

ECOTOXILAB

ECOTOXICITY TESTS

- **Daphnia sp. Acute Immobilization Test
OECD 202**

RESULTS REPORT

REPORT No. 2

Toxicity analysis according to the ecotoxicity standards for aquatic organisms OECD 201 and **OECD 202**; and on terrestrial organisms OECD 207 and OECD 208. Tests carried out with the sample:

-Sample 1: Ecofire forest ("water-based organic filler solution")

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2. Daphnia sp. ACUTE IMMOBILIZATION TEST (OECD Standard 202)

2.1- TEST SAMPLE

- Sample 1: Ecofire forest

Sample characteristics: Water-based organic filter solution (data provided by the client).

2.2 –TEST DESCRIPTION

The assay consists of the detection of immobilization of the freshwater microcrustacean *Daphnia magna* at 24 and 48h, when exposed to different dilutions of the aqueous sample. The test has been carried out in accordance with the OECD 202 standard (Ed. November 23, 2004).

2.3- SPECIES USED IN THE TEST

- Species: *Daphnia magna*, Strauss
- Source: Microbiotests Inc. (Ghent, Belgium). Batch: DM121022
- Origin: Neonates <24h. of life from ehipias
- Breeding temperature: $22 \pm 1^\circ\text{C}$
- Hatching temperature: $20 \pm 1^\circ\text{C}$
- Photoperiod: 12h:12h (light : dark) / 24h during hatching
- Feeding: For 1h prior to the trial with the alga *Pseudokirchneriella subcapitata*

2.4- TEST CONDITIONS

- Number of animals in the test: A total of 20 animals per dilution (4 replicates x 5 individuals) were used. Loading ratio: 5 mL dilution/organism.

- Preconditioning of the sample: The sample was left to decant for 24h at 4°C in a refrigerator, the supernatant was centrifuged at 2000 rpm. The pH of the sample was not adjusted.

- Final dilutions tested:

- 3.12, 1.56, 0.78, 0.19 and 0.097% dilution of the original sample
- Control (blank) with culture medium

- Exposure mode: 30-well polystyrene multiwell plates.

- **Origin of the dilution medium**: Reconstituted water according to the composition described in the OECD 202 standard with the following general chemical characteristics:

- pH: 7.7 ± 0.1
- Temperature: $22 \pm 1^\circ\text{C}$
- Dissolved oxygen: 100% saturation by continuous bubbling prior to the test

- **Method of preparation of dilutions**: Dilutions, with reconstituted and aerated culture medium, prepared immediately before the test from the suitably conditioned sample.

- **Lighting in the test**: Cold light. Intensity 4000lux. Photoperiod 16:8 h. (light darkness)

- **Calculation of EC50**: Mortality values in each dilution were adjusted by probit regression following the method described by Finney (1952) (Finney, D.J. (1952). Probit analysis: A statistical treatment of the sigmoid response curve (2nd ed.). Cambridge University Press.).

- **Quality control and acceptability criteria**: Internal control with reference toxicant Potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$). $\text{EC}_{50-24\text{h}} = 1.21$ ppm. Acceptability range for $\text{EC}_{50-24\text{h}}$ according to ISO 6341: 0.6-2.1 mg/L.

The test acceptability criterion establishes a maximum mortality in the negative controls of up to 20% of the organisms initially exposed.

2.5- RESULTS AFTER 24 HOURS OF EXPOSURE

2.5.1 - PERCENTAGES OF IMMOBILITY AFTER 24 HOURS OF EXPOSURE

Reference sample	Dilution (%)					
	Blank	3.12	1.56	0.78	0.19	0.097
Ecofire	0	100	70	15	10	0

2.5.2. – ECOTOXICOLOGICAL PARAMETERS

Reference Sample	EC 50-24h (% dilution)/ (mg/L)	95% CI* (% dilution)
eco fire	1,016/ 12700	na na

*Confidence Interval ($\alpha = 0.05$)
na: not possible to determine

2.5.3. – RESULT IN TOXIC UNITS (old UT Equitox index/m3)

Reference Sample	UT	95% CI*
eco fire	98.4	na na

2.6- RESULTS AFTER 48 HOURS OF EXPOSURE

2.6.1 - PERCENTAGES OF IMMOBILITY AFTER 48 HOURS OF EXPOSURE

Reference sample	Dilution (%)					
	Blank	3.12	1.56	0.78	0.19	0.097
Ecofire	0	100	90	25	15	5

2.6.2. – ECOTOXICOLOGICAL PARAMETERS

Reference Sample	EC 50-48h (% dilution)/ (mg/L)	95% CI* (% dilution)
eco fire	0.74/ 9250	na na

*Confidence Interval ($\alpha = 0.05$)

na : cannot be determined

2.6.3. – RESULT IN TOXIC UNITS (old UT Equitox index/m3)

Reference Sample	UT	95% CI*
eco fire	135.1	na na

2.7- COMMENT ON RESULTS AND INCIDENTS

Test based on the OECD 202 standard. During the test there were no notable technical incidents.

The values of toxicity in the tested sample have been EC 50-24 h of 1.016 (% dilution) and EC-50-48 h of 0.74 (% dilution). They correspond to a weight / volume concentration of the ECOFIRE product of 12,700mg L-1 and 9,250 mg L-1 respectively.

The commercial product called **ECOFIRE forest is outside the classification criteria for Acute Aquatic Toxicity of the Globally Harmonized System of Classification and Labeling of Chemicals (GHS)**, which establishes its lowest category (Category Acute 3 for Daphnia) between 10 and 100 mg L -1. The same criteria is applied from Regulation (EC) No. 1272/2008 of the European Parliament and of the Council, of December 16, 2008, on the classification, labeling and packaging of substances and mixtures, commonly known as the CLP Regulation.

Dr. Technician

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Sgd. Gerardo Mengs González