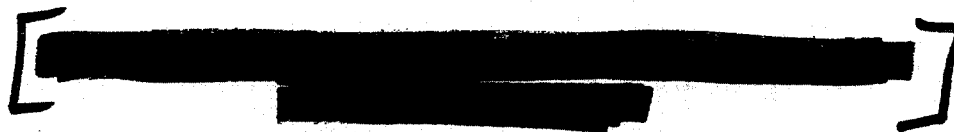


AR 226 - 3278



Earthworm (*Eisenia fetida*), Acute Toxicity Test in Artificial Soil

acc. to OECD Guideline 207 for Testing of Chemicals
(adopted April 04, 1984)

Sponsor

[REDACTED]
ASAHI GLASS
CLARIANT
DAIKIN INDUSTRIES LTD
DUPONT

Author

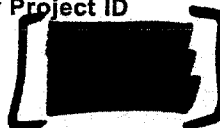
Martina Noack

Test Facility

DR.U.NOACK-LABORATORIUM
FÜR ANGEWANDTE BIOLOGIE
Käthe-Paulus-Str. 1
D-31157 Sarstedt

Laboratory Project ID

Project-No.
Study-No.
Study Plan-No.



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Date

2003-04-14

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Earthworm (*Eisenia fetida*), Acute Toxicity Test in Artificial Soil,
according to OECD Guideline 207

Project-No. [REDACTED]
Study-No. [REDACTED]

Statement of GLP Compliance

Title

Earthworm (*Eisenia fetida*), Acute Toxicity Test in Artificial Soil

Guideline

OECD No. 207 (April 4, 1984) for Testing of Chemicals

Test Item

[REDACTED]
(Batch: P00/001)

Test Facility

DR.U.NOACK-LABORATORIUM FÜR ANGEWANDTE BIOLOGIE

Phone: (+49) 050 66 / 706 70, Fax: (+49) 050 66 / 706 789

Deviations from
GLP Principles

None

We declare that this study was conducted and reported in compliance with the actual OECD principles of Good Laboratory Practice and the national GLP regulations as specified in the law in force, except deviations mentioned above.

2003-04-14
(Date)

[REDACTED]
.....
(M. Noack, Study Director)

2003-04-14
(Date)

[REDACTED]
.....
(Dr. Udo Noack, Head of Testing Facility)

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Report

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Earthworm (*Eisenia fetida*), Acute Toxicity Test in Artificial Soil,
according to OECD Guideline 207Project-No. [REDACTED]
Study-No. [REDACTED]

Statement of the Quality Assurance Unit

Title

Earthworm (*Eisenia fetida*), Acute Toxicity Test in Artificial Soil

Guideline

OECD No. 207 (April 4, 1984) for Testing of Chemicals

Test Item

(Batch: P00/001)

Study Director

Martina Noack

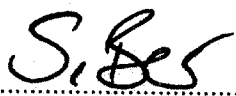
The study was verified as follows:

inspection	dates	date of report
study plan	2002-11-11	2002-11-11
	2002-11-13	2002-11-13
process based	2002-11-07	2002-11-07
report	2003-01-14	2003-01-14
	2003-01-30	2003-01-30
	2003-03-06	2003-03-07
	2003-04-10	2003-04-10

The reported results accurately and completely reflect the raw data of the study. Also methods, procedures, and observations are accurately and completely described in the report.

The accordance of the study with its study plan and the principles of Good Laboratory Practice is guaranteed.

2003-04-14

...pp. 
Gudrun Möhrmann-Kalabokidis

Report

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Earthworm (*Eisenia fetida*), Acute Toxicity Test in Artificial Soil,
according to OECD Guideline 207

Project-No. [REDACTED]
Study-No. [REDACTED]

Personnel Involved

Study Director:

Martina Noack
(Biologist)

Technical Staff:

Kirsten McVean
Gwendolin Kraetzig
Petra Makus
Barbara Wienker

Quality Assurance Unit:

Gudrun Möhrmann-Kalabokidis
(Biologist)

Deputy:

Susanne Becker
(Biologist)

Head of
Test Facility:

Dr. Udo Noack
(Biologist)

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Earthworm (*Eisenia fetida*), Acute Toxicity Test in Artificial Soil according to OECD Guideline 207Project-No. [REDACTED]
Study-No. [REDACTED]

1 Summary

The acute effects of [REDACTED] (batch no. P00/001) on earthworm *Eisenia fetida* Savigny were determined according to OECD Guideline No. 207 at DR.U.NOACK-LABORATORIUM FÜR ANGEWANDTE BIOLOGIE in 31157 Sarstedt, Germany from 2002-12-13 to 2002-12-27.

The study was conducted under static conditions over a duration of 14 days. The test item was applied once at the test start. Forty test organisms with an individual weight of 0.30 to 0.60 g, divided into four replicates, were tested per concentration, solvent control and control in artificial soil as described by the guideline.

The study was conducted with the nominal concentrations:

10 - 32 - 100 - 320 - 1000 mg/kg *).

*) All data given in mg/kg refer to dry weight of artificial soil

No significant mortality was seen in the concentrations 10 - 320 mg/kg after 14 days of exposure. The concentration 1000 mg/kg resulted in a mean mortality of 45 % after 14 days. The LC₅₀-value was greater than the nominal test item concentration of 1000 mg/kg dry weight of artificial soil.

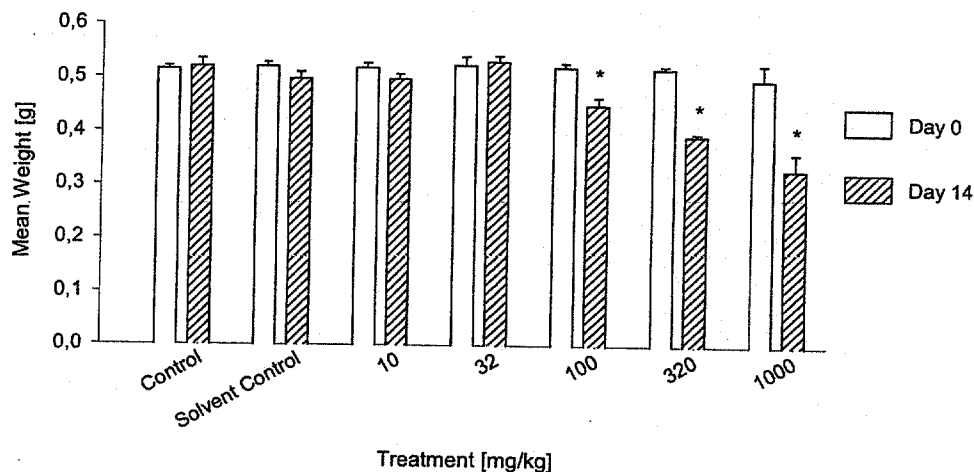


Figure 1: Course of Live Weights

* statistically significant difference (ANOVA, DUNNETT'S Method, $p < 0.001$)

