[Chronic toxicity test report]. 1989

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1.0 INTRODUCTION

A static renewal, freshwater toxicity test was conducted at the Hunter/ESE, Inc. Midwest Regional Aquatic Toxicology Laboratory in St. Louis, Missouri, to determine the acute and chronic toxicity of synthetically prepared effluent from the Kennecott Project, Ladysmith, Wisconsin. The effluent was prepared to simulate actual effluent when operations commence. The tests were conducted on an invertebrate species, a water flea known as *Ceriodaphnia dubia* (*C. dubia*) and a vertebrate, species, the fathead minnow known as *Pimephales promelas (P. promelas)*. The criteria for effect were survival and reproduction of *C. dubia* and survival and growth (dry weight) of *P. promelas*.

All raw data related to this study are maintained at Hunter/ESE's St. Louis, Missouri location.

2.0 MATERIALS AND METHODS

2.1 TEST WATERS

2.1.1 Test Effluent

The synthetic effluent samples were prepared on May 22, 24 and 26, 1989 by Foth and Van Dyke, Green Bay, Wisconsin. Two types of synthetic effluent were prepared. The first simulated a lime treatment of the wastewater and the second simulated a lime and sulfide treatment. The wastewater for both samples was prepared based upon the waste characterization studies and bench scale testing performed by Foth and Van Dyke. Appendix E contains the chemical composition of each synthetic effluent stream.

Effluent samples were then shipped via Federal Express overnight delivery, received on ice, and stored at 4°C until needed. The only sample adjustment required before use was acclimation to test temperature.

Test concentrations are reported as percent effluent on a volume:volume basis.

2.1.2 Dilution Water

Flambeau River water, collected as grab samples on May 22, 24 and 26, 1989 upstream from the Kennecott Project site, was used as dilution water where necessary for the chronic tests.

2.1.3 Control Water

Three control treatments were utilized. One consisted of Flambeau River water. A second control water consisted of standard deionized laboratory culture water. A third (synthetic) non-deionized control water was prepared by Foth and Van Dyke (see Appendix E).

2.2 TEST ORGANISMS

2.2.1 *Ceriodaphnia dubia*

Gravid adult *C. dubia* were isolated from laboratory cultures (originally obtained from U.S. EPA, Cincinnati, Ohio the day prior to test initiation. Less than 24-hour old neonates released from the isolated adults during a six hour period were used to initiate testing.

2.2.2 *Pimephales promelas*

Larval (<24 hour old) *P. promelas* were obtained from Florida Bioassay Supply, Gainesville, Florida.

2.3 TEST METHODS

Methods for the 7-day static renewal *Ceriodaphnia* and *Pimephales* toxicity tests followed procedures outlined in "Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" (U.S. Environmental Protection Agency, 1985).

2.3.1 *Ceriodaphnia dubia*

C. dubia were tested on 30-mL plastic cups containing 15 mL of test solution or control water. One animal was tested per treatment container and ten containers were tested per treatment. Feeding consisted of a 0.1 mL food suspension (yeast-CEROPHYLO-trout chow) supplemented with *Selenastrum capricornutum* added daily to test chambers. Young produced were counted and removed daily. Test animals were transferred into new test solution or control water daily. The test was conducted at 24.4 to 25.7°C under ambient fluorescent lighting on a 16 hour light:8-hour dark photoperiod.

Ceriodaphnids were tested from May 23 to May 30, 1989 at treated synthetic effluent concentrations of 1.0, 3.0, 10.0, 30.0, and 100 percent. This dilution series is based on a standard logarithmic scale. Test solutions were prepared by mixing appropriate volumes of dilution water and synthetic effluent. The two treated synthetic effluent streams, the lime treatment and lime treatment/sulfide treatment were tested. The three control treatments, were tested concurrently.

2.3.2 *Pimephales promelas*

Pimephales promelas were tested in 1-liter beakers containing 500 mL of test solution or control waters. Ten animals were tested per test container and four containers per treatment. Fish were fed <24 hour old *Artemia salina* twice daily. Test solutions were siphoned down to a depth of approximately 0.6 cm being careful not to remove test animals. New test solution (500 mL) was added slowly by pouring down the side of the test container to avoid excessive turbulence. The test was conducted at 24.4 to 25.7°C under ambient fluorescent lighting on a 16-hour light:8-hour dark photoperiod. At test termination, *P. promelas* were preserved in 4 percent formalin for dry weight determination. Mean dry weight per replicate was determined by drying all surviving larvae for each replicate on pre-weighed foil at 100°C for 2 hours, re-weighing, and dividing the weight by the number of surviving larvae.

P. promelas were tested from May 23 to May 30, 1989. Like the *C. dubia*, the *P. promelas* were exposed to the two treated synthetic effluent concentrations of 1.0, 3.0, 10.0, 30.0, and 100 percent as well as the control waters.

2.4 QUALITY ASSURANCE

A reference toxicant test using sodium dodecylsulfate (SDS) was conducted with neonate *C. dubia*. The static test was conducted for 48 hours at test concentrations of 0.5, 1.0, 2.5, 5.0, and 10.0 mg/L. A control treatment consisting of reconstituted freshwater was also run. Dilution water was laboratory reconstituted freshwater. *Ceriodaphnia* were tested in glass crystallizing dishes containing 200 mL of test solution. Ten animals were tested per test container. The test was documented at 22±2°C. *Ceriodaphnia* were tested May 18 to May 20, 1989 and were from the same culture used to obtain test organisms.

A reference toxicant using SDS was conducted from April 25-27, 1989 using <24-hour old *P. promelas*. The static test was conducted for 48 hours at test concentrations of 1.0, 2.5, 5.0, 10.0, and 20.0 mg/L. A control treatment consisting of reconstituted freshwater was run concurrently. *P. promelas* were tested in glass beakers with 500 mL of test solution. The test was conducted at 22±2°C.

2.5 STATISTICAL ANALYSIS

C. dubia reproduction data were determined to be normally distributed by Bartlett's test for homogeneity of variance. *C. dubia* reproduction data were analyzed using Steel's Many-One Rank Test and Kruskal-Wallis ANOVA by Ranks and Dunns Multiple Comparison.

Growth data from the *P. promelas* chronic test were analyzed using Analysis of Variance (ANOVA) and Dunnett's procedure. Transformation of raw *P. promelas* data was not required as the raw data was normally distributed with homogeneity of variance as determined by Chi square test for normality and Bartlett's test for homogeneity of variance.

