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Ciba File N°: 329351/324

## CGA 329351

### STUDY TITLE

**Report on the test for Activated Sludge Respiration  
Inhibition of CGA 329351 (Enantiomer of CGA 48988)**

### DATA REQUIREMENT

OECD - Guideline No.: 209 (Paris 1984)

### AUTHOR

Dr. XXXXXXXXXX

### STUDY COMPLETED ON

January 10, 1996

### PERFORMING LABORATORY

*CIBA-GEIGY Limited*  
Crop Protection Division  
CH-4002 Basle, Switzerland  
Product Safety  
Ecotoxicology Department

### LABORATORY PROJECT IDENTIFICATION

Project Number: **953610**

### SPONSOR

*CIBA-GEIGY Limited*  
Crop Protection Division  
Ecotoxicology Department  
CH-4002 Basle, Switzerland







**Quality Assurance Statement**  
Ciba-Geigy Ltd., GLP Quality Assurance Product Safety, 4002 Basel

Project 953610  
Test Substance CGA 329351  
Study Title Activated sludge respiration inhibition of CGA 329351  
(Enantiomer of CGA 48988)  
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	August 30, 1995	November 02, 1995
Protocol Audit	November 15, 1995	November 15, 1995
Final Report Audit	January 09, 1996	January 10, 1996

January 10, 1996  
Date  
Form QSSTAT12

5.1.2.e Woo

Inspector Quality Assurance

**LIST OF AMENDMENTS TO PROTOCOL**

During the experimental period of the study no amendments to the study protocol were reported.

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## 1. Summary

Study : Determination of the inhibitory concentration of a chemical substance on the respiration of aerobic waste water bacteria

Test system : Activated sludge from a sewage treatment plant.

Duration : 3 hours

Guideline : OECD Guideline No. 209 (April 1984)

Deviations : Instead of a centrifuged sludge a settled sludge was used.

Design: The respiration rate of an activated sludge fed with a standard amount of synthetic sewage feed is measured after a contact time of 3 hours.  
The respiration rate of the same activated sludge is also measured in the presence of 1.0, 3.2, 10.0, 32.0 and 100 mg test substance/l, respectively

Test substance : Identification Code: CGA 329351  
Batch No.: Op.4  
Purity: 97.1 %

Results : The inhibitory effect of the test substance is expressed as a percentage of the mean respiration rates of two controls. Following values were based on nominal concentrations:

EC50(3h) : >100 mg/l

EC20(3h) : >100 mg/l

EC80(3h) : >100 mg/l

Conclusion: Based on the results , EC 50 (3h) >100 mg/l, CGA 329351 is:  
"not toxic to bacteria"



## 2. Introduction

- 2.1. **Purpose :** This report describes the experimental techniques and the results obtained in this study to determine the inhibitory concentration of the test substance on aerobic waste-water bacteria. 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. 209 (April 1984)
- 2.3. **Deviations :** Instead of a centrifuged sludge a settled sludge was used.
- 2.4. **Testing Facility :** CIBA-GEIGY Ltd.  
Product Safety  
Ecotoxicology  
CH-4002 Basel / Switzerland
- 2.5. **Archives :** CIBA-GEIGY Ltd.  
Product Safety  
Ecotoxicology  
CH-4002 Basel

2.6. Personnel

• Study director : ..... Date : January 10, 1996  
Dr. 5.1.2.e Woo

• Test Facility Management  
Dr. 5.1.2.e Woo Date : Jan. 10, 1996

Technical personnel : Mrs. 5.1.2.e Woo  
Mr. 5.1.2.e Woo

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) : November 10, 1995  
Experimental start : November 15, 1995  
Experimental end : December 07, 1995  
Study completed : see page 1

2.8. Distribution :  
Sponsor  
Quality assurance unit  
Archives

### 3. Materials and Methods

#### 3.1. Test substance

Identification Code : CGA 329351  
Generic/Trade name : -  
Batch No.: Op.4  
Appearance : brown liquid  
Purity : 97.1 %  
Solubility (in water) : 26 g/l  
Received : 14/09/95  
Storage : room temperature  
Stability : 04/1998

#### 3.2. Reference substance

Identification : 3,5-Dichlorophenol  
ALDRICH D 7,060-0  
Stability : 12/99  
Storage : room temperature

#### 3.3. Test system / inoculum

Activated sludge collected from the sewage treatment plant of CH-4153 Reinach on 06/12/95. The pH after collection was 8.2.  
The preparation was carried out according to the method described in the guideline.  
The sludge was separated from the aqueous layer by settling instead of centrifugation.  
The pH of the sludge before use was 8.4.

