

<b>Annex</b>	<b>II</b>	<b>Effects on earthworms - Acute toxicity</b>
<b>Point addressed</b>	<b>8.4.1</b>	

1.2	<b>Title</b>	Acute toxicity test of CGA 329351 (enantiomer of CGA 48988) to the earthworm ( <i>Eisenia foetida</i> )
1.3	<b>Report and/or project N°</b> <b>Ciba File N° (Desire)</b>	973501 329351/689
1.4	<b>Lab. Report N°</b>	973501
1.5	<b>Cross reference to original study / report</b>	8.4.1/02
1.6	<b>Authors</b>	Report: [redacted] Summary: [redacted]
1.7	<b>Date of report</b>	28 July, 1997
1.8	<b>Published / owner</b>	Unpublished / Novartis Crop Protection AG
2.1	<b>Testing facility</b>	Novartis Crop Protection AG, Environmental Safety, Ecotoxicology, CH-4002 Basel, Switzerland.
2.2	<b>Dates of experimental work</b>	24 March 1997 - 28 July, 1997
3.	<b>Objectives</b>	To assess the toxicity of technical CGA 329351 to the earthworm ( <i>Eisenia foetida</i> ).
4.1	<b>Test substance</b>	CGA 329351 technical
4.2	<b>Specification</b>	Batch No.: Op. 4, purity 97.1%.
4.3	<b>Storage stability</b>	The test substance was reported stable to April, 1998
4.4	<b>Stability in vehicle</b>	Not specified in the report.
4.5	<b>Homogeneity in vehicle</b>	The test substance was mixed in water with no reported problems with solubility.
4.6	<b>Validity</b>	Not applicable.
5	<b>Vehicle / solvent</b>	Water, added to artificial soil mix [10% sphagnum peat (% related to dry weight), 20% kaolin clay, 69% industrial sand, approx. 1% CaCO <sub>3</sub> (to adjust the pH to 5.5 - 6.5)]
6	<b>Physical form</b>	Brown liquid
7.1	<b>Test method</b>	OECD Guidelines for testing of chemicals, No. 207 (April 1984), Earthworm, Acute Toxicity Test
7.2	<b>Justification</b>	Not applicable.
7.3	<b>Copy of method</b>	Available on request.
8	<b>Choice of method</b>	Not applicable.
9	<b>Deviations</b>	- The water content was adjusted to reach approx. 60% of the dry weight of the soil (Approx. 40% of total weight of soil). This higher water content corresponds to the conditions prescribed for the sublethal worm study (40 - 60% of water holding capacity) and seems to represent test conditions which are more adequate for the test organism based on our experience.  -Temperature: 22.0 - 23.0°C due to season.
10.1	<b>Certified laboratory</b>	Not specified in the report.

<b>10.2 Certifying authority</b>	Not applicable.
<b>10.3 GLP</b>	The study was conducted in compliance with the Procedures and Principles of Good Laboratory Practice (March 1986) issued by the Swiss Federal Department of the Interior and 40 CFR, Part 160
<b>10.4 Justification</b>	Not applicable
<b>11.1 GEP</b>	Not applicable.
<b>11.2 Type of facility (official or officially recognised)</b>	Not applicable.
<b>11.3 Justification</b>	Not applicable.
<b>12 Test system</b>	<p>Test species: Earthworm (<i>Eisenia foetida</i>)</p> <p>Age: Mature clitellate adults (greater than two months old)</p> <p>Supplied by: <span style="background-color: grey; color: white;">31.2.5.Woo</span></p> <p>No. of animals: 240: 40 per concentration and control (4 replicates of 10 worms each)</p> <p>Average weight: 342 mg (330 - 360 mg per concentration and control) at start of exposure)</p> <p>Acclimatisation: Earthworms were gradually acclimated to the test conditions, and were held for 41 days prior to testing.</p> <p>Test soil: 10% sphagnum peat (% related to dry weight), 20% kaolin clay, 69% industrial sand, approx. 1% CaCO<sub>3</sub> (to adjust the pH to 5.5 - 6.5)</p> <p>Moisture content (% of dry wt. of soil): 37 - 38% (at start of exposure); 35 - 36% (at end of exposure)</p> <p>pH: 6.3 - 6.5 (at start of exposure)</p> <p>Temperature: 22.0 - 23.0°C</p> <p>Photoperiod: Continuous illumination at 400 - 800 lux with cold white fluorescent light</p> <p>Concentrations: 0 (blank control), 12.3, 37, 111, 333 and 1000 mg/kg dry weight soil (nominal concentrations).</p> <p>Application: The test substance was mixed with water, and the required amounts added to the dry premixed artificial soil. The soil was then mixed thoroughly.</p> <p>Duration of exposure: 14 days</p> <p>Assessments: At the start of the test, the number of worms which are still on the soil surface 10, 30, &amp; 60 min after the initial placing on the soil surface (burrowing time). Mortality, flaccidity, and abnormal behavioural activity and stress after 7 and 14 days of exposure. Body weight at the beginning (day 0) and end (day 14) of the test.</p>

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**13 Findings**

After 14 days exposure, mortality in the blank control and in the concentrations 12.3, 37, 111, 333 and 1000 mg/kg was 0, 0, 0, 0, 0, and 93%, respectively. The LC 50 (14 d) graphically determined was 830 mg/kg. A decrease of the LC50 value from 950 to 830 mg/kg was observed from 7 to 14 days exposure. the LC0 (14 d) was 333 and the LC 100 (14 d) > 1000 mg/kg.

