

# International Molybdenum Association

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26th February 1996



FYI-96-001265  
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FYI Co-ordinator, FYI Submissions  
Office of Toxic Substances (TS-778)  
U.S. Environmental Protection Agency  
401 M Street, SW  
Washington DC 20460, USA



84960000007

*FYI-0396-001265*

Dear Sir

## Testing for Skin Sensitisation of Molybdenum Compounds

In 1991 and 1994, this Association published reports on the acute toxicity and ecotoxicity of certain molybdenum compounds.

The Association has now completed another stage in its test programme and I am enclosing reports on tests for skin sensitisation of the same molybdenum compounds.

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

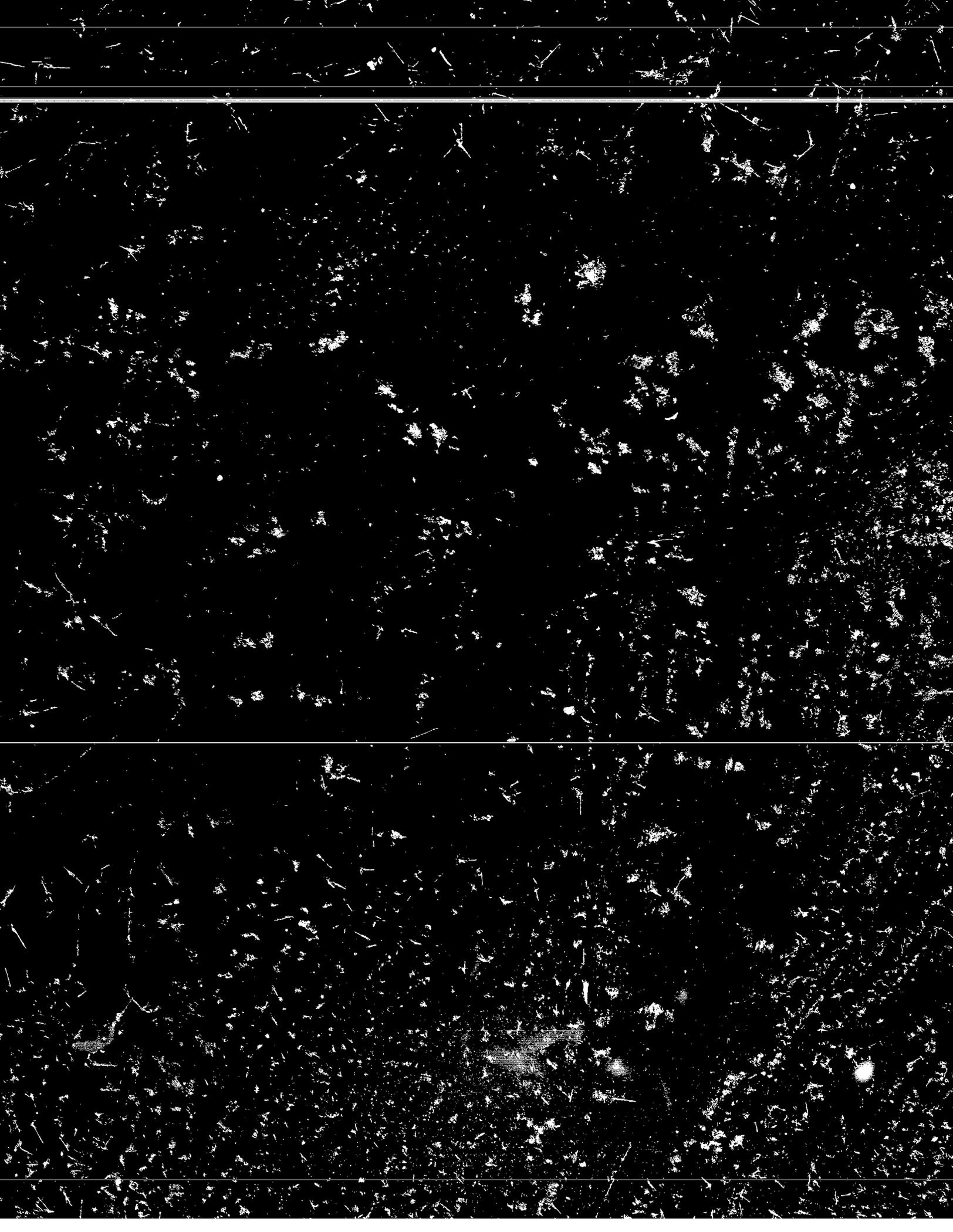
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**Huntingdon**  
Life Sciences

Report

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**MOLYBDENUM DYES (TECHNICAL)**

**SKIN SENSITISATION OF  
THE GUINEA-PIG**

Huntingdon Research Centre Limited changed its name to Huntingdon Life Sciences Limited  
with effect from 21 November 1995

**Sponsor**

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

**Testing facility**

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Report issued 23 January 1996

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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 I consider the data generated to be valid.

Good Laboratory Practice, The United Kingdom Compliance Programme, Department of Health & Social Security 1986 and subsequent revision, Department of Health 1989.

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).

*SA*

Sarah A. Allan, B.Sc. (Hons.), C.Biol., M.I.Biol.,  
Study Director,  
Huntingdon Life Sciences Ltd.

*23 June 96*

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

9 - 16 October 1992

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

16 October 1992

Date of reporting audit findings to the  
Study Director and Management

12 November 1992



Peter Watson,  
Quality Assurance Unit Head,  
Department of Quality Assurance,  
Huntingdon Life Sciences Ltd.

19.1.96

Date

920887D/IMA 15a/SS

**RESPONSIBLE PERSONNEL**

Sarah A. Allan, B.Sc. (Hons.), C.Biol., M.I.Biol.,  
Study Director,  
Department of Industrial Toxicology.

Handwritten signature of Sarah A. Allan, consisting of a stylized 'S' and 'A' followed by a horizontal line.

**SUMMARY**

A study was performed to assess the skin sensitisation potential of Molybdenum oxide (technical) using the guinea-pig. The method followed was that described in:

OECD Guideline for Testing of Chemicals No. 406 "Skin Sensitisation". Adopted: 12 May 1981.

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: 5% w/w in water for irrigation

Topical application: 70% w/w in distilled water

Challenge application: 70 and 35% w/w in distilled water

Twenty test and twenty control guinea-pigs were used in this study.

In this study performed using twenty guinea pigs Molybdenum oxide (technical) did not produce evidence of skin sensitisation (delayed contact hypersensitivity) in any of the test animals.

## INTRODUCTION

The study was designed to assess the skin sensitisation potential of Molybdenum oxide (technical) 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: 12 May 1981.

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 twenty test and twenty 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 the Study Director and HRC Management on 27 July 1992 and by the Sponsor on 31 July 1992.

The experimental phase was undertaken between 15 September and 17 October 1992.

**TEST SUBSTANCE**

**Identity:** Molybdenum oxide (technical)

**Chemical name:** Molybdenum oxide

**Lot number:** C

**Expiry:** Indefinite

**Purity:** 90 % MoO<sub>3</sub>

**Appearance:** Grey powder

**Storage conditions:** Room temperature

**Date received:** 8 June 1990

**EXPERIMENTAL PROCEDURE****ANIMAL MANAGEMENT**

Forty healthy female nulliparous and non-pregnant albino guinea-pigs of the Dunkin/Hartley strain were obtained from D. Hall, Newchurch, Staffordshire, England.

The animals were in the weight range of 295 to 353 g on arrival and approximately six to seven weeks of age. All the guinea-pigs were acclimatised to the experimental environment for twelve days prior to allocation to the main study.

An additional four 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	20	3950 to 3969
Test animals	20	3970 to 3989

The guinea-pigs were housed in groups of ten in suspended metal cages with wire mesh floors in Building R 17 Room 13.

A vitamin C enriched guinea-pig diet FD1 and drinking water were provided *ad libitum*. Hay was given weekly.

The batch of diet used for the study was not analysed for nutrients, possible contaminants or micro-organisms.

Results of routine physical and chemical examination of drinking water at source, as conducted usually weekly by the supplier, are made available to Huntingdon Research Centre Ltd. as quarterly summaries.

Animal room temperature was maintained at approximately 21°C and relative humidity at 30 - 70%. 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 HRC Industrial 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 HRC with formalin, a known sensitiser. 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 the appropriate vehicle.

The absorption of the test substance was not determined.

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

## **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 will 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.

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 oxide (technical) were selected:

**Induction intradermal injection** - 5% w/w in water for irrigation

**Induction topical application** - 70% w/w in distilled water

**Topical challenge** - 70 and 35% w/w in distilled water

From preliminary investigations 70% w/w in distilled water 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 x 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 2 x 4 cm 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 oxide (technical), 5% w/w in water for irrigation.
3. Molybdenum oxide (technical), 5% w/w in a 50 : 50 mixture of Freund's complete adjuvant and water for irrigation.

#### **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 x 60 mm interscapular area was clipped and shaved free of hair and the site was pre-treated by gentle rubbing with 0.2 ml per site of 10% w/w sodium lauryl sulphate in petrolatum. Twenty-four hours later a 20 x 40 mm patch of Whatman No. 3 paper was saturated with approximately 0.4 ml of Molybdenum oxide (technical), 70% w/w in distilled water. 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.

#### **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.

\* Difco Laboratories, Detroit 1, Michigan, U.S.A.

**Challenge****Challenge - control and test animals**

The control and test animals were challenged topically two weeks after the topical induction application using Molybdenum oxide (technical), 70 and 35% w/w in distilled water.

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 oxide (technical), 70% w/w in distilled water and applied to an anterior site on the flank. Molybdenum oxide (technical), 35% w/w in distilled water 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 are 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 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

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

Any other lesion not covered by this scoring system was described.

The challenge sites were evaluated 24, 48 and 72 hours after removal of the patches.

#### INTERPRETATION OF 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 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.

## RESULTS

### CLINICAL SIGNS

No signs of ill health or toxicity were recorded.