#### 3.4. Design and procedure

Vessels : 250 ml BOD flasks with gas inlet  
Water : Dechlorinated drinking water  
Temperature :  $20 \pm 2^\circ\text{C}$   
Duration : 3 hours  
Nutrient solution : 16 g Peptone  
11 g Meat extract  
3.0 g Urea  
0.7 g NaCl  
0.4 g  $\text{CaCl}_2 \cdot 2 \text{H}_2\text{O}$   
0.2 g  $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$   
2.8 g  $\text{K}_2\text{HPO}_4$

were dissolved and the volume made up to one liter with dechlorinated drinking water.

### 3.5. Stock solution and test solutions

500.2 mg test substance were mixed and made up to 1000 ml with water. (stock solution for test concentration 100 mg/l)

160 ml of the stock solution were mixed and made up to 500 ml with water. (dilution 160 mg/l for test concentration 32 mg/l)

50 ml of the stock solution were mixed and made up to 500 ml with water (dilution 50 mg/l for test concentration 10 mg/l)

50 ml of dilution 160 mg/l were mixed and made up to 500 ml with water. (dilution 16 mg/l for test concentration 3.2 mg/l).

50 ml of dilution 50 mg/l were mixed and made up to 500 ml with water. (dilution 5 mg/l for test concentration 1 mg/l).

The test solution was freshly prepared at the start of the test.

### 3.6. Test concentrations

Test substance : 100.0, 32.0, 10.0, 3.2 and 1.0 mg/l

Reference substance : 32.0, 10.0 and 3.2 mg/l

6.4 ml of the nutrient solution, 74 ml water, 40 ml of the test solutions and 80 ml of the sludge were mixed in 250 ml BOD flasks and aerated immediately.

### 3.7. Measurements

Oxygen consumption per hour in mg/liter with a ORION -Electrode Type 97-08, ORION Microprocessor Ionizer 901 and plotted on a recorder.

The sludge concentration in the test bottles was 1.48 g/l (dry weight).

### 3.8. Calculations/Statistical Analysis

The inhibitory values were calculated on the basis of the measured time depend oxygen consumption of a Blank and Test solution.

The Results ( ECxx Values ) were determined after calculation the linear regression.

The following formulas were used.

c = concentration  
i = inhibition  
n = number of values

$$A = \frac{\sum(\text{Ln}(c) * i) - (\sum(\text{Ln}(c)) * \sum(i) / (n-1))}{\sum(\text{Ln}(c))^2 - (\sum(\text{Ln}(c)) * \sum(\text{Ln}(c)) / (n-1))}$$

$$B = \sum(i) / n - A * \sum(\text{Ln}(c)) / (n-1)$$

$$\text{ECxx} = \text{Exp}((xx - B) / A)$$

## 4. Results

The inhibitory effect of the test substance is expressed as a percentage of the mean respiration rates of two controls. Following values were calculated based on nominal concentrations:

### 4.1. Values

For the test substance (tested nominal concentrations: 1.0, 3.2, 10.0, 32.0 and 100 mg/l):

EC50(3h) : >100 mg/l

EC20(3h) : >100 mg/l

EC80(3h) : >100 mg/l

For the reference substance (tested nominal concentrations: 3.2, 10.0 and 32.0 mg/l):

EC50(3h) : 18.2 mg/l

EC20(3h) : 8.3 mg/l

EC80(3h) : 40.0 mg/l

### 4.2. Conclusion

Based on the results, EC 50 (3h) > 100 mg/l, CGA 329351 is:

