

Ultimate Products (Aust) Pty Ltd

**Toxicity Assessment:
Hydra-Gyp**



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Report Date: 16th November 2010
Report Number: UL101104
Copy Number: *electronic*

| Name | Date |
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1. Executive Summary

A series of toxicity tests was conducted on various levels of freshwater aquatic organisms. The species tested were the green alga *Selenastrum capricornutum*, the freshwater aquatic duckweed *Lemna minor*, the freshwater cladoceran *Ceriodaphnia cf dubia*, and the freshwater rainbowfish *Melanotaenia splendid*.

The concentration of Hydra-Gyp at which 50% of test organisms was estimated to exhibit a specified sub-lethal or inhibition effect within the specified time frame was 1000 ppm or greater for all species tested.

The lowest observed effect concentration (LOEC) for all species tested was 1000 ppm or greater, and the highest Hydra-Gyp concentration in which the values for the measured response were not statistically significantly different from those in the control was 333 ppm for an effect on the reproduction of *Ceriodaphnia*, and 1000 ppm or greater for all other tests.

2. Introduction

An aquatic toxicity test is a procedure in which the responses of aquatic organisms are used to measure the effect of a particular substance in the environment. Toxicity testing is useful in water quality evaluations where chemical interactions in complex matrices make it difficult to determine the effect on aquatic biota from chemical and physical tests alone. Different species of aquatic organisms are not equally effected by a toxic substance, nor are organisms equally effected at varying points in their life cycle. Exposing a variety of organisms under both short- and long- term conditions gives a broader understanding of the effect of a particular toxicant on an environment. When using the results for regulatory assessments, it is necessary to use toxicity data in conjunction with receiving-water and site-specific discharge data on volumes, dilution rates and exposure times.

3. Scope and Limitations

Ultimate Products (Aust) Pty Ltd (the client) submitted a sample of a technical grade water flocculant named Hydra-Gyp for assessment as to its toxicity to aquatic lifeforms. VGT engaged the laboratory Ecotox Services Australasia to undertake the following tests as agreed with the client:

| Test Name | Type of test | Type of organism |
|---|---|----------------------------|
| 72 hr growth inhibition test using <i>Selenastrum capricornutum</i> | Reduction in growth rate relative to a control | Freshwater algae |
| 7 day growth inhibition test with <i>Lemna sp</i> | Reduction in growth rate relative to a control | Freshwater flowering plant |
| 48 hr acute toxicity test with <i>Ceriodaphnia dubia</i> | Short term lethal effects | Freshwater crustacean |
| 7 day chronic test with <i>Ceriodaphnia dubia</i> | Long effects, eg reduced growth, reduced reproduction | Freshwater crustacean |
| 96 hr Fish Imbalance test with rainbow fish | Short term lethal effects | Freshwater fish |

The range of tests covers both acute and chronic toxicity to varying levels of aquatic lifeforms in fresh water environments. In order to expediate results a range-finding test was not used, and concentrations for a definitive test were determined using the expected upper limit for which the product is designed to be used. Therefore tests were limited to an upper dilution of 1000 parts per million (ppm) which is equivalent to 1000 litres per million litres.

4. Terminology

The following terms are used in this document. ^{Ref. 1}

Response – the measured biological effect of the variable tested. In acute toxicity tests the response usually is death or immobilisation. In plant toxicity tests, the response can be death, growth inhibition, or reproductive inhibition.

Control – treatment in a toxicity test that duplicates all the conditions of the exposure treatment but contains no test material.

Range-finding test – preliminary test designed to establish approximate toxicity of a solution.

Screening test – toxicity test to determine if an impact is likely to be observed; test design incorporates one concentration, multiple replicates, exposure 24 to 96 hr.

Definitive test – toxicity test designed to establish concentration at which a particular end point occurs. Exposures for these tests are longer than for range-finding tests, including multiple concentrations at closer intervals and multiple replicates.

Dose – amount of toxicant that enters the organism. Dose and concentration are not interchangeable.

Toxicity – potential or capacity of a test material to cause adverse effects on a living organism. Toxicity is result of dose and exposure time.

Exposure Time – time of exposure of test organism to test solution.

Acute Toxicity – relatively short-term lethal, or other, effect. Usually within 4 days for fish and 2 days for organism with shorter life spans.

Chronic Toxicity – toxicity involving a stimulus that lingers for a relatively long period of time, and is a relative term depending on the lifespan of the organism. A chronic toxic effect can be measured in terms of reduced growth, reduced reproductivity, etc, in addition to death.

Lethal Concentration (LC_p) – toxicant concentration estimated to produce death in a specified proportion (p) of test organisms. Usually defined as LC₅₀, that is concentration of toxicant that kills 50% of exposed organisms at specific time of observation, for example 96-hr LC₅₀.

