Determination of the Toxicity of Products towards green Alga

1	**Sample designation:**	**„Dryflex 1“**
1.1	Sample received on:	25-Apr-2008
1.2	Storage conditions:	room temperature
2	**Sponsor:**	Drytech Italia s.r.l.
2.1	Address:	Via Ravona 1H, I-20220 San Fermo, Italy
3	**Testing facility:**	Intitut Fresenius Chemische und Biologische Laboratorien AG
3.1	Address:	Im Maisel 14, D-65232 Taunusstein-Neuhof, Germany
3.2	Study director:	Dr. H. Lebertz
4	**Test method:**	Alga Growth Inhibition Test, OECD 201 (Version dated 23.03.06)
4.1	Test system:	*Desmodesmus subspicatus CHODAT*
4.1.1	Origin:	Institute of plant physiology at University of Göttingen, strain-No.: 8681
4.2	Test conditions:	Static test within 72 hours; incubator: WTW Binder, Type KB 240 with illumination equipment (intensity >120 µE/m^{2}s and a temperature-range of 24 ± 1 °C)

5 Method description:

The test algae were exposed to the test item at different concentrations of up to 2.165 mg per litre of test medium (final concentration, main test) for a period of 72 hours. For this purpose, the test item was dissolved in "Ultrapure water" (Seral, Purelab plus). By the addition of the respective stock-solutions with the mineral nutrients necessary for the growth of the alga, and the amount of algal inoculation the final concentrations of 0.135, 0.270, 0.542, 1.082, and 2.165 mg/L were achieved. For Blank correction, respective controls were prepared in the same way, but without algal inoculation containing algal test medium instead of the inoculation. In the same way, a set of test solutions without any test item were prepared as the unaffected controls. For statistical purpose, 7 inoculated test solutions with the test item were prepared at each concentration, and 7 unaffected controls with inoculation were prepared in parallel. For "back-correction", three non-inoculated test solutions were prepared per test concentration, respectively. The test solutions were then incubated at a temperature of 24 ± 1 °C at constant illumination. In order to assure the constant distribution of the algal cells in the test medium the test solutions were stirred for 15 min per hour of incubation on a multi-magnetic-stirrer. After 24, 48 and 72 hours a quantification of the alga in the test solutions was performed using a photometer. The inhibition of the algal growth is given as a comparison with the unaffected control. As a further control, pH-value was determined in the test solutions at the start of the test and after 72h of incubation.

5.1 Treatment of results:

Inhibition values were calculated according to OECD 201 using the commercial computer program ToxRatPro Version 2.09 (08.11.2006). For this purpose, extinction values were converted into cell numbers using a calibration curve where cell numbers are plotted against the respective extinction value at 578 nm. For the generation of the calibration curve, cell numbers had been counted via microscope using a "Thoma chamber".

All necessary parameters E_YC_{50}, E_rC_{50} as well as NOEC and LOEC were determined by the computer program. The specific growth rates were determined separately for each intermediate time period and calculated as the section-by-section growth rates.

5.1.1 Definitions and Formula for Growth rates and „Yield"

The mean specific growth rate of the respective period was calculated as a logarithmic increase in biomass for each test vessel according:

$$\mu = \frac{\ln X_j - \ln X_i}{t_j - t_i} \ (day^{-1}) \quad \text{where}$$

μ_{i-j} is the mean growth rate between time i to j
X_i is the biomass at time i
X_j is the biomass at time j

The percent inhibition was calculated according

$$\%I_r = \frac{\mu_c - \mu_t}{\mu_c} \times 100 \quad \text{where}$$

$\%I_r$ is the percent inhibition of the mean growth rate
μ_c is the mean specific growth rate (μ) in the controls
μ_t is the mean growth rate (μ) of the parallels with the test item

The yield is calculated as the biomass at the end of the test minus the biomass at the beginninmg of the incubation for each control and each treatment with the test item. For each concentration and each control the mean of the yield including variances were calculated The percent inhibition ($\%I_y$) was calculated for each concentration according:

$$\%I_y = \frac{(Y_c - Y_t)}{Y_c} \times 100 \quad \text{where}$$

$\%I_y$ is the percent inhibition of the yield
Y_c is the mean value for yield in the controls
Y_t is the value for yield of the parallels with the test item

6 Criteria for Validity of the test

According to OECD 201, the factor of the biomass parameter, measured in the control between 0 and 72 h, must be at least 16. With the current test it was found to be 108.0. The test fulfils this validity criterion.

