TRAC

ACUTE TOXICITY OF ALKYLBENZENE SULFONATE TO *HYALELLA AZTECA* IN SEDIMENT: EFFECT OF MANIPULATION OF SEDIMENT ORGANIC CONTENT WITH HUMIC ACID

Toxicity Test Report

Submitted to:

Dr. Richard Sedlack
The Soap and Detergent Association
475 Park Ave. South
New York, NY 10016

Report Number B-393
October 1, 1993

TRAC Laboratories, Inc.
113 Cedar St.
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1.0 INTRODUCTION

The objective of this study was to examine the behavior of a test system in relation to the addition of organic carbon. The test system consisted of a test organism (the amphipod *Hyallela azteca*) exposed for 48 h to the control substance (reference sediment) spiked with the test substance (alkyl benzene sulfonate). The organic carbon of the test system was manipulated by adding humic acid (Aldrich Technical grade, sodium salt).

All biological and analytical testing was conducted at TRAC Laboratories, Inc. 113 Cedar St., Denton, TX 76201.

2.0 MATERIALS AND METHODS

Documentation of sediment toxicity test procedures was per Good Laboratory Practices, 40CFR Part 792, August, 1989. This documentation is provided as part of the raw data (Appendix A).

2.1 Organisms

Organisms used in the tests were *Hyallela azteca* obtained from in-house cultures (Appendix B). Organisms used in the tests were 14 -20 d of age.

2.2 Reference Sediment

Reference sediment for the tests consisted of sandy loam topsoil obtained from a commercial supplier. The soil was sifted through a $1mm^2$ mesh sieve. Before being used in toxicity tests, the soil was tested for suitability as a reference sediment by exposing test organisms to it in a 10 d chronic growth and survival test.

2.3 Humic Acid and Reference Sediment

Procedures for preparing the humic acid/ABS mixtures in the reference sediment are provided in Appendix C. The toxicity tests were begun on 8/17/93 and ended on 8/19/93.

3.0 RESULTS AND DISCUSSION

3.1 Toxicity Tests

Results of toxicity tests are provided in Table 3.1. The toxicity test on reference sediment without humic acid showed acceptable control performance and a clear monotonic dose response. Tests in which humic acid was added to the reference

sediment did not show adequate control survival. Mortality in the controls of these tests was related to the amount of humic acid added, with nearly complete mortality occurring in the highest (1.2%) humic acid addition.

3.2 Analytical Testing

No interstitial water (IW) could be collected from the treatments containing humic acid. The IW samplers used in previous testing and specified in the experimental protocol were not effective in collecting IW. Sediment was collected from the test vessels and centrifuged at 3000 rpm for up to 1 h, but no clear separation of IW and sediment occurred. Sediment was pressure filtered (up to 80 psi) using large (245 mm diameter) filters of up to 5μ pore size, but no IW could be collected. Due to the poor organism performance in the treatments containing humic acid, and the difficulty in collecting IW samples from those treatments, further analytical testing of OW and sediment was not conducted.

Table 3.1. Results of toxicity tests on reference sediment/humic acid mixtures spiked with alkyl benzene sulfonate. Values are % survival (n = 30) after 48 h.

Exposure (mg/Kg ABS)	Treatment (% Humic Acid)			
	0	0.6	1.2	3.0
Control	100	70	30	3
250	100	53	33	0
500	77	87	27	0
1000	10	37	20	0
2000	7	17	20	0
4000	0	0	3	0

REPORT PREPARED BY: Patrick Downey

Patrick S Downey Oct 4, 1993

Study Director Date

Statement of Quality Assurance

This study was reviewed by the Quality Assurance Officer to insure the methods, standard operating procedures, and protocol used in the performance of this study were followed. The final report is an accurate reflection of raw data.

BIOASSAY SECTION

Raw Data audit: BV

Final report audit: BV

B L Venables 10/5/93

Barney Venables, Ph.D. Date
Quality Assurance Officer

B-393

HYALELLA AZTECA
ACUTE SEDIMENT TEST SURVIVAL DATA

TRAC ID TB-422 Sponsor: SOAP AND DETERGENT ASSN.

Test Substance: Alkylbenzene sulfonate

Reference sediment w/ No humic acid Age 14-30 d Brood H-049,050

Exposure (mg/Kg)	Rep	# Organisms Alive			Alive	Dead
		0	24	48		
CONTROL	1	15		15		
	2	15		15	30	0
250	1	15		15		
	2	15		15	30	0
500	1	15		12 (3)		
	2	15		11 (3)	23	7
1000	1	15		2 (13)		
	2	15		1 (14)	3	27
2000	1	15		1 (14)		
	2	15		0 (15)	2	28
4000	1	15		0 (15)		
	2	15		0 (15)	0	30
Operator	Load	MP				
	Load Check	LT				

Comments

Begin	End
8/17/93	8/19/93
1130	1200

SEDIMENT TEST CHEMISTRY

TRAC ID TB-422 Species: *H. azteca*

Exposure	Dissolved Oxygen (ppm)		
	0	24	48
Control	5.2	5.1	5.2
250	5.1	5.1	5.2
500	5.1	5.1	5.1
1000	5.3	5.2	5.2
2000	5.7	5.4	5.2
4000	5.9	5.5	5.2
CONTROL DUP	5.3	5.1	5.5
Operator	LT/PD	LT/PD	MP/LT
Time	1030	1000	0945
Meter #	#1	#1	#1
Exposure	Temperature °C		
	0	24	48
Control	25.2	25.7	24.6
250	25.2	25.7	25.4
500	25.2	25.4	25.4
1000	25.2	25.4	25.7
2000	25.1	25.4	25.7
4000	25.2	25.3	26.1
250 - dup	25.2	25.7	25.2
Operator	LT/PD	LT/PD	MP/LT
Time	1020	950	950
Meter #	#2	#2	#2

