



Microtox Toxicity Test

Introduction

Microtox is an *in vitro* testing system which utilises the bioluminescent bacteria *Aliivibrio fischeri* to detect toxic substances in different substrates.

Aliivibrio fischeri are non-pathogenic, marine, bacteria that luminesce as a natural part of their metabolism. When exposed to a toxic substance, the respiratory process of the bacteria is disrupted, reducing light output. *Aliivibrio fischeri* have demonstrated high sensitivity across a wide variety of toxic substances. Response to toxicity is observed as a change in luminescence, which is a by-product of cellular respiration. This change can be used to calculate a percent inhibition of *Aliivibrio fischeri* that directly correlates to toxicity. ALS has a range of tools to assess whether chemicals, effluents or wastewaters discharged into water will be toxic to aquatic biota. These tools include sensitive chemical analyses and bioassay based toxicity test- Microtox.

ALS provides NATA accredited analysis of Microtox in waters (ground, surface and potable), wastewaters, recycled water and sediments.

Guidelines

Australian water quality guidelines for fresh and marine water provides guidance on acceptable concentrations of recognised toxicants.

Microtox has emerged as an important screening tool for toxicity assessments for regulatory compliance and to rapidly monitor the health hazards and risks of chemicals in aquatic environment.

METHOD AND LOR INFORMATION

METHOD CODE

MP564

LIMITS OF REPORTING (LOR)

0.1 mg/L

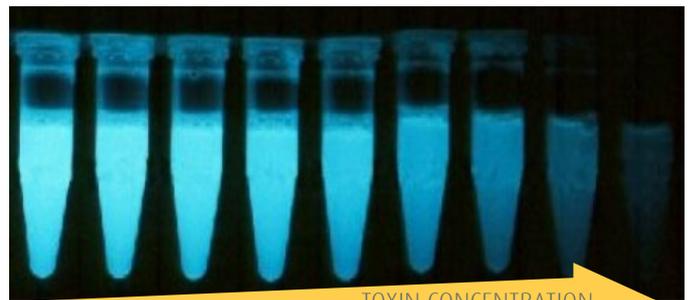
METHOD REFERENCE

ISO 11348-3: 2007

Principle

The Microtox toxicity test exposes *Aliivibrio fischeri* to a sample for analysis. *Aliivibrio fischeri* has the property of emitting part of the energy released in the metabolic reaction (ascorbic acid cycle) as light. Any disruption in this metabolic reaction as a result of the presence of toxic substances will result in a change in the amount of light emitted. The inhibition of light emission correlates with the degree of toxicity.

The reduction in light can be quantitatively determined using a luminescence meter (Microtox Model 500 Analyser). The toxicity of a sample is expressed as the concentration at which 50% reduction in luminescence occurs relative to the blank (EC50).



Water Industry Applications

Microtox can be applied to a variety of matrices including drinking water, storm water run-off, effluents, aqueous extracts from sediments and industrial discharges. A sample pre-treatment step also allows the toxicity analysis of turbid and coloured samples.

A number of water industry applications are:

- » Wastewater treatment plants effluent testing for protection of receiving water and influent testing for protection of activated sludge;
- » Toxicity Reduction Evaluations (TRES) and Toxicity Identification Evaluations (TIEs);
- » Surface water monitoring for identification of point source and non-point source pollution;
- » Monitoring of remediation process;
- » Biocide monitoring of industrial processed waters;
- » Drinking water surveillance for regular monitoring at strategic points.

Right Solutions • Right Partner

Brisbane • Sydney • Melbourne (Springvale) • Perth • Newcastle • Roma • Darwin • Adelaide • Townsville • Mackay • Gladstone • Wollongong Nowra • Mudgee • Chinchilla • Emerald Water Resources Group: Canberra • Bendigo • Geelong • Melbourne (Scoresby) • Wangaratta • Traralgon

