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Evaluation of the Toxicity of Los Alamos National Laboratory Sediments to the Larval Insect *Chironomus tentans*

(Samples Collected September 21, 2001)

9863R

Prepared For

Los Alamos National Laboratory
Bldg SM30 Bikini Atoll Rd
Los Alamos, NM 35232

Prepared by

Pacific EcoRisk
835 Arnold Dr., Suite 104
Martinez, CA 94553

FIELD COPY

Karen
Report Sent To: _____

Approved By: _____

Date Sent: _____

October 2001

3618

PACIFIC ECORISK
ENVIRONMENTAL CONSULTING & TESTING

RECEIVED
JAN 31 2003
By RPY-

PACIFIC ECORISK ENVIRONMENTAL CONSULTING & TESTING

Oct 23, 2001

Steve Bolivar
Los Alamos National Laboratory
Bldg SM30 Bikini Atoll Rd
Los Alamos, NM 87545

Dear Mr. Bolivar:

I have enclosed two (2) copies of our report describing the toxicity testing of the four sediment samples that were collected on September 21, 2001. As you will recall, these sediments were tested using the larval insect *Chironomus tentans*. The results of these tests are summarized below:

Table 1. Effects of Los Alamos sediment samples on *Chironomus tentans* survival and growth

Sample ID	Mean % Survival	Mean Growth (mg, ash-free dry wt)
Control	97.5	0.32
Sediment 3283	22.5 *	0.68
Sediment 3285	68.75 *	0.37
Sediment 3287	86.25 *	0.39
Sediment 3289	82.5 *	0.44

These test results indicate that all four of the sediment samples resulted in statistically significant reductions in *Chironomus* survival; none of the sediments resulted in any reduction in *Chironomus* growth.

If you have any questions regarding the performance and interpretation of these tests, please give me a call. We look forward to hearing from you.

Sincerely,

Scott Ogle

Scott Ogle, Ph.D.
Special Projects Director

835 Arnold Drive, Suite 104 Martinez, CA 94553 Phone: 925.313.8080 Fax: 925.313.8089

Evaluation of the Toxicity of Los Alamos National Laboratory Sediments to the Larval Insect *Chironomus tentans*

(Samples Collected September 21, 2001)

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Appendix F Test Data & Summary of Statistics for the Reference Toxicant Evaluation of the *Chironomus tentans*

Evaluation of the Toxicity of Los Alamos National Laboratory Sediments to the Larval Insect *Chironomus tentans*

1.0 INTRODUCTION

The Los Alamos National Laboratory (LANL) has contracted Pacific EcoRisk (PER) to perform toxicity evaluations of several freshwater sediments. These evaluations consist of performing the U.S. EPA 10-day survival and growth sediment toxicity test with the larval insect *Chironomus tentans*. These tests were conducted for four sediment samples that were collected on September 21, 2001. This report describes the performance and results of these tests.

2.0 TEST PROCEDURES

The methods used in conducting these tests followed the guidelines established by the U.S. EPA:

- "Methods for Measuring the Toxicity and Bioaccumulation of Sediment-Associated Contaminants with Freshwater Invertebrates" (EPA/600/R-99/064).

2.1 Receipt and Handling of the Surface Water and Sediment Samples

On September 21, 2001, grab samples of overlying water and sediment were collected from four sites by LANL staff into appropriately-cleaned sample containers. These samples were placed "on ice" in ice-chest coolers and held over the week-end, after which they were shipped via overnight delivery, under chain of custody, to the PER testing laboratory in Martinez, CA (Chain-of-Custody documentation is provided in Appendix A). Upon receipt at the laboratory, an aliquot of each surface water sample was removed for analysis of initial water quality characteristics (Table 1), with the remainder of the surface water and sediment samples being stored at 4°C until needed.

Table 1. Water quality characteristics of the Los Alamos National Laboratory surface water samples

Sample ID	Temp. (°C)	pH	D.O. (mg/L)	Alkalinity (mg/L)	Hardness (mg/L)	Conductivity (µS/cm)	Total Ammonia (mg/L N)
3274/3282	10.1	6.96	6.3	81	74	186	<0.01
3276/3284	7.7	7.78	8.9	62	63	163	<0.01
3278/3286	8.5	7.83	8.2	101	94	194	<0.01
3280/3288	4.5	7.52	8.3	58	63	147	<0.01

Immediately prior to the start of the tests, each sediment was homogenized and aliquot of each was centrifuged at 2500 g for 15 minutes for collection of sediment porewater and determination of the initial porewater chemistries (Table 2).

Table 2. Sediment porewater initial water quality characteristics.

Sample ID	PH	Total Ammonia (mg/L N)	Total Sulfide (mg/L)
Sediment 3283	6.86	6.85	0.34
Sediment 3285	7.28	0.28	0.09
Sediment 3287	7.66	<0.01	0.54
Sediment 3289	7.72	<0.01	0.35

2.2 Test Organisms

At approximately 3 weeks and again at 2 weeks prior to the start of these tests, *Chironomus tentans* egg cases were obtained from a commercial supplier (Aquatic Biosystems, Ft. Collins, CO). These eggs were incubated in EPA synthetic moderately hard water at 23°C. Upon hatching, the larvae were maintained in the same water at 23°C, and were provided shredded paper towel substrate and were fed YCT and *Selenastrum capricornutum*, as per EPA guidelines. Immediately prior to the start of the tests, healthy third-instar larvae were collected from these cultures.

2.3 Sediment Toxicity Testing with *Chironomus tentans*

The freshwater sediment toxicity test with *Chironomus tentans* consists of exposing the larval insects to the sediment for 10 days, after which effects on survival and growth are evaluated. The specific procedures used in this test are described below.

Each of the four site sediments was tested at the 100% concentration only. The Control treatment sediment consisted of the same reference soil that is used as the routine control sediment by the National Biological Survey (Columbia, MO) in their freshwater sediment toxicity tests. There were eight replicates for each of the test treatments. Each replicate container consisted of a 300 mL tall-form glass beaker with a 3 cm ribbon of 540 µm mesh NITEX attached to the top of the beaker with silicone sealant. Each of the sediment samples was re-homogenized immediately prior to introduction of the sediments into the test replicates. Approximately 100 mL of sediment was loaded into each of the test replicate containers. Each of the Control treatment test replicates was then carefully filled with clean overlying water (synthetic Moderately Hard water, modified for use with *Chironomus* as per the EPA test guidelines), and each of the four site sediments were similarly filled with site-specific "surface water" collected from the same sites as the sediments. The replicates with sediments and clean overlying water were established 24 hrs prior to the introduction of the larval insects.

After this initial 24 hrs period, the overlying water in each replicate was flushed with one volume of the corresponding treatment-specific water (approximately 150 mL) using a modified Zumwalt

delivery system (Zumwalt et al. 1994). A small aliquot of the renewed overlying water in each of the eight replicates per treatment was then collected and composited for measurement of "initial" water quality characteristics (pH, D.O., conductivity, alkalinity, hardness, and total ammonia). Then 10 third-instar *Chironomus* were randomly allocated into each replicate, followed by the addition of 1.5 mL of YCT food. The test replicates were then returned to the water baths.

