

Test No.: 933720  
Test substance : CGA 329351

1/18

Report on the growth inhibition test  
of CGA 329351 techn. (Enantiomer of CGA 48988)  
to Green Algae (*Scenedesmus subspicatus*)

Study Director : Dr. 5.1.2.e Woo  
Testing Facility : CIBA-GEIGY Ltd.  
Product Safety  
Ecotoxicology  
CH-4002 Basel / Switzerland  
Test Guideline : OECD - Guideline No.: 201, Paris 1984  
Study completed : 01/12/94  
Sponsor : CIBA-GEIGY Ltd  
PP - Division  
CH-4002 Basel / Switzerland  
represented by : Dr. 5.1.2.e Woo  
Project No. of Sponsor : 933720

This report contains 18 pages.

European Registration Dossier  
Dossier File N°: 8.2.6/5  
Ciba File N°: 329351/27

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### 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

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Reserved page for flagging statements

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## Quality Assurance Statement

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

Project 933720  
 Test Substance CGA 329351  
 Study Title Growth inhibition test of CGA 329351 techn. (Enantiomer of CGA 48988) to Green Algae (*Scenedesmus subspicatus*)  
 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

5.1.2.e Woo

Inspector Quality Assurance

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## 1. Summary

Study : Determination of EbC 50 (0-72 h) :  
Concentration at which the growth of algae  
is reduced by 50% in relation to control.

Test system : Green Algae (*Scenedesmus subspicatus*)

Duration : 72 hours

Guideline : OECD - Guideline No.: 201, Paris 1984

Deviations : None

Test substance : Identification Code : CGA 329351  
Batch No. : KGL 4643/6  
Purity : 97.3 %

Design : After a preculture period of 3 days,  
*Scenedesmus subspicatus* (initial 10600  
cells/ml) was exposed to the nominal  
concentrations 4.4, 9.6, 21, 46 and 100 mg  
test substance/l.

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

EbC 50 (0-72 h) : 36 mg/l  
95 % confidence limit : 28-44 mg/l  
slope : 1.65

NOEbC (0-72 h) : 9.6 mg/l  
(5 % level)

Conclusion : Based on the EbC50 (0-72h) the  
ecotoxicological classification is according  
to the 7th Amendment to Directive 67/548/EEC,  
i.e. Directive 92/32/EEC:  
harmful to algae

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## 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 algae.

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.: 201, Paris 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-1066.142  
CH-4002 Basel



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## 2.6. Personnel

- Study director : ..... Date : 01/12/94..  
Dr. 5.12.e Woo
- Scientist contributing to this report  
[ ] 5.12.e Woo Date : 1 Dec. 1994  
Dr. 5.12.e Woo
- Test Facility Management :  
5.12.e Woo Date : 7-12-94  
Dr. 5.12.e Woo
- Technical personnel : 5.12.e Woo (technician)

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
  - Experimental start : 24/05/94
  - Experimental end : 25/11/94
  - Study completed : see page 1

## 2.8. Distribution :

Sponsor  
Quality assurance unit  
Archives

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### 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

Strain : Green Algae (*Scenedesmus subspicatus*)

Origin : Collection of algae cultures  
Pflanzenphysiologisches Institut  
University  
Nikolausberger Weg 18  
D-3400 Göttingen  
Germany

Initial cell dens.: 10600 cells/ml

Culture : According to the guideline cited.

Preculture : 3 days under test conditions.

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### 3.3. Design and procedure

Vessels : 100 ml Erlenmeyer flasks, cotton stoppers,  
on Lab-shaker, 50 ml test solution per flask

Water : Composition according to the guideline.

Temperature :  $22 \pm 1^\circ\text{C}$

Lighting : Continuous illumination,  
cold white fluorescent light,  
 $115 \mu\text{E}/\text{m}^2 \text{ sec} \pm 20\%$  (approx. 8000 lux)

Duration : 72 hours

3.4. Stock solution 100.3 mg test substance were mixed and made up  
to 1000 ml with water and homogenized by  
ultrasonification.

### 3.5. Test concentrations

Nominal : 4.4, 9.6, 21, 46 and 100 mg test substance/l

Controls :  
Blank : water

Replicates : Each test concentration was tested in  
3 replicates, the blank control in 6.

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

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#### Sampling

for analysis : Composite samples of each test concentration were drawn by mixing identical volumes of the test solutions taken from of the test vessels. They were taken immediately before exposure and after 72 hours exposure and kept at  $-18^{\circ}\text{C}$  to  $-22^{\circ}\text{C}$  until analysis.

### 3.6. Measurements

Cell densities were measured at 24, 48 and 72 hours exposure on a "TOA" cell counter. For the results see tables 1 to 4 and figure 1.

Temperature was continuously measured and maintained at  $22 \pm 1^{\circ}\text{C}$ .

pH was measured at 0h and 72h exposure; for results see table 5.

Analytical determination of test substance concentrations see appendix.

