

Test No.: 933719
Test substance : CGA 329351

Report on the Acute Toxicity Test
of CGA 329351 techn.(Enantiomer of CGA 48988)
on Daphnia (Daphnia magna Straus 1820)

Study Director : Dr. **5.2.e Woo**

Testing Facility : CIBA-GEIGY Ltd.
Product Safety
Ecotoxicology
CH-4002 Basel / Switzerland

Test Guideline : OECD-Guideline No. 202, Part I, 1984

Study completed
(day/month/year) : 01/12/94

Sponsor : CIBA-GEIGY Ltd
PP - Division
CH-4002 Basel / Switzerland

represented by : Dr. **5.2.e Woo**

Project No. of Sponsor : 933719

This report contains 17 pages.

European Registration Dossier Dossier File N°: 8.2.4/7 Ciba File N°: 329351/26
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Test No.: 933719
 Test substance : CGA 329351

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 this page of this report, 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 : Dr.

5.1.2.e Woo

Signature : ...

Date : 29.11.94

Statement of Compliance with Good Laboratory Practices

This study has been performed in compliance with Good Laboratory Practice (GLP) in Switzerland, Procedures and Principles, March 1986 (Verfahren und Grundsätze der Guten Laborpraxis (GLP) in der Schweiz), 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.

5.1.2.e Woo

Signature : ...

Date : 28/11/94

Test No.: 933719
Test substance : CGA 329351

Reserved page for flagging statements

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Quality Assurance Statement

Ciba-Geigy Ltd., Toxicology Services, Quality Assurance (GLP), 4002 Basel

Project 933719
Test Substance CGA 329351
Study Title Acute Toxicity Test of CGA 329351 techn. (Enantiomer of CGA 48988) on Daphnia (Daphnia magna Straus 1820)
Study Director Dr. 5.1.2.e Woo
QA-Inspector 5.1.2.e Woo

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

Activity	Performed	Reported
Facility Inspection	March 07, 1994	April 14, 1994
Facility Inspection	May 05, 1994	May 09, 1994
Protocol Audit	May 24, 1994	May 24, 1994
Final Report Audit	November 29, 1994	November 29, 1994

Date
Form QSSTAT12

December 09, 1994

5.1.2.e Woo

Inspector Quality Assurance

Test No.: 933719
 Test substance : CGA 329351

Table of contents

	Page
Proprietary information	2
Statement of compliance with GLP	3
Reserved page for Flagging Statement	4
Quality Assurance Statement	5
1. Summary	7
2. Introduction	8
2.1. Purpose	8
2.2. Guideline	8
2.3. Deviations	8
2.4. Testing facility	8
2.5. Archives	8
2.6. Personnel	9
2.7. Dates	9
2.8. Distribution	9
3. Materials and Methods	10
3.1. Test substance	10
3.2. Test system / species	10
3.3. Design and procedure	11
3.4. Stock solution	11
3.5. Test concentrations	11
3.6. Observations	12
3.7. Measurements	12
3.8. Calculations / statistical analysis	12
4. Results	13
4.1. Values calculated	13
4.2. Values graphically determined	13
4.3. Values observed	13
4.4. Controls	13
4.5. Conclusion	13
5. Tables	14 - 15
Appendix : Analysis report on test No. 933719	16 - 17

Test No.: 933719
 Test substance : CGA 329351

1. Summary

Study : Determination of EC 50 (24 h and 48 h) :
 Concentration at which 50% of the daphnia population is immobilized.

Test system : Daphnia (Daphnia magna Straus 1820)

Duration : 48 hours

Guideline: OECD-Guideline No. 202, Part I, 1984

Deviations: None

Design: 20 daphnids (0 - 24 hours old) were exposed to nominal test concentrations of 10, 18, 32, 58 and 100 mg test substance/l respectively.
 A static system was used for this test.