All analyses were conducted at a probability level of ≤ 0.05.

3.0 RESULTS AND DISCUSSION

3.1 EFFLUENT TEST RESULTS

3.1.1 Acute Toxicity Result

Under DNR regulations (NR 106), a determination of acute toxicity can be made from observing species mortality in a chronic test. For aquatic invertebrates, acute toxicity is determined to be less than 50 percent survival in 100 percent effluent for 48 hours. For aquatic vertebrates acute toxicity is determined to be less than 50 percent survival in 100 percent effluent for 96 hours. Utilizing this test, there was no acute toxicity reported for any species for any of the effluent tested or control waters tested.

Vertebrate Species Results.

The 96-hour mortality in 100 percent lime treatment effluent concentrations was 10 percent (see Table A-1) and 5 percent in 100 percent lime/sulfide treatment (see Table A-2). All of these results show survival well above the 50 percent level required. The 96-hour mortality for the control tests was 5 percent in the Flambeau River, 0 percent in the laboratory control and 5 percent in the Foth and Van Dyke control, respectively (see Table A-3).

Although mortality for the entire 7-day period is not a criteria for passing the acute test, it should be noted that final mortality in the lime/sulfide treatment ranged from 5 percent in the 100 percent effluent to 42.5 percent in the 30 percent effluent (see Table A-2) which compared favorably to the Flambeau River control mortality of 37.5 percent.

Invertebrate Species Results.

The 48-hour tests of the invertebrate *C. dubia* showed 0 percent mortality (100 percent survival) in 100 percent effluent from both the lime treatment and in the lime/sulfide treatment (Table A-4 and A-5, respectively). The three control waters also showed 0 percent mortality in the first 48 hours.

Again, while not part of the acute test, it should be noted that survival of *C. dubia* over the entire 7 day test period was 100 percent in the lime treatment except at the 100 percent effluent level where survival was 11.1. In the lime/sulfide treatment survival ranged from 90 percent in the 1.0 percent and 3.0 percent effluent to 100 percent in the 10.0, 30.0 and 100 percent effluent concentrations (which was better than the 70 percent survival in the Flambeau River control sample).

3.1.2 Chronic Toxicity Test

The determination of chronic toxicity involves more detailed analysis. Under standard EPA test methods and DNR regulations (NR 106), and identification of chronic toxicity is made by a determination that there is a statistically significant adverse affect on the test species exposed to an effluent concentration equal to the instream waste concentration (IWC) for a seven day period when compared to the control group population. The criteria for determining an adverse chronic affect were reproduction of *C. dubia* and growth (dry weight) of *P. promelas*.

Under DNR regulations (NR 106), the instream waste concentration is the projected annual average wastewater flow divided by one fourth of the 7Q10 of the receiving water. (7Q10 is the average minimum 7-day flow which occurs once in 10 years.) The projected IWC here is projected to be 1.06 percent (see Appendix D).

Utilizing these tests, there were no significant adverse chronic affects on any of the species for any of the effluent concentrations tested which approximate the projected instream waste concentration. The few statistically significant affects which were observed did not occur until 30 percent or 100 percent effluent was used--which is one to two orders of magnitude above the applicable IWC level.

Vertebrate Species Results

Chronic effects of the tested synthetic effluents were based upon the growth of *P. promelas* in each effluent concentration and treatment. Mean dry weight of larvae exposed to the Flambeau River, laboratory and synthetic control treatments ranged from 0.28 to 0.38 mg, 0.36 to 0.39 mg, and 0.26 to 0.40 mg, respectively (see Table A-6). Statistical analysis of the lime treatment synthetic effluent indicates that exposure of *P. promelas* to the effluent resulted no significant impact on growth when compared to the Flambeau River control except at the 100 percent level. Statistical analysis of the lime treatment/sulfide treatment synthetic effluent indicates no significant impact on growth occurred except when *P. promelas* were exposed at 30 percent effluent level. All other lime treatment/sulfide treatment synthetic effluent concentrations, including the 100 percent concentration, did not result in less growth when compared to the Flambeau River control.

Invertebrate Species Results

The total number of young produced in the control tests was 21.4 per surviving adult in the laboratory control, 23.0 per surviving adult in the Flambeau River control, and 22.0 per surviving adult in the Foth and Van Dyke synthetic control (see Table A-7). The average production of young in the lime treatment synthetic effluent was 24.6, 22.9, 23.2, 22.8, and 2.0 per surviving adult in the 1.0, 3.0, 10.0, 30.0, and 100 percent effluents, respectively (see Table A-4). Within the lime treatment/sulfide treatment test dilutions, *C. dubia* production was 22.6, 21.9, 23.9, 24.1, and 3.3 per surviving adult in the 1.0, 3.0, 10.0, 30.0, and 100 percent effluents, respectively.

Statistical analysis of the lime treatment concentrations did not include the 100 percent lime treatment concentration due to the mortality to *C. dubia* at this concentration. Analysis of the other concentrations (1.0, 3.0, 10.0, and 3.0 percent) indicates that exposure of *C. dubia* to these concentrations of lime treatment synthetic effluent did not result in significantly less neonate production compared to neonate production in the Flambeau River control.

Statistical analysis indicates that exposure of *C. dubia* to the lime treatment/ sulfide treatment resulted in no significant impact on neonate production when compared to the Flambeau River control except at the 100 percent level.

Water quality parameters remained within the acceptable ranges throughout the test (Appendix B).

3.2 QUALITY ASSURANCE TEST RESULTS

Mortality of *C. dubia* during the 48-hour exposure to SDS is reported in Table A-8. The 48-hour LC_{50} for *C. dubia* was 2.3 mg/L with 95 percent confidence limits of 1.6 to 3.4 mg/L.

Mortality of *P. promelas* during the 48-hour exposure to SDS is reported in Table A-9. The 48-hour LC_{50} for *P. promelas* was 5.0 mg/L with 95 percent confidence limits of 2.5 to 20.0 mg/L. These results fall within expected values for reference toxicant tests conducted at Hunter/ESE.

3.3 TEST ANOMALIES

The tray holding the Foth and Van Dyke synthetic water control *C. dubia* test chambers broke on Day 4 resulting in the loss of 9 of the 10 replicates. This did not significantly impact the test results because the Foth and Van Dyke control was one of three control samples and correlated with the other control samples until the accident on Day 4.