Each day, for the following nine days, the test replicates were pulled from the water bath, and each replicate was examined for the presence of any dead *Chironomus*. A small aliquot of the overlying water in each of the eight replicates was then collected and composited as before for measurement of "old" D.O., after which each replicate was flushed with one volume of fresh treatment water. Another small aliquot of the overlying water in each of the eight replicates was then collected and composited as before for measurement of "new" D.O., after which each replicate was fed 1.5 mL of YCT, and then replaced within the water bath.

After 10 days exposure, the replicate containers were pulled from the water bath, and an aliquot of overlying water was collected from each replicate and composited for analysis of the "final" water quality characteristics. The sediments in each replicate container were then carefully washed out and sieved using a #40 (425 um mesh) stainless steel sieve, and the number of surviving *Chironomus* determined. The surviving *Chironomus* from each replicate were then rinsed with de-ionized water, and transferred to a pre-dried and pre-tared drying pan. These were then dried at 100°C for 24 hrs and re-weighed to determine the mean "dry" weight per individual *Chironomus*. Each replicate pan was then placed into a muffle furnace and ashed at 550°C for two hrs, after which the pans were placed within a desiccator. After the pans had cooled, they were each re-weighed to determine the mean ash-free dry wt (= final ashed weight minus "dry" weight divided by the number of organisms in the pan). The data for each sediment treatment were analyzed and compared to the Control treatment to determine whether or not any statistically significant reductions in survival and/or growth were observed. All statistical analyses were performed using the ToxCalc statistical package (Version 5.0, TidePool Scientific, McKinleyville, CA).

2.3.1 Reference Toxicant Test Procedures with *Chironomus tentans*

In order to validate that the *Chironomus* used in these tests were responding in a typical fashion to chemical stress, a reference toxicant test was run. The reference toxicant test consists of an acute 96 hour static toxicity test with copper (as $CuSO_4$), at concentrations of 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 mg/L. There were two replicates at each treatment level, each replicate consisting of 400 mL of test media in a 600 mL glass beaker. The test was initiated by randomly allocating ten chironomids into each of the replicate beakers. The beakers were placed in a temperature-controlled water bath at 23°C under continual darkness.

Each replicate container was examined daily, and the number of live chironomids in each was recorded at this time. Routine water quality characteristics (D.O., pH and temperature) of the treatment waters were measured and recorded for one randomly selected replicate per treatment each day. After 96 hrs, the number of live organisms in each replicate beaker was determined. The test

response data were then compared to the ongoing database of response data from previous reference toxicant tests performed in this lab.

3.0 RESULTS

The results of the toxicity evaluations of the Los Alamos National Laboratory sediments are presented below.

3.1 Sediment Toxicity to *Chironomus tentans*

3.1.1 Toxicity of Sediment 3283

The survival results of this test are summarized below in Table 3. Briefly, there was 97.5% survival in the Control sediment, and 22.5% survival in the Sediment 3283 treatment, which was significantly less than the Control.

The growth results of this test are also summarized below in Table 2. Briefly, the mean ash-free dry weight was 0.32 mg at the Control treatment, and was 0.68 mg at the Sediment 3283 treatment, which was not significantly less than the Control.

The test data & summary of statistical analyses for this test are presented in Appendix B.

Table 3. Toxicity of Sediment 3283 to *Chironomus tentans* survival and growth.

Treatment	Rep A	Rep B	Rep C	Rep D	Rep E	Rep F	Rep G	Rep H	Mean
	% Survival								
Control	100	100	90	100	100	100	90	100	97.5
Sed-3283	30	20	20	50	0	20	10	30	22.5 *
	Mean *Chironomus* Weight (ash-free dry wt, mg)								
Control	0.34	0.33	0.38	0.28	0.31	0.32	0.35	0.29	0.32
Sed-3283	0.58	0.64	0.80	0.63	-	0.52	1.07	0.53	0.68

* - Significantly less than the Control treatment response at $p < 0.05$.

3.1.2 Toxicity of Sediment 3285

The survival results of this test are summarized below in Table 4. Briefly, there was 97.5% survival in the Control sediment, and 68.75% survival in the Sediment 3285 treatment, which was significantly less than the Control.

The growth results of this test are also summarized below in Table 4. Briefly, the mean ash-free dry weight was 0.32 mg at the Control treatment, and was 0.37 mg at the Sediment 3285 treatment, which was not significantly less than the Control.

The test data & summary of statistical analyses for this test are presented in Appendix C.

Table 4. Toxicity of Sediment 3285 to *Chironomus tentans* survival and growth.

Treatment	Rep A	Rep B	Rep C	Rep D	Rep E	Rep F	Rep G	Rep H	Mean
	% Survival								
Control	100	100	90	100	100	100	90	100	97.5
Sed-3285	80	80	80	70	60	60	90	30	68.75 *
	Mean *Chironomus* Weight (ash-free dry wt, mg)								
Control	0.34	0.33	0.38	0.28	0.31	0.32	0.35	0.29	0.32
Sed-3285	0.37	0.44	0.34	0.35	0.37	0.40	0.35	0.38	0.37

* - Significantly less than the Control treatment response at $p < 0.05$.

3.1.3 Toxicity of Sediment 3287

The survival results of this test are summarized below in Table 5. Briefly, there was 97.5% survival in the Control sediment, and 86.25% survival in the Sediment 3287 treatment, which was significantly less than the Control.

The growth results of this test are also summarized below in Table 5. Briefly, the mean ash-free dry weight was 0.32 mg at the Control treatment, and was 0.39 mg at the Sediment 3287 treatment, which was not significantly less than the Control.

The test data & summary of statistical analyses for this test are presented in Appendix D.

Table 5. Toxicity of Sediment 3287 to *Chironomus tentans* survival and growth.

Treatment	Rep A	Rep B	Rep C	Rep D	Rep E	Rep F	Rep G	Rep H	Mean
	% Survival								
Control	100	100	90	100	100	100	90	100	97.5
Sed-3287	90	90	80	80	90	90	70	100	86.25 *
	Mean *Chironomus* Weight (ash-free dry wt, mg)								
Control	0.34	0.33	0.38	0.28	0.31	0.32	0.35	0.29	0.32
Sed-3287	0.39	0.35	0.39	0.50	0.37	0.36	0.42	0.38	0.39

* - Significantly less than the Control treatment response at $p < 0.05$.

3.1.4 Toxicity of Sediment 3289

The survival results of this test are summarized below in Table 6. Briefly, there was 97.5% survival in the Control sediment, and 82.5% survival in the Sediment 3289 treatment, which was significantly less than the Control.

The growth results of this test are also summarized below in Table 6. Briefly, the mean ash-free dry weight was 0.32 mg at the Control treatment, and was 0.44 mg at the Sediment 3289 treatment, which was not significantly less than the Control.

The test data & summary of statistical analyses for this test are presented in Appendix E.

Table 6. Toxicity of Sediment 3289 to *Chironomus tentans* survival and growth.

Treatment	Rep A	Rep B	Rep C	Rep D	Rep E	Rep F	Rep G	Rep H	Mean
	% Survival								
Control	100	100	90	100	100	100	90	100	97.5
Sed-3289	90	90	90	70	60	90	90	80	82.5 *
	Mean *Chironomus* Weight (ash-free dry wt, mg)								
Control	0.34	0.33	0.38	0.28	0.31	0.32	0.35	0.29	0.32
Sed-3289	0.37	0.34	0.45	0.47	0.52	0.44	0.43	0.46	0.44

* - Significantly less than the Control treatment response at $p < 0.05$.