No increases burrowing time was observed at any concentration - no worms were seen at the surface of the soil after 10 min in the control and all test concentrations. After 14 d exposure, flaccidity was observed at concentration 1000 mg/kg with 100% effect on the surviving organisms. The average live weight of the earthworms in the control and at concentrations 12.3, 37, 111, 333 and 1000 mg/kg changed to a weight of 106, 104, 107, 101, 90, and 68% of the initial weight during the 14 days exposure, respectively. The decrease of the average live weight was statistically significant at concentrations 333 and 1000 mg/kg (P<0.05). Thus, the highest concentration with no sublethal or lethal effects was 111 mg/kg.

Using the ecotoxicological classification range proposed by IPS, CGA 329351 is "non-toxic to earthworms" based on the inherent toxicity of this test alone.

Table 1

Average live weight of test organisms before and after exposure to CGA 329351

Nominal concentration mg/kg dry weight soil	Average live weight (mg)*	
	Day 0	Day 14
0 (control)	250	373
12.3	333	345
37	340	365
111	340	343
333	360	323
1000	330	225

\* Based on 10 worms per concentration and control

The 14-day LC<sub>50</sub> for CGA 329351 to earthworms was 830 mg/kg soil. The 14-day LC<sub>0</sub> and LC<sub>100</sub> values were 333 and >1000 mg/kg soil, respectively.

**14 Statistics**

Procedures: Results of the toxicity test were interpreted by standard statistical techniques using SAS Version 6.11 (SAS 1990 a) and the procedure "ECOS" by 5.1.2.e Woo 1996 and "PROPRO" by Fisch, 1996 using SAS PROC GLM and PROC NLIN (SAS 1990b).

LC-50: the LC50 values were calculated according to the maximum likelihood method, probit model (Finney, D. J., 1971).

NOEC: For the determination of NOEC's in regard to abnormalities (lethal and sublethal effects), Dunnett's multiple means comparison test (Dunnett, 1955 & 1989) was performed.

**15 References (published)**

None.

**16 Unpublished data**

None.

**CGA 329351**

**STUDY TITLE**

**Acute Toxicity Test of CGA 329351 (Enantiomer of CGA 48988) to the Earthworm (*Eisenia foetida*)**

**GUIDELINE**

OECD - Guideline No. 207, 1984: Earthworm, Acute Toxicity Test

**AUTHOR**

Dr. 

**STUDY COMPLETED ON**

July 28, 1997

**PERFORMING LABORATORY**

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Sector Unit R&D  
Ecotoxicology Department  
CH-4002 Basle, Switzerland

**LABORATORY PROJECT IDENTIFICATION**

Project Number : 973501

**SPONSOR**

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Sector Unit R&D  
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CH-4002 Basle, Switzerland

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No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10 (d)(1)(A), (B), or (C).

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CERTIFICATE OF AUTHENTICITY

Project 973501  
Test Substance CGA 329351  
Study Director Dr. [Redacted]  
Study Title Acute Toxicity Test of CGA 329351 (Enantiomer of CGA 48988) to the Earthworm (*Eisenia foetida*)

This is an unaltered copy of the final report received from the following laboratory:

Novartis Crop Protection AG  
Sector Unit R&D  
Ecotoxicology Department  
CH-4002 Basle, Switzerland

Company: .....  
Representative: .....  
Signature: .....  
Date: .....

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# Quality Assurance Statement

Novartis Crop Protection AG GLP Quality Assurance, R & D Services, 4002 Basel  
(Successor in business of Ciba-Geigy Ltd. and Sandoz Ltd.)

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Project	973501
Test substance	CGA 329351
Study Title	Acute Toxicity Test of CGA 329351 (Enantiomer of CGA 48988) to the Earthworm ( <i>Eisenia foetida</i> )
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 Based Inspection	January 30, 1997	February 10, 1997
Process Based Inspection	March 19, 1997	March 19, 1997
Protocol Audit	March 24, 1997	March 24, 1997
Final Report Audit	July 21, 1997	July 25, 1997

Date

August 05, 1997

5.1.2.e Woo

Quality Assurance Inspector



**LIST OF AMENDMENTS TO PROTOCOL**

Amendment No.	Date	Concerning	Reason for Alteration
None			

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### GENERAL INFORMATION

#### Study Signatures

- Study Director :

Date :

*July 21, 1999*

Dr. [Redacted]

- Test Facility Management :

Date :

*Aug 9, 1999*

Dr. [Redacted]

Technical personnel :

[Redacted] (principal coworker)

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

**Guideline:**

OECD - Guideline No.: 207, 1984: Earthworm, Acute Toxicity Test

**Deviations to Guideline:**

- The water content was adjusted to reach approx. 60% of the dry weight of the soil (approx. 40% of total weight of soil). This higher water content corresponds to the conditions prescribed for the sublethal worm study (40-60% of water holding capacity) and seems to represent test conditions which are more adequate for the test organism based on our experience.
- Temperature: 22.0-23.0°C due to season.

**Sponsor:**

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Represented by:

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**Archives:**

Novartis Crop Protection AG  
Sector Unit R&D  
Ecotoxicology Department  
CH-4002 Basel, Switzerland

**Dates:**

Begin of test (Study plan) :	March 24, 1997
Experimental start :	April 2, 1997
Experimental end :	April 16, 1997
Study completed :	see page 1

<sup>1</sup>Novartis Crop Protection AG is partial successor in business of Sandoz Ltd. and Ciba-Geigy Ltd. This applies also to all aspects concerned with the requirements of Good Laboratory Practice.

