agriculture, Forestry and Fisheries, Requirements for Safety Evaluation of Agricultural Chemicals and Testing Guidelines for Toxicology Studies, Acute Oral Toxicity Study, 59 NohSan No. 4200, Agricultural Production Bureau, January 28, 1985.

A group of ten fasted rats (five males and five females) received a single oral gavage dose of the test substance, formulated in corn oil and administered at a dose level of 5000 mg/kg bodyweight. This dosage was selected on the basis of results from a preliminary investigation. All surviving animals were killed and examined macroscopically on Day 15, the end of the observation period.

One male died within approximately 9 minutes of dosing. Macroscopic examination of this animal revealed congestive changes in some tissues and congestion and red fluid contents in the lungs. Necropsy findings were considered to indicate the death of this animal was a result of intubation error during dosing and not related to the toxicity of the test material.

Clinical signs of reaction to treatment included piloerection, hunched posture, waddling/unsteady gait, abnormal faeces, ungroomed appearance and dark colouring to eyes, seen in both males and females. In addition, lethargy, abnormal respiration, pallid extremities, walking on toes, increased sensitivity to touch, thin appearance and prostration were seen in females only, with protruding eyes noted in one male only. Recovery of surviving rats was complete in all animals by Day 8.

All animals were considered to have achieved satisfactory bodyweight gains throughout the study.

No macroscopic abnormalities were observed for animals killed at study termination on Day 15.

The acute median lethal oral dose (LD₅₀) to rats of Cerium sulphide was demonstrated to be greater than 5000 mg/kg bodyweight.

INTRODUCTION

This study was designed to assess the toxicity of the test substance following a single oral dose in the rat. The rats were dosed by oral gavage as the test substance may be ingested accidentally.

The study was conducted in compliance with:

EPA Health Effects Testing Guidelines, Subpart B - General Toxicity Testing § 798.1175 Acute oral toxicity, September 27, 1985 (described in Federal Register Vol. 50, No. 188) and subsequent revisions. Subpart B provides detailed information relating to data requirements of 40 CFR Part 798 and supports the Toxic Substances Control Act (TSCA).

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The rat was chosen as the test species as it has been shown to be a suitable model for this type of study and is one of the animals recommended by the test guidelines.

The dose level for the study was chosen in compliance with the guidelines and on the basis of a preliminary investigation.

The protocol was approved by Huntingdon Life Sciences Management on 5 June 1998, by the Sponsor on 9 June 1998 and by the Study Director on 18 August 1998.

The experimental phase of the study was conducted between 18 August and 11 September 1998.

TEST SUBSTANCE

Identity:	Cerium sulphide
Intended use:	Component of pigment
Appearance:	Dark red powder
Storage conditions:	Room temperature
Lot number:	97PLV556
Expiry:	Not advised, but assumed stable for six months from date of receipt
Purity:	>95%
Sample received:	29 July 1998

EXPERIMENTAL PROCEDURE

ANIMAL MANAGEMENT

Animals chosen for this study were selected from a stock supply of healthy male and female CD rats of Sprague-Dawley origin (Hsd:Sprague-Dawley(CD)) obtained from Harlan U.K. Ltd, Bicester, Oxon, England.

Animals in the main study were in the weight range of 222 to 273 g and approximately eight to ten weeks of age prior to dosing (Day 1). These rats were acclimatised to the experimental environment for a period of 8 days prior to the start of the study.

Rats were allocated without conscious bias to cages within the treatment group and housed in groups of five rats of the same sex in metal cages with wire mesh floors in Building R14 Room 7.

A standard laboratory rodent diet (Special Diet Services RM1(E) SQC expanded pellet) and drinking water were provided *ad libitum*. Access to food only was prevented overnight prior to and for approximately 4 hours after dosing.

The batch of diet used for the study was analysed for certain nutrients, possible contaminants and micro-organisms.

Results of routine physical and chemical examination of drinking water, as conducted by the supplier, are made available to Huntingdon Life Sciences Ltd. as quarterly summaries.

Animal room temperature was in the range 20 to 23.5°C and relative humidity was in the range 41 - 61%. Permanent daily recordings of these parameters were made and these are archived with other Department raw data. Lighting was controlled by means of a time switch to provide 12 hours of artificial light (0700 - 1900 hours) in each 24-hour period.

Each animal was identified by cage number and ear punching. Each cage was identified by a coloured label displaying the dose level, study schedule number, animal mark and the initials of the Study Director and Home Office licensee.

TEST SUBSTANCE PREPARATION

In the main study, Cerium sulphide was formulated at a concentration of 50% w/v in corn oil and administered at a volume of 10 ml/kg bodyweight.

The test substance was prepared on the day of dosing.

The absorption of the test substance was not determined.

Characterisation of the homogeneity, stability and purity of the test substance was not undertaken in this study and remains the responsibility of the Sponsor.

TREATMENT PROCEDURE

Preliminary study

In the absence of precise toxicological information, a group of two rats (one male and one female) was dosed at 2000 mg/kg bodyweight.

Main study

A group of ten rats (five males and five females) was treated at 5000 mg/kg bodyweight. This dose level was selected on the basis of results from the preliminary study and in compliance with the study guideline.

Control animals

No control animals were included in this study.

ADMINISTRATION OF THE TEST SUBSTANCE

The appropriate dose volume of the test substance was administered to each rat by oral gavage using a plastic syringe and catheter (8 choke).

The day of dosing was designated Day 1.

OBSERVATIONS

Mortality

Cages of rats were checked at least twice daily for any mortalities.

Clinical signs

Animals were observed soon after dosing and at frequent intervals for the remainder of Day 1. On subsequent days, surviving animals in the main study were observed once in the morning and again at the end of the experimental day (with the exception of Day 15 - morning only). The nature and severity of the clinical signs and time were recorded at each observation.