Test substance : Identification code : CGA 329351
 Batch No. : KGL 4634/6
 Purity : 97.3 %

Results : The measured test substance concentrations were within the range 100 ± 20 % of the nominal concentrations, therefore values based on nominal concentrations.

EC 50 (48 h) calculated : >100 mg/l

95 % confidence limit : none

EC 50 (24 h) calculated : >100 mg/l

95 % confidence limit : none

NOEC (48 h) : 100 mg/l

EC 0 (48 h) : 100 mg/l

EC 100 (48 h) : >100 mg/l

Conclusion: The EC50 (48h) is >100 mg/l. According to the 7th Amendment to Directive 67/548/EEC, i.e. Directive 92/32/EEC, the ecotoxicological classification is "not toxic to daphnia"

Test No.: 933719
Test substance : CGA 329351

2. Introduction

- 2.1. Purpose : At the request of the sponsor an acute toxicity study was conducted. This report describes the experimental techniques and the results obtained in this study to determine the acute toxicity of the test substance on daphnia.
Results from pretests for this study or studies not fulfilling the validity criteria are not reported but documented in the raw data.
- 2.2. Guideline : OECD-Guideline No. 202, Part I, 1984
- 2.3. Deviations : None
- 2.4. Testing Facility : CIBA-GEIGY Ltd.
Product Safety
Ecotoxicology
R-1066.P.
CH-4002 Basel / Switzerland
- 2.5. Archives : CIBA-GEIGY Ltd.
R-1066.1.42
CH-4002 Basel

Test No.: 933719
 Test substance : CGA 329351

2.6. Personnel

- Study director : ... **5.1.2.e Woo** Date : 01/12/94
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- Analytical scientist contributing to this report :
 Dr. **5.1.2.e Woo** Date : 1 Dec. 1994
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- Test Facility Management :
 Dr. **5.1.2.e Woo** Date : 7-12-1994
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- Technical personnel : **5.1.2.e Woo** (technician)
 (assistant)

The job descriptions and the summaries of training and professional experience for all personnel participating in this study are archived in the test facility.

2.7. Dates : Begin of test (Study plan): 18/05/94
 (d/m/y) Experimental start : 24/05/94
 Experimental end : 25/11/94
 Study completed : see page 1

2.8. Distribution : Sponsor
 Quality assurance unit
 Archives

Test No.: 933719
 Test substance : CGA 329351

3. Materials and Methods

3.1. Test substance

Identification Code : CGA 329351
 Generic/Trade name : -
 Batch No.: KGL 4634/6
 Appearance : yellow liquid
 Purity : 97.3 %
 Solubility (in water) : -
 Received : 28/02/94
 Storage : room temperature
 Stability : 02/98

3.2. Test system / species

Test system : *Daphnia magna*
 Strain : Straus 1820
 Source : CIBA-GEIGY Ltd., testing facility
 Number of daphnia : 20 per concentration and control.
 4 replicates of 5 daphnia each.
 Feeding : None during the test.
 Breeding : Cultures of daphnia are maintained in glass vessels containing approx. 2.5 l of reconstituted water (see below) at 20 ± 1 °C (water is renewed partially thrice weekly). At each renewal the daphnia are fed with a suspension of green algae (*Scenedesmus subspicatus*) supplemented by a suspension of TETRAMIN-extract in such quantities that the food is consumed after 24h.
 Pretreatment : 24 hours before the begin of the exposure reproductive daphnia are separated from the young by sieving all individuals through a 800 µm sieve. This operation is repeated immediately before the exposure and the young (0-24h of age) are retained for the test.