**Good Laboratory Practice:** The study was 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, März 1986], issued by the Swiss Federal Department of Interior and the Intercantonal Office for the Control of Medicaments, Switzerland.

These procedures are based on the OECD Principles of Good Laboratory Practice, adopted May 12, 1981 by Decision of the OECD Council [C(81)30 (Final)] concerning Mutual Acceptance of Data in the Assessment of Chemicals and comply with the Council Directive 87/18/EEC of December 18, 1986.

These procedures are in essence consistent with the following Good Laboratory Practice Standards and Regulations:

- Food and Drug Administration, Department of Health and Human Services: 21 CFR Part 58, USA: September 4, 1987
- Environmental Protection Agency  
Federal Insecticide, Fungicide and Rodenticide Act (FIFRA): 40 CFR Part 160, USA: August 17, 1989
- Environmental Protection Agency  
Toxic Substances Control Act (TSCA): 40 CFR Part 792  
USA: August 17, 1989
- Ministry of Agriculture, Forestry and Earthwormeries (MAFF): 59 NohSan No. 3850, Japan: August 10, 1984
- Ministry of Health and Welfare (MHW)
- Ministry of International Trade and Industry (MITI)  
Japan: October 1, 1985

**Quality Assurance:** Novartis Crop Protection AG  
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Quality Assurance  
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## SUMMARY

**Report:** Acute Toxicity Test of CGA 329351 (Enantiomer of CGA 48988) to the Earthworm (*Eisenia foetida*)

**Guideline:** OECD - Guideline No.: 207, 1984: Earthworm, Acute Toxicity Test

**Deviations:**

- The water content was adjusted to reach approx. 60% of the dry weight of the soil (approx. 40% of total weight of soil). This higher water content corresponds to the conditions prescribed for the sublethal worm study (40-60% of water holding capacity) and seems to represent test conditions which are more adequate for the test organism based on our experience.
- Temperature: 22.0-23.0°C due to season.

**GLP:** Certified GLP-laboratory

### Material and Methods :

**Test substance:** Identification Code: CGA 329351  
 Batch No.: Op. 4  
 Purity: 97.1%  
 Appearance: brown liquid  
 Stability: 04/98

Based on the experience with crop protection products of this chemical class, the following nominal concentrations were chosen for the main test: 12.3, 37, 111, 333, 1000 mg test substance/kg (dry weight of soil). The test was performed exposing 4 replicates of 10 worms per concentration and control in polystyrol beakers of 1 liter (with plastic covers). The beakers were filled with 750 g soil (wet weight).

### Findings :

The pH of the soil was determined to be 6.3-6.5 at the start of the test. The moisture content was 37-38% and 35-36% of the total weight of the soil at the start and the end of the exposure, respectively. Continuous fluorescent light of 400-800 Lux was used throughout the period of the test. Temperature was maintained at 22.0-23.0°C. The nominal concentrations were used to express toxicity. After 14 days exposure, mortality in the blank control and in the concentrations 12.3, 37, 111, 333 and 1000 mg/kg was 0, 0, 0, 0, 0 and 93%, respectively.

Exposure period days	LC50 (95 % Confidence limit) mg/kg	Slope mg/kg	Goodness of fit		
			X <sup>2</sup>	df	p
14	830 (none)	7.80	0.0	3	1.00
7	950 (none)	6.66	0.0	3	1.00

A decrease of the LC50-value from 950 to 830 mg/kg was observed from 7 to 14 days exposure. The LC0 (14 d) was 333 mg/kg and the LC 100 (14 d) >1000 mg/kg. No increased burrowing time was observed at any concentration - no worms were seen at the surface of the soil after 10 min in the control and all test concentrations. After 14 d exposure, flaccidity was observed at concentration 1000 mg/kg with 100% effect on the surviving organisms. The average live weight of the earthworms in the control and at concentrations 12.3, 37, 111, 333 and 1000 mg/kg changed to a weight of 106, 104, 107, 101, 90 and 68% of the initial weight during the 14 days exposure, respectively. The decrease of the average live weight was statistically significant at concentrations 333 and 1000 mg/kg (P<0.05). Thus, the highest concentration with no lethal or sublethal effects was 111 mg/kg.



## 1. Introduction

This report describes the experimental techniques and the results obtained to assess the potential impact of CGA 329351 to Earthworm (*Eisenia foetida*). The effect assessment is based on the time distribution of mortalities and sublethal effects recorded in weekly intervals.

Results from any pretests for this study or studies not fulfilling the validity criteria are not reported but documented in the raw data.

## 2. Materials and Methods

### 2.1. Test Substance

Identification Code	CGA 329351
Appearance	brown liquid
Batch No.	Op. 4
Purity :	97.1%
	see Appendix A
Received	September 14, 1995 <sup>2</sup>
Storage	room temperature
Stability	04/98

### 2.2. Test Organism

Test species: Earthworm (*Eisenia foetida*)

Origin : Supplier: 5.2.e.Woo

Acclimation : Earthworms were gradually acclimated to the test conditions, and were held for 41 days prior to testing.

