TRAC

ACUTE TOXICITY OF ALKYLBENZENE SULFONATE TO *HYALLELA AZTECA*: EFFECT OF MANIPULATION OF SEDIMENT ORGANIC CARBON

Final Report

Submitted to:

Soap and Detergent Association
475 Park Ave. South
New York, NY 10016

Report Number B-463
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TRAC Laboratories, Inc.
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TABLE OF CONTENTS

LIST OF TABLES

1.0 INTRODUCTION

The objective of this study was to examine the effect of manipulation of sediment organic carbon on the toxicity of alkylbenzene sulfonate (ABS) in reference sediment. ABS used in the study was provided by Monsanto Co. (St. Louis) and was identified as "Branched Detergent Slurry" NBP4138558 (Lot No. CC8439) with an active content of 20.2%.

Toxicity was evaluated in five test systems. The first test system consisted of a test organism (the amphipod *Hyallela azteca*) exposed for 48 h to ABS spiked control substance (reference sediment). The remaining four systems consisted of *H. azteca* exposed for 48 h to reference sediment spiked with ABS at each of four concentrations of organic carbon. Organic carbon in the form of peat moss was added to the reference sediment to produce target total organic carbon concentrations of 0.3, 0.6, 1.2 and 3.0% in sediment. The test system behaviors which were of interest were 1) Toxicity of ABS to *H. azteca* at the various organic carbon levels, and 2) ABS concentrations and organic carbon concentrations in interstitial water (IW), overlying water (OW), and sediment (S).

All biological and analytical testing was done at TRAC Laboratories, Inc., 113 Cedar St., Denton, TX 76201, except where noted below.

2.0 MATERIALS AND METHODS

Documentation of sediment toxicity tests was per Good Laboratory Practices, 40 CFR Part 792, August, 1989. This documentation is provided as part of the raw data (Appendix A). Procedures for exposure preparation and sample collection are to be found in the test protocol provided in Appendix B.

2.1 Organisms

Organisms used in the study were *H. azteca* obtained from in-house cultures. Culture procedures are presented in Appendix C. Organisms used in the test were 15-19 d of age.

2.2 Reference Sediment

Reference sediment was sandy loam top soil (Lot #1) obtained locally. The sediment has been shown to be free of lethal or sub-lethal toxic effects to *H. azteca* (TRAC Report B-450, March 23, 1994). The soil was sifted through a 1 mm mesh screen and air dried. Water content was 3% at the time of testing. Organic carbon content was determined before testing began to be 0.02% by dry weight.

2.3 Peat Moss

Commercial grade peat moss (Signing Hills Michigan Peat, Michigan Peat Co., Houston, TX 77098) was sifted through a 1 mm sieve and air dried. Water content was < 1% at the time of testing. Organic carbon content was 43.5% by dry weight.

2.4 Test Substance (ABS)

ABS slurry was delivered to TRAC on 09/13/89 (TRAC ID T-1343). This slurry was prepared by Monsanto (LOT # CC 8439) and reported to have an ABS content of 20.2%.

2.5 Analytical Procedures

Sample collection for analysis of ABS in IW, OW and S is described in the test protocol (Appendix B). Samples were collected at the beginning of each bioassay (at the beginning of the 48 h exposure but after the 24 h equilibration period; see Appendix B). ABS concentrations in IW and OW were estimated using EPA Method 425.1 (colorimetric MBAS). IW and OW were analyzed for total organic carbon (TOC) according to EPA 415.1. Dissolved organic carbon was estimated in OW by filtering an aliquot of the OW through acid washed 0.45 µ membrane filters and analyzing the filtrate according to EPA 415.1. ABS concentrations in S were estimated by methanol extraction followed by cation exchange cartridge cleanup and colorimetric MBAS analysis (Proctor and Gamble memo from B.L. Moore to C. Pittinger, October 10, 1989 and TRAC Report "Evaluation of MBAS analysis for use in sediment bioassays", January, 1990). Un-spiked S and selected spiked S exposures were analyzed for total organic carbon (EPA Method 9060).

2.6 Toxicity Test Procedures

Toxicity test procedures, including preparation of test exposures, are provided in Appendix B. Target sediment ABS levels were determined from range finding tests conducted previously (TRAC Report B-450, March 23, 1994). The amount of peat moss to be added to the reference sediment to produce the desired sediment organic carbon levels was determined based on analytical determinations of organic carbon content of the peat moss and reference sediment. The appropriate amount of peat moss was then combined with reference sediment to produce 7 kg of each sediment for testing.

2.7 Data Analysis

Analysis of acute toxicity data included the calculation of LC50 values. Computer programs were used to estimate the LC50 according to the trimmed Spearman-Karber method (Hamilton et al, 1977). LC50 values for sediment bioassays were calculated for nominal and measured S concentrations and measured IW and OW concentrations.

3.0 RESULTS AND DISCUSSION

3.1 Toxicity Tests and Analytical results

Results of each toxicity test (survival and ABS concentrations in IW, OW and S) are summarized in Tables 1 through 5. LC50 values are summarized in Table 6. Results of TOC analyses are presented in Table 7. A summary of ambient physical/chemical conditions present in the test exposures is presented in Table 8. A QA summary for MBAS and TOC analyses are provided in Tables 9 and 10.

Test temperature was outside the target range ($25 \pm 1^{o}C$) once during the tests using the reference sediment only and the 3.0% organic carbon level (Table 7). This deviation was slight (0.1 to $0.6^{o}C$) and does not confound the results of the tests.

Due to the values obtained at the at the 200 and 400 mg/Kg exposures the measured IW MBAS values were not monotonically increasing with sediment concentration (Table 1). The IW samples were reanalyzed and a similar result was obtained.

There was not close agreement between the analytical estimate of organic carbon content in the reference sediment before testing (0.02%) and the estimate obtained from the reference control test exposure (0.35%). Since the measured organic carbon content of the controls of the 0.3, 0.6 and 1.2% organic carbon concentrations all exceed the target (nominal) value by 0.24 to 0.35%, the measured values shown in Table 8 may more closely reflect the true organic carbon content of the test systems.

3.2 Effect of Organic Carbon Concentration

Control survival was 100% at all organic carbon concentrations. MBAS control values were unaffected by organic carbon in the IW compartment but increased slightly with increasing organic carbon in the OW and S compartments. Control DO and pH dropped slightly with increasing organic carbon concentration while conductivity increased. Measured control TOC content increased with increasing nominal organic carbon concentration in all compartments. DOC in OW paralleled the TOC in OW at slightly reduced concentrations.

3.3 Effect of ABS Concentrations

Toxicity of ABS in reference sediment was similar to that seen in previous experiments (TRAC Report B-319, April 23, 1993). Increasing the organic concentration from 0.35% to 4.6% increased the LC50 by 4.5 and 4.6 times based on nominal and measured sediment ABS concentrations, respectively. LC50 estimates based on measured ABS concentrations in IW and OW were independent of organic carbon concentrations.

Table 1. Results of toxicity test in reference sediment without peat moss. IW = interstitial water, OW = overlying water, S = sediment. Where applicable, values are presented as means of replicate analyses. ND = No data.