3.2 Results of the Reference Toxicant Evaluation of *Chironomus tentans*

The results of the reference toxicant evaluation of the *Chironomus* used in these tests are presented in Table 7. There was a mean of 95% survival at the Control treatment, and 90% survival at the 0.1 mg/L Cu treatment. At 0.2 mg/L Cu, survival decreased to 50% which was significantly less than the Control. Statistical analysis of the survival data indicated that NOEC was 0.1 mg/L Cu and the LOEC was 0.2 mg/L Cu. The EC50 was 0.29 mg/L Cu.

This EC50 value is consistent with previous performance of this test in our laboratory, indicating that these test organisms were responding to toxic stress in a typical fashion. The test data sheets and the summary of statistical analyses for this test are presented in Appendix F.

Table 7. Reference toxicant testing: Effects of copper on survival of *Chironomus tentans.*

Copper Treatment (mg/L)	% Survival in Test Replicates		**Overall Mean % Survival**
	Rep A	Rep B	
Control	90	100	95
0.1	90	90	90
0.2*	**80**	**20**	**50***
0.4*	**50**	**30**	**40***
0.8*	**20**	**0**	**10***
1.6*	**0**	**20**	**10***
3.2*	**0**	**0**	**0 ***
NOEC =	0.1 mg/L Cu		
LOEC =	0.2 mg/L Cu		
EC50 =	0.29 mg/L Cu		

* - Survival at this treatment level was significantly less than the Control at $p < 0.05$.

4.0 SUMMARY AND CONCLUSIONS

Results for the sediment toxicity evaluations of the Los Alamos freshwater sediment (and overlying site water) samples indicated that there was statistically significant reductions in *Chironomus tentans* survival for all of the sites tested (Table 8); however, there were no significant reductions in *Chironomus* growth in any of the four sediments.

Table 8. Effects of Los Alamos sediment samples on *Chironomus tentans* survival and growth.

Sample ID	Mean % Survival	Mean Weight (mg, ash-free dry wt)
Control	97.5	0.32
Sediment 3283	22.5 *	0.68
Sediment 3285	68.75 *	0.37
Sediment 3287	86.25 *	0.39
Sediment 3289	82.5 *	0.44

* - Significantly less than the Control treatment response at $p < 0.05$.

4.1 QA/QC SUMMARY

Test Conditions - Test conditions (pH, D.O., temperature, etc.) were within acceptable limits for these test organisms. All such analyses were performed according the laboratory Standard Operating Procedures.

Negative Control - The biological responses for each of the various test organisms at the Control treatment were within acceptable limits.

Positive Control - The results for the concurrent reference toxicant test were consistent with the reference toxicant test database, indicating that these test organisms were responding to toxic stress in a typical fashion. A summary of in-house reference toxicant database values are presented in Table 9.

Table 9. Summary of Reference Toxicant Database for *Chironomus tentans.*

Mean	Standard Deviation	Upper Limit (mean + 2SD)	Lower Limit (mean - 2SD)	Current Result
0.72 mg/L	0.36 mg/L	1.44 mg/L	0	0.29 mg/L

APPENDIX A

Chain-of-Custody Records for the Los Alamos National Laboratory Surface Water and Sediment Samples

Tuesday, September 25, 2001

REQUEST NUMBER: 9863R
ANALYSIS TYPE: INORG

Los Alamos
NATIONAL LABORATORY

ATTN: SCOTT OLGE
PACIFIC ECORISK
835 ARNOLD DR. SUITE 104
MARTINEZ, CA 94553

Please analyze the enclosed samples according to the schedule indicated:

SHIP DATE: 9/25/2001
REPORT DUE: 10/25/2001
TURN AROUND REQ'D: 30 days

These samples are on:

LANL Request Number: 9863R
Per Agreement Number: 35232-001-01-A
Project Cost Code: MR3A0331WEH0

RAD SCREENING: Not Required
COMMENTS: 16 - 1082 , WN;

LANL ER SMO CONTACT: Joylene Valdez MS H865 5056659968
Signature: Joylene Valdez

ANALYSIS ORDER CODE	ANALYTE(S)	SAMPLE ID	CONT ID	SAMPLE MATRIX	DATE SAMPLED	COMMENTS
NH4	3274 =	RE16-01-3282	01	W	9/21/2001	> 3274
N		RE16-01-3283	01	SS	9/21/2001	
NH4	3276	RE16-01-3284	01	W	9/21/2001	> 3284 (3276? ~~3284~~?)
NH4		RE16-01-3285	01	SS	9/21/2001	
NH4	3278 =	RE16-01-3286	01	W	9/21/2001	> 3278
NH4		RE16-01-3287	01	SS	9/21/2001	
NH4	3280 =	RE16-01-3288	01	W	9/21/2001	> 3280
NH4		RE16-01-3289	01	SS	9/21/2001	

Final Page of REQUEST NUMBER 9863R

Client wants
EPA 100.2 (Chironomus)
tests (as per 9/26/01
conversation)

Tuesday, September 25, 2001

CHAIN OF CUSTODY DOCUMENT NUMBER: 9863RC

Los Alamos
NATIONAL LABORATORY

REQUEST NUMBER: 9863R
ANALYSIS TYPE: INORG

ATTN: SCOTT OLGE
PACIFIC ECORISK
835 ARNOLD DR. SUITE 104
MARTINEZ, CA 94553

SAMPLE ID	CONT ID	CONTAINER DESCRIPTION	ANALYSIS ORDER CODE	PRESERVATIVE	MATRIX
RE16-01-3282	01	Bioassay Container	NH4	Ice	W
RE16-01-3283	01	Bioassay Container	NH4	Ice	SS
RE16-01-3284	01	Bioassay Container	NH4	Ice	W
RE16-01-3285	01	Bioassay Container	NH4	Ice	SS
RE16-01-3286	01	Bioassay Container	NH4	Ice	W
RE16-01-3287	01	Bioassay Container	NH4	Ice	SS
RE16-01-3288	01	Bioassay Container	NH4	Ice	W
RE16-01-3289	01	Bioassay Container	NH4	Ice	SS

Final Page of CHAIN OF CUSTODY DOCUMENT FOR REQUEST NUMBER 9863R

Relinquished By:		Date	Time
[illegible]	[illegible]	9-25-01	1500
PRINTED NAME	SIGNATURE		
PRINTED NAME	SIGNATURE		
PRINTED NAME	SIGNATURE		

Received By:		Date	Time
Karine Rios		9-26-01	1000
PRINTED NAME	SIGNATURE		
PRINTED NAME	SIGNATURE		
PRINTED NAME	SIGNATURE		

Received for DISPOSAL By:		Date	Time
PRINTED NAME	SIGNATURE		

Remarks:

APPENDIX B

Test Data and Summary of Statistics for the Evaluation of Sediment 3283 Toxicity to *Chironomus tentans*

10 Day Freshwater Sediment Test-Proportion Survived

tart Date:	04 Oct-01 00:00	Test ID:	6417	Sample ID:	Los Alamos Sediment 3283
nd Date:	14 Oct-01 00:00	Lab ID:	CAPER-Pacific EcoRisk	Sample Type:	FS-Freshwater Sediment
ample Date:		Protocol:	EPA 2000-Freshwater Sedim	Test Species:	CT-Chironomus tentans
omments:	Toxicity of Los Alamos Sediment 3283 to Chironomus tentans.				