Additionally, replicate A of the fathead minnow chronic lime treatment effluent test was dropped because of unexplained mortality in 24 hours possibly due to acetone contamination of the glassware.

3.4 DISCUSSION

Analysis of the *C. dubia* test results indicates no acute toxicity affects for either the lime or lime and sulfide treatment effluent. In addition, the 1.0, 3.0, 10.0, and 30.0 percent effluent dilutions for both synthetic effluent treatments produced no significant chronic toxicity (reproductive) affects when compared to the Flambeau River control. Since the applicable IWC is 1.06 percent, no chronic toxicity is expected from the effluent. Only at the 100 percent effluent level were significant chronic affects observed.

P. promelas test results also indicate no acute toxicity affects for either synthetic effluent. There were no significant chronic toxicity (growth) at levels approximating the IWC for either effluent treatment.

Only two chronic affects were observed and these were at levels one or two orders of magnitude above the IWC. In the lime treatment there were significant affects at the 100 percent concentration.

In the lime treatment/sulfide treatment synthetic effluent, the 30 percent effluent produced significant chronic toxicity. This result may be an outlier as the 100 percent lime treatment/sulfide treatment synthetic effluent did not produce significant chronic toxicity. This is particularly apparent in that minimal acute toxicity to *P. promelas* was produced in this sample in all concentrations of the lime treatment/sulfide treatment effluent. Additionally, some acute mortality was observed in the Flambeau River control samples which may have influenced the results of the test.

In summary, these tests show no acute toxicity affects or chronic toxicity affects at the IWC from either synthetic effluent. The fact that observable chronic toxicity affects did not occur until the effluent concentration was increased by one to two orders of magnitude also allows a considerable "safety" factor in projecting these results from the synthetic effluent to actual treated effluent.

APPENDIX A

Results

Table A-1. Cumulative Mortality of the Fathead Minnow (*Pimephales promelas*) During a 7-day Exposure to Synthetic (Lime Treatment) Effluent of the Kennecott Project, Ladysmith, Wisconsin, May 23-30, 1989

Substance	Replicate	N	Percent Mortality Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Total Mortality
LT 1.0%	B	10	0	0	0	0	0	50	50	
	C	10	0	0	0	0	0	80	80	
	D	10	0	0	0	0	0	20	20	
						0*				50.0%
LT 3.0%	A	10	0	0	10	10	10	10	10	
	B	10	0	0	0	0	0	10	10	
	C	10	0	0	0	0	0	40	40	
	D	10	0	0	10	20	30	30	30	
						7.5*				22.5%
LT 10%	A	10	0	0	0	0	0	0	10	
	B	10	0	0	0	0	10	50	50	
	C	10	0	10	10	10	10	80	80	
	D	10	0	0	0	0	10	70	70	
						2.5*				52.5%
LT 30%	A	10	10	10	10	20	40	40	40	
	B	10	0	0	20	30	50	50	50	
	C	10	0	0	0	0	10	30	30	
	D	10	0	0	0	20	20	20	20	
						17.5*				35.0%
LT 100%	A	10	0	0	0	0	10	20	30	
	B	10	0	0	0	0	20	40	40	
	C	10	0	10	10	20	40	50	60	
	D	10	0	0	10	20	30	50	80	
						10*				52.5%

* 96-hour mortality (percent).

Source: Hunter/ESE, 1989.

Table A-2. Cumulative Mortality of the Fathead Minnow (*Pimephales promelas*) During a 7-day Exposure to Synthetic (Lime Treatment/Sulfide Treatment) Effluent of the Kennecott Project, Ladysmith, Wisconsin, May 23-30, 1989

			Percent Mortality							
Substance	Replicate	N	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Total Mortality
LTST 1.0%	A	10	10	10	10	20	20	20	20	
	B	10	0	0	0	0	0	0	10	
	C	10	10	10	10	10	10	30	30	
	D	10	0	10	10	20	20	40	40	
						12.5*				25.0%
LTST 3.0%	A	10	0	10	10	10	10	10	10	
	B	10	0	0	0	0	0	20	20	
	C	10	0	0	0	20	30	50	50	
	D	10	0	0	0	10	10	10	10	
						10*				22.5%
LTST 10%	A	10	0	0	0	10	10	40	40	
	B	10	0	0	0	0	0	50	50	
	C	10	0	0	0	0	10	10	10	
	D	10	0	0	0	0	10	40	40	
						2.5*				35.0%
LTST 30%	A	10	0	0	0	0	0	40	40	
	B	10	20	20	20	20	20	60	60	
	C	10	10	10	10	20	30	40	40	
	D	10	0	0	0	0	20	20	30	
						10*				42.5%
LTST 100%	A	10	0	0	0	0	0	0	0	
	B	10	0	10	10	10	10	10	10	
	C	10	0	0	0	0	0	0	0	
	D	10	0	0	10	10	10	10	10	
						5*				5.0%

* 96-hour mortality (percent).

Source: Hunter/ESE, 1989.

Table A-3. Cumulative Mortality of the Fathead Minnow (*Pimephales promelas*) During a 7-day Exposure to Control Samples for the Kennecott Project, Ladysmith, Wisconsin, May 23-30, 1989

Substance	Replicate	N	Percent Mortality Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Total Mortality
Flambeau River Control	A	10	0	0	10	10	10	10	10	
	B	10	0	0	0	0	0	30	30	
	C	10	0	0	0	0	0	60	60	
	D	10	0	0	0	10	50	50	50	
						5*				37.5%
Laboratory Water Control	A	10	0	0	0	0	10	10	10	
	B	10	0	0	0	0	0	10	10	
	C	10	0	0	0	0	0	0	0	
	D	10	0	0	0	0	0	0	0	
						0*				5%
Foth and Van Dyke Control	A	10	10	10	10	10	10	10	10	
	B	10	0	0	0	0	0	0	0	
	C	10	0	0	10	10	10	10	10	
	D	10	0	0	0	0	0	0	0	
						5*				5%

* 96-hour mortality (percent).

Source: Hunter/ESE, 1989.

