

International Molybdenum Association

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FYI - 0598 - 1265
8496000007

28th April 1998

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98 MAY 15 AM 11:37

FYI Co-ordinator, FYI Submissions
Office of Toxic Substances (TS-778)
U.S. Environmental Protection Agency
401 M Street, SW
Washington DC 20460, USA



FYI-96-001265

Dear Sir

Testing of Molybdenum Compounds

In 1991 and 1994, this Association published reports on the acute toxicity and ecotoxicity of certain molybdenum compounds. In 1996, reports on tests for skin sensitisation of the same molybdenum compounds were sent to you and, in 1997, a report was sent to you on a further test conducted on sodium molybdate in relation to algal growth inhibition.

The Association has since conducted tests on molybdenum disulphide and the following reports are enclosed:

- skin sensitisation in the guinea-pig
- skin irritation to the rabbit
- acute dermal toxicity to the rat
- eye irritation to the rabbit
- acute oral toxicity to the rat
- acute (4 hour) inhalation study in rats

Contains No CB:

I would be grateful if you would ensure that the test results are fed into existing databases and given as wide a distribution as possible.

Yours faithfully

Michael Maby

Michael Maby
Secretary-General



8536000011

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98 MAY 21 AM 9:18

MOLYBDENUM DISULPHIDE

SKIN SENSITISATION IN THE GUINEA PIG

Report

CONFIDENTIAL

IMA 025/973001/SS

**MOLYBDENUM DISULPHIDE
SKIN SENSITISATION IN THE GUINEA-PIG**

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MPT/PPS
98 MAY 15 AM 11:38

Sponsor

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Research Laboratory

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Report issued 13 February 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.

Good Laboratory Practice, The United Kingdom Compliance Programme, Department of Health 1989, and subsequently the United Kingdom Good Laboratory Practice Regulations 1997, Statutory Instrument No. 654.

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

Good Laboratory Practice in the testing of Chemicals OECD, ISBN 92-64-12367-9, Paris 1982, subsequently republished OECD Environment Monograph No. 45, 1992.

United States Environmental Protection Agency (TSCA), Title 40 Code of Federal Regulations Part 792, Federal Register, 29 November 1983 and subsequent amendment Federal Register 17 August 1989.

Japan Ministry of International Trade and Industry, Directive 31 March 1984 (Kanpogyo No. 39 Environmental Agency, Kikyoku No. 85 MITI).

The study complied fully with OECD and EEC Good Laboratory Practice Standards. However, although procedures were inspected on this type of study during the same period and the report was reviewed by the QA Unit, no inspections were carried out on this specific study. I do not consider that this affects the integrity or validity of the study.

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

In line with normal practice in this type of short term study, the protocol did not require chemical analysis of formulated test and control articles for determination of stability, homogeneity and concentration.


.....
David G. Coleman, B.Sc. (Hons.),
Study Director,
Huntingdon Life Sciences Ltd.

13 February 1998
Date

QUALITY ASSURANCE STATEMENT

This report has been audited by Huntingdon Life Sciences Quality Assurance Department (Huntingdon). The methods, practices and procedures reported herein are an accurate description of those employed at Huntingdon Life Sciences during the course of the study. Observations and results presented in this final report form a true and accurate representation of the raw data generated during the conduct of the study at Huntingdon Life Sciences.

Certain studies such as that described in this report, are conducted at Huntingdon Life Sciences in a setting which involves frequent repetition of similar or identical procedures. At or about the time the study described in this report was in progress, 'process-based' inspections were made by the Quality Assurance Department of critical procedures relevant to this study type. The findings of these inspections were reported promptly to the Study Director and to Huntingdon Life Sciences Management.

Date(s) of inspection 29 July to 8 August 1997

Date(s) of reporting inspection findings to the Study Director and Huntingdon Life Sciences Management 13 August 1997

Date of reporting audit findings to the Study Director and Huntingdon Life Sciences Management 27 August 1997

Chris Wright
Chris Wright
Audit Team Supervisor,
Department of Quality Assurance,
Huntingdon Life Sciences Ltd.

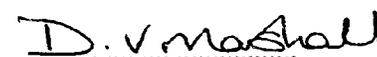
10/2/98
Date

RESPONSIBLE PERSONNEL

David G. Coleman B.Sc. (Hons.),
Study Director.
Department of Acute Toxicology



Deirdre V. Marshall, B.Sc. (Hons.), M.Sc.,
Study Supervisor,
Department of Acute Toxicology



SUMMARY

This study was performed to assess the skin sensitisation potential of Molybdenum disulphide using the guinea-pig. The method followed was that described in:

OECD Guideline for Testing of Chemicals No. 406 "Skin Sensitisation". Adopted: 17 July 1992.

MAGNUSSON, B. And KLIGMAN, A.M. (1970) *Allergic Contact Dermatitis in the Guinea-pig: Identification of contact allergens*, Thomas, C.C., Springfield, Illinois, U.S.A.

Based on the results of a preliminary study and in compliance with the guideline, the following dose levels were selected:

- Intradermal injection: 10% w/v in Alembicol D
- Topical application: 70% w/v in Alembicol D
- Challenge application: 70 and 35% w/v in Alembicol D

Ten test and five control guinea-pigs were used in this study.

In this study Molybdenum disulphide did not produce evidence of skin sensitisation (delayed contact hypersensitivity) in any of the ten test animals.

Molybdenum disulphide does not require labelling with the risk phrase R43 "May cause sensitisation by skin contact" in accordance with Commission Directive 93/21/EEC.

INTRODUCTION

This study was designed to assess the skin sensitisation potential of Molybdenum disulphide using the guinea-pig. The test substance may come into contact with skin during handling or use.

The study was conducted in compliance with OECD Guideline for Testing of Chemicals No. 406 "Skin Sensitisation". Adopted: 17 July 1992.

The method used was the guinea-pig maximisation test described by MAGNUSSON, B. And KLIGMAN, A.M. (1970) *Allergic Contact Dermatitis in the Guinea-pig: Identification of contact allergens*, Thomas, C.C., Springfield, Illinois, U.S.A.

On this occasion ten test and five control animals were used.

The albino guinea-pig was chosen as the test species as it had been shown to be a suitable model for skin sensitisation studies and is the animal recommended in the test guideline.

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

The protocol was approved by Huntingdon Life Sciences Management on 7 November 1996, by the Sponsor on 4 June 1997 and by the Study Director on 3 July 1997.

The experimental phase of the study was undertaken between 9 July and 9 August 1997.

TEST SUBSTANCE

Identity: Molybdenum disulphide

Intended use: Lubricant and Corrosion inhibitor

Appearance: Dark grey powder

Storage conditions: Room temperature

Lot number: IMOA 0597

Expiry: Not advised

Purity: >99%

Date received: 17 June 1997

EXPERIMENTAL PROCEDURE

ANIMAL MANAGEMENT

Fifteen healthy male albino guinea-pigs of the Dunkin/Hartley strain were obtained from D. Hall, Newchurch, Staffordshire, England.

The animals were in the weight range of 338 to 368 g on arrival and approximately four to seven weeks of age. All the guinea-pigs were acclimatised to the experimental environment for six days prior to the start of the main study.

Additional animals from the same supplier were used for the preliminary investigations.

The animals on the main study were allocated without conscious bias to two groups as follows:

Group	Number of animals	Animal numbers
Control animals	5	3210 to 3214
Test animals	10	3215 to 3224

The guinea-pigs were housed in groups of five in suspended metal cages with wire mesh floors in Building R17 Room 14.

A vitamin C enriched guinea-pig diet (Harlan Teklad 9600 FD2 SQC) and drinking water were provided *ad libitum*. Hay was given weekly.

The batch of diet used for the study was analysed for nutrients, possible contaminants or micro-organisms, likely to be present in the diet, and which, if in excess, may have had an undesirable effect on the test system. The certificates of analyses were lodged in Huntingdon Life Sciences Limited Archives. There were no known contaminants presented in the diet which were expected to be capable of interfering with the study outcome.

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 controlled within the range 19.5 to 25.0 °C and relative humidity within the range 45 to 66%. These environmental parameters were recorded daily. Air exchange was maintained at approximately 15 air changes per hour and lighting was controlled by means of a time switch to give 12 hours of artificial light (0700 - 1900 hours) in each 24 hours period.

Each animal was identified by ear tattoo number. This number was unique within the Huntingdon Life Sciences Acute Toxicology Department throughout the duration of the study. Each cage was identified by a coloured label displaying the study schedule number, animal numbers and the initials of the Study Director and Home Office licensee.



POSITIVE CONTROL

The sensitivity of the guinea-pig strain used is checked periodically at Huntingdon Life Sciences with known sensitisers hexyl cinnamic aldehyde (HCA), Benzocaine and 2-mercaptobenzothiazole (MBT). The results of recent tests are presented in Appendix 3.

TEST SUBSTANCE PREPARATION

The test substance was prepared prior to each application on the day of dosing in Alembicol D[#]. The concentrations used are described in the treatment procedure.

The absorption of the test substance was not determined.

The homogeneity, stability and purity of the test substance were the responsibility of the Sponsor.

[#] *A product of coconut oil, supplied by Alembic Products, Saltney, Chester, England*

TREATMENT PROCEDURE**Preliminary study**

The intradermal and topical irritancy of a range of dilutions of the test substance was investigated to identify where possible (a) concentrations of the test substance that would produce irritation suitable for the induction phase of the main study and (b) a maximum non-irritant concentration by the topical route of administration for the challenge phase.

Animals for these investigations were pre-treated with an intradermal injection of Freund's complete adjuvant, 50 : 50 with water for irrigation (Ph.Eur.), approximately two weeks prior to the start of the preliminary investigations.

The numerical values given to the dermal reactions observed in the preliminary tests are shown in Appendix 2.

Selection of concentrations of test substance for the main study

Based on the results of the preliminary investigations, the following concentrations of Molybdenum disulphide were selected:

Induction intradermal injection - 10% w/v in Alembicol D

This was the maximum practical concentration for intradermal administration and caused irritation but did not adversely affect the animals.

Induction topical application - 70% w/v in Alembicol D**Topical challenge - 70 and 35% w/v in Alembicol D**

From preliminary investigations 70% w/v in Alembicol D was the maximum practical concentration and did not give rise to irritating effects.

Main study

The procedure may be considered in two parts, Induction and Challenge.

Induction**Induction intradermal injections - test animals**

A 40 × 60 mm area of dorsal skin on the scapular region of the guinea-pig was clipped free of hair with electric clippers. Three pairs of intradermal injections were made into a 20 × 40 mm area within the clipped area as shown in Figure 1.

Injectables for the test animals were prepared as follows:

1. Freund's complete adjuvant** was diluted with an equal volume of water for irrigation (Ph.Eur.).
2. Molybdenum disulphide, 10% w/v in Alembicol D.
3. Molybdenum disulphide, 10% w/v in a 50 mm width of complete adjuvant and Alembicol D.

Induction topical application - test animals

The preliminary investigations indicated that the maximum practical concentration of the test substance for topical application (70%) did not produce skin irritation. Therefore, six days after the injections, the same 40 × 60 mm interscapular area was clipped and shaved free of hair and the site was pre-treated by gentle rubbing with 0.5 ml per site of 10% w/w sodium lauryl sulphate in petrolatum. Twenty-four hours later a 20 × 40 mm patch of Whatman No. 3 paper was saturated with approximately 0.4 ml of Molybdenum disulphide, 70% w/v in Alembicol D. The patch was placed on the skin of the test animals and covered by a length of impermeable plastic adhesive tape (50 mm width "Blenderm"). This in turn was firmly secured by elastic adhesive bandage (50 mm width "Elastoplast") wound round the torso of the animal and fixed with "Sleek" impervious plastic adhesive tape. The dressing was left in place for 48 hours.

** Difco Laboratories, Detroit, Michigan, U.S.A.

Induction - control animals

During the induction phase, the control animals were treated similarly to the test animals with the exception that the test substance was omitted from the intradermal injections and topical application.

The dermal reactions observed after each induction phase in both control and test animals by group are shown in Table 1.

Challenge**Challenge - control and test animals**

The control and test animals were challenged topically two weeks after the topical induction application using Molybdenum disulphide, 70 and 35% w/v in Alembicol D.

Hair was removed by clipping and then shaving from an area on the left flank of each guinea-pig. A 20 x 20 mm patch of Whatman No. 3 paper was saturated with approximately 0.2 ml of Molybdenum disulphide, 70% w/v in Alembicol D and applied to an anterior site on the flank. Molybdenum disulphide, 35% w/v in Alembicol D was applied in a similar manner to a posterior site. The patches were sealed to the flank for 24 hours under strips of "Blenderm" covered by "Elastoplast" wound round the trunk and secured with "Sleek".