### BODYWEIGHT

Individual bodyweights are shown in Appendix 1.

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 oxide (technical), 5% w/w in water for irrigation and no irritation was observed in control animals receiving water for irrigation.

#### Topical application

Staining (grey) of the test sites prevented assessment of irritation in all test animals following topical application with Molybdenum oxide (technical), 70% w/w in distilled water.

Slight erythema was seen in the control guinea-pigs.

### CHALLENGE

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

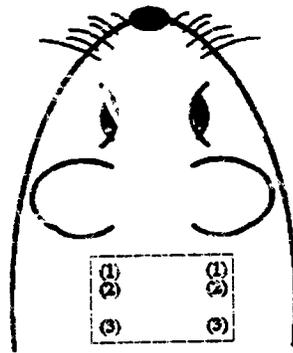
The dermal responses seen in the test animals were similar to those of the controls.

**CONCLUSION**

**In this test, performed in twenty albino guinea-pigs Molybdenum oxide (technical) did not produce evidence of skin sensitisation (delayed contact hypersensitivity) in any of the animals.**

FIGURE 1

Position of intradermal injections and topical induction application



The rectangle outlines the 2 x 4 cm clipped scapular area in which injections are made and to which the topical induction application is 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 water for irrigation.
- (3) 0.1 ml of Freund's complete adjuvant 50 : 50 with water for irrigation.

**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 oxide (technical), 5% w/w in water for irrigation.
- (3) 0.1 ml of Molybdenum oxide (technical), 5% w/w in a 50 : 50 mixture of water for irrigation and Freund's complete adjuvant.

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

TABLE I

Dermal reactions observed after each induction

Site	Intradermal injection		Topical application	
	Test animals	Control animals	Test animals	Control animals
1	Necrosis	Necrosis	G	Slight erythema
2	Slight irritation <sup>g</sup>	No irritation		
3	Necrosis <sup>g</sup>	Necrosis		

<sup>g</sup> Slight grey staining

G Unable to assess irritation due to grey staining

TABLE 2

Dermal reactions observed after the challenge application with  
Molybdenum oxide (technical)

Freund's treated controls

Guinea-pig number	E = Erythema O = Oedema	Score					
		24 Hours		48 Hours		72 Hours	
		A	P	A	P	A	P
3950	E	0	0	0*	0	0*	0
	O	0	0	0	0	0	0
3951	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
3952	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
3953	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
3954	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
3955	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
3956	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
3957	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
3958	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
3959	E	0	0	0*	0	0	0*
	O	0	0	0	0	0	0

\* Dryness and sloughing of the epidermis

A Anterior site, exposed to Molybdenum oxide (technical), 70% w/w in distilled water

P Posterior site, exposed to Molybdenum oxide (technical), 35% w/w in distilled water

Slight grey staining was observed on all sites

TABLE 2

(continued)

Freund's treated controls

Guinea-pig number	E = Erythema O = Oedema	Score					
		24 Hours		48 Hours		72 Hours	
		A	P	A	P	A	P
3960	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
3961	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
3962	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
3963	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
3964	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
3965	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
3966	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
3967	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
3968	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
3969	E	0	0	0	0	0	0
	O	0	0	0	0	0	0

A Anterior site, exposed to Molybdenum oxide (technical), 70% w/w in distilled water  
 P Posterior site, exposed to Molybdenum oxide (technical), 35% w/w in distilled water

Slight grey staining was observed on all sites

TABLE 2

(continued)

Test animals

Guinea-pig number	E = Erythema O = Oedema	Score						Results Positive (+) Negative (-) Inconclusive (±)
		24 Hours		48 Hours		72 Hours		
		A	P	A	P	A	P	
3970	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	
3971	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	
3972	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	
3973	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	
3974	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	
3975	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	
3976	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	
3977	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	
3978	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	
3979	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	

A Anterior site, exposed to Molybdenum oxide (technical), 70% w/w in distilled water

P Posterior site, exposed to Molybdenum oxide (technical), 35% w/w in distilled water

Slight grey staining was observed on all sites

TABLE 2

(continued)

## Test animals

Guinea-pig number	E = Erythema O = Oedema	Score						Results Positive (+) Negative (-) Inconclusive (±)
		24 Hours		48 Hours		72 Hours		
		A	P	A	P	A	P	
3980	E O	0 0	0 0	0 0	0 0	0 0	0 0	-
3981	E O	0 0	0 0	0 0	0 0	0 0	0 0	-
3982	E O	0 0	0 0	0 0	0 0	0 0	0 0	-
3983	E O	0 0	0 0	0 0	0 0	0 0	0 0	-
3984	E O	0 0	0 0	0 0	0 0	0 0	0 0	-
3985	E O	0 0	0 0	0 0	0 0	0 0	0 0	-
3986	E O	0 0	0 0	0 0	0 0	0 0	0 0	-
3987	E O	0 0	0 0	0* 0	0 0	0* 0	0 0	-
3988	E O	0 0	0 0	0* 0	0 0	0* 0	0 0	-
3989	E O	0 0	0 0	0 0	0 0	0 0	0 0	-

\* Dryness and sloughing of the epidermis

A Anterior site, exposed to Molybdenum oxide (technical), 70% w/w in distilled water

P Posterior site, exposed to Molybdenum oxide (technical), 35% w/w in distilled water

Slight grey staining was observed on all sites

## APPENDIX 1

## Individual bodyweights (g)

Group	Guinea-pig number	Day 1 22 September 1992	Last day of study 17 October 1992
Control	3950	377	498
	3951	375	490
	3952	409	522
	3953	432	576
	3954	419	578
	3955	428	599
	3956	412	597
	3957	418	519
	3958	441	557
	3959	385	481
	3960	379	503
	3961	386	591
	3962	396	562
	3963	400	565
	3964	423	541
	3965	445	593
3966	377	541	
3967	402	631	
3968	431	513	
3969	426	510	
Test	3970	434	573
	3971	413	546
	3972	410	586
	3973	408	559
	3974	419	586
	3975	444	606
	3976	399	512
	3977	395	545
	3978	407	602
	3979	381	574
	3980	426	579
	3981	446	637
	3982	421	527
	3983	443	610
	3984	393	537
	3985	395	602
3986	435	604	
3987	399	516	
3988	397	536	
3989	405	589	

## APPENDIX 2

## Results of preliminary investigations with Molybdenum oxide (technical)

## Intradermal injections

Vehicle: water for irrigation

Guinea-pig Number	Concentration % w/w	Score		
		Hours	24	72
3853	10.0	D	12	12
		E	NG	NG
		O	2	2
	7.5	D	12	10
		E	NG	NG
		O	2	2
	5.0	D	8	6
		E	G2	G2
		O	2	2
2.5	D	8	6	
	E	G2	G2	
	O	2	1	
1.0	D	6	5	
	E	G2	G2	
	O	2	1	
0.5	D	5	5	
	E	G2	G1	
	O	1	1	
0.25	D	5	4	
	E	1	1	
	O	1	1	
0.1	D	4	2	
	E	1	1	
	O	1	0	
Vehicle control	D	3	2	
	E	1	0	
	O	1	0	

Guinea-pig Number	Concentration % w/w	Score		
		Hours	24	72
3855	10.0	D	12	12
		E	NG	NG
		O	2	2
	7.5	D	12	10
		E	NG	NG
		O	2	2
	5.0	D	9	8
		E	G2	G2
		O	2	2
2.5	D	8	6	
	E	G2	G2	
	O	2	1	
1.0	D	6	5	
	E	G2	G2	
	O	2	1	
0.5	D	5	4	
	E	G2	G1	
	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	0	
	E	1	0	
	O	1	0	

## Key:

- D Diameter (mm)
- E Erythema (0-4 numerical scores)
- O Oedema (0-4 numerical scores)
- N Necrosis
- G Slight grey staining

## APPENDIX 2

(continued)

## Topical application

Vehicle: distilled water

Guinea-pig number	Concentration % w/w	Score					
		0 Hours		24 Hours		48 Hours	
		E	O	E	O	E	O
3856	70	0	0	0	0	0	0
	60	0	0	0	0	0	0
	50	0	0	0	0	0	0
	30	0	0	0	0	0	0
3857	70	0	0	0	0	0	0
	60	0	0	0	0	0	0
	50	0	0	0	0	0	0
	30	0	0	0	0	0	0
3858	70	0	0	0	0	0	0
	60	0	0	0	0	0	0
	50	0	0	0	0	0	0
	30	0	0	0	0	0	0
3859	70	0	0	0	0	0	0
	60	0	0	0	0	0	0
	50	0	0	0	0	0	0
	30	0	0	0	0	0	0

E Erythema (0-4 numerical scores)

O Oedema (0-4 numerical scores)

Slight grey staining was observed on all dose sites

## APPENDIX 3

Summary of positive control data  
Magnesium and Klignan test using formalin

M&K RAD No.	Number of female animals		Dates of study		Dates of receipt of Formalin	Dose levels % (aqueous dilutions)		Challenge	Results		
	Test	Control	Start	Finish		Intradermal	Topical		Positive	Inconclusive	Negative
26	10	10	18.01.88	12.02.88	05.08.87	0.1	10	5 and 1	10/10	0/10	0/10
27	10	10	21.07.88	15.08.88	13.05.88	0.1	10	5 and 1	10/10	0/10	0/10
28	10	10	10.01.89	04.02.89	16.12.88	0.1	10	5 and 1	10/10	0/10	0/10
29	10	10	17.07.89	11.08.89	15.05.89	0.1	10	5 and 1	10/10	0/10	0/10
30	1	10	31.10.89	25.11.89	15.05.89	0.1	10	5 and 1	10/10	0/10	0/10
31	10	10	16.01.90	10.02.90	29.11.89	0.1	10	5 and 1	10/10	0/10	0/10
32	10	10	23.07.90	24.09.90	04.07.90	0.05	5	3 and 1, 5 5 and 3**	5/10	0/10	5/10
33	10	10	30.10.90	24.11.90	04.07.90	0.1	10	5 and 1	10/10	0/10	0/10
34	10	10	15.01.91	09.02.91	09.01.91	0.1	10	5 and 1	8/10	1/10	1/10
35	10	10	06.03.91	31.03.91	09.01.91	0.1	10	5 and 1	10/10	0/10	0/10
36	10	10	12.03.91	06.04.91	09.01.91	0.1	10	5 and 1	10/10	0/10	0/10
37	10	10	15.08.91	09.09.91	19.06.91	0.1	10	5 and 1	10/10	0/10	0/10
38	10	10	26.11.91	21.12.91	19.06.91	0.1	10	5 and 1	10/10	0/10	0/10
39	10	10	10.02.92	13.03.92	09.01.92	0.1	10	5 and 1	7/10	0/10	3/10
40	10	10	13.08.92	08.09.92	29.06.92	0.1	10	5 and 1**	10/10	0/10	0/10

