1075974 - R8 SDMS

Title: Toxicity o f Libby Asbestos Superfund Site (OU3) Water to Rainbow Trout (*Oncorhynchus mykiss*) Under Static-Renewal Test Conditions

Testing Facility: Parametrix Environmental Research Laboratory (PERL)
33972 Texas Street SW
Albany, OR 97321

Study Sponsor: Remedium Group, Inc.
6401 Poplar Ave., Suite 301
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Project Officer: Robert R. Marriam

Study Director: Eric Van Genderen

Proposed Experimental Start Date: April 2008

Proposed Experimental Termination Date: July 2008

1.0 INTRODUCTION

1.1 Objective

To determine the early life-stage toxicity of Libby Asbestos Superfund Site (OU3) water to *Oncorhynchus mykiss* under static-renewal test conditions.

1.2 Experimental Approach

O. mykiss will be continuously exposed to Libby water from newly hatched sac-fry through 42 d following test initiation.

1.3 Test Substance

All water for testing will be received as one batch and combined into a composite vessel for mixing and use throughout testing. Water will be supplied by Study Sponsor from a selected location at the site.

2.0 BASIS AND TEST SYSTEM

2.1 Basis

This protocol is designed to comply with Section 7 of the Phase IIA Sampling and Analysis Plan for the Libby Asbestos Superfund Site: Operable Unit 3. Specific methods follow those reported by Belanger et al. (1986) and Belanger (1985), and general testing methods based on ASTM (2002) test guidelines.

2.2 Test Organism

1. Species – *O. mykiss*
2. Number - A total of 45 organisms will be tested in three replicates for each treatment and control.
3. Age – *O. mykiss* will start as freshly hatched sac-fry. All sac-fry used for testing will be hatched within a 24 h period and no more than 48 h post-hatch upon test initiation.
4. Source – *O. mykiss* will be obtained from the Sinnhuber Aquatic Research Laboratory (SARL; Corvallis, OR) or Troutlodge (Sumner, WA).
5. Culture/Holding Water – Prior to the start of testing, *O. mykiss* will be maintained in a continuous flow-through system with natural water specific to the hatchery from which eggs are obtained. Upon arrival at PERL, eyed eggs will be maintained in hatching trays under flow-through conditions with well water. Eggs will be slowly acclimated to PERL water temperature and water conditions upon receipt.
6. Feeding – Post swim-up, each chamber will be fed freshly-hatched brine shrimp daily at a rate of 0.05 grams of brine shrimp per gram of fish. Mass of fish at

swim-up will be estimated using a surrogate batch of fish that have been maintained in similar conditions as fish used in the study. Food should be offered to all treatments once fish begin to swim-up to determine each chamber's readiness to eat. During testing, the appropriate amount of food offered to each chamber will be determined relative to the amount of uneaten food present in each chamber from the previous feeding. Fish will not be fed during the last 24 hours of testing.

7. Procedure for identification: *O. mykiss* are routinely verified to species by a certified laboratory.
8. Justification: Use of this test system is in compliance with ASTM testing guidelines.

2.3 Test Diet

Brine shrimp nauplii will be prepared using cysts (Brine Shrimp Direct, Ogden, UT) and reconstituted sea water to produce a slurry for feeding. A sample of brine shrimp is routinely analyzed for total metals, organochlorine pesticides, and PCBs per PERL Standard Operation Procedures (SOPs).

3.0 EXPOSURE SYSTEM

3.1 Route of Administration

Method: Mixtures of Libby water will be directly delivered to the test chambers.

Equipment: Mixtures of toxicant and dilution water will be mixed using a geometric dilution series and delivered to exposure chambers (2.5-gal glass aquaria submerged in a temperature-controlled water bath). Water within chambers will be continuously recirculated to provide sufficient mixing and aeration. All construction and materials in contact with test water will be glass, stainless steel, or plastic.

Frequency: Control and treatment solutions will be prepared on a specified interval (every ten days during sac-fry exposure and every three days following swim-up) and delivered directly to exposure chambers. Prior to renewal, approximately 80% of the solution volume within each chamber will be siphoned (removing waste material and any other debris).