Feeding : The earthworms were fed with kitchen wastes, supplemented by cattle manure, until 24 hours prior to the test.

Treatment : Earthworm were not treated against a disease during holding.

Handling : Care was taken to minimise possible stress due to handling. Worms which were damaged or dropped during the transfer were not used for the exposure.

Adaptation : 24 hours conditioning in soil used for the test.

Randomization : The groups of ten earthworms each were allocated at random to the test vessels

<sup>2</sup> The test substance was delivered from 'Entwicklungszentrum Agro' EZA, Novartis Crop Protection AG, Muenchwilen, Switzerland



## 2.3. Experimental Design

### 2.3.1. Stock Solution

Preparation of stock solution / Test initiation :

The stock solution was prepared the following way:

4.11 g test substance were mixed with and made up to 1000 ml with water (additional ultrasonication for 30 min).

For each concentration, the required amount of water to obtain the specified moisture content was added to the calculated amount of stock solution. The resulting solution was added to the dry premixed artificial soil by a BAER RN20 - blender and the moist soil was dispensed into the test vessels. These were then transferred to the exposure room, where the earthworms were added (start of exposure).

### 2.3.2. Test Concentrations

Range finding test :

No range finding test was performed. Based on the experience with crop protection products of this chemical class, the following nominal concentrations were chosen.

Definitive test :

Concentrations : nominal: 12.3, 37, 111, 333, 1000 mg/kg (dry weight of soil)

Controls Blank : Artificial soil without test substance

### 2.3.3. Test Procedure

Duration :

14 days

Test organism :

Earthworm (*Eisenia foetida*)

Number of earthworms : 40 per concentration and control  
10 per vessel

Body weight at start (mean) : 342 mg (range 330-360 mg per concentration and control)

Feeding :

None.

Vessels :

Polystyrol beakers of 1 liter (with plastic covers) filled with 750 g soil (wet weight).

Replicates :

Performed with 4 replicates per concentration and control

Artificial soil :

10% sphagnum peat (% related to dry weight)  
20% kaolin clay  
69% industrial sand  
approx. 1% CaCO<sub>3</sub>, to adjust the pH to 5.5-6.5

Temperature :

22.0-23.0°C maintained by room temperature

Lighting :

Continuous fluorescent light of 400-800 Lux

## 2.4. Sampling, Measurements and Observations

### 2.4.1. Soil Quality Measurements and Observations

The pH was measured at the start of the test in each concentration. In addition, the moisture content was determined at the start and the end of the exposure using a Mettler LP 15 infrared drying oven. The temperature (°C) was recorded continuously by a Haenni thermo-hygrograph. The pH was measured with a Methrom pH-meter 632 using a combined glass electrode of Orion Ross Sure-Flow.

### 2.4.2. Biological Observations

At the start of the test, the number of worms which are still on the soil surface 10, 30 and 60 min after the initial placing on the soil surface was recorded in each replicate (burrowing time). After 7 and 14 days exposure, observations of mortality, and sublethal symptoms, such as flaccidity, abnormal behavioural activity and stress were made. Dead earthworm, if any, were removed from the test concentrations at least at the above mentioned intervals.

At test termination, the earthworms of one test vessel per concentration and control were retained for measurements of weight.

## 2.5. Calculations / Statistical Analysis

### Procedures

Results of the toxicity test were interpreted by standard statistical techniques using SAS Version 6.11. (SAS 1990 a) and the procedure "ECOS" by Fisch and Strehlau, 1996 and "PROPRO" by Fisch, 1996 using SAS PROC GLM and PROC NLIN (SAS 1990b).

### LC-50

The LC-50 values were calculated according to the maximum likelihood method, probit model (Finney, D.J., 1971).

### NOEC

For the determination of NOECs in regard to abnormalities (lethal and sublethal effects), Dunnett's multiple means comparison test (Dunnett, 1955 & 1989) was performed.

### 3. Results and Discussion

#### 3.1. Physico-Chemical Data

##### 3.1.1. Soil Quality Data

The pH of the soil was determined to be 6.3-6.5 at the start of the test. The moisture content was 37-38% and 35-36% of the total weight of the soil at the start and the end of the exposure, respectively (Table 4).

##### 3.1.2. Temperature and Light

The temperature was maintained at 22.0-23.0°C. Continuous fluorescent light of 400-800 Lux was used throughout the period of the test.

#### 3.2. Biological Results

The nominal concentrations were used to express toxicity.

##### 3.2.1. Lethal Effects

After 14 days exposure, mortality in the blank control and in the concentrations 12.3, 37, 111, 333 and 1000 mg/kg was 0, 0, 0, 0, 0 and 93%, respectively (Table 1).

Exposure period days	LC 50 (95% Confidence limit) mg/kg	Slope mg/kg	Goodness of fit		
			X <sup>2</sup>	df	p
14	830 (none)	7.80	0.0	3	1.00
7	950 (none)	6.66	0.0	3	1.00

n.d.: not determined

The LC 50 (14 d) graphically determined was 830 mg/kg, see Figure 1. A decrease of the LC50-value from 950 to 830 mg/kg was observed from 7 to 14 days exposure. The LC0 (14 d) was 333 mg/kg and the LC100 (14 d) >1000 mg/kg (see Table 1).

