Test No.: 933719
Test substance : CGA 329351

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

3.6. **Observations**

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

3.7. **Measurements**

The oxygen content, pH and temperature were measured at 0 and 48 hours. For the values see table 2.
Analytical determination of test substance concentrations see appendix.

3.8. **Calculations / Statistical Analysis**

none

Test No.: 933719
Test substance : CGA 329351

4. Results

The test substance was homogeneously distributed in the test vessels at all test times and test concentrations.
Analytical results and method are presented in the appendix.
Based on nominal concentrations the following values were calculated:

4.1. Values calculated

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

4.2. Values graphically determined

EC 50 (48 h)	**: not determined**

4.3. Values observed

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

4.4. Controls

Immobilizations in blank : 0

4.5. Conclusion

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

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5. Tables

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

Conc. nominal mg/l	Number of daphnia immobilized after 24 h exposure					
Vessel:	1	2	3	4	Total	%
blank	0	0	0	0	0	0
10	0	0	0	0	0	0
18	0	0	0	0	0	0
32	0	0	0	0	0	0
58	0	0	0	0	0	0
100	0	0	0	0	0	0

Conc. nominal mg/l	Number of daphnia immobilized after 48 h exposure					
Vessel:	1	2	3	4	Total	%
blank	0	0	0	0	0	0
10	0	0	0	0	0	0
18	0	0	0	0	0	0
32	0	0	0	0	0	0
58	0	0	0	0	0	0
100	0	0	0	0	0	0

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Table 2 Measurements

Conc. nominal mg/l	0h* pH	0h* %O2	0h* °C	Final measurements** Vessel 1 pH	Final measurements** Vessel 1 %O2	Final measurements** Vessel 1 °C
blank	7.9	100	21	8.0	104	23
10	8.0	99	21	8.0	103	23
18	8.0	99	21	8.0	104	23
32	8.0	100	21	8.0	103	23
58	8.0	99	21	8.0	104	23
100	8.0	100	21	8.0	103	23

* : measurements in separate vessels without daphnia.

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

Test No.: 933719
Test substance : CGA 329351

Appendix

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

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

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

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

Abstract of the method:
HPLC with UV detection: the injected specimen is preconcentrated and precleaned on a short column (C_{18}) and then transferred onto the analytical HPLC column (C_{18}). The substance is eluted with water-acetonitrile (65 vol. + 35 vol.) and detected at 240 nm.

Details of the method:
The HPLC system is equipped with a short column (1 cm length, 4 mm i.d., packed with Nucleosil 100 C_{18} 5 µm), a switching valve and an analytical column (12 cm length, 4 mm i.d., packed with Nucleosil 100 C_{18} 5 µm). 1 mL of the water specimen (appropriately diluted with water if necessary) is injected and transferred onto the short column, where it is preconcentrated and precleaned by washing with water. By means of the switching valve and water-acetonitrile (65 vol. + 35 vol.) as the mobile phase the substance is eluted from the short column and transferred onto the analytical column, from where it is eluted by the mobile phase and detected with an UV detector at 240 nm. CGA 329351 is used as the reference substance.
Quantitation: by alternate injections of water specimens and of reference substance solutions. Interpolation by method of weighted least squares of peak heights, regression of 1st order. From the measured contents of CGA 329351 the corresponding values of the test product were calculated (the product contains 97.3% CGA 329351).
The procedure was checked with recovery experiments at two spike levels. 4.5 ml of the control specimen was spiked with 0.5 ml of an appropriate standard solution of CGA 329351 in water.

Test No.: 933719
Test substance : CGA 329351

Appendix (continued)

3. RESULTS

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

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

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

Analyst: 5.1.2.e Woo

25 NOV 1994
date

5.1.2.e Woo
(principal investigator for analytics)

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

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