TEST SPECIFIC CHECKLIST

Revised: June 1997

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

Parameter	Specification	Met Specifics Y	N	NA
Sample Preparation				
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.	☐	☐	☐
Salinity Adjustment	Sample can be salinity adjust to 2 % with MOAS (1 part MOAS/10 parts sample) if conc. of > 45% are required.	☐	☐	☐
Test Conditions				
Test Species	*Photobacterium phosphoreum*, 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	Disposable glass incubation cuvettes (12 x 50 mm).	☐	☐	☐
Solution Volume	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.	☐	☐	☐

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Observations & Measurements				
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).	☐	☐	☐
Test Organisms				
Source.	Microbics.	☐	☐	☐
Age.	Exponential phase of growth; lyophilized.	☐	☐	☐
	Used within 2 h of reconstitution **(Must GM)**.	☐	☐	☐
Lot #.	Traceable to known source.	☐	☐	☐
QA/QC				
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.	☐	☐	☐
Test Report				
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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Parameter	Specification	Met Specifics Y	N	NA
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 *Analyzer*	☐	☐	☐
	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)	☐	☐	☐