Conc-%	1	2	3	4	5	6	7	8
Control	1.0000	1.0000	0.9000	1.0000	1.0000	1.0000	0.9000	1.0000
Sed 3283	0.3000	0.2000	0.2000	0.5000	0.0000	0.2000	0.1000	0.3000

			Transform: Arcsin Square Root						1-Tailed	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
Control	0.9750	1.0000	1.3713	1.2490	1.4120	5.501	8			
*Sed 3283	0.2250	0.2308	0.4770	0.1588	0.7854	38.985	8	12.604	1.761	0.0089

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9097	0.844	-0.2288	2.0921
F-Test indicates equal variances (p = 0.03)	6.0765	8.8853		

Hypothesis Test (1-tail, 0.05)

Homoscedastic t Test indicates significant differences

ToxCalc v5.0

Reviewed by: [signature]

10 Day Freshwater Sediment Test-Growth(wt)

Start Date:	04 Oct-01 00:00	Test ID:	6417	Sample ID:	Los Alamos Sediment 3283
End Date:	14 Oct-01 00:00	Lab ID:	CAPER-Pacific EcoRisk	Sample Type:	FS-Freshwater Sediment
Sample Date:		Protocol:	EPA 2000-Freshwater Sedim	Test Species:	CT-Chironomus tentans

Comments: Toxicity of Los Alamos Sediment 3283 to Chironomus tentans.

Conc-%	1	2	3	4	5	6	7	8
Control	0.3380	0.3320	0.3811	0.2760	0.3100	0.3180	0.3456	0.2910
Sed 3283	0.5800	0.6350	0.8050	0.6340	0.5250	1.0700	0.5300	

			Transform: Untransformed					Rank	1-Tailed
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical
Control	0.3240	1.0000	0.3240	0.2760	0.3811	10.179	8		
Sed 3283	0.6827	2.1074	0.6827	0.5250	1.0700	28.574	7	84.00	41.00

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.8173	0.835	1.9277	5.5246
F-Test indicates unequal variances (p = 1.42E-04)	35	9.1554		

Hypothesis Test (1-tail, 0.05)

Wilcoxon Two-Sample Test indicates no significant differences

ToxCalc v5.0

Reviewed by: [illegible]

10 Day Freshwater Sediment Toxicity Test Data

Client: Los Alamos Test ID#: 6417 Date (day 0): 10-4-01

Species: *Chironomus tentans* Organism Log#: — Organism Age/Size: 3RD INSTAR

DAY	DATE	TEMP	CONTROL D.O. (OLD)	CONTROL D.O. (NEW)	3283 D.O. (OLD)	3283 D.O. (NEW)	SIGN-OFF
1	10-5-01	22.7	5.43	7.03	3.60	6.30	[illegible] AG
			A 0 B 0 C 0 D 0		A 0 B 0 C 0 D 0		
			E 0 F 0 G 0 H 0		E 0 F 0 G 0 H 0		
2	10-6-01	22.8	7.22	7.34	6.02	6.34	[illegible]
			A 0 B 0 C 0 D 0		A 0 B 0 C 0 D 0		
			E 0 F 0 G 0 H 0		E 0 F 0 G 0 H 0		
3	10-7-01	22.8	6.78	7.68	4.72	6.96	SC JB MM
			A 0 B 0 C 0 D 0		A 0 B 0 C 0 D 0		
			E 0 F 0 G 0 H 0		E 0 F 0 G 0 H 0		
4	10-8-01	22.8	4.73	6.37	4.13	5.75	MC JB
			A 0 B 0 C 0 D 0		A 0 B 0 C 0 D 0		
			E 0 F 0 G 0 H 0		E 0 F 0 G 0 H 0		
5	10-9-01	22.7	6.40	7.22	5.97	6.46	[illegible] MC
			A 0 B 0 C 0 D 0		A 0 B 0 C 0 D 0		
			E 0 F 0 G 0 H 0		E 0 F 0 G 0 H 0		
6	10-10-01	22.8	6.17	7.20	4.70	6.80	[illegible]
			A 0 B 0 C 0 D 0		A 0 B 0 C 0 D 0		
			E 0 F 0 G 0 H 0		E 0 F 1 G 0 H 0		
7	10-11-01	22.8	5.60	7.94	4.10	6.41	MC
			A 0 B 0 C 0 D 0		A 0 B 0 C 0 D 0		
			E 0 F 0 G 0 H 0		E 0 F 0 G 0 H 0		
8	10-12-01	22.7	5.64	7.05	4.26	6.00	[illegible] KC
			A 0 B 0 C 0 D 0		A 0 B 0 C 0 D 0		
			E 0 F 0 G 0 H 0		E 0 F 0 G 0 H 0		
9	10-13-01	22.8	5.17	6.52	3.90	6.02	[illegible]
			A 0 B 0 C 0 D 0		A 0 B 0 C 0 D 0		
			E 0 F 0 G 0 H 0		E 0 F 0 G 0 H 0		
10	10-14-01	22.7	# Alive Organisms/replicate		# Alive Organisms/replicate		[illegible]
			A 10 B 10 C 9 D 10		A 3 B 2 C 2 D 5		
			E 10 F 10 G 9 H 10		E 0 F 2 G 1 H 3		

Chironomus Weight Data Sheets

Client: Los Alamos Species: *Chironomus tentans* Test Date: 10/4/01
Sample ID: Sediment 3283 Investigator: AJ Test ID: 6417

PAN Dry Weight Calculations

Pan	Concentration Replicate		Initial Weight. PAN WEIGHT (mg)	Final Weight. DRY (mg)	# organisms	Ave Dry Weight (mg)
1	Control	A	33.58	40.04	10	0.646
2		B	29.84	35.75	10	0.591
3		C	34.66	40.96	9	0.700
4		D	30.34	35.71	10	0.537
5		E	32.86	38.98	10	0.612
6		F	31.65	37.21	10	0.556
7		G	34.64	41.46	9	0.758
8		H	36.82	41.79	10	0.497
33	3283	A	33.00	35.60	3	0.867
34		B	32.28	34.43	2	1.075
35		C	35.71	38.08	2	1.185
36		D	34.10	39.66	5	1.112
37		E	28.86	—	0	—
38		F	31.18	32.82	2	0.820
39		G	32.64	34.36	1	1.720
40		H	30.49	33.03	3	0.847
QA 1			31.01	31.01		
QA 2			29.70	29.73		

Ash Free Dry Weight Calculations

Pan	Concentration Replicate		Dry Weight. (mg)	Ash ~~Free~~ Dry Weight. + PAN (mg)	# organisms	Ave. Ash Free Dry Weight (mg)
1	Control	A	40.04	36.66	10	0.338
2		B	35.75	32.43	10	0.332
3		C	40.96	37.53	9	0.381
4		D	35.71	32.95	10	0.276
5		E	38.98	35.88	10	0.310
6		F	37.21	34.03	10	0.318
7		G	41.46	38.35	9	0.346
8		H	41.79	38.88	10	~~0.346~~ 0.291
33	3283	A	35.60	33.86	3	0.580
34		B	34.43	33.16	2	0.635
35		C	38.08	36.47	2	0.805
36		D	39.66	36.49	5	0.634
37		E	—	—	0	—
38		F	32.82	31.77	2	0.525
39		G	34.36	33.29	1	1.070
40		H	33.03	31.44	3	0.530
QA						
QA						

APPENDIX C

Test Data and Summary of Statistics for the Evaluation of Sediment 3285 Toxicity to *Chironomus tentans*

10 Day Freshwater Sediment Test-Proportion Survived

Start Date:	04 Oct-01 00:00	Test ID:	6418	Sample ID:	Los Alamos Sediment 3285
End Date:	14 Oct-01 00:00	Lab ID:	CAPER-Pacific EcoRisk	Sample Type:	FS-Freshwater Sediment
Sample Date:		Protocol:	EPA 2000-EPA Freshwater S	Test Species:	CT-Chironomus tentans

Comments: Toxicity of Los Alamos Sediment 3285 to Chironomus tentans.