3.2 Dilution Water

Dilutions of the site water shall be prepared using laboratory water that is prepared to have hardness and alkalinity (pH) that are matched to the site water selected for toxicity testing. Dilution water will be water prepared as reconstituted laboratory water from reagent grade chemicals ($CaSO_4 \cdot 2H_2O$, $MgSO_4$, KCl, and $NaHCO_3$). In addition, the Ca/Mg ratio will be

matched to the conditions of the site water. New batches of dilution water will be prepared as needed or after 14 days from preparation date following PERL SOPs.

3.3 Test Temperature

Test temperature will be 12 ± 2°C. During testing, temperature will be maintained within 1 °C. Testing will be conducted in a temperature-controlled water bath.

3.4 Test Containers

Test containers will be 2.5-gal rectangular glass chambers. Test chambers will contain approximately 4-L (1-gal) of test media.

3.5 Photoperiod

The photoperiod will be 16-hours light and 8-hours dark.

3.6 Dissolved Oxygen Concentrations

Dissolved oxygen concentrations will be maintained at ≥ 60 percent of saturation. If the dissolved oxygen concentration in test solutions approaches this level, all test solutions will be aerated for the duration of the test.

4.0 TEST DESIGN

4.1 Test Concentrations/Dosages

Six test treatments and a dilution water control will be tested using a geometric dilution scheme. The nominal test treatments will be determined based on measured asbestos concentrations and target to cover a range from the initial (undiluted) concentration down to about 0.001 million fibers per liter. Selection of test treatments is based on literature data for similar exposures (Belanger, 1985).

4.2 Number of Test Organisms

A total of 45 organisms will be tested in three replicates for each treatment and control.

4.3 Bias Control

To control bias, test chambers will be numbered according to a 3 X 7 randomization sheet and placed into the water bath. Test chambers will also be labeled with the test treatment and replicate number for identification during renewal.

4.4 Test Initiation

Sac-fry will be distributed to plastic cups and then randomly placed in numerical order (based on randomization sheet) into each test chamber to initiate testing.

4.5 Chemical and Physical Monitoring

At a minimum, the following measurements will be made according to the methods laid out in PERL SOPs:

1. Hardness, alkalinity, dissolved oxygen, temperature, conductivity, total ammonia, total residual chlorine, pH, calcium, magnesium, sodium, potassium, chloride, sulfate, and dissolved organic carbon will be measured in the control/dilution water at test initiation.
2. Dissolved oxygen, temperature, conductivity, and pH will be measured daily in each treatment.
3. Hardness and alkalinity will be measured weekly in each treatment.

4.6 Biological Monitoring

Mortalities observations will be recorded twice daily and dead fish will be removed immediately. Behavioral effects (frequency and duration of swimming events of the larvae, swimming and feeding behavior of the fry, etc.) will be noted daily on the data package, but fish should not be removed until they are considered dead. During the study, dead fish will be removed and preserved for histopathological examination. At the end of the study, all fish will be measured for length, wet weight (blot dried), and preserved for histopathological examination.

4.7 Analytical Chemistry

For each round of static renewal, one composite sample of each dilution will be prepared by withdrawing and combining 33 mL from each of the three replicate test aquaria for each dilution. One composite per dilution will be collected shortly after the start of each renewal cycle, and one composite shall be collected at the end of the cycle shortly before performing the renewal. For convenience, the samples will be identified by the dilution level and the static renewal cycle number and the designation "New" to indicate the sample taken at the start of the cycle and "Old" to designate the sample taken near the end of the cycle. These samples will be placed into 100 mL plastic bottles and stored in the dark at 4°C at the toxicity testing laboratory. EPA will designate samples that will be sent from the toxicity testing laboratory for analysis. Initially, the samples that will be sent for analysis of LA are as follows:

Cycle 1, Dilutions 1 to 7, new and old (N = 14)
Cycle 7, Dilutions 1 to 7, new and old (N = 14)

From Section 7 of the Phase IIA Sampling and Analysis Plan:

Aliquots of the site water will be removed from the mixing vessel and sent for analysis of the following (sample labels will be provided by EPA):

Libby amphibole asbestos (three 100 mL HDPE bottles) will be sent to:

EMSL Analytical
107 West 4th Street
Libby, MT 59923

Metals and metalloids (two 8-oz glass bottles) will be sent to:

Energy Laboratories
1120 South 27th Street
Billings, MT 59107-0916

4.8 Test Duration

The test duration will be 42 days.