Nominal Exposure (mg/Kg)	Measured MBAS Concentrations			Number Surviving (n = 20)
	IW (mg/L)	OW (mg/L)	S (mg/Kg)	
Control	ND	0.10	2.0	20
100	11.8	3.97	63.6	19
200	57.1	6.85	122	15
400	42.9	10.2	215	11
800	124	15.7	548	3
1600	138	21.5	803	0

Table 2. Results of toxicity test in reference sediment with 0.3 % organic carbon as peat moss. IW = interstitial water, OW = overlying water, S = sediment. Where applicable, values are presented as means of replicate analyses.

Nominal Exposure (mg/Kg)	Measured MBAS Concentrations			Number Surviving (n = 20)
	IW (mg/L)	OW (mg/L)	S (mg/Kg)	
Control	< 0.01	0.10	3.5	20
200	32.0	5.35	125	11
400	43.3	10.3	297	8
800	73.0	12.8	600	5
1600	153	22.5	1304	0
3200	181	41.3	2632	0

Table 3. Results of toxicity test in reference sediment with 0.6 % organic carbon as peat moss. IW = interstitial water, OW = overlying water, S = sediment. Where applicable, values are presented as means of replicate analyses.

Nominal Exposure (mg/Kg)	Measured MBAS Concentrations			Number Surviving (n = 20)
	IW (mg/L)	OW (mg/L)	S (mg/Kg)	
Control	< 0.01	0.01	4.3	20
200	33.1	3.57	131	13
400	63.3	7.09	134	8
800	71.6	12.2	546	7
1600	176	21.1	1169	0
3200	208	35.0	3364	0

Table 4. Results of toxicity test in reference sediment with 1.2 % organic carbon as peat moss. IW = interstitial water, OW = overlying water, S = sediment. Where applicable, values are presented as means of replicate analyses. ND = No data.

Nominal Exposure (mg/Kg)	Measured MBAS Concentrations			Number Surviving (n = 20)
	IW (mg/L)	OW (mg/L)	S (mg/Kg)	
Control	< 0.01	0.27	ND	20
400	29.8	3.79	600	13
800	76.5	6.45	146	12
1600	133	13.1	1576	3
3200	166	23.4	679	0
6400	167	38.6	6228	0

Table 5. Results of toxicity test in reference sediment with 3.0 % organic carbon as peat moss. IW = interstitial water, OW = overlying water, S = sediment. Where applicable, values are presented as means of replicate analyses.

Nominal Exposure (mg/Kg)	Measured MBAS Concentrations			Number Surviving (n = 20)
	IW (mg/L)	OW (mg/L)	S (mg/Kg)	
Control	< .01	0.40	9.3	20
800	21.9	2.98	535	17
1600	55.4	7.02	890	12
3200	85.2	18.5	2319	1
6400	96.8	35.3	4919	0
9600	119	44.3	7449	0

Table 6. Summary of LC50 values (mg/L ABS as estimated from MBAS analysis) for each level of sediment organic carbon content (nominal) based on measured and nominal exposures from sediment tests. Values in parentheses = 95% confidence interval.

Partition	% (weight/weight) Sediment Organic Carbon (nominal)				
	0.02 (Reference)	0.3	0.6	1.2	3.0
Sediment (Nominal)	380 (294/491)	252 (177/541)	317 (172/587)	887 (526/1490)	1700 (1350/2130)
Sediment (Measured)	229 (178/296)	167 (64.3/434)	149 (72.5/307)	617 (594/641)	1045 (825/1323)
Overlying Water (Measured)	9.82 (8.37/11.5)	6.66 (3.23/13.7)	5.57 (3.23/9.62)	7.27 (4.65/11.4)	7.77 (5.75/10.5)
Interstitial Water (Measured)	56.8 (44.3/72.8)	35.9 (25.4/49.4)	48.8 (33.8/70.4)	80.4 (43.7/148)	52.6 (41.5/66.6)

Table 7. Mean and range (minimum/maximum) values for routine physical-chemical measurements in overlying water. DO = dissolved oxygen (mg/L), Temp = temperature (°C), Cond = specific conductance (µmhos/cm). See raw data (Appendix A) for DO, temp, pH and cond values at other exposures.

Organic Carbon Level	Exposure (Nominal)	DO	Temp	pH	Cond.
Reference	Control	6.9 6.0/8.2	25.1 24.4/25.4	7.7 7.6/7.8	402 379/415
	1600 mg/Kg	6.9 6.2/8.1	25.9 25.0/26.6	7.8 7.7/7.9	451 400/500
0.3%	Control	6.6 5.8/7.7	25.1 24.6/25.3	7.7 7.6/7.7	408 390/420
	3200 mg/Kg	6.6 5.8/7.7	25.2 24.6/25.5	7.8 7.7/7.9	464 425/495
0.6%	Control	6.7 6.2/7.7	24.9 24.2/25.4	7.6 7.6/7.6	430 390/480
	3200 mg/Kg	7.5 7.1/8.3	24.7 24.1/25.4	7.8 7.7/7.9	451 411/482
1.2%	Control mg/Kg	6.6 5.7/7.9	25.1 24.8/25.5	7.6 7.5/7.6	425 390/450
	6400 mg/Kg	7.3 6.7/8.4	24.9 24.1/25.5	7.7 7.6/7.8	480 409/550
3.0%	Control	5.9 5.5/6.5	25.7 25.0/26.1	7.4 7.4/7.5	553 465/605
	9600 mg/Kg	6.9 6.4/7.4	25.3 24.5/25.8	7.6 7.5/7.6	527 430/600

Table 8. Total organic carbon content of IW, OW and S in toxicity tests using reference and Red River sediment. IW = interstitial water, OW = overlying water, S = sediment, ND = not determined. Where applicable, values are presented as means of replicate analyses.

Organic Carbon Level	Test Exposure (Nominal Sediment ABS)	Overlying Water (mg/L)		Interstitial Water TOC (mg/L)	Sediment TOC (% by weight)
		DOC	TOC		
Reference	Control	3.65	4.08	9.07	0.35
	100	7.70	7.94	23.3	
	200	11.1	11.4	66.0	
	400	10.7	11.7	49.4	
	800	14.8	16.0	92.3	
	1600	19.4	19.5	97.9	0.46
0.3%	Control	4.64	4.66	9.94	0.65
	200	8.24	8.32	49.4	
	400	9.54	10.7	55.5	
	800	13.6	14.4	96.5	
	1600	20.7	21.1	156	
	3200	28.6	30.2	325	0.99
0.6%	Control	4.79	4.99	18.0	0.84
	200	6.90	7.84	73.1	
	400	9.13	10.0	107	
	800	11.7	13.0	109	
	1600	17.4	18.6	233	
	3200	24.8	26.1	286	1.25

Table 8 (Continued). Total organic carbon content of IW, OW and S in toxicity tests using reference and Red River sediment. IW = interstitial water, OW = overlying water, S = sediment, ND = not determined. Where applicable, values are presented as means of replicate analyses.

Organic Carbon Level	Test Exposure (Nominal Sediment ABS)	Overlying Water (mg/L)		Interstitial Water TOC (mg/L)	Sediment TOC (% by weight)
		DOC	TOC		
1.2%	Control	6.09	5.93	25.3	1.47
	400	7.98	8.01	83.8	
	800	9.49	9.55	186	
	1600	15.4	15.6	242	
	3200	20.3	20.6	319	
	6400	29.6	29.7	537	1.36
3.0	Control	7.44	9.06	35.7	4.64
	800	9.65	9.93	233	
	1600	11.7	12.0	327	
	3200	18.6	19.8	408	
	6400	31.2	30.2	526	
	9600	34.0	34.4	719	4.55

Table 9. Summary of QC results for MBAS (methylene blue active substance) analyses. IW = interstitial water, OW = overlying water, S = sediment, ND = no data.