### 3.7. Calculations/Statistical Analysis

The EbC-50 values were calculated according to the maximum likelihood method, logit model (Mc Cullagh, P., Nelder, J.A., 1983: Generalized linear models, Chapman & Hall, London)

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#### 4. Results

##### 4.1. Values calculated

The test substance was homogeneously distributed in the test vessels at all test times and test concentrations. The measured concentrations were within the range  $100 \pm 20\%$  of the nominal concentrations (see analytical results page 17) therefore the following values were based on nominal concentrations.

##### a) Inhibition, areas under growth curves

EbC 50 (0-72 h) : 36 mg/l  
slope :  
95 % confidence limit : 28-44 mg/l

NOEbC (0-72 h) (5 % level): 9.6 mg/l

##### b) Inhibition, growth rates

ErC 50 (0-72 h) : 103 mg/l  
slope : 1.259  
95 % confidence limit : 93-117 mg/l

NOErC (0-72 h) (5 % level) : 9.6 mg/l

##### 4.2. Values observed

none

##### 4.3. Conclusion

Based on the EbC 50 (0-72 h) the ecotoxicological classification is according to Directive 67/548/EEC, i.e. Directive 92/32/EEC:  
harmful to algae

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5. Tables and Figures

Initial cell density: 10600 cells/ml

Multiplication factor N72h / N 0h: 59

Table 1: Cell densities after 24h exposure

Conc. nominal mg/l	Dilut. Factor 1:	Cell Densities/sample cells/ml*10000						Mean Dens. N24h cells/ml*10000
		1	2	3	4	5	6	
Blank	10	2.6	2.4	2.2	2.2	2.2	2.1	2.3
4.4	10	2.3	2.6	2.5				2.5
9.6	10	2.4	2.7	2.6				2.5
21	10	2.4	2.3	2.7				2.5
46	10	2.8	2.9	2.6				2.7
100	10	2.6	2.5	2.5				2.5

Table 2: Cell densities after 48h exposure

Conc. nominal mg/l	Dilut. Factor 1:	Cell Densities/sample cells/ml*10000						Mean Dens. N48h cells/ml*10000
		1	2	3	4	5	6	
Blank	10	10.8	10.3	12.6	10.5	13.6	14.5	12.0
4.4	10	7.2	7.1	7.7				7.3
9.6	10	9.9	9.3	9.7				9.6
21	10	13.1	10.0	13.9				12.3
46	10	4.4	4.1	4.1				4.2
100	10	3.8	4.0	4.1				4.0

Table 3: Cell densities after 72h exposure

Conc. nominal mg/l	Dilut. Factor 1:	Cell Densities/sample cells/ml*10000						Mean Dens. N72h cells/ml*10000
		1	2	3	4	5	6	
Blank	100	72.5	73.5	54.5	53.5	51.0	68.5	62.3
4.4	100	56.5	64.5	69.0				63.3
9.6	100	52.0	59.0	68.0				59.7
21	100	37.0	34.5	39.5				37.0
46	100	22.0	21.5	20.0				21.2
100	100	8.5	9.0	9.0				8.8

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Table 4a: Inhibition

Conc. nominal mg/l	Areas under Growth Curves (A) *10000						Mean A	Inhibition * IA 0-72h (%)
	1	2	3	4	5	6		
Blank	1127	1123	944	882	926	1154	1026	0.0
4.4	842	943	1008				931	9.3
9.6	853	931	1045				943	8.1
21	751	646	809				735	28.4
46	371	361	336				356	65.3
100	191	199	202				197	80.8

Table 4b: Inhibition

Conc. nominal mg/l	Growth Rates ( $\mu$ ) *0.001						Mean $\mu$	Inhibition * I $\mu$ 0-72h (%)
	1	2	3	4	5	6		
Blank	59	59	55	54	54	58	56	0.0
4.4	55	57	58				57	0.0
9.6	54	56	58				56	0.9
21	49	48	50				49	12.6
46	42	42	41				42	26.3
100	29	30	30				29	47.8