The course of live weights is given in Figure 1. The percent loss in live weight of the surviving adults came to a maximum of 4 % in the solvent control group and to 4 - 34 % in the treatment groups. In the control group there was no loss in live weight (Table 4, Figure 1)

The mean live weights of the surviving earthworms of the treatment groups were compared by a suitable statistical method (ANOVA, One Way Analysis of Variance, DUNNETT's test) for significance of differences ($\alpha = 0.05$) from controls. A statistically significant difference in live weight was found for the test concentrations ≥ 100 mg/kg when compared to the control ($P = < 0.001$), (Table 5).

Other effects (pathological symptoms, changes in behaviour of the worms) of the test item were reported after 7 and 14 days of exposure. Pathological symptoms were found for concentrations ≥ 100 mg/kg (Table 3).

A summary of all significant effects in the test concentrations is given in Table 1.

Table 1: Summary of All Significant Effects in the Test Concentrations

Effects	Concentrations [mg/kg]				
	10	32	100	320	1000
Mortality	---	---	---	---	+++
Mean loss of live weight	---	---	---	+++	+++
Mean live weight compared to control	---	---	+++	+++	+++
Other symptoms (behaviour and pathological symptoms)	---	---	+++	+++	+++
Summary of all test effects	---	---*	+++ [#]	+++	+++

--- = no significant difference

+++ = significant difference

* = NOEC

= LOEC

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Earthworm (*Eisenia fetida*), Acute Toxicity Test in Artificial Soil, according to OECD Guideline 207

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The no observed effect concentration (NOEC) for

with respect to mortality, live weight and other symptoms of *Eisenia fetida* was found to be 32 mg/kg after 14 days of exposure. The lowest observed effect concentration (LOEC) was 100 mg/kg (Table 1).

Results of the reference test with 2-Chloroacetamide performed from 2001-11-30 to 2001-12-14 showed that the method is sensitive and valid according to the guidelines DIN ISO 11268-1 (1997) and OECD No. 207 (1984) (Part 10). The LC_{50} of the reference item 2-Chloroacetamide was in the range 15 - 60 mg/kg.

Validity criteria required by the guideline were fulfilled. Percent mortality in the control group in the reference test and the final definitive test was within the required value of $\leq 10\%$. The average loss of biomass of the worms in the control group did not exceed 20 %.

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Earthworm (*Eisenia fetida*), Acute Toxicity Test in Artificial Soil,
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2 Characterisation Data of the Test Item

TEST ITEM	[REDACTED]
Batch Number	P00/001
CAS RN	[REDACTED]
Chemical characterization	[REDACTED] (EINECS)
Purity	[REDACTED]
Appearance	Yellowish solid wax
Water solubility	134 µg/L at 12 degree C, Std 29 µg/L, n=8 (DuPont, 2002) 137 µg/L at 25 degree C, Std 53 µg/L, n=17 (DuPont, 2002)
Stability in water	Stable at test conditions
Density	Ca. 1.7 g/cm ³
Expiry date	Unlimited if stored under the recommended conditions.
Recommended storage	Room temperature (20 °C), protected from moisture.
Storage at testing facility	Room temperature, protected from moisture and light.
Retention	At least 1 g has been retained.
Identification parameter at testing facility	Name, batch number and colour.

The test item and the information concerning the test item were provided by the sponsor.

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Report [REDACTED]

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Earthworm (*Eisenia fetida*), Acute Toxicity Test in Artificial Soil according to OECD Guideline 207

Project-No. [REDACTED]
Study-No. [REDACTED]

3 Method

GUIDELINE

OECD Guideline No. 207, adopted April 4, 1984.

TYPE AND PURPOSE OF THE STUDY

Determination of the effects of the test item on mortality and live weight development of the earthworm species *Eisenia fetida* in artificial soil.

TEST SYSTEM

Eisenia fetida Savigny 1826 (Annelida, Lumbricidae)
Subspecies: *Eisenia fetida andrei*

Reason for the selection of the test system

According to the guideline *Eisenia fetida* is suitable for this kind of study.

Source

Biologische Bundesanstalt für Land- und Forstwirtschaft,
Messeweg 11/12, D-38104 Braunschweig

Culture

Organisms of the species *Eisenia fetida* are cultured at the test facility in covered plastic vessels (volume 60 L) containing potting compost at a temperature of $20 \pm 2^\circ\text{C}$ in the dark.

Feeding

The earthworms were fed with dried litter of stinging nettle and porridge oats portioned according to the feeding rate and to the density of worms in the vessels.

TEST ITEM

[REDACTED]

Pretreatment

The test item was heated to 50°C , weighed out, dissolved in *tert.*-methyl-butyl-ether and mixed with 10 g quartz sand. After the solvent had volatilized out of the quartz sand, the sand was mixed into the artificial soil to produce the desired test item concentrations.

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Earthworm (*Eisenia fetida*), Acute Toxicity Test in Artificial Soil,
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Test concentrations

1000 - 320 - 100 - 32 - 10 mg/kg dry weight (factor $\sqrt{10}$)

Test item content [g]	Test Concentration [mg/kg]				
	10	32	100	320	1000
nominal	23	73.6	230	736	2300
actual	23.1	73.5	230.1	735.7	2300.3

Vehicle

10 g quartz sand

Solvent

tert.-methyl-butyl-ether

Solvent [mL]	Test Concentration [mg/kg]					
	Solvent control	10	32	100	320	1000
actual	15	3	4	6	10	15

CONTROL

Artificial soil and 10 g quartz sand moistened with demineralized water (621 mL) without test or reference item was used as control medium.

SOLVENT CONTROL

Artificial soil and 10 g quartz sand treated with *tert.*-methyl-butyl-ether in a manner similar to the test item treatments and moistened with demineralized water without test item was used as solvent control medium.

REFERENCE ITEM

2-Chloroacetamide (FLUKA)

Item purity

99.7 % (HPLC)

Batch No.