Compartment	Organic Carbon Level	Replicate Analyses (% deviation)	Spike Recovery (%)
IW	Reference	0.4	99.3
	0.3%	ND	115
	0.6%	0.00	81
	1.2%	ND	80
	3.0%	5.0	44.2
OW	Reference	0.98	84.8
	0.3%	ND	98.4
	0.6%	3.7	96.8
	1.2%	ND	97.0
	3.0%	0.00	91.0
S	Reference	0.00, 0.62, 14.4	96.8, 120
	0.3%	1.15	105
	0.6%	1.90	120
	1.2%	2.07	135
	3.0%	3.45	ND

Table 10. Summary of QC results for total organic carbon analyses. IW = interstitial water, OW = overlying water, ND = no data.

Compartment	Organic Carbon Level	Replicate Analyses (% deviation)	Spike Recovery (%)
IW	Reference	2.26	ND
	3.0%	0.43	101
OW	Reference	1.71	99.3
	0.3%	0.71	100
	0.6%	1.28	103
	1.2%	0.50	104
	3.0%	1.48	102

4.0 REFERENCES

Hamilton, M.A., R.C. Russo, and R.V. Thurston. 1977. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Env. Sci. Tech.11:714-719.

APPENDIX A: Toxicity Test Information

I. Toxicity Test Compound and Test Identification

Compound: Alkylbenzene sulfonate, Lot No. CC 8439

Formulation or Trade Name: Branched detergent slurry, NBP 4138558

Manufacturer and Location: Monsanto Co.
St. Louis, MO

TRAC ID: TB-1343

Date shipped: 09/12/89

Date Received: 09/13/89

Test Material Storage: 4°C

Test Material Volume or Weight: 3.8 L

Sample Time in Transit: 1 d

Shipping Route: Air

Shipped by: Federal Express

Received by: TRAC Laboratories, Inc.

Test Material Appearance: Yellowish white, soft, viscous

II. Test Organisms

Test Organism Culture Method: Appendix C

Test Organism Culture Medium: Appendix C

Organism Feeding During Test: None

III. Toxicity Test Specifications

Test Protocol Followed: Appendix B

Test Endpoint: Mortality

Carrier Solvent Used: None

Nominal or Measured Test Concentrations: Both

Photoperiod: DAY/NIGHT = 16 h/8 h

Light Intensity: 50 - 100 foot-candles

Test Containers:

Type: Pyrex™

Size: 1 L

Dilution Water Source: TRAC

IV. *In situ* Measurements:

Dissolved Oxygen: Cole Parmer meter; Model 9070 (TRAC SOP 400.1)

pH: Cole Parmer meter; Model 5994-10 (TRAC SOP 401.1)

Temperature: Cole Parmer meter; Model 9070 (TRAC SOP 403.1)

Conductivity: Cole Parmer meter; Model 1481-55 (TRAC SOP 402.1)

Hardness: Titration, Standard Methods (Method 314B, APHA, 1985)

V. Raw Data

Raw data sheet numbers

REPORT PREPARED BY: Patrick Downey

Patrick J Downey 01/18/95

Principal Investigator Date

STUDY DIRECTOR: Patrick Downey

Patrick J Downey 01/18/95

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 the same as those specified herein, except as where noted.

Raw data audit: BV

Final report audit: BV

Barney Venables 1/18/95

Barney Venables, Ph.D. Date

Quality Assurance Officer

HYALELLA AZTECA
ACUTE SEDIMENT TEST SURVIVAL DATA

TRAC ID: TB-469

Sponsor: SOAP AND DETERGENT ASSN.

Test Substance: Alkylbenzene sulfonate
Reference Sediment

Age 15-19d Brood H-069A,B,CD

Exposure (mg/Kg)	Rep	# Organisms Alive		Alive	Dead
		0	48		
CONTROL	1	10	10		
	2	10	10	20	0
100	1	10	10		
	2	10	9	19	1(0)
200	1	10	7(3)		
	2	10	8(2)	15	5(5)
400	1	10	3(7)		
	2	10	8(2)	11	9(9)
800	1	10	0(10)		
	2	10	3(7)	3	17(17)
1600	1	10	0(10)		
	2	10	0(10)	0	20(20)
Operator	Load	NR	NR		
	Load Check	NR	NR		

Comments
Counts in parenthesis = # bodies found

Begin	End
3/7/94	3/4/94
1430	1600

SEDIMENT TEST CHEMISTRY

TRAC ID: TB-469

Species: *H. azteca*

Exposure	Dissolved Oxygen (ppm)		
	0	24	48
Control	8.2	6.0	6.5
100	8.1	6.2	6.5
200	8.2	6.4	6.7
400	8.2	6.6	6.9
800	8.1	6.5	6.8
1600	8.1	6.3	6.2
Operator	MP	MP	
Time	1030	1300	
Meter #	3	3	

Exposure	Temperature °C		
	0	24	48
Control 24.4	~~22.4~~	25.4	25.4
100 24.4	~~22.3~~	25.4	25.4
200 24.4	~~22.3~~	25.9	25.9
400 24.4	~~22.4~~	25.9	26.1
800 24.9	~~23.0~~	25.9	26.1
1600 25.0	~~23.0~~	26.0	26.6
Operator	MP	MP	LT/JT
Time 1423	~~1033~~	1302	100
Meter #	3	3	3

Initial temps out of range. Test grading delayed until initial temp w/in range
RD 3/2/94

TRAC ID: TB-469

SEDIMENT TEST CHEMISTRY

Species: *H. azteca*

Exposure	pH		
	0	24	48
Control	7.6	7.6	7.8
100	7.6	7.7	7.8
200	7.6	7.7	7.8
400	7.7	7.8	7.8
800	7.7	7.9	7.9
1600	7.7	7.9	7.9
Operator	MP	MP	
Time	1036	① 1813	
Meter #	1	1	

Exposure	Conductivity (µmhos/cm)		
	0	24	48
Control	379	415	412
100	380	420	426
200	385	425	430
400	389	429	432
800	391	439	447
1600	400	500	454
Operator	MP	MP	LT/JT
Time	1038	1306	1300
Meter #	3	3	3

① IE 3/3 MP

A7

HYALELLA AZTECA
ACUTE SEDIMENT TEST SURVIVAL DATA

TRAC ID: TB-470

Sponsor: SOAP AND DETERGENT ASSN.

