

METHOD STATEMENT

Determinand:

Determination of the percentage toxicity factor (%TF) or EC₅₀

Matrix:

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

Principle of Method:

The Microtox test organism is a specially selected strain of the marine bacterium, *Vibrio fischeri*. The bacterium produces bioluminescence or 'light output', under normal conditions, which is detected in a luminometer. Toxic substances that affect the metabolic system of this bacterium reduce the light output over the selected time periods.

The method determines the effect of a sample on the bioluminescence or light output of *Vibrio fischeri* under the conditions defined by this method. Reduction in the bacterial light output in the presence of the sample, compared with the non-toxic control, indicates toxicity and is referred to as the 'toxicity factor' or %TF.

Any significant % TF detected in the sample(s) and above any observed in the controls can indicate potential contamination in the water supply.

The minimum test sample dilution is x2.0 (50.0% sample concentration), with the toxicity factor calculated from the lower limit of detection at 5% up to 100% when compared with a non-toxic control.

Light output readings are taken at 5, 15, 30, 60 and 120 minutes exposure times.

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 H₂SO₄ 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 test sample dilution is x2.0 (50.0% sample concentration), with the toxicity factor calculated from the lower limit of detection at 5% up to 100% when compared with a non-toxic control.

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