\* promotion of algal growth in regard to control is indicated as 0

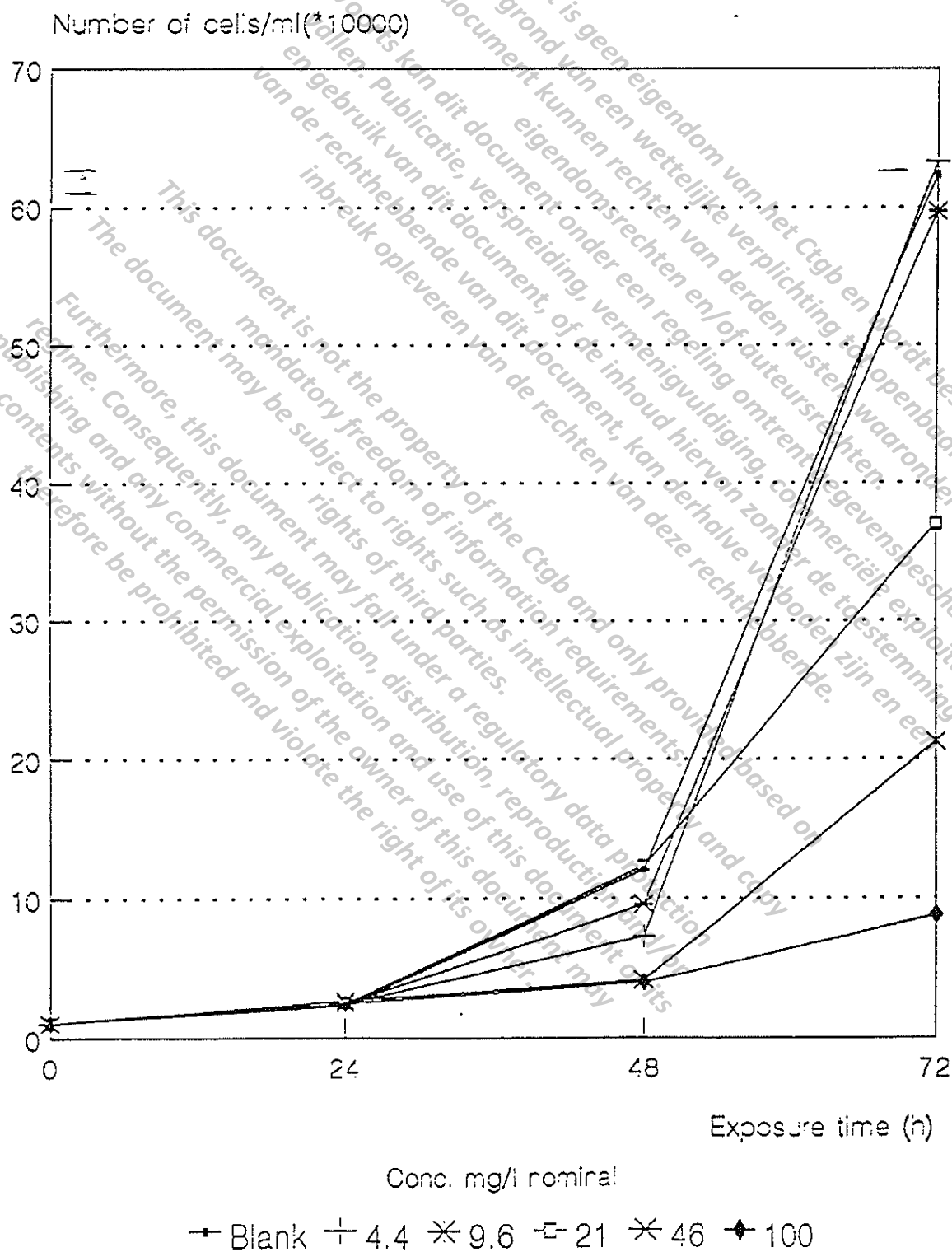
Table 5: pH-values

Conc. nominal mg/l	pH	
	0 h	72 h
Blank	7.7	8.0
4.4	7.8	8.0
9.6	7.8	8.0
21	7.8	8.2
46	7.8	8.2
100	7.8	8.0

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Figure 1: Growth curves





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## Appendix

Ciba

Crop Protection/Residue Analysis

Basel/Switzerland

ANALYSIS REPORT ON TEST NO. 933720 (PROJECT NO. OF SPONSOR: 933720)  
 CGA 329351 WATER

### DETERMINATION OF CGA 329351 IN WATER SPECIMENS FROM GROWTH INHIBITION TEST TO GREEN ALGAE

#### 1. DESCRIPTION OF SPECIMENS

Refer to protocol of project.  
 Arrival of specimens: 30 May 1994.  
 Storage: at -20°C until analysis.  
 Analysis: 29 Sep 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 (C<sub>18</sub>) and then transferred onto the analytical HPLC column (C<sub>18</sub>). 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 C<sub>18</sub>, 5 µm), a switching valve and an analytical column (12 cm length, 4 mm i.d., packed with Nucleosil 100 C<sub>18</sub>, 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 1<sup>st</sup> 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.

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Crop Protection/Residue Analysis

Basel/Switzerland  
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## 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	4.4	4.13	4.10	93
24 May 94 0 h	9.6	8.91	8.85	92
24 May 94 0 h	21	20.0	19.9	95
24 May 94 0 h	46	43.6	43.3	94
24 May 94 0 h	100	97.0	95.3	95
27 May 94 72 h	4.4	3.94	3.91	89
27 May 94 72 h	9.6	8.38	8.32	87
27 May 94 72 h	21	18.3	18.2	87
27 May 94 72 h	46	40.5	40.2	87
27 May 94 72 h	100	88.0	87.4	87
24 May 94 0 h	control	<1.00	<0.99	-
27 May 94 72 h	control	<1.00	<0.99	-

## Remarks:

- conc. found (corr.): these results are corrected for an average recovery of 103.5 %.

## Recoveries:

Spike level 2.0 mg/L CGA 329351 (2.1 mg/L CGA 329351 techn.): 103%

Spike level 8.0 mg/L CGA 329351 (8.2 mg/L CGA 329351 techn.): 104%

Analyst: 5.12.e Woo

25 NOV 1994

date

(principal investigator for analytics)

Distribution: Dr. 5.12.e Woo (study director)

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