Test Substance: Alkylbenzene sulfonate

0.3% Organic Carbon

Age ~~21 days~~ 15-19 days Brood ~~H-069B~~ H-069A, B, C, D

Exposure (mg/Kg)	Rep	# Organisms Alive		Alive	Dead
		0	48		
CONTROL	1	10	10		
	2	10	10	20	0
200	1	10	4(2)		
	2	10	7(0)	11	9(2)
400	1	10	5(3)		
	2	10	3(4)	8	12(7)
800	1	10	2(5)		
	2	10	3(7)	5	15(12)
1600	1	10	0(10)		
	2	10	0(10)	0	20(20)
3200	1	10	0(10)		
	2	10	0(10)	0	20(20)
Operator	Load	MP			
	Load Check	MP	MP/PM		

Comments

Begin	End
3-2-94	3/4/94
1530	~~1730~~ 1730

TRAC ID: TB-470

SEDIMENT TEST CHEMISTRY

Species: *H. azteca*

Exposure	Dissolved Oxygen (ppm)		
	0	24	48
Control	7.7	5.8	6.3
200	7.8	6.5	6.6
400	8.0	6.3	6.6
800	7.7	6.4	6.7
1600	8.0	6.8	6.8
3200	8.0	7.1	6.9
2ow Duplicate			6.6
Operator	MP	MP	
Time	1113	1308	
Meter #	3	3	

Exposure	Temperature °C		
	0	24	48
Control 24.6	~~23.1~~	25.3	25.3
200 24.6	~~23.1~~	25.5	25.1
400 24.6	~~23.0~~	25.5	25.2
800 24.6	~~23.0~~	25.5	25.1
1600 24.6	~~23.0~~	25.5	25.4
3200 24.6	~~23.0~~	25.5	25.4
Operator	MP	MP	
Time 1425	~~1116~~	1310	
Meter #	3	3	

TRAC ID: TB-470

SEDIMENT TEST CHEMISTRY

Species: *H. azteca*

Exposure	pH		
	0	24	48
Control	7.6	7.6	7.7
200	7.6	7.7	7.7
400	7.6	7.7	7.7
800	7.6	7.7	7.8
1600	7.6	7.8	7.8
3200	7.7	7.9	7.8
Operator	MP	MP	
Time	1118	1312	
Meter #	1	1	

Exposure	Conductivity (µmhos/cm)		
	0	24	48
Control	390	415	420
200	395	429	440
400	399	430	439
800	401	440	455
1600	415	460	473
3200	425	472	495
Operator	MP	MP	
Time	1120	1314	
Meter #	3	3	

HYALELLA AZTECA
ACUTE SEDIMENT TEST SURVIVAL DATA

TRAC ID: TB-471

Sponsor: SOAP AND DETERGENT ASSN.

Test Substance: Alkylbenzene sulfonate

0.6% Organic Carbon

Age ~~21 days~~ 15-19 days Brood ~~H-069B~~ H-069A, B, C, D

Exposure (mg/Kg)	Rep	# Organisms Alive		Alive	Dead
		0	48		
CONTROL	1	10	10		
	2	10	10	20	0
200	1	10	7(0)		
	2	10	6(2)	8 13	7(2)
400	1	10	3(6)		
	2	10	5(3)	8	12(9)
800	1	10	4(6)		
	2	10	3(2)	7	~~3~~ 5(8)
1600	1	10	0(10)		
	2	10	0(9)	0	20(19)
3200	1	10	0(10)		
	2	10	0(10)	0	20(19)
Operator	Load	MP			
	Load Check	MP	PM		

Comments

Counts in parentheses = babies found

Begin	End
3-2-94	3/4/94
1600	1520

SEDIMENT TEST CHEMISTRY

TRAC ID: TB-471

Species: *H. azteca*

Exposure	Dissolved Oxygen (ppm)		
	0	24	48
Control	7.7	6.3	6.2
200	8.0	6.5	6.5
400	8.2	6.5	6.8
800	8.2	6.5	7.0
1600	8.3	6.5	7.1
3200	8.3	7.0	7.1
Blank duplicate			6.1
Operator	MP	MP	
Time	1100	1316	
Meter #	3	3	

Exposure	Temperature °C		
	0	24	48
Control 24.2	~~22.5~~	25.0	25.4
200 24.2	~~22.5~~	25.0	25.4
400 24.1	~~22.6~~	25.0	25.4
800 24.1	~~22.5~~	25.0	25.4
1600 24.1	~~22.5~~	24.8	25.4
3200 24.1	~~22.5~~	24.6	25.4
Operator	MP	MP	
Time 1427	~~1103~~	1318	
Meter #	3	3	

TRAC ID: TB-471

SEDIMENT TEST CHEMISTRY

Species: *H. azteca*

Exposure	pH		
	0	24	48
Control	7.6	7.6	7.6
200	7.6	7.7	7.7
400	7.6	7.7	7.7
800	7.6	7.8	7.7
1600	7.7	7.8	7.7
3200	7.7	7.9	7.8
Operator	mp	mp	
Time	1105	1320	
Meter #	1	1	

Exposure	Conductivity (µmhos/cm)		
	0	24	48
Control	390	420	480
200	395	425	435
400	398	429	440
800	399	435	448
1600	405	449	460
3200	411	460	482
Operator	mp	mp	
Time	1110	1322	
Meter #	3	3	

HYALELLA AZTECA
ACUTE SEDIMENT TEST SURVIVAL DATA

TRAC ID: TB-472

Sponsor: SOAP AND DETERGENT ASSN.

Test Substance: Alkylbenzene sulfonate

1.2% Organic carbon

Age ~~21 day~~ 15-19 day Brood ~~H-069C~~ H-069A, B, C, D

Exposure (mg/Kg)	Rep	# Organisms Alive		Alive	Dead
		0	48		
CONTROL	1	10	10		
	2	10	10	20	0
400	1	10	7(3)		
	2	10	6(1)	13	7(4)
800	1	10	8(2)		
	2	10	4(6)	12	8(8)
1600	1	10	2(8)		
	2	10	1(9)	3	17(17)
3200	1	10	0(7)		
	2	10	0(8)	0	20(15)
6400	1	10	0(9)		
	2	10	0(8)	0	20(17)
Operator	Load	SMP			
	Load Check	SMP	MP		

Comments Counts in parenthesis = # of bodies

Begin	End
3-2-94	3/4/94
1630	1530

TRAC ID: TB-472

SEDIMENT TEST CHEMISTRY

Species: *H. azteca*

Exposure	Dissolved Oxygen (ppm)		
	0	24	48
Control	7.9	6.1	5.7
400	8.3	6.1	6.2
800	8.3	6.4	6.5
1600	8.4	6.5	6.6
3200	8.4	6.7	6.9
6400	8.4	6.7	6.8
Operator	MP	MP	
Time	1040	1324	
Meter #	3	3	

Exposure	Temperature °C		
	0	24	48
Control 24.8	~~22.0~~	25.0	25.5
400 ~~24.6~~	~~22.0~~	25.0	25.5
800 24.0	~~22.0~~	25.0	25.5
1600 23.8	~~22.0~~	25.0	25.5
3200 24.0	~~22.0~~	25.0	25.5
6400 24.1	~~22.0~~	25.1	25.5
Operator	MP	MP	
Time 1429	~~1049~~	1326	
Meter #	3	3	

TRAC ID: TB-472

SEDIMENT TEST CHEMISTRY

Species: *H. azteca*

Exposure	pH		
	0	24	48
Control	7.5	7.6	7.6
400	7.5	7.6	7.6
800	7.5	7.7	7.6
1600	7.5	7.7	7.6
3200	7.6	7.8	7.7
6400	7.6	7.8	7.7
Operator	mp	mp	
Time	1052	1328	
Meter # ①	31	31	

Exposure	Conductivity (µmhos/cm)		
	0	24	48
Control	390	435	450
400	398	445	460
800	400	448	465
1600	405	460	485
3200	405	465	490
6400	409	480	550
Operator	mp	mp	
Time	1055	1330	
Meter #	3	3	

① IE 3/3 mp

A16

TRAC ID: TB-473

SEDIMENT TEST CHEMISTRY

Species: *H. azteca*

Exposure	Dissolved Oxygen (ppm)		
	0	24	48
Control	6.5	5.5	5.8
800	7.1	5.9	5.6
1600	7.2	6.0	6.0
3200	7.5	6.3	6.4
6400	7.4	6.4	6.5
9600	7.6	6.5	6.6
800 Duplicate			5.7
Operator	MP	MP	
Time	1122	1332	
Meter #	3	3	