Conc-%	1	2	3	4	5	6	7	8
Control	1.0000	1.0000	0.9000	1.0000	1.0000	1.0000	0.9000	1.0000
Sed 3285	0.8000	0.8000	0.8000	0.7000	0.6000	0.6000	0.9000	0.3000

			Transform: Arcsin Square Root						1-Tailed	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
Control	0.9750	1.0000	1.3713	1.2490	1.4120	5.501	8			
*Sed 3285	0.6875	0.7051	0.9892	0.5796	1.2490	20.868	8	4.917	1.761	0.0106

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.8804	0.844	-1.163	2.9511
F-Test indicates equal variances (p = 0.02)	7.4868	8.8853		

Hypothesis Test (1-tail, 0.05)

Homoscedastic t Test indicates significant differences

ToxCalc v5.0

Reviewed by:

10 Day Freshwater Sediment Test-Growth(wt)

Start Date:	04 Oct-01 00:00	Test ID:	6418	Sample ID:	Los Alamos Sediment 3285
End Date:	14 Oct-01 00:00	Lab ID:	CAPER-Pacific EcoRisk	Sample Type:	FS-Freshwater Sediment
Sample Date:		Protocol:	EPAFS 94-EPA Freshwater S	Test Species:	CT-Chironomus tentans

Comments: Toxicity of Los Alamos Sediment 3285 to Chironomus tentans.

Conc-%	1	2	3	4	5	6	7	8
Control	0.3380	0.3320	0.3811	0.2760	0.3100	0.3180	0.3456	0.2910
Sed 3285	0.3700	0.4375	0.3388	0.3471	0.3683	0.3983	0.3522	0.3833

			Transform: Untransformed						1-Tailed	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
Control	0.3240	1.0000	0.3240	0.2760	0.3811	10.179	8			
Sed 3285	0.3745	1.1559	0.3745	0.3388	0.4375	8.563	8	-3.105	1.761	0.0005

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9547	0.844	0.5866	-0.0282
F-Test indicates equal variances (p = 0.94)	1.0577	8.8853		

Hypothesis Test (1-tail, 0.05)

Homoscedastic t Test indicates no significant differences

ToxCalc v5.0

Reviewed by: [signature]

10 Day Freshwater Sediment Toxicity Test Data

Client: Los Alamos Test ID#: 6418 Date (day 0): 10-4-01

Species: *Chironomus tentans* Organism Log#: — Organism Age/Size: 3rd INSTAR

DAY	DATE	TEMP	CONTROL		3285		SIGN-OFF
			D.O. (OLD)	D.O. (NEW)	D.O. (OLD)	D.O. (NEW)	
1	10-5-01	22.7	5.43	7.03	4.20	7.02	CL
			A 0 B 0 C 0 D 0		A 0 B 0 C 0 D 0		
			E 0 F 0 G 0 H 0		E 0 F 0 G 0 H 0		
2	10-6-01	22.8	7.22	7.34	6.89	7.81	CL
			A 0 B 0 C 0 D 0		A 0 B 0 C 0 D 0		
			E 0 F 0 G 0 H 0		E 0 F 0 G 0 H 0		
3	10-7-01	22.8	6.78	7.68	6.42	7.94	SC MM
			A 0 B 0 C 0 D 0		A 0 B 0 C 0 D 0		
			E 0 F 0 G 0 H 0		E 0 F 0 G 0 H 0		
4	10-8-01	22.8	4.73	6.37	5.11	7.31	mc DB
			A 0 B 0 C 0 D 0		A 0 B 0 C 0 D 0		
			E 0 F 0 G 0 H 0		E 0 F 0 G 0 H 0		
5	10-9-01	22.7	6.40	7.22	6.25	7.86	CL mc
			A 0 B 0 C 0 D 0		A 0 B 0 C 0 D 0		
			E 0 F 0 G 0 H 0		E 0 F 0 G 0 H 0		
6	10-10-01	22.8	6.17	7.20	6.05	7.89	CL
			A 0 B 0 C 0 D 0		A 0 B 0 C 0 D 0		
			E 0 F 0 G 0 H 0		E 0 F 0 G 0 H 0		
7	10-11-01	22.8	5.60	7.94	5.32	7.98	mc
			A 0 B 0 C 0 D 0		A 0 B 0 C 0 D 0		
			E 0 F 0 G 0 H 0		E 0 F 0 G 0 H 0		
8	10-12-01	22.7	5.64	7.05	6.18	7.00	CL ke
			A 0 B 0 C 0 D 0		A 0 B 0 C 0 D 0		
			E 0 F 0 G 0 H 0		E 0 F 0 G 0 H 0		
9	10-13-01	22.8	5.17	6.52	4.25	7.29	CL
			A 0 B 0 C 0 D 0		A 0 B 0 C 0 D 0		
			E 0 F 0 G 0 H 0		E 0 F 0 G 0 H 0		
10	10-14-01	22.7	# Alive Organisms/replicate		# Alive Organisms/replicate		DB
			A 10 B 10 C 9 D 10		A 8 B 8 C 8 D 7		
			E 10 F 10 G 9 H 10		E 6 F 6 G 9 H 3		

Chironomus Weight Data Sheets

Client: Los Alamos
Species: *Chironomus tentans* Test Date: 10/4/01
Sample ID: Sediment 3285
Investigator: AB Test ID: 6418

Dry Weight Calculations

Pan	Concentration	Replicate	Initial Weight. (mg)	Final Weight. (mg)	# organisms	Ave Dry Weight (mg)
1	Control	A	33.58	40.04	10	0.646
2		B	29.84	35.75	10	0.591
3		C	34.66	40.96	9	0.700
4		D	30.34	35.71	10	0.537
5		E	32.86	38.98	10	0.612
6		F	31.65	37.21	10	0.556
7		G	34.64	41.46	9	0.758
8		H	36.82	41.79	10	0.497
17	3285	A	32.10	38.30	8	0.775
18		B	32.11	39.28	8	0.896
19		C	32.74	38.18	8	0.680
20		D	33.29	38.59	7	0.757
21		E	31.57	35.49	6	0.653
22		F	31.64	36.80	6	0.860
23		G	30.78	37.60	9	0.758
24		H	33.93	36.61	3	0.893
QA 1			31.01	31.01		
QA 3			33.45	33.46		

Ash Free Dry Weight Calculations

Pan	Concentration	Replicate	Dry Weight. (mg)	Ash Free Dry Weight. (mg)	# organisms	Ave. Ash Free Dry Weight (mg)
1	Control	A	40.04	36.66	10	0.338
2		B	35.75	32.43	10	0.332
3		C	40.96	37.53	9	0.381
4		D	35.71	32.95	10	0.276
5		E	38.98	35.88	10	0.310
6		F	37.21	34.03	10	0.318
7		G	41.46	38.35	9	0.346
8		H	41.79	38.88	10	0.291
17	3285	A	38.30	35.34	8	~~0.338~~ 0.370
18		B	39.28	35.78	8	0.438
19		C	38.18	35.47	8	0.339
20		D	38.59	36.16	7	0.347
21		E	~~36.80~~ 35.49	33.28	6	0.368
22		F	~~37.60~~ 36.80	34.41	6	0.398
23		G	37.60	34.43	9	0.352
24		H	36.61	35.46	3	0.383
QA						
QA						