“not toxic to bacteria”

## 5. Tables and Figures

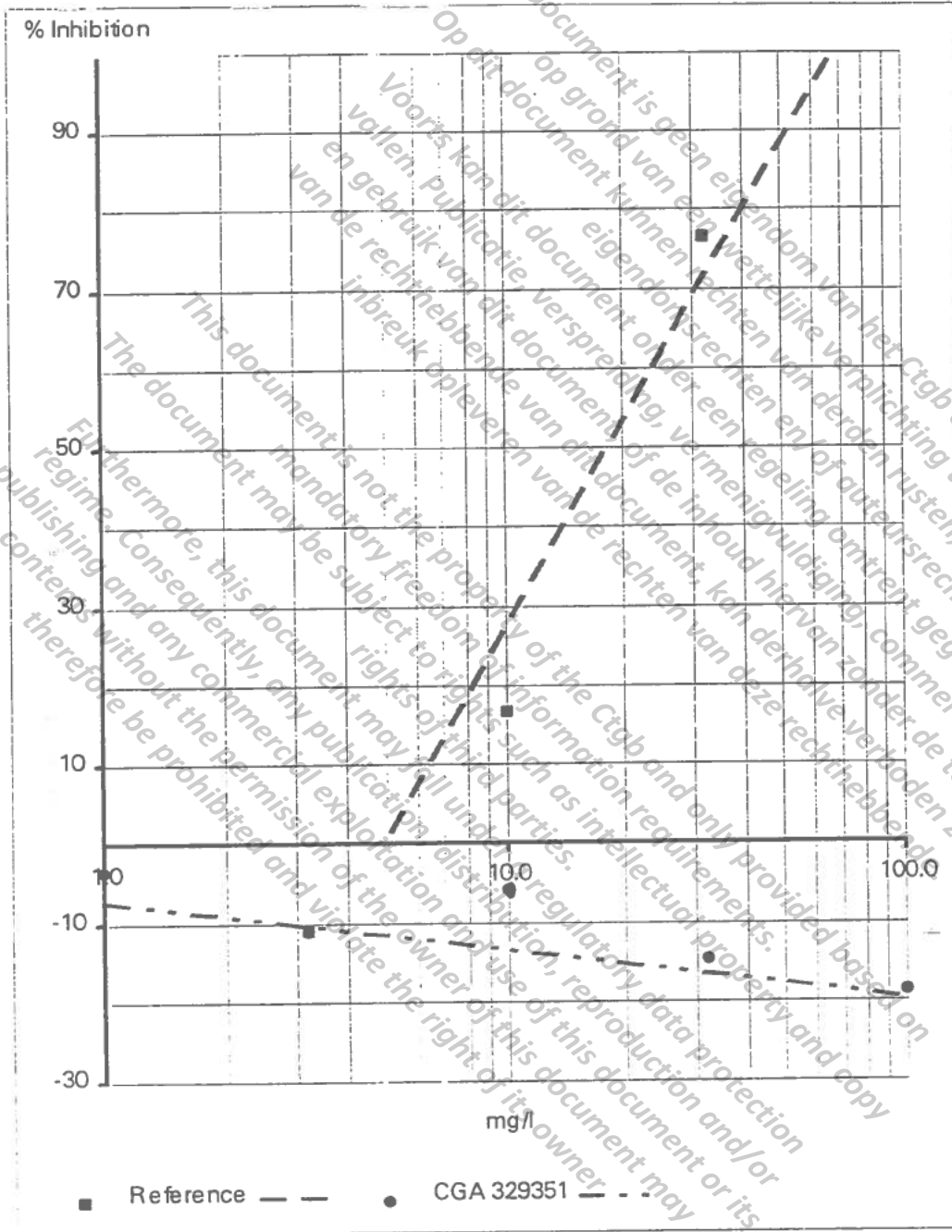
**Table: Consumption rate and inhibitory effects of the test substance and reference**

Sample	Conc. [mg/l]	Consumption rate [mg/l/h]	Inhibition [%]	pH
Blank 1	0.0	69.8	-3	8.5
Blank 2	0.0	65.2	3	8.6
Reference 1	32.0	15.8	77	8.6
Reference 2	10.0	56.3	17	8.5
Reference 3	3.2	75.0	-11	8.5
Test conc. 1	100.0	80.0	-19	8.6
Test conc. 2	32.0	77.4	-15	8.5
Test conc. 3	10.0	71.4	-6	8.5
Test conc. 4	3.2	75.0	-11	8.5
Test conc. 5	1.0	69.8	-3	8.5

The pH was measured after 3h aeration.