Table A-4. Daily Survival and Reproduction Results from ***Ceriodaphnia*** Exposed to Synthetic (Lime Treatment) Effluent the Kennecott Project, Ladysmith, Wisconsin, May 23-30, 1989 (Page 1 of 2)

Effluent Concentration	Days No.	Replicate A	B	C	D	E	F	G	H	I	J	Total Live Young	No. of Live Adults
LT 1%	0	0	0	0	0	0	0	0	0	0	0	0	10
	1	0	0	0	0	0	0	0	0	0	0	0	10
	2	0	0	0	0	0	0	0	0	0	0	0	10 (100††)
	3	0	0	0	0	0	0	0	0	3	0	3	10
	4	4	4	4	4	4	4	4	0	3	3	34	10
	5	0	0	9	0	0	7	0	9	6	0	31	10
	6	7	6	0	7	6	6	13	11	6	8	70	10
	7	10	12	15	12	14	13	8	0	12	12	108	10
Total		21	22	28	23	24	30	25	20	30	23	246	100** (24.6 per surviving adult)
LT 3%	0	0	0	0	0	0	0	0	0	0	0	0	10
	1	0	0	0	0	0	0	0	0	0	0	0	10
	2	0	0	0	0	0	0	0	0	0	0	0	10 (100††)
	3	0	0	0	0	0	0	0	0	0	0	0	10
	4	4	4	4	5	4	4	4	4	4	4	41	10
	5	0	0	0	0	0	6	8	8	6	0	28	10
	6	5	7	6	8	0	6	0	6	0	5	43	10
	7	12	9	10	12	15	10	10	12	15	12	117	10
Total		21	20	20	25	19	26	22	30	25	21	229	100** (22.9 per surviving adult)
LT 10%	0	0	0	0	0	0	0	0	0	0	0	0	10
	1	0	0	0	0	0	0	0	0	0	0	0	10
	2	0	0	0	0	0	0	0	0	0	0	0	10 (100††)
	3	0	0	0	0	0	2	0	0	0	0	2	10
	4	3	4	4	4	3	0	3	5	5	4	35	10
	5	0	0	8	6	6	12	0	0	10	0	42	10
	6	7	7	0	0	0	0	7	7	0	8	36	10
	7	12	13	14	12	11	10	12	10	11	12	117	10
Total		22	24	26	22	20	24	22	22	26	24	232	100** (23.2 per surviving adult)
LT 30%	0	0	0	0	0	0	0	0	0	0	0	0	10
	1	0	0	0	0	0	0	0	0	0	0	0	10
	2	0	0	0	0	0	0	0	0	0	0	0	10 (100††)
	3	6	0	0	0	0	0	0	0	0	0	6	10
	4	0	1	1	3	2	2	0	0	3	5	17	10
	5	9	0	8	10	0	0	7	12	0	6	52	10
	6	13	4	0	0	8	8	0	0	8	6	47	10
	7	1	12	10	14	10	13	9	13	12	12	106	10
Total		29	17	19	27	20	23	16	25	23	29	228	100** (22.8 per surviving adult)

Table A-4. Daily Survival and Reproduction Results from ***Ceriodaphnia*** Exposed to Synthetic (Lime Treatment) Effluent the Kennecott Project, Ladysmith, Wisconsin, May 23-30, 1989 (Page 2 of 2)

Effluent Concentration	Days No.	Replicate A	B	C	D	E	F	G	H	I	J	Total Live Young	No. of Live Adults
LT 100%	0	0	0	0	0	0	0	0	0	0	0	0	10
	1	0	0	0	0	0	0	0	0	0	0	0	10
	2	0	0	0	0	0	0	+	0	0	0	0	9 (100††)
	3	0	0	0	0	0	0	--	0	0	0	0	9
	4	0	*	0	0	0	0	--	0	0	0	0	8
	5	0	--	*	0	0	0	--	*	0	*	0	5
	6	0	--	--	0	*	*	--	--	0	--	0	3
	7	8*	--	--	2	--	--	--	--	*	--	10	1
Total		8	0	0	2	0	0	0	0	0	0	10	11.1** (2.0 per surviving adult)

* Adult not living by the test reading.
** Survival (percent).
\+ Chamber accidentally spilled--replicate lost, n=9.
†† 48-hour survival (percent).

Source: Hunter/ESE, 1989.

Table A-5. Daily Survival and Reproduction Results from ***Ceriodaphnia*** Exposed to Synthetic (Lime Treatment/Sulfide Treatment) of the Kennecott Project, Ladysmith, Wisconsin, May 23-30, 1989 (Page 1 of 2)

Effluent Concentration	Days No.	Replicate A	B	C	D	E	F	G	H	I	J	Total Live Young	No. of Live Adults
LTST 1%	0	0	0	0	0	0	0	0	0	0	0	0	10
	1	0	0	0	0	0	0	0	0	0	0	0	10
	2	0	0	0	0	0	0	0	0	0	0	0	10 (100††)
	3	0	0	0	0	0	0	0	0	0	0	0	10
	4	4	4	4	4	4	4	4	0	5	4	37	10
	5	9	7	8	9	8	8	8	6	8	8	75	10
	6	0	0	0	0	0	13	0	0	+	0	13	9
	7	14	11	16	12	12	0	4	12	--	10	91	9
Total		27	22	28	25	24	25	16	18	13	22	216	90** (22.6 per surviving adult)
LTST 3%	0	0	0	0	0	0	0	0	0	0	0	0	10
	1	0	0	0	0	0	0	0	0	0	0	0	10
	2	0	0	0	0	0	0	0	0	0	0	0	10 (100††)
	3	0	4	0	0	5	0	0	4	0	0	13	10
	4	6	0	6	4	0	4	3	0	5	3	31	10
	5	6	8	8	0	4	6	10	7	8+	8	65	10
	6	3	0	0	7	11	0	0	10	*	0	31	9
	7	5	13	12	8	0	8	12	0	--	12	70	9
Total		20	25	26	19	20	18	25	21	13	23	210	90** (21.9 per surviving adult)
LTST 10%	0	0	0	0	0	0	0	0	0	0	0	0	10
	1	0	0	0	0	0	0	0	0	0	0	0	10
	2	0	0	0	0	0	0	0	0	0	0	0	10 (100††)
	3	0	0	0	4	0	0	0	0	0	0	4	10
	4	4	4	3	0	5	4	4	4	5	5	38	10
	5	6	10	7	8	7	7	6	6	8	8	73	10
	6	0	0	0	13	0	6	0	0	0	0	19	10
	7	12	12	12	0	13	11	13	10	12	10	105	10
Total		22	26	22	25	25	28	23	20	25	23	239	100** (23.9 per surviving adult)
LTST 30%	0	0	0	0	0	0	0	0	0	0	0	0	10
	1	0	0	0	0	0	0	0	0	0	0	0	10
	2	0	0	0	0	0	0	0	0	0	0	0	10 (100††)
	3	6	0	0	0	0	5	0	4	0	0	15	10
	4	0	3	3	4	4	2	4	0	4	4	28	10
	5	9	6	8	8	7	7	8	10	8	12	83	10
	6	14	5	0	0	1	12	0	0	0	0	32	10
	7	0	10	12	11	12	0	14	11	0	13	83	10
Total		29	24	23	23	24	26	26	25	12	29	241	100** (24.1 per surviving adult)