OBSERVATIONS**Clinical signs**

All animals were observed daily for signs of ill health or toxicity.

Bodyweight

The bodyweight of each guinea-pig on the main study was recorded on Day 1 (day of intradermal injections) and on the last day observations were made of dermal responses to the challenge applications.

Dermal responses

The dermal reactions resulting from intradermal injection and topical application on the preliminary study, and topical application at the challenge were assessed using the following numerical system:

Erythema and eschar formation:

No erythema	0
Slight erythema	1
Well-defined erythema	2
Moderate erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4

Oedema formation:	
No oedema	0
Slight oedema	1
Well-defined oedema (edges of swelling defined by definite riasing)	2
Moderate oedema (raised approx. 2 to 4 mm diameter)	3
Severe oedema (raised more than 4 mm diameter and extending beyond the area of exposure)	4

The approximate diameter (mm) of the dermal response at the intradermal injection site was recorded in the preliminary study only to assist in the choice of concentrations for the main study.

Any other lesions not covered by this scoring system, was described.

The challenge sites were evaluated 24 and 48 hours after removal of the patches.

INTERPRETATION OF THE RESULTS

Dermal reactions in the test animals elicited by the challenge application were compared with the findings simultaneously obtained in the control animals.

A test animal was considered to show positive evidence of delayed contact hypersensitivity if the observed dermal reaction at challenge was definitely more marked and/or persistent than the maximum reaction seen in animals of the control group.

If the dermal reaction seen in a test animal at challenge was slightly more marked and/or persistent than (but not clearly distinguishable from) the maximum reaction seen in control animals, the result for that test animal was classified as inconclusive.

A test animal was considered to show no evidence of delayed contact hypersensitivity if the dermal reaction resulting from the challenge application was the same as, or less marked and/or persistent than the maximum reaction seen in animals of the control group.

ARCHIVES

All raw data and study related documents generated during the course of the study at Huntingdon Life Sciences, 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

The batch of diet used in this study was analysed for nutrients, possible contaminants and micro-organisms. There were no other deviations from the protocol.

RESULTS**CLINICAL SIGNS**

One animal (no. 3212) from the control group was killed for humane reasons following the induction topical application. The animal had a broken hind leg and this finding was considered to be incidental and not related to treatment.

For the remaining animals, no signs of ill health or toxicity were recorded.

BODYWEIGHT

Individual bodyweights are shown in Appendix I.

Bodyweight increases were recorded for all guinea-pigs over the period of the study.

INDUCTION

Dermal reactions seen following the induction applications are summarised in Table 1.

Intradermal injections

Necrosis was recorded at sites receiving Freund's Complete Adjuvant in test and control animals.

Slight irritation was seen in test animals at sites receiving Molybdenum disulphide, 10% w/v in Alembicol D and slight irritation was observed in control animals receiving Alembicol D.

Topical application

Slight erythema was observed in test animals following topical application with Molybdenum disulphide, 70% w/v in Alembicol D.

Slight erythema was seen in the control guinea-pigs.

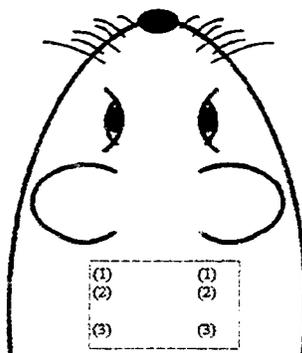
CHALLENGE

The numerical values given to the dermal reactions elicited by the challenge applications are shown in Table 2.

There were no dermal reactions seen in any of the test or control animals, therefore all ten test animals gave negative responses. Grey staining was noted on all dose sites, however, this did not interfere with scoring.

CONCLUSION

In this study, Molybdenum disulphide did not produce evidence of skin sensitisation (delayed contact hypersensitivity) in any of the ten test animals.

FIGURE 1**Position of intradermal injections and topical induction application**

The rectangle outlines the 20 × 40 mm clipped scapular area in which injections were made and to which the topical induction application was made one week later.

Control animals:

- (1) 0.1 ml of Freund's complete adjuvant 50 : 50 with water for irrigation (Ph.Eur.).
- (2) 0.1 ml of Alembicol D.
- (3) 0.1 ml of Freund's complete adjuvant 50 : 50 with Alembicol D.

Test animals:

- (1) 0.1 ml of Freund's complete adjuvant 50 : 50 with water for irrigation (Ph.Eur.).
- (2) 0.1 ml of Molybdenum disulphide, 10% w/v in Alembicol D.
- (3) 0.1 ml of Molybdenum disulphide, 10% w/v in a 50 : 50 mixture of Alembicol D and Freund's complete adjuvant.

A volume of 0.1 ml was injected into both the left and right injection sites.

TABLE 1**Dermal reactions observed after each induction**

Site	Intradermal injection		Topical application	
	Test animals	Control animals	Test animals	Control animals
1	Necrosis	Necrosis	Slight erythema*	Slight erythema
2	Slight irritation#	Slight irritation		
3	Necrosis	Necrosis		

Grey centre observed on intradermal sites 2 and 3

* Grey stain observed on topical sites 2 and 3

TABLE 2

Dermal reactions observed after the challenge application with Molybdenum disulphide

Freund's treated controls

Guinea-pig number	E = Erythema O = Oedema	Score			
		24 Hours		48 Hours	
		Ag	Pg	Ag	Pg
3210	E	0	0	0	0
	O	0	0	0	0
3211	E	0	0	0	0
	O	0	0	0	0
3212*	E				
	O				
3213	E	0	0	0	0
	O	0	0	0	0
3214	E	0	0	0	0
	O	0	0	0	0

- A Anterior site, exposed to Molybdenum disulphide, 70% w/v in Alembicol D
- P Posterior site, exposed to Molybdenum disulphide, 35% w/v in Alembicol D
- * Animal killed in extremis
- g Grey stain observed on dose sites

TABLE 2
(continued)
Test animals

Guinea-pig number	E = Erythema O = Oedema	Score				Results Positive (+) Negative (-) Inconclusive (±)
		24 Hours		48 Hours		
		Ag	Pg	Ag	Pg	
3215	E	0	0	0	0	-
	O	0	0	0	0	
3216	E	0	0	0	0	-
	O	0	0	0	0	
3217	E	0	0	0	0	-
	O	0	0	0	0	
3218	E	0	0	0	0	-
	O	0	0	0	0	
3219	E	0	0	0	0	-
	O	0	0	0	0	
3220	E	0	0	0	0	-
	O	0	0	0	0	
3221	E	0	0	0	0	-
	O	0	0	0	0	
3222	E	0	0	0	0	-
	O	0	0	0	0	
3223	E	0	0	0	0	-
	O	0	0	0	0	
3224	E	0	0	0	0	-
	O	0	0	0	0	

A Anterior site, exposed to Molybdenum disulphide, 70% w/v in Alembicol D
P Posterior site, exposed to Molybdenum disulphide, 35% w/v in Alembicol D
g Grey stain observed on dose sites

APPENDIX 1

Individual bodyweights (g)

Group	Guinea-pig number	Day 1 16 July 1997	Last observation day 9 August 1997
Control	3210	425	710
	3211	421	616
	3212	*	*
	3213	428	696
	3214	433	699
Test	3215	431	697
	3216	396	585
	3217	434	698
	3218	425	667
	3219	411	568
	3220	417	617
	3221	422	632
	3222	421	630
	3223	436	724
	3224	407	574

* Animal killed in extremis

APPENDIX 2

Results of preliminary investigations with Molybdenum disulphide

Intradermal injections

Vehicle: Alembicol D

Guinea-pig number	Concentration % w/v	Score		
		Hours	24	72
24	10.0	D	10	10
		E	Gc	Gc
		O	2	2
	7.5	D	10	10
		E	Gc	Gc
		O	2	2
	5.0	D	8	8
		E	Gc	Gc
		O	2	2
	2.5	D	6	6
		E	Gc	Gc
		O	2	2
1.0	D	6	6	
	E	Gc	Gc	
	O	2	2	
0.5	D	5	4	
	E	1	1	
	O	1	1	
0.25	D	5	4	
	E	1	1	
	O	1	1	
0.1	D	4	3	
	E	1	1	
	O	1	1	
Vehicle control	D	2	2	
	E	1	1	
	O	1	1	

Guinea-pig number	Concentration % w/v	Score		
		Hours	24	72
25	10.0	D	10	10
		E	Gc	Gc
		O	2	2
	7.5	D	10	10
		E	Gc	Gc
		O	2	2
	5.0	D	8	8
		E	Gc	Gc
		O	2	2
	2.5	D	6	6
		E	Gc	Gc
		O	2	2
1.0	D	6	6	
	E	Gc	Gc	
	O	2	2	
0.5	D	5	4	
	E	1	1	
	O	1	1	
0.25	D	5	5	
	E	1	1	
	O	1	1	
0.1	D	4	4	
	E	1	1	
	O	1	1	
Vehicle control	D	2	2	
	E	1	1	
	O	1	1	

Key:

- D Diameter (mm)
- E Erythema (0 - 4 numerical scores)
- O Oedema (0 - 4 numerical scores)
- Gc Grey centre observed on intradermal sites

APPENDIX 2

(continued)

Topical application:

Vehicle: Alembicol D

Guinea-pig number	Concentration % w/v	Score					
		0 Hours		24 Hours		48 Hours	
		Eg	Og	Eg	Og	Eg	Og
26	70	0	0	0	0	0	0
	50	0	0	0	0	0	0
	40	0	0	0	0	0	0
	20	0	0	0	0	0	0
27	70	0	0	0	0	0	0
	50	0	0	0	0	0	0
	40	0	0	0	0	0	0
	20	0	0	0	0	0	0
28	70	0	0	0	0	0	0
	50	0	0	0	0	0	0
	40	0	0	0	0	0	0
	20	0	0	0	0	0	0
29	70	0	0	0	0	0	0
	50	0	0	0	0	0	0
	40	0	0	0	0	0	0
	20	0	0	0	0	0	0

- E Erythema (0 - 4 numerical scores)
- O Oedema (0 - 4 numerical scores)
- g Grey stain observed on dose sites - all sites washed with distilled water at zero hours

APPENDIX 3

**Summary of positive control data
Magnusson and Kligman test method**

Schedule number	Number of animals		Dates of study		Test Substance	Dose levels % v/v (MBT %w/v)			Results		
						Induction		Challenge	Positive	Inconclusive	Negative
	Test	Control	Start	Finish		Intradermal	Topical				
XXX/1	10M	10M	13.3.96	10.4.96	HCA	10	As supplied	As supplied and 50	10/10	0/10	0/10
XXX/5	10M	10M	29.10.96	22.11.96	MBT	10	83.33	83.33 and 40	10/10	0/10	0/10
XXX/8	10M	10M	08.04.97	02.05.97	HCA	10	As supplied	As supplied and 50	10/10	0/10	0/10
XXX/10	10M	5M	04.06.97	29.06.97	MBT	10	83.33	83.33 and 40	9/10	1/10	0/10

Animals supplied by D. Hall, Newchurch, Staffordshire, England

Hexyl cinnamic aldehyde (HCA) obtained from Aldrich Chemicals Co., England

Benzocaine and mercaptobenzothiazole (MBT) obtained from SIGMA Chemicals, St Louis, USA

APPENDIX 4

Certificate of analysis

Werkzeugnis 2.2
 Test Report 2.2
 gemäß/per EN 10204
 Datum/Date 16.06.97/AS AZWI2202

Kunde/Customer
 IMO A

Produkt/Product
 Molybdenumdisulphide

Ihre Bestell-Nr vom/Your Order No. Dated
 IMO A Enquiry No. 14189

Unsere Auftrags-Nr./Our Order No.

Liefermenge/Quantity Delivered
 1.5 kg

Lot-Nr./Lot No.
 IMO A 0597

Analyseergebnisse/Analytical Results

Analyseergebnisse nach vorschriftsmäßiger Probenahme und Prüfung/Analytical results by specified sampling and testing

CHEMICAL ANALYSIS

Fe	0,03	%
Cu	0,005	%
Pb	0,008	%
Si	< 0,001	%
MoO ₃	< 0,01	%
Water	< 0,01	%
Oil	0,03	%

PHYSICAL ANALYSIS

FSSS 5,1 %

Particle Size Distribution
 (Sympatec HELOS)

X ₁₀	1,23	µm
X ₅₀	18,71	µm
X ₉₀	53,71	µm

Bemerkungen/Remarks

D 01

Huntingdon
Life Sciences

MOLYBDENUM DISULFIDE
SKIN IRRITATION TO THE RABBIT

Report

D 02

CONFIDENTIAL

IMA 023/973124/SE

**MOLYBDENUM DISULPHIDE
SKIN IRRITATION TO THE RABBIT**

RECEIVED
15 MAY 1998
11:30 AM

Sponsor

International Molybdenum Association
Unit 7
Hackford Walk
119-123 Hackford Road
London SW9 0QT
ENGLAND

Research Laboratory

Huntingdon Life Sciences Ltd
P.O. Box 2
Huntingdon
Cambridgeshire
PE18 6ES
ENGLAND

Report issued 13 February 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, with the exception noted below, and I consider the data generated to be valid.

