

TEST SPECIFIC CHECKLIST

Revised: June 1997

Toxicity Test Using Luminescent Bacteria *Photobacterium phosphoreum* (GM)

Page 1 / 3

Parameter	Specification	Met Specifics		
		Y	N	NA
<u>Sample Preparation</u>				
Pre-Aeration.	Normally none required however if D.O. < 40% or > 100% saturation, aeration of all test solutions for ≤ 20 min is optional.
pH Adjustment.	No adjustment if pH of sample is within range of 6.0 - 8.5; adjustment optional outside that range.
Colour/Solids/ Floatables.	Use colour-correction technique for high colour and/or dark solids; remove light-coloured solids; for floatables, test underlying liquid.	Y
Salinity Adjustment.	Sample can be salinity adjust to 2 % with MOAS (1 part MOAS/10 parts sample) if conc. of > 45% are required.
<u>Test Conditions</u>				
Test Species.	<i>Photobacterium phosphoreum</i> , strain NRRL B-11177.
Test Type.	Static.
Test Duration.	5 and 15 min, to 30 or 60 min if necessary.
Test T°.	15 ± 0.3°C.
pH Range.	6.0 - 8.5.
D.O. Range.	Not specified.
Aeration.	None.
Vessel Size & Type. Solution Volume.	Disposable glass incubation cuvettes (12 x 50 mm). 1 mL.
Dilution Water (Diluent).	Equivalent to 2% NaCl in purified water; pH 6.0 - 8.5; sucrose osmotic adjustment method used for increased sensitivity to ammonia and some metals; may be stored for ≤ 1 year at 2 - 8°C. Be kept from freezing (Must GM).
Reconstitution Solution.	Distilled water, free of toxic material; may be stored for ≤ 1 year at 2 - 8°C. Be kept from freezing (Must GM).
Microtox Osmotic Adjustment Solution.	22% solution of NaCl; may be stored for ≤ 1 year at 2 - 8°C. Be kept from freezing (Must GM).
Microtox Reagent & Bacterial Reagent.	Can be stored in freezer (-20°C) for at least 1 year; should be used within 2 h of reconstitution.
# Conc.	≥ 4 plus a control to calculate IC50; 6 conc. plus a control is recommended; a sample conc. of 45% is the highest obtainable by means of basic technique; lower conc. in two-fold dilutions.
# Replicates/Conc.	Only 1 replicate per test conc. required; recommend 2 replicates for control.
# Organisms/Vessel.	10 µL Reconstitution Reagent (~1 million bacteria).
Photometers.	Variety of photometers can be used; Microbics Toxicity Analyzers Models 2055 and 500 are recommended.
Vessel Cleaning.	None, cuvettes are disposable.
Endpoint.	Conc. of sample estimated to cause 50 % inhibition of light production by bacteria (IC50, 95% confidence limits) after 5, 15, or 30 min.

This checklist is a summary of the requirements and recommendations in the Environment Canada test method. As a summary, it will not contain all supplementary information. If there is a discrepancy between the checklist and the Environment Canada test method, the test method is taken as the definitive source.

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Page 2 / 3

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<u>Observations & Measurements</u>				
Time-Zero Light Reading.	Measured 15 min after Bacterial Reagent added to Diluent and before sample is added (allowing light production to reach relatively steady state) (Must GM) . Light production at time zero should be in the range of 80 - 100.
Subsequent Light Readings.	Each cuvette should be read exactly 5 min and 15 min after solution mixed; readings at 30 min and 60 min is frequently desirable to determine if light emission is still decreasing appreciably (> 10% change since previous reading).
<u>Test Organisms</u>				
Source.	Microbics.
Age.	Exponential phase of growth; lyophilized. Used within 2 h of reconstitution (Must GM)
Lot #.	Traceable to known source.
<u>QA/QC</u>				
Acceptability Criterion.	Valid numerical estimate of ICp based on conc. showing light inhibition both greater than and less than the inhibition at the ICp.
Reference Toxicant Data.	Within acceptable limits; conducted for every new batch of test organisms and at least once per month.
Controls.	Run in duplicate; consists of Diluent plus Reconstituted Reagent (bacteria)..
Sample Holding Time.	Test to be initiated within 72 h after sampling (liquid) or 2 - 6 w after sampling (solid) (Must GM) Recommend test initiation within 24 h after sampling.
Sample Volume.	500 mL - 1 L.
<u>Test Report</u>				
Sample Data.	Type of sample. Sampling location. Sampling method. Nature, appearance and other properties. Volume and/or weight. Information on labelling or coding of the test substance. Transport and storage conditions (time, T°). Person providing/collecting sample. Date and time of sample collection. Any physico-chemical measurements on sample.
Test Organisms.	Batch or lot # of organisms used. Date obtained and T° of holding.
Test Facilities & Apparatus.	Name and address of testing laboratory. Person(s) performing the test. Model # of Analyzer and description of any non-standard items of apparatus..

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Page 3 / 3

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Test Methods & Conditions.....	Date and time for start of definitive tests.
	Brief description of method, if standard.
	D.O. of sample before and after initial aeration, if any.
	Aeration of solutions before test (rate, duration, manner of application).
	Chemical analyses of stock or test solution and analytical procedures used..
	Procedures used in preparing stock and/or test solutions of chemicals.
	Details of any salinity adjustment of samples..
	Sampling and storage details (if clean seawater was used for dilution of samples)..
	Conc. tested and # of cuvettes at each conc..
	Appearance of test solutions and any changes during test.
	T° as monitored in the incubation section of the <i>Analyzer</i>
	pH of sample and description of adjustment.
	Observation times during test..
	Test Results.	Results for range-finding test (if conducted).
Code used for identifying chart recording, if used.
Time-zero readings of light emission.
% light inhibition in each test solution (including control) at each observation time..
Results of "colour-correction" test (loss of light transmission caused by colour and other attributes of sample)..
Any IC50s (and 95% confidence limits), with method of calculation and values for important intermediate variables..
IC50 and 95% confidence limits for the reference toxicant(s).
Chemicals (reference toxicant); date test initiated (must be within one month of the test, or when a new batch of Bacterial Reagent was first used), historic geometric mean IC50 and warning limits (± 2 SD)..

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