SEDIMENT TEST CHEMISTRY

TRAC ID TO-422

Species: *H. azteca*

Exposure	pH		
	0	24	48
Control	7.8	7.7	7.8
250	7.8	7.7	7.7
500	7.8	7.7	7.7
1000	7.8	7.7	7.7
2000	7.9	7.7	7.7
4000	7.9	7.7	7.7
2000-dup	7.8	7.8	7.6
Operator	LT/PD	LT/PD	MP/LT
Time	1045	930	1000
Meter #	1	#1	#1

Exposure	Conductivity (µmhos/cm)		
	0	24	48
Control	410	410	400
250	410	410	410
500	410	410	410
1000	410	410	450
2000	410	410	450
4000	410	420	490
500-dup	410	400	410
Operator	LT/PD	LT/PD	MP/LT
Time	1050	1010	1015
Meter #	#3	#3	#3

HYALELLA AZTECA
ACUTE SEDIMENT TEST SURVIVAL DATA

TRAC ID TB-423 Sponsor: SOAP AND DETERGENT ASSN.

Test Substance: Alkylbenzene sulfonate

0.6 % Organic carbon as humic acid Age 14-30 d Brood H049-H050

Exposure (mg/Kg)	Rep	# Organisms Alive 0	24	48	Alive	Dead
CONTROL	1	15		13		
	2	15		8	21	9
250	1	15		9 (1)		
	2	15		7 (6)	16 ~~26~~	14
500	1	15		12		
	2	15		14	26	4
1000	1	15		8 (1)		
	2	15		3 (9)	11	19
2000	1	15		1 (13)		
	2	15		4 (8)	5	25
4000	1	15		0 (14)		
	2	15		0 (15)	0	30
Operator	Load	PJD				
	Load Check	LT				

incorrect addition pass

Comments

Begin	End
8/17/93	9/9/93
11:30	1400

SEDIMENT TEST CHEMISTRY

TRAC ID TB-423 Species: *H. azteca*

Exposure	Dissolved Oxygen (ppm)		
	0	24	48
Control	5.3	5.1	4.4
250	5.4	5.1	4.5
500	5.5	5.1	4.6
1000	5.4	5.1	4.7
2000	5.5	5.1	4.9
4000	5.7	3.3*	4.4
500-dup	5.9	5.0	3.9
Operator	LT/PD	LT/PD	mp/LT
Time	1030	1000	950
Meter #	#1	#1	#1

Exposure	Temperature °C		
	0	24	48
Control	25.0	25.7	25.8
250	25.0	25.7	25.6
500	25.0	26.0	25.9
1000	24.6	25.4	25.7
2000	24.7	25.8	25.5
4000	24.8	26.0	25.6
Control-dup	25.2	26.0	25.7
Operator	LT/PD	LT/PD	mp/LT
Time	1020	950	955
Meter #	#2	#2	#2

* Reading in Rep 2 is 4.2

SEDIMENT TEST CHEMISTRY

TRAC ID TB-423 Species: *H. azteca*

Exposure	pH		
	0	24	48
Control	8.3	8.1	8.2
250	8.3	8.1	8.2
500	8.3	8.1	8.2
1000	8.3	8.1	8.2
2000	8.3	8.0	8.2
4000	8.3	8.0	8.2
CONTROL-dup	8.3	8.2	8.2
Operator	LT/PD	LT/PD	MP/LT
Time	1045	930	1000
Meter #	#1	#1	#1

Exposure	Conductivity (µmhos/cm)		
	0	24	48
Control	500	550	610
250	500	550	600
500	500	550	600
1000	510	590	650
2000	500	550	600
4000	500	590	610
2000-dup	500	550	610
Operator	LT/PD	LT/PD	MP/LT
Time	11.05	1010	1020
Meter #	#3	#3	#3

HYALELLA AZTECA
ACUTE SEDIMENT TEST SURVIVAL DATA

TRAC ID TB-424 Sponsor: SOAP AND DETERGENT ASSN.

Test Substance: Alkylbenzene sulfonate
1.2 % C-ganic carbon as humic acid

Age 14-20d Brood H619, H630

Exposure (mg/Kg)	Rep	# Organisms Alive			Alive	Dead
		0	24	48		
CONTROL	1	15		5 (6)		
	2	15		4 (8)	9	21
250	1	15		4 (7)		
	2	15		6 (9)	10	20
500	1	15		6 (4)		
	2	15		2 (12)	8	22
1000	1	15		3 (11)		
	2	15		3 (12)	6	24
2000	1	15		2 (11)		
	2	15		4 (9)	6	24
4000	1	15		1 (14)		
	2	15		0 (14)	1	29
Operator	Load	LT				
	Load Check	PM				

Comments

Begin	End

SEDIMENT TEST CHEMISTRY

TRAC ID TD-424 Species: *H. azteca*

Exposure	Dissolved Oxygen (ppm)		
	0	24	48
Control	5.4	4.8	4.4
250	5.2	4.4	3.6
500	4.9	4.4	3.1
1000	5.0	4.0	3.2
2000	5.0	4.0	3.3
4000	4.8	4.6	3.4
2000 - dup	5.2	4.8	3.5
Operator	LT/PD	LT/PD	MP/LT
Time	1030	1000	1000
Meter #	#1	#1	#1