Test No.: 933719
 Test substance : CGA 329351

3.3 Design and procedure

Vessels : Beakers covered with watch glasses 100 ml solution per beaker
 Water : Reconstituted water containing
 65 mg NaHCO_3
 294 mg $\text{CaCl}_2 \cdot 2 \text{H}_2\text{O}$
 123 mg $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$
 6 mg KCl
 in 1000 ml bidistilled water.
 Total hardness : 240 mg CaCO_3/l .
 The water was aerated with clean air for at least 24 hours before use.
 Temperature : $20 \pm 1^\circ\text{C}$
 Aeration : none
 Lighting : Fluorescent light, 16 hours daily approx. 1500 lux
 Duration : 48 hours

3.4 Stock solution

200.7 mg test substance were mixed with 1500 ml water by polytron for 10 minutes, made up to 2000 ml with water and homogenized by ultrasonification.

3.5. Test concentrations

Nominal : 10, 18, 32, 58 and 100 mg test substance/l

Controls :

Blank : water

Remarks : Calculated amounts of the stock solution to produce the desired test concentrations were given into the water and were homogeneously distributed. The daphnia were then transferred into the beakers.

Test No.: 933719
Test substance : CGA 329351

Sampling
for analysis :

Composite samples of each test concentration were drawn by mixing identical volumes of the test solutions taken from the approximate center of the test vessels. They were taken immediately before exposure and after 48 hours exposure and kept at - 18°C to - 22°C until analysis.

3.6. Observations

Immobilizations were recorded after 24 and 48 hours exposure and given on table 1.

3.7. Measurements

The oxygen content, pH and temperature were measured at 0 and 48 hours. For the values see table 2.

Analytical determination of test substance concentrations see appendix.

3.8. Calculations / Statistical Analysis

none

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Test No.: 933719
 Test substance : CGA 329351

4. Results

The test substance was homogeneously distributed in the test vessels at all test times and test concentrations.

Analytical results and method are presented in the appendix.

Based on nominal concentrations the following values were calculated:

4.1. Values calculated

EC 50 (48 h)	: >100 mg/l
95 % confidence limit	: none
EC 50 (24 h)	: >100 mg/l
95 % confidence limit	: none

4.2. Values graphically determined

EC 50 (48 h)	: not determined
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4.3. Values observed

NOEC (48 h)	: 100 mg/l
EC 0 (48 h)	: 100 mg/l
EC 100 (48 h)	: >100 mg/l

4.4. Controls

Immobilizations in blank	: 0
--------------------------	-----

4.5. Conclusion

The EC 50 (48h) is >100 mg/l. According to the 7th Amendment to Directive (67/548/EEC, i.e. Directive 92/32/EEC, the ecotoxicological classification is "not toxic to dahpnia"

Test No.: 933719
 Test substance : CGA 329351

5. Tables

Table 1 Immobilization
 (Initial numbers of daphnia = 20/test conc.)

Conc. nominal mg/l	Number of daphnia immobilized after 24 h exposure				Total	%
	Vessel:	1	2	3		
blank		0	0	0	0	0
10		0	0	0	0	0
18		0	0	0	0	0
32		0	0	0	0	0
58		0	0	0	0	0
100		0	0	0	0	0

Conc. nominal mg/l	Number of daphnia immobilized after 48 h exposure				Total	%
	Vessel:	1	2	3		
blank		0	0	0	0	0
10		0	0	0	0	0
18		0	0	0	0	0
32		0	0	0	0	0
58		0	0	0	0	0
100		0	0	0	0	0

Test No.: 933719
 Test substance : CGA 329351

Table 2 Measurements

Conc nominal mg/l	0h*			Final measurements**		
	pH	%O ₂	°C	Vessel 1 pH	%O ₂	°C
blank	7.9	100	21	8.0	104	23
10	8.0	99	21	8.0	103	23
18	8.0	99	21	8.0	104	23
32	8.0	100	21	8.0	103	23
58	8.0	99	21	8.0	104	23
100	8.0	100	21	8.0	103	23

* : measurements in separate vessels without daphnia.

** : measurements after the end of exposure. Temperature listed corresponding to the situation at the time of measurements and not identical with temperature during exposure.

