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European Registration Dossier
Dossier File N°: 5.2.6 / 01
Ciba File N°: 329351/8

Skin Sensitisation Test in the Guinea Pig

Maximisation Test

Test No. 943030

CGA 329351 tech.

Report

Study Director: Dr. med.vet. [redacted]

Testing Facility: CIBA-GEIGY Limited
Toxicology Services
Short-term Toxicology
4332 Stein / Switzerland

Test Guideline: OECD 406; 92/69/EEC, B.6.

Date of protocol: May 25, 1994

Completion date: August 16, 1994

Sponsor: CIBA-GEIGY Limited
Plant Protection
4002 Basel / Switzerland

This report contains: 30 pages

Certification of GLP and verification of the report

(Certification of Good Laboratory Practice and verification of a complete and unaltered copy of the report by the sponsor)

The Statement of Compliance with Good Laboratory Practice found on page 4, and signed by the Study Director is truthful and accurate. This report as provided by the testing facility is complete and unaltered.

For the Sponsor:

Signature: [redacted] 5.1.2.e Woo .... Date: August 27, 1994

Statement of compliance with Good Laboratory Practice

This study has been performed in compliance with Good Laboratory Practice (GLP) in Switzerland (Verfahren und Grundsaetze der Guten Laborpraxis (GLP) in der Schweiz), Procedures and Principles, March 1986, issued by the Swiss Federal Department of the Interior and the Intercantonal Office for the Control of Medicaments. These procedures are in essence consistent with:

- OECD Principles of Good Laboratory Practice (Council Decision 81/30, adopted on May 12, 1981, and the OECD Recommendation 83/95 concerning the 'Mutual Recognition of Compliance with Good Laboratory Practice', adopted on July 26, 1983).
- United States Environmental Protection Agency, Title 40 Code of Federal Regulations Part 160 (FIFRA); Federal Register, August 17, 1989.
- United States Environmental Protection Agency, Title 40 Code of Federal Regulations Part 792 (TSCA); Federal Register, August 17, 1989.
- Japan Ministry of Agriculture, Forestry and Fisheries, NohSan, Notification No. 3850, Agricultural Production Bureau, August 10, 1984.

Study director: Dr. med.vet. 5.1.2.e Woo

Signature: 5.1.2.e Woo ... Date: August 16, 1994

Signatures

This report represents the results of the investigations compiled by the undersigned:

Study director: Dr. med.vet. [redacted]

Signature: ...[redacted]. Date: August 16, 1994

for Dr. med.vet. [redacted] D.A.B.T (absent)
Head Shortterm and Reproduction Toxicology

Signature: [redacted].. Date: August 16, 1994

Reserved page for flagging statements

Quality assurance statement

Test Article:	**CGA 329351 tech.**
Study Title:	**Skin Sensitisation Test in the Guinea Pig**
Test Number:	**943030**
Study Director:	**Dr. med.vet.** [redacted]

I hereby certify that the following Quality Assurance activities were performed:

QA-Activity	Date performed	Date reported
Facility Inspection	March 23, 1994	April 08, 1994
Protocol Audit	May 27, 1994	May 27, 1994
Final Report Audit	August 11, 1994	August 12, 1994

Quality Assurance Inspector: [redacted]

Signature: .... Date: August 17, 1994

1. SUMMARY AND CONCLUSION

Under the experimental conditions employed, 5% of the animals of the test group showed skin reactions 24 and 48 hours after removing the dressings, respectively.

According to the maximisation grading of Magnusson and Kligman CGA 329351 tech. showed a weak grade of skin-sensitising (contact allergenic) potential in albino guinea pigs.

2. GENERAL

2.1. Introduction

At the request of the Plant Protection of CIBA-GEIGY Limited, a sensitisation test in albino guinea pigs was performed to determine the contact allergenic potency of CGA 329351 tech. in albino guinea pigs.

This test was based on the OECD Guideline No. 406, adopted May 12, 1981, adapted July 17, 1992, by the OECD council, and on Annex V, Part B of Council Directive 67/548/EEC (Commission Directive 92/69/EEC of July 31, 1992).

Experimental starting date:	May 30, 1994
Experimental termination date:	June 23, 1994
Testing facility:	CIBA-GEIGY Limited Toxicology Services Short-term Toxicology 4332 Stein/Switzerland

2.2. Archives

Archives are located at CIBA-GEIGY Limited, Werk Stein, CH-4332 Stein, Switzerland. Raw data, protocol and report will be stored at this location.