Exposure	Temperature °C		
	0	24	48
Control	25.2	25.4	25.4
250	25.2	25.8	25.4
500	25.0	25.9	25.5
1000	25.1	26.0	25.6
2000	25.3	25.8	25.9
4000	25.5	26.0	26.0
2000 - dup	25.6	25.8	26.1
Operator	LT/PD	LT/PD	LT/MP
Time	1020	950	1005
Meter #	#2	#2	#2

SEDIMENT TEST CHEMISTRY

TRAC ID TB-424 Species: *H. azteca*

Exposure	pH		
	0	24	48
Control	8.6	8.4	8.5
250	8.6	8.4	8.5
500	8.6	8.3	8.5
1000	8.7	8.4	8.5
2000	8.6	8.4	8.5
4000	8.6	8.3	8.5
2000 - dup	8.6	8.4	8.5
Operator	LT/PD	LT/PD	MP/LT
Time	1045	940	1012
Meter #	#1	#1	#1

Exposure	Conductivity (µmhos/cm)		
	0	24	48
Control	590	700	850
250	590	700	800
500	600	700	810
1000	600	700	900
2000	600	700	890
4000	600	710	890
2000 - dup	600	700	800
Operator	LT/PD	LT/PD	MP/LT
Time	1050	1010	1025
Meter #	3	3	3

HYALELLA AZTECA
ACUTE SEDIMENT TEST SURVIVAL DATA

TRAC ID TG-425 Sponsor: SOAP AND DETERGENT ASSN.

Test Substance: Alkylbenzene sulfonate
3% Organic carbon as humic acid Age 14-20d Brood H049, H050

Exposure (mg/Kg)	Rep	# Organisms Alive			Alive	Dead
		0	24	48		
CONTROL	1	15		1 (14)		
	2	15		0 (15)	1	29
250	1	15		0 (15)		
	2	15		0 (15)	0	30
500	1	15		0 (15)		
	2	15		0 (15)	0	30
1000	1	15		0 (12)		
	2	15		0 (15)	0	30
2000	1	15		0 (15)		
	2	15		0 (15)	0	30
4000	1	15		0 (13)		
	2	15		0 (10)	0	30
Operator	Load	MD				
	Load Check	CT				

Comments

Begin	End
3/17/93	3/24/93
11:30	1600

SEDIMENT TEST CHEMISTRY

TRAC ID TB-425 Species: *H. azteca*

Exposure	Dissolved Oxygen (ppm)		
	0	24	48
Control	5.2	4.0	3.3
250	5.3	4.4	3.4
500	5.4	4.4	2.2*
1000	5.3	3.2*	3.2
2000	5.2	4.1	2.9
4000	5.3	3.8	3.2
CONTROL-dup	5.4	4.1	3.0
Operator	LT/PD	LT/PD	MP/LT
Time	1030	1000	1000
Meter #	1	#1	#1

Exposure	Temperature °C		
	0	24	48
Control	24.6	25.1	25.1
250	24.6	25.4	25.2
500	24.6	24.8	25.4
1000	24.6	25.5	25.5
2000	24.6	25.9	25.5
4000	24.8	26.4	25.9
250-dup	24.3	25.5	25.1
Operator	LT/PD	LT/PD	MP/LT
Time	1020	950	1010
Meter #	#2	#2	#2

* Reading in Rep 2 is 4.2

* Reading in Rep 2 is 3.5, 48 hrs 500 EXPOSURE!

SEDIMENT TEST CHEMISTRY

TRAC ID TB-425 Species: *H. azteca*

Exposure	pH		
	0	24	48
Control	9.0	8.8	8.9
250	8.9	8.7	8.9
500	9.1	8.8	8.9
1000	9.1	8.8	8.9
2000	9.0	8.8	8.9
4000	9.0	8.7	~~8.9~~ 9.0
500-dup	9.0	8.8	8.9
Operator	LT/PD	LT/PD	mp/LT
Time	1045	940	1015
Meter #	#1	#1	#1

① IE 8/19/93 mp

Exposure	Conductivity (µmhos/cm)		
	0	24	48
Control	800	990	1400
250	750	990	1500
500	950	1190*	1600
1000	900	1100	1450
2000	890	1150	1490
4000	850	1050	1450
1000-dup	890	1090	1400
Operator	LT/PD	LT/PD	mp/LT
Time	1105	1010	1030
Meter #	#3①	#3①	#3

* Rep 2 is 1000

① W.O LT 8/15/93

F1.2

GENERAL DATA SHEET

TRAC ID TB-422 423 424 425 SPONSOR SDA

Humic acid spike PD 5/16/93

Spike 7kg of sediment to target values of 0.6, 1.2, & 3% OC using Aldrich Na salt of humic acid = 38% OC

= 1g OC / 2.63g humic acid (HA)

Tst	g OC needed	g HA to add
0.6%	$\frac{0.006}{.006 \times 7000g}$ = 420	420 × 2.63 = 110.4
.2	.012 × 7000 = 84	= ~~200~~ 221
3	.03 × 7000g = 210	210 × 2.63 = 552 = 276 × 2

GENERAL DATA SHEET

TRAC ID TB-422, 423, 424, 425 SPONSOR SDA

Preparation of 100 g/L ABS slurry PD 3/19/92

balance calibration: wt. of std = 20.0 g
Balance reading = 20.0 g

25 g ABS slurry weighed into 100 mL beaker.
Transfered to 250 mL Volumetric flask. After bubbles had settled, flask was brought to volume.
First 250 mL stock used to prepare TB-423 & TB-424, a second stock was prepared for TB 425. There was insufficient amount of first stock to prepare all 3 sets of spiked exposures.