##### 3.2.2. Sublethal Effects

No increased burrowing time was observed at any concentration - no worms were seen at the surface of the soil after 10 min in the control and all test concentrations. After 14 d exposure, flaccidity was observed at concentration 1000 mg/kg with 100% effect on the surviving organisms (Table 2). The average live weight of the earthworms in the control and at concentrations 12.3, 37, 111, 333 and 1000 mg/kg changed to a weight of 106, 104, 107, 101, 90 and 68% of the initial weight during the 14 days exposure, respectively (Table 3). The decrease of the average live weight was statistically significant at concentrations 333 and 1000 mg/kg (P<0.05). Thus, the highest concentration with no sublethal or lethal effects was 111 mg/kg.

### 3.3. Results of Tests with Chloracetamide as Reference Substance

The LC50 (14d) - values of tests with Chloracetamide as reference substance fluctuated within a range of 13 to 33 mg/kg in the time period of March 1985 to February 1997 (Appendix D). Compared to the values of the ring test in 1984, our results of 33, 14, 17, 32, 26, 25 and 13 mg/kg were below the mean LC50 (14d) of 38.5 mg/kg, but all values were within the 95%-confidence interval of 18.5 - 58.6 mg/kg, except for the results of 1990 and 1997 (Appendix D). Hence, our earthworm population and laboratory procedure used was more sensitive to Chloracetamide than the majority of the populations and procedures used in the ring test.

### 4. Conclusion

The LC50 (14d) of CGA 329351 was determined to be 830 mg/kg (dry weight of soil) based on nominal concentrations. Using the ecotoxicological classification range proposed by IPS, CGA 329351 is "non-toxic to earthworms" based on the inherent toxicity of this test alone. After 14 d exposure, the highest concentration with no sublethal or lethal effects was 111 mg/kg.

### 5. References

- Dunnett, C.W., 1955: Multiple comparisons procedure for comparing several treatments with a control, JASA 50, 1096-1121.
- Dunnett, C.W., 1980: Pairwise multiple comparisons in the homogeneous variance, unequal sample size case, JASA 75, 789-795.
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- SAS 1990 b: SAS / GRAPH Software, Version 6, Vol. 1+2, SAS Institute Inc., Cary, NC

**6. Tables and Figures**

**Table 1: Mortalities**

Initial number of earthworms: 40 per test concentration

Concentration nominal mg/kg dry weight of soil	Cumulated dead after:			
	7 days		14 days	
	number	%	number	%
Blank	0	0	0	0
12.3	0	0	0	0
37	0	0	0	0
111	0	0	0	0
333	0	0	0	0
1000	25	63	37	93

**Table 2: Observations and Symptoms of Surviving Organisms**

Concentration nominal mg/kg dry weight of soil	Flaccidity			
	after 7 days		after 14 days	
	number	%*	number	%*
Blank	0	0	0	0
12.3	0	0	0	0
37	0	0	0	0
111	0	0	0	0
333	0	0	0	0
1000	11	73	3	100

\* based on surviving organisms

**Table 3 : Average Live Weight of Test Organisms**

Concentration nominal mg/kg dry weight of soil	Exposure		Average live weight (mg)		% of initial weight	
	Start per container	End per conc. per container	Start per container	End per conc. per container	Start per container	End per conc. per container
Blank	310	360	310	360	116	
	380	390	380	390	103	
	320	340	320	340	106	
	390	400	390	400	103	
12.3		350	350	373		106
	360	330	360	330	92	
	340	350	340	350	103	
	300	360	300	360	120	
	330	340	330	340	103	
37		333	333	345		104
	350	360	350	360	103	
	350	390	350	390	111	
	320	350	320	350	109	
	340	360	340	360	106	
111		340	340	365		107
	330	330	330	330	100	
	340	360	340	360	106	
	360	340	360	340	94	
	330	340	330	340	103	
333		340	340	343		101
	370	300	370	300	81	
	360	350	360	350	97	
	370	310	370	310	84	
	340	330	340	330	97	
1000		360	360	323		90
	320	250	320	250	83	
	300	200	300	200	61	
	330	200	330	200	61	
	370	200	370	200	61	
	330	225	330	225		68 <sup>s</sup>
mean		342	329		94	*
min		330	225		68	*
max		360	373		107	*

\* values of the mean ' % of initial weight ' are based on the values of the concentrations only, without those of the control

<sup>s</sup> statistically significant decrease compared to control (P<0.05)

Table 4 : pH and Moisture Content of Soil

Concentration nominal mg/kg dry weight of soil	pH at start of exposure	moisture content*	
		at start %	at end of exposure %
Blank	6.5	38	36
12.3	6.4	37	35
37	6.4	37	35
111	6.4	37	35
333	6.3	37	36
1000	6.3	38	35
mean	6.4	37	35
min	6.3	37	35
max	6.5	38	36

