PMTurkeyCOLNPEm Resource

From: Labiosa, Rochelle G [Rochelle.Labiosa@pnl.gov]
Sent: Wednesday, October 27, 2010 2:23 PM
To: Gary Rand
Cc: Kugler, Andrew; Masnik, Michael; Haque, Mohammad; Vail, Lance W; Bryce, Robert W; Ward, Jeffrey A; Thorne, Paul D
Subject: RE: wastewater aquatic toxicity test plan
Attachments: WASD_Tox_Test_Matrix-2[1].docx

Thanks so much, Dr. Rand. It was great talking to you yesterday. We are writing up our notes and will send them to you soon to get your comments and edits.

Thanks again,
Rochelle

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From: Gary Rand [mailto:randg@fiu.edu]
Sent: Tuesday, October 26, 2010 5:03 PM
To: Labiosa, Rochelle G
Subject: wastewater aquatic toxicity test plan

Attached is a summary table of the toxicity tests for the MD-WASD. This was forwarded to stakeholders today.
GM Rand

Hearing Identifier: TurkeyPoint_COL_NonPublic
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Subject: RE: wastewater aquatic toxicity test plan
Sent Date: 10/27/2010 2:22:30 PM
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From: Labiosa, Rochelle G

Created By: Rochelle.Labiosa@pnl.gov

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Aquatic Toxicity Testing Plan (Addendum): GM Rand, Florida International University

Table: Acute and chronic aquatic laboratory toxicity testing matrix with fresh- and salt-water organisms in water and sediment for the treatment trains.

The table below is a summary of the aquatic toxicity tests in fresh- and salt-water to be conducted by Florida International University for Miami-Dade County WASD as part of the Coastal Wetlands Wastewater Rehydration Project. All tests will follow standardized U.S.EPA methodology or a modification based on use of native species. Because it is anticipated that there will be three treatment trains, all test organisms at the beginning will first be exposed to 100% strength water -no dilution- of each of the treatment trains. At 100% strength, if exposed organisms elicit mortality at > 10% of controls the Phase I-Acute Testing will be initiated. At 100% strength, if exposed organisms elicit mortality at ≤ 10% of controls the Phase I-Short-Term Chronic Testing will be initiated. To-date this is the most comprehensive aquatic toxicity program to assess potential impact of pharmaceuticals and personal care products in reuse water since it will include: standard as well native species from different trophic groups in the water column and sediment (epibenthic, infaunal), acute and chronic toxicity assessment, evaluating typical regulatory biological endpoints (mortality, growth, development and reproduction), and endocrine specific endpoints.

Table. Acute and chronic aquatic laboratory toxicity testing matrix[1] with fresh- and salt-water organisms in water and sediment for the treatment trains.

Phase- Exposure Type[2]	Trophic Test Group[3]	Test Conditions					Biological Endpoints	Exposure Duration
		Exposure System[4]	No. organisms /chamber	No. reps./concentration	No. test concentrations	Sample volume treatment train water		
PHASE I **Water**								
Acute	Bacteria (Microtox®)	S	—	2	5	1L	EC50[6] (Light Inhibition)	5-30 min
	Invertebrate (4)[5]	SR	5-10	2-4	5	20L	LC50[6](Mortality)	96 h
	Fish (4)	SR	10	2-4	5	90L	LC50 (Mortality)	96 h
	Phytoplankton (4)	S	~10^4 cells/ml	4	5	20L	EC50 (Growth; Cell Counts, Chlorophyll Fluorescence, Absorbance, or Biomass)	96 h
	Vascular Plants[7](4)	SR	10-20	2-4	5	100L	IC50[6], EC50 (Growth, Sexual Reproduction)	1-2 wk

	Frog Embryo Assay[8] (1)	SR or FT	25	2	5	5L	LC50 (Embryo death), EC50 (malformations)	96 h
Short-Term Chronic	Invertebrate (4)	SR	1	10	5	175L	LC50 and EC50; NOECs[6] & LOECs[6] (Mortality, Growth, Fecundity)	7 d
	Fish (4)	SR	10	4	5	350L	LC50 and EC50; NOECs & LOECs (Mortality, Growth)	7 d
Sediment Acute	Benthic Invertebrates (4)	SR	10	8	5	20L	LC50 and EC50 (Mortality, Growth)	10 d
PHASE II **Water** Full Chronic Invert. and Partial (e.g., fish ELS) Chronic Fish	Invertebrate (4)	FT	10	4	5	4000L	LC50 and EC50; NOECs & LOECs (Mortality, Growth,	28 d