APPENDIX D

Test Data and Summary of Statistics for the Evaluation of Sediment 3287 Toxicity to *Chironomus tentans*

10 Day Freshwater Sediment Test-Proportion Survived

tart Date:	04 Oct-01 00:00	Test ID:	6419	Sample ID:	Los Alamos Sediment 3287
nd Date:	14 Oct-01 00:00	Lab ID:	CAPER-Pacific EcoRisk	Sample Type:	FS-Freshwater Sediment
ample Date:		Protocol:	EPAFS 94-EPA Freshwater S	Test Species:	CT-Chironomus tentans
omments:	Toxicity of Los Alamos Sediment 3287 to Chironomus tentans.				

Conc-%	1	2	3	4	5	6	7	8
Control	1.0000	1.0000	0.9000	1.0000	1.0000	1.0000	0.9000	1.0000
Sed 3287	0.9000	0.9000	0.8000	0.8000	0.9000	0.9000	0.7000	1.0000

			Transform: Arcsin Square Root					Rank	1-Tailed
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical
Control	0.9750	1.0000	1.3713	1.2490	1.4120	5.501	8		
*Sed 3287	0.8625	0.8846	1.2017	0.9912	1.4120	10.685	8	45.00	51.00

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.8319	0.844	-0.3289	0.5368
F-Test indicates equal variances (p = 0.18)	2.8969	8.8853		

Hypothesis Test (1-tail, 0.05)

Wilcoxon Two-Sample Test indicates significant differences

ToxCalc v5.0

Reviewed by:

10 Day Freshwater Sediment Test-Growth(wt)

Start Date:	04 Oct-01 00:00	Test ID:	6419	Sample ID:	Los Alamos Sediment 3287
End Date:	14 Oct-01 00:00	Lab ID:	CAPER-Pacific EcoRisk	Sample Type:	FS-Freshwater Sediment
Sample Date:		Protocol:	EPA 2000-Freshwater Sedim	Test Species:	CT-Chironomus tentans

Comments: Toxicity of Los Alamos Sediment 3287 to Chironomus tentans.

Conc-%	1	2	3	4	5	6	7	8
Control	0.3380	0.3320	0.3811	0.2760	0.3100	0.3180	0.3456	0.2910
Sed 3287	0.3933	0.3533	0.3888	0.5000	0.3744	0.3589	0.4157	0.3750

			Transform: Untransformed						1-Tailed	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
Control	0.3240	1.0000	0.3240	0.2760	0.3811	10.179	8			
Sed 3287	0.3949	1.2191	0.3949	0.3533	0.5000	11.860	8	-3.504	1.761	0.0007

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.8947	0.844	1.3786	2.431
F-Test indicates equal variances (p = 0.37)	2.0179	8.8853		

Hypothesis Test (1-tail, 0.05)

Homoscedastic t Test indicates no significant differences

ToxCalc v5.0

Reviewed by: [illegible]

10 Day Freshwater Sediment Toxicity Test Data

Client: Los Alamos Test ID#: 6419 Date (day 0): 10-4-01

Species: *Chironomus tentans* Organism Log#: — Organism Age/Size: 3rd INSTAR

DAY	DATE	TEMP	CONTROL		3287		SIGN-OFF
			D.O. (OLD)	D.O. (NEW)	D.O. (OLD)	D.O. (NEW)	
1	10-5-01	22.7	5.43	7.03	5.70	7.40	[illegible]
			A 0 B 0 C 0 D 0		A 0 B 0 C 0 D 0		
			E 0 F 0 G 0 H 0		E 0 F 0 G 0 H 0		
2	10-6-01	22.8	7.22	7.34	6.59	7.66	[illegible]
			A 0 B 0 C 0 D 0		A 0 B 0 C 0 D 0		
			E 0 F 0 G 0 H 0		E 0 F 0 G 0 H 0		
3	10-7-01	22.8	6.78	7.68	6.59	7.38	[illegible]
			A 0 B 0 C 0 D 0		A 0 B 0 C 0 D 0		
			E 0 F 0 G 0 H 0		E 0 F 0 G 0 H 0		
4	10-8-01	22.8	4.73	6.37	4.85	7.64	[illegible]
			A 0 B 0 C 0 D 0		A 0 B 0 C 0 D 0		
			E 0 F 0 G 0 H 0		E 0 F 0 G 0 H 0		
5	10-9-01	22.7	6.40	7.22	6.59	7.81	[illegible]
			A 0 B 0 C 0 D 0		A 0 B 0 C 0 D 0		
			E 0 F 0 G 0 H 0		E 0 F 0 G 0 H 0		
6	10-10-01	22.8	6.17	7.20	6.26	8.03	[illegible]
			A 0 B 0 C 0 D 0		A 0 B 0 C 0 D 0		
			E 0 F 0 G 0 H 0		E 0 F 0 G 0 H 0		
7	10-11-01	22.8	5.60	7.94	5.69	7.67	[illegible]
			A 0 B 0 C 0 D 0		A 0 B 0 C 0 D 0		
			E 0 F 0 G 0 H 0		E 0 F 0 G 0 H 0		
8	10-12-01	22.7	5.64	7.05	5.98	~~6.00~~ 6.13	[illegible]
			A 0 B 0 C 0 D 0		A 0 B 0 C 0 D 0		
			E 0 F 0 G 0 H 0		E 0 F 0 G 0 H 0		
9	10-13-01	22.9	5.17	6.52	4.36	7.44	[illegible]
			A 0 B 0 C 0 D 0		A 0 B 0 C 0 D 0		
			E 0 F 0 G 0 H 0		E 0 F 0 G 0 H 0		
10	10-14-01	22.7	# Alive Organisms/replicate		# Alive Organisms/replicate		[illegible]
			A 10 B 10 C 9 D 10		A 9 B 9 C 8 D 8		
			E 10 F 10 G 9 H 10		E 9 F 9 G 7 H 10		

Chironomus Weight Data Sheets

Client: Los Alamos Species: *Chironomus tentans* Test Date: 10/4/01
Sample ID: Sediment 3287 Investigator: [illegible] Test ID: 6419

Dry Weight Calculations

Pan	Concentration	Replicate	Initial Weight (mg)	Final Weight (mg)	# organisms	Ave Dry Weight (mg)
1	Control	A	33.58	40.04	10	0.646
2		B	29.84	35.75	10	0.591
3		C	34.66	40.96	9	0.700
4		D	30.34	35.71	10	0.537
5		E	32.86	38.98	10	0.612
6		F	31.65	37.21	10	0.556
7		G	34.64	41.46	9	0.758
8		H	36.82	41.79	10	0.497
25	3287	A	31.73	39.86	9	0.903
26		B	30.05	37.47	9	0.824
27		C	32.04	39.06	8	0.878
28		D	30.41	39.37	8	1.120
29		E	34.86	43.67	9	0.979
30		F	31.28	39.25	9	0.886
31		G	35.98	43.41	7	1.061
32		H	28.61	37.50	10	0.889
QA 1			31.01	31.01		
QA 4			30.37	30.37		