DEVIATIONS FROM PROTOCOL

TRAC ID TB-422, 423, 424, 425 SPONSOR SPA

DATE 8/16/93

DEVIATION: Measured amount of organic carbon not known @ time of sediment spiking w/ humic acid.

REASON FOR DEVIATION: Analytical results from laboratory not available.

CORRECTIVE ACTION: Sediments were spiked to the target concentration of organic carbon as if no background existed. Background is probably low (~1-3%). Humic acid spikes will still provide ~~as~~ "High" "Medium" & "Low" organic carbon matrices. PWD 8/16/93

GENERAL DATA SHEET

TRAC ID TC-423, 424, 425 SPONSOR SDA

8/17/93

Humic acid appeared to go into solution into OW upon addition of OW. A layer of darkly stained OW formed (~ 2 cm) above the sediment.

To produce a uniform OW the OW was gently stirred to mix the humic acid layer through the OW. The mixing turned the entire OW column a very dark color, especially in the 3% humic acid test. Aerial will be difficult to see.

8/18/93 Collection of sediment samples for centrifugation:

Siphoned OW from beaker until 2 cm overlying water remaining. The OW used to OW sample (containing OW siphoned off along w/ a small amount of sediment until no OW remaining.

DEVIATIONS FROM PROTOCOL

TRAC ID TG423, 424, 425 SPONSOR SDA

DATE 8/13/93

DEVIATION: IW samples collected using centrifugation.

REASON FOR DEVIATION:

IW will not penetrate liner surrounding fitted gloves end of IW samplers in lab. w/ humic acid. Samplers as prepared a/o humic acid (TG-422) collected adequate amounts of IW.

CORRECTIVE ACTION:

Attempt to collect IW by centrifugation.

DEVIATIONS FROM PROTOCOL

TRAC ID TB-422, 424, 425 SPONSOR SDA

DATE 8/19/93, 8/20/93, 8/23/93

DEVIATION:

IW sample cannot be collected by centrifugation from exposures w/ humic acid added.

REASON FOR DEVIATION:

A clear separation of IW & sediment did not develop.

CORRECTIVE ACTION:

① – Try pressure filtration through 140 mm filter (0.45 μ)
Result – no IW collected in exposures w/ humic acid.

② – Tried pressure filtration through 1.2 μ filter
Result – IW collected from TB-422 exposures.
Result – No IW collected.

③ – Tried pressure filtration through 5 μ filter
Result – Very little IW collected.

A18

DEVIATIONS FROM PROTOCOL

TRAC ID 76423,424,425 SPONSOR SDA

DATE 8/24/93

DEVIATION: No IV samples collected from exposures containing humic acid.

REASON FOR DEVIATION: Adequate sample volume of acceptable IV could not be collected from exposures containing humic acid.

CORRECTIVE ACTION:

DUTIES OF PERSONNEL

TEST SUBSTANCE: Branched detergent slurry; alkylbenzene sulfonate (ABS; T-1343).

TRAC ID: TB-422, TB-423, TB-424, TB-425

SPONSOR: Soap and Detergent Association

STUDY DIRECTOR: Patrick Downey

QUALITY ASSURANCE OFFICER: Barney Venables

LABORATORY ASSISTANT: Melody Pride, Nhung Tran

TEST SYSTEM: *Hyallela azteca* in reference sediment + humic acid and dechlorinated tap water.

STUDY DIRECTOR: Patrick Downey (PJD)

1) Responsible for conduct of all phases of the test.

2) Coordinate toxicity and analytical section.

3) Supervise laboratory assistants.

4) Prepare data sheets, master checklist and test protocol.

5) Prepare ABS stock solutions.

6) Supervise and assist in spiking sediment with ABS.

7) Supervise and assist in preparation of test vessels including placement of IW samplers and addition of OW.

8) Supervise and assist in test startup, including initial *in situ* measurements, addition of organisms, and collection of IW, OW and sediment samples.

9) Supervise and assist in collection of IW, OW and sediment samples.

10) Transfer custody of samples to analytical section for analysis.

11) Supervise and assist in maintenance of test.

12) Supervise and assist in ending of test.

13) Preparation of final report.

QUALITY ASSURANCE OFFICER: Barney Venables (BJV)

1) Conduct QA reviews of test.

2) Submit QA reports to Study Director.

LABORATORY ASSISTANT: Melody Pride (MPT), Nhung tran (LT)

Melody Pride:

1) Maintain *H. azteca* culture

2) Sift topsoil through 1 mm sieve to produce TRAC reference sediment, Lot #1.

3) Prepare organisms for testing.

4) Assist in sediment exposure preparation.

5) Assist in startup of test (addition of overlying water, addion of organisms to test vessels, record initial *in situ* measurments.

6) Assist in test maintenance.

Nhung Tran:

1) Assist in sediment exposure preparation.

2) Assist in startup of test (addition of overlying water, addion of organisms to test vessels, record initial *in situ* measurments.

3) Assist in test maintenance.