I. Animals supplied by Interfauna, Huntingdon, Cambridgeshire, England  
 All other animals supplied by D. Hall, Newchurch, Staffordshire, England  
 (Formalin obtained from Savory and Moore, Huntingdon, Cambridgeshire, England or from 1991, supplied by Thornton and Ross, Huddersfield, England)  
 \*\* Second challenge

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**Huntingdon  
Life Sciences**

**Report**

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**CONFIDENTIAL**

**920916D/IMA 15d/SS**

**AMMONIUM DIMOLYBDATE**

**SKIN SENSITISATION IN  
THE GUINEA-PIG**

Huntingdon Research Centre Limited changed its name to Huntingdon Life Sciences Limited  
with effect from 21 November 1995

**Sponsor**

International Molybdenum Association,  
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Report issued 23 January 1996

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0 0 3 5

**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.

Good Laboratory Practice, The United Kingdom Compliance Programme, Department of Health & Social Security 1986 and subsequent revision, Department of Health 1989.

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).



Sarah A. Allan, B.Sc. (Hons.), C.Biol., M.I.Biol.,  
Study Director,  
Huntingdon Life Sciences Ltd.

23 Mar 96  
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

9 - 16 October 1992

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

16 October 1992

Date of reporting audit findings to the  
Study Director and Management

12 November 1992



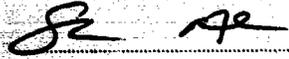
Peter Watson,  
Quality Assurance Unit Head,  
Department of Quality Assurance,  
Huntingdon Life Sciences Ltd.

19.1.96

Date

**RESPONSIBLE PERSONNEL**

Sarah A. Allan, B.Sc. (Hons.), C.Biol., M.I.Biol.,  
Study Director,  
Department of Industrial Toxicology.

A handwritten signature in black ink, appearing to be 'SA Allan', written over a horizontal dotted line.

**SUMMARY**

A study was performed to assess the skin sensitisation potential of Ammonium dimolybdate using the guinea-pig. The method followed was that described in:

OECD Guideline for Testing of Chemicals No. 406 "Skin Sensitisation". Adopted: 12 May 1981.

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: 0.1% w/w in water for irrigation
- Topical application: 90% w/w in distilled water
- Challenge application: 90 and 45% w/w in distilled water

Twenty test and twenty control guinea-pigs were used in this study.

In this study performed using twenty guinea pigs Ammonium dimolybdate produced evidence of skin sensitisation (delayed contact hypersensitivity) in four test animals. A second challenge application confirmed this result for one animal only.

## INTRODUCTION

The study was designed to assess the skin sensitisation potential of Ammonium dimolybdate 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: 12 May 1981.

The method used was the guinea-pig maximisation test described by MAGNUSSON, B. and KLIEMAN, 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 twenty test and twenty 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 the Study Director and HRC Management on 27 July 1992 and by the Sponsor on 31 July 1992.

The experimental phase of the main study was undertaken between 16 September and 31 October 1992.

920916D/IMA 15d/SS

**TEST SUBSTANCE**

**Identity:** Ammonium dimolybdate

**Chemical name:** Ammonium dimolybdate

**Lot number:** 35

**Expiry:** Not available to HRC

**Composition:** Mo: 56.4%  
NH<sub>4</sub>: 10.5%

**Appearance:** White powder

**Storage conditions:** Room temperature

**Date received:** 8 June 1990

0 0 4 1

**EXPERIMENTAL PROCEDURE****ANIMAL MANAGEMENT**

Forty healthy female nulliparous and non-pregnant albino guinea-pigs of the Dunkin/Hartley strain were obtained from D. Hall, Newchurch, Staffordshire, England.

The animals were in the weight range of 280 to 332 g on arrival and approximately six to seven weeks of age. All the guinea-pigs were acclimatised to the experimental environment for nineteen days prior to allocation to the main study.

An additional six 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	20	4030 to 4049
Test animals	20	4050 to 4069

The guinea-pigs were housed in groups of ten in suspended metal cages with wire mesh floors in Building R 17 Room 13.

A vitamin C enriched guinea-pig diet FD1 and drinking water were provided *ad libitum*. Hay was given weekly.

The batch of diet used for the study was not analysed for nutrients, possible contaminants or micro-organisms.

Results of routine physical and chemical examination of drinking water at source, as conducted usually weekly by the supplier, are made available to Huntingdon Research Centre Ltd. as quarterly summaries.

Animal room temperature was maintained at approximately 21°C and relative humidity at 30 - 70%. 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 HRC Industrial 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 HRC with formalin, a known sensitiser. 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 the appropriate vehicle.

The absorption of the test substance was not determined.

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

### **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 will 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.

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 Ammonium dimolybdate were selected:

**Induction intradermal injection** - 0.1% w/w in water for irrigation

This was the maximum practical concentration that could be prepared and dosed intradermally.

**Induction topical application** - 90% w/w in distilled water

**Topical challenge** - 90 and 45% w/w in distilled water

From preliminary investigations 90% w/w in distilled water 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 x 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 2 x 4 cm 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. Ammonium dimolybdate, 0.1% w/w in water for irrigation.
3. Ammonium dimolybdate, 0.1% w/w in a 50 : 50 mixture of Freund's complete adjuvant and water for irrigation.

**Induction topical application - test animals**

The preliminary investigations indicated that the maximum practical concentration of the test substance for topical application (90%) did not produce skin irritation. Therefore, six days after the injections, the same 40 x 60 mm interscapular area was clipped and shaved free of hair and the site was pre-treated by gentle rubbing with 0.2 ml per site of 10% w/w sodium lauryl sulphate in petrolatum. Twenty-four hours later a 20 x 40 mm patch of Whatman No. 3 paper was saturated with approximately 0.4 ml of Ammonium dimolybdate, 90% w/w in distilled water. 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.

**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.

\* Difco Laboratories, Detroit 1, Michigan, U.S.A.

8-044

**Challenge****Challenge - control and test animals**

The control and test animals were challenged topically two weeks after the topical induction application using Ammonium dimolybdate, 90 and 45% w/w in distilled water.

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 Ammonium dimolybdate, 90% w/w in distilled water and applied to an anterior site on the flank. Ammonium dimolybdate, 45% w/w in distilled water 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 "Steek".

A second challenge application was made one week later to differentiate between irritation and allergic responses following observation of irritant reactions in the control animals after the first challenge. The method employed was similar to that described above, with the exception that on this occasion Ammonium dimolybdate, 50 and 25% w/w in distilled water, was applied to the right flank of all the control and test animals.

**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 are 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 challenges 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 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

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

Any other lesion not covered by this scoring system was described.

The challenge sites were evaluated 24, 48 and 72 hours after removal of the patches.

**INTERPRETATION OF 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 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.

## RESULTS

### CLINICAL SIGNS

No signs of ill health or toxicity were recorded.

### BODYWEIGHT

Individual bodyweights are shown in Appendix 1.

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 Ammonium dimolybdate, 0.1% w/w in water for irrigation and no irritation was observed in control animals receiving water for irrigation.

#### Topical application

Slight erythema was observed in test animals following topical application with Ammonium dimolybdate, 90% w/w in distilled water.

Very slight erythema was seen in the control guinea-pigs.

### CHALLENGE

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

The dermal responses seen at the anterior sites (90% w/w in distilled water) for control and test animals were similar, irritation (well-defined to moderate in some cases) was recorded for most animals. Results were assessed using the posterior site only (virtually no dermal irritation for controls) and the responses seen were more marked for four of the twenty test animals than those of the controls.

### **SECOND CHALLENGE**

The numerical values given to the dermal reactions elicited by the second challenge application are shown in Table 3.

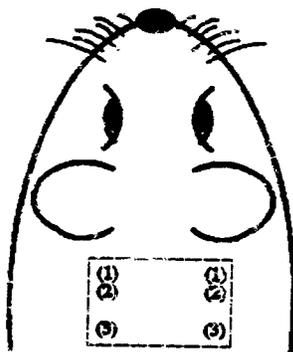
The dermal response seen for one test animal was more marked than those of the controls. This animal had showed a positive response after the first challenge application. One further animal showed an inconclusive response.

### **CONCLUSION**

In this test, performed in twenty albino guinea-pigs Ammonium dimolybdate produced evidence of skin sensitisation (delayed contact hypersensitivity) in four animals. This was confirmed by a second challenge application for one animal only.

FIGURE 1

Position of intradermal injections and topical induction application



The rectangle outlines the 2 x 4 cm clipped scapular area in which injections are made and to which the topical induction application is 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 water for irrigation.
- (3) 0.1 ml of Freund's complete adjuvant 50 : 50 with water for irrigation.

**Test animals:**

- (1) 0.1 ml of Freund's complete adjuvant 50 : 50 with water for irrigation (Ph. Eur.).
- (2) 0.1 ml of Ammonium dimolybdate, 0.1% w/w in water for irrigation.
- (3) 0.1 ml of Ammonium dimolybdate, 0.1% w/w in a 50 : 50 mixture of water for irrigation and Freund's complete adjuvant.