United States Environmental Protection Agency, (TSCA), Title 40 Code of Federal Regulations Part 792, Federal Register, 29 November 1983, and subsequent amendment Federal Register 17 August, 1989.

Japanese Ministry of International Trade and Industry, Directive 31 March 1984 (Kanpogyo No.39 Environmental Agency, Kikyoku No. 85 MITI).

Good Laboratory Practice in the testing of Chemicals OECD, ISBN 92-64-12367-9, Paris 1982, subsequently republished OECD Environment Monograph No. 45, 1992.

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

EC Council Directive, 87/18 EEC of 18 December 1986, (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.

The study complied fully with OECD and EEC Good Laboratory Practice standards. However, although procedures were inspected on this type of study during the same period and the report was reviewed by the QA Unit, no inspections were carried out on this specific study. I do not consider that this affects the integrity or validity of the study.



Brenda I. Parcell, M.I.A.T.,
Study Director,
Huntingdon Life Sciences Ltd.

13.2.98

Date

QUALITY ASSURANCE STATEMENT

This report has been audited by Huntingdon Life Sciences Quality Assurance Department (Huntingdon). The methods, practices and procedures reported herein are an accurate description of those employed at Huntingdon Life Sciences during the course of the study. Observations and results presented in this final report form a true and accurate representation of the raw data generated during the conduct of the study at Huntingdon Life Sciences .

Certain studies such as that described in this report, are conducted at Huntingdon Life Sciences in a setting which involves frequent repetition of similar or identical procedures. At or about the time the study described in this report was in progress, 'process-based' inspections were made by the Quality Assurance Department of critical procedures relevant to this study type. The findings of these inspections were reported promptly to the Study Director and to Huntingdon Life Sciences Management.

Date(s) of inspection

2 - 13 June 1997

Date(s) of reporting inspection findings to the Study Director and Huntingdon Life Sciences Management

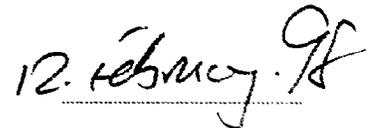
16 June 1997

Date of reporting audit findings to the Study Director and Huntingdon Life Sciences Management

11 August 1997



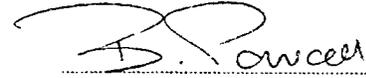
Kevin P. de-Salis, B.A. (Hons.), C.Biol., M.I.Biol., Dip.R.Q.A.,
Quality Assurance Unit Head,
Department of Quality Assurance,
Huntingdon Life Sciences Ltd.



Date

RESPONSIBLE PERSONNEL

Brenda I. Parcell, M.I.A.T.,
Study Director,
Department of Acute Toxicology.



B. I. Parcell

SUMMARY

A study was performed to assess the skin irritation potential of Molybdenum disulphide to the rabbit. The method followed was that described in OECD Guideline for Testing of Chemicals No. 404 "Acute Dermal Irritation/Corrosion". Adopted: 17 July 1992.

Three rabbits were each administered a single dermal dose of 0.5 g of the test substance and observed for four days.

No dermal reactions were observed following a single semi-occlusive application of Molybdenum disulphide to intact rabbit skin for four hours.

Molybdenum disulphide will not require labelling with the risk phrase R38 "Irritating to skin", in accordance with Commission Directive 93/21/EEC.

INTRODUCTION

The study was designed to assess skin irritation potential of Molybdenum disulphide following a single dermal application to rabbits. The test substance may come into contact with skin during handling or use.

The study was conducted in compliance with OECD Guideline for Testing of Chemicals No. 404 "Acute Dermal Irritation/Corrosion". Adopted: 17 July 1992.

The albino rabbit was chosen as it has been shown to be a suitable model for skin irritation studies and is the animal recommended in the test guideline.

The amount of test substance administered was chosen in compliance with the guideline.

The protocol was approved by Huntingdon Life Sciences Management on 7 November 1996, by the Sponsor on 4 June 1997 and by the Study Director on 30 June 1997.

The experimental phase of the study was undertaken between 8 and 11 July 1997.

TEST SUBSTANCE

Identity: Molybdenum disulphide

Intended use: Lubricant and Corrosion Inhibitor

Appearance: Dark grey powder

Storage conditions: Room temperature

Lot number: IMOA 0597

Expiry: Not advised

Purity: > 99%

Date received: 17 June 1997

EXPERIMENTAL PROCEDURE

ANIMAL MANAGEMENT

Three healthy adult rabbits of the New Zealand White strain were obtained from Harlan Interfauna (UK) Ltd., Huntingdon, Cambs., England.

They were in the weight range of 2.6 to 3.1 kg and approximately 11 to 13 weeks of age, prior to treatment (Day 1). All rabbits were acclimatised to the experimental environment.

The rabbits were selected without conscious bias for the study. They were housed individually in stainless steel cages with perforated floors in Building R14 Room 5.

A standard laboratory diet SDS Stanrab (P) Rabbit Diet and drinking water were provided *ad libitum*.

The batch of diet used for the study was analysed for nutrients, 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 maintained at 16 to 26°C and relative humidity at 53 - 74%. These environmental parameters were recorded daily. Air exchange was maintained at approximately 19 air changes per hour and lighting was controlled by means of a time switch to give 12 hours of artificial light (0700 - 1900 hours) in each 24 hours period.

Each animal was identified by a numbered aluminium tag placed through the edge of one ear. This number was unique within the Huntingdon Life Sciences Acute Toxicology Department throughout the duration of the study. Each cage was identified by a coloured label displaying the study schedule number, animal number and initials of the Study Director and Home Office licensee.

TEST SUBSTANCE PREPARATION

Molybdenum disulphide was administered as supplied by the Sponsor.

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

Approximately 24 hours prior to application of the test substance, hair was removed with electric clippers from the dorso-lumbar region of each rabbit exposing an area of skin approximately 100 mm x 100 mm.

Approximately 0.5 g of the test substance was applied under a 25 mm x 25 mm gauze pad which was then moistened with 0.5 ml distilled water, to one intact skin site on each animal.

Each treatment site was covered with "Elastoplast" elastic adhesive dressing for four hours. The animals were not restrained during the exposure period and were returned to their cages immediately after treatment.

At the end of the exposure period, the semi-occlusive dressing and gauze pad were removed and the treatment site was washed with warm water (30° to 40°C) to remove any residual test substance. The treated area was blotted dry with absorbent paper.

OBSERVATIONS

Clinical signs

All animals were observed daily for signs of ill health or toxicity.

Dermal responses

Examination of the treated skin was made on Day 1 (*ie* approximately 60 minutes after removal of the dressings) and on Days 2, 3 and 4 (equivalent to 24, 48 and 72 hours after exposure).

Local dermal irritation was assessed using the prescribed numerical system:

Erythema and eschar formation:

No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth) preventing erythema reading	4

Oedema formation:

No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre and extending beyond the area of exposure)	4

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 that were considered to have affected the integrity or validity of the study. However, the following deviations were noted:

The upper value for humidity recorded was 74%. This exceeded the expected high of 70% stated in the protocol.

The diet was analysed for nutrients, possible contaminants or micro-organisms. This was at variance with the protocol that stated that it would not be analysed.

There were no other deviations from the protocol.

RESULTS

CLINICAL SIGNS

There were no signs of toxicity or ill health in any rabbit during the observation period.

DERMAL RESPONSES

The numerical values given to the dermal reactions elicited by Molybdenum disulphide are shown in Table I.

No dermal reaction to treatment was observed in any animal throughout the study.

CONCLUSION

A single semi-occlusive application of Molybdenum disulphide to intact rabbit skin for four hours elicited no dermal irritation.

TABLE 1
Dermal reactions

Rabbit no. & sex	E = Erythema O = Oedema	Day			
		1*	2	3	4
1941 Female	E	0	0	0	0
	O	0	0	0	0
1942 Female	E	0	0	0	0
	O	0	0	0	0
1943 Female	E	0	0	0	0
	O	0	0	0	0

* Approximately 60 minutes after removal of the dressing

APPENDIX 1

Certificate of analysis

Werkszeugnis 2.2
Test Report 2.2
gemäß/per EN 10204

Datum/Date 16.06.97/AS AZWI2202

Kunde/Customer
IMOA

Produkt/Product
Molybdenumdisulfide

Ihre Bestell-Nr. vom/Your Order No. Dated
IMOA Enquiry No. 14189

Unsere Auftrags-Nr./Our Order No.

Liefermenge/Quantity Delivered
1,5 kg

Lot-Nr./Lot No.
IMOA 0597

Analysenergebnisse/Analytical Results

Analysenergebnisse nach vorschriftsmäßiger Probenahme und Prüfung/Analytical results by specified sampling and testing

CHEMICAL ANALYSIS

Fe	0,03	%
Cu	0,005	%
Pb	0,008	%
Si	< 0,001	%
MoO ₃	< 0,01	%
Water	< 0,01	%
Oil	0,03	%

PHYSICAL ANALYSIS

FSSS 5,1 µm

Particle Size Distribution:
(Sympatec HELOS)

X ₁₀	1,22	µm
X ₅₀	18,73	µm
X ₉₀	53,54	µm

Bemerkungen/Remarks

E 02

MOYBDENUM DISULPHIDE
ACUTE DERMAL TOXICITY TO THE RAT

Report

CONFIDENTIAL

IMA 022/973192/AC

RECEIVED
DEPT. OF
98 MAY 15 AM 11:38

**MOLYBDENUM DISULPHIDE
ACUTE DERMAL TOXICITY TO THE RAT**

Sponsor

International Molybdenum Association
Unit 7
Hackford Walk
119 - 123 Hackford Road
London SW9 0QT
ENGLAND

Research Laboratory

Huntingdon Life Sciences Ltd
PO Box 2
Huntingdon
Cambridgeshire
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ENGLAND

Report issued 13 February 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.

United States Environmental Protection Agency, (TSCA), Title 40 Code of Federal Regulations Part 792, Federal Register, 29 November 1983 and subsequent amendment Federal Register 17 August, 1989.

Good Laboratory Practice in the testing of Chemicals OECD, ISBN 92-64-12367-9, Paris 1982, subsequently republished OECD Environment Monograph No. 45, 1992.

Japan Ministry of International Trade and Industry, Directive 31 March 1984 (Kanpogyo No. 39 Environmental Agency, Kikyoku No. 85 MITI).

UK Good Laboratory Practice Regulations 1997, Statutory Instrument No. 654.

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

Information regarding test substance characterisation, namely expiry, was not made available to Huntingdon Life Sciences as required for compliance with 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.

The study complied fully with OECD and EEC Good Laboratory Practice standards. However, although procedures were inspected on this type of study during the same period and the report was reviewed by the QA Unit, no inspections were carried out on this specific study. I do not consider that this affects the integrity or validity of the study



.....
Lewis A. McRae, H.N.C., M.I.A.T., M.I.Sc.T.,
Study Director,
Huntingdon Life Sciences Ltd.

13 February 1998

Date

QUALITY ASSURANCE STATEMENT

This report has been audited by Huntingdon Life Sciences Quality Assurance Department (Huntingdon). The methods, practices and procedures reported herein are an accurate description of those employed at Huntingdon Life Sciences during the course of the study. Observations and results presented in this final report form a true and accurate representation of the raw data generated during the conduct of the study at Huntingdon Life Sciences.

Certain studies such as that described in this report, are conducted at Huntingdon Life Sciences in a setting which involves frequent repetition of similar or identical procedures. At or about the time the study described in this report was in progress, 'process-based' inspections were made by the Quality Assurance Department of critical procedures relevant to this study type. The findings of these inspections were reported promptly to the Study Director and to Huntingdon Life Sciences Management.

Date(s) of inspection 30 July to 1 August 1997

Date(s) of reporting inspection findings to the Study Director and Huntingdon Life Sciences Management 4 August 1997

Date of reporting audit findings to the Study Director and Huntingdon Life Sciences Management 19 August 1997

Chris Wright
Chris Wright,
Audit Team Supervisor,
Department of Quality Assurance,
Huntingdon Life Sciences Ltd.

19/2/98
Date

RESPONSIBLE PERSONNEL

Lewis A. McRae, H.N.C., M.I.A.T., M.I.Sc.T.,
Study Director,
Department of Acute Toxicology.