Exposure	Temperature °C		
	0	24	48
Control 25.0	~~22.9~~	26.0	26.1
800 25.0	~~22.9~~	25.8	26.1
1600 24.7	~~22.8~~	25.8	25.9
3200 24.6	~~22.8~~	25.7	25.8
6400 24.5	~~22.8~~	25.6	25.8
9600 24.5	~~22.8~~	25.6	25.8
Operator	MP	MP	
Time 1442	~~1124~~	1334	
Meter #	3	3	

TRAC ID: TB-473

SEDIMENT TEST CHEMISTRY

Species: *H. azteca*

Exposure	pH		
	0	24	48
Control	7.4	7.4	7.5
800	7.4	7.5	7.5
1600	7.4	7.5	7.5
3200	7.5	7.6	7.5
6400	7.5	7.6	7.6
9600	7.5	7.6	7.6
Operator	MP	MP	
Time	1127	1336	
Meter #	1	1	

Exposure	Conductivity (µmhos/cm)		
	0	24	48
Control	465	605	590
800	420	595	520
1600	415	510	530
3200	420	515	580
6400	435	590	610
9600	430	550	600
Operator	MP	MP	
Time	1131	1338	
Meter #	3	3	

GENERAL DATA SHEET

TRAC ID TB-469 – 473 SPONSOR SDA

2/25/96

ABS Stock Prep

Prepare 500 mL of 100 g ABS/L

Slurry is 20.2% active ABS

→ $\frac{1}{0.202}$ = 4.95 g Slurry/1 g ABS

100 g/L = 50 g/500 mL = 50 × 4.95

= 248 g Slurry/500 mL

De Chlorinated H_2O.

GENERAL DATA SHEET

TRAC ID TB 469-473 SPONSOR SDA

Reference Sediment & Peat Mass Mixing

Peat mass = 43.5% OC

$= \frac{2.3\text{ g Peat mass}}{1\text{ g OC}}$

Prepare 7 kg of each OC level

OC level	g OC needed	g Peat mass needed
0.3 %	0.003 × 7000 = 21	21 × 2.3 = 48.3
0.6 %	42	96.6
1.2	84	193.2
3.0	210	483

DEVIATIONS FROM PROTOCOL

TRAC ID TB-469, 473 SPONSOR SDA

DATE 3/4/94

DEVIATION: Temperature outside specified range on 1 Control & 800 mg exposure of TB-473, 400, 800 & 1600 mg exposure of TB-469 at end of test

REASON FOR DEVIATION: Uneven heating of test room

CORRECTIVE ACTION: None. Occurred @ 48 h = end of test

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

Ref Sed
Measured IW

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 3/2/94 TEST NUMBER: TB-469 DURATION: 48 H
CHEMICAL: ABS SPECIES: H. AZTECA

RAW DATA:

CONCENTRATION(MG/L)	11.80	42.90	57.10	124.00	138.00
NUMBER EXPOSED:	20	20	20	20	20
MORTALITIES:	1	9	5	17	20

SPEARMAN-KARBER TRIM: 5.00%

SPEARMAN-KARBER ESTIMATES: LC50: (56.82)
95% LOWER CONFIDENCE: 44.33
95% UPPER CONFIDENCE: 72.82

NOTE: MORTALITY PROPORTIONS WERE NOT MONOTONICALLY INCREASING.
ADJUSTMENTS WERE MADE PRIOR TO SPEARMAN-KARBER ESTIMATION.

Measured IW
0.3%

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 3/2/94 TEST NUMBER: TB470 DURATION: 48 H
CHEMICAL: ABS; IW SPECIES: H. AZTECA

RAW DATA:

CONCENTRATION(MG/L)	32.00	43.30	73.00	153.00	181.00
NUMBER EXPOSED:	20	20	20	20	20
MORTALITIES:	9	12	15	20	20

SPEARMAN-KARBER TRIM: 45.00%

SPEARMAN-KARBER ESTIMATES: LC50: (35.39)
95% LOWER CONFIDENCE: 25.36
95% UPPER CONFIDENCE: 49.40

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

Measured IW
0.6%

DATE: 3/2/94 TEST NUMBER: TB-471 DURATION: 48 H
CHEMICAL: ABS MEASURED SPECIES: H. AZTECA

RAW DATA:

CONCENTRATION(PPM IW)	33.10	63.30	71.60	176.00	208.00
NUMBER EXPOSED:	20	20	20	20	20
MORTALITIES:	7	12	13	20	20

SPEARMAN-KARBER TRIM: 35.00%

SPEARMAN-KARBER ESTIMATES: LC50: 48.81
95% LOWER CONFIDENCE: 33.83
95% UPPER CONFIDENCE: 70.43

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

Measured IW
1.2%

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 3/2/94 TEST NUMBER: TB-472 DURATION: 48 H
CHEMICAL: ABS MEASURED SPECIES: H. AZTECA

RAW DATA:

CONCENTRATION(MG/L)	29.80	76.50	133.00	162.00	166.00
NUMBER EXPOSED:	20	20	20	20	20
MORTALITIES:	7	8	17	20	20

SPEARMAN-KARBER TRIM: 35.00%

SPEARMAN-KARBER ESTIMATES: LC50: 80.38
95% LOWER CONFIDENCE: 43.72
95% UPPER CONFIDENCE: 147.77

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

Measured IW

37

DATE: 3/2/94 TEST NUMBER: TB-473 DURATION: 48 H
CHEMICAL: ABS; IW SPECIES: H. AZTECA

RAW DATA:

CONCENTRATION(MG/L MEA)		21.90	55.40	85.20	96.80	119.00
NUMBER EXPOSED:	20	20	20	20	20	
MORTALITIES:	3	8	19	20	20	

SPEARMAN-KARBER TRIM: 15.00%

SPEARMAN-KARBER ESTIMATES: LC50: 52.56
95% LOWER CONFIDENCE: 41.50
95% UPPER CONFIDENCE: 66.58

A25

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

Ref Sed
Nominal Sediment
Conc

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 3/2/94 TEST NUMBER: TB-469 DURATION: 48 H
CHEMICAL: ABS SPECIES: H. AZTECA

RAW DATA:

CONCENTRATION(MG/KG	NO)	100.00	200.00	400.00	800.00	1600.00
NUMBER EXPOSED:		20	20	20	20	20
MORTALITIES:		1	5	9	17	20

SPEARMAN-KARBER TRIM: 5.00%

SPEARMAN-KARBER ESTIMATES: LC50: 379.98
95% LOWER CONFIDENCE: 293.61
95% UPPER CONFIDENCE: 491.77

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

~~0.03~~
0.37
Nominal Sed

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 3/2/94 TEST NUMBER: TB-470 DURATION: 48 H
CHEMICAL: ABS SPECIES: H. AZTECA

RAW DATA:

CONCENTRATION(MG/KG	NO)	200.00	400.00	800.00	1600.00	3200.00
NUMBER EXPOSED:		20	20	20	20	20
MORTALITIES:		9	12	15	20	20

SPEARMAN-KARBER TRIM: 45.00%

SPEARMAN-KARBER ESTIMATES: LC50: 251.98
95% LOWER CONFIDENCE: 117.38
95% UPPER CONFIDENCE: 540.96

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

Nominal Sediment Conc.
0.6%

DATE: 3/2/94 TEST NUMBER: TB-471 DURATION: 48 H
CHEMICAL: ABS SPECIES: H. AZTECA

RAW DATA:

CONCENTRATION(MG/KG NO)	200.00	400.00	800.00	1600.00	3200.00
NUMBER EXPOSED:	20	20	20	20	20
MORTALITIES:	7	12	13	20	20

SPEARMAN-KARBER TRIM: 35.00%

SPEARMAN-KARBER ESTIMATES: LC50: 317.48
95% LOWER CONFIDENCE: 171.72
95% UPPER CONFIDENCE: 586.97

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

Nominal Sediment Conc.
1.2%

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 3/2/94 TEST NUMBER: TB-472 DURATION: 48 H
CHEMICAL: ABS SPECIES: H. AZTECA

RAW DATA:

CONCENTRATION(MG/KG NO)	400.00	800.00	1600.00	3200.00	6400.00
NUMBER EXPOSED:	20	20	20	20	20
MORTALITIES:	7	8	17	20	20

SPEARMAN-KARBER TRIM: 35.00%

SPEARMAN-KARBER ESTIMATES: LC50: 886.52
95% LOWER CONFIDENCE: 526.20
95% UPPER CONFIDENCE: 1493.57

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

Nominal Sediment Conc.

3.7

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 3/2/94 TEST NUMBER: TB-473 DURATION: 48 H
CHEMICAL: ABS SPECIES: H. AZTECA

RAW DATA:

CONCENTRATION(MG/KG NO)	800.00	1600.00	3200.00	6400.00	9600.00	
NUMBER EXPOSED:	20	20	20	20	20	
MORTALITIES:	3	8	19	20	20	

SPEARMAN-KARBER TRIM: 15.00%

SPEARMAN-KARBER ESTIMATES: LC50: 1696.41
95% LOWER CONFIDENCE: 1348.10
95% UPPER CONFIDENCE: 2134.72

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

Ref Seed
Measured OW

DATE: 3/2/94 TEST NUMBER: TB-469 DURATION: 48 H
CHEMICAL: ABS MEASURED SPECIES: H. AZTECA

RAW DATA:

CONCENTRATION(MG/L OW)	3.97	6.85	10.20	15.70	21.50
NUMBER EXPOSED:	20	20	20	20	20
MORTALITIES:	1	5	9	17	20

SPEARMAN-KARBER TRIM: 5.00%

SPEARMAN-KARBER ESTIMATES: LC50: 9.82
95% LOWER CONFIDENCE: 8.37
95% UPPER CONFIDENCE: 11.53

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

Measured Seed

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 3/2/94 TEST NUMBER: TB-469 DURATION: 48 H
CHEMICAL: ABS MEASURED SPECIES: H. AZTECA

RAW DATA:

CONCENTRATION(MG/KG SE)	63.60	122.00	215.00	548.00	803.00
NUMBER EXPOSED:	20	20	20	20	20
MORTALITIES:	1	5	9	17	20

SPEARMAN-KARBER TRIM: 5.00%

SPEARMAN-KARBER ESTIMATES: LC50: 229.39
95% LOWER CONFIDENCE: 177.52
95% UPPER CONFIDENCE: 296.40

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

0.3 %

Measured OW

DATE: 3/2/94 TEST NUMBER: TB-470 DURATION: 48 H
CHEMICAL: ABS MEASURED SPECIES: H. AZTECA

RAW DATA:

CONCENTRATION(MG/L OW)	5.35	10.30	12.80	22.50	41.30
NUMBER EXPOSED:	20	20	20	20	20
MORTALITIES:	9	12	15	20	20

SPEARMAN-KARBER TRIM: 45.00%

SPEARMAN-KARBER ESTIMATES: LC50: 6.66
95% LOWER CONFIDENCE: 3.23
95% UPPER CONFIDENCE: 13.70

0.3 %

Measured Sed

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 3/2/94 TEST NUMBER: TB-470 DURATION: 48 H
CHEMICAL: ABS MEASURED SPECIES: H. AZTECA

RAW DATA:

CONCENTRATION(MG/KG SE)	125.00	297.00	600.00	1304.00	2632.00
NUMBER EXPOSED:	20	20	20	20	20
MORTALITIES:	9	12	15	20	20

SPEARMAN-KARBER TRIM: 45.00%

SPEARMAN-KARBER ESTIMATES: LC50: 166.80
95% LOWER CONFIDENCE: 64.26
95% UPPER CONFIDENCE: 432.96

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

0.67 Measured OW

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 3/2/94 TEST NUMBER: TB-471 DURATION: 48 H
CHEMICAL: ABS MEASURED SPECIES: H. AZTECA

RAW DATA:

CONCENTRATION(MG/L OW)	3.57	7.09	12.20	21.10	35.00
NUMBER EXPOSED:	20	20	20	20	20
MORTALITIES:	7	12	13	20	20

SPEARMAN-KARBER TRIM: 35.00%

SPEARMAN-KARBER ESTIMATES: LC50: 5.57
95% LOWER CONFIDENCE: 3.23
95% UPPER CONFIDENCE: 9.62

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

Measured Sed

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 3/2/94 TEST NUMBER: TB-471 DURATION: 48 H
CHEMICAL: ABS; SED SPECIES: H. AZTECA

RAW DATA:

CONCENTRATION(MG/KG ME)	131.00	134.00	546.00	1169.00	3364.00	
NUMBER EXPOSED:	20	20	20	20	20	
MORTALITIES:	7	12	13	20	20	

SPEARMAN-KARBER TRIM: 35.00%

SPEARMAN-KARBER ESTIMATES: LC50: 149.23
95% LOWER CONFIDENCE: 72.50
95% UPPER CONFIDENCE: 307.16

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

1.27 Measured OW

DATE: 3/2/94 TEST NUMBER: TB-472 DURATION: 48 H
CHEMICAL: ABS MEASURED SPECIES: H. AZTECA

RAW DATA:

CONCENTRATION(MG/L OW)	3.79	6.45	13.10	23.40	38.60
NUMBER EXPOSED:	20	20	20	20	20
MORTALITIES:	7	8	17	20	20

SPEARMAN-KARBER TRIM: 35.00%

SPEARMAN-KARBER ESTIMATES: LC50: 7.27
95% LOWER CONFIDENCE: 4.65
95% UPPER CONFIDENCE: 11.38

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

Measured Sed

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 3/2/94 TEST NUMBER: TB-472 DURATION: 48 H
CHEMICAL: ABS MEASURED SPECIES: H. AZTECA

RAW DATA:

CONCENTRATION(MG/KG SE)	146.00	600.00	679.00	1576.00	6228.00
NUMBER EXPOSED:	20	20	20	20	20
MORTALITIES:	8	7	20	17	20

SPEARMAN-KARBER TRIM: 37.50%

SPEARMAN-KARBER ESTIMATES: LC50: 617.11
95% LOWER CONFIDENCE: 594.03
95% UPPER CONFIDENCE: 641.07

NOTE: MORTALITY PROPORTIONS WERE NOT MONOTONICALLY INCREASING.
ADJUSTMENTS WERE MADE PRIOR TO SPEARMAN-KARBER ESTIMATION.