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

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

Site	Intradermal injection		Topical application	
	Test animals	Control animals	Test animals	Control animals
1	Necrosis	Necrosis		
2	Slight irritation	No irritation	Slight erythema <sup>a</sup>	Very slight erythema
3	Necrosis	Necrosis		

<sup>a</sup> Slight grey staining

TABLE 2

Dermal reactions observed after the challenge application with  
Ammonium dimolybdate

Freund's treated controls

Guinea-pig number	E = Erythema O = Oedema	Score					
		24 Hours		48 Hours		72 Hours	
		A	P	A	P	A	P
4030	E	2	0	Ø2	0*	Ø2	0*
	O	1	0	1	0	1	0
4031	E	L2	0	L2	0*	L1	0*
	O	NP0	0	NP0	0	NP0	0
4032	E	2	0	Ø2	0*	Ø2	0*
	O	NP1	0	NP1	0	NP1	0
4033	E	N	0	ØN	0*	ØN	0*
	O	2	0	3	0	3	0
4034	E	0	0	1*	0*	L1*	0*
	O	0	0	0	0	0	0
4035	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
4036	E	2	0	ØN	0*	ØN	0*
	O	NP1	0	2	0	2	0
4037	E	2	0	ØN	0*	ØN	0*
	O	2	0	2	0	2	0
4038	E	L2	0	L1*	0*	0*	0*
	O	0	0	0	0	0	0
4039	E	L1	0	L2	0	L1	0
	O	0	0	0	0	0	0

L Localised dermal reaction (restricted to a small area of the challenge site)

N Necrosis

NP Necrotic patch

\* Dryness and sloughing of the epidermis

Ø Thickening, dryness and sloughing of the epidermis

A Anterior site, exposed to Ammonium dimolybdate, 90% w/w in distilled water

P Posterior site, exposed to Ammonium dimolybdate, 45% w/w in distilled water

TABLE 2

(continued)

Freund's treated controls

Guinea-pig number	E = Erythema O = Oedema	Score					
		24 Hours		48 Hours		72 Hours	
		A	P	A	P	A	P
4040	E	0	L1	0*	0*	0*	0*
	O	0	0	0	0	0	0
4041	E	2	0	Ø2	0	Ø2	0
	O	NP1	0	NP1	0	NP1	0
4042	E	0	0	0*	0*	0*	0*
	O	0	0	0	0	0	0
4043	E	0	0	0*	0*	0*	0
	O	0	0	0	0	0	0
4044	E	0	0	0*	0	0*	0
	O	0	0	0	0	0	0
4045	E	0	0	L1*	0	0*	0
	O	0	0	0	0	0	0
4046	E	0	0	0	0*	0	0*
	O	0	0	0	0	0	0
4047	E	L1	0	Ø1	0	Ø1	0
	O	0	0	1	0	1	0
4048	E	0	0	0*	0	0*	0
	O	0	0	0	0	0	0
4049	E	0	0	0	0	0	0
	O	0	0	0	0	0	0

- L Localised dermal reaction (restricted to a small area of the challenge site)  
 \* Dryness and sloughing of the epidermis  
 Ø Thickening, dryness and sloughing of the epidermis  
 A Anterior site, exposed to Ammonium dimolybdate, 90% w/w in distilled water  
 P Posterior site, exposed to Ammonium dimolybdate, 45% w/w in distilled water

TABLE 2

(continued)

Test animals

Guinea-pig number	E = Erythema O = Oedema	Score						Results <sup>†</sup> Positive (+) Negative (-) Inconclusive (±)
		24 Hours		48 Hours		72 Hours		
		A	P	A	P	A	P	
4050	E	2	0	ØN	0*	ØN	0*	-
	O	1	0	2	0	2	0	
4051	E	2	0	ØN	0	ØN	0	-
	O	2	0	2	0	2	0	
4052	E	0	0	L1*	0	0*	0	-
	O	0	0	0	0	0	0	
4053	E	1	0	Ø2	0	Ø2	0	-
	O	0	0	1	0	1	0	
4054	E	2	0	2	0	2	0	-
	O	2	0	NP2	0	NP2	0	
4055	E	2	0	ØØ2	0*	Ø2	0*	-
	O	2	0	2	0	2	0	
4056	E	2	0	Ø2	0*	Ø2	0*	-
	O	1	0	2	0	2	0	
4057	E	L2	L2	Ø2	L2*	Ø2	L2*	+
	O	1	0	1	0	2	0	
4058	E	0	0	Ø2	0	Ø2	0	-
	O	0	0	1	0	2	0	
4059	E	L2	0	Ø2	0*	Ø2	0*	-
	O	0	0	1	0	1	0	

L Localised dermal reaction (restricted to a small area of the challenge site)

N Necrosis

NP Necrotic patch

\* Dryness and sloughing of the epidermis

Ø Thickening, dryness and sloughing of the epidermis

A Anterior site, exposed to Ammonium dimolybdate, 90% w/w in distilled water

P Posterior site, exposed to Ammonium dimolybdate, 45% w/w in distilled water

† Result assessed on the posterior site only

TABLE 2

(continued)

## Test animals

Guinea-pig number	E = Erythema O = Oedema	Score						Results† Positive (+) Negative (-) Inconclusive (±)
		24 Hours		48 Hours		72 Hours		
		A	P	A	P	A	P	
4060	E	2	0	Ø2	0	Ø2	0	-
	O	1	0	2	0	2	0	
4061	E	2	0	ØN	0	ØN	0	-
	O	NP3	0	3	0	3	0	
4062	E	2	0	Ø2	0	Ø2	0	-
	O	NP1	0	NP2	0	NP2	0	
4063	E	L1	0	L1*	0	L1*	0	-
	O	0	0	0	0	0	0	
4064	E	0	L1	L1*	L1*	L1*	L1*	+
	O	0	0	0	0	0	0	
4065	E	2	0	Ø2	0*	Ø2	0*	-
	O	2	0	2	0	2	0	
4066	E	2	L2	ØN	L2*	ØN	2*	+
	O	NP2	0	2	0	2	0	
4067	E	0	0	1*	0*	L2*	0*	-
	O	0	0	0	0	0	0	
4068	E	2	1	ØN	Ø2	ØN	Ø2	+
	O	2	1	3	1	3	1	
4069	E	2	0	Ø2	0*	Ø2	0*	-
	O	1	0	1	0	1	0	

- L Localised dermal reaction (restricted to a small area of the challenge site)  
 N Necrosis  
 NP Necrotic patch  
 \* Dryness and sloughing of the epidermis  
 Ø Thickening, dryness and sloughing of the epidermis  
 A Anterior site, exposed to Ammonium dimolybdate, 90% w/w in distilled water  
 P Posterior site, exposed to Ammonium dimolybdate, 45% w/w in distilled water  
 † Result assessed on the posterior site only

0054

TABLE 3

Dermal reactions observed after the second challenge application with  
Ammonium dimolybdate

Freund's treated controls

Guinea-pig number	E = Erythema O = Oedema	Score					
		24 Hours		48 Hours		72 Hours	
		A	P	A	P	A	P
4030	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
4031	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
4032	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
4033	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
4034	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
4035	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
4036	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
4037	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
4038	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
4039	E	0	0	0	0	0	0
	O	0	0	0	0	0	0

A Anterior site, exposed to Ammonium dimolybdate, 50% w/w in distilled water  
P Posterior site, exposed to Ammonium dimolybdate, 25% w/w in distilled water

TABLE 3

(continued)

Freund's treated controls

Guinea-pig number	E = Erythema O = Oedema	Score					
		24 Hours		48 Hours		72 Hours	
		A	P	A	P	A	P
4040	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
4041	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
4042	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
4043	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
4044	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
4045	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
4046	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
4047	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
4048	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
4049	E	0	0	0	0	0	0
	O	0	0	0	0	0	0

A Anterior site, exposed to Ammonium dimolybdate, 50% w/w in distilled water  
 P Posterior site, exposed to Ammonium dimolybdate, 25% w/w in distilled water

TABLE 3

(continued)

Test animals

Guinea-pig number	E = Erythema O = Oedema	Score						Results Positive (+) Negative (-) Inconclusive (±)
		24 Hours		48 Hours		72 Hours		
		A	P	A	P	A	P	
4050	E	0*	0*	0*	0*	0	0*	-
	O	0	0	0	0	0	0	
4051	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	
4052	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	
4053	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	
4054	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	
4055	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	
4056	E	1	0	0	0	0	0	±
	O	0	0	0	0	0	0	
4057	E	L2	0	L1	0	L1	0	+
	O	0	0	0	0	0	0	
4058	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	
4059	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	

L Localised dermal reaction (restricted to a small area of the challenge site)

\* Dryness and sloughing of the epidermis

A Anterior site, exposed to Ammonium dimolybdate, 50% w/w in distilled water

P Posterior site, exposed to Ammonium dimolybdate, 25% w/w in distilled water

TABLE 3

(continued)

## Test animals

Guinea-pig number	E = Erythema O = Oedema	Score						Results Positive (+) Negative (-) Inconclusive (±)
		24 Hours		48 Hours		72 Hours		
		A	P	A	P	A	P	
4060	E O	0	0	0	0	0	0	-
4061	E O	0	0	0	0	0	0	-
4062	E O	0	0	0	0	0	0	-
4063	E O	0	0	0	0	0	0	-
4064	E O	0	0	0	0	0	0	-
4065	E O	L1	0	0	0	0	0	-
4066	E O	0	0	0	0	0	0	-
4067	E O	0	0	0	0	0	0	-
4068	E O	0	0	0	0	0	0	-
4069	E O	0	0	0	0	0	0	-

- L Localised dermal reaction (restricted to a small area of the challenge site)  
 A Anterior site, exposed to Ammonium dimolybdate, 50% w/w in distilled water  
 P Posterior site, exposed to Ammonium dimolybdate, 25% w/w in distilled water