A handwritten signature in black ink, appearing to read 'L. McRae', is written over a horizontal dotted line.

SUMMARY

A study was performed to assess the acute dermal toxicity of Molybdenum Disulphide to the rat. The method followed was that described in the OECD Guideline for Testing of Chemicals No. 402 "Acute Dermal Toxicity". Adopted: 24 February 1987.

A group of ten rats (five males and five females) received a single topical application of the test substance, formulated at a maximum practical concentration of 100% in 1% w/v aqueous methylcellulose, and administered at a dose level of 2000 mg/kg bodyweight

There was no systemic response in any animal throughout the study.

Slight to well-defined dermal irritation (Grade 1 or 2 for erythema and oedema) was observed in four rats following removal of the dressings, resolving completely by Day 4. No dermal reactions were noted for the remaining six animals.

Notably low bodyweight gains were evident in two females (Nos 7 and 8) on Day 8. All other rats were considered to have achieved satisfactory bodyweight gains throughout the study.

All animals were killed and examined macroscopically on Day 15, the end of the observation period. This examination revealed no abnormalities.

The acute lethal dermal dose to rats of Molybdenum Disulphide was demonstrated to be greater than 2000 mg/kg bodyweight.

Molybdenum disulphide will not require labelling with the risk phase R21 "Harmful in contact with skin", in accordance with Commission Directive 93/21/EEC.

INTRODUCTION

The study was designed to assess the toxicity of Molybdenum Disulphide following a single dermal dose to the rat. The rats were dosed by topical application as the test substance may come in contact with the skin during handling or use.

The study was conducted in compliance with the OECD Guideline for Testing of Chemicals No. 402 "Acute Dermal Toxicity". Adopted: 24 February 1987.

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

The dose level for the study was chosen in compliance with the study guideline.

The protocol was approved by Huntingdon Life Sciences Management on 7 November 1996, by the Sponsor on 4 June 1997 and by the Study Director on 4 July 1997.

The experimental phase of the study was undertaken between 7 and 21 July 1997.

TEST SUBSTANCE

Identity:	Molybdenum disulphide
Intended use:	Lubricant and Corrosion Inhibitor
Appearance:	Dark grey powder
Storage conditions:	Room temperature
Lot number:	IMOA 0597
Expiry:	Not advised
Purity:	> 99%
Date received:	17 June 1997

EXPERIMENTAL PROCEDURE

ANIMAL MANAGEMENT

The 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.

They were in the weight range of 238 to 286 g and approximately eight to 11 weeks of age prior to dosing (Day 1). All the rats were acclimatised to the experimental environment for a period of 11 days prior to the start of the study.

Rats were allocated without conscious bias to cages within the treatment group. They were housed individually in metal cages with wire mesh floors in Building R14 Room 6.

A standard laboratory rodent diet (Special Diet Services RM1(E) SQC expanded pellet) and drinking water were provided *ad libitum*.

Each 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 25°C and relative humidity was in the range 40 - 63%. Permanent daily recordings of these parameters were made and these are archived with other Department raw data. Air exchange was maintained at 10 to 15 air changes per hour and lighting 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

Molybdenum Disulphide was formulated at a maximum practical concentration of 100% w/v in 1% w/v aqueous methylcellulose and administered at a volume of 2.0 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 were not undertaken in this study and remained the responsibility of the Sponsor.

ADMINISTRATION OF TEST SUBSTANCE

A group of ten rats (five males and five females) was treated at 2000 mg/kg bodyweight.

One day prior to treatment, hair was removed from the dorso-lumbar region of each rat with electric clippers taking care to avoid damaging the skin, exposing an area equivalent to approximately 10% of the total body surface area.

The test substance was applied by spreading it evenly over the prepared skin. The treatment area (approximately 50 mm x 50 mm) was covered with porous gauze held in place with a non irritating dressing, and further covered by a waterproof dressing encircled firmly around the trunk of the animal.

Treatment in this manner was performed on Day 1 (day of dosing) of the study only.

At the end of the 24 hours exposure period the dressings was carefully removed and the treated area of skin was washed with warm water (30° to 40°C) to remove any residual test substance. The treated area was blotted dry with absorbent paper.

Control animals

No control animals were included in this study.

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 (a period of five hours). On subsequent days 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.

All animals were observed for 14 days after dosing.

Dermal responses

Local dermal irritation at the treatment site was assessed daily using the following numerical scoring system:

Erythema and eschar formation:

No erythema	0
Slight erythema	1
Well defined erythema	2
Moderate erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4

Oedema formation:

No oedema	0
Slight oedema	1
Well-defined oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre and extending beyond the area of exposure)	4

Bodyweight

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

TERMINAL STUDIES**Termination**

All animals were killed on Day 15 by carbon dioxide asphyxiation.

Macroscopic pathology

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

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**MAIN STUDY**

There were no deaths and no evidence of a systemic response in any animal throughout the study following a single dermal application of Molybdenum Disulphide to a group of ten rats (five males and five females) at a dose level of 2000 mg/kg bodyweight.

DERMAL RESPONSES (Table 1)

Slight to well-defined dermal irritation (Grade 1 or 2 for erythema and oedema) was observed in one male and three females following removal of the dressings, resolving completely by Day 4. No dermal reactions were noted for the remaining six animals.

BODYWEIGHT (Tables 2 and 3)

Notably low bodyweight gains were evident in two females (Nos 7 and 8) on Day 8. All other rats were considered to have achieved satisfactory bodyweight gains throughout the study.

MACROSCOPIC EXAMINATION

No abnormalities were recorded at the macroscopic examination on Day 15.

CONCLUSION

The acute lethal dermal dose to rats of Molybdenum Disulphide was demonstrated to be greater than 2000 mg/kg bodyweight.

TABLE I
Dermal reactions

Dose (mg/kg)	Sex	Animal No.	E=Erythema O=Oedema	Days after dosing		
				2	3	4 to 15
2000	Male	1	E	0	0	0
			O	0	0	0
		2	E	0	0	0
			O	0	0	0
		3	E	0	0	0
	O		0	0	0	
	4	E	1	0	0	
		O	0	0	0	
	5	E	0	0	0	
		O	0	0	0	
Female	6	E	1	0	0	
		O	0	0	0	
	7	E	1	0	0	
		O	0	0	0	
	8	E	0	0	0	
O		0	0	0		
9	E	0	0	0		
	O	0	0	0		
10	E	1	1	0		
	O	2	0	0		

TABLE 2
Individual and group mean bodyweights (g)

Dose (mg/kg)	Sex	Animal Number	Bodyweight (g) at Day		
			1	8	15
2000	Male	1	271	313	359
		2	265	315	359
		3	286	326	374
		4	272	314	357
		5	272	301	339
		Mean	273	314	358
	Female	6	244	257	277
		7	256	258	282
		8	239	244	257
		9	239	270	303
10		238	253	268	
	Mean	243	256	277	

TABLE 3
Individual bodyweight changes (g)

Dose (mg/kg)	Sex	Animal Number	Bodyweight changes at Day (g)	
			8	15
2000	Male	1	42	46
		2	50	44
		3	40	48
		4	42	43
		5	29	38
	Female	6	13	20
		7	2	24
		8	5	13
		9	31	33
		10	15	15

APPENDIX 1

Certificate of analysis

Werkszeugnis 2.2
Test Report 2.2
gemäß/per EN 10204

Datum/Date 16.06.97/AS AZWI2202

Kunde/Customor
IMO A

Produkt/Product
Molybdenumdisulphide

Ihre Bestell-Nr. vom/Your Order No. Dated
IMO A Enquiry No. 14189

Unsere Auftrags-Nr./Our Order No.
-

Liefermenge/Quantity Delivered
1,5 kg

Lot-Nr./Lot No.
IMO A 0597

Analysenergebnisse/Analytical Results

Analysenergebnisse nach vorschriftsmäßiger Probenahme und Prüfung/Analytical results by specified sampling and testing

CHEMICAL ANALYSIS

Fe	0,03	%
Cu	0,005	%
Pb	0,008	%
Si	< 0,001	%
MoO ₃	< 0,01	%
Water	< 0,01	%
Oil	0,03	%

PHYSICAL ANALYSIS

FSSS 5,1 µm

Particle Size Distribution:
(Sympatec HELOS)

X ₁₀	1,22	µm
X ₅₀	13,73	µm
X ₉₀	53,54	µm

Bemerkungen/Remarks

MOLYBDENUM DISULPHIDE
EYE IRRITATION TO THE RABBIT

Report

CONFIDENTIAL

IMA 024/973189/SE

MOLYBDENUM DISULPHIDE
EYE IRRITATION TO THE RABBIT

RECEIVED
OFFICE
98 MAY 15 AM 11:38

Sponsor

International Molybdenum Association
Unit 7
Hackford Walk
119 - 123 Hackford Road
London SW9 0QT
ENGLAND

Research Laboratory

Huntingdon Life Sciences Ltd
P.O. Box 2
Huntingdon
Cambridgeshire
PE18 6ES
ENGLAND

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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 United Kingdom Good Laboratory Practice Regulations 1997, Statutory Instrument No. 654.

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

Good Laboratory Practice in the testing of Chemicals OECD, ISBN 92-64-12367-9, Paris 1982, subsequently republished OECD Environment Monograph No. 45, 1992.

United-States Environmental Protection Agency, (TSCA), Title 40 Code of Federal Regulations Part 792, Federal Register, 29 November 1983 and subsequent amendment Federal Register 17 August, 1989.

Japan Ministry of International Trade and Industry, Directive 31 March 1984 (Kanpogyo No. 39 Environmental Agency, Kikyoku No. 85 MITI).

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.

The study complied fully with OECD and EEC Good Laboratory Practice standards. However, although procedures were inspected on this type of study during the same period and the report was reviewed by the QA Unit, no inspections were carried out on this specific study. I do not consider that this affects the integrity or validity of the study.



Brenda I. Parcell, M.I.A.T.,
Study Director,
Huntingdon Life Sciences Ltd.

13.2.98

Date

QUALITY ASSURANCE STATEMENT

This report has been audited by Huntingdon Life Sciences Quality Assurance Department (Huntingdon). The methods, practices and procedures reported herein are an accurate description of those employed at Huntingdon Life Sciences during the course of the study. Observations and results presented in this final report form a true and accurate representation of the raw data generated during the conduct of the study at Huntingdon Life Sciences.

Certain studies such as that described in this report, are conducted at Huntingdon Life Sciences in a setting which involves frequent repetition of similar or identical procedures. At or about the time the study described in this report was in progress, 'process-based' inspections were made by the Quality Assurance Department of critical procedures relevant to this study type. The findings of these inspections were reported promptly to the Study Director and to Huntingdon Life Sciences Management.-

Date(s) of inspection 2 - 13 June 1997

Date(s) of reporting inspection findings
to the Study Director and Huntingdon Life Sciences Management 16 June 1997

Date of reporting audit findings to the
Study Director and Huntingdon Life Sciences Management 1 August 1997


.....


.....

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

A 06

IMA 024/973189/SE

RESPONSIBLE PERSONNEL

Brenda I. Parcell, M.I.A.T.,
Study Director,
Department of Acute Toxicology.



SUMMARY

A study was performed to assess the eye irritation potential of Molybdenum disulphide to the rabbit. The method followed was that described in the OECD Guideline for Testing of Chemicals No. 405, "Acute Eye Irritation/Corrosion", Adopted: 24 February 1987.

As the weight of a volume of 0.1 ml test material exceeded 100 mg, in compliance with the study guideline approximately 100 mg of Molybdenum disulphide was instilled into the eyes of three rabbits. The animals were observed for three days after treatment.

A single instillation of Molybdenum disulphide into the eye of the rabbit elicited transient very slight conjunctival irritation only.

Molybdenum disulphide will not require labelling with the risk phrase R36 "Irritating to eyes", in accordance with Commission Directive 93/21/EEC.

INTRODUCTION

The study was designed to assess eye irritation potential of Molybdenum disulphide following a single instillation into the eye of the rabbit. The test substance may come into contact with the eye during handling or use.

The study was conducted in compliance with the OECD Guideline for Testing of Chemicals No. 405, "Acute Eye Irritation/Corrosion", Adopted: 24 February 1987.

The albino rabbit was chosen as it has been shown to be a suitable model for eye irritation studies and is the animal recommended in the test guideline.

The amount of test substance instilled was chosen in compliance with the guideline.

The protocol was approved by Huntingdon Life Sciences Management on 7 November 1996, by the Sponsor on 4 June 1997 and by the Study Director on 3 July 1997.

The experimental phase of the study was undertaken between 14 and 19 July 1997.