Table A-5. Daily Survival and Reproduction Results from ***Ceriodaphnia*** Exposed to Synthetic (Lime Treatment/Sulfide Treatment) of the Kennecott Project, Ladysmith, Wisconsin, May 23-30, 1989 (Page 2 of 2)

Effluent Concentration	Days No.	Replicate A	B	C	D	E	F	G	H	I	J	Total Live Young	No. of Live Adults
LTST 100%	0	0	0	0	0	0	0	0	0	0	0	0	10
	1	0	0	0	0	0	0	0	0	0	0	0	10
	2	0	0	0	0	0	0	0	0	0	0	0	10 (100††)
	3	0	0	0	0	0	0	0	0	2	0	2	10
	4	0	0	0	0	0	2	0	0	0	0	2	10
	5	0	0	0	0	0	0	0	0	0	0	0	10
	6	3	2	2	2	0	2	0	0	0	6	17	10
	7	2	0	0	0	0	3	0	0	0	7	12	10
Total		5	2	2	2	0	7	0	0	2	13	33	100** (3.3 per surviving adult)

* Adult not living by the test reading.
** Survival (percent).
\+ Adult damaged during renewal--discarded.
†† 48-hour survival (percent).

Source: Hunter/ESE, 1989.

Table A-6. Individual Mean Dry Weight (mg) of *Pimephales promelas* Larvae Exposed to Kennecott Project Synthetic Effluent, Ladysmith, Wisconsin, May 23-30, 1989

Effluent Concentration	Replicate A	Replicate B	Replicate C	Replicate D
Flambeau River Control	0.37	0.38	0.35	0.28
Culture Water Control	0.38	0.36	0.38	0.39
Foth-Van Dyke Control	0.40	0.26	0.27	0.37
Lime Treatment Synthetic Effluent				
1.0%	--	0.40	0.35	0.29
3.0%	0.33	0.36	0.30	0.17
10%	0.21	0.30	0.35	0.23
30%	0.25	0.26	0.26	0.30
100%	0.16	0.22	0.15	0.15
Lime Treatment/Sulfide Treatment Synthetic Effluent				
1.0%	0.35	0.32	0.34	0.28
3.0%	0.33	0.30	0.32	0.22
10%	0.32	0.28	0.26	0.28
30%	0.30	0.28	0.22	0.24
100%	0.32	0.33	0.33	0.29

Source: Hunter/ESE, 1989.

Table A-7. Daily Survival and Reproduction Results from *Ceriodaphnia* Exposed to Control Samples for the Kennecott Project, Ladysmith, Wisconsin, May 23-30, 1989

Effluent Concentration	Days No.	Replicate A	B	C	D	E	F	G	H	I	J	Total Live Young	No. of Live Adults
Laboratory Control	0	0	0	0	0	0	0	0	0	0	0	0	10
	1	0	0	0	0	0	0	0	0	0	0	0	10
	2	0	0	0	0	0	0	0	0	0	0	0	10 (100†)
	3	0	0	0	0	0	0	0	0	0	0	0	10
	4	3	5	4	*	*	4	3	4	5	5	33	8
	5	0	6	0	--	--	9	8	4	6	6	39	8
	6	+	0	5	--	--	0	0	4	0	0	9	7
	7	--	10	11	--	--	11	8	8	12	10	70	7
Total		3	21	20	0	0	24	19	20	23	21	151	70** (21.4 per surviving adult)
Flambeau River Control	0	0	0	0	0	0	0	0	0	0	0	0	10
	1	0	0	0	0	0	0	0	0	0	0	0	10
	2	0	0	0	0	0	0	0	0	0	0	0	10 (100†)
	3	0	0	0	0	0	++	0	0	0	2	2	9
	4	6	6	4	4	4	--	4	3	++	1	32	8
	5	8	8	0	0	0	--	0	0	--	6	22	8
	6	0	4	7	7	7	--	5	4	--	10	44	8
	7	14	13	12	12	15	--	10	8	--	0	84	8
Total		28	31	23	23	26	0	19	15	0	19	184	80** (23.0 per surviving adult)
Foth and Van Dyke Control	0	0	0	0	0	0	0	0	0	0	0	0	10
	1	0	0	0	0	0	0	0	0	0	0	0	10
	2	0	0	0	0	0	0	0	0	0	0	0	10 (100†)
	3	0	0	0	0	0	0	0	0	3	0	3	10
	4	++	2	++	++	++	++	++	++	++	++	2	1
	5	--	8	--	--	--	--	--	--	--	--	8	1
	6	--	0	--	--	--	--	--	--	--	--	0	1
	7	--	12	--	--	--	--	--	--	--	--	12	1
Total		0	22	0	0	0	0	0	0	3	0	25	100** (22.0 per surviving adult)

* Adult not living by the test reading.
** Survival (percent).
\+ Test chamber washed out by trough water--replicate lost.
++ Carry tray broke resulting in the loss of the chambers and replicates. In the Foth and Van Dyke control, all adults appeared healthy and had eyed up young prior to the loss of the replicates, (n=1).
† 48-hour survival (percent).

Source: Hunter/ESE, 1989.

Table A-8. Mortality of *Ceriodaphnia dubia* Exposed to the Reference Toxicant, SDS, for 48 Hours, May 18-20, 1989

Nominal Concentration (mg/L)	Cumulative Mortality (%)	
	24 Hour	48 Hour
Control	0	0
0.5	0	0
1.0	20	20
2.5	50	50
5.0	80	80
10.0	100	100

Table A-9. Mortality of *Pimephales promelas* Larvae Exposed to the Reference Toxicant, SDS, for 48 Hours, April 25-27, 1989

Nominal Concentration (mg/L)	Cumulative Mortality (%)	
	24 Hour	48 Hour
Control	0	0
1.0	0	0
2.5	0	0
5.0	0	50
10.0	30	70
20.0	100	100

Source: Hunter/ESE, 1989.