374979/1 14199

Stability

2002-06-07

Test concentrations

15 - 30 - 60 mg/kg dry mass

Test duration

2001-11-30 to 2001-12-14.

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Earthworm (*Eisenia fetida*), Acute Toxicity Test in Artificial Soil, according to OECD Guideline 207

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TEST METHOD

Replicates	Four replicates were used per concentration of the test item and per control treatment.
Number and age of the earthworms	10 adult earthworms (with clitellum) were used per replicate. They had been acclimatised to artificial soil two days prior to test start. At start of the test, they had an individual live weight of 0.37 to 0.60 g.
Test medium	Artificial soil was used as described by the test guideline. It consisted of the following components: - 10.5 % peat (air dried and finely ground) - 20 % kaolin (kaolinite content 84 - 87 %) - 69 % quartz sand (sand with particle size of 50-200 µm: > 50 %) - 0.5 % calcium carbonate (CaCO₃) Demineralized water was added (1080 mL) to give an overall moisture content of 27 % of the dry weight (about 25 % of the max. water capacity) of the soil. The definitive moisture content, determined the day before application of the test item by drying a sample at 105 °C and re-weighing, was 26 %. The measured pH-value of 5.94 was in the recommended range of 6.0 ± 0.5.
Test container	Glass dishes (volume 1.5 L) were used as test vessels. During the test they were covered with perforated plastic film to prevent the test substrate from drying.
Application technique	Test item was incorporated into the artificial soil 24 h prior to test start. The concentrations of the test item are related to the dry weight of the soil. The test item was weighed out and dissolved in <i>tert.</i>-methyl-butyl-ether. The test item stock solution was mixed with 10 g quartz sand. After the solvent had volatilized out of the quartz sand, the treated quartz sand was carefully mixed into the artificial soil with a mixer to insure a homogeneous distribution of the test item in the soil. The amount of quartz sand added was the same for each test concentration, solvent control and control treatment. After 24 h the test substrate was added to the test vessels.
Feeding	No food was added to the test vessels during the toxicity test.

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Earthworm (*Eisenia fetida*), Acute Toxicity Test in Artificial Soil, according to OECD Guideline 207

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Temperature 18 - 22 °C
Photoperiod 16 h
Light intensity 400 - 800 lx

TYPE AND FREQUENCY OF MEASUREMENTS AND OBSERVATIONS

The maximum water holding capacity of the soil was determined before adaptation of the earthworms (actual 106 %, according to ISO DIS 1128-2 / Annex C). At the start and at the end of the test, pH and moisture content of the test substrate were determined in every treatment and control using mixed samples of all replicates.

The live weight of the earthworms was measured individually on the day of application and on day 14.

Mortality, behaviour and morphological changes of the earthworms were recorded 7 and 14 days after application.

TEST DESCRIPTION

A preliminary NON-GLP test was performed with 3 concentrations (10 - 100 - 1000 mg/kg). Effects on mortality and the mean live weight of the earthworms were evaluated. Control replicates were tested under the same conditions as the test groups. Based on the results of the preliminary test, the main study was performed with five concentrations from 10 to 1000 mg/kg with a geometric factor of $\sqrt{10}$.

Preparations:

Artificial soil was produced and moistened with demineralized water to about 25 % of the maximum water holding capacity of the artificial soil. It was adjusted to a pH of 5.94.

Day -2:

Earthworms were placed on the artificial soil separately moistened to about 35 % of the soil dry weight (adaptation).

Day -1:

The test item was heated to 50 °C, weighed out, dissolved in *tert.*-methyl-butyl-ether and mixed with 10 g quartz sand. After the solvent was volatilized out of the quartz sand, the treated quartz sand was mixed with the artificial soil.

Start:

The treated test substrate was added to the test vessels.

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Earthworm (*Eisenia fetida*), Acute Toxicity Test in Artificial Soil, according to OECD Guideline 207

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Earthworms were washed in dechlorinated tap water, weighed individually and placed on the surface of the test medium. The pH and moisture content were determined using pooled samples of each treatment and control group. The test vessels were covered with perforated plastic film.

Day 7:

Earthworms were taken out of the test vessels and observed with respect to mortality and morphological and behavioural changes. Thereafter, live earthworms were replaced on the surface of the test substrate and test vessels were covered with perforated film.

Day 14:

Earthworms were taken out of the test vessels and observed with respect to mortality and morphological and behavioural changes. The individual live weight of the survivors was measured. Soil pH and moisture content of every test item treatment and of the controls were measured using pooled samples.

VALIDITY CRITERIA

The mortality in the control group should not exceed 10 % at the end of the test.

The average loss of biomass of the worms in the control groups should not exceed 20 %.

STATISTICS AND CALCULATIONS

NOEC, live weights

Significant differences in worm live weight after 14 days were determined in comparison with the control group using One Way Analysis of Variance and the Multiple Comparison Method of DUNNETT'S. When running a One Way Analysis of Variances a Normality and Equal Variance Test were done first. P-Values for both Normality and Equal Variance Test were < 0.05 . The α -value for ANOVA and DUNNETT'S test was $\alpha=0.05$. The calculations were carried out using SigmaStat rel. 2.03 (1992-1997), SPSS INC.

LC₅₀-values and confidence intervals for mortality

Calculations for the reference item were carried out via probit analysis using software SigmaPlot rel. 2000 (2000), SPSS CORPORATION.

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Earthworm (*Eisenia fetida*), Acute Toxicity Test in Artificial Soil, according to OECD Guideline 207

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Probit values according to WEBER (1986). Calculation of the confidence intervals for LC_{50} were carried out using standard procedures according to BREITIG & TÖMPLING (1982).

The LC_{50} -value for the test item control could not be determined at the tested concentration range.

TIME OF THE STUDY

The study was carried out from 2002-12-13 to 2002-12-27.

DEVIATIONS FROM THE GUIDELINE

None

DEVIATIONS FROM THE STUDY PLAN

None

REPORTING

The final report is issued as paper copy (5 originals) and an electronic record (1 original).

ARCHIVING

The following will be retained in the archive of the test facility for the period as specified in the operative national GLP regulations:

- all raw data
- study plan (Original 1 of 2)
- final report paper copy (Original 1 of 5)
- all records performed by the quality assurance programme including master schedules
- samples of test and reference items

Microfilms will be retained in a safe-deposit by Volksbank Sarstedt, D-31157 Sarstedt.