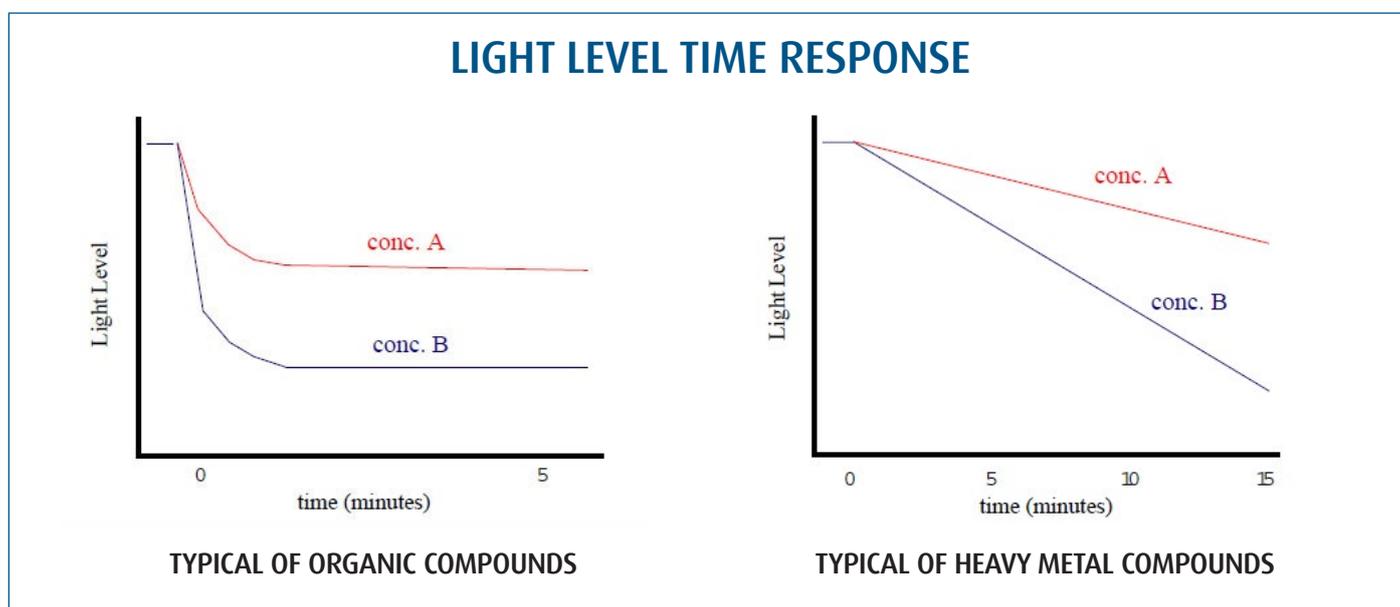
Toxicity Analysis

Microtox is an in vitro test system based on changes in light production of the marine bioluminescent bacterium *Allivibrio fischeri*. Toxicity reduces enzyme activity of this bacteria and thus reduces light output. The Microtox photometer, a temperature controlled analyser, measures the light levels before and after addition of the sample. The reduction in intensity of light emitted from the bacteria is measured along with standard solutions and control samples. The change in light output and concentration of the toxicant produces a dose/response relationship. EC50 - effective concentration at which a 50 % reduction of the light emission was observed after a certain exposure time, is calculated based on dose response curve.

Different chemicals affect living organisms at different rates, reflecting modifications in the mechanism of action and producing distinctly different Light Level-Time Response curves. For some chemicals (like organic compounds) the effect on light output is complete in 5 minutes whereas decay rate of light is slower with heavy metals compounds. Due to different response curves, multiple light readings (5, 15 & 30 minutes) are taken for sample analysis. Different dilutions of sample are tested to determine EC50 values.

This test approach is rapid, sensitive, uses small sample size and has large sample throughput capabilities. Statistically, the Microtox system gives better results because the response of a larger number of organisms, about one million bacteria is observed.

This in vitro bioassay provides an alternate to traditional & complex methods and detects unknown pollutants and effects of mixtures (synergistic effects).



Sampling Requirements

Holding Time	48 hrs for unrefrigerated samples; one week for samples stored at 3°C ± 2°C
Turnaround Time	Standard: 5 days (<24hrs available if required)
Sample Volume	100mL amber bottle
Sample Shipping & Storage	Transport sample on ice or in a refrigeration unit.

For further information or to order sample containers please contact your local ALS client services team.

REFERENCES

- » ISO 11348-3. 2007. Water quality-Determination of inhibitory effect of water samples on the light emission of *Vibrio fischeri*, Part 3 method with freeze dried bacteria.
- » WCMUC 1994. Standard Procedure for Microtox® Analysis, ed. Irene Gaudet, Alberta Environmental Centre

Right Solutions • Right Partner

Brisbane • Sydney • Melbourne (Springvale) • Perth • Newcastle • Roma • Darwin • Adelaide • Townsville • Mackay • Gladstone • Wollongong Nowra • Mudgee • Chinchilla • Emerald Water Resources Group: Canberra • Bendigo • Geelong • Melbourne (Scoresby) • Wangaratta • Traralgon