MASTER CHECKLIST

DATE	ACTIVITY	OPERATOR
BT	Clean IW samplers and assure that they are functioning properly.	MP
BT	Homogenize and air dry reference sediment	POD
22 d BT	Remove neonates from *H. azteca* culture.	MP / PMD
14 - 21 d BT	Remove neonates from culture and reserve for testing.	MP / PMD
BT	Prepare glassware for tests.	POD / LT
BT	Prepare glasware for TOC and DOC samples.	POD / LT
Day 1	Prepare ABS stock.	PMD
BT	Approval test of protocol by Study Director upon review by QA officer. Study officially begins.	POD / LCV
Day 1	Prepare sediment-humic acid mixtures (0.6, 1.2, 3% organic carbon)	PMD / MP / LT
	Collect and submit samples for analysis of cation exchange capacity and particle-size distribution.	
	Prepare sediment exposures.	PMD / LT / MP
	Place sediment in beakers with IW samplers.	LT / MP
	Introduce OW.	LT
	QA review. QA report.	
Day 2	Take initial *in situ* measurements.	POD / LT
	Introduce organisms to test vessels.	POD / LT / MP
	Take IW, OW and sediment samples for TOC, DOC, ABS.	(1)
	Transfer custody of IW, OW and sediment samples to analytical section.	
	QA review.	
Day 3	Take 24 h *in situ* measurements and make observations of test.	POD / LT
Day 4	Take final *in situ* measurements.	MP / LT
	Remove organisms and make final counts.	MP / LT / PMD
	QA review. QA report.	

BT = before test.

(1) See write up for IW sampling.

MASTER CHECKLIST

DATE	ACTIVITY	OPERATOR
AT	Collate data from toxicity test.	
AT	Collate analytical results.	
AT	Conduct data analysis.	
AT	Begin preparation for second series of tests.	

AT = after test

GENERAL DATA SHEET

TRAC ID 3425 SPONSOR SDA

Organism count worksheet

3 Toluic acid

Exposure	Count Alive	Count Dead
500-1	0	
2	0	
1000-1	0	
-2	0	
2000-1	0	
	0	

GENERAL DATA SHEET

TRAC ID TT-425 SPONSOR SDA

39% humic Acid spike

Exposure	Count	
	Dead	Alive
Control-1	~~HH HH~~ IIII	
C-2	~~HH HH~~ IIII	
25%-1	~~HH HH~~ IIII	
25%-2	~~HH HH~~	
50%-1	~~HH HH~~	
50%-2	~~HH~~ IIII	

GENERAL DATA SHEET

TRAC ID TB-424 SPONSOR SDA

Organism count worksheet

1.2 % humic acid spike

Exposure	Counts	
	Alive	Dead
C-1	5	6
C-2	4	8
250-1	4	7
250-2	6	2
500-1	6	4
500-2	2	12
1000-1	6	11
1000-2	7	12
2000-2	2	11
2000-1	4	9
4000-1	1	14
4000-2		14

GENERAL DATA SHEET

TRAC ID TB-422 SPONSOR SDA

Organism Count Worksheet

No Humic Acid

Exposure	Count
Control 1	𝍸 𝍸 𝍸 𝍸 𝍸 𝍸
3	
250:1	𝍸 𝍸 𝍸 𝍸 𝍸 𝍸
3	
500:1	𝍸 𝍸 II
3	𝍸 𝍸 I → 3 Dead
1000:1	II → 13 Dead
1000:3	I → 14 DEAD
2000:1	I → 14 Dead
2000_3	I → 14 Dead
4000 1	15 DEAD
4000_2	15 DEAD

GENERAL DATA SHEET

TRAC ID 5-423 SPONSOR SDA

Organism count worksheet

0.6% formic acid spike

Exposure	Counts
C-1	
C 2	
250-1	
2	
500-1	
-2	
1000-1	
-2	
2000-1	
2	
4000-1	
2	

H. AZTECA
~~P. PROMELAS~~ DRY WT.

TRAC ID TB-427 N = number of fish weighed

Conc.	Pan	Pan wt.	Pan + fish	Difference	N	Wt/fish (mg)
UNT 1	50	1.30730	1.31103	.00373	10	.373
2	51	1.34315	1.34677	.00362	10	.362
3	52	1.33229	1.33613	.00384	10	.384
TRAC 1	53	1.33706	1.34012	.00306	10	.306
2	54	1.32400	1.32714	.00314	10	.314
3	55	1.31918	1.32579	.00661	10	.661 *

	Date	Time	Operator
Tare weights	8-11-93	1521	MP
Fish placed in oven	8-11-93	1630	LT
Fish removed from oven	8-12-93	1300	LT
Final weights	8-12-93	1510	LT

Fish preserved: Yes ____ No ____ Checked By: ____

* Note:
There were some sediment and debris that dried along w/ organism and may cause this larger weight. Tried to scrap out as much of sediment as possible w/out damaging the dried

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TOXICITY TEST SURVIVAL SPECIES Hyalella

TRAC ID TB427 SPONSOR TRAC

SAMPLE ID UNT Reference Sed / 14 TRAC Reference Sed AGE 12d BROOD

16 d. Survival BEGIN END

DATE

TIME

Conc. (%)	REP	Number Alive 0	24	48	72	96	120	144	168	ALIVE	DEAD
UNT	1	10	10								
	2	10	10								
	3	10	10								
	4										
TRAC	1	10	10								
	2	10	9								
	3	10	10								
	4										
	1										
	2										
	3										
	4										
	1										
	2										
	3										
	4										
	1										
	2										
	3										
	4										
	1										
	2										
	3										
	4										
COUNT											
RENEWAL											

LOADING CHECK _____ COMMENTS:

DISSOLVED OXYGEN AND TEMPERATURE

TRAC ID TB-427 Species TRAC

	Conc (%)	DISSOLVED OXYGEN (mg/L)							
		10 d Final	24	48	72	96	120	144	168
INITIAL	C								
	UNT	1.2							
	TRAC	2.9							
FINAL	C								