2.3. Distribution

Sponsor (Dr. [redacted] 5.1.2.e Woo
Archives

2.4. Test material

Test article:	CGA 329351 tech.
Batch No.:	KGL 4634/6
Contents/Purity:	97.30%
Physical properties:	viscous
Storage conditions:	room temperature
Validity:	February, 1998
Test article received:	March 16, 1994

2.5. Auxiliary compounds

- Physiological saline (0.9 %), sterile solution ([redacted], St. Gallen, Switzerland)
- Bacto Adjuvant, Complete, [redacted] (Difco Lab. Detroit, Michigan USA)
- Vaseline (white petrolatum) Ph. [redacted] (Siegfried AG, Zofingen, Switzerland)
- Oleum arachidis Ph. [redacted] (Siegfried AG, Zofingen, Switzerland

2.6. Test System

The albino guinea pig is the recommended species for skin sensitisation studies.

Animal strain:	Pirbright White Strain (Tif: DHP)
Breeder:	CIBA-GEIGY Limited Animal Production 4332 Stein / Switzerland
Date of acclimatisation:	May 25, 1994

2.7. Group Size and Husbandry

The test was performed on a total of 10 male and 10 female guinea pigs in the test group and 5 males and 5 females in the control group, respectively, initially weighing between 304 to 407 g.

The animals were housed individually in Macrolon cages (Type 3), assigned to the different groups by means of random numbers generated by the random number generator, identified by individual ear tags, kept at a constant room temperature of 22±3°C, at a relative humidity of 30 to 70% and a 12 hours light cycle day.

The animals received ad libitum standard guinea pig pellets - NAFAG No. 845, Gossau SG and fresh water.

All batches of the diet are assayed for nutritive ingredients and contamination level by the manufacturer. Analytical results are available at the animal supply office.

The drinking water quality fulfilled the critical parameters in the specifications of the "Schweizerisches Lebensmittelbuch" (Edition 1972). The results of the routine chemical examination of water at source (Grundwasserfassung Stein) as conducted periodically by the water authority (Baudepartement des Kantons Aargau, Abteilung Gewaesserschutz) are available to CIBA-GEIGY Limited, as well as the results of inhouse chemical analysis by the analytical laboratories of the Pharmaceutical Division, CIBA-GEIGY Limited.

2.8. Sensitivity of strain

The sensitivity of the strain is checked once or twice a year with a known mild to moderate sensitiser, such as mercaptobenzothiazole, hexyl cinnamic aldehyde or potassiumdichromate.

The results of the latest positive control test are presented in Appendix 3 of this report.

3. METHODS

3.1. Reason for selection

The maximisation test has been selected, because it is one of the recommended tests in the OECD guideline 406, adopted May 12, 1981, adapted July 17, 1992, as well as in Annex V, Part B of Council Directive 67/548/EEC (Commission Directive 92/69/EEC of July 31, 1992), and because the sensitivity of the method is well known. The test has been performed in essence according to the original protocol of 5.1.2.e Woo and 5.1.2.e Woo (J. invest. Dermatol. 52, 268-276, 1969; Contact Dermatitis 6, 46-50, 1980).

3.2. Test procedure and concentrations used

3.2.1. Pretests

Intradermal Induction

The concentration for the intradermal injections was selected on account of the solubility of the test article in standard vehicles and its local and systemic tolerability in a pretest. The following concentration of test article has been used for intradermal injection:

5% in Oleum arachidis (w/v).

Since 5% CGA 329351 tech. in Oleum arachidis could be injected and was well tolerated, this concentration was used for the intradermal induction.

Epidermal Applications (induction and challenge)

The concentrations for the epidermal applications were selected on account of the primary irritation potential of the test article. The following concentrations of CGA 329351 tech. have been examined on separate animals for the determination of the maximum subirritant concentration (see also Table 4):

20, 30, and 50% in vaseline and the undiluted test article.

50% was the highest possible concentration of the test article in vaseline.

Reactions were observed with 50% CGA 329351 tech. in vaseline and with the undiluted test aricle.