A 09

IMA 024/973189/SE

TEST SUBSTANCE

Identity:	Molybdenum disulphide
Intended use:	Lubricant and Corrosion Inhibitor
Appearance:	Dark grey powder
Storage conditions:	Room temperature
Lot number:	IMOA 0597
Expiry:	Not advised
Purity:	> 99%
Date received:	17 June 1997

EXPERIMENTAL PROCEDURE

ANIMAL MANAGEMENT

Three healthy adult rabbits of the New Zealand White strain were obtained from Harlan Interfauna (UK) Ltd, Huntingdon, Cambridgeshire, England.

The animals were in the weight range of 2.5 to 2.9 kg and approximately 11 to 12 weeks of age, prior to treatment (Day 1). All rabbits were acclimatised to the experimental environment.

The rabbits were selected without conscious bias for the study. They were housed individually in metal cages with perforated floors in Building R14 Room 5.

A standard laboratory diet SDS Stanrab (P) SQC Rabbit Diet and drinking water were provided *ad libitum*.

The batch of diet used for the study was analysed for nutrient contaminants or 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 maintained at 16 to 22°C and relative humidity at 54 - 74%. These environmental parameters were recorded daily. Air exchange was maintained at approximately 10 air changes per hour and lighting was controlled by means of a time switch to give 12 hours of artificial light (0700 - 1900 hours) in each 24 hours period.

Each animal was identified by a numbered aluminium tag placed through the edge of one ear. This number was unique within the Huntingdon Life Sciences Acute Toxicology Department throughout the duration of the study. Each cage, was identified by a coloured label displaying the study schedule number, animal number and initials of the Study Director and Home Office licensee.

TEST SUBSTANCE PREPARATION

Molybdenum disulphide was administered as supplied by the Sponsor.

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

The eyes of each animal were examined prior to instillation of the test substance to ensure that there was no pre-existing corneal damage, iridial or conjunctival inflammation.

One animal was treated in advance of the others, to ensure that if a severe response was produced, no further animals would be exposed (pilot animal see Table I).

In compliance with the study guideline, the weight of the test substance which when gently compacted occupied a volume of 0.1 ml was measured as below;

Weight of 1 ml syringe (g)	Weight of syringe + 0.1 ml test material (g)	Weight of 0.1 ml test material (mg)
2.291	2.482	191
2.237	2.406	169
2.255	2.440	185
Mean weight of 0.1 ml test substance		182

On this occasion, 100 mg of the test substance was placed into the lower everted lid of one eye of each animal as the volume of 0.1 ml of the test substance (approximately 181 mg) exceeded the 100 mg limit stated in the test guideline.

The eyelid was then gently held together for one second before releasing. The contralateral eye remained untreated.

OBSERVATIONS**Clinical signs**

All animals were observed daily for signs of ill health or toxicity.

Ocular responses

Examination of the eyes was made after 1 hour and 1, 2, 3 days (equivalent to 24, 48 and 72 hours) after instillation. Observation of the eyes was aided by the use of a handheld light.

Ocular irritation was assessed using the prescribed numerical system:

Cornea

Opacity: degree of density (area most dense taken for reading)

No ulceration or opacity	0
Scattered or diffuse areas of opacity (other than slight dulling of normal lustre), details of iris clearly visible	1
Easily discernible translucent areas, details of iris slightly obscured	2
Nacreous areas, no details of iris visible, size of pupil barely discernible	3
Opaque cornea, iris not discernible through the opacity	4

A.12

IMA 024/973189/SE

Area of cornea involved

One quarter (or less) but not zero	1
Greater than one quarter, but less than half	2
Greater than half, but less than three quarters	3
Greater than three quarters, up to whole area	4

Iris

Normal	0
Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperaemia or injection, any of these or combination of any thereof, iris still reacting to light (sluggish reaction is possible)	1
No reaction to light, haemorrhage, gross destruction (any or all of these)	2

Conjunctivae

Redness (refers to palpebral and bulbar conjunctivae, excluding cornea and iris)

Blood vessels normal	0
Some blood vessels definitely hyperaemic (injected)	1
Diffuse, crimson colour, individual vessels not easily discernible	2
Diffuse beefy red	3

Chemosis (lids and/or nictating membranes)

No swelling	0
Any swelling above normal (includes nictating membranes)	1
Obvious swelling with partial eversion of lids	2
Swelling with lids about half-closed	3
Swelling with lids more than half-closed	4

ARCHIVES

All raw data and study related documents generated during the course of the study at Huntingdon Life Sciences, 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

The upper value for humidity recorded was 74%. This exceeded the expected high of 70% stated in the protocol. This deviation is not thought to have affected the integrity or validity of the study.

There were no other deviations from the protocol.

A.13.

IMA 024/973189/SE

RESULTS

CLINICAL SIGNS

There were no signs of toxicity or ill health in any rabbit during the observation period.

OCULAR RESPONSES

The numerical values given to the ocular reactions elicited by Molybdenum disulphide are shown in Table I.

No corneal damage or iridial inflammation was observed.

Transient hyperaemia of blood vessels with slight conjunctival swelling was seen in all animals. All reactions had resolved completely by two days after instillation.

CONCLUSION

Instillation of Molybdenum disulphide into the rabbit eye elicited transient very slight conjunctival irritation only.

TABLE I
Ocular reactions

Rabbit no. and sex	Region of eye		One hour	Day after instillation		
				1	2	3
1982 Female*	Cornea	Density	0	0	0	0
		Area	0	0	0	0
	Iris		0	0	0	0
	Conjunctiva	Redness	1	0	0	0
		Chemosis	1	0	0	0
	1983 Female	Cornea	Density	0	0	0
Area			0	0	0	0
Iris		0	0	0	0	
Conjunctiva		Redness	1	1	0	0
		Chemosis	1	0	0	0
1984 Female		Cornea	Density	0	0	0
	Area		0	0	0	0
	Iris		0	0	0	0
	Conjunctiva	Redness	1	1	0	0
		Chemosis	1	0	0	0

* Pilot animal

B 01 .

IMA 024/973189/SE

APPENDIX 1

Certificate of analysis

Werkszeugnis 2.2

Test Report 2.2

gemäß/par EN 10204

Datum/Date 16.06.97/AS AZWI2202

Kunde/Customer
IMOA

Produkt/Product
Molybdenumdisulphide

Ihre Bestell-Nr. vom/Your Order No. Dated
IMOA Enquiry.No. 14189

Unsere Auftrags-Nr./Our Order No.

Liefermenge/Quantity Delivered
1.5 kg

Lot-Nr./Lot No.
IMOA 0597

Analysenergebnisse/Analytical Results

Analysenergebnisse nach vorschriftsmäßiger Probenahme und Prüfung/Analytical results by specified sampling and testing

CHEMICAL ANALYSIS

Fe	0,03	%
Cu	0,005	%
Pb	0,008	%
Si	< 0,001	%
MoO ₃	< 0,01	%
Water	< 0,01	%
Oil	0,03	%

PHYSICAL ANALYSIS

FSSS 5,1 μm

Particle Size Distribution:
(Sympatec HELOS)

x ₁₀	1,22	μm
x ₅₀	18,73	μm
x ₉₀	53,54	μm

Bemerkungen/Remarks

●

B 02

C 01

Huntingdon
Life Sciences

MOLYBDENUM DISULPHIDE
ACUTE ORAL TOXICITY TO THE RAT

Report

C 02

CONFIDENTIAL

IMA 021/973206/AC

MOLYBDENUM DISULPHIDE
ACUTE ORAL TOXICITY TO THE RAT

RECEIVED
08 MAY 15 AM 11:38

Sponsor

International Molybdenum Association
Unit 7
Hackford Walk
119 - 123 Hackford Road
London SW9 0QT
ENGLAND

Research Laboratory

Huntingdon Life Sciences Ltd
PO Box 2
Huntingdon
Cambridgeshire
PE18 6ES
ENGLAND

Report issued 13 February 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, with the exception noted below, and I consider the data generated to be valid.

United States Environmental Protection Agency, (TSCA), Title 40 Code of Federal Regulations Part 792, Federal Register, 29 November 1983 and subsequent amendment Federal Register 17 August, 1989.

Good Laboratory Practice in the testing of Chemicals OECD, ISBN 92-64-12367-9, Paris 1982, subsequently republished OECD Environment Monograph No. 45, 1992.

Japan Ministry of International Trade and Industry, Directive 31 March 1984 (Kanpogyo No. 39 Environmental Agency, Kikyoku No. 85 MITI).

The United Kingdom Good Laboratory Practice Regulations 1997, Statutory Instrument No. 654.

EC Council Directive, 87/18 EEC of 18 December 1986, (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.

The study complied fully with OECD and EEC Good Laboratory Practice standards. However, although procedures were inspected on this type of study during the same period and the report was reviewed by the QA Unit, no inspections were carried out on this specific study. I do not consider that this affects the integrity or validity of the study.



Lewis A. McRae, H.N.C., M.I.A.T., M.I.Sc.T.,
Study Director,
Huntingdon Life Sciences Ltd.

13 February 1998

Date

QUALITY ASSURANCE STATEMENT

This report has been audited by Huntingdon Life Sciences Quality Assurance Department (Huntingdon). The methods, practices and procedures reported herein are an accurate description of those employed at Huntingdon Life Sciences during the course of the study. Observations and results presented in this final report form a true and accurate representation of the raw data generated during the conduct of the study at Huntingdon Life Sciences.

Certain studies such as that described in this report, are conducted at Huntingdon Life Sciences in a setting which involves frequent repetition of similar or identical procedures. At or about the time the study described in this report was in progress, 'process-based' inspections were made by the Quality Assurance Department of critical procedures relevant to this study type. The findings of these inspections were reported promptly to the Study Director and to Huntingdon Life Sciences Management.

Date(s) of inspection

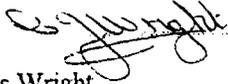
30 July - 1 August 1997

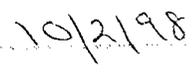
Date(s) of reporting inspection findings to the Study Director and Huntingdon Life Sciences Management

4 August 1997

Date of reporting audit findings to the Study Director and Huntingdon Life Sciences Management

11 August 1997


Chris Wright,
Audit Team Supervisor
Department of Quality Assurance,
Huntingdon Life Sciences Ltd.


Date

C 06

IMA 021/973206/AC

RESPONSIBLE PERSONNEL

Lewis A. McRae, H.N.C., M.I.A.T., M.I.Sc.T.,
Study Director,
Department of Acute Toxicology.



SUMMARY

A study was performed to assess the acute oral toxicity of Molybdenum disulphide to the rat. The method followed was that described in the OECD Guideline for Testing of Chemicals No. 401 "Acute Oral Toxicity". Adopted: 24 February 1987.

A group of ten fasted rats (five males and five females) received a single oral gavage dose of the test substance formulated in 1% w/v aqueous methylcellulose and administered at a dose level of 2000 mg/kg bodyweight. This dose level was selected on the basis of results from preliminary study investigations.

There were no deaths. Clinical signs of reaction to treatment in the main study comprised piloerection, hunched posture and ungroomed appearance observed in all rats. There were no other clinical signs and recovery was complete in all instances by Day 4.

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

All animals were killed and examined macroscopically on Day 15, the end of the observation period. This examination revealed no abnormalities.

The acute lethal oral dose to rats of Molybdenum disulphide was demonstrated to be greater than 2000 mg/kg bodyweight.

Molybdenum disulphide will not require labelling with the risk phrase R22, "Harmful if swallowed", in accordance with Commission Directive 93/21/EEC.

INTRODUCTION

The study was designed to assess the toxicity of Molybdenum disulphide 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 the OECD Guideline for Testing of Chemicals No. 401 "Acute Oral Toxicity". Adopted: 24 February 1987.

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

The dose level for the study was selected on the basis of results from the preliminary study and in compliance with the study guideline.

The protocol was approved by Huntingdon Life Sciences Management on 7 November 1996, by the Sponsor on 4 June 1997 and by the Study Director on 30 June 1997.

The experimental phase of the study was undertaken between 7 and 23 July 1997.

TEST SUBSTANCE

Identity: Molybdenum disulphide

Intended use: Lubricant and Corrosion Inhibitor

Appearance: Dark grey powder

Storage conditions: Room temperature

Lot number: IMO A 0597

Expiry: Not advised

Purity: > 99%

Date received: 17 June 1997

EXPERIMENTAL PROCEDURE

ANIMAL MANAGEMENT

The 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.

The animals in the main study were in the weight range of 87 to 102 g and approximately four to seven weeks of age prior to dosing (Day 1). All the rats were acclimatised to the experimental environment for a period of six days prior to the start of the study.

Rats were allocated without conscious bias to cages within the treatment group. They were housed in groups of five rats of the same sex in metal cages with wire mesh floors in Building R14 Room 6.

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.