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

3%
Measured OW

DATE: 3/2/94 TEST NUMBER: TB-473 DURATION: 48 H
CHEMICAL: ABS MEASURED SPECIES: H. AZTECA

RAW DATA:

CONCENTRATION(MG/L OW)	2.98	7.02	18.50	35.30	44.30
NUMBER EXPOSED:	20	20	20	20	20
MORTALITIES:	3	8	19	20	20

SPEARMAN-KARBER TRIM: 15.00%

SPEARMAN-KARBER ESTIMATES: LC50: 7.77
95% LOWER CONFIDENCE: 5.75
95% UPPER CONFIDENCE: 10.50

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

Measured Sed

DATE: 3/2/94 TEST NUMBER: TB-473 DURATION: 48 H
CHEMICAL: ABS MEASURED SPECIES: H. AZTECA

RAW DATA:

CONCENTRATION(MG/L SED)	535.00	890.00	2319.00	4919.00	7449.00
NUMBER EXPOSED:	20	20	20	20	20
MORTALITIES:	3	8	19	20	20

SPEARMAN-KARBER TRIM: 15.00%

SPEARMAN-KARBER ESTIMATES: LC50: 1045.44
95% LOWER CONFIDENCE: 825.50
95% UPPER CONFIDENCE: 1323.97

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

TB-~~446~~ 469 IW

DATE: 3/2/94 TEST NUMBER: TB-469 DURATION: 48 H
CHEMICAL: ABS SPECIES: H. AZTECA

assuming labels of 200 & 400 mg/kg exposures were switched

RAW DATA:

CONCENTRATION(MG/L)	11.80	42.90	57.10	124.00	138.00
NUMBER EXPOSED:	20	20	20	20	20
MORTALITIES:	1	5	9	17	20

SPEARMAN-KARBER TRIM: 5.00%

SPEARMAN-KARBER ESTIMATES: LC50: 58.47
95% LOWER CONFIDENCE: 46.07
95% UPPER CONFIDENCE: 74.21

QUALITY ASSURANCE REVIEW

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

TRAC ID: TB-469, TB-470, TB-471, TB-472, TB-473

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 + peat moss and dechlorinated tap water.

Attach comments on a separate page.

ITEM	INITIAL	DATE
Calibration logs complete and calibrations satisfactory: End of test.	BV	3/07/94
Correct number of organisms added to test vessels.	BV	3/02/94
Operator and time of operation indicated with all observations.	BV	3/07/94

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-469, TB-470, TB-471, TB-472, TB-473

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 + peat moss and dechlorinated tap water.

Written: February 25, 1994

Reviewed: February 27, 1994

Approved

Patrick Downey 2/28/94

Patrick Downey, Study Director

B J Venables 2/28/94

Barney Venables, Quality Assurance Officer

D:\SDA\SOP.3

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-469: Exposure concentrations will be prepared by mixing ABS with TRAC reference sediment (Lot #1).

2) Test TB-470: Exposure concentrations will be prepared by mixing ABS with TRAC reference sediment (Lot #1) which has been mixed with peat moss to produce sediment with a target total organic carbon (TOC) content of 0.3%.

3) Test TB-471: Prepared as TB-470 except with a target sediment TOC content of 0.6%.

4) Test TB-472 Prepared as TB-470 except with a target sediment TOC content of 1.2%.

5) Test TB-473 Prepared as TB-470 except with a target sediment TOC content of 3.0%.

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 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 Peat Moss

Analytical determination of TOC in TRAC reference sediment (Lot #1) has been determined to be 0.02% and is considered to be negligible relative to the added amounts. An amount of peat moss will be added to 7 Kg (dry wt.) portions of the reference sediment to produce four sediment matrices with TOC content of 0.3, 0.6, 1.2 and 3%.

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 248 g ABS slurry into 500 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 range finding tests (TRAC Report B-450) indicate preparation of exposures as in Table 4.1. The ABS is to be loaded on the sediment as follows. For each exposure the appropriate amount of ABS stock (Table 4.2) is combined with dechlorinated tap

water to produce 200 mL total volume. One Kg of reference sediment is placed in a glass bowl. The 200 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. Definitve test exposures.

Organic Carbon Level (% Organic Carbon as Peat Moss, by Weight)	Test Concentrations (mg Active ABS/Kg Sediment)
0.02 (Reference)	100, 200, 400, 800, 1600
0.3	200, 400, 800, 1600, 3200
0.6	200, 400, 800, 1600, 3200
1.2	400, 800, 1600, 3200, 6400
3.0	800, 1600, 3200, 6400, 9600

Table 4.2 Volumes of ABS stock (100 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
100	1.0
200	2.0
400	4.0
800	8.0
1600	16
3200	32
6400	64
9600	96

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 samplers 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 remaining 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^{TM} 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 an additional 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 10 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	10
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 approximately 20 mL of IW is collected from each exposure, the flasks are swirled to thoroughly mix the contents. One 10 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) 25 mL test tube for TOC analysis.

5.3.2. Collection and Handling of OW Samples

After the IW samples are collected, the OW is removed by siphoning 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 this sample will be filtered through acid washed 0.45 μ membrane filters and preserved for analysis of DOC.

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.

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

Pittinger, C.A., V.C. Hand, J.A. Masters and L.F. Davidson. 1988. Interstitial water sampling in ecotoxicological testing: Partitioning of a cationic surfactant. In: W.J. Adams, G.A. Chapman and W.G. Landis, Eds, Aquatic Toxicology and Hazard Assessment: 10th Volume, ASTM STP 971, pp. 138-148

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.

Figure C1. QA control chart for *H. azteca* acute reference tests in copper sulfate.

SOP 400.1: DISSOLVED OXYGEN METER CALIBRATION, USE, AND MAINTENANCE

Reference: MODEL 9070 Oxygen Meter Operating Instructions

Cole-Parmer Model 9070 Dissolved Oxygen Meter:

OBJECTIVE

To obtain accurate and repeatable measurements of dissolved oxygen (DO) in aqueous samples. Measurements are deemed acceptable if final calibration readings are within 10% of initial settings.

METHOD

A. CALIBRATION

1. Connect probe to meter.

2. Set "MODE" to "ZERO" and set display to 000 with "ZERO" control knob.

3. Set "MODE" to "%" and allow electrode to polarize. This will take about 10 minutes. To prevent waiting each time for the electrode to polarize each time the instrument is used, it should be stored with the electrode permanently connected. The instrument will keep the electrode polarized even when switched "OFF". If instrument has been so stored, ignore this step.

4. After polarization, set the display to 100 with the "SLOPE" control knob. The electrode should be in air saturated with water vapor for this adjustment. To achieve this, hold probe close to, but not touching (about 1 cm above), a clean sample of water.

5. The instrument can be calibrated with the electrode in dry air but a small error will occur (approx 2% of reading). This can often be ignored in practice.

6. The instrument is now ready for use in either % saturation or mg/L without further calibration. No calibration is required for Temp. measurements.

B. MEASUREMENT OF OXYGEN

1. mg/L: This Mode gives the amount of oxygen in the water directly in mg/L or ppm. In this mode, the electrode compensates for a change in solubility of oxygen in water with temperature.

2. Select the Mode required and immerse the probe in the sample to be measured. The probe must be immersed at least 40mm. The probe should be lightly shaken so that errors can be avoided due to oxygen starvation of the membrane.