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Earthworm (*Eisenia fetida*), Acute Toxicity Test in Artificial Soil,
according to OECD Guideline 207

Project-No. [REDACTED]
Study-No. [REDACTED]

4 Results

4.1 Mortality

The percentage mortality after 7 and 14 days of all replicates is given in Table 4.

Table 2: Earthworm Mortality in [%] after 7 and 14 Days of Exposure
4 replicates (n = 40)

M O R T A L I T Y [%]										
Nominal conc. [mg/kg]	7 day					14 day				
	1	2	3	4	MV	1	2	3	4	MV
Control	0	0	0	0	0	0	0	0	0	0
Solvent control	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0	0	0
320	0	0	0	0	0	10	0	0	0	2.5
1000	0	0	0	0	0	40	40	50	50	45

MV = mean value

No significant mortality was seen in the tested concentrations 10 - 320 mg/kg after 14 days of exposure. The highest tested concentration of 1000 mg/kg resulted in a mortality of 45 %. The LC₅₀-value was not determinable.

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Earthworm (*Eisenia fetida*), Acute Toxicity Test in Artificial Soil,
according to OECD Guideline 207

Project-No. [REDACTED]
Study-No. [REDACTED]

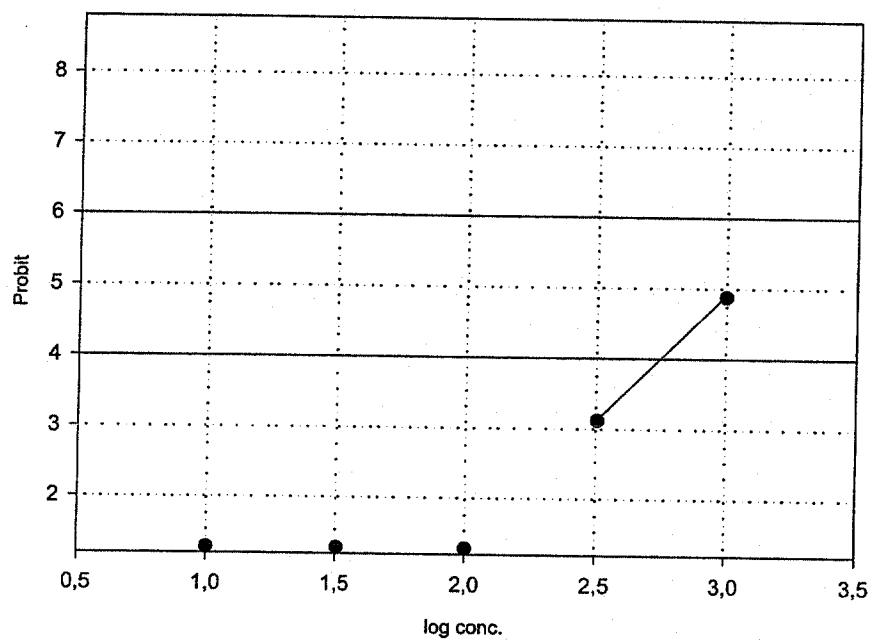


Figure 2: Concentration-Effect Relationship after 14 Days of Exposure

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Earthworm (*Eisenia fetida*), Acute Toxicity Test in Artificial Soil,
according to OECD Guideline 207

Project-No. [REDACTED]
Study-No. [REDACTED]

4.2 Behaviour and Pathological Symptoms

The earthworms in the control, the solvent control and the tested concentrations 10 to 32 mg/kg showed normal behaviour with normal reactions to mechanical stimulus and negative phototactic reactions after placing them on the substrate surface after 7 and 14 days of exposure.

In the concentrations 100 to 1000 mg/kg pathological symptoms and abnormal reactions were observed at 7 and 14 days.

The data are given in Table 3.

Table 3: Earthworm Behaviour and Pathological Symptoms after 7 and 14 Days of Exposure
4 replicates (n = 40)

Conc. [mg/kg]	Effect	DAY 7 Replicate				DAY 14 Replicate			
		1	2	3	4	1	2	3	4
Control	a)	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
Solvent control	a)	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
10	a)	10/10	10/10	10/10	10/10	10/10	9/10	10/10	10/10
	e)	-	-	-	-	-	1/10	-	-
32	a)	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
100	a)	7/10	5/10	4/10	5/10	-	-	-	-
	c)	-	-	1/10	-	-	-	*	-
	g)	2/10	2/10	1/10	1/10	2/10	2/10	1/10	1/10
	h)	2/10	4/10	6/10	4/10	10/10	10/10	10/10	10/10
320	e)	-	-	-	-	1/10	-	-	-
	g)	1/10	-	1/10	2/10	2/10	1/10	1/10	2/10
	h)	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
1000	c)	-	1/10	-	-	-	-	*	-
	e)	-	-	-	-	5/10	4/10	6/10	-
	g)	2/10	1/10	-	1/10	1/10	3/10	3/10	2/10
	h)	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10

a) no obvious pathological symptoms

e) spontaneous segmentation and separation

h) worms hard, not elastic

* after 14 days no worm was on the surface of the test medium

c) no negative phototactic reaction
g) ulcer and skin bleeding

Earthworm (*Eisenia fetida*), Acute Toxicity Test in Artificial Soil, according to OECD Guideline 207

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4.3 Live Weight of the Earthworms

The worms had an individual weight of 0.37 to 0.60 g at test start in all concentrations and control groups. Therefore, all weights were in the recommended range. The data of the treatment, solvent control and control groups are given in Tables 6 to 12.

The mean weights across replicates for each concentration and control and the pooled residual standard deviation were calculated for data at 14 days.

The tables 15 to 21 present data for individual weight of the earthworms on day 14 as well as the mean values and the standard deviations for all replicates of test and control groups.

The percent loss of live weight was 4 % in the solvent control group and 4 to 34 % in the treatment groups. Only in the tested concentrations ≥ 320 mg/kg the loss of live weight was ≥ 20 %. The live weight of the control group remained at the starting level (Table 4).