Negative inhibition results as obtained in this test, are a consequence of the comparison of oxygen consumption values obtained in the blank, and the higher oxygen consumption values obtained in the presence of the test substance, which are due to biological variations between the various test samples.

Figure: Inhibitory concentration of the test substance



**APPENDICES:**

**A. Study Protocol**

**Study Protocol**

CGA 329351

**STUDY TITLE**

Study protocol for the test for activated sludge respiration inhibition of CGA 329351 (Enantiomer of CGA 48988)

**DATA REQUIREMENT**

OECD Guideline No. 209 (April 1984)

**SPONSOR**

CIBA-GEIGY Limited  
Crop Protection Division  
CH-4002 Basle, Switzerland  
Product Safety  
Ecotoxicology Department

**TESTING FACILITY**

CIBA-GEIGY Limited  
Crop Protection Division  
CH-4002 Basle, Switzerland  
Product Safety  
Ecotoxicology Department

**LABORATORY PROJECT IDENTIFICATION**

Project Number: 953610

**PROPOSED EXPERIMENTAL START**

November 1995

**PROPOSED EXPERIMENTAL TERMINATION DATE**

December 1995

Total Number of Pages: 9



Ciba Crop Protection  
Ecotoxicology

Test substance: CGA 329351

Project No: 953610 - Page 2 of 9

GENERAL INFORMATION

Study Signatures

Study Director :

Date: November 10, 1995

D. 512e Woo

Test Facility Management :

Date: November 10, 1995

D. 512e Woo

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Ciba Crop Protection  
Ecotoxicology

Test substance: CGA 329351

Project No: 953610 - Page 3 of 9

**Guidelines** OECD Guideline No. 209 (April 1984)

**Sponsor** CIBA-GEIGY Limited  
Crop Protection Division  
CH-4002 Basle, Switzerland  
Product Safety  
Ecotoxicology Department

**represented by** Dr. 5.1.2.e Woo

**Testing Facilities** CIBA-GEIGY Limited  
Crop Protection Division  
CH-4002 Basle, Switzerland  
Product Safety  
Ecotoxicology Department

**Study Director** Dr. 5.1.2.e Woo

**Technical Personnel** 5.1.2.e Woo

**Analytical Laboratory** none

**Analytical Scientist** none

**Supplier of the Test Substances** CIBA-GEIGY Limited  
Crop Protection Division  
CH-4002 Basle, Switzerland  
Product Safety  
Safety Assessment

**represented by** Dr. 5.1.2.e Woo

**Testing Period (proposed)** Start of Experiments: November 1995  
Date of Termination: December 1995  
Date of Completion: December 1995

**Amendment Procedure** This protocol can be amended at the discretion of the study director. Detailed descriptions of all amendments will be signed by the study director. The amendment will be effective at the time of study directors signature. The amendment will be distributed and added to all copies of the protocol.

**Archives** Protocols, raw data, correspondence, calculations, and the final report will be archived for at least ten years in the test facilities at:

CIBA-GEIGY Limited  
Crop Protection Division  
CH-4002 Basle, Switzerland  
Product Safety  
Ecotoxicology Department

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Ecotoxicology

Test substance: CGA 329351

Project No: 953610 - Page 6 of 9

### 1. Purpose and Objective

The purpose of this test is to provide a method for screening of substances for determination of the inhibitory effect on the respiration of aerobic waste-water bacteria aqueous system. Substances which may adversely affect aerobic microbial treatment plants can be identified.