3.2.2. Test procedure

DAY 0: INDUCTION, intradermal injections

Three pairs of intradermal injections (0.1 ml per injection) were made simultaneously into the left and right side of the shaved neck of the test and control group animals.

Test group:

- adjuvant/saline mixture 1:1 (v/v)
- 5% CGA 329351 tech. in Oleum arachidis (w/v)
- 5% CGA 329351 tech. in the adjuvant/saline mixture (w/v)

Control group:

- adjuvant/saline mixture 1:1 (v/v)
- adjuvant/saline mixture 1:1 (v/v)
- Oleum arachidis

DAY 8: INDUCTION, epidermal application

In the test group CGA 329351 tech. applied on a filterpaper patch to the neck of the animals (patch 2x4 cm; approx. 0.4 g per patch; occluded administration for 48 hours).
The control group was treated with vaseline only.

Test group:

- CGA 329351 tech. used undiluted

Control group:

- vaseline only

DAY 21: Challenge

The test and control group animals were tested on one flank with CGA 329351 tech. in vaseline (w/w) and on the other flank with the vehicle alone (patch 2x2 cm; approx. 0.2 g per patch; occluded administration for 24 hours).

Test and control group:

- 30% CGA 329351 tech. in vaseline
- vaseline only

3.3. Observations and records

Induction reactions

After removal of the dressing on day 10, irritation of the epidermal application site was observed in 20/20 test group animals.

Challenge reactions

Twenty four and forty eight hours after removing the dressings, the challenge reactions were graded according to the Draize scoring scale (Appendix 1).

General

The body weight was recorded at start and end of the test.

3.4. Interpretation of results

The sensitising potential of CGA 329351 tech. was classified according to the grading of Magnusson and Kligman (Appendix 2).

According to the guide to the labelling of dangerous substances and the criteria for the choice of sentences indicating particular hazards (R sentences) attributed to dangerous substances (Commission Directive 93/21/EEC, April 27, 1993) a test article was classified as a sensitiser in the case where a positive response was noted in at least 30 % of the animals.

4. RESULTS

The incidence of positive animals per group, the individual challenge reactions and the evaluation of the primary skin irritation potential are listed in Tables 1, 2, 3 and 4.

The individual animal weights at start and end of the test are listed in Table 5.
Body weights were not affected by treatment.

Under the experimental conditions employed, 5% of the animals of the test group showed skin reactions 24 and 48 hours after removing the dressings, respectively.

CGA 329351 tech. is, therefore, classified as a weak sensitiser in albino guinea pigs according to the grading of Magnusson and Kligman.

According to the EEC classification criteria (Commission Directive 93/21/EEC, April 27, 1993) CGA 329351 tech. did not show a skin-sensitising (contact allergenic) potential in albino guinea pigs.

5. TABLES

TABLE 1

Number of positive animals per group after occlusive epidermal application

Control group:

	after 24 hours	after 48 hours
vehicle control	0/10	0/10
test article	0/10	0/10

Test group:

	after 24 hours	after 48 hours
vehicle control	0/20	0/20
test article	1/20	1/20

TABLE 2

Challenge reactions after epidermal application (CONTROL GROUP)

DRAIZE Score 24 hours after removal of the dressing

Vehicle control

Male animals	01	02	03	04	05
Erythema score:	0	0	0	0	0
Edema score:	0	0	0	0	0

Female animals	16	17	18	19	20
Erythema score:	0	0	0	0	0
Edema score:	0	0	0	0	0

Test article control

Male animals	01	02	03	04	05
Erythema score:	0	0	0	0	0
Edema score:	0	0	0	0	0

Female animals	16	17	18	19	20
Erythema score:	0	0	0	0	0
Edema score:	0	0	0	0	0

TABLE 2 cont.