## APPENDIX 1

## Individual bodyweights (g)

Group	Guinea-pig number	Day 1 29 September 1992	Last day of study 31 October 1992
Control	4030	496	700
	4031	482	660
	4032	499	606
	4033	458	602
	4034	490	668
	4035	476	669
	4036	482	670
	4037	485	626
	4038	489	636
	4039	469	621
	4040	443	636
	4041	516	678
	4042	491	600
	4043	497	649
	4044	455	601
	4045	474	646
	4046	478	651
4047	494	668	
4048	452	599	
4049	461	586	
Test	4050	434	572
	4051	478	598
	4052	447	530
	4053	497	601
	4054	466	592
	4055	448	596
	4056	444	598
	4057	463	616
	4058	459	642
	4059	480	660
	4060	482	701
	4061	463	612
	4062	419	596
	4063	488	699
	4064	516	704
	4065	474	672
	4066	443	531
4067	458	598	
4068	482	679	
4069	404	592	

## APPENDIX 2

## Results of preliminary investigations with Ammonium dimolybdate:

## Intradermal injections

Vehicle: water for irrigation

Guinea-pig Number	Concentration % w/w	Score		
		Hours	24	72
3690	10.0	D	12	12
		E	N	N
		O	2	2
	7.5	D	10	10
		E	N	N
		O	2	2
	5.0	D	10	10
		E	N	N
		O	2	2
2.5	D	10	9	
	E	N	N	
	O	2	2	
1.0	D	8	8	
	E	N	N	
	O	2	2	
0.5	D	8	8	
	E	N	N	
	O	2	2	
0.25	D	8	6	
	E	SN	SN	
	O	2	2	
0.1	D	6	6	
	E	2	2	
	O	2	2	
Vehicle control	D	2	0	
	E	1	0	
	O	1	0	

Guinea-pig Number	Concentration % w/w	Score		
		Hours	24	72
3691	10.0	D	15	14
		E	N	N
		O	2	2
	7.5	D	12	12
		E	N	N
		O	2	2
	5.0	D	10	10
		E	N	N
		O	2	2
2.5	D	8	8	
	E	N	N	
	O	2	2	
1.0	D	8	8	
	E	N	N	
	O	2	2	
0.5	D	8	8	
	E	N	N	
	O	2	2	
0.25	D	8	6	
	E	SN	SN	
	O	2	2	
0.1	D	6	6	
	E	2	2	
	O	2	2	
Vehicle control	D	3	0	
	E	1	0	
	O	1	0	

Key:

- D Diameter (mm)
- E Erythema (0-4 numerical scores)
- O Oedema (0-4 numerical scores)
- N Necrosis
- SN Slight necrosis

## APPENDIX 2

(continued)

## Topical application

Vehicle: distilled water

Guinea-pig number	Concentration % w/w	Score					
		0 Hours		24 Hours		48 Hours	
		E	O	E	O	E	O
3692	90	0	0	0	0	0	0
	70	0	0	0	0	0	0
	50	0	0	0	0	0	0
	30	0	0	0	0	0	0
3693	90	0	0	0	0	0	0
	70	0	0	0	0	0	0
	50	0	0	0	0	0	0
	30	0	0	0	0	0	0
3694	90	0	0	0	0	0	0
	70	0	0	0	0	0	0
	50	0	0	0	0	0	0
	30	0	0	0	0	0	0
3695	90	0	0	0	0	0	0
	70	0	0	0	0	0	0
	50	0	0	0	0	0	0
	30	0	0	0	0	0	0

E Erythema (0-4 numerical scores)

O Oedema (0-4 numerical scores)

**APPENDIX 3**  
**Summary of positive control data**  
**Magnason and Kligman test using formalin**

M&K R&D No.	Number of female animals		Dates of study:		Dates of receipt of Formalin	Dose levels % (aqueous dilutions)		Challenge	Results		
	Test	Control	Start	Finish		Induction			Positive	Inoculative	Negative
						Intradermal	Topical				
26	10	10	18.01.88	12.02.88	05.08.87	0.1	10	5 and 1	10/10	0/10	0/10
27	10	10	21.07.88	15.08.88	13.05.88	0.1	10	5 and 1	10/10	0/10	0/10
28	10	10	10.01.89	0.02.89	16.12.88	0.1	10	5 and 1	10/10	0/10	0/10
29	10	10	17.07.89	11.08.89	15.05.89	0.1	10	5 and 1	10/10	0/10	0/10
30	1	10	31.10.89	25.11.89	15.05.89	0.1	10	5 and 1	10/10	0/10	0/10
31	10	10	16.01.90	10.02.90	29.11.89	0.1	10	5 and 1	10/10	0/10	0/10
32	10	10	23.07.90	24.09.90	04.07.90	0.05	5	3 and 1.5	5/10	0/10	0/10
33	10	10	30.10.90	24.11.90	04.07.90	0.1	10	5 and 1	10/10	0/10	0/10
34	10	10	15.01.91	09.02.91	09.01.91	0.1	10	5 and 1	8/10	1/10	1/10
35	10	10	06.03.91	31.03.91	09.01.91	0.1	10	5 and 1	10/10	0/10	0/10
36	10	10	12.03.91	06.04.91	09.01.91	0.1	10	5 and 1	10/10	0/10	0/10
37	10	10	15.08.91	09.09.91	19.06.91	0.1	10	5 and 1	10/10	0/10	0/10
38	10	10	26.11.91	21.12.91	19.06.91	0.1	10	5 and 1	10/10	0/10	0/10
39	10	10	10.02.92	13.03.92	09.01.92	0.1	10	5 and 1	7/10	0/10	3/10
40	10	10	13.08.92	08.09.92	29.06.92	0.1	10	5 and 1**	10/10	0/10	0/10

1. Animals supplied by Interfarma, Huntingdon, Cambridgeshire, England  
 All other animals supplied by D. Hall, Newchurch, Salfordshire, England  
 (Formalin obtained from Severy and Moore, Huntingdon, Cambridgeshire, England or from 1991, supplied by Thornton and Ross, Huddersfield, England)  
 \*\* Second challenge

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**Huntingdon  
Life Sciences**

**Report**

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**CONFIDENTIAL**

**IMA 16a/930983/SS**

**SODIUM MOLYBDATE 241/32**

**SKIN SENSITISATION IN  
THE GUINEA-PIG**

Huntingdon Research Centre Limited changed its name to Huntingdon Life Sciences Limited  
with effect from 2<sup>1</sup> November 1995

**Sponsor**

International Molybdenum Association,  
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Report issued 23 January 1996

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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 I consider the data generated to be valid.

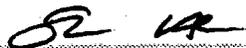
Good Laboratory Practice, The United Kingdom Compliance Programme, Department of Health & Social Security 1986 and subsequent revision, Department of Health 1989.

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).



Sarah A. Allan, B.Sc. (Hons.), C.Biol., M.I.Biol.,  
Study Director,  
Huntingdon Life Sciences Ltd.

  
Date

**QUALITY ASSURANCE STATEMENT**

Certain studies such as that described in this report, are conducted at HRC 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 HRC Management.

This report has been audited by the Huntingdon Research Centre Quality Assurance Department. The methods, practices and procedures reported herein are an accurate description of those employed at HRC 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 HRC.

Date(s) of inspection

8-12 March 1993

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

12 March 1993

Date of reporting audit findings to the  
Study Director and HRC Management

28 June 1993

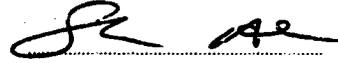
  
\_\_\_\_\_  
P. Watson,  
Systems Compliance Auditor,  
Department of Quality Assurance,  
Huntingdon Research Centre Ltd.

30.6.93

IMA 16a/930983/SS

**RESPONSIBLE PERSONNEL**

**Sarah A. Allan, B.Sc. (Hons.), C.Biol., M.I.Biol.,  
Study Director,  
Department of Industrial Toxicology.**

Handwritten signature of Sarah A. Allan, consisting of a stylized 'S' and 'A'.

**SUMMARY**

A study was performed to assess the skin sensitisation potential of Sodium molybdate 241/32 using the guinea-pig. The method followed was that described

OECD Guideline for Testing of Chemicals No. 406 "Skin Sensitisation". Adopted: 12 May 1981.

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: 5% w/w in water for irrigation
- Topical application: 10% w/w in distilled water
- Challenge application: 70 and 35% w/w in distilled water

Twenty test and twenty control guinea-pigs were used in this study.

In this study Sodium molybdate 241/32 did not produce evidence of skin sensitisation (delayed contact hypersensitivity) in any of the test animals.

## INTRODUCTION

The study was designed to assess the skin sensitisation potential of Sodium molybdate 241/32 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: 12 May 1981.

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 twenty test and twenty 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 the Study Director and HRC Management on 31 March 1993 and by the Sponsor on 2 April 1993.

The experimental phase of the study was undertaken between 30 April and 4 June 1993.

**TEST SUBSTANCE**

**Identity:** Sodium molybdate 241/32

**Chemical name:** Not available to HRC

**Batch number:** 241/32

**Expiry:** Not available to HRC

**Purity/Composition:** See certificate of analysis (Appendix 4)

**Appearance:** White powder

**Storage conditions:** Room temperature

**Date received:** 24 March 1993

**EXPERIMENTAL PROCEDURE****ANIMAL MANAGEMENT**

Forty healthy female nulliparous and non-pregnant albino guinea-pigs of the Dunkin/Hartley strain were obtained from D. Hall, Newchurch, Staffordshire, England.

The animals were in the weight range of 286 to 377 g on arrival and approximately six to seven weeks of age. All the guinea-pigs were acclimatised to the experimental environment for 4 days prior to allocation to the main study.

An additional six 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	20	1550 to 1569
Test animals	20	1570 to 1589

The guinea-pigs were housed in groups of ten in suspended metal cages with wire mesh floors in Building R 17 Room 13.