Effective Concentration (EC_p) – toxicant concentration estimated to cause a specified effect in a specified proportion (p) of test organisms. The effect is usually sub-lethal, such as a change in respiration rate or loss of equilibrium. For example 48-hr EC₅₀ for loss of equilibrium is the effective concentration at which 50% of organisms exhibited this effect after 48 hours.

Inhibition Concentration (IC_p) – toxicant concentration estimated to cause a specified percentage (p) inhibition or impairment in a qualitative biological function. For example, a 72-hr IC₅₀ could be the concentration estimated to cause a 50% reduction in number of algal cells after 72 hours of exposure.

No-observed-effect Concentration (NOEC) – in a full- or partial-lifecycle test, the highest toxicant concentration in which the values for the measured response are not statistically significantly different from those in the control.

Lowest-observed-effect Concentration (LOEC) – in a full- or partial-lifecycle test, the lowest toxicant concentration in which the values for the measured response are statistically significantly different from those in the control.

5. Results

As previously mentioned, no range-finding tests were undertaken, and so all tests were performed at the specified highest concentration of 1000 parts per million (ppm) of Hydra-Gyp to diluents. Further testing was undertaken at serial dilutions from the highest, ie 333.3 ppm, 111.1 ppm, 37 ppm, 12.3 ppm and 4.1 ppm. These results do not take into account the effects of any site-specific discharge or receiving waters.

a. 72 hr growth inhibition test with *Selenastrum capricornutum*

The test was performed using the green alga *Selenastrum capricornutum*. There was a slight reduction in the rate of cell growth in the highest concentration sample, but no statistical difference from the control in the other dilutions. The 72-hr IC50 was not achieved, that is after 72 hours even the highest concentration of Hydra-Gyp had greater than 50% growth rate. There was no statistically significant difference between the controls and the dosed solutions, and therefore the NOEC is reported at 1000 ppm and the LOEC is > 1000 ppm.

Therefore at concentrations of 1000 ppm or below in growth media, Hydra-Gyp is not toxic to green alga.

b. 7 day growth inhibition test with *Lemna sp*

The test was performed using the freshwater duckweed *Lemna minor*. In this case there was no statistically significant reduction in the growth rate of the aquatic flowering plant after 7 days. The 7-day IC50 was >1000ppm, the NOEC was 1000ppm and the LOEC was > 1000ppm.

Therefore at concentrations of 1000 ppm or below in growth media, Hydra-Gyp is not chronically or acutely toxic to freshwater aquatic duckweed.

c. 48 hr acute toxicity test with *Ceriodaphnia Dubia*

The test was performed using the freshwater cladoceran *Ceriodaphnia cf Dubia*. After 48 hours there was a 100% survival rate of the organisms at all concentrations tested. The 48-hr EC50 and LOEC were all >1000 ppm, and the NOEC was 1000 ppm.

Therefore after 48 hours at concentrations of less than 1000ppm Hydra-Gyp shows no acute toxic effect on freshwater crustaceans *Ceriodaphnia Dubia*.

d. 7 day chronic test with *Ceriodaphnia dubia*

This partial life-cycle test was also performed using the freshwater cladoceran *Ceriodaphnia cf Dubia*. After 7 days, there was still a 100% survival rate of the organisms at all concentrations tested. The 7-d EC50 (survival), and the LOEC were all > 1000ppm and the NOEC was 1000 ppm.

There was a statistically significant reduced number of young in the highest concentration sample solution. The NOEC was therefore 333.3 ppm and the LOEC was 1000 ppm. The 7-day IC50 was >1000ppm.

e. 96 hr Fish Imbalance test with rainbow fish

This toxicity test was conducted using *Melanotaenia splendida*, rainbow fish. The 111.1ppm dilution sample resulted in 65% of the test organisms unaffected by the Hydra-Gyp concentration. All other samples, including those of higher concentrations were not significantly different from the control. The 96hr EC50 and LOEC are greater than 1000 ppm, and the NOEC is equal to 1000 ppm.

6. Summary

| Species | Time | Effective / Inhibition Concentration (ppm) 50% | No-observed-effect Concentration | Lowest-observed-effect Concentration |
|-------------|--------------------|--|----------------------------------|--------------------------------------|
| Algae | 72-hr | >1000 | 1000 | >1000 |
| Duckweed | 7-day | >1000 | 1000 | >1000 |
| Cladoceran | 48-hr | >1000 | 1000 | >1000 |
| Cladoceran | 7-day survival | >1000 | 1000 | >1000 |
| Cladoceran | 7-day reproduction | >1000 | 333.3 | 1000 |
| Rainbowfish | 96-hr | >1000 | 1000 | >1000 |

The lowest observed effect concentration for all species tested was 1000 parts per million Hydra-Gyp in water.

7. References

Ref. 1. Standard Methods for the Examination of Water and Wastewater, APHA, AWWA, WEF.

8. Appendix: Ecotox Services Report Number TR0627