DRAIZE Score 48 hours after removal of the dressing

Vehicle control

Male animals	01	02	03	04	05
Erythema score:	0	0	0	0	0
Edema score:	0	0	0	0	0

Female animals	16	17	18	19	20
Erythema score:	0	0	0	0	0
Edema score:	0	0	0	0	0

Test article control

Male animals	01	02	03	04	05
Erythema score:	0	0	0	0	0
Edema score:	0	0	0	0	0

Female animals	16	17	18	19	20
Erythema score:	0	0	0	0	0
Edema score:	0	0	0	0	0

TABLE 3

Challenge reactions after epidermal application (TEST GROUP)

DRAIZE Score 24 hours after removal of the dressing

Vehicle control

Male animals	06	07	08	09	10	11	12	13	14	15
Erythema score:	0	0	0	0	0	0	0	0	0	0
Edema score:	0	0	0	0	0	0	0	0	0	0

Female animals	21	22	23	24	25	26	27	28	29	30
Erythema score:	0	0	0	0	0	0	0	0	0	0
Edema score:	0	0	0	0	0	0	0	0	0	0

Test article

Male animals	06	07	08	09	10	11	12	13	14	15
Erythema score:	0	0	0	0	0	0	1	0	0	0
Edema score:	0	0	0	0	0	0	0	0	0	0

Female animals	21	22	23	24	25	26	27	28	29	30
Erythema score:	0	0	0	0	0	0	0	0	0	0
Edema score:	0	0	0	0	0	0	0	0	0	0

TABLE 3 cont.

DRAIZE Score 48 hours after removal of the dressing

Vehicle control

Male animals	06	07	08	09	10	11	12	13	14	15
Erythema score:	0	0	0	0	0	0	0	0	0	0
Edema score:	0	0	0	0	0	0	0	0	0	0

Female animals	21	22	23	24	25	26	27	28	29	30
Erythema score:	0	0	0	0	0	0	0	0	0	0
Edema score:	0	0	0	0	0	0	0	0	0	0

Test article

Male animals	06	07	08	09	10	11	12	13	14	15
Erythema score:	0	0	0	0	0	0	1	0	0	0
Edema score:	0	0	0	0	0	0	0	0	0	0

Female animals	21	22	23	24	25	26	27	28	29	30
Erythema score:	0	0	0	0	0	0	0	0	0	0
Edema score:	0	0	0	0	0	0	0	0	0	0

TABLE 4

Evaluation of the primary skin irritation potential

Procedure: On each animal 2 concentrations of CGA 329351 tech. were applied simultaneously on the left and right flank. A naive skin site served as control (not reported).
7 days before application of CGA 329351 tech. two pairs of intradermal injections of an adjuvant/saline mixture 1:1 (v/v; 0.1 ml per injection) were made simultaneously into the shaved neck of the guinea pigs.

score 24 hours | **score 48 hours**
after removing the dressing

concentrations of CGA 329351 tech. in vaseline (w/v;%) and CGA 329351 tech. used undiluted

Animal No. / sex	50 % er/ed (24 h)	100% er/ed (24 h)	50 % er/ed (48 h)	100% er/ed (48 h)
1 male	1/0	1/0	1/0	1/0
2 female	0/0	1/0	0/0	0/0

Animal No. / sex	20 % er/ed (24 h)	30 % er/ed (24 h)	20 % er/ed (48 h)	30 % er/ed (48 h)
3 male	0/0	0/0	0/0	0/0
4 female	0/0	0/0	0/0	0/0

er = erythema, ed = edema

TABLE 5

Individual animal bodyweights in g - males

CONTROL GROUP Animal No.	weight at start	weight at end	TEST GROUP Animal No.	weight at start	weight at end
01	379	505	06	304	474
02	370	504	07	382	499
03	404	586	08	387	526
04	348	495	09	383	571
05	340	477	10	364	448
			11	386	490
			12	320	482
			13	372	519
			14	385	572
			15	363	516
Mean	368	513		365	510
Std.Dev.	25.5	42.1		29.3	40.0

Individual animal bodyweights in g - females

CONTROL GROUP Animal No.	weight at start	weight at end	TEST GROUP Animal No.	weight at start	weight at end
16	334	436	21	338	422
17	364	510	22	407	529
18	389	510	23	394	534
19	355	483	24	338	497
20	391	500	25	364	446
			26	361	456
			27	385	501
			28	338	430
			29	338	440
			30	364	501
Mean	367	488		363	476
Std.Dev.	24.0	31.0		25.6	41.5

6. APPENDICES

APPENDIX 1

Evaluation of skin reactions

Evaluation of skin reactions according to Draize in Appraisal of the Safety of chemicals in Foods, Drugs and Cosmetics (1959), The US Association of Food and Drug Officials (AFDO).