Ash Free Dry Weight Calculations

Pan	Concentration	Replicate	Dry Weight (mg)	Ash Free Dry Weight (mg)	# organisms	Ave. Ash Free Dry Weight (mg)
1	Control	A	40.04	36.66	10	0.338
2		B	35.75	32.43	10	0.332
3		C	40.96	37.53	9	0.381
4		D	35.71	32.95	10	0.276
5		E	38.98	35.88	10	0.310
6		F	37.21	34.03	10	0.318
7		G	41.46	38.35	9	0.346
8		H	41.79	38.88	10	0.346
25	3287	A	39.86	36.32	9	0.393
26		B	37.47	34.29	9	0.353
27		C	39.06	35.95	8	0.389
28		D	39.37	35.37	8	~~0.459~~ 0.500
29		E	43.67	40.30	9	0.374
30		F	39.25	36.02	9	0.359
31		G	43.41	40.50	7	0.416
32		H	37.50	33.75	10	0.375
QA						
QA						

APPENDIX E

Test Data and Summary of Statistics for the Evaluation of Sediment 3289 Toxicity to *Chironomus tentans*

10 Day Freshwater Sediment Test-Proportion Survived

Start Date:	04 Oct-01 00:00	Test ID:	6420	Sample ID:	Los Alamos Sediment 3289
End Date:	14 Oct-01 00:00	Lab ID:	CAPER-Pacific EcoRisk	Sample Type:	FS-Freshwater Sediment
Sample Date:		Protocol:	EPA 2000-Freshwater Sedim	Test Species:	CT-Chironomus tentans

Comments: Toxicity of Los Alamos Sediment 3289 to Chironomus tentans.

Conc-%	1	2	3	4	5	6	7	8
Control	1.0000	1.0000	0.9000	1.0000	1.0000	1.0000	0.9000	1.0000
Sed 3289	0.9000	0.9000	0.9000	0.7000	0.6000	0.9000	0.9000	0.8000

			Transform: Arcsin Square Root					Rank	1-Tailed
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical
Control	0.9750	1.0000	1.3713	1.2490	1.4120	5.501	8		
*Sed 3289	0.8250	0.8462	1.1537	0.8861	1.2490	12.504	8	41.00	51.00

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.8029	0.844	-1.2452	0.6617
F-Test indicates equal variances (p = 0.11)	3.6563	8.8853		

Hypothesis Test (1-tail, 0.05)

Wilcoxon Two-Sample Test indicates significant differences

ToxCalc v5.0

Reviewed by: [illegible]

10 Day Freshwater Sediment Test-Growth(wt)

art Date:	04 Oct-01 00:00	Test ID:	6420	Sample ID:	Los Alamos Sediment 3289
nd Date:	14 Oct-01 00:00	Lab ID:	CAPER-Pacific EcoRisk	Sample Type:	FS-Freshwater Sediment
ample Date:		Protocol:	EPAFS 94-EPA Freshwater S	Test Species:	CT-Chironomus tentans

omments: Toxicity of Los Alamos Sediment 3289 to Chironomus tentans.

Conc-%	1	2	3	4	5	6	7	8
Control	0.3380	0.3320	0.3811	0.2760	0.3100	0.3180	0.3456	0.2910
Sed 3289	0.3678	0.3389	0.4544	0.4714	0.5217	0.4400	0.4322	0.4563

			Transform: Untransformed						1-Tailed	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
Control	0.3240	1.0000	0.3240	0.2760	0.3811	10.179	8			
Sed 3289	0.4353	1.3438	0.4353	0.3389	0.5217	13.297	8	-4.729	1.761	0.0010

uxiliary Tests	Statistic	Critical	Skew	Kurt
hapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.973	0.844	-0.3619	0.527
-Test indicates equal variances (p = 0.16)	3.0817	8.8853		

ypothesis Test (1-tail, 0.05)

omoscedastic t Test indicates no significant differences

ToxCalc v5.0

Reviewed by: [signature]

10 Day Freshwater Sediment Toxicity Test Data

Client: Los Alamos Test ID#: 6420 Date (day 0): 10-4-01

Species: *Chironomus tentans* Organism Log#: — Organism Age/Size: 3rd INSTAR

DAY	DATE	TEMP	CONTROL D.O. (OLD)	CONTROL D.O. (NEW)	3289 D.O. (OLD)	3289 D.O. (NEW)	SIGN-OFF
1	10-5-01	22.7	5.43 A 0 B 0 C 0 D 0 E 0 F 0 G 0 H 0	7.03	5.38 A 0 B 0 C 0 D 0 E 0 F 0 G 0 H 0	6.20	OL
2	10-6-01	22.8	7.22 A 0 B 0 C 0 D 0 E 0 F 0 G 0 H 0	7.34	6.73 A 0 B 0 C 0 D 0 E 0 F 0 G 0 H 0	7.60	OL
3	10-7-01	22.8	6.78 A 0 B 0 C 0 D 0 E 0 F 0 G 0 H 0	7.68	7.28 A 0 B 0 C 0 D 0 E 0 F 0 G 0 H 0	8.23	DD & MM
4	10-8-01	22.8	4.73 A 0 B 0 C 0 D 0 E 0 F 0 G 0 H 0	6.37	5.61 A 0 B 0 C 0 D 0 E 0 F 0 G 0 H 0	7.74	MC JB
5	10-9-01	22.7	6.40 A 0 B 0 C 0 D 0 E 0 F 0 G 0 H 0	7.22	MC ~~6.95~~ 6.45 A 0 B 0 C 0 D 0 E 0 F 0 G 0 H 0	7.66	OL MC
6	10-10-01	22.8	6.17 A 0 B 0 C 0 D 0 E 0 F 0 G 0 H 0	7.20	6.71 A 0 B 0 C 0 D 0 E 0 F 0 G 0 H 0	8.16	OL
7	10-11-01	22.8	5.60 A 0 B 0 C 0 D 0 E 0 F 0 G 0 H 0	7.94	5.93 A 0 B 0 C 0 D 0 E 0 F 0 G 0 H 0	8.11	MC
8	10-12-01	22.7	5.64 A 0 B 0 C 0 D 0 E 0 F 0 G 0 H 0	7.05	5.38 A 0 B 0 C 0 D 0 E 0 F 0 G 0 H 0	7.32	OL KW
9	10-13-01	22.8	5.17 A 0 B 0 C 0 D 0 E 0 F 0 G 0 H 0	6.52	5.05 A 0 B 0 C 0 D 0 E 0 F 0 G 0 H 0	7.31	OL
10	10-14-01	22.7	# Alive Organisms/replicate A 10 B 10 C 9 D 10 E 10 F 10 G 9 H 10		# Alive Organisms/replicate A 9 B 9 C 9 D 7 E 6 F 9 G 9 H 8		DD

Chironomus Weight Data Sheets

Client: Los Alamos
Sample ID: Sediment 3289
Species: *Chironomus tentans* Test Date: 10/4/01
Investigator: AJ Test ID: 6420