Evaluation of the section-by-section growth rates:

Arithmetic means of the control replicates from 0 h to 72 h were: Replicate 1: 1.536; Replicate

2: 1.544; Replicate 3: 1.552; Replicate 4: 1.579; Replicate 5: 1.560; Replicate 6: 1.560; Replicate 7: 1.584. [1/d]

Coefficients of variation in control replicates from 0 to 72 h were:

Replicate 1: 25.4%; Replicate 2: 27.8%; Replicate 3: 31.1%; Replicate 4: 33.0%; Replicate 5: 33.4%; Replicate 6: 31.4%; Replicate 7: 28.9%.

The mean of the replicate coefficients of variation in the section-by-section growth rate was: 30.1%.

According to OECD 201, the mean coefficient of variation, measured in the control from 0 to 72 h, must not be higher than 35%. The test fulfils this validity criterion.

The coefficient of variation of the mean specific growth rate replicates in the control between 0 and 72 h was: 1.1%.

According to OECD 201, the coefficient of variation of the mean specific growth rate, measured in the control from 0 to 72 h, must not exceed 7%. The test fulfils this validity criterion.

Thus, all criteria for validity of the test were met.

7 Test Report:

Pretreatment of the test item:

The test item was dissolved in "Ultrapure water" (Seral, Purelab plus). By the addition of the respective stock-solutions with the mineral nutrients necessary for the growth of the alga, and the amount of algal inoculation the final nominal concentrations of 0.135, 0.270, 0.542, 1.082, and 2.165 mg/L were achieved.

8 Results

8.1 Table 1: Determination of Cell Numbers

Test Concentration (mg/L)	Mean Cell Numbers in the Test Solutions (n/mL)			
	t_0	t_{24h}	t_{48h}	t_{72h}
0 (Control)	$2.640*10^3$	$1.94*10^4$	$9.75*10^4$	$2.84*10^5$
0.135	$2.640*10^3$	$1.78*10^4$	$6.85*10^4$	$2.45*10^5$
0.270	$2.640*10^3$	$1.22*10^4$	$5.37*10^4$	$2.15*10^5$
0.542	$2.640*10^3$	$1.28*10^4$	$1.94*10^4$	$1.44*10^5$
1.082	$2.640*10^3$	$9.45*10^3$	$8.56*10^3$	$5.34*10^4$
2.165	$2.640*10^3$	$8.01*10^3$	$4.63*10^3$	$1.56*10^4$

Table 2: Inhibition Values

From the results of the main test following effect concentrations were determined:

On the Basis of the nominal Concentrations [mg/L]

Yield (0 - 72 h)		
	EC10	0.149
95%-CL	lower	0.082
	upper	0.209
	EC20	0.226
95%-CL	lower	0.147
	upper	0.294
	EC50	0.501
95%-CL	lower	0.405
	upper	0.620
Yield	LOEC	<0.135
	NOEC	<0.135
Growth rate (0 - 72 h)		
	EC10	0.429
95%-CL	lower	0.316
	upper	0.529
	EC20	0.656
95%-CL	lower	0.533
	upper	0.763
	EC50	1.470
95%-CL	lower	1.312
	upper	1.672

Wachstumsrate	LOEC	0.542
	NOEC	0.270
Section-by-section growth rate (48 - 72 h)		
	EC10	n.d.
95%-CL	lower	n.d.
	upper	n.d.
	EC20	n.d.
95%-CL	lower	n.d.
	upper	n.d.
	EC50	n.d.
95%-CL	lower	n.d.
	upper	n.d.
Section-by-section growth rate		
	LOEC	>2.165
	NOEC	≥ 2.165

n.d.: not determined due to mathemetical reasons

8 Remarks:

This main test was carried out without chemical analyses (quantification of the test substance in the test solutions) as the concentrations were too low for the chosen analytical method. Within a first main test using higher concentrations of the test item, according to DOC determinations performed with test solutions, it was shown to be stable within 72h of incubation.

9 Discussion:

The test is considered valid as all criteria for validity of the test were met.

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Taunusstein, 16-Jul-2008

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