Table 4: Mean Loss of Live Weight: Comparing Test Start to Test End

Concentration, [mg/kg]	Mean live weight		
	Test start [g/worm]	Test end [g/worm]	Loss* [%]
Control	0.52	0.52	0
Solvent Control	0.52	0.50	4
10	0.52	0.50	4
32	0.52	0.53	-2
100	0.52	0.45	13
320	0.52	0.39	25
1000	0.50	0.33	34

* loss ≤ 20 % biologically not significant (according to DIN ISO 11268-1 (1997))

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Earthworm (*Eisenia fetida*), Acute Toxicity Test in Artificial Soil, according to OECD Guideline 207

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The mean live weights of the surviving earthworms after 14 days of exposure of the treatment groups were compared to the control by One Way Analysis of Variance for significance of differences ($\alpha = 0.05$).

In the One Way Analysis of Variance the differences in the mean values among the treatment groups were greater than would be expected by chance. To isolate the groups that differ from the others the multiple comparison method of DUNNETT's was used. There was a statistically significant difference at the concentrations ≥ 100 mg/kg ($P < 0.001$, Table 5).

Table 5: Mean Live Weight on Day 14 Compared to Control Group
4 replicates, n=40

Conc. [mg/kg]	Mean live weight [g] Replicate					Comparison versus control	
	1	2	3	4	Overall MV $\pm$ SD	CV	Statistically significant*
Control	0.51	0.52	0.54	0.51	0.52 ± 0.05	9.29	—
Solvent Control	0.50	0.50	0.51	0.48	0.50 ± 0.05	9.95	No
10	0.49	0.49	0.51	0.50	0.50 ± 0.06	11.25	No
32	0.52	0.52	0.53	0.54	0.53 ± 0.06	11.45	No
100	0.45	0.43	0.46	0.46	0.45 ± 0.06	13.17	Yes
320	0.39	0.39	0.40	0.39	0.39 ± 0.05	12.99	Yes
1000	0.36	0.35	0.32	0.29	0.33 ± 0.04	13.12	Yes

* statistically significant difference (ANOVA, DUNNETT's Method, $p < 0.001$)

4.4 Physical Data

Physical data are given in part 9.

Moisture content of test medium was 51.6 - 53.4 % of dry weight at test start and 50.4 - 51.5 % at test end (Table 20).

Values for pH were 5.08 - 5.58 at test start and 5.45 - 5.75 at test end (Table 20).
During the test the temperature in the incubation room remained in the range 20 ± 2 ° C.

Testing was done with a mean light intensity of 491 lx for a photoperiod of 16 h (Table 21).

5 Validity Criteria

Conditions for the validity of the test were as follows:

The mortality in the control group did not exceed 10 % at the end of the test.

The average loss of biomass in the control group did not exceed 20 % at the end of the test.

6 Discussion and Conclusions

Mortality was seen at the test concentrations of 320 and 1000 mg/kg after 14 days of exposure (Table 2).

The live weight was statistically significantly decreased at concentrations ≥ 100 mg/kg when compared to control group during the 14 days of exposure (ANOVA, DUNNETT's Method, $p < 0.001$). Pathological symptoms were seen at concentrations ≥ 100 mg/kg (Table 5).

No test item related mortality, pathological symptoms and reduction in live weight were seen at concentrations ≤ 32 mg/kg.

Finally the highest tested concentration with no effect (NOEC) of the test item with respect to mortality, body weight and other symptoms was found to be 32 mg/kg.

The lowest observed effect concentration (LOEC) was found to be 100 mg/kg.

Effects of the reference item **2-Chloroacetamide** on earthworms show the method is sensitive and valid (Part 10).

7 Literature / References

- (1) BBA (1984)-Verfahrensvorschlag: „Toxizitätstest am Regenwurm *Eisenia fetida* (Savigny) 1826 in künstlichem Boden" (LC 50, 14 Tage), Braunschweig.
- (2) BREITIG, G., TÜMLING, W. (editors, 1982): Methoden der Wasseruntersuchungen Bd.II. VEB GUSTAV FISCHER VERLAG, Jena.
- (3) DIN ISO 11268-1 (APRIL 1997), WIRKUNGEN VON SCHADSTOFFEN AUF REGENWÜRMER.
- (4) OECD Guideline 207 for Testing of Chemicals: Earthworm, Acute Toxicity Test adopted April 4, 1984.
- (5) WEBER, E., Grundriß der biologischen Statistik, 9. Auflage (1986).

(6)

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Earthworm (*Eisenia fetida*), Acute Toxicity Test in Artificial Soil,
according to OECD Guideline 207

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8 Live Weight Data of the Earthworms

Table 6: Live Weight on Day 0 (Control)

Rep.	WEIGHT g OF EARTHWORM NO.										sum	n	mean	std. dev.
	1	2	3	4	5	6	7	8	9	10				
1	0.42	0.49	0.51	0.51	0.52	0.60	0.55	0.57	0.52	0.52	5.21	10	0.52	0.05
2	0.60	0.47	0.44	0.47	0.54	0.51	0.51	0.46	0.52	0.55	5.07	10	0.51	0.05
3	0.44	0.51	0.50	0.48	0.58	0.60	0.49	0.57	0.46	0.53	5.16	10	0.52	0.05
4	0.48	0.49	0.51	0.41	0.43	0.55	0.51	0.47	0.60	0.60	5.05	10	0.51	0.06
											20.5	40	0.52	

Table 7: Live Weight on Day 0 (Solvent control)

Rep.	WEIGHT g OF EARTHWORM NO.										sum	n	mean	std. dev.
	1	2	3	4	5	6	7	8	9	10				
1	0.57	0.48	0.46	0.49	0.51	0.60	0.56	0.53	0.47	0.52	5.19	10	0.52	0.05
2	0.40	0.60	0.51	0.50	0.42	0.53	0.50	0.59	0.45	0.55	5.05	10	0.51	0.07
3	0.44	0.50	0.44	0.56	0.56	0.48	0.56	0.59	0.58	0.54	5.25	10	0.53	0.06
4	0.48	0.49	0.46	0.47	0.58	0.54	0.53	0.53	0.59	0.51	5.18	10	0.52	0.04
											20.8	40	0.52	

Table 8: Live Weight on Day 0 (10 mg/kg)

Rep.	WEIGHT g OF EARTHWORM NO.										sum	n	mean	std. dev.
	1	2	3	4	5	6	7	8	9	10				
1	0.49	0.44	0.46	0.55	0.60	0.57	0.48	0.55	0.60	0.51	5.25	10	0.53	0.06
2	0.50	0.45	0.45	0.60	0.46	0.52	0.47	0.50	0.56	0.60	5.11	10	0.51	0.06
3	0.49	0.45	0.46	0.53	0.60	0.47	0.48	0.52	0.55	0.60	5.15	10	0.52	0.05
4	0.48	0.51	0.44	0.60	0.49	0.42	0.46	0.50	0.60	0.59	5.09	10	0.51	0.07
											20.6	40	0.52	