Dry Weight Calculations

Pan	Concentration Replicate		Initial Weight. (mg)	Final Weight. (mg)	# organisms	Ave Dry Weight (mg)
1	Control	A	33.58	40.04	10	0.646
2		B	29.84	35.75	10	0.591
3		C	34.66	40.96	9	0.700
4		D	30.34	35.71	10	0.537
5		E	32.86	38.98	10	0.612
6		F	31.65	37.21	10	0.556
7		G	34.64	41.46	9	0.758
8		H	36.82	41.79	10	0.497
9	3289	A	30.25	37.91	9	0.851
10		B	33.06	41.07	9	0.890
11		C	27.92	37.34	9	1.047
12		D	30.11	37.51	7	1.057
13		E	34.08	41.21	6	1.188
14		F	31.88	40.34	9	0.946
15		G	34.24	43.58	9	1.038
16		H	31.74	40.25	8	1.064
QA 1			~~38.~~31.01	31.01		
QA 2			29.70	29.73		

Ash Free Dry Weight Calculations

Pan	Concentration Replicate		Dry Weight. (mg)	Ash Free Dry Weight. (mg)	# organisms	Ave. Ash Free Dry Weight (mg)
1	Control	A	40.04	36.66	10	0.338
2		B	35.75	32.43	10	0.332
3		C	40.96	37.53	9	0.381
4		D	35.71	32.95	10	0.276
5		E	38.98	35.88	10	0.310
6		F	37.21	34.03	10	0.318
7		G	41.46	38.35	9	0.346
8		H	41.79	38.88	10	0.346
9	3289	A	37.91	34.60	9	0.368
10		B	41.07	38.02	9	0.339
11		C	37.34	33.25	9	0.454
12		D	37.51	34.21	7	0.471
13		E	41.21	38.08	6	0.522
14		F	40.34	36.38	9	0.440
15		G	43.58	39.69	9	0.432
16		H	40.25	36.60	8	0.456
QA						
QA						

APPENDIX F

Test Data and Summary of Statistics for the Reference Toxicant Evaluation of the *Chironomus tentans*

Acute Test-48 Hr Survival

Start Date:	04 Oct-01 00:00	Test ID:	6421	Sample ID:	REF-Ref Toxicant
End Date:	06 Oct-01 00:00	Lab ID:	CAPER-Pacific EcoRisk	Sample Type:	CUSO-Copper sulfate
Sample Date:		Protocol:	EPAFS 94-EPA Freshwater S	Test Species:	CT-Chironomus tentans
Comments:					

Conc-mg/L	1	2
Control	0.9000	1.0000
100	0.9000	0.9000
200	0.8000	0.2000
400	0.5000	0.3000
800	0.2000	0.0000
1600	0.0000	0.2000
3200	0.0000	0.0000

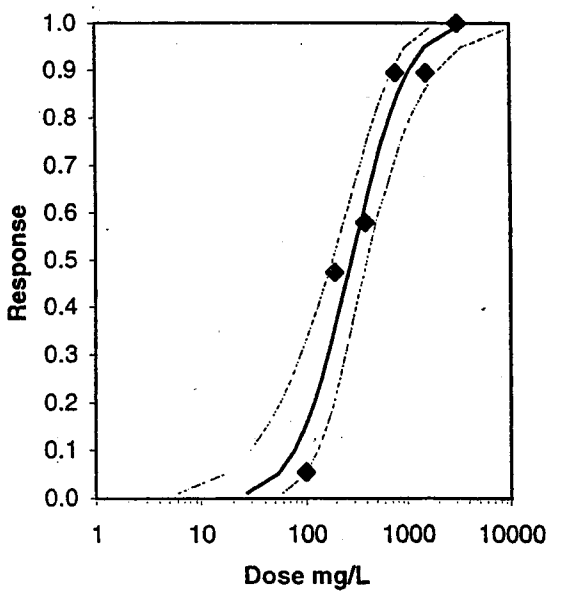
Conc-mg/L	Mean	N-Mean		Affected	Not Affected	Total	N	Fisher's Exact P	1-Tailed Critical		Number Resp	Total Number
Control	0.9500	1.0000	1.3305	1	19	20	2				1	20
100	0.9000	0.9474	1.249	2	18	20	2	0.5000	0.0500	0.6681	2	20
*200	0.5000	0.5263	0.7854	10	10	20	2	0.0017	0.0500	0.6681	10	20
*400	0.4000	0.4211	0.6825	12	8	20	2	0.0002	0.0500	0.6681	12	20
*800	0.1000	0.1053	0.3112	18	2	20	2	0.0000	0.0500	0.6681	18	20
*1600	0.1000	0.1053	0.3112	18	2	20	2	0.0000	0.0500	0.6681	18	20
3200	0.0000	0.0000	0.1588	20	0	20	2				20	20

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Fisher's Exact Test	100	200	141.42	

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	2.2583	0.4026	1.4692	3.0474	0.05	4.2027	13.277	0.38	2.4651	0.4428	6
Intercept	-0.5671	1.0521	-2.6292	1.495							
TSCR	0.0421	0.0443	-0.0448	0.129							

Point	Probits	mg/L	95% Fiducial Limits	
EC01	2.674	27.228	5.9091	58.556
EC05	3.355	54.549	16.892	99.747
EC10	3.718	79.006	29.417	133.19
EC15	3.964	101.44	42.624	162.43
EC20	4.158	123.73	57.066	190.75
EC25	4.326	146.71	73.091	219.58
EC40	4.747	225.4	133.9	318.79
EC50	5.000	291.84	188.87	407.09
EC60	5.253	377.85	260.25	532.17
EC75	5.674	580.51	416.24	884.97
EC80	5.842	688.36	491.47	1105
EC85	6.036	839.62	590.04	1447.1
EC90	6.282	1078	733.63	2056.7
EC95	6.645	1561.3	997.08	3519
EC99	7.326	3128	1726.3	9896.8



ToxCalc v5.0

Reviewed by: RB

48 Hour Acute *Chironomus* Toxicity Test Data

Client: HESE
Test ID #: 6421
Date: 10/4/01

Test Species: *Chironomus tentans*
Test Material: Copper sulfate
Control/Dilution Water: 80:20

Treatment (µgCu/L)	Temp	pH Old	pH New	D.O. Old	D.O. New	Conductivity (µS/cm)	# Live Animals A	# Live Animals B	Sign-Off
Control	23.2		8.24		8.2	180.2	10	10	Time:
100	23.2		8.28		8.2	179.4	10	10	1530
200	23.2		8.27		8.2	179.2	10	10	Date:
400	23.2		8.27		8.2	179.4	10	10	10/4/01
800	23.2		8.22		8.2	179.5	10	10	
1600	23.2		8.12		8.2	180.1	10	10	ID:
3200	23.2		7.85		8.2	182.1	10	10	MC KC
Control	23.0	7.48		5.6			10	10	Time:
100	23.0	7.90		6.0			10	9	1000
00	23.0	8.05		6.8			10	10	Date:
400	23.0	8.24		7.6			10	10	MC 10/15/01
800	23.0	8.31		7.9			10	8	
1600	23.0	8.30		8.0			8	7	ID:
3200	23.0	8.23		8.0			5	7	MC KC
Control	23.1	7.78		7.1		191.7	9	10	Time:
100	23.1	7.78		5.9		192.0	9	9	1615
200	23.1	7.78		6.0		193.0	8	2	Date:
400	23.1	7.86		5.3		191.7	5	3	10/6/01
800	23.1	8.27		8.0		192.3	2	0	
1600	23.1	8.35		8.3		192.5	0	2	ID:
3200	23.1	8.34		8.4		192.5	0	0	MC MN