

## METHOD STATEMENT

### **Determinand:**

Determination of the percentage toxicity factor (%TF) or EC<sub>50</sub>

### **Matrix:**

Sample Type: Waters using the marine bacterium *Vibrio fischeri*

### **Principle of Method:**

The method determines the effect of a sample on the light output of *Vibrio fischeri* under the conditions defined by this method. Reduction in bacterial luminescence in the presence of the sample, compared with the non-toxic control, indicates toxicity.

The EC<sub>50</sub> is the effective concentration of the sample which causes 50% reduction in light output from the test organism in a set period of time (5, 15, 30, 60 and 120 minutes).

The Microtox organism is a specially selected strain of the marine bacterium, *Vibrio fischeri*, which is sensitive to toxic materials. The bacterium emits light, which is detected in a luminometer. Toxic materials that affect the metabolic system of this bacterium reduce the light output. The period of incubation will be 5, 15, 30, 60 and 120 minutes as required.

### **Interferences:**

Turbid samples should be allowed to settle prior to analysis.

To prevent contamination of glassware it must be well washed after use with warm water and detergent.

If the sample pH value is not within the pH range of 6-8 it is likely to affect the result. Neutralisation of the sample to pH 6-8 is carried out using 1M NaOH or 1M H<sub>2</sub>SO<sub>4</sub> is carried out prior to analysis unless the customer specifies otherwise.

Divalent metals may take longer than organic compounds to diffuse into the bacterial cells and affect light loss.

### **Performance of Method:**

#### ***Range of Application:***

The minimum sample dilution is 2.0 (50.0%), with the toxicity factor measured at 0 to 100% when compared with a non-toxic control. The limit of detection will be stated as 5% toxicity factor. If the toxicity is greater than 50% it is usually appropriate to determine the EC<sub>50</sub> for the sample using appropriate sample dilutions

#### ***Limit of Detection:***

The limit of detection will be stated as 5% toxicity factor.

#### ***Uncertainty of measurement:***

The uncertainty of measurement cannot be calculated for this method.

### **References:**

Microtox Manuals, Microbics Corporation, USA, 1995