APPENDIX B

Water Quality

Table B-1. Conductivity, Hardness, and Alkalinity Measurements for Water Samples Used in Static Renewal Toxicity Tests Conducted for Kennecott, Ladysmith, Wisconsin, May 23-30, 1989

Substance	Sample No.	Hardness*	Alkalinity*	Conductivity+
Flambeau River	1	26	30	93
	2	24	26	92
	3	32	32	90
	4	22	30	90
Synthetic water control	1	26	28	130
	2	32	30	158
	3	26	30	157
Lime treatment	1	28	32	192
	2	26	30	189
	3	24	28	186
Lime-sulfite treatment	1	30	30	185
	2	30	32	177
	3	28	28	176

* mg/L as $CaCO_3$
+ microhms/cm

Source: Hunter/ESE, 1989.

Table B-2. Daily Temperature (°C) Measurements for Fathead Minnow Larvae Exposed to Synthetic Effluents, Kennecott, Ladysmith, Wisconsin, May 23-30, 1989

	Laboratory Control				Synthetic Control				River Control			
	A	B	C	D	A	B	C	D	A	B	C	D
Day 0-New	25.7	25.7	25.7	25.7	25.7	25.7	25.7	25.7	25.7	25.7	25.7	25.7
Day 1-New	25.4	25.4	25.4	25.4	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5
Day 1-Old	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4
Day 2-New	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4
Day 2-Old	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5
Day 3-New	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4
Day 3-Old	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4
Day 4-New	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7
Day 4-Old	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7
Day 5-New	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4
Day 5-Old	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4
Day 6-New	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7
Day 6-Old	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7
Day 7-Old	25.6	25.6	25.6	25.6	25.6	25.6	25.6	25.6	25.6	25.6	25.6	25.6

Source: Hunter-ESE, 1989.

Table B-3. Daily Temperature (°C) Measurements for Fathead Minnow Larvae Exposed to Synthetic Effluents, Kennecott, Ladysmith, Wisconsin, May 23-30, 1989

	1% LT				3% LT				10% LT				30% LT			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Day 0-New	25.7	25.7	25.7	25.7	25.7	25.7	25.7	25.7	25.7	25.7	25.7	25.7	25.7	25.7	25.7	25.7
Day 1-New	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5
Day 1-Old	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4
Day 2-New	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4
Day 2-Old	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5
Day 3-New	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4
Day 3-Old	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4
Day 4-New	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7
Day 4-Old	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7
Day 5-New	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4
Day 5-Old	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4
Day 6-New	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7
Day 6-Old	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.7
Day 7-Old	25.6	25.6	25.6	25.6	25.6	25.6	25.6	25.6	25.6	25.6	25.6	25.6	25.6	25.6	25.6	25.6

Source: Hunter-ESE, 1989.

Table B-4. Daily Temperature (°C) Measurements for Fathead Minnow Larvae Exposed to Synthetic Effluents, Kennecott, Ladysmith, Wisconsin, May 23-30, 1989

	100% LT				1% LTST				3% LTST				10% LTST			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Day 0-New	25.7	25.7	25.7	25.7	25.7	25.7	25.7	25.7	25.7	25.7	25.7	25.7	25.7	25.7	25.7	25.7
Day 1-New	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5
Day 1-Old	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4
Day 2-New	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4
Day 2-Old	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5
Day 3-New	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4
Day 3-Old	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4
Day 4-New	24.6	24.6	24.6	24.6	24.6	24.6	24.6	24.6	24.6	24.6	24.6	24.6	24.6	24.6	24.6	24.6
Day 4-Old	24.6	24.6	24.6	24.6	24.6	24.6	24.6	24.6	24.6	24.6	24.6	24.6	24.6	24.6	24.6	24.6
Day 5-New	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4
Day 5-Old	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4
Day 6-New	24.7	24.7	24.7	24.7	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Day 6-Old	24.7	24.7	24.7	24.7	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Day 7-Old	25.6	25.6	25.6	25.6	25.6	25.6	25.6	25.6	25.6	25.6	25.6	25.6	25.6	25.6	25.6	25.6

Source: Hunter-ESE, 1989.

Table B-5. Daily Temperature (°C) Measurements for Fathead Minnow Larvae Exposed to Synthetic Effluents, Kennecott, Ladysmith, Wisconsin, May 23-30, 1989

	30% LTST				100% LTST			
	A	B	C	D	A	B	C	D
Day 0-New	25.7	25.7	25.7	25.7	25.7	25.7	25.7	25.7
Day 1-New	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5
Day 1-Old	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4
Day 2-New	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4
Day 2-Old	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5
Day 3-New	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4
Day 3-Old	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4
Day 4-New	24.6	24.6	24.6	24.6	24.6	24.6	24.6	24.6
Day 4-Old	24.6	24.6	24.6	24.6	24.6	24.6	24.6	24.6
Day 5-New	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4
Day 5-Old	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4
Day 6-New	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Day 6-Old	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Day 7-Old	25.6	25.6	25.6	25.6	25.6	25.6	25.6	25.6

Source: Hunter-ESE, 1989.

Table B-6. Daily Dissolved Oxygen (ppm) Measurements for Fathead Minnow Larvae Exposed to Synthetic Effluents, Kennecott, Ladysmith, Wisconsin, May 23-30, 1989

	Laboratory Control				Synthetic Control				River Control			
	A	B	C	D	A	B	C	D	A	B	C	D
Day 0-New	8.2	8.2	8.2	8.2	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0
Day 1-New	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	9.2	9.2	9.2	9.2
Day 1-Old	6.3	6.3	6.3	6.3	6.4	6.4	6.4	6.4	6.5	6.5	6.5	6.5
Day 2-New	8.1	8.1	8.1	8.1	8.4	8.4	8.4	8.4	9.6	9.6	9.6	9.6
Day 2-Old	5.2	5.2	5.5	5.9	5.0	4.7	4.9	5.3	4.3	4.2	4.5	4.4
Day 3-New	7.9	7.9	7.9	7.9	8.6	8.6	8.6	8.6	9.0	9.0	9.0	9.0
Day 3-Old	2.7	5.4	5.9	5.7	3.3	4.7	4.6	4.4	4.1	4.1	3.5	3.5
Day 4-New	7.5	7.5	7.5	7.5	8.6	8.6	8.6	8.6	8.3	8.3	8.3	8.3
Day 4-Old	4.0	5.6	5.0	5.7	4.9	5.2	5.1	5.5	4.9	4.9	4.5	4.6
Day 5-New	7.4	7.4	7.4	7.4	9.2	9.2	9.2	9.2	8.6	8.6	8.6	8.6
Day 5-Old	5.4	5.6	5.7	5.6	5.6	5.2	5.4	5.4	5.4	4.9	5.0	5.0
Day 6-New	8.0	8.0	8.0	8.0	9.4	9.4	9.4	9.4	8.7	8.7	8.7	8.7
Day 6-Old	7.2	7.2	7.2	7.3	7.0	7.0	7.0	7.0	7.1	7.1	6.7	6.8
Day 7-Old	4.6	3.8	4.3	4.6	4.7	5.0	3.9	3.8	4.7	4.9	4.9	4.9

Source: Hunter-ESE, 1989.