Each 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 25°C and relative humidity was in the range 40 - 63%. Permanent daily recordings of these parameters were made and these are archived with other Department raw data. Air exchange was maintained at 10 to 15 air changes per hour and lighting 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

Molybdenum disulphide was formulated at a concentration of 20% w/v in 1% w/v aqueous methylcellulose and administered at a volume of 10 ml/kg bodyweight in the main study.

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 1600 mg/kg bodyweight to establish a dosing regime for the main study.

Main study

A group of ten rats (five males and five females) was treated at 2000 mg/kg bodyweight.

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 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 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 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 animals in the preliminary and main studies were observed for 7 or 14 days respectively after dosing.

Bodyweight

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

TERMINAL STUDIES

Termination

All animals 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.

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

In the interests of animal welfare, the preliminary study was carried out using a group of two rats (one male and one female), rather than a group of four rats (two males and two females) as stated in the protocol. This deviation was not considered to have affected the integrity or validity of the study.

There were no other deviations from the protocol.

RESULTS

PRELIMINARY STUDY (Table 1)

A group of two rats (one male and one female) was dosed at 1600 mg/kg bodyweight. There were no deaths. Clinical signs included piloerection, hunched posture and waddling gait, seen in all animals. In addition, lethargy, pallor of extremities and increased respiration were observed in the male only and protruding eyes was noted for the female only. Bodyweight gains were considered satisfactory for studies of this nature and duration and no macroscopic abnormalities were noted at the terminal necropsy on Day 8.

The above results were considered sufficient to permit confidence in progression to the main study at a limit dosage of 2000 mg/kg.

MAIN STUDY

There were no deaths following a single oral dose of Molybdenum disulphide to a group of ten rats (five males and five females) at a dosage of 2000 mg/kg bodyweight.

CLINICAL SIGNS (Table 1)

Piloerection was observed in all rats within six minutes of dosing. This sign persisted and was accompanied in all rats during the study by hunched posture and ungroomed appearance. All but piloerection had resolved by Day 2 and recovery was complete in all instances by Day 4.

BODYWEIGHT (Tables 2 and 3)

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

MACROSCOPIC EXAMINATION

Macroscopic examination of animals killed on Day 15 revealed no abnormalities.

CONCLUSION

The acute lethal oral dose to rats of Molybdenum disulphide was demonstrated to be greater than 2000 mg/kg bodyweight.

TABLE 1
Signs of reaction to treatment

Signs	No. of rats in group of 1* or 5 showing signs			
	Dose (mg/kg)			
	1600*		2000	
	Male	Female	Male	Female
Piloerection	1	1	5	5
Hunched posture	1	1	5	5
Waddling gait	1	1	0	0
Lethargy	1	0	0	0
Pallor of extremities	1	0	0	0
Increased respiration	1	0	0	0
Protruding eye	0	1	0	0
Ungroomed appearance	0	0	5	5

* - Preliminary investigation comprised 1 male and 1 female

TABLE 2
Individual and group mean bodyweights (g)

Dose (mg/kg)	Sex	Animal Number	Bodyweight (g) at			
			Day 1*	Day 8	Day 15	
2000	Male	11	95	150	197	
		12	93	143	219	
		13	89	153	204	
		14	96	159	206	
		15	92	159	181	
		Mean		93	153	201
	Female	16	96	152	182	
		17	102	151	175	
		18	102	149	175	
		19	87	120	150	
20		90	139	163		
	Mean		95	142	169	

* Prior to dosing

TABLE 3
Individual body weight changes (g)

Dose (mg/kg)	Sex	Animal Number	Bodyweight gains (g) at	
			Day 8	Day 15
2000	Male	11	55	47
		12	50	76
		13	64	51
		14	63	47
		15	67	22
	Female	16	56	30
		17	49	24
		18	47	26
		19	33	30
		20	49	24

APPENDIX 1

Certificate of analysis

Werkzeugnis 2.2
Test Report 2.2
gemäß/per EN 10204

Datum/Date 16.06.97/AS AZWI2202

Kunde/Customer
IMOA

Produkt/Product
Molybdenumdisulphide

Ihre Bestell-Nr. vom/Your Order No. Dated
IMOA Enquiry No. 14189

Unsere Auftrags-Nr./Our Order No.

Liefermenge/Quantity Delivered
1,5 kg

Lot-Nr./Lot No.
IMOA 0597

Analysenergebnisse/Analytical Results

Analysenergebnisse nach vorschriftsmäßiger Probenahme und Prüfung/Analytical results by specified sampling and testing

CHEMICAL ANALYSIS

Fe	0,03	%
Cu	0,005	%
Pb	0,008	%
Si	< 0,001	%
MoO ₃	< 0,01	%
Water	< 0,01	%
Oil	0,03	%

PHYSICAL ANALYSIS

FSSS 5,1 μm

Particle Size Distribution:
(Sympatec HELOS)

X ₁₀	1,22	μm
X ₅₀	18,73	μm
X ₉₀	53,54	μm

Bemerkungen/Remarks

D 04

E 01

Huntingdon
Life Sciences

VOXYDENUM DISULPHIDE

ACUTE (FOUR HOUR) INHALATION STUDY IN RATS

Report

CONFIDENTIAL

IMA 026/972902

MOLYBDENUM DISULPHIDE
ACUTE (FOUR - HOUR) INHALATION STUDY IN RATS

RECEIVED
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99 MAR 15 AM 11:39

Sponsor

International Molybdenum Association,
Unit 7 Hackford Walk,
119 - 123 Hackford Road,
London,
SW9 0QT,
ENGLAND.

Research Laboratory

Huntingdon Life Sciences Ltd.,
P.O. Box 2,
Huntingdon,
Cambridgeshire,
PE18 6ES,
ENGLAND.

Report issued: 11 March 1998

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1. Certificate of Analysis	31

COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

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

The United Kingdom Good Laboratory Practice Regulations, 1997.

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

Good Laboratory Practice in the testing of Chemicals OECD, ISBN 92-64-12367-9, Paris 1982, subsequently republished OECD Environment Monograph No. 45, 1992.

The above Good Laboratory Practice Standards are equivalent to the following:

United States Environmental Protection Agency, (FIFRA), Title 40 Code of Federal Regulations Part 160 (FIFRA) or Part 792 (TSCA), Federal Register, 29 November 1983 and subsequent amendment Federal Register 17 August 1989.

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

G. C. Jackson

11 March 1998

Graham C. Jackson, B.A. (Hons.), L.R.S.C.,
Study Director,
Huntingdon Life Sciences Ltd.

Date

QUALITY ASSURANCE STATEMENT

This report has been audited by Huntingdon Life Sciences Quality Assurance Department (Huntingdon). The methods, practices and procedures reported herein are an accurate description of those employed at Huntingdon during the course of the study. Observations and results presented in this final report form a true and accurate representation of the raw data generated during the conduct of the study at Huntingdon.

Certain studies such as that described in this report, are conducted at Huntingdon in a setting which involves frequent repetition of similar or identical procedures. At or about the time the study described in this report was in progress, 'process-based' inspections were made by the Quality Assurance Department of critical procedures relevant to this study type. The findings of these inspections were reported promptly to the Study Director and to Management, Huntingdon Life Sciences.

Date(s) of inspection

16 & 18 June 1997

Date(s) of reporting inspection findings to the Study Director and Management

20 June 1997

Date of reporting audit findings to the Study Director and Management

29 September 1997



Mark Somerset,
Audit Team Supervisor,
Department of Quality Assurance,
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11 March 1998

Date

RESPONSIBLE PERSONNEL

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SUMMARY**Test substance**

A dark grey powder identified as Molybdenum disulphide.

Test animals

Albino rats (Sprague-Dawley in origin). One control group and 1 test group each of 5 male and 5 female rats.

Route of Administration

By inhalation of a test atmosphere containing a respirable particulate aerosol generated from the test substance.

Duration of exposure

Four hour continuous snout-only exposure.

Observation period

Fourteen days post exposure.

Exposure levels and mortality

There were no deaths following exposure of rats to a particulate aerosol of Molybdenum disulphide at a concentration of 2.82 mg/l of air.

Clinical signs

During the exposure, an accumulation of the test substance was seen on the tail and fur of all test rats.

Residues of the test substance were evident as grey staining on the fur and tail on all test rats during the observation period. Grey staining on the tail persisted until the end of the observation period. A matted appearance of the fur was noted in 1 test female immediately following exposure.

Soiling of the fur with excreta was noted in all test and control rats during and immediately following exposure. This sign is attributed to the method of restraint.

Bodyweight

The rate of bodyweight gain in test rats exposed to Molybdenum disulphide was similar to that of the controls.

Food and water consumption

Food and water consumption in the test rats was similar to that of the control rats.

Lung weight to bodyweight ratio

The lung weight to bodyweight ratios for all test rats exposed to Molybdenum disulphide were similar to the ratios found for the control rats.

Macroscopic pathology

A grey appearance of the lungs was noted for all test rats at *post mortem* and this finding was considered to be treatment-related. The toxicological importance of the finding can not be determined without histopathological examination of the lung tissues.

There were no other macroscopic abnormalities.

Conclusion

The LC₅₀ (4 hour) for Molybdenum disulphide is in excess of 2.82 mg/l in air. The highest attainable concentration. The inhalation hazard associated with acute exposure to with Molybdenum disulphide is considered to be low. In the absence of toxic effects following exposure, labelling of the test substance with the risk phrase R20 (Harmful by inhalation) is not indicated.

INTRODUCTION

The acute inhalation toxicity of Molybdenum disulphide was assessed by exposing a group of rats, for a period of 4 hours, to a particulate aerosol produced from the test substance. The concentration of Molybdenum disulphide was 2.82 mg/l of air this was the highest attainable concentration. A further group, acting as a control was exposed to clean air only.

The study was conducted at Huntingdon Life Sciences during the period 2 July to 24 July 1997. The protocol for the study was approved by the Study Director and Huntingdon Management on 25 June 1997 and approved by the Sponsor's Agent on 26 June 1997.

The study design was in compliance with US-EPA, EEC, OECD and JMITI test guidelines for acute inhalation studies.

On completion of the study all data relating to the study, and a copy of the final report, were lodged in the Huntingdon Life Sciences Archives, Huntingdon, Cambridgeshire, England. The data will be retained in Archives for at least 5 years from the completion date for the study.

TEST SUBSTANCE

Identity: Molybdenum disulphide

Intended use: Lubricant and Corrosion Inhibitor

Appearance: Dark grey powder

Storage conditions: Room temperature

Lot number: IMOA 0597

Expiry: Not advised

Purity: >99%

Date received: 17 June 1997

The complete description of the chemical and physical properties of the test substance are the responsibility of the Sponsor.

A small sample (~1 g) was taken, sealed in an suitable container and stored in Archives at an appropriate temperature.

MATERIALS AND METHODS**ANIMALS AND MAINTENANCE**

Ten male and 10 female albino rats (Sprague-Dawley in origin) were selected from a consignment of rats obtained from Charles River UK Limited, Manston Road, Margate, Kent, England 2 July 1997. The rats were selected so that males and females would be between 7 and 8 weeks old on the day of arrival.

On arrival the rats were allocated to 1 of 2 groups of 5 males and 5 females and were identified individually by a number tattooed on the ear pinnae. The rats were housed by sex in groups of 5 and acclimatised to laboratory conditions for at least 5 days before the day of exposure.

The holding cages (size 35cm x 53 cm x 25 cm height) were made of stainless steel sheet and wire mesh and were suspended on a movable rack. While in their cages all rats had free access to a measured excess amount of SDS rat and mouse diet (RM1) and tap water. Food and water were analysed routinely to determine the levels of chemical or microbiological contaminants. Room lighting was by artificial light between 8 am and 8 pm daily and controlled automatically.

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

The temperature and relative humidity of the holding room air was recorded continuously using a Kent Clearspan thermohydrograph.

The temperature and relative humidity of the holding area during the study remained within the range of $22^{\circ}\text{C} \pm 3^{\circ}\text{C}$ and the relative humidity was between the limits 40% and 70%.

INHALATION EXPOSURES

A single group of rats was exposed continuously for 4 hours to a test atmosphere containing a particulate aerosol generated from Molybdenum disulphide.

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

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

Group 1 (Control): 10 July 1997
Group 2 (Test): 10 July 1997

The mean concentration of the test aerosol for the test group is given in the **RESULTS** section of this report.

EXPOSURE SYSTEM**Wright dust generator**

A Wright dust generator ⁽¹⁾ was used to produce the test atmospheres containing the dust of Molybdenum disulphide.

The construction of the dust generator is shown in Figure 1. The generator was designed to produce and maintain atmospheres containing dust by suspending the material scraped from the surface of a compressed powder in a stream of dry air. The concentration of dust in the air is determined by the rate at which the scraper blade is advanced into the compressed powder.