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Earthworm (*Eisenia fetida*), Acute Toxicity Test in Artificial Soil, according to OECD Guideline 207Project-No.
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Table 9: Live Weight on Day 0 (32 mg/kg)

Rep.	WEIGHT g OF EARTHWORM NO.										sum	n	mean	std. dev.
	1	2	3	4	5	6	7	8	9	10				
1	0.40	0.46	0.58	0.55	0.59	0.54	0.43	0.44	0.51	0.45	4.95	10	0.50	0.06
2	0.57	0.43	0.48	0.60	0.60	0.55	0.50	0.56	0.55	0.52	5.36	10	0.54	0.05
3	0.49	0.60	0.58	0.43	0.55	0.45	0.48	0.55	0.43	0.60	5.16	10	0.52	0.07
4	0.50	0.58	0.55	0.50	0.57	0.52	0.55	0.46	0.59	0.45	5.27	10	0.53	0.05
											20.7	40	0.52	

Table 10: Live Weight on Day 0 (100 mg/kg)

Rep.	WEIGHT g OF EARTHWORM NO.										sum	n	mean	std. dev.
	1	2	3	4	5	6	7	8	9	10				
1	0.52	0.57	0.42	0.40	0.53	0.46	0.60	0.55	0.60	0.60	5.25	10	0.53	0.05
2	0.48	0.47	0.60	0.57	0.60	0.43	0.47	0.53	0.55	0.52	5.22	10	0.52	0.06
3	0.52	0.52	0.53	0.51	0.49	0.51	0.50	0.49	0.56	0.53	5.16	10	0.52	0.02
4	0.46	0.45	0.55	0.52	0.41	0.52	0.58	0.59	0.48	0.51	5.07	10	0.51	0.06
											20.7	40	0.52	

Table 11: Live Weight on Day 0 (320 mg/kg)

Rep.	WEIGHT g OF EARTHWORM NO.										sum	n	mean	std. dev.
	1	2	3	4	5	6	7	8	9	10				
1	0.45	0.57	0.49	0.45	0.60	0.58	0.53	0.52	0.50	0.50	5.19	10	0.52	0.05
2	0.41	0.50	0.60	0.59	0.57	0.59	0.44	0.44	0.54	0.51	5.19	10	0.52	0.07
3	0.50	0.46	0.51	0.51	0.51	0.58	0.46	0.56	0.47	0.60	5.16	10	0.52	0.05
4	0.56	0.45	0.47	0.53	0.55	0.52	0.47	0.45	0.48	0.57	5.05	10	0.51	0.05
											20.6	40	0.52	

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Earthworm (*Eisenia fetida*), Acute Toxicity Test in Artificial Soil according to OECD Guideline 207Project-No.
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Table 12: Live Weight on Day 0 (1000 mg/kg)

Rep.	WEIGHT g OF EARTHWORM NO.										sum	n	mean	std. dev.
	1	2	3	4	5	6	7	8	9	10				
1	0.53	0.49	0.51	0.48	0.60	0.52	0.60	0.46	0.49	0.57	5.25	10	0.53	0.05
2	0.40	0.43	0.51	0.50	0.51	0.43	0.52	0.53	0.53	0.60	4.96	10	0.50	0.06
3	0.45	0.55	0.51	0.46	0.52	0.51	0.60	0.49	0.46	0.45	5.00	10	0.50	0.05
4	0.42	0.44	0.43	0.60	0.48	0.60	0.37	0.40	0.40	0.44	4.58	10	0.46	0.08
											19.8	40	0.50	

Table 13: Live Weight on Day 14 (Control)

Rep.	WEIGHT g OF EARTHWORM NO.										sum	n	mean	std. dev.
	1	2	3	4	5	6	7	8	9	10				
1	0.58	0.54	0.47	0.62	0.48	0.49	0.54	0.47	0.49	0.43	5.11	10	0.51	0.06
2	0.53	0.48	0.55	0.45	0.49	0.52	0.54	0.61	0.54	0.47	5.18	10	0.52	0.05
3	0.51	0.57	0.47	0.61	0.52	0.56	0.57	0.58	0.51	0.46	5.36	10	0.54	0.05
4	0.58	0.43	0.52	0.47	0.52	0.54	0.52	0.50	0.50	0.53	5.11	10	0.51	0.04
											20.8	40	0.52	

Table 14: Live Weight on Day 14 (Solvent control)

Rep.	WEIGHT g OF EARTHWORM NO.										sum	n	mean	std. dev.
	1	2	3	4	5	6	7	8	9	10				
1	0.45	0.47	0.52	0.52	0.46	0.54	0.52	0.55	0.45	0.54	5.02	10	0.50	0.04
2	0.48	0.54	0.57	0.47	0.49	0.46	0.59	0.37	0.52	0.47	4.96	10	0.50	0.06
3	0.61	0.54	0.43	0.55	0.52	0.54	0.44	0.45	0.51	0.51	5.10	10	0.51	0.06
4	0.48	0.53	0.44	0.46	0.49	0.49	0.52	0.46	0.43	0.45	4.75	10	0.48	0.03
											19.8	40	0.50	

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Earthworm (*Eisenia fetida*), Acute Toxicity Test in Artificial Soil according to OECD Guideline 207Project-No. [REDACTED]
Study-No. [REDACTED]

Table 15: Live Weight on Day 14 (10 mg/kg)

Rep.	WEIGHT g OF EARTHWORM NO.										Sum	n	mean	std. dev.
	1	2	3	4	5	6	7	8	9	10				
1	0.49	0.47	0.52	0.44	0.37	0.59	0.43	0.52	0.52	0.51	4.86	10	0.49	0.06
2	0.39	0.46	0.47	0.49	0.49	0.55	0.60	0.49	0.49	0.51	4.94	10	0.49	0.06
3	0.42	0.48	0.53	0.51	0.48	0.48	0.58	0.61	0.43	0.54	5.06	10	0.51	0.06
4	0.44	0.49	0.45	0.55	0.57	0.53	0.44	0.44	0.56	0.50	4.97	10	0.50	0.05
											19.8	40	0.50	

Table 16: Live Weight on Day 14 (32 mg/kg)