Erythema and eschar formation

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

Edema formation

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

APPENDIX 2

Maximisation grading (according to Magnusson and Kligman)

Sensitisation rate (%)	Grade	Classification
0 - 8	I	weak
9 - 28	II	mild
29 - 64	III	moderate
65 - 80	IV	strong
81 - 100	V	extreme

APPENDIX 3

Reference values with 2-Mercaptobenzothiazole puriss.

Test No. 930018

Experimental starting date: January 3, 1994
Experimental completion date: January 27, 1994

The following concentrations of the reference compound and vehicles were used:

Intradermal induction

Concentration of compound: 5%
Vehicle: Oleum arachidis

Epidermal induction

Concentration of compound: 50%
Vehicle: vaseline

Epidermal challenge

Concentration of compound: 30%
Vehicle: vaseline

APPENDIX 3 cont.

Number of positive animals per group after occlusive epidermal application

Control group:

	after 24 hours	after 48 hours
vehicle control	0/10	0/10
test article	0/10	0/10

Test group:

	after 24 hours	after 48 hours
vehicle control	0/20	0/20
test article	20/20	20/20

Test and results fullfill the requirements for reliability check of the OECD Guideline 406 (page 2, paragraph 10 and 11).

APPENDIX 3 cont.

Challenge reactions after epidermal application (CONTROL GROUP)

DRAIZE Score 24 hours after removal of the dressing

Vehicle control

Male animals	31	32	33	34	35
Erythema score:	0	0	0	0	0
Edema score:	0	0	0	0	0

Female animals	46	47	48	49	50
Erythema score:	0	0	0	0	0
Edema score:	0	0	0	0	0

Test article control

Male animals	31	32	33	34	35
Erythema score:	0	0	0	0	0
Edema score:	0	0	0	0	0

Female animals	46	47	48	49	50
Erythema score:	0	0	0	0	0
Edema score:	0	0	0	0	0

APPENDIX 3 cont.

DRAIZE Score 48 hours after removal of the dressing

Vehicle control

Male animals	31	32	33	34	35
Erythema score:	0	0	0	0	0
Edema score:	0	0	0	0	0

Female animals	46	47	48	49	50
Erythema score:	0	0	0	0	0
Edema score:	0	0	0	0	0

Test article control

Male animals	31	32	33	34	35
Erythema score:	0	0	0	0	0
Edema score:	0	0	0	0	0

Female animals	46	47	48	49	50
Erythema score:	0	0	0	0	0
Edema score:	0	0	0	0	0

APPENDIX 3 cont.

Challenge reactions after epidermal application (TEST GROUP)

DRAIZE Score 24 hours after removal of the dressing

Vehicle control

Male animals	36	37	38	39	40	41	42	43	44	45
Erythema score:	0	0	0	0	0	0	0	0	0	0
Edema score:	0	0	0	0	0	0	0	0	0	0

Female animals	51	52	53	54	55	56	57	58	59	60
Erythema score:	0	0	0	0	0	0	0	0	0	0
Edema score:	0	0	0	0	0	0	0	0	0	0

Test article

Male animals	36	37	38	39	40	41	42	43	44	45
Erythema score:	2	2	1	2	1	2	1	2	1	2
Edema score:	1	2	1	2	1	2	1	2	1	1

Female animals	51	52	53	54	55	56	57	58	59	60
Erythema score:	2	1	1	2	1	2	2	1	2	2
Edema score:	1	1	0	1	1	2	2	0	2	2

APPENDIX 3 cont.

DRAIZE Score 48 hours after removal of the dressing

Vehicle control

Male animals	36	37	38	39	40	41	42	43	44	45
Erythema score:	0	0	0	0	0	0	0	0	0	0
Edema score:	0	0	0	0	0	0	0	0	0	0

Female animals	51	52	53	54	55	56	57	58	59	60
Erythema score:	0	0	0	0	0	0	0	0	0	0
Edema score:	0	0	0	0	0	0	0	0	0	0

Test article

Male animals	36	37	38	39	40	41	42	43	44	45
Erythema score:	2	2s	1	2s	1s	1s	1s	2s	2s	2s
Edema score:	1	2	0	2	0	1	1	2	2	2

Female animals	51	52	53	54	55	56	57	58	59	60
Erythema score:	2s	1s	1s	2s	1s	2s	2s	1s	2s	2s
Edema score:	1	0	1	2	1	2	2	0	2	2

s = scaling

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