Aerosol conditioning

The test atmosphere was passed through an elutriation column to reduce, by sedimentation, the amount of non-respirable particulate in the test atmosphere.

Exposure chambers

The snout-only exposure chambers (ADG Developments) used for the exposures were of cylindrical form (30 cm i.d., 45 cm height) and made of aluminium alloy ⁽²⁾. The chambers had an enclosed volume of approximately 30 litres. The rats were held for exposure in moulded polycarbonate restraining tubes which were attached at evenly spaced ports in the cylindrical section of the chamber, and were designed to allow only the snout to project into the chamber. Each rat was restrained in a forward position by an adjustable foamed plastic stopper which also provided a seal for the tube.

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

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

PROCEDURE

The test substance was packed into the container of the Wright dust generator using a hydraulic bench press to assist packing. Even density of the powder was achieved by packing the container in stages and applying a force of 1.0 tonnes weight ⁽³⁾.

⁽¹⁾ Wright, B.M., *J. Scient. Instruments*, 27, (1) 1958, p12

⁽²⁾ The internal surfaces of the chamber have a conformal chemically resistant coating

⁽³⁾ The applied force must be sufficient to prevent the disintegration of the packed powder during the generation procedure

The dust generator was positioned on a stand at the side of the exposure chamber and the output was connected to the top inlet port of the chamber via the elutriation column. The speed controller of the generator mechanism was set to give the maximum attainable concentrations of dust ⁽¹⁾.

A supply of clean dried compressed air was connected to the dust generator and the supply pressure was adjusted to give an initial flow rate of 15 litres per minute measured at the generator outlet nozzle.

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

The powder container of the Wright dust generator was advanced manually until a trace of suspended dust was seen in the elutriation column. The gearing on the generator was then engaged and the generator motor switched on to start the exposure. After a 5-minute ⁽²⁾ equilibration period, the exposure was timed for 4 hours. The generator was then switched off and the chamber allowed to clear before the rats were removed for examination.

After 4 hours the test substance was discontinued and the exposure chamber was allowed to clear before the rats were removed for examination.

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

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

CHAMBER ATMOSPHERE ANALYSIS

Five air samples were taken from the chamber during the exposure and the collected material was weighed to determine the concentration of Molybdenum disulphide in the chamber air. The samples were taken at 30, 60, 120, 180 and 230 minutes after the start of exposure.

Each air sample was withdrawn, at 2 litres per minute, through a weighed glass fibre filter (Whatman GF/A) mounted in an open face filter holder.

The volume of the air sample was measured with a wet-type gas meter.

⁽¹⁾ The performance of the dust generator with this test substance was assessed in a preliminary experiment. A setting of 80% of the maximum was the highest at which the mechanism would operate reliably.

⁽²⁾ 5 minutes is the theoretical time required for the concentration of aerosol to reach 90% of its final value under the conditions of the exposure employed.

Two additional air samples were taken during the exposure at a sampling rate of 2 litres/minute using a Marple cascade impactor ⁽¹⁾. The samples were taken at 90 and 210 minutes after the start of exposure.

The material collected on the stages of the sampler was weighed to determine the mass median aerodynamic diameter (MMAD) and the particle size distribution of Molybdenum disulphide in the test atmosphere.

The collection characteristics for the Marple sampler are shown in Table 2.

NOMINAL CONCENTRATION

The nominal concentration of the test substance in the exposure chamber was calculated from the amount of Molybdenum disulphide dispersed by the generator and the total volume of air supplied to the exposure system during the exposure period.

CHAMBER AIR TEMPERATURE AND RELATIVE HUMIDITY

The air temperature in the exposure chamber was measured with a thermometer and the relative humidity was measured using a Casella type T6900 relative humidity meter. The temperature and humidity were recorded at the start of exposure and then at 30-minute intervals during the 4-hour exposure.

OBSERVATIONS

Clinical signs

The rats were observed continuously for signs of reaction to the test substance during exposure and at least twice daily throughout the observation period. The clinical signs were recorded at the end of the chamber equilibration period, at 0.25, 0.5 and 1.0 hours then at hourly intervals during the exposure. During the observation period, the clinical signs were recorded once in the morning and then as necessary following a later check for clinical signs.

Bodyweight

All rats were weighed daily from the day of delivery to the Huntingdon Life Sciences until the end of the observation period.

⁽¹⁾ Model 296, Andersen Samplers Inc., Atlanta, GA, USA

Food and water consumption

The amount of food and water consumed by each cage of rats was measured daily from the day of arrival. The daily mean intakes of food and water for each cage were calculated from the recorded data.

TERMINAL STUDIES

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

All rats were subjected to a detailed macroscopic examination. The lungs, liver and kidneys were removed, dissected clear of surrounding tissue. The lungs were weighed in order to calculate the lung weight to bodyweight ratio.

The lungs were infused with, and preserved in, neutral buffered 10% formalin together with samples of the liver and kidneys for possible future microscopic examination. The tissues were scheduled for disposal following the completion date for the study.

RESULTS

CHAMBER ATMOSPHERE CONDITIONS

Concentration of Molybdenum disulphide

The gravimetric results for the air samples taken during the exposure are shown in Table 1.

The mean concentration of Molybdenum disulphide in the chamber air and the standard deviation (sd) were:

Molybdenum Disulphide (mg/l)	sd
2.82	0.226

The concentration of Molybdenum disulphide was the maximum attainable concentration. The Wright dust generator was the preferred apparatus for the generation of the test atmosphere from Molybdenum disulphide and there were no alternative procedures to increase the concentration of aerosol.

The nominal concentration, calculated from the amount of Molybdenum disulphide dispersed and the total volume of air supplied to the exposure system, was 93.6 mg/l.

Particle size distribution

The results for the air samples taken for determination of the particle size distribution of Molybdenum disulphide shown in Table 2. The particle size data are summarised below:

Group	MMAD (μm)	σ_g	% respirable ($<7 \mu\text{m}$)
2	4.7	2.67	65

MMAD Mass median aerodynamic diameter

σ_g Standard geometric deviation

The MMAD was slightly above the ideal size range ($1 \mu\text{m}$ to $4 \mu\text{m}$) for an acute inhalation study.

Chamber air temperature and relative humidity

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

Group	Temperature (°C)		Relative Humidity (%)	
	Mean	sd	Mean	sd
1 (Control)	21.3	0.25	38.7	1.41
2 (Test)	21.0	0.35	40.2	3.49

There were no extremes of chamber air temperature or humidity considered likely to have influenced the results of the study.

CLINICAL OBSERVATIONS

Mortality

There were no deaths following exposure of rats to an aerosol of Molybdenum disulphide at a concentration of 2.82 mg/l of air. This was the highest attainable concentration.

Clinical signs

During the exposure - The incidence of clinical signs noted during the exposure period is shown in Table 3. There were no clinical signs attributable to exposure to Molybdenum disulphide. An accumulation of the test substance was seen on the tail and fur of all test rats.

During the observation period - The incidence of clinical signs seen during the observation period is shown in Table 4. Residues of the test substance were evident, as grey staining on the fur and tail, on all test rats during the observation period. Grey staining on the fur persisted until the end of the observation period. A matted appearance of the fur was noted in one test female immediately following exposure.

Soiling of the fur with excreta was noted in all control and test animals immediately following exposure. This sign was attributed to the method of restraint.

Bodyweight

The group mean and individual bodyweights are shown in Table 5. The group mean bodyweights are also shown in Figure 3.

The rate of bodyweight gain in rats exposed to Molybdenum disulphide was similar to that of the control rats.

Food and water consumption.

The food consumption data is presented in Tables 6 and 7 respectively.

Food and water consumption in test rats was similar to that of the controls.

TERMINAL STUDIES**Lung weight to bodyweight ratio**

The lung weight to bodyweight ratios for individual rats are shown in Table 8.

The lung weight to bodyweight ratios for test rats exposed to Molybdenum disulphide were similar to the control values.

Macroscopic pathology

The macroscopic pathological findings for individual rats are shown in Table 9.

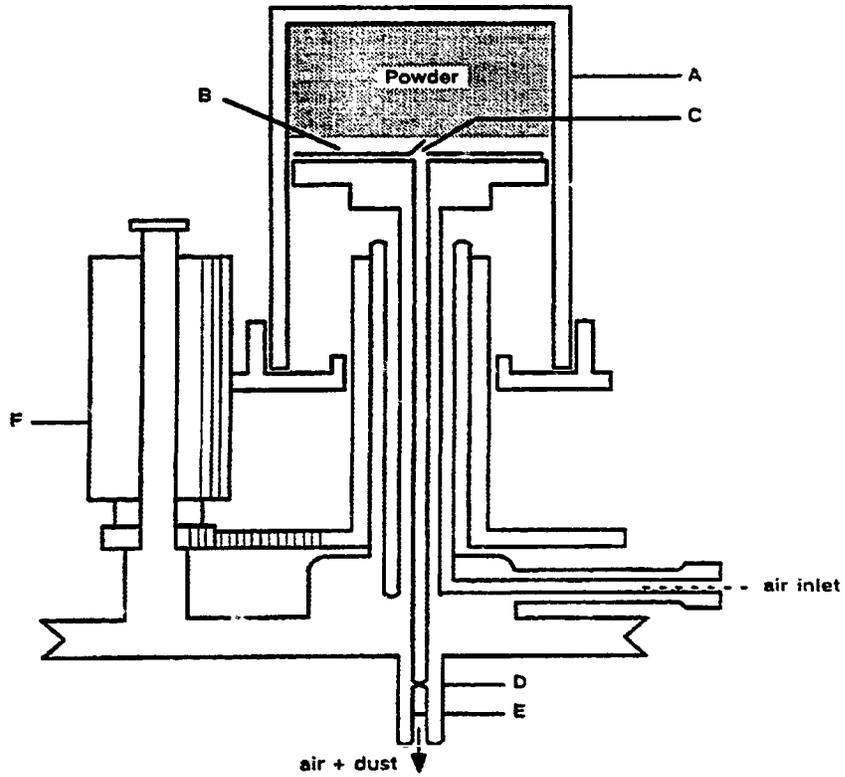
A grey appearance of the lungs was noted for all test rats at *post mortem*. This finding was considered to be treatment-related. The toxicological importance of the finding can not be determined without histopathological examination of the lung tissues.

There were no other macroscopic abnormalities.

Estimation of the LC₅₀ (4-hour) for MOLYBDENUM DISULPHIDE

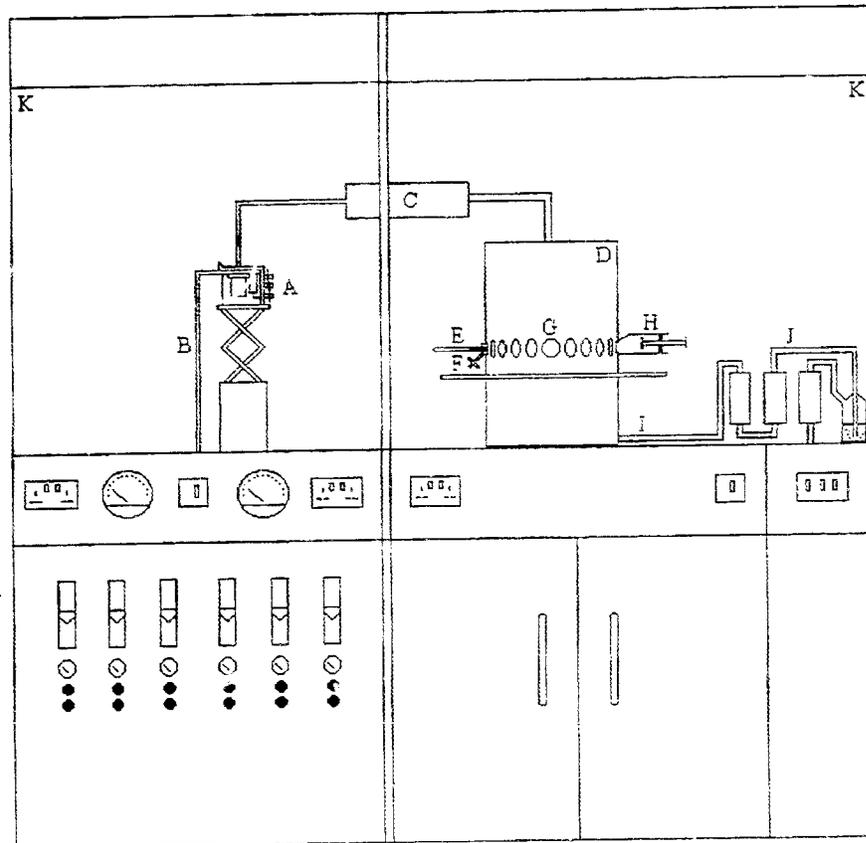
There were no deaths within 14-days following a single 4-hour exposure of rats to a particulate aerosol of Molybdenum disulphide at a concentration in air of 2.82 mg/l. The LC₅₀ (4-hour) for Molybdenum disulphide is therefore in excess of 2.82 mg/l of air. The concentration of Molybdenum disulphide was the highest attainable concentration.