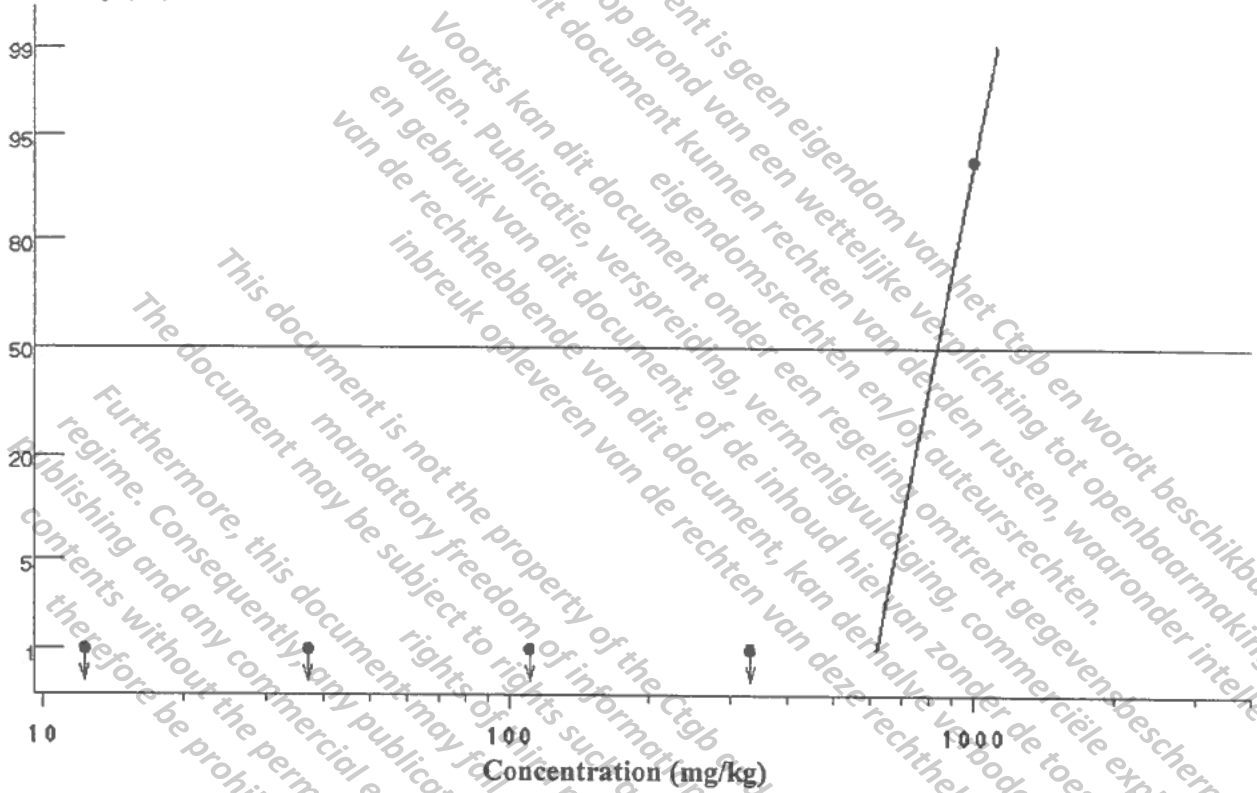
\* based on total weight of soil

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Figure 1: Graphical Determination of LC 50 (14d)

The graphically determined LC 50 (14d) was 830 mg/kg based on nominal concentrations.

Mortality (%)





## Appendix A: Certificate of Chemical Composition

# 5.1.1.0 Woo

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## Appendix B: Study Protocol

### Study Protocol

CGA 329351

### Study Title

Acute Toxicity Test of CGA 329351 (Enantiomer of CGA 48988) to the Earthworm (*Eisenia foetida*)

### Guidelines

OECD - Guideline No. 207, 1984: Earthworm, Acute Toxicity Test

### Sponsor

Novartis Crop Protection AG  
Sector Unit R&D  
Ecotoxicology Department  
CH-4002 Basle, Switzerland

### Testing Facility

Novartis Crop Protection AG  
Sector Unit R&D  
Ecotoxicology Department  
CH-4002 Basle, Switzerland

### Project Number

973501

### Proposed Experimental Start Date

April 1997

### Proposed Experimental Termination Date

Juni 1997

Total Number of Pages: 9

Test No.: 973501  
Test substance: CGA 329351

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GENERAL INFORMATION

Study Signatures

Study Director:

March 24, 1997  
Date

Sponsor

March 27, 1997  
Date

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5.1.2.e W00

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**Guidelines** OECD - Guideline No.: 207, 1984, Earthworm, Acute Toxicity Test

**Sponsor** Novartis Crop Protection AG<sup>1</sup>  
Sector Unit R&D  
Ecotoxicology Department  
CH-4002 Basle, Switzerland

**represented by** Dr. [REDACTED]

**Testing Facilities** Novartis Crop Protection AG  
Sector Unit R&D  
Ecotoxicology Department  
CH-4002 Basle, Switzerland

**Study Director** 5.1.2.e Wood Dr. sc. nat. ETH

**Responsible for Conductance of Practical Study** Mr. 5.1.2.e Wood

**Supplier of the Test Substances** Novartis Crop Protection AG  
Sector Unit R&D  
CH-4002 Basle, Switzerland

**Testing Period (proposed)** Start of Experiments: April 1997  
Date of Termination: Juni 1997  
Date of Completion: July 1997

**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, and the final report will be archived for at least ten years in the test facilities at:  
Novartis Crop Protection AG  
Sector Unit R&D  
Ecotoxicology Department  
CH-4002 Basle, Switzerland

<sup>1</sup>Novartis Crop Protection AG is partial successor in business of Sandoz Ltd. and Ciba-Geigy Ltd. This applies also to all aspects concerned with the requirements of Good Laboratory Practice.

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*Good Laboratory Practice*

The study will be 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, März 1986], issued by the Swiss Federal Department of Interior and the Intercantonal Office for the Control of Medicaments, Switzerland.