Rep.	WEIGHT g OF EARTHWORM NO.										sum	n	mean	std. dev.
	1	2	3	4	5	6	7	8	9	10				
1	0.48	0.47	0.64	0.58	0.44	0.59	0.40	0.55	0.55	0.49	5.19	10	0.52	0.07
2	0.50	0.54	0.55	0.45	0.44	0.57	0.53	0.54	0.56	0.55	5.23	10	0.52	0.05
3	0.57	0.49	0.50	0.67	0.57	0.61	0.48	0.44	0.46	0.56	5.35	10	0.54	0.07
4	0.60	0.55	0.57	0.57	0.58	0.42	0.51	0.49	0.58	0.49	5.36	10	0.54	0.06
											21.1	40	0.53	

Table 17: Live Weight on Day 14 (100 mg/kg)

Rep.	WEIGHT g OF EARTHWORM NO.										sum	n	mean	std. dev.
	1	2	3	4	5	6	7	8	9	10				
1	0.45	0.47	0.38	0.31	0.51	0.33	0.51	0.50	0.52	0.50	4.48	10	0.45	0.08
2	0.35	0.41	0.45	0.45	0.47	0.51	0.39	0.44	0.34	0.48	4.29	10	0.43	0.06
3	0.43	0.43	0.48	0.42	0.50	0.46	0.41	0.49	0.51	0.50	4.63	10	0.46	0.04
4	0.51	0.42	0.42	0.44	0.36	0.50	0.41	0.57	0.47	0.48	4.58	10	0.46	0.06
											18.0	40	0.45	

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Earthworm (*Eisenia fetida*), Acute Toxicity Test in Artificial Soil according to OECD Guideline 207

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Table 18: Live Weight on Day 14 (320 mg/kg)

Rep.	WEIGHT g OF EARTHWORM NO.										sum	n	mean	std. dev.
	1	2	3	4	5	6	7	8	9	10				
1	0.45	0.46	0.42	0.34	0.41	0.40	0.35	0.47	0.37	(0.22)	3.89	10	0.39	0.07
2	0.34	0.45	0.39	0.40	0.38	0.34	0.36	0.33	0.44	0.47	3.90	10	0.39	0.05
3	0.34	0.43	0.40	0.41	0.40	0.38	0.43	0.37	0.49	0.37	4.02	10	0.40	0.04
4	0.33	0.35	0.42	0.37	0.37	0.42	0.41	0.37	0.45	0.41	3.90	10	0.39	0.04
											15.7	40	0.39	

() = earthworm dead, but could be found, not decomposed, included in calculations

Table 19: Live Weight on Day 14 (1000 mg/kg)

Rep.	WEIGHT g OF EARTHWORM NO.										sum	n	mean	std. dev.
	1	2	3	4	5	6	7	8	9	10				
1	0.38	0.40	0.41	0.33	0.32	0.31	-	-	-	-	2.15	6	0.36	0.04
2	0.30	0.34	0.40	0.39	0.36	0.29	-	-	-	-	2.08	6	0.35	0.05
3	0.32	0.36	0.29	0.31	0.31	-	-	-	-	-	1.59	5	0.32	0.03
4	0.33	0.29	0.28	0.30	0.26	-	-	-	-	-	1.46	5	0.29	0.03
											7.28	22	0.33	

- not determinable, earthworms decomposed

Company Sanitized. Does not contain TSCA CBI

9 Physical Data

Measurement of pH values and moisture content of test medium was based on pooled samples of all replicates per treatment. Moisture values are given in % of the dry weight of the test medium.

Table 20: pH-Values and Moisture of the Test Media

Conc. [mg/kg]	pH-value		Moisture [%] of dry weight	
	day 0	day 14	day 0	day 14
Control	5.20	5.75	53.3	50.7
Solvent control	5.08	5.45	52.4	50.5
10	5.31	5.45	53.4	50.4
32	5.58	5.74	52.8	51.4
100	5.18	5.57	52.8	51.5
320	5.26	5.47	51.6	51.1
1000	5.29	5.46	53.1	51.1

Table 21: Light Intensity in the Incubation Room
(Measurement in [lx])

Measurement on day	Measurements [lx]				Mean [lx]
	1	2	3	4	
2002-12-16	512	432	421	598	491

Earthworm (*Eisenia fetida*), Acute Toxicity Test in Artificial Soil, according to OECD Guideline 207

Project-No.
Study-No.

10 Data of the Reference Item

2-Chloroacetamide is used as reference item in the Acute Toxicity Test in Artificial Soil with earthworms. The reference test was performed at the test facility from 2001-11-30 to 2001-12-14. The study no. was [REDACTED]

The reference item was applied once at test start. The study was conducted with the nominal concentrations:

15 - 30 - 60 mg/kg

The mortality for the reference item was determined after 7 and 14 days in percent (Table 22).

Table 22: Reference Item: Mortality of the Earthworms after 7 and 14 Days

MORTALITY [%]										
Conc. [mg/kg]	7 d					14 d				
	1	2	3	4	MV	1	2	3	4	MV
Control	0	0	0	0	0	0	0	0	0	0
15	0	0	0	10	2.5	0	0	0	10	2.5
30	100	80	90	60	82.5	100	90	90	60	85
60	100	100	100	100	100	100	100	100	100	100

The lowest tested concentration resulting in 100 % mortality (14d LC₁₀₀) after 14 days of exposure was determined directly from the test results to be 60 mg/kg.

The mean lethal concentration (14 d LC₅₀) was determined to be 23.5 mg/kg with a confidence interval ranging from 21.8 to 25.2 mg/kg estimated by probit analysis, so that the mean lethal concentration was in the recommended range of the guidelines (DIN ISO 11268-1; OECD 207) (Table 23).

Finally, the concentration with no effect (NOEC) of the reference item with respect to mortality and other effects was found to be 15 mg/kg after 14 days of exposure.

Earthworm (*Eisenia fetida*), Acute Toxicity Test in Artificial Soil, according to OECD Guideline 207

Project-No. [REDACTED]
Study-No. [REDACTED]

Table 23: LC-Values after 14 d for 2-Chloroacetamide

LC - Values	Concentration [mg/kg]	Confidence interval [mg/kg]
LC ₁₀	17.3	---
LC ₅₀	23.5	21.8 - 25.2
LC ₁₀₀	60.0	---

--- = not determined

The earthworms in the control and test group 15 mg/kg showed normal behaviour with normal reactions to mechanical stimulus and negative phototactic reactions after placing them on the substrate surface. Obvious pathological symptoms were observed at 30 mg/kg.