Test No.: 933719
 Test substance : CGA 329351

Appendix

Ciba

Crop Protection/Residue Analysis

Basel/Switzerland

ANALYSIS REPORT ON TEST NO. 933719 (PROJECT NO. OF SPONSOR: 933719)
 CGA 329351 WATER

DETERMINATION OF CGA 329351 IN WATER SPECIMENS FROM ACUTE TOXICITY TEST ON DAPHNIA

1. DESCRIPTION OF SPECIMENS

Refer to protocol of project.
 Arrival of specimens: 30 May 1994.
 Storage: at -20°C until analysis.
 Analysis: 30 Sep 1994 - 3 Oct 1994.

2. ANALYTICAL METHOD

General Analytical Method for "Test Substances Used for Ecotoxicity Studies", Residue Analysis,
 8 Feb. 1988.
 Calculations according to General Calculation Method REM 119.04.

Abstract of the method:

HPLC with UV detection: the injected specimen is preconcentrated and precleaned on a short column (C₁₈) and then transferred onto the analytical HPLC column (C₁₈). The substance is eluted with water-acetonitrile (65 vol. + 35 vol.) and detected at 240 nm.

Details of the method:

The HPLC system is equipped with a short column (1 cm length, 4 mm i.d., packed with Nucleosil 100 C₁₈ 5 µm), a switching valve and an analytical column (12 cm length, 4 mm i.d., packed with Nucleosil 100 C₁₈ 5 µm). 1 mL of the water specimen (appropriately diluted with water if necessary) is injected and transferred onto the short column, where it is preconcentrated and precleaned by washing with water. By means of the switching valve and water-acetonitrile (65 vol. + 35 vol.) as the mobile phase the substance is eluted from the short column and transferred onto the analytical column, from where it is eluted by the mobile phase and detected with an UV detector at 240 nm. CGA 329351 is used as the reference substance.

Quantitation: by alternate injections of water specimens and of reference substance solutions. Interpolation by method of weighted least squares of peak heights, regression of 1st order. From the measured contents of CGA 329351 the corresponding values of the test product were calculated (the product contains 97.3% CGA 329351).

The procedure was checked with recovery experiments at two spike levels. 4.5 ml of the control specimen was spiked with 0.5 ml of an appropriate standard solution of CGA 329351 in water.

Test No.: 933719
 Test substance : CGA 329351

Appendix (continued)

Ciba
 Test No. 933719

Crop Protection/Residue Analysis

Basel/Switzerland
 page 2 of 2

3. RESULTS

specimen description	nominal conc. CGA 329351 techn. [mg/L]	conc. found CGA 329351 [mg/L]	conc. found (corr.) CGA 329351 techn. [mg/L]	conc. found (corr.) relative to conc. nominal [%]
24 May 94 0 h	10	9.36	9.03	90
24 May 94 0 h	18	16.7	16.1	90
24 May 94 0 h	32	30.9	29.8	93
24 May 94 0 h	58	55.8	53.8	93
24 May 94 0 h	100	97.1	93.7	94
26 May 94 48 h	10	9.21	8.89	89
26 May 94 48 h	18	17.7	17.1	95
26 May 94 48 h	32	31.3	30.2	94
26 May 94 48 h	58	58.6	56.6	98
26 May 94 48 h	100	97.9	94.5	94
24 May 94 0 h	control	<1.00	<0.97	-
26 May 94 48 h	control	<1.00	<0.97	-

Remarks:

• conc. found (corr.): these results are corrected for an average recovery of 106.5 %.

Recoveries:

Spike level 2.0 mg/L CGA 329351 (2.1 mg/L CGA 329351 techn.): 105%
 Spike level 8.0 mg/L CGA 329351 (8.2 mg/L CGA 329351 techn.): 108 %

Analyst:

5.1.2.e Woo

25 NOV 1994

date

5.1.2.e Woo
 (principal investigator for analytics)

Distribution: Dr. 5.1.2.e Woo (study director)

Original report and raw data in archives of Residue Analysis, PP 2.53.

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