These procedures are based on the OECD Principles of Good Laboratory Practice, adopted May 12, 1981 by Decision of the OECD Council [C(81)30 (Final)] concerning Mutual Acceptance of Data in the Assessment of Chemicals and comply with the Council Directive 87/18/EEC of December 18, 1986.

These procedures are in essence consistent with the following Good Laboratory Practice Standards and Regulations:

- Food and Drug Administration  
Department of Health and Human Services: 21 CFR Part 58  
USA: September 4, 1987
- Environmental Protection Agency  
Federal Insecticide, Fungicide and Rodenticide Act (FIFRA): 40 CFR Part 160  
USA: August 17, 1989
- Environmental Protection Agency  
Toxic Substances Control Act (TSCA): 40 CFR Part 792  
USA: August 17, 1989
- Ministry of Agriculture, Forestry and Fisheries (MAFF):  
59 NohSan No. 3850  
Japan: August 10, 1984
- Ministry of Health and Welfare (MHW)  
Ministry of International Trade and Industry (MITI)  
Japan: October 1, 1985

*Quality Assurance*

Novartis Crop Protection AG  
Sector Unit R&D  
Quality Assurance Unit  
CH-4002 Basle, Switzerland

*Pre-test*

Any pre-tests to assist in the selection of the test concentrations, are performed prior to the experimental start of the study. Pre-tests are conducted using widely spaced test concentrations. If necessary, data on the solubility, stability and behaviour of the test substance under test conditions are determined during the pre-test.

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## 1. PURPOSE AND OBJECTIVE

The purpose of this test will be the determination of the 14-days acute toxicity (LC 50 (14 days): concentration at which 50% of the worm population dies) of CGA 329351 to the earthworm (*Eisenia foerida*).

## 2. MATERIALS AND METHODS

The test system will fulfil the specifications of the cited guidelines and SOP No.: 803000.

### 2.1 Test substance

Identification Code	CGA 329351
Appearance	brown liquid
Batch No	Op.4
Purity	97.1%
Solubility (in water)	Not yet known. Will be reported in the raw data and the final report.
Received	September 14, 1995
Storage	room temperature
Stability	04/98
Safety	The test substance will, unless otherwise specified, be handled with routine hygiene procedures to maintain both human and environmental safety.
Test concentrations	Based on the experience with substances of this chemical class, the test concentrations will be: 12.3, 37, 111, 353, 1000 mg/kg dry weight of soil.
Solvent Control (Vehicle)	As specified in the amendment or in the final report.

### 2.2 Test Organisms

Test organisms: Adult earthworms of the species (*Eisenia foerida*), at least 2 month old with a clitellum and a wet mass between 300 and 600 mg.

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<i>Origin and Acclimation</i>	The worms will either be from the culture of Novartis Crop Protection AG or from a reliable commercial supplier. Worms will be conditioned in an artificial soil before use.
<i>Feeding</i>	None
<i>Handling</i>	It will be taken care to minimise possible stress due to handling. Worms which are damaged or dropped during the transfer will not be used for the exposure.
<b>2.3 Test System</b>	
<i>Test chambers</i>	Plastic containers filled with 400-500 g dry weight of soil (18.5 x 13.6 x 6 cm).
<i>Replicates / Number of Worms per Concentration</i>	Test will be performed with 4 replicates. Each test container will contain 10 worms per concentration and control.
<i>Artificial soil</i>	Artificial soil is prepared by wetting the soil substrate consisting of: Sphagnum peat 10% Kaolinite clay 20% Industrial quartz sand 68-69% Calcium carbonate approx. 1-2 % to reach a pH of 6±0.5 The water content will be adjusted with deionised or distilled water to reach approx. 60% of the dry weight of the soil (approx. 40% of total weight of the soil and 40-60% of water holding capacity, as prescribed for the sublethal worm study)
<i>Water content</i>	
<b>2.4 Test Conditions</b>	
<i>Temperature</i>	20°C within ± 2 °C maintained by the control of the room temperature
<i>Lighting</i>	Continuous fluorescent light, 400-800 lux
<i>Test Initiation</i>	After the preparation of the artificial soil and the addition and mixing in of the calculated amounts of water and stock solution (or mixture) by a BÄR RN20-blender, the test organisms will be added and allowed to enter the soil (any organisms not entering the soil will be replaced). Thereafter the test containers are transferred to the exposure chamber (start of exposure)
<i>Duration</i>	14 days
<b>2.5 Sampling and Observations</b>	
<i>Sampling for analysis</i>	None



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**Soil Quality Measurements** The following measurements will be made:  
• at the start of the test: water content and pH of each concentration  
• at the end of the test: water content in each container

**Biological Observations** Measurements of weight and observations on burrowing time will be recorded for each replicate at the start of the test. Observations on mortality and sublethal symptoms, such as abnormal behavioural and flaccidity will be made after 7 days and at the end of the test, when measurements of weights for each replicate will be repeated. Dead organisms, if any, will be removed from the test soil at least at the above mentioned intervals.

At the start and the end of the exposure the adults will be weighed. All test organisms will be incinerated at the conclusion of the exposure.

**Acceptability Criterion** The study will be repeated, if  $\geq 10\%$  of the organisms in the control die.

## 2.6 Statistical Analysis

### Procedures

Results of the toxicity test will be interpreted by standard statistical techniques using SAS Version 6.11. (SAS 1990 a) and the procedure "ECOS" by Fisch and Strehlau, 1996 using SAS PROC GLM and PROC NLIN (SAS 1990b) and/or the procedure "AQUA" (Kremers, 1991).