The worms had an individual weight of 0.30 to 0.60 g at test start in all concentration and control groups. Therefore all weights were in the recommended range. The mean live weights across replicates for each concentration and control were calculated (Table 24).

During the test the weight of the earthworms decreased in the treatment and control groups due to the fact, that there was no feeding at all in the test period. The percent loss in live weight came to 8 % in the control, to 6 % in the concentration 15 mg/kg and up to 37 % in the concentration 30 mg/kg. The loss of live weight was significant in the tested concentration 30 mg/kg (> 20%), (Table 24).

Table 24: Mean Loss of Live Weight

Concentration [mg/kg]	Mean Live weight			Significance*
	Test start [g/worm]	Test end [g/worm]	Loss [%]	
Control	0.49	0.45	8	n.s.
15	0.49	0.46	6	n.s.
30	0.49	0.31	37	sig.
60	0.49	---	---	---

--- = dead

* = live weight loss > 20 %

n.s. = not significant (loss ≤ 20 %)

sig. = significant (loss > 20 %)

Company Sanitized. Does not contain TSCA CBI

Earthworm (*Eisenia fetida*), Acute Toxicity Test in Aqueous Solution according to OECD Guideline 207

Project-No. [REDACTED]
Study-No. [REDACTED]

The mean live weight after 14 days of the concentrations 15 and 30 mg/kg was then compared with the control mean using One Way Analysis of Variance. The differences in the mean values among the treatment group 30 mg/kg were determined to be statistically significant, $P < 0.001$ (One Way Analysis of Variances, Dunnett's Method), Table 25.

Table 25: Reference item: Mean Live Weight on Day 14 Compared to Control Group

Conc. [mg/kg]	Mean live weight [g]					Comparison versus Control Significance*
	Replicate					
	1	2	3	4	Ø	
Control	0.45	0.45	0.44	0.45	0.45	-
15	0.46	0.47	0.45	0.45	0.46	no
30	0.42	0.35	0.27	---	0.31	yes
60	---	---	---	---	---	---

--- = dead

* = One Way Analysis of Variance (DUNNETT'S Method, $p < 0.001$)

no = no statistically significant difference

The environmental conditions were as follows:

Moisture content of test medium was 32.7 - 33.6 % of dry weight at the test start and 31.5 - 32.5 % at the test end.

Values of pH were 5.52 - 5.62 at the test start and 5.20 - 5.29 at the test end.

During the test the temperature was in the range of 18 - 20 °C.

Testing was done with a light intensity of 428 to 535 lx (mean of 753 lx) for a photoperiod of 16 h.

Company Sanitized. Does not contain TSCA CBI

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Earthworm (*Eisenia fetida*), Acute Toxicity Test in Artificial Soil,
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11 Certificate of Test Item Analysis

Clariant GmbH, Werk Gendorf
CQG / Qualitätsmanagement
D-44504 Burgkirchen
Fax: ++49 8679 / 2676



Inspection certificate
according to EN10204-3.1B

Date: 13.11.2001
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Our Consignment
Material

Material-no.
Batch No.

On the batch, of which the consignment is a part, the following values
were determined. They conform to the agreed product specification.

Inspection characteristics-method	Specification	Result
-----------------------------------	---------------	--------

[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]

The above particulars do not release the customer from the obligation to
carry out an inspection of goods received.

Ms. Baumgartner - Worksinspector

This report is not to be signed

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Earthworm (*Eisenia fetida*), Acute Toxicity Test in Artificial Soil,
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12 GLP Certificate of DR.U.NOACK-LABORATORIUM



Niedersächsisches
Umweltministerium

Gute Laborpraxis/Good Laboratory Practice

GLP-Bescheinigung/Statement of GLP Compliance

(gemäß/according to § 19 b Abs.1 Chemikaliengesetz)

Eine GLP-Inspektion zur Überwachung der
Einhaltung der GLP-Grundsätze gemäß
Chemikaliengesetz bzw. Richtlinie 88/320/EEG
wurde durchgeführt in:

Assessment of conformity with GLP according to
Chemikaliengesetz and Directive 88/320/EEC at:

☒ Prüfeinrichtung / Test facility

☐ Prüfstandort / Test site

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(Unverwechselbare Bezeichnung und Adresse/Unequivocal name and address)

Prüfungen nach Kategorien/Areas of Expertise

(gemäß/according ChemVwV-GLP Nr. 5.3/OECD guidance)

- 1 - Prüfungen zur Bestimmung der physikalisch-chemischen Eigenschaften und Gehaltsbestimmungen
- 3 - Prüfungen zur Bestimmung der erbgutverändernden Eigenschaften (in vitro und in vivo)
- 4 - Umwelttoxikologische Prüfungen zu Auswirkungen auf aquatische und terrestrische Organismen
- 5 - Prüfungen zum Verhalten im Boden, im Wasser und in der Luft, Prüfungen zur Bioakkumulation und zur Metabolisierung
- 6 - Prüfungen zur Bestimmung von Rückständen
- 7 - Prüfungen zur Bestimmung der Auswirkungen auf Mesokosmen und natürliche Ökosysteme

Datum der Inspektion / Date of Inspection

(Tag, Monat, Jahr / day, month, year)

25. - 27. Juni 2001 / June 25th - 27th, 2001

29. August und 21. September 2001 / August 29th and September 21st, 2001

Die genannte Prüfeinrichtung/der genannte Prüfstandort
befindet sich im nationalen GLP-
Überwachungsverfahren und wird regelmäßig auf
Einhaltung der GLP-Grundsätze überwacht.

The above mentioned test facility / test-site is included in
the national GLP Compliance Programme and is
inspected on a regular basis.

Auf der Grundlage des Inspektionsberichtes wird hiermit
bestätigt, dass in dieser Prüfeinrichtung/diesem
Prüfstandort die oben genannten Prüfungen unter
Einhaltung der GLP-Grundsätze durchgeführt werden
können.

Based on the inspection report it can be confirmed, that
this test facility / test-site is able to conduct the
aforementioned studies in compliance with the Principles
of GLP.

Niedersächsisches Umweltministerium
Referat 33
Archivstraße 2
30169 Hannover



11. März 2002
Im Auftrage

Dr. Braedt

Company Sanitized. Does not contain TSCA CBI