

TOXICITY TEST REPORT

For:
Fireblock cc

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Survey:
2024-11

Report reference:
FRB-FFG-A-24_TOX

Revision:
0

Project:
Fireblock Firefighting Gel Ecotoxicity

Samples:
Fireblock Firefighting Gel

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1. ANALYSES REQUESTED AND SAMPLE INFORMATION

Enclosed please find Test Certificate of analysis number FRB-FFG-A-24_TOX. The results relate only to the sample tested. BioToxLab does not accept responsibility for any matters arising from the further use of the results. Tests marked "Not SANAS accredited" (NA or OS) in this Certificate of Analyses are not included in the SANAS Schedule of Accreditation for this Laboratory.

No part of this Certificate of Analyses may be quoted in isolation of the rest of the text without the written permission of BioToxLab. Opinions and Interpretations expressed herein are outside the scope of SANAS accreditation.

Please contact the Laboratory if further information is required.

Table 1: Analyses requested and description for the different samples, including sampling and delivery dates.

| Sample name | Sampling date | Sample type (water, sediment, product etc) | Sampled by | Delivery date | Delivered by | Additional comments (sample description or deviations) | Oxygenation required for sample, please tick (lab use): ✓ ✗ N/A | Tests requested - Marked with X | | | | | | | | | | | | |
|----------------------------|---------------|--|--------------|---------------|--------------|--|--|---------------------------------|------------|---------------|---------------------|---------------------|---------------------------|----------------------|-------------|----------|--|--|--|--|
| | | | | | | | | Screening | Definitive | Water | | | | | | Sediment | | | | |
| | | | | | | | | | | Daphnia magna | Poecilia reticulata | Allivibrio fischeri | Selenastrum capricornutum | Spirodela polyrrhiza | Phyto seeds | Ostracod | | | | |
| Fireblock Firefighting Gel | 2024.11.18 | Gel | Fireblock CC | 2024.11.19 | Fireblock CC | Sample is a gel | N/A | X | | | | | | | | | | | | |

Screening = 100% (undiluted) sample tested only

Definitive = Series of sample dilutions tested to enhance classification accuracy and to determine safe dilution

2. METHODOLOGY

Sampling and sample handling

The sample was analysed as received from the Client. The sample received from Fireblock CC was exposed as a screen on 1 trophic level (Phytotox) to assess the effect of the product on the receiving vegetation.

Test Conditions

The toxicity test was conducted in environmentally controlled rooms using standard techniques.

Quality Assurance

The BioToxLab Aquatic Toxicology Laboratory's Policy and Quality Manual, intended to support and maintain all aspects of the Quality System, is based on the application of ISO/IEC 17025. The following Quality Assurance information can be made available on request (1) inhouse reference toxicant test data and control charts (2) Proficiency Testing Scheme (PTS) test data (3) lot and batch numbers (4) raw toxicity test data.

Assessments

Given the limitations of substance-specific assessments, and the risk of allowing ecological toxicity hazards to go unchecked/undetected, water resource managers and scientists have for some time called for methodologies that will allow more complete assessments of ecological toxicity hazards to be used in addition to the substance-specific approach. The National Water Act (Act no. 36 of 1998), providing for water in sufficient quantity and in sufficient quality for basic human needs and for maintenance of aquatic ecosystem function, implemented an approach known as the Direct Estimation of Ecological Effect Potential (DEEEP) protocol as a means of circumventing the shortcomings of direct toxicant monitoring. This protocol normally consists of a battery of tests to directly assess lethal (acute) and sub-lethal (chronic) toxicity, using test organisms from a range of trophic levels. These toxicity tests can demonstrate whether contaminants are bioavailable, it can evaluate the aggregate toxic effects of all contaminants in the medium and it can evaluate the toxicity of substances whose biological effects may not have been well characterized.

Lethal or sub-lethal toxicity testing (as applied for this assessment) is applied by exposing the plant seeds to the sample source to determine the potential risk of such samples to the biological integrity of the receiving water bodies and the general environment. A risk category is determined based on the percentage germination (lethal) or growth inhibition (sub-lethal) of the exposed seeds. It is important to note that the hazard classification is based on the selected test materials and therefore represents the risk/hazard towards similar materials in the receiving environment. The toxicity hazard is therefore in terms of the aquatic biotic integrity and does in no way represent toxicology towards humans or other mammals. Standard, internationally accepted methods and materials were applied to conduct lethal and sub-lethal toxicity testing.

Phytotox seed germination and early growth toxicity test with higher plants (A)

| | |
|---|---|
| BioToxLab method number: | QM7.2/TMH-11 |
| Deviation from the method: | None |
| Test endpoint: | % germination (lethal); % growth inhibition (sub-lethal) |
| Exposure period: | 72-hours |
| Test chamber type: | Polyvinylchloride (PVD) transparent test plates (21x15.5x0.8cm) |
| Number of replicates per sample: | 3 (3 types of seeds, thus 6 test plates inoculated with each type of seed – 3 plates with test soil & 3 plates with control soil) |
| Number of seeds per chamber: | 10 seeds of each different type of seed |
| Test temperature (24-26°C): | 25°C |
| Test seeds names & origin | <i>Lepidium sativum</i> (dicotyl garden cress) <i>Sinapis alba</i> (dicotyl mustard) <i>Sorghum saccharatum</i> (monocotyl Sorgho) Obtained from Microbiotests |
| Cyst batch number(s): | LES061223; SIA030524; SOS291023 |
| Statistical methods used: | Microsoft Excel® spreadsheet formulated by supplier (MicroBioTests Inc., Belgium) – RegTox and Regression analysis |
| Date(s) of performance of the test(s): | 2024.11.25 |
| Uncertainty of measurement: | Available on request |
| Validity criteria (mean % germination ≥70%/ mean root length in controls of ≥30 mm for the 3 test species): | 83.3% / 70 mm |