A vitamin C enriched guinea-pig diet FD1 and drinking water were provided *ad libitum*. Hay was given weekly.

The batch of diet used for the study was not analysed for nutrients, possible contaminants or micro-organisms.

Results of routine physical and chemical examination of drinking water at source, as conducted usually weekly by the supplier, are made available to Huntingdon Research Centre Ltd. as quarterly summaries.

Animal room temperature was maintained at approximately 21°C and relative humidity at 30 - 70%. 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 HRC Industrial 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 HRC with formalin, a known sensitiser. 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 water for irrigation. The absorption of the test substance was not determined.

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

### **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.

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 Sodium molybdate 241/32 were selected:

**Induction intradermal injection** - 5% w/w in water for irrigation

**Induction topical application** - 70% w/w in distilled water

This was the maximum practical concentration that could be prepared and dosed topically and did not give rise to irritating effects.

**Topical challenge** - 70 and 35% w/w in distilled water

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

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### Main study

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

### Induction

#### Induction intradermal injections - test animals

A 40 x 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 2 x 4 cm 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. Sodium molybdate 241/32, 5% w/w in water for irrigation.
3. Sodium molybdate 241/32, 5% w/w in a 50 : 50 mixture of Freund's complete adjuvant and water for irrigation.

#### 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 x 60 mm interscapular area was clipped and shaved free of hair and the site was pre-treated by gentle rubbing with 0.2 ml per site of 10% w/w sodium lauryl sulphate in petrolatum. Twenty-four hours later a 20 x 40 mm patch of Whatman No. 3 paper was saturated with approximately 0.4 ml of Sodium molybdate 241/32, 70% w/w in distilled water. 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.

#### 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.

\* Difco Laboratories, Detroit 1, Michigan, U.S.A.

**Challenge**

**Challenge - control and test animals**

The control and test animals were challenged topically two weeks after the topical induction application using Sodium molybdate 241/32, 70 and 35% w/w in distilled water.

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 Sodium molybdate 241/32, 70% w/w in distilled water and applied to an anterior site on the flank. Sodium molybdate 241/32, 35% w/w in distilled water 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 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

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

Any other lesion not covered by this scoring system was described.

The challenge sites were evaluated 24, 48 and 72 hours after removal of the patches.

#### INTERPRETATION OF 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 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.

## RESULTS

### CLINICAL SIGNS

No signs of ill health or toxicity were recorded.

### BODYWEIGHT

Individual bodyweights are shown in Appendix 1.

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 Sodium molybdate 241/32, 5% w/w in water for irrigation. No irritation was observed in the control animals receiving water for irrigation.

#### Topical application

Slight erythema was observed in test animals following topical application with Sodium molybdate 241/32, 70% w/w in distilled water.

Very slight erythema was seen in the control guinea-pigs.

### CHALLENGE

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

There were no dermal reactions seen in the test animals that were considered to be evidence of a skin sensitisation response.

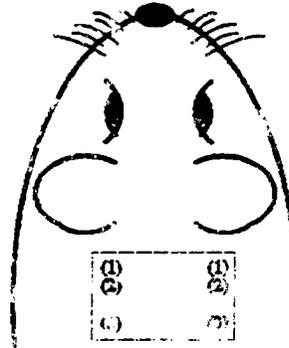
IMA 16a/930983/SS

### CONCLUSION

In this study, Sodium molybdate 241/32 did not produce evidence of skin sensitisation (delayed contact hypersensitivity) in any of the test animals.

FIGURE 1

Position of intradermal injections and topical induction application



The rectangle outlines the 2 x 4 cm clipped scapular area in which injections are made and to which the topical induction application is 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 water for irrigation.
- (3) 0.1 ml of Freund's complete adjuvant 50 : 50 with water for irrigation.

**Test animals:**

- (1) 0.1 ml of Freund's complete adjuvant 50 : 50 with water for irrigation (Ph. Eur.).
- (2) 0.1 ml of Sodium molybdate 241/32, 5% w/w in water for irrigation.
- (3) 0.1 ml of Sodium molybdate 241/32, 5% w/w in a 50 : 50 mixture of water for irrigation 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	Very slight erythema
2	Slight irritation	No irritation		
3	Necrosis	Necrosis		

**TABLE 2**

**Dermal reactions observed after the challenge application with Sodium molybdate 241/32**

Freund's treated controls

Guinea-pig number	E = Erythema O = Oedema	Score					
		24 Hours		48 Hours		72 Hours	
		A	P	A	P	A	P
1550	E	0	0	0	0	0	0
	O	0	0	0		0	0
1551	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
1552	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
1553	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
1554	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
1555	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
1556	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
1557	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
1558	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
1559	E	0	0	0	0	0	0
	O	0	0	0	0	0	0

A Anterior site, exposed to Sodium molybdate 241/32, 70% w/w in distilled water  
 P Posterior site, exposed to Sodium molybdate 241/32, 35% w/w in distilled water

**TABLE 2**

(continued)

Freund's treated controls

Guinea-pig number	E = Erythema O = Oedema	Score					
		24 Hours		48 Hours		72 Hours	
		A	P	A	P	A	P
1560	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
1561	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
1562	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
1563	E	0*	0	0*	0	0*	0
	O	0	0	0	0	0	0
1564	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
1565	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
1566	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
1567	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
1568	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
1569	E	0	0	0	0	0	0
	O	0	0	0	0	0	0

- \* Dryness and sloughing of the epidermis
- A Anterior site, exposed to Sodium molybdate 241/32, 70% w/w in distilled water
- P Posterior site, exposed to Sodium molybdate 241/32, 35% w/w in distilled water

TABLE 2

(continued)

Test animals

Guinea-pig number	E = Erythema O = Oedema	Score						Results Positive (+) Negative (-) Inconclusive (±)
		24 Hours		48 Hours		72 Hours		
		A	P	A	P	A	P	
1570	E O	0 0	0 0	0 0	0 0	0 0	0 0	-
1571	E O	0 0	0 0	0 0	0 0	0 0	0 0	-
1572	E O	0 0	0 0	0 0	0* 0	0 0	0* 0	-
1573	E O	0 0	0 0	0 0	0 0	0 0	0 0	-
1574	E O	0 0	0 0	0 0	0 0	0 0	0 0	-
1575	E O	0 0	0 0	0 0	0 0	0 0	0 0	-
1576	E O	0 0	0 0	0 0	0* 0	0 0	0* 0	-
1577	E O	0* 0	0 0	0* 0	0 0	0* 0	0 0	-
1578	E O	0 0	0 0	0 0	0 0	0 0	0 0	-
1579	E O	0 0	0 0	0 0	0 0	0 0	0 0	-

\* Dryness and sloughing of the epidermis

A Anterior site, exposed to Sodium molybdate 241/32, 70% w/w in distilled water

P Posterior site, exposed to Sodium molybdate 241/32, 35% w/w in distilled water

**TABLE 2**

(continued)

Test animals

Guinea-pig number	E = Erythema O = Oedema	Score						Results Positive (+) Negative (-) Inconclusive (±)
		24 Hours		48 Hours		72 Hours		
		A	P	A	P	A	P	
1580	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	
1581	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	
1582	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	
1583	E	0	0	0*	0	0*	0	-
	O	0	0	0	0	0	0	
1584	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	
1585	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	
1586	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	
1587	E	0	0	0	0*	0	0*	-
	O	0	0	0	0	0	0	
1588	E	0	0	0*	0	0*	0	-
	O	0	0	0	0	0	0	
1589	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	

\* Dryness and sloughing of the epidermis

A Anterior site, exposed to Sodium molybdate 241/32, 70% w/w in distilled water

P Posterior site, exposed to Sodium molybdate 241/32, 35% w/w in distilled water

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## APPENDIX 1

## Individual bodyweights (g)

Group	Guinea-pig number	Day 1 10 May 1993	Last observation day 4 June 1993
Control	1550	414	612
	1551	376	540
	1552	357	514
	1553	378	573
	1554	366	596
	1555	357	525
	1556	347	473
	1557	388	450
	1558	370	479
	1559	363	505
	1560	351	518
	1561	337	520
	1562	335	449
	1563	347	441
	1564	348	533
	1565	337	468
	1566	340	494
1567	349	556	
1568	348	482	
1569	359	537	
Test	1570	378	522
	1571	372	502
	1572	367	514
	1573	360	523
	1574	361	522
	1575	341	548
	1576	335	499
	1577	355	500
	1578	351	527
	1579	353	525
	1580	344	498
	1581	344	498
	1582	381	557
	1583	359	549
	1584	347	529
	1585	347	456
	1586	346	514
1587	349	551	
1588	351	458	
1589	354	459	

APPENDIX 2

Results of preliminary investigations with Sodium molybdate 241/32

Intradermal injections

Vehicle: water for irrigation

Guinea-pig Number	Concentration % w/w	Score		
		Hours	24	72
1330	10.0	D	6	10
		E	2	N
		O	1	2
	7.5	D	4	6
		E	2	N
		O	1	1
	5.0	D	4	6
		E	2	2
		O	1	1
2.5	D	0	0	
	E	0	0	
	O	0	0	
1.0	D	0	0	
	E	0	0	
	O	0	0	
0.5	D	0	0	
	E	0	0	
	O	0	0	
0.25	D	0	0	
	E	0	0	
	O	0	0	
0.1	D	0	0	
	E	0	0	
	O	0	0	
Vehicle control	D	0	0	
	E	0	0	
	O	0	0	

Guinea-pig Number	Concentration % w/w	Score		
		Hours	24	72
1332	10.0	D	8	10
		E	N	N
		O	2	2
	7.5	D	4	6
		E	2	N
		O	1	2
	5.0	D	4	4
		E	2	1
		O	1	1
2.5	D	0	0	
	E	0	0	
	O	0	0	
1.0	D	0	0	
	E	0	0	
	O	0	0	
0.5	D	0	0	
	E	0	0	
	O	0	0	
0.25	D	0	0	
	E	0	0	
	O	0	0	
0.1	D	0	0	
	E	0	0	
	O	0	0	
Vehicle control	D	0	0	
	E	0	0	
	O	0	0	