### LC<sub>50</sub>

The LC<sub>50</sub> values will be calculated either according to the maximum likelihood method, probit model (Finney, D.J., 1971) or the method of Berkson (Berkson, 1953). The method selected is determined by the data base (i.e., presence or absence of 100% response, number of partial responses, etc.). An LC<sub>50</sub> cannot be calculated if the mortality data derived is insufficient according to any of the above mentioned statistical methods. The program provides 95% confidence-intervals and a statistical test to evaluate goodness-of-fit ( $\chi^2$ ).

### NOEC

For the determination of NOECs in regard to abnormalities (lethal and non-lethal effects), Dunnett's multiple means comparison test (Dunnett, 1955 & 1989) will be performed or the Cochran-Armitage test (repeatedly for the first 2, 3, ... n concentrations). The methods provide the Lowest-Observed-Effect-Concentrations LOEC's and the No-Observed-Effect-Concentrations NOEC's.

## 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

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- the objectives of the study
- the description of the test substance
- the description of the test system
- the results of the measurements of the soil characteristics (e.g. pH, water content)
- the experimental design
- the test conditions (lighting, photoperiod, etc.)
- the description of all methods and procedures used
- the experimental findings including the results of non-GLP-studies with the reference substance chloracetamide and comparison to the results of the ring test
- 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 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

- Berkson, J., 1953: A Statistical Precise and Relatively Simple Method of Estimating the Bioassay with Quantal Response, Based on the Logistic function. *JASA* 48, 565-599.
- Dunnnett, C.W., 1955: Multiple comparisons procedure for comparing several treatments with a control, *JASA* 50, 1096-1121.
- Dunnnett, C.W., 1980: Pairwise multiple comparisons in the homogeneous variance, unequal sample size case, *JASA* 75, 789-795.
- \_\_\_\_\_, 1971: Probit Analysis, 3rd edition, London: Cambridge University Press.
- 5.1.2.e Woo and 5.1.2.g, 1996: ECOS: Statistische Analyse von Oekotoxikologie-Daten. Mathematical Applications in Biology and Surveys, CIBA-GEIGY AG
- 5.1.2.e Woo 1991: SAS Program for Analysis of Aquatic Data, CIBA-GEIGY AG
- OECD, 1984: OECD Guidelines for Testing of Chemicals, Section 2: Effects on Biotic Systems. Method 207. Earthworm, Acute Toxicity Test, Adopted 4 April 1984.
- SAS 1990 a: SAS / STAT Users Guide, Version 6, Vol. 2, SAS Institute Inc., Cary, NC
- SAS 1990 b: SAS / GRAPH Software, Version 6, Vol. 1+2, SAS Institute Inc., Cary, NC

**Appendix C: Deviations to Protocol (not amended)**

Deviation No.	Concerning
1	Water solubility: not reported (not: will be reported in the raw data and the final report)
2	Temperature: 22.0-23.0°C due to season (not: 20.0±2.0°C)

Deviations did not influence the results and the integrity of the study.

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### Appendix D: Results of Tests with Chloracetamide as Reference Substance

The LC50 (14d) - values of tests with Chloracetamide as reference substance fluctuated within a range of 13 to 33 mg/kg in the time period of March 1985 to February 1997, thus by a factor of approximately two.

Results of tests with Chloracetamide as reference substance:

Experimental dates	LC 50 (14d)* (mg/kg)	95 % Confidence limit (mg/kg)	Slope (mg/kg)	Goodness of fit		
				X <sup>2</sup>	df	p
March 1985	33	29 - 37	4.57	7.193	3	0.0660
March 2-16, 1990	14	11 - 17	3.09	19.650	3	0.0002
March 15-29, 1990	17	14 - 20	3.45	10.105	3	0.0177
February 6-20, 1991	32	29 - 36	4.49	4.937	3	0.1765
June 10-24, 1996	26	22 - 30	4.64	16.456	3	0.0009
October 17-31, 1996	25	22 - 29	4.61	12.037	3	0.0073
February 6-20, 1997	13	11 - 16	3.10	18.468	3	0.0004

\* The LC-50 values were calculated according to the method of estimating the bioassay with quantal response, based on the logistic function (Berkson, 1953).

Compared to the values of the ring test in 1984, our results of 33, 14, 17, 32, 26, 25 and 13 mg/kg were below the mean LC50 (14d) of 38.5 mg/kg. However, all values were within the 95%-confidence interval of 18.5 - 58.6 mg/kg, except for the results of 1990 and 1997. Hence, our earthworm population and laboratory procedure used was more sensitive to Chloracetamide than the majority of the populations and procedures used in the ring test.

Results of the ring test (Edwards, 1984)

Mean LC 50 (14d) (mg/kg)	Median LC 50 (14d) (mg/kg)	95 % Confidence limit (mg/kg)	Reproducibility standard deviation (mg/kg)
38.5	38.0	18.5 - 58.6	40.3

### References

- Berkson, J., 1953: A Statistical Precise and Relatively Simple Method of Estimating the Bioassay with Quantal Response, Based on the Logistic function. JASA 48, 565 - 599.
- Edwards, C.A., 1984: Report of the second stage in development of a standardized laboratory method for assessing the toxicity of chemical substances to earthworms. CEC, Brussels, EUR 9360 EN, 98pp.