Table B-7. Daily Dissolved Oxygen (ppm) Measurements for Fathead Minnow Larvae Exposed to Synthetic Effluents, Kennecott, Ladysmith, Wisconsin, May 23-30, 1989

	1% LT				3% LT				10% LT				30% LT			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Day 0-New	9.1	9.1	9.1	9.1	8.9	8.9	8.9	8.9	9.2	9.2	9.2	9.2	9.0	9.0	9.0	9.0
Day 1-New	--	8.8	8.8	8.8	8.8	8.8	8.8	8.8	8.6	8.6	8.6	8.6	8.5	8.5	8.5	8.5
Day 1-Old	6.6	6.5	6.5	6.5	6.6	6.6	6.6	6.6	5.2	5.2	5.2	5.2	5.6	5.6	5.6	5.6
Day 2-New	--	9.8	9.8	9.8	9.9	9.9	9.9	9.9	9.8	9.8	9.8	9.8	9.9	9.9	9.9	9.9
Day 2-Old	--	4.1	4.1	4.1	4.3	4.2	4.4	4.1	4.1	3.8	4.0	4.3	3.8	3.9	4.1	4.4
Day 3-New	--	9.0	9.0	9.0	9.2	9.2	9.2	9.2	9.1	9.1	9.1	9.1	9.0	9.0	9.0	9.0
Day 3-Old	--	3.9	3.7	3.9	3.9	4.2	4.2	3.6	3.7	3.5	3.6	3.6	3.8	2.8	2.9	4.4
Day 4-New	--	8.7	8.7	8.7	8.3	8.3	8.3	8.3	8.6	8.6	8.6	8.6	8.2	8.2	8.2	8.2
Day 4-Old	--	4.9	5.0	4.7	4.6	4.7	4.9	5.2	6.2	5.8	5.0	5.0	5.4	4.6	4.9	5.4
Day 5-New	--	8.4	8.4	8.4	8.3	8.3	8.3	8.3	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2
Day 5-Old	--	5.1	5.3	5.1	5.2	4.9	5.2	5.0	5.6	5.5	4.8	5.0	5.5	5.4	5.2	5.3
Day 6-New	--	8.4	8.4	8.4	8.5	8.5	8.5	8.5	8.6	8.6	8.6	8.6	8.5	8.5	8.5	8.5
Day 6-Old	--	6.6	6.7	6.8	7.0	6.9	6.9	6.9	7.0	7.1	6.9	6.7	7.0	7.0	6.9	6.8
Day 7-Old	--	4.3	4.4	3.9	4.0	4.2	3.2	3.8	4.4	4.3	5.0	4.7	4.3	5.0	4.6	5.0

Source: Hunter-ESE, 1989.

Table B-8. Daily Dissolved Oxygen (ppm) Measurements for Fathead Minnow Larvae Exposed to Synthetic Effluents, Kennecott, Ladysmith, Wisconsin, May 23-30, 1989

	100% LT				1% LTST				3% LTST				10% LTST			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Day 0-New	9.1	9.1	9.1	9.1	9.2	9.2	9.2	9.2	9.3	9.3	9.3	9.3	9.3	9.3	9.3	9.3
Day 1-New	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3
Day 1-Old	6.2	6.2	6.2	6.2	6.6	6.6	6.6	6.6	6.1	6.1	6.1	6.1	5.8	5.8	5.8	5.8
Day 2-New	9.4	9.4	9.4	9.4	9.7	9.7	9.7	9.7	10.0	10.0	10.0	10.0	9.5	9.5	9.5	9.5
Day 2-Old	4.2	4.6	4.6	4.3	4.4	3.9	4.1	3.8	3.9	4.1	4.0	4.0	4.2	3.9	3.8	4.3
Day 3-New	8.9	8.9	8.9	8.9	9.1	9.1	9.1	9.1	9.3	9.3	9.3	9.3	9.2	9.2	9.2	9.2
Day 3-Old	4.9	4.9	5.6	5.3	3.8	4.1	3.9	3.9	3.6	3.5	4.1	4.1	3.1	2.7	4.2	4.1
Day 4-New	8.6	8.6	8.6	8.6	8.1	8.1	8.1	8.1	8.0	8.0	8.0	8.0	8.3	8.3	8.3	8.3
Day 4-Old	5.9	5.6	6.1	5.6	4.1	4.7	4.5	4.6	3.3	4.4	4.9	5.1	4.5	4.8	4.9	5.1
Day 5-New	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.4	8.4	8.4	8.4	8.0	8.0	8.0	8.0
Day 5-Old	6.2	6.1	6.2	6.2	5.0	5.1	5.0	5.0	4.8	5.2	5.1	5.2	4.9	4.5	4.3	4.5
Day 6-New	9.0	9.0	9.0	9.0	8.3	8.3	8.3	8.3	8.4	8.4	8.4	8.4	8.5	8.5	8.5	8.5
Day 6-Old	7.3	7.3	7.5	7.4	6.9	7.0	7.0	7.0	6.9	6.8	6.9	7.0	7.0	6.9	6.7	6.8
Day 7-Old	5.4	5.6	5.7	5.4	4.9	4.2	4.7	4.5	4.5	4.4	4.9	4.6	4.1	4.6	4.3	4.6

Source: Hunter-ESE, 1989.