Key:

- D Diameter (mm)
- E Erythema (0-4 numerical scores)
- O Oedema (0-4 numerical scores)
- N Necrosis

APPENDIX 2

(continued)

Topical application

Vehicle: distilled water

Guinea-pig number	Concentration % w/w	Score					
		0 Hours		24 Hours		48 Hours	
		E	O	E	O	E	O
1333	70	0	0	0	0	0	0
	60	0	0	0	0	0	0
	50	0	0	0	0	0	0
	30	0	0	0	0	0	0
1334	70	0	0	0	0	0	0
	60	0	0	0	0	0	0
	50	0	0	0	0	0	0
	30	0	0	0	0	0	0

- E Erythema (0-4 numerical scores)
- O Oedema (0-4 numerical scores)
- L Localised dermal reaction

**APPENDIX 3**  
**Summary of positive control data**  
**Magnusson and Klugman test using formalin**

M&K R&D No.	Number of female animals		Dates of study		Dates of receipt of Formalin	Dose levels % (aqueous dilutions)			Challenge	Results		
	Test	Control	Start	Finish		Induction		Positive		Inconclusive	Negative	
						Intradermal	Topical					
26	10	10	18.01.88	12.02.88	05.08.87	0.1	10	5 and 1	10/10	0/10	0/10	
27	10	10	21.07.88	15.08.88	13.05.88	0.1	10	5 and 1	10/10	0/10	0/10	
28	10	10	10.01.89	04.02.89	16.12.88	0.1	10	5 and 1	10/10	0/10	0/10	
29	10	10	17.07.89	11.08.89	15.05.89	0.1	10	5 and 1	10/10	0/10	0/10	
30	10	10	31.10.89	25.11.89	15.05.89	0.1	10	5 and 1	10/10	0/10	0/10	
31	10	10	16.01.90	10.02.90	29.1.89	0.1	10	5 and 1	10/10	0/10	0/10	
32	10	10	23.07.90	24.09.90	04.07.90	0.05	5	3 and 1.5 5 and 3*	5/10 10/10	0/10 0/10	0/10 0/10	
33	10	10	30.10.90	24.11.90	04.07.90	0.1	10	5 and 1	10/10	0/10	0/10	
34	10	10	15.01.91	09.02.91	09.01.91	0.1	10	5 and 1	8/10	1/10	1/10	
35	10	10	06.03.91	31.03.91	09.01.91	0.1	10	5 and 1	10/10	0/10	0/10	
36	10	10	12.03.91	06.04.91	09.01.91	0.1	10	5 and 1	10/10	0/10	0/10	
37	10	10	15.08.91	09.09.91	19.06.91	0.1	10	5 and 1	10/10	0/10	0/10	
38	10	10	26.11.91	21.12.91	19.06.91	C.1	10	5 and 1	10/10	0/10	0/10	
39	10	10	10.02.92	13.03.92	09.01.92	0.1	10	5 and 1	7/10	0/10	3/10	
40	10	10	13.08.92	08.09.92	29.06.92	0.1	10	5 and 1*	10/10	0/10	0/10	

† Animals supplied by Interfauna, Huntingdon, Cambridgeshire, England  
 All other animals supplied by D. Hall, Newchurch, Staffordshire, England  
 (Formalin obtained from Savory and Moore, Huntingdon, Cambridgeshire, England or from 1991, supplied by Thornton and Ross, Huddersfield, England)  
 \* Second challenge

APPENDIX 4

Certificate of analysis

CERTIFICATE OF ASSAY		<b>K</b>																																																																																																																																																		
ALFRED H. KNIGHT																																																																																																																																																				
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REMARKS		LABORATORY																																																																																																																																																		
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Huntingdon  
Life Sciences

Report

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**CONFIDENTIAL**

**920889D/IMA 15c/SS**

**MOLYBDENUM OXIDE (PURE)**

**SKIN SENSITISATION IN  
THE GUINEA-PIG**

Huntingdon Research Centre Limited changed its name to Huntingdon Life Sciences Limited  
with effect from 21 November 1995

**Sponsor**

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

**Testing facility**

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

Report issued 23 January 1996

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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 I consider the data generated to be valid.

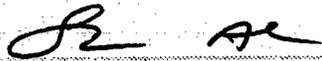
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EC Council Directive, 87/18 EEC of 18 December 1986, (No. L 15/29).

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).



Sarah A. Allan, B.Sc. (Hons.), C.Biol., M.I.Biol.,  
Study Director,  
Huntingdon Life Sciences Ltd.

23 January 96

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

9 - 16 October 1992

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

16 October 1992

Date of reporting audit findings to the  
Study Director and Management

12 November 1992



Peter Watson,  
Quality Assurance Unit Head,  
Department of Quality Assurance,  
Huntingdon Life Sciences Ltd.

19.1.96  
Date

**RESPONSIBLE PERSONNEL**

**Sarah A. Allan, B.Sc. (Hons.), C.Biol., M.I.Biol.,  
Study Director,  
Department of Industrial Toxicology.**



**SUMMARY**

A study was performed to assess the skin sensitisation potential of Molybdenum oxide (pure) using the guinea-pig. The method followed was that described in:

OECD Guideline for Testing of Chemicals No. 406 "Skin Sensitisation". Adopted: 12 May 1981.

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: 7.5% w/w in Alembicol D
- Topical application: 70% w/w in Alembicol D
- Challenge application: 70 and 35% w/w in Alembicol D

Twenty test and twenty control guinea-pigs were used in this study.

In this study performed using twenty guinea pigs Molybdenum oxide (pure) did not produce evidence of skin sensitisation (delayed contact hypersensitivity) in any of the test animals.

## INTRODUCTION

The study was designed to assess the skin sensitisation potential of Molybdenum oxide (pure) 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: 12 May 1981.

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 twenty test and twenty 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 the Study Director and HRC Management on 27 July 1992 and by the Sponsor on 31 July 1992.

The experimental phase was undertaken between 15 September and 24 October 1992.

920889D/IMA 15c/SS

**TEST SUBSTANCE**

**Identity:** Molybdenum oxide (pure)

**Chemical name:** Molybdenum trioxide

**Batch number:** Not available to HRC

**Expiry:** Not available to HRC

**Purity:** MoO<sub>3</sub>: 90%  
Mo: 57%

**Appearance:** White crystalline powder

**Storage conditions:** Room temperature

**Date received:** 8 June 1990

**EXPERIMENTAL PROCEDURE****ANIMAL MANAGEMENT**

Forty healthy female nulliparous and non-pregnant albino guinea-pigs of the Dunkin/Hartley strain were obtained from D. Hall, Newchurch, Staffordshire, England.

The animals were in the weight range of 276 to 340 g on arrival and approximately six to seven weeks of age. All the guinea-pigs were acclimatised to the experimental environment for nineteen days prior to allocation to the main study.

An additional four 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	20	4090 to 4109
Test animals	20	4110 to 4129

The guinea-pigs were housed in groups of ten in suspended metal cages with wire mesh floors in Building R 17 Room 14.

A vitamin C enriched guinea-pig diet FD1 and drinking water were provided *ad libitum*. Hay was given weekly.

The batch of diet used for the study was not analysed for nutrients, possible contaminants or micro-organisms.

Results of routine physical and chemical examination of drinking water at source, as conducted usually weekly by the supplier, are made available to Huntingdon Research Centre Ltd. as quarterly summaries.

Animal room temperature was maintained at approximately 21 °C and relative humidity at 30 - 70%. 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 HRC Industrial 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 licensees.

## **POSITIVE CONTROL**

The sensitivity of the guinea-pig strain used is checked periodically at HRC with formalin, a known sensitiser. 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.

## **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 will 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.

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 oxide (pure) were selected:

**Induction intradermal injection** - 7.5% w/w in Alembicol D

**Induction topical application** - 70% w/w in Alembicol D

This was the maximum practical concentration that could be prepared and dosed topically and did not give rise to irritating effects.

**Topical challenge** - 70 and 35% w/w in Alembicol D

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

\* A product of coconut oil, supplied by Alembic Products, Saltney, Chester, England

**Main study**

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

**Induction****Induction intradermal injections - test animals**

A 40 x 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 2 x 4 cm 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 oxide (pure), 7.5% w/w in Alembicol D.
3. Molybdenum oxide (pure), 7.5% w/w in a 50 : 50 mixture of Freund's 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 x 60 mm interscapular area was clipped and shaved free of hair and the site was pre-treated by gentle rubbing with 0.2 ml per site of 10% w/w sodium lauryl sulphate in petrolatum. Twenty-four hours later a 20 x 40 mm patch of Whatman No. 3 paper was saturated with approximately 0.4 ml of Molybdenum oxide (pure), 70% w/w 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.

**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.

\*\* Difco Laboratories, Detroit 1, Michigan, U.S.A.

**Challenge****Challenge - control and test animals**

The control and test animals were challenged topically two weeks after the topical induction application using Molybdenum oxide (pure), 70 and 35% w/w 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 oxide (pure), 70% w/w in Alembicol D and applied to an anterior site on the flank. Molybdenum oxide (pure), 35% w/w 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 are 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 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

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

Any other lesion not covered by this scoring system was described.

The challenge sites were evaluated 24, 48 and 72 hours after removal of the patches.

#### **INTERPRETATION OF 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 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.

## RESULTS

### CLINICAL SIGNS

No signs of ill health or toxicity were recorded.

### BODYWEIGHT

Individual bodyweights are shown in Appendix 1.

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 oxide (pure), 7.5% w/w in Alembicol D and very slight irritation was observed in control animals receiving Alembicol D.

#### Topical application

Slight erythema was observed in test animals following topical application with Molybdenum oxide (pure), 70% w/w 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 application are shown in Table 2.

