

Ultimate Products (Aust) Pty Ltd

**Toxicity Assessment:
Triple Strike**





Environmental
Compliance
Solutions

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Ultimate Products (Aust) Pty Ltd

Toxicity Assessment: Triple Strike

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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 cladoceran *Ceriodaphnia cf dubia*, and the freshwater rainbowfish *Melanotaenia splendida*.

The different species showed varied effect to the Triple Strike, with some toxicity being shown at less than 1000 mg/L (or ppm) in all cases. In the acute tests, *Selenastrum* 72hr IC50 was 70 mg/L, and *Ceriodaphnia* 48hr EC50 was >1000 mg/L. In the chronic tests, *Ceriodaphnia* 7day EC50 survival was 177 mg/L, 7day IC50 reproduction was 122 mg/L, and the *Melanotaenia splendid* 96hr EC50 imbalance test result was 521 mg/L.

The tests were limited to specific dilutions of 4.1, 12.3, 37.0, 111.1, 333.3, and 1000 mg/L, thus the NOEC (No Observed Effect Concentration) and LOEC (Lowest Observed Effect Concentration) results are limited to these values. An NOEC of 37.0 mg/L and an LOEC of 111.1 mg/L was reported for algae 72hr, cladoceran 48hr survival and 7day reproduction. The NOEC for cladoceran 7day survival and rainbow fish 96hr imbalance test was 111.1 mg/L and the LOEC was 333.3 mg/L.

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 algae treatment and water flocculant named Triple Strike 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
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 mg/L (ppm). Serial dilutions were made from this upper dilution of 333.3 mg/L, 111.1 mg/L, 37.0 mg/L, 12.3 mg/L, and 4.1 mg/L.

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.

mg/L – Concentration is expressed in mg/L as the dilutions were made using a weight rather than a volume. This concentration can also be expressed as ppm, or parts per million, and is approximately equivalent to Kg per 1000m³ and litres per mega litre.

5. Results

As previously mentioned, no range-finding tests were undertaken, and so all tests were performed at the specified concentrations of 1000 mg/L of Triple Strike in diluent, 333.3 mg/L, 111.1 mg/L, 37.0 mg/L, 12.3 mg/L and 4.1 mg/L. 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 no statistically significant effect at 37.0 mg/L (NOEC) and the lowest concentration that did show an effect was 111.1 mg/L (LOEC). The 72-hr IC50 growth inhibition (the estimated Triple Strike concentration to cause a 50% reduction in number of algal cells after 72 hours of exposure) was calculated to be 70 mg/L.

b. 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 dilutions of 4.1, 12.3, and 37.0 mg/L Triple Strike in dilute mineral water. There was 60% survival rate at concentrations 111.1, 333.3, and 1000 mg/L. The NOEC is therefore 37.0 mg/L and the LOEC is 111.1 mg/L. The effective concentration at which less than 50% of organisms survived after 48 hours (48 hr EC50 survival) was not reached at 1000 mg/L.

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

c. 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 100% survival rate at 111.1 mg/L, and 0% at 333.3 and 1000 mg/L. Therefore the NOEC was 111.1 mg/L, LOEC was 333.3 mg/L. The Triple Strike concentration at which 50% of the organisms could be expected to survive after 7 days (7 day EC50 survival) was calculated at 177 mg/L.

There was a statistically significant reduced number of young in the 111.1 mg/L dilution of Triple Strike in dilute mineral water after 7 days. The NOEC was therefore 37.0 mg/L and the LOEC was 111.1 mg/L. The 7-day IC50 (reproduction) was calculated at 122 mg/L.

d. 96 hr Fish Imbalance test with rainbow fish *Melanotaenia splendida*

This toxicity test was conducted using *Melanotaenia splendida*, rainbow fish. The 111.1 mg/L dilution sample resulted in 75% of the test organisms being unaffected by the Triple Strike concentration, this was calculated as significant and therefore the NOEC for rainbow fish is 111.1 mg/L and the LOEC is 333.3 mg/L. The effective Triple Strike concentration at which 50% of rainbow fish exhibited some imbalance after 96 hours (96-hr EC50) was calculated at 521 mg/L.

6. Summary

Species	Time	Effective / Inhibition Concentration 50%	No-observed-effect Concentration NOEC	Lowest-observed-effect Concentration LOEC
Algae: <i>Selenastrum</i>	72-hr IC50	70	37	111
Cladoceran: <i>Ceriodaphnia</i>	48-hr survival (acute) EC50	>1000	37	111
Cladoceran: <i>Ceriodaphnia</i>	7-day survival (chronic) EC50	177	111	333
Cladoceran: <i>Ceriodaphnia</i>	7-day reproduction (chronic) IC50	122	37	111
Rainbowfish: <i>Melanotaenia</i>	96-hr EC50	521	111	333

7. References

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

8. Appendix: Ecotox Services Report Number PR0706