METHOD STATEMENT

Determinand:
Determination of the percentage toxicity factor (%TF) or EC_{50}

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 ouput', 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 and 30 minutes (also 60 and 120 minutes if required) exposure times.

Sampling and Sample Preparation:
When the Model 500 Analyzer is switched on, the red "Temperature Warning Indicator Light" will appear on the front panel, and it will remain on until the incubator wells reach their operating temperature in about 10 minutes. When the green 'ready' light appears, the Model 500 is ready to run tests.
The sample should be received in a sodium thiosulphate dosed bottle to neutralise any chlorine in the sample. If not the sample must be dechlorinated by adding 50ul of 1% w/v sodium thiosulphate to 50ml of sample.
Obtain a sample of reception area cooler drinking water in a 500ml thiosulphate dosed bottle to be used as an uncontaminated drinking water reference control.
Prepare 50ml volumes of each sample(s), and cooler water and osmotically adjust to 2% saline by adding 1.0g of sodium chloride to all respective sample tubes.

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 with 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 Coporation, USA, 1995