	Conc (%)	TEMPERATURE (°C)							
		0	24	48	72	96	120	144	168
INITIAL	C								
FINAL	C								
I = Initial F = Final		I	F/I	F/I	F/I	F/I	F/I	F/I	F/I
Operator									
Time									

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pH AND CONDUCTIVITY

TRAC ID TB. 427 Species H. azteca

	Conc (%)	pH							
		Final 10 d	24	48	72	96	120	144	168
INITIAL	C								
	UNT	7.2							
	TRAC	7.0							
FINAL	C								

	Conc (%)	CONDUCTIVITY (umhos/cm)							
		0	24	48	72	96	120	144	168
INITIAL	C								

I = Initial F = Final	I	F/I	F/I	F/I	F/I	F/I	F/I	F/I
Operator								
Time								
Sample								
Prep								

APPENDIX B: Organism Culture Procedures

SOP 105.1: CULTURE PROCEDURE FOR *HYALELLA AZTECA*

OBJECTIVE:

To provide a healthy culture of *H. azteca* suitable for testing.

METHOD:

A. Handling Tools

1. 5mm glass tubing cut into 15 cm sections, fire polished on both ends
2. Small pipette bulb.
3. Wash bottle filled with culture water (DeCl-Dechlorinated tap water).

B. Substrate

1. 1mm mesh Nytex(TM) screen cut into 10 cm squares.
3. Use 2 squares per jar.

C. Culture Jars

1. 1 gallon (3.785L) glass jars.
2. Acid and acetone rinse new glassware before using.
3. Fill each culture jar with 1L of DeCl and make a mark on the side of the jar to avoid measuring 1L at each renewal.

D. Feeding

1. Feed each jar (60 organisms) $1X10^7$ cells *Selenastrum capricornutum* + 10 mL YCT three times per week.

E. Starting A Culture

1. Put 1L DeCl and 2 squares of 1mm mesh Nytex(TM) screen into jar.
2. Add 10ml YCT and $1X10^7$ cells *Selenastrum capricornutum*.
3. Place 60 *H. azteca* into each jar.

4. Aerate the culture jar gently. Aggressive aeration leads to increased evaporation and "floaters"-animals trapped in the surface tension of the water.

F. Harvesting Neonates

Neonates are harvested once a week from each culture jar. This provides a brood of neonates each week that are 0-7 days old. These neonates are then reared to provide test organisms in known age lots of 7-14 days old or 14-21 days old. This method can be modified to provide a smaller age span, for example, harvesting can be done twice a week which yields organisms in a 3 or 4 day window. Fewer young are harvested each time but the window is tighter.

1. Remove the 2 squares of 1mm mesh screen, shaking gently to dislodge all of the organisms, and place the squares into a rinse of DeCl, set aside.

2. Gently pour contents of mature culture through 1mm mesh sieve, this traps the adult *H. azteca* but allows the neonates to pass through, and into a collecting pan (a shallow enamel or stainless steal pan-the darker the color the better).

3. Rinse the sides of the jar with the wash bottle to free any organisms that may stick to the side of the jar.

4. Rinse out the culture jar with DI water.

5. Refill the jar to the mark with DeCl.

6. Replace the 2 squares of 1mm mesh Nytex(TM) screen.

7. Feed the culture (D. 1).

8. Return adults that were collected in 1mm mesh sieve to culture jar using the glass pipette. Discharge the *H. azteca* carefully underwater, to avoid capturing air under the carapace-resulting in floaters.

9. Count adult *H. azteca* to keep track of brood stock survival recording the total number alive and the number of mating pairs. Count the neonates to monitor reproduction. As reproduction drops, replace brood stock with new animals. Record all data in appropriate log.

10. Neonates will now be left in the counting pan. There are two options at this point.

    (1) Use 0-7 day old neonates in testing.

(2) Place collected neonates into culture jar (E. 1-2). Give neonates a brood number and record it in the Log Book. Rear these organisms to the 7-14 day old stage for testing. Feed this jar at the same rate as the culture stock jars.

A good source of new brood stock are animals that have been matured to 7-14 days old but are not used in testing. At 14 days these animals should be thinned to 50 per jar and allowed to mature for breeding purposes at this density. Mating pairs will become evident at about 18-21 days of age.

G. Log Book Maintenance

Each time the culture is handled, the log book must be filled out. The maintenance records help to troubleshoot culture deficiencies. The following are recorded in the *H. azteca* culture log.

1. Place the date on the next available line for the stock jar you are renewing (each stock jar has a separate page).

2. Record feeding by placing the YCT batch number and your initials under the heading "YCT", and your initials under the heading "Alga" for each time you feed them.

3. Record the amount of water renewed under the appropriate heading (ex. 100%).

4. Record the number of neonates produced at each renewal. All neonates collected at each renewal are placed into a single jar and given a brood number. This number is recorded in a separated section of the log book titled "Broods".

5. Record the number of surviving adults at each renewal.

6. Record the number of mating pairs at each renewal.

APPENDIX C: Toxicity Test Protocol

TEST PROTOCOL FOR CONDUCT OF SEDIMENT TOXICITY TESTS WITH *HYALELLA AZTECA* IN REFERENCE SEDIMENT: ORGANIC CARBON MANIPULATIONS

TEST SUBSTANCE: Branched detergent slurry; alkylbenzene sulfonate (ABS; T-1343).