3. HAZARD CLASSIFICATION METHODOLOGY

A risk/hazard category is determined by using a hazard classification system developed by Persoone *et al.* (2003) whereby one can classify sites using the toxicity data of the non-diluted samples. The percentage effect (PE) of toxicity (germination, growth inhibition) is used to rank the sample into one of five classes (Table 2 –screenings) based on the highest toxic response obtained in at least one of the tests applied.

Table 2: Hazard classification system for natural water samples (Persoone *et al.* (2003))

| Class | Symbol | Hazard rating | PE | Percentage effect |
|-------|--------|------------------------------------|------------------------------|---|
| I | ☺ | No lethal/sub-lethal hazard | ≤10/20% | None of the tests show a toxic effect (i.e. an effect value that is significantly higher than that noted in the controls) |
| II | ☹ | Slight lethal/sub-lethal hazard | 10/20%≤PE<50% | A statistically significant (P<0.05) PE is reached in at least one test, but the effect level is below 50% |
| III | ☠ | Lethal/sub-lethal hazard | 50%≤PE<100% | The PE50 is reached or exceeded in at least one test but the effect level is below 100% |
| IV | ☠☠ | High lethal/sub-lethal hazard | PE 100% in at least one test | The PE100 is reached or exceeded in at least one test |
| V | ☠☠☠ | Very high lethal/sub-lethal hazard | PE 100% in all tests | The PE100 is reached in all the tests |

EP (Percentage effect) = an effect measured either as mortality or inhibition (depending on the type of test). A >10% effect is regarded as slight lethal toxicity for Phytotox, while a >20% effect is regarded as slight sub-lethal toxicity for Phytotox. A 50% effect is regarded as a lethal/sub-lethal toxicity for the Phytotox test

4. RESULTS AND HAZARD CLASSIFICATION DATA

Table 3: Hazard classification of sediment samples

| Site/sample | Phytotox (A) | | | |
|----------------------------|--|------------|---|------------|
| | <i>L. sativum, S. alba, S. saccharatum</i> | | | |
| | % effect Lethal (germination) | Test score | % effect Sub-lethal (growth inhibition) | Test score |
| Fireblock Firefighting Gel | -8 | 0 | -10 | 0 |

Table 4: Site hazard classification of sediment samples

| Results | | Fireblock Firefighting Gel |
|---------------------------------------|--|---------------------------------------|
| Phytotox (seed germination) (A) | Date test performed | 2024.11.25 |
| | *72hour inhibition (-) / stimulation (+) (%) | -10 |
| | 72hour Germination success (% relative to control) | -8 |
| | Description | no lethal/sub-lethal hazard |
| Overall classification - Hazard class | | Class I - No lethal/sub-lethal hazard |

* = The sub-lethal toxicity interpretation relies on the inhibition/stimulation result of this test. The lethal toxicity interpretation relies on germination success.

| Site | Hazard classification | Percentage Effect |
|----------------------------|--|---|
| Fireblock Firefighting Gel | I ☺ No lethal (germination inhibition) or sub-lethal (growth inhibition) environmental toxicity hazard | None of the tests show a toxic effect (i.e. an effect value that is significantly higher than that noted in the controls) |

5. COMMENTS

One gel-like product sample was delivered to the BioToxLab office on 2024.11.19. The client (Fireblock CC) noted that this product is used to fight fires on grass, forest, coal and lithium batteries and that the effect on natural vegetation needs to be assessed. Based on the constituency of the product and the method applied for this test, it was agreed to dilute the sample 1:4 to enable testing according to ISO18763 (Soil quality – determination of the toxic effects of pollutants on germination and early growth of higher plants).

Any queries regarding the results can be lodged with Lizet Swart within 14 days from the date of receiving this report after which the samples will be discarded. It is not advised to use these samples for any retesting other than range confirmation of chemical parameters – re-sampling must be done in the case of any queries relating to the results associated with the samples.

6. REFERENCES

INTERNATIONAL STANDARD ISO 18763 (2016). Soil quality – Determination of the toxic effects of pollutants on germination and early growth of higher plants.

PERSOONE G, MARSALEK B, BLINOVA I, TÖRÖKNE A, ZARINA T, MANUSADZIANAS L, NALECZ-JAWECKI G, TOFAN L, STEPANOVA L, TOTHOVA L, KOLAR B (2003). A practical and user-friendly toxicity classification system with Microbiotests for natural waters and wastewaters (personal communication).

SOUTH AFRICAN NATIONAL STANDARD, (SANS), ISO/IEC 17025:2017. General requirements for the competence of testing and calibration laboratories. 3rd Edition. South African Bureau of Standards, Pretoria.

END OF REPORT