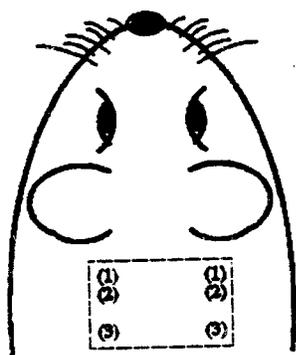
No dermal responses were seen in any of the test or control animals.

**CONCLUSION**

In this test, performed in twenty albino guinea-pigs Molybdenum oxide (pure) did not produce evidence of skin sensitization (delayed contact hypersensitivity) in any of the animals.

FIGURE 1

Position of intradermal injections and topical induction application



The rectangle outlines the 2 x 4 cm clipped scapular area in which injections are made and to which the topical induction application is 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 oxide (pure), 7.5% w/w in Alembicol D.
- (3) 0.1 ml of Molybdenum oxide (pure), 7.5% w/w 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 I

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	Very slight irritation		
3	Necrosis	Necrosis		

TABLE 2

Dermal reactions observed after the challenge application with  
Molybdenum oxide (pure)

Freund's treated controls

Guinea-pig number	E = Erythema O = Oedema	Score					
		24 Hours		48 Hours		72 Hours	
		A	P	A	P	A	P
4090	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
4091	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
4092	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
4093	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
4094	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
4095	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
4096	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
4097	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
4098	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
4099	E	0	0	0	0	0	0
	O	0	0	0	0	0	0

A Anterior site, exposed to Molybdenum oxide (pure), 70% w/w in Alembic D  
P Posterior site, exposed to Molybdenum oxide (pure), 35% w/w in Alembic D

Slight grey staining was observed at all sites

TABLE 2

(continued)

Freund's treated controls

Guinea-pig number	E = Erythema O = Oedema	Score					
		24 Hours		48 Hours		72 Hours	
		A	P	A	P	A	P
4100	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
4101	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
4102	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
4103	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
4104	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
4105	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
4106	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
4107	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
4108	E	0	0	0	0	0	0
	O	0	0	0	0	0	0
4109	E	0	0	0	0	0	0
	O	0	0	0	0	0	0

A Anterior site, exposed to Molybdenum oxide (pure), 70% w/w in Alembicol D  
 P Posterior site, exposed to Molybdenum oxide (pure), 35% w/w in Alembicol D

Slight grey staining was observed at all sites

TABLE 2

(continued)

Test animals

Guinea-pig number	E = Erythema O = Oedema	Score						Results Positive (+) Negative (-) Inconclusive (±)
		24 Hours		48 Hours		72 Hours		
		A	P	A	P	A	P	
4110	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	
4111	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	
4112	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	
4113	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	
4114	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	
4115	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	
4116	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	
4117	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	
4118	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	
4119	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	

A Anterior site, exposed to Molybdenum oxide (pure), 70% w/w in Alembicol D  
 P Posterior site, exposed to Molybdenum oxide (pure), 35% w/w in Alembicol D

Slight grey staining was observed at all sites

01114

TABLE 2

(continued)

## Test animals

Guinea-pig number	E = Erythema O = Oedema	Score						Results Positive (+) Negative (-) Inconclusive (±)
		24 Hours		48 Hours		72 Hours		
		A	P	A	P	A	P	
4120	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	
4121	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	
4122	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	
4123	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	
4124	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	
4125	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	
4126	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	
4127	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	
4128	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	
4129	E	0	0	0	0	0	0	-
	O	0	0	0	0	0	0	

A Anterior site, exposed to Molybdenum oxide (pure), 70% w/w in Alembicol D  
 P Posterior site, exposed to Molybdenum oxide (pure), 35% w/w in Alembicol D

Slight grey staining was observed at all sites

0115

## APPENDIX 1

## Individual bodyweights (g)

Group	Guinea-pig number	Day 1 29 September 1992	Last day of study 24 October 1992
Control	4090	479	616
	4091	471	600
	4092	452	614
	4093	495	672
	4094	450	598
	4095	494	609
	4096	434	594
	4097	453	582
	4098	427	567
	4099	414	546
	4100	430	577
	4101	453	609
	4102	454	569
	4103	443	605
	4104	425	546
	4105	462	633
	4106	488	598
4107	472	607	
4108	471	597	
4109	442	607	
Test	4110	489	680
	4111	422	572
	4112	492	685
	4113	470	639
	4114	469	632
	4115	461	623
	4116	459	595
	4117	449	573
	4118	461	669
	4119	472	664
	4120	473	619
	4121	497	635
	4122	486	527
	4123	373	651
	4124	480	679
	4125	443	578
	4126	416	544
4127	443	584	
4128	427	577	
4129	496	627	

## APPENDIX 2

## Results of preliminary investigations with Molybdenum oxide (pure)

## Intradermal injections

Vehicle: Alcohol D

Guinea- pig Number	Concentration % w/w	Score		
		Hours	24	72
3847	10.0	D	10	10
		E	N	N
		O	2	2
	7.5	D	8	8
		E	2	2
		O	2	2
	5.0	D	8	6
		E	2	2
		O	2	2
	2.5	D	6	6
E		2	2	
O		1	1	
1.0	D	5	5	
	E	1	1	
	O	1	1	
0.5	D	4	4	
	E	1	1	
	O	1	1	
0.25	D	4	4	
	E	1	1	
	O	1	0	
0.1	D	3	2	
	E	1	1	
	O	1	0	
Vehicle control	D	2	2	
	E	1	1	
	O	1	0	

Guinea- pig Number	Concentration % w/w	Score		
		Hours	24	72
3848	10.0	D	10	10
		E	N	N
		O	2	2
	7.5	D	8	8
		E	2	2
		O	2	2
	5.0	D	8	6
		E	2	2
		O	2	2
	2.5	D	6	6
E		2	2	
O		1	1	
1.0	D	5	5	
	E	1	1	
	O	1	1	
0.5	D	4	4	
	E	1	1	
	O	1	1	
0.25	D	4	4	
	E	1	1	
	O	1	1	
0.1	D	3	3	
	E	1	1	
	O	1	0	
Vehicle control	D	2	2	
	E	1	1	
	O	1	0	

Key:

- D Diameter (mm)  
 E Erythema (0-4 numerical scores)  
 O Oedema (0-4 numerical scores)  
 N Necrosis

## APPENDIX 2

(continued)

## Topical application

Vehicle: Alembicol D

Guinea-pig number	Concentration % w/w	Score					
		0 Hours		24 Hours		48 Hours	
		E*	O	E	O	E	O
3849	70	0	0	0	0	0	0
	60	0	0	0	0	0	0
	50	0	0	0	0	0	0
	30	0	0	0	0	0	0
3850	70	0	0	0	0	0	0
	60	0	0	0	0	0	0
	50	0	0	0	0	0	0
	30	0	0	0	0	0	0
3851	70	0	0	0	0	0	0
	60	0	0	0	0	0	0
	50	0	0	0	0	0	0
	30	0	0	0	0	0	0
3852	70	0	0	0	0	0	0
	60	0	0	0	0	0	0
	50	0	0	0	0	0	0
	30	0	0	0	0	0	0

E Erythema (0-4 numerical scores)

O Oedema (0-4 numerical scores)

L Localised dermal reaction

g Slight grey staining observed at all sites

## APPENDIX 3

Summary of positive control data  
Magnumson and Kilgus test using formalin

M&K R&D No.	Number of female animals		Dates of study		Dates of recep. of Formalin	Dose levels % (aqueous dilutions)		Challenges	Results		
	Test	Control	Start	Finish		Intradermal	Topical		Positive	Inconclusive	Negative
26	10	10	18.01.88	12.02.88	05.08.87	0.1	10	5 and 1	10/10	0/10	0/10
27	10	10	21.07.88	15.08.88	13.05.88	0.1	10	5 and 1	10/10	0/10	0/10
28	10	10	10.01.89	04.02.89	16.12.88	0.1	10	5 and 1	10/10	0/10	0/10
29	10	10	17.07.89	11.08.89	15.05.89	0.1	10	5 and 1	10/10	0/10	0/10
30	1	10	31.10.89	25.11.89	15.05.89	0.1	10	5 and 1	10/10	0/10	0/10
31	10	10	16.01.90	10.02.90	29.11.89	0.1	10	5 and 1	10/10	0/10	0/10
32	10	10	23.07.90	24.09.90	04.07.90	0.05	5	3 and 1.5 5 and 3 <sup>+</sup>	5/10 10/10	0/10 0/10	5/10 0/10
33	10	10	30.10.90	24.11.90	04.07.90	0.1	10	5 and 1	10/10	0/10	0/10
34	10	10	15.01.91	09.02.91	09.01.91	0.1	10	5 and 1	8/10	1/10	1/10
35	10	10	06.03.91	31.03.91	09.01.91	0.1	10	5 and 1	10/10	0/10	0/10
36	10	10	12.03.91	06.04.91	09.01.91	0.1	10	5 and 1	10/10	0/10	0/10
37	10	10	15.08.91	09.09.91	19.06.91	0.1	10	5 and 1	10/10	0/10	0/10
38	10	10	26.11.91	21.12.91	19.06.91	0.1	10	5 and 1	10/10	0/10	0/10
39	10	10	10.02.92	13.03.92	09.01.92	0.1	10	5 and 1 5 and 1 <sup>+</sup>	7/10 10/10	0/10 0/10	3/10 0/10
40	10	10	13.08.92	08.09.92	19.06.92	0.1	10	5 and 1	10/10	0/10	0/10

1. Animals supplied by Interfarms, Huntingdon, Cambridgeshire, England  
 All other animals supplied by D. Hall, Newchurch, Staffordshire, England  
 (Formalins obtained from Severy and Moore, Huntingdon, Cambridgeshire, England or from 1991, supplied by Thornton and Ross, Huddersfield, England)  
 + 8 second challenges



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