TRAC ID: TB-422, TB-423, TB-424, TB-425

SPONSOR: Soap and Detergent Association

STUDY DIRECTOR: Patrick Downey

QUALITY ASSURANCE OFFICER: Barney Venables

LABORATORY ASSISTANT: Melody Pride, Nhung Tran

TEST SYSTEM: *Hyallela azteca* in reference sediment + humic acid and dechlorinated tap water.

Written August 2, 1993
Revised August 12, 1993

Reviewed

Approved

_____

Patrick Downey, Study Director

_____

Barney Venables, Quality Assurance Officer

1.0 INTRODUCTION

This protocol is written specifically for the "Sediment/ABS" project Sponsored by the Soap and Detergent Association (475 Park Ave. South, New York, NY 10016). Documentation of test conduct is to be carried out per "Good Laboratory Practices; 40 CFR Part 792, August 1989".

All applicable documentation of activities associated with this test is found in the raw data.

Three series of tests will be conducted. Each series will include four simultaneous toxicity tests. Each toxicity test will consist of five alkylbenzene sulfonate (ABS) concentrations and a control. Each toxicity test will be prepared as follows.

1) Test TB-422: Exposure concentrations will be prepared by mixing ABS with TRAC reference sediment (Lot #1).

2) Test TB-423: Exposure concentrations will be prepared by mixing ABS with TRAC reference sediment (Lot #1) which has been mixed with humic acid (technical sodium salt, Aldrich Catalog No. H1675-2, Lot No. JF 01828JZ) to produce sediment with a target total organic carbon (TOC) content of 0.6%.

3) Test TB-424: Prepared as TB-423 except with a target sediment TOC content of 1.2%.

3) Test TB-425: Prepared as TB-423 except with a target sediment TOC content of 3%.

Three replicates of each exposure will be prepared (See below). One replicate will contain the interstitial water (IW) sampler. The same replicate will be sacrificed at the beginning of the test to provide samples for analytical determination of methylene blue active substance (MBAS) and TOC. The two remaining replicates of each exposure will contain organisms for the toxicity test.

The following analytical determinations will be made:

1) MBAS concentration in IW, overlying water (OW) and sediment of each exposure and control of each test.

2) TOC in sediment of the unspiked control and the highest test exposure.

3) TOC and dissolved organic carbon (DOC) in the IW and OW of each exposure.

4) Particle size distribution (per Gee and Bauder, 1986) and cation exchange capacity (per Plumb, 1981) will be determine on TRAC Reference Sediment, Lot #1.

The toxicity test will begin and IW, OW and sediment samples will be collected after the overlying water and the sediment loaded with ABS have been in contact for 24 h. The toxicity test will last 48 h with no renewal.

2.0 SOURCE OF ORGANISMS

The amphipod *Hyalella azteca* is cultured in-house according to SOP 105.1.

3.0 PREPARATION OF ORGANISMS

Organisms to be used for this project will be *H. azteca* neonates 14 - 20 d of age. Neonates are removed from each culture jar 20 d before the beginning of the test. Neonates are again removed from the culture jars twice at three day interval. These neonates are maintained in dechlorinated tap water until used in the tests.

4.0 PREPARATION OF EXPOSURES

4.1 Addition of Humic Acid

Analytical determination of TOC in TRAC reference sediment (Lot #1) will be made. According to the manufacturer, assays of the technical grade humic acid indicate that it contains 38% $\pm$ 4% organic carbon. An amount of humic acid will be added to 7 Kg (dry wt.) portions of the reference sediment to produce three sediment matrices with TOC content of 0.6, 1.2 and 3%, including the organic carbon already present.

4.2 Stock ABS solution

ABS slurry (TRAC ID T-1343) was delivered to TRAC on 09/13/89. This slurry was prepared by Monsanto and reported to have an MBAS content of 20.2%. A stock ABS solution containing 100 g/L ABS slurry will be prepared by dissolving 25 g ABS slurry into 250 mL dechlorinated tap water. Errors in preparation may result from foaming of the solution during preparation. These errors are minimized by allowing the foam to dissipate before bringing the solution to final volume. This solution is stored at 4° C before it is used.

4.3 Loading Sediment With ABS

Previous testing using ABS indicates that the appropriate ABS slurry concentrations in the sediment are 250, 500, 1000, 2000, and 4000 mg slurry/Kg sediment. The ABS is to be loaded on the sediment as follows. For each exposure the appropriate amount of

ABS stock (Table 4.1) is combined with dechlorinated tap water to produce 50 mL total volume. One Kg of reference sediment is placed in a glass bowl. The 50 mL sample is then added to the sediment and thoroughly mixed by hand with a Teflon™ spoon. The sediment is then ready to place in the test vessels.

Table 4.1 Volumes of ABS stock (25 g/L) to be added to 1 Kg sediment to achieve the indicated exposures.

ABS Slurry Sediment Concentration (mg/Kg)	mL ABS Stock Added to 1 kg Sediment
250	2.5
500	5.0
1000	10.0
2000	20.0
4000	40.0

4.4 Preparation of IW Samplers

One 47 mm glass fiber filter (Gelman A/E) is placed over the fritted end of each sampler (Pittinger, et al; 1988) and secured with a rubber band. A small mark is made on the sampler 1.5 cm above the fritted glass bottom using a permanent marker. The open end of each sampler is then plugged with a rubber stopper.