FIGURE 1
Wright dust generator



- A Canister
- B Scraper
- C Outlet tube
- D Jet
- E Baffle plate
- F Drive gear

FIGURE 2
Exposure system



- A Wright dust feed mechanism
- B Dust feed air supply
- C Elutriator
- D Exposure chamber (30 litres)
- E Thermometer
- F Sample line to water vapour analyser

- G Animal exposure/sampling port
- H Rat holding tube
- I Extract from exposure chamber
- J Filter/extract unit
- K Air extraction cabinet

FIGURE 3
Group mean bodyweights

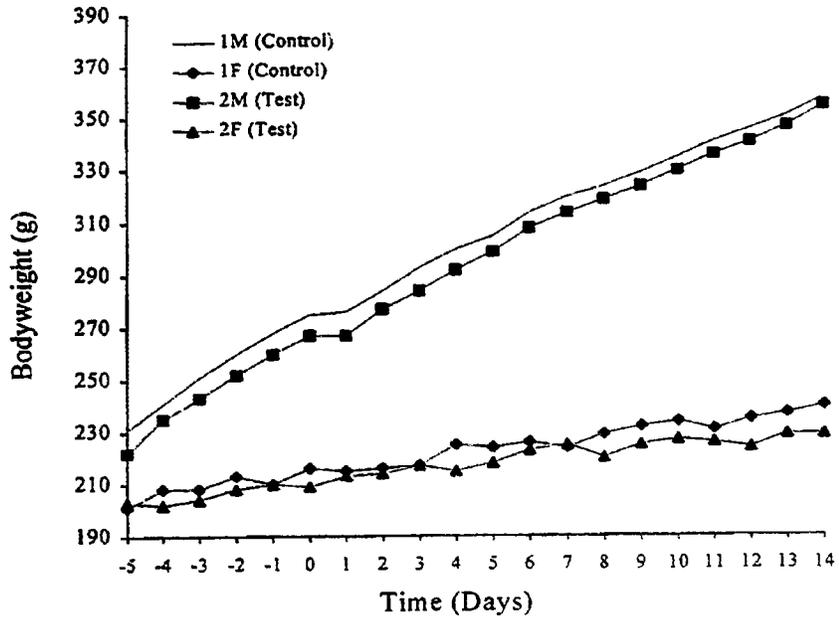


TABLE 1

Concentrations of Molybdenum disulphide

Gravimetric results

Group	Sample	Time taken	Amount in air (mg/l)	Nominal concentration (1) (mg/l)
2 (Test)	1	0h:30m	2.58	
	2	1h:00m	2.57	
	3	2h:00m	2.99	
	4	3h:00m	2.90	
	5	3h:50m	3.04	
		Mean		2.82
	sd		0.226	

(1) Calculated from the weight of test substance dispersed and the total volume of air supplied to the exposure system

sd Standard deviation

TABLE 2

Particle size distribution of Molybdenum disulphide

Gravimetric analysis

Sample	Time taken	Stage	Cut-off size (μm)	Amount collected (mg)	
				PSD1	PSD2
PSD 1	1h:30m	3	9.8	0.38	0.60
PSD 2	3h:30m	4	6.0	0.38	0.78
		5	3.5	0.40	0.79
		6	1.55	0.58	0.92
		7	0.93	0.11	0.17
		8	0.52	0.02	0.05
		Filter	0.0	0.06	0.07
		Totals			1.93

Calculations

Cut-off size (μm)	% less than size (cumulative)
9.8	81.4
6.0	59.6
3.5	37.2
1.55	9.0
0.93	3.7
0.52	2.4
MMAD	4.7
σ	2.67
% respirable (<7 μm)	65

MMAD Mass median aerodynamic diameter
 σ Standard geometric deviation

TABLE 3
Clinical signs during exposure

Group	Signs	Number showing signs						
		Time in hours						
		0*	0.25	0.5	1.0	2.0	3.0	4.0
1M (Control)	Normal appearance and behaviour	5						
	Fur soiled with excreta			5	5	5	5	5
1F (Control)	Normal appearance and behaviour	5						
	Fur soiled with excreta			5	5	5	5	5
2M (Test)	Normal appearance and behaviour	5						
	Test material on tail (grey)					5	5	5
	Test material on fur					5	5	5
2F (Test)	Normal appearance and behaviour	5						
	Test material on tail (grey)					5	5	5
	Test material on fur					5	5	5

* Clinical signs recorded during the 5-minute equilibration period

TABLE 4
Clinical signs during observation period

Group	Signs	Day of observation period																
		0*	1hr*	2hr*	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1M (Control)	Normal appearance and behaviour																	
	Fur soiled with excreta	5	5	5														
1F (Control)	Normal appearance and behaviour																	
	Fur soiled with excreta	5	5	5														
2M (Test)	Fur soiled with excreta	5	5	5														
	Test material on fur	5	5	5														
	Test material on tail	5	5	5														
2F (Test)	Fur soiled with excreta	5	5	5														
	Test material on fur	5	5	5														
	Matted fur Test material on tail	5	5	5														

* Clinical signs recorded after exposure on the day of exposure

TABLE 5
Individual and group mean bodyweights (g)

Group	Rat	Day of observation																			
		-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
IF (Control)	21	235	246	247	263	270	276	277	284	293	300	304	313	314	322	319	323	329	332	335	341
	22	235	247	249	265	272	280	281	293	303	314	317	327	334	341	353	359	369	376	383	388
	23	230	238	254	259	264	272	273	278	287	294	302	304	311	311	311	320	321	322	325	333
	24	227	233	255	256	265	271	273	281	289	292	301	311	318	323	332	339	345	352	355	367
	25	230	240	250	259	267	275	278	284	293	299	303	314	321	324	329	335	341	347	355	361
	Mean	231	241	251	260	268	275	276	284	293	300	305	314	320	324	329	335	341	346	351	358
IF (Control)	26	207	213	215	221	216	222	221	223	218	227	229	234	229	231	237	236	234	237	240	244
	27	207	216	218	225	218	224	226	230	229	237	236	240	235	242	244	251	241	247	253	257
	28	192	197	197	207	194	203	204	201	203	210	210	212	207	214	216	218	215	220	222	223
	29	201	211	211	215	213	217	214	215	216	227	225	227	222	231	232	238	231	236	240	242
	30	197	202	200	198	208	213	210	211	221	223	221	218	227	228	231	228	232	233	232	233
	Mean	201	208	208	213	210	216	215	216	217	225	224	226	224	229	232	234	231	235	237	240

0 = Day of exposure

TABLE 5
(Individual and group mean bodyweights - continued)

Group	Rat	Day of observation																			
		-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
2M (Test)	31	217	227	236	241	248	255	254	265	270	277	284	291	298	301	308	316	319	325	333	337
	32	224	236	243	253	260	267	268	277	286	291	296	307	314	319	322	330	332	338	347	350
	33	221	231	243	252	262	268	272	282	289	298	307	317	321	328	335	339	348	356	361	371
	34	232	244	254	264	274	282	280	294	298	312	321	332	337	345	349	357	371	375	380	390
	35	218	239	241	249	257	263	259	268	275	281	288	293	299	302	306	307	309	312	316	325
	Mean	222	235	243	252	260	267	267	277	284	292	299	308	314	319	324	330	336	341	347	355
2F (Test)	36	202	207	202	210	211	214	217	218	222	221	222	234	235	233	229	239	240	239	236	245
	37	211	202	206	210	214	208	214	220	220	212	222	226	224	219	228	231	229	226	233	236
	38	209	202	208	215	213	208	214	218	218	213	221	227	227	221	229	227	226	220	233	232
	39	200	203	202	200	206	210	213	206	215	219	213	212	221	219	223	218	219	222	223	210
	40	195	196	200	206	208	206	206	206	210	208	211	216	219	209	218	220	216	214	219	220
	Mean	203	202	204	208	210	209	213	214	217	215	218	223	225	220	225	227	226	224	229	229

0 = Day of exposure

TABLE 6
Group mean daily food consumption (g/rat)

Group	Pre-exposure											Post exposure													
	-5	-4	-3	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14						
1M (Control)	33	33	32	33	33	28	32	31	32	31	31	31	30	31	31	30	31	29	29	30					
1F (Control)	24	24	24	18	22	21	22	20	24	22	21	20	22	22	23	19	21	21	21	21					
2M (Test)	33	33	33	32	32	26	31	31	33	32	32	31	32	31	31	32	31	31	31	31					
2F (Test)	21	23	22	20	20	21	19	22	21	21	22	23	19	22	21	21	18	22	20	20					

TABLE 7
Group mean daily water consumption (g/rat)

Group	Days																			
	Pre-exposure					Post exposure														
	-5	-4	-3	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
1M (Control)	36	35	34	34	34	33	33	32	32	31	31	31	31	31	31	32	32	32	32	32
1F (Control)	25	24	25	19	14	25	23	21	26	23	23	20	26	25	25	20	24	24	24	24
2M (Test)	36	34	35	35	33	34	34	31	33	31	31	30	30	29	31	28	31	32	30	30
2F (Test)	24	27	27	25	23	32	24	28	24	25	24	27	24	26	25	25	22	27	25	25

TABLE 8
Lung weight to bodyweight ratios

Group	Rat	Bodyweight (g)	Lungs	
			Weight (g)	LW:BW ratio
1M (Control)	21	341	1.50	0.44
	22	388	1.55	0.40
	23	333	1.51	0.45
	24	367	1.54	0.42
	25	361	1.61	0.45
			Mean	0.43
			sd	0.022
1F (Control)	26	244	1.55	0.55
	27	257	1.22	0.47
	28	223	1.42	0.64
	29	242	1.17	0.48
	30	233	1.29	0.55
			Mean	0.54
			sd	0.068
2M (Test)	31	337	1.40	0.42
	32	350	1.52	0.43
	33	371	1.64	0.44
	34	390	1.89	0.48
	35	325	1.45	0.44
			Mean	0.44
			sd	0.023
2F (Test)	36	245	1.39	0.57
	37	236	1.35	0.57
	38	232	1.34	0.58
	39	210	1.43	0.68
	40	220	1.41	0.64
			Mean	0.61
			sd	0.050

sd Standard deviation

Lung weight to bodyweight ratio calculated as follows:

$$\text{LW:BW ratio} = \frac{\text{lung weight} \times 100}{\text{bodyweight}}$$

TABLE 9
Macroscopic pathology

Group	Rat	Observation
1M (Control)	21	No abnormalities detected
	22	No abnormalities detected
	23	No abnormalities detected
	24	No abnormalities detected
	25	No abnormalities detected
1F (Control)	26	No abnormalities detected
	27	No abnormalities detected
	28	No abnormalities detected
	29	No abnormalities detected
	30	No abnormalities detected
2M (Test)	31	Lungs appear grey in colour
	32	Lungs appear grey in colour
	33	Lungs appear grey in colour
	34	Lungs appear grey in colour
	35	Lungs appear grey in colour
2F (Test)	36	Lungs appear grey in colour
	37	Lungs appear grey in colour
	38	Lungs appear grey in colour
	39	Lungs appear grey in colour
	40	Lungs appear grey in colour

APPENDIX 1

Certificate of Analysis

Werkzeugnis 2.2
Test Report 2.2
gemäß/per EN 10204
Datum/Date 16.06.97/AS AZWI2202

Kunde/Customer IMOA
Produkt/Product Molybdenumdisulphide

Ihre Bestell-Nr. vom/Your Order No. Dated IMOA Enquiry No. 14189
Unsere Auftrags-Nr./Our Order No.

Liefermenge/Quantity Delivered 1,5 kg
Lot-Nr./Lot No IMOA 0097

Analysenergebnisse/Analytical Results
Analysenergebnisse nach vorschriftsmäßiger Probenahme und Prüfung/Analytical results by specified sampling and testing

CHEMICAL ANALYSIS

Fe	0,03	%
Cu	0,003	%
Pb	0,008	%
Si	0,001	%
MoO ₃	< 0,01	%
Water	< 0,01	%
Oil	0,03	%

PHYSICAL ANALYSIS

FSSS 5,1 μm

Particle Size Distribution:
(Sympatec HELOS)

X ₁₀	1,22	μm
X ₅₀	18,73	μm
X ₉₀	53,54	μm

Bemerkungen/Remarks