Furthermore the test indicates suitable non-inhibitory concentrations of test substances to be used in biodegradability tests.

The method assesses the effect of a test substance on micro-organisms by measuring the respiration rate under defined conditions in the presence of different concentrations of the test substance. The respiration rate is the oxygen consumption of aerobic sludge or waste-water micro-organisms expressed as mg O<sub>2</sub> per litre per hour.

### 2. Materials and Methods

The test system will fulfill the specifications of the cited protocol and SOP No.: 801701.

#### 2.1. Test substance

Company Code	CGA 329351 (Enantiomer of CGA 48988)
Generic/Trade name	-
Summary Formula	C <sub>15</sub> H <sub>11</sub> NO <sub>2</sub>
Appearance	brown liquid
Batch No.	Op.4
Purity	97.1 %
Solubility (in water)	not yet known
Received	14/09/1995
Storage	room temperature
Stability	04/1998
Safety	The test substance will, unless otherwise specified, be handled with routine hygiene procedures to maintain both human and environmental safety.
Test concentrations	blank (two replicates), reference 3.2, 10 and 32 mg/l test substance: 1.0, 3.2, 10, 32 and 100 mg/l
Vehicle	If possible, no vehicle is used.
Reference	3,5 Dichloro-phenol

#### 2.2. Test Organisms

**Test organisms** Activated sludge from a sewage treatment plant is used as the microbial inoculum for the test.

**Justification of species** Activated sludge is obtained from a sewage work treating predominantly domestic sewage. Results obtained with activated sludge from works treating industrial waste waters may be atypical.



Ciba Crop Protection  
Ecotoxicology

Test substance: CGA 329351

Project No: 953610 - Page 8 of 9

## 2.5. Sampling and Observations

### Sampling for analysis

After three hours 100 ml of the sample will be poured into the measuring apparatus (100 ml BOD<sub>5</sub> flask) and the respiration rate will be measured over a period up to 10 minutes

### Water Quality Measurements

pH of the sludge before start and of the test sample at the end of exposure

### Biological Observations

none

### Acceptability Criteria

If the respiration rates of the two controls are not within 15 per cent of each other or the reference substance is not in the accepted range (5 to 30 mg/l), the test is invalid and must be repeated.

## 2.6. Statistical Analysis

### Calculation of Results:

In order to calculate the inhibitory effect of the test substance at a particular concentration, the respiration rate is expressed as a percentage of the mean of the two control respiration rates:

$$1 - 2R_t / (R_{c1} + R_{c2})$$

R<sub>t</sub>: oxygen-consumption rate at tested concentration

R<sub>c1</sub> and R<sub>c2</sub>: oxygen-consumption rate of control 1 and 2

The per cent inhibition will be calculated at each test concentration as above. The per cent inhibition will be plotted against concentration on log paper and a linear regression calculated. The EC 50 (if possible EC 20 and EC 80) value will be reported.

## 3. Reporting

The final reports will contain but not be limited to the following information:

- the name, address, and signature of the study director
- the names of all technical personnel participating in this study
- the name and address of the testing facilities
- the testing period
- the objectives of the study
- the description of the test substance
- the description of the test system
- the results of the water chemistry (e.g., hardness)
- the experimental design
- the test conditions (lighting, photoperiod, etc.)
- the description of all methods and procedures used
- the experimental findings
- the description of all circumstances that may have affected the quality or integrity of the data
- the description of all operations, calculations, and transformations that were performed on the data presented, including detection limits and examples of raw data copies
- a summary of the results obtained
- a conclusion from the results obtained
- the results of the analytical determination of the test concentrations
- the analytical method

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Ciba Crop Protection  
Ecotoxicology

Test substance: CGA 329351

Project No: 953610 - Page 9 of 9

- the locations where protocols, correspondence, raw data, and the final report are archived
- a GLP Compliance Statement
- a Quality Assurance Statement
- a Certification of Authenticity

#### 4. References

OECD Guideline for testing of chemicals, 4 April 1984.

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## B.Deviations to Protocol

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## C. Report on chemical Composition

# 5.1.1.c Woo

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