4.9 Quality Criterion

- The test will not be considered valid if control mortality exceeds 20%.
- The dissolved oxygen concentration must be > 60 percent saturation.
- There must be evidence that the temperature, dissolved oxygen, and concentration of the test substance being tested has been satisfactorily maintained, based on time-weighted averages, over the test period.
- The instantaneous loading within each test chamber should not exceed 10 g organism/liter.

5.0 DATA ANALYSIS

All hypothesis testing and point estimation will be conducted using average asbestos concentrations measured in each treatment. Statistical analysis (hypothesis testing) of the test data will be conducted using a computer program. A statistical test (as determined by the USEPA Decision Tree [USEPA, 2002]) will be used to test for significant differences in the survival, growth and behavior among test treatments and controls. The no observable effect concentration (NOEC) and lowest observable effect concentration (LOEC) will be calculated on the basis of survival, growth and behavior ($p < 0.05$). In addition, a median lethal concentration (LC50) and growth inhibition concentrations or behavioral effect concentrations (e.g., EC10 and EC20) will be calculated along with the determination of outliers and the need for data transformation (i.e., arc sine, square root, logarithmic, etc.).

6.0 TEST REPORT

The report will be a typed document describing the results of the test and will be signed by the Study Director, Quality Assurance Unit, and Study Sponsor. The report will include, but not be limited to, the following:

- Name and address of the test facility;
- Dates of test initiation, completion, and/or termination;
- Objectives of the study as stated in the test protocol, including any changes from the protocol;
- Statistical methods used in data analysis;
- Identification of the test, control, and reference substances (by name, CAS number, or code number) and description of substance purity, strength, composition, stability, solubility, and/or other appropriate characteristics documented by the Study Sponsor (location of documentation shall be specified);
- A description of the methods used during testing;
- A description of the test system used including, where applicable, number of animals used, sex, body weight range, source of supply, species, strain, substrain, age, and procedure for identification;
- A description of the dosage, dosage regimen, route of administration, and duration;
- All analytical results concerning test treatments and water quality;
- A description of all circumstances that may have affected the quality or integrity of the data;
- The name of the Study Director and the names of other scientists, professionals, or supervisory personnel (e.g., task manager, senior biomonitoring technician) involved in the study;
- A description of the methods of data analysis; a summary and analysis of the data, and a statement of the conclusions drawn from the analysis;
- Signature and date of the Study Director and/or other professionals involved in the study as required by the testing facility or Sponsor;
- The location (s) where all specimens, raw data, and final report are to be stored;
- A statement of Quality Assurance

7.0 RECORD RETENTION

All records will be maintained and archived at PERL in accordance with appropriate SOP.

8.0 PROTOCOL AMENDMENTS AND DEVIATIONS

All changes (i.e., amendments, deviations, and final report revisions) of the approved protocol, plus the reasons for the changes, must be documented in writing. The changes will be signed

and dated by the Study Director and maintained with the protocol. All protocol amendments must be authorized in advance by the Sponsor.

9.0 LITERATURE CITED

ASTM. 2002. Standard Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians. E729-96 (2002). In: 2002 ASTM Annual Book of Standards, Volume 11.05.

Belanger SE. 1985. Functional and pathological responses of selected aquatic organisms to chrysotile asbestos. Dissertation; Virginia Polytechnic Institute and State University.

Belanger SE, Schurr K, Allen DJ, Gohara AF. 1986. Effects of chrysotile asbestos on coho salmon and green sunfish: Evidence for behavioral and pathological stress. Environmental Research 39:74-85.

USEPA. 2002. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms. Fourth Edition. EPA-821-R-02-013.

10.0 SPONSOR AND STUDY DIRECTOR APPROVAL

Sponsor Approval: _____ Date: _____

Study Director: _____ Date: 21 April 2008

and dated by the Study Director and maintained with the protocol. All protocol amendments must be authorized in advance by the Sponsor.

9.0 LITERATURE CITED

ASTM. 2002. Standard Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians. E729-96 (2002). In: 2002 ASTM Annual Book of Standards, Volume 11.05.

Belanger SE. 1985. Functional and pathological responses of selected aquatic organisms to chrysotile asbestos. Dissertation; Virginia Polytechnic Institute and State University.

Belanger SE, Schurr K, Allen DJ, Gohara AF. 1986. Effects of chrysotile asbestos on coho salmon and green sunfish: Evidence for behavioral and pathological stress. Environmental Research 39:74-85.