All surviving animals in the main study were observed for 14 days after dosing.

Bodyweight

The bodyweight of each rat was recorded on Days 1 (prior to dosing), 8 and 15, or at death. Individual weekly bodyweight changes and group mean bodyweights were calculated.

TERMINAL STUDIES

Termination

All surviving animals in the main study were killed on Day 15 by carbon dioxide asphyxiation.

CONFIDENTIAL

RNP 590/984377/AC

CERIUM SULPHIDE
ACUTE ORAL TOXICITY TO THE RAT

Sponsor

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Report issued 26 October 1998

Macroscopic pathology

All animals were subjected to a macroscopic examination which consisted of opening the cranial, thoracic and abdominal cavities. The macroscopic appearance of all examined organs was recorded.

ARCHIVES

All raw data and study related documents generated during the course of the study at Huntingdon Life Sciences Ltd, together with a copy of the final report are lodged in the Huntingdon Life Sciences Ltd Archive, Huntingdon.

Such records will be retained for a minimum period of five years from the date of issue of the final report. At the end of the five year retention period the Sponsor will be contacted and advice sought on the future requirements. Under no circumstances will any item be discarded without the Sponsor's knowledge.

DEVIATIONS FROM PROTOCOL

There were no deviations from the protocol.

RESULTS

PRELIMINARY STUDY (Table 1)

In the absence of precise toxicological information, a group of two rats (one male and one female) was dosed at 2000 mg/kg bodyweight. There were no deaths and clinical signs among these animals were confined to piloerection and hunched posture, seen in both rats with waddling/unsteady gait also seen in the female. Bodyweight gain was considered satisfactory for studies of this nature and duration, and no macroscopic abnormalities were recorded at the terminal necropsy on Day 8.

On the basis of results from this preliminary study, 5000 mg/kg was selected as a suitable dose level for the main study.

MAIN STUDY

A group of ten rats (five males and five females) received a single dose by oral gavage of Cerium sulphide, formulated at a concentration of 50% w/v in corn oil and administered at a dose level of 5000 mg/kg bodyweight.

MORTALITY (Table 1)

One male died within approximately 9 minutes of dosing. Macroscopic examination of this animal revealed congestive changes (characterised by dark tissue) in the brain, heart, liver and spleen. In addition, congestion with red fluid contents was seen in the lungs and thoracic cavity, with red fluid contents also seen in the stomach. These findings were considered to indicate the death of this animal was due to intubation error during dosing, rather than to be related to the toxicity of the test material.

CLINICAL SIGNS (Table 2)

Piloerection was observed in surviving all rats soon after dosing. This sign persisted and was accompanied in surviving male and female animals later during the study by hunched posture, waddling/unsteady gait, abnormal faeces and ungroomed appearance. In addition, dark colouring to eyes was observed in two males and two females, protruding eyes was seen in one male, with lethargy, abnormal respiration, pallid extremities, walking on toes, increased sensitivity to touch, thin appearance and prostration seen in females only. There were no other clinical signs and recovery of surviving rats was complete by Day 8.

BODYWEIGHT (Tables 3 and 4)

All surviving animals were considered to have achieved satisfactory bodyweight gains throughout the study.

MACROSCOPIC EXAMINATION

No macroscopic abnormalities were observed for animals killed at study termination on Day 15.

CONCLUSION

The acute median lethal oral dose (LD₅₀) to rats of Cerium sulphide was demonstrated to be greater than 5000 mg/kg bodyweight.

TABLE 1
Mortality data

Study	Sex	Dose (mg/kg)	Number of deaths	Day		
				1		
				Time after dosing (min)		
			<5	5	9	
Preliminary	Male	2000	0/1			
	Female		0/1			
Main	Male	5000	1/5		1	
	Female		0/5			

TABLE 2

Signs of reaction to treatment

Signs	No. of rats showing signs			
	2000 mg/kg (prelim)		5000 mg/kg (main)	
	Male	Female	Male	Female
Piloerection	1	1	4	5
Hunched posture	1	1	4	5
Waddling/unsteady gait	0	1	4	5
Lethargy	0	0	0	1
Abnormal respiration*	0	0	0	2
Pallid extremities	0	0	0	1
Walking on toes	0	0	0	3
Abnormal faeces**	0	0	4	5
Increased sensitivity to touch	0	0	0	1
Thin appearance	0	0	0	1
Ungroomed appearance***	0	0	4	5
Protruding eyes	0	0	1	0
Prostration (collapsed state)	0	0	0	1
Dark colouring to eyes	0	0	2	2

Preliminary study comprised one male and one female

Main study comprised five males and five females

* Characterised by gasping/noisy and decreased respiration

** Characterised by soft to liquid faeces

*** Characterised by soiled/stained fur over all or most of body

TABLE 3

Individual and group mean bodyweights (g)

Dose (mg/kg)	Sex	Animal Number	Bodyweight (g) at Day			
			1*	8	15	
5000	Male	11	250	-	-	
		12	257	297	328	
		13	252	284	319	
		14	245	289	323	
		15	273	324	362	
	Mean			255	299	333
	Female	16	222	253	272	
		17	215	228	243	
		18	227	243	265	
		19	229	253	272	
20		228	243	267		
Mean			224	244	264	

* prior to dosing

TABLE 4

Individual bodyweight changes (g)

Dose (mg/kg)	Sex	Animal Number	Bodyweight gains (g) at Day	
			8	15
5000	Male	11	-	-
		12	40	31
		13	32	35
		14	44	34
		15	51	38
	Female	16	31	19
		17	13	15
		18	16	22
		19	24	19
		20	15	24

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