C. MEASUREMENT OF TEMPERATURE

1. Set Mode to ^{o}C, immerse the temperature sensor into the sample. Read the temperature directly in ^{o}C.

D. MAINTENANCE AND STORAGE

1. In order to prevent the membrane from drying, store the probe in distilled water.

2. If the instrument is to be stored for more than 24 hours, store the membrane itself in distilled water to prevent the electrode from drying out as the membrane is porous to water vapor as well as oxygen.

3. When the "BAT" symbol appears in the top left hand corner of the display, the battery needs replacing. Remove the battery cover plate on the rear of the instrument to gain access to the batteries. Use only battery types PP3, 6FR, or MN1604.

E. LOG BOOK

1. Every time the Oxygen Meter is used, the calibration must be recorded in the Log book marked D.O. Log Book. Record the **Date**, the **Test Numbers** of the tests checked, **whether** Initial and/or Final measurements were taken, the **Meter** number, the **Slope/Zero** displayed on the meter after checking the dissolved oxygen, and the initials of the **Operators** who took the measurements.

SOP 401.1: pH METER CALIBRATION USE AND MAINTENANCE

OBJECTIVE

To obtain accurate and repeatable measurements of pH in aqueous samples. Measurements are deemed acceptable if final calibration reading are within 10% of initial settings.

METHOD

Reference: Digisense pH Meter Operating Manual

Cole-Parmer Model No. 5994

A. Calibration

1. Use fresh pH buffer every time the meter is to be calibrated. Make sure depth of solution is sufficient to cover the end of the probe.

2. Rinse probe with distilled water and blot dry with soft, lint-free napkin or cloth.

3. Uncover electrode filling hole by rolling down plastic cover. Immerse pH probe in standard buffer solution, pH 7. Measuring the temperature of the buffer solution, set the temperature of the pH meter to that of the temperature of the solution using the TEMP ^{o}C control to adjust the temperature.

4. Using the standardize control, cause the display to indicate the pH of the standardized buffer, pH 7.

5. Remove probe from the buffer solution. Rinse probe with distilled water, and blot dry.

6. Place probe in second pH buffer solution pH 10, if the expected value is greater than 7, or use pH 4 buffer if the expected value less than 7. Adjust the slope control in the side of the meter with screw driver until display indicates the pH of the second buffer solution.

7. Remove probe from buffer solution, rinse with distilled water, and blot dry. The meter is now ready to measure the pH of samples.

B. Measurement of pH:

1. Place probe in solution, making sure the sample depth is sufficiently covering the end of the probe.

2. Take temperature of solution. Adjust the TEMP ^{o}C control the pH meter to that of the solution.

3. Agitate probe and wait until the display indication to stabilize. Read pH of sample.

4. Remove probe from sample solution, rinse with distilled water and blot dry. Cover up electrode filling hole by rolling up plastic cover.

C. Routine Maintenance and Storage:

Storage of Electrode:

1. Short term storage: Immerse electrode tip in 2M KCl solution. Cover electrode filling hole with plastic cover.

2. Long term storage: Fill lower plastic cap with 2M KCl and replace it on the electrode tip. Replace electrode in shipping box. Cover electrode filling hole with plastic cover.

Routine Maintenance of pH Meter:

1. Long term storage of meter: For prolonged storage, it is suggested that the probe be disconnected, the batteries removed, and placed in a plastic bag and kept in a dry $0^{o}C$ to $70^{o}C$ environment.

2. Keep the instrument case clean and free of dust. Wipe clean with soft, lint-free cloth or napkin that has been dampened with a mild detergent and distilled water. Wipe dry.

3. Periodically inspect receptacle and controls for loosening. tighten with proper tools as necessary. If receptacles display corrosion, return the instrument for repair service.

4. Approximately every two months, or as needed, replace battery. (one nine volt alkaline battery).

5. As the instrument is designed with no other user serviceable components other than the battery, if malfunctioning or failure occurs, return instrument for repair. Make sure problem is isolated to instrument, checking battery, buffers and probes first.

D. Maintenance of pH Log book:

1. Every time the pH meter is used, the calibration must be recorded in the Log Book marked "pH Log Book". Record the **Date**, the **Sample** or **Test** numbers that were checked, whether Initial and/or Final measurements were taken, the **Meter** number, the date the **Buffer** was changed, the display **After reading** and the **Operator** who took the measurements.

402.1 CONDUCTIVITY METER CALIBRATION AND MAINTENANCE

OBJECTIVE

To obtain an estimate specific conductivity in aqueous samples. Measurements are deemed acceptable if final calibration reading are within 10% of initial settings.

METHOD

Reference: Owners Manual: Cole-Parmer 1481-55 Meter

A. Calibration:

1. To calibrate the conductivity meter use a standard calibration solution. The conductivity solution (0.1 M KCl) has a conductivity value of 12.88 mS/cm at 25°C. Use enough solution that at least 1 cm of the probe will be covered, but not to much that the liquid will cover the holes.

2. Press On switch.

3. Press the COND/TEMP switch to make the °C symbol appear on the display.

4. Measure the temperature of the calibration solution and adjust the temperature knob until the same temperature appears on the display. For best accuracy, calibrate the instrument at a temperature within +/- 2°C of the measurement temperature.

5. Press the COND/TEMP switch to make the °+-C symbol disappear.

6. Select the range.

7. Stir the liquid and tap the electrode against the bottom of the tube so that all air bubbles come out of the electrode through the holes in the sleeve.

8. Adjust the calibration screw located on the left side of the meter, until the display reads the conductivity value of the solution.

9. When taking the measurements, it may be necessary to change the range, this can be done by just pressing a different range control button as needed.

B. Routine Maintenance and Storage:

1. When not in use, keep the probe dry, and do not expose to high temperatures.

2. After use, rinse the electrode with D.I. water. For a thorough cleaning, pull off sleeve and clean electrode with cloth or mild detergent. Do not use abrasives. Note: Before removing the sleeve, note location of holes, and do not replace sleeve in the reverse direction, as inaccurate readings will result.

3. If the battery light appears, replace the battery. Use one 9 volt alkaline battery.

C. Log Book:

1. Every time the conductivity meter is used, the conductivity meter log book must be likewise filled out.
2. Enter the **Date**, the **Sample** number that is being measured, the **meter** number, the calibration readings **Before/After** the measurements were taken. Also the initials of the **operator** must be entered.

403.1 TEMPERATURE METER USE AND MAINTENANCE

OBJECTIVE

To obtain an estimate temperature in aqueous samples.

METHOD

Reference: Owner's manual, Digi-Sense Model 8528-20

1. Turn on power.
2. Change from ^{o}F to ^{o}C with control button.
3. Change thermocouple control to match color of thermocouple cord.
4. Take readings.
5. Store dry, and do not expose to high temperatures.
6. Reading are compared to an NBS traceable mercury thermometer once every three months.

SOP 800.1: WASHING OF GLASSWARE

OBJECTIVE

To provide clean glassware for use in toxicity tests and associated activities.

METHOD

A. Wash Procedure

1) Scrub with detergent in hot tap water.

2) Rinse 3X with tap water.

3) Rinse 3X with technical grade acetone.

4) Rinse 1X with tap water.

5) Rinse 3X with 5% technical grade HCl.

6) Rinse 3X with Type II deionized water.

B. New glassware which will come in direct or indirect contact with organisms must be soaked for a minimum of 24 h in 10% HCl before use.

C. Plastic cups to be used in toxicity tests (eg *C. dubia* chronic tests) must be rinsed with Type II deionized water before use.

D. Graduated cylinders, volumetric flasks, and volumetric pipets are to be washed after use.