USEPA. 2002. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms. Fourth Edition. EPA-821-R-02-013.

10.0 SPONSOR AND STUDY DIRECTOR APPROVAL

Sponsor Approval: _____ Date: 4/25/08

Study Director: _____ Date: 21 April 2008

EPA Approval: _____ 4/24/08

SUBJECT: MORTALITY LOG FOR RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) EMBRYOS AND FRY

Project Number:

Treatment:

Day	Replicate 1 (Obs 1 / Obs 2)			Replicate 2 (Obs 1 / Obs 2)			Replicate 3 (Obs 1 / Obs 2)		
	# Alive	# Dead	Notes	# Alive	# Dead	Notes	# Alive	# Dead	Notes
1	/	/		/	/		/	/	
2	/	/		/	/		/	/	
3	/	/		/	/		/	/	
4	/	/		/	/		/	/	
5	/	/		/	/		/	/	
6	/	/		/	/		/	/	
7	/	/		/	/		/	/	
8	/	/		/	/		/	/	
9	/	/		/	/		/	/	
10	/	/		/	/		/	/	
11	/	/		/	/		/	/	
12	/	/		/	/		/	/	
13	/	/		/	/		/	/	
14	/	/		/	/		/	/	
15	/	/		/	/		/	/	
16	/	/		/	/		/	/	
17	/	/		/	/		/	/	
18	/	/		/	/		/	/	
19	/	/		/	/		/	/	
20	/	/		/	/		/	/	
21	/	/		/	/		/	/	
22	/	/		/	/		/	/	

SUBJECT: MORTALITY LOG FOR RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) EMBRYOS AND FRY (CONT.)

Project Number:									
Treatment:									
Day	Replicate 1 (Obs 1 / Obs 2)			Replicate 2 (Obs 1 / Obs 2)			Replicate 3 (Obs 1 / Obs 2)		
	# Alive	# Dead	Notes	# Alive	# Dead	Notes	# Alive	# Dead	Notes
23	/	/		/	/		/	/	
24	/	/		/	/		/	/	
25	/	/		/	/		/	/	
26	/	/		/	/		/	/	
27	/	/		/	/		/	/	
28	/	/		/	/		/	/	
29	/	/		/	/		/	/	
30	/	/		/	/		/	/	
31	/	/		/	/		/	/	
32	/	/		/	/		/	/	
33	/	/		/	/		/	/	
34	/	/		/	/		/	/	
35	/	/		/	/		/	/	
36	/	/		/	/		/	/	
37	/	/		/	/		/	/	
38	/	/		/	/		/	/	
39	/	/		/	/		/	/	
40	/	/		/	/		/	/	
41	/	/		/	/		/	/	
42	/	/		/	/		/	/	

SUBJECT: BEHAVIOR LOG FOR FISH

Project Number:	
Treatment:	Replicate:
Day	Observation
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
11	
12	
13	
14	
15	
16	
17	
18	
19	
20	
21	
22	

SUBJECT: BEHAVIOR LOG FOR FISH (CONT.)

Project Number:	
Treatment:	Replicate:
Day	Observation
23	
24	
25	
26	
27	
28	
29	
30	
31	
32	
33	
34	
35	
36	
37	
38	
39	
40	
41	
42	

SUBJECT: GROWTH LOG FOR RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) EMBRYOS AND FRY

Project Number:

Treatment:

Fish	Replicate 1			Replicate 2			Replicate 3		
	Day of Death	Length (mm)	Wet Weight (mg)	Day of Death	Length (mm)	Wet Weight (mg)	Day of Death	Length (mm)	Wet Weight (mg)
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14									
15									

SUBJECT: DAILY TOXICITY TEST LOG

Project Number:			
Test Species (Circle): *C. dubia* *D. magna* *D. pulex* *P. promelas* *O. mykiss* Other (Specify):			
General Comments		Feeding	Initials/Date
Test Day 0	Test Solution Mixed at: Test Organisms Added at:		
Test Day	Biological observations made at:		
Test Day	Biological observations made at:		
Test Day	Biological observations made at:		
Test Day	Biological observations made at:		
Test Day	Biological observations made at:		
Test Day	Biological observations made at:		
Test Day	Biological observations made at:		
Test Day	Biological observations made at:		