							Reproduction)	
	Fish ELS[9] (4)	FT	20	3	5	4000L	LC50 and EC50; NOECs & LOECs (Embryo/fry Mortality, Growth, Development)	30-90 d
Fish Screening & Amphibian Metamorphosis Assays	Fish Screening (or Short-term Chronic Repro.) (2) [10]	FT	4 female/2 male	4	3	4000L	Fecundity, Secondary Sex Characteristics, VTG[10], GSI[10]	21-35 d
	Frog (1)[11]	SR or FT	20	4	3	600L	Growth, Development (malformations), Thyroid Histology	21 d
Special Acute								
Sea Urchin Reproduction [12]	Echinoderm (1)	SR	2000 eggs/5million sperm	4	5	1-2L	EC50 (Egg fertilization)	96 h
` Bacterial Inhibition[13]	Bacteria (2)[13]	S	$\geq 10^3$ to 10^8 cells/ml	5-8	5	1L	Biomass, Growth Rates, MIC[6], IC50[6]	1 d
Biofilms[14]	Algae, Bacteria,	S	$\geq 10^3$ to 10^8	3-4	5	2-4L	Community	1 wk-4

	and Cyanobacteria (2)		cells/ml				Composition/ Structure, Biomass, Carbon Utilization	wk
PHASE III								
Fish Full Chronic	Fish (2)[15]	FT	20	3-4	5	50,000-60,000L	F_0 generation: Survival of embryos, time required to hatch, hatching success, total number of larvae, including dead and deformed, length and weight of fry, spawning and fecundity data; F_1 generation: Survival of embryos, time required to hatch, hatching success, total number of larvae, including dead and deformed, length and weight of fry	6-9 mo

Laboratory-Microcosm [16]	Phytoplankton, Zooplankton, Macro-invertebrates, Fish, Vascular Plants	SR or FT	-------	3-4	5	18,000-22,000L	Population dynamics, primary productivity	~1-12 wk

[1]All toxicity tests will be conducted according to standard U.S.EPA protocols. When mortality is > 10% of controls in test organisms in tests after exposure to 100% strength water (i.e., to any of the three treatment trains), the acute toxicity (in Phase I) test matrix will be initiated for that train in this table followed by long-term testing (Phase II). However, when mortality is ≤ 10% of controls after exposure to 100% strength water, the short-term chronic toxicity test matrix (in Phase I) will be initiated. Chemical analyses of substances for: acute tests (96h) and short-term chronics (7d) will be at the beginning and at the end of the test, for chronics (3 wks or >) will be at beginning, weekly and at the end of the test.

[2] Most aquatic toxicity tests will be conducted in static-renewal (i.e., of water) exposure systems in water-only (or sediment-water) tests and include 5 treatment concentrations ([0.5 dilution series]; at 100%, 50%, 25%, 12.5%, and 6.25% treated water with the remainder as diluent water (i.e., fresh-or salt-water making up the remainder depending on test). Plus an untreated control. Exposure type indicates either: acute, short-term chronic, or full-chronic time period exposures with constant exposure concentrations with static-renewal exposure systems.

[3] Tests will be conducted with freshwater and saltwater organisms from each trophic group.

[4]System indicates static-renewal (SR) or flow-through (FT) exposures.

[5]Indicates the number of different species used for each of the battery of fresh- and salt-water tests.

[6]EC50: median effective concentration; concentration in water that is estimated to be effective in producing some response (other than mortality) in 50% of the test organisms; LC50: median lethal concentration; concentration in water that is estimated to be effective in producing some mortality in 50% of the test organisms; IC50: median inhibitory concentration; concentration that would cause a given percentage reduction (50%) in a nonlethal biological measurement of the test organisms such as growth or reproduction; NOEC: no observed effect concentration; highest concentration of a test material that has no statistically significant adverse effect on the exposed

population of test organisms compared to control; LOEC: lowest observed effect concentration; lowest concentration of a test material that has a statistically significant adverse effect on the exposed population of test organisms compared to control; MIC: minimum inhibitory concentration; IC50: that concentration of the test substance giving 50% inhibition of the endpoint being measured.

[7]Both floating and rooted vascular plants will be considered.

[8]Frog embryo assay (FETAX) will be conducted with 1 freshwater species. Conducted mainly to assess developmental malformations.

[9]ELS (early life stage)

[10]To identify endocrine active substances in sexually dimorphic fish to detect substances that affect estrogenic and/or androgenic activities. The assay measures several core biological endpoints as indicators and reflective of the reproductive endocrine system including secondary sexual characteristics, plasma concentrations of vitellogenin (VTG) and the gonadal-somatic index (GSI). One fresh- and one salt-water fish will be used for this test.

[11]The amphibian metamorphosis test will be conducted with one freshwater frog species to determine if substances are present which may interfere with the normal functioning of the hypothalamo-pituitary-thyroid axis.

[12]This test is primarily for substances that are particularly toxic to sperm. It is rapid and requires small test volumes. It is a widely-used standardized marine toxicity test.

[13]Bacterial inhibition studies to assess the toxicity of any substances to bacteria using growth rate as an endpoint. One fresh- and one salt-water study will be conducted.

[14]Microorganisms, particularly those growing as biofilms are important in terms of fundamental processes such as biogeochemical cycling and biodegradation activity and are also an important source of food. Microbial communities are good indicators of changes in aquatic ecosystems. This test could be used to take the place of bacterial inhibition since it includes this group.

[15] One fresh- and one salt-water fish full chronic study will be conducted.

[16]One laboratory microcosm study in fresh-and salt-water will be conducted.