4.5 Preparation of Test Vessels (Day 1)

Test vessels are 1 L glass beakers. The test vessels which are to contain the IW samplers are prepared before the addition of sediment. Three hundred and fifty g of the appropriate loaded sediment are placed in the beaker and spread evenly on the bottom. Using a pipet, 50 mL dechlorinated tap water are then poured evenly over the sediment. The sides of the beaker are tapped gently with a finger to settle the sediment. Using rubber bands, the samplers are affixed to a horizontal rod which places the samples in the middle of the beakers. Each IW sampler is then slowly lowered into the sediment until the mark on the sampler is even with the surface of the sediment. After the samplers are in place, sediment is then introduced into the test vessels without IW samplers exactly as above.

After sediment has been introduced into all test vessels, 800 mL dechlorinated tap water

are gently siphoned into each vessel using a glass pipet and Tygon™ tubing. The test vessels are allowed to stand for 24 h before organisms are introduced.

5.0 BEGINNING THE TEST (Day 2)

5.1 Initial *In Situ* Measurements

On the day the test is to begin (Day 2), initial measurements of temperature (SOP 403.1), dissolved oxygen (SOP 400.1), pH (SOP 401.1), and conductivity (SOP 402.1) are taken from the replicate of each exposure containing the sediment samplers. In addition, these measurements are taken from one replicate of each exposure in which organisms are to be placed and in both replicates of the 4000 mg/Kg exposure. If all *in situ* measurements are within prescribed limits (Table 5.1), as verified by the QA officer, the organisms may be added to the test vessels.

5.2 Adding Organisms to Test Vessels

H. azteca neonates 14 - 21 d old are removed from the holding vessel using a glass pipet. Five neonates are placed in each of 14 30-mL beakers containing 10 mL dechlorinated tap water. The number of organisms in each beaker is verified by the QA officer. The organisms are then transferred by pipet to the appropriate test vessel and the time of day is recorded. This procedure is repeated twice until 15 organisms have been placed in each test vessel.

Table 5.1. Experimental conditions of test.

Temperature	25 ± 1°C
Minimum Dissolved Oxygen	4.0 mg/L
pH Range	6.5 - 8.5
Light Quality	Ambient laboratory illumination
Photoperiod	16 h light, 8 h dark
Test Vessel	1000 mL glass beaker
Replicates per Concentration	2
Organisms per Replicate	15
Duration of Test	48 h
Endpoint	Death; no movement when gently disturbed

5.3 Collection and Handling of IW, OW, and Sediment Samples.

Collection of aqueous samples will follow procedures recommended in EPA Method 425.1 for MBAS analysis and APHA 5310C for TOC and DOC. Treatment of glassware for collection of MBAS samples will be per routine cleaning procedures (SOP 800.1). Cleaning of glassware for storage of both aqueous and sediment samples for TOC and/or DOC analysis involves submerging an amber glass bottle overnight in 1:1 nitric acid. The bottles are then sealed with aluminum foil and placed in an oven at 400°C for at least one h. Flasks to be used in the collection of organic carbon samples are to be similarly prepared.

5.3.1. Collection and Handling of IW Samples

At the time the last organisms are added to the vessels, the stoppers are removed from the IW samplers. After the samplers have filled completely, the IW sample is removed using a pipet and bulb and placed in an appropriately cleaned (see above) 250 mL Erlenmeyer flask. After 145 mL of IW is collected from each exposure, the flasks are swirled to thoroughly mix the contents. One 20 mL aliquot of IW sample is placed in a 25 mL test tube for MBAS analysis. The remaining IW sample is placed in an appropriately cleaned (See APHA 5310C) 125 mL amber glass bottle with a Teflon™ cap. One half of the 125 mL sample is filtered through an appropriately washed 0.45 µ filter (APHA 5310C). The filtered and unfiltered IW samples in the amber bottles are then preserved with sulfuric acid for organic carbon analysis.

5.3.2. Collection and Handling of OW Samples

After the IW samples are collected, the OW is removed by siphon into a beaker. The OW is then swirled gently to thoroughly mix the contents. A 250 mL aliquot is then transferred to a clean (APHA 5310C) amber glass bottle. One half of the OW sample is then filtered (APHA 5310C) and both the filtered and unfiltered OW samples are preserved with sulfuric acid.

5.3.3 Collection and Handling of Sediment Samples

The test vessels containing sediment are covered with aluminum foil. The IW, OW, and sediment samples are then submitted to the analytical section for analysis. Approximately 25 g of sediment plus associated IW are placed in a polyethylene centrifuge tube and centrifuged at 3000 rpm for 1 h. The supernatant water is then removed with suction. The sediment samples are then submitted to the analytical section for analysis. An additional sample of sediment from the highest exposure of each test is in a clean amber glass jar for analysis of sediment TOC. This analysis will be performed by an outside laboratory.

Three 1 Kg samples for analysis of particle size distribution and cation exchange capacity

will be collected in a polyethylene bag.

6.0 TEST MAINTENANCE

Measurements of temperature, dissolved oxygen, pH, and conductivity are taken in one replicate of each exposure and in both replicates of the 4000 mg/Kg exposure at 24 h. Measurements which are outside specified limits are reported to the QA officer. In addition, the following additional observations are made: OW appearance (clear, turbid) and level of activity shown by organisms observed in the test vessels (swimming in OW, moving about sediment surface, not moving).

7.0 ENDING THE TEST

The test is ended 48 ± 2 h after the first organism was introduced. Final *in situ* measurements are taken. Organisms and OW are removed from the test vessels by siphon. All OW and the top 1 cm of sediment are removed from the test vessel and filtered through 0.25 mm mesh screen. All living and dead organisms are counted. An attempt is made to account for all organisms which were placed in the test vessel.

8.0 REFERENCES

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