Table B-9. Daily Dissolved Oxygen (ppm) Measurements for Fathead Minnow Larvae Exposed to Synthetic Effluents, Kennecott, Ladysmith, Wisconsin, May 23-30, 1989

	30% LTST				100% LTST			
	A	B	C	D	A	B	C	D
Day 0-New	9.4	9.4	9.4	9.4	9.6	9.6	9.6	9.6
Day 1-New	8.4	8.4	8.4	8.4	8.6	8.6	8.6	8.6
Day 1-Old	6.2	6.2	6.2	6.2	6.4	6.4	6.4	6.4
Day 2-New	9.8	9.8	9.8	9.8	10.2	10.2	10.2	10.2
Day 2-Old	3.7	4.1	4.1	3.9	4.6	4.8	4.4	4.9
Day 3-New	9.2	9.2	9.2	9.2	9.0	9.0	9.0	9.0
Day 3-Old	3.8	3.9	3.5	4.0	4.6	5.4	5.5	5.1
Day 4-New	8.1	8.1	8.1	8.1	8.4	8.4	8.4	8.4
Day 4-Old	5.1	4.8	4.9	5.1	5.5	5.5	5.4	5.0
Day 5-New	8.1	8.1	8.1	8.1	8.3	8.3	8.3	8.3
Day 5-Old	4.4	4.3	4.7	4.8	5.3	5.2	5.2	5.5
Day 6-New	8.3	8.3	8.3	8.3	9.2	9.2	9.2	9.2
Day 6-Old	6.9	6.7	6.7	6.9	7.0	7.0	7.1	7.1
Day 7-Old	4.8	4.7	4.7	4.8	5.2	5.1	5.3	5.2

Source: Hunter-ESE, 1989.

Table B-10. Daily pH Measurements for Fathead Minnow Larvae Exposed to Synthetic Effluents, Kennecott, Ladysmith, Wisconsin, May 23-30, 1989

	Laboratory Control				Synthetic Control				River Control			
	A	B	C	D	A	B	C	D	A	B	C	D
Day 0-New	8.0	8.0	8.0	8.0	7.5	7.5	7.5	7.5	7.1	7.1	7.1	7.1
Day 1-New	8.0	8.0	8.0	8.0	7.3	7.3	7.3	7.3	7.0	7.0	7.0	7.0
Day 1-Old	7.8	8.0	8.0	8.0	7.4	7.4	7.5	7.5	7.3	7.3	7.3	7.3
Day 2-New	8.2	8.2	8.2	8.2	7.4	7.4	7.4	7.4	7.2	7.2	7.2	7.2
Day 2-Old	7.5	7.5	7.5	7.6	6.7	6.8	6.8	6.9	6.8	6.8	6.8	6.8
Day 3-New	8.1	8.1	8.1	8.1	7.4	7.4	7.4	7.4	7.2	7.2	7.2	7.2
Day 3-Old	7.2	7.5	7.6	7.6	6.7	6.9	6.9	6.9	6.9	6.8	6.8	6.8
Day 4-New	8.2	8.2	8.2	8.2	7.6	7.6	7.6	7.6	7.1	7.1	7.1	7.1
Day 4-Old	7.6	7.8	7.8	7.7	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1
Day 5-New	8.1	8.1	8.1	8.1	7.5	7.5	7.5	7.5	6.9	6.9	6.9	6.9
Day 5-Old	7.7	7.8	7.8	7.8	7.1	7.1	7.1	7.1	7.1	7.0	7.1	7.1
Day 6-New	8.1	8.1	8.1	8.1	7.4	7.4	7.4	7.4	7.1	7.1	7.1	7.1
Day 6-Old	8.1	8.2	8.2	8.2	7.5	7.5	7.5	7.5	7.6	7.5	7.5	7.4
Day 7-Old	7.7	7.7	7.7	7.7	7.7	7.6	7.5	7.5	7.4	7.4	7.4	7.3

Source: Hunter-ESE, 1989.

Table B-11. Daily pH Measurements for Fathead Minnow Larvae Exposed to Synthetic Effluents, Kennecott, Ladysmith, Wisconsin, May 23-30, 1989

	1% LT				3% LT				10% LT				30% LT			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Day 0-New	6.6	6.6	6.6	6.6	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8	7.0	7.0	7.0	7.0
Day 1-New	--	7.0	7.0	7.0	7.1	7.1	7.1	7.1	7.2	7.2	7.2	7.2	7.3	7.3	7.3	7.3
Day 1-Old	7.5	7.3	7.3	7.3	7.3	7.2	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.4	7.3	7.3
Day 2-New	--	7.1	7.1	7.1	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2
Day 2-Old	--	6.9	6.8	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	7.0
Day 3-New	--	7.2	7.2	7.2	7.2	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3
Day 3-Old	--	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	7.0	6.9	6.9	7.0
Day 4-New	--	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.3	7.3	7.3	7.3
Day 4-Old	--	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.3	7.2	7.1	7.1	7.2	7.1	7.1	7.2
Day 5-New	--	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.1	7.1	7.1	7.1	7.2	7.2	7.2	7.2
Day 5-Old	--	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.2	7.2	7.1	7.2	7.2	7.2	7.2	7.2
Day 6-New	--	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.2	7.2	7.2	7.2	7.3	7.3	7.3	7.3
Day 6-Old	--	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.5	7.5	7.4	7.4	7.5	7.5	7.5	7.5
Day 7-Old	--	7.3	7.3	7.3	7.3	7.3	7.2	7.2	7.3	7.2	7.3	7.3	7.3	7.3	7.3	7.3

Source: Hunter-ESE, 1989.

Table B-12. Daily pH Measurements for Fathead Minnow Larvae Exposed to Synthetic Effluents, Kennecott, Ladysmith, Wisconsin, May 23-30, 1989

	100% LT				1% LTST				3% LTST				10% LTST			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Day 0-New	7.2	7.2	7.2	7.2	6.7	6.7	6.7	6.7	6.8	6.8	6.8	6.8	6.9	6.9	6.9	6.9
Day 1-New	7.7	7.7	7.7	7.7	7.0	7.0	7.0	7.0	7.1	7.1	7.1	7.1	7.2	7.2	7.2	7.2
Day 1-Old	7.6	7.6	7.6	7.5	7.3	7.3	7.3	7.4	7.3	7.3	7.4	7.3	7.4	7.4	7.4	7.4
Day 2-New	7.6	7.6	7.6	7.6	7.2	7.2	7.2	7.2	7.1	7.1	7.1	7.1	7.2	7.2	7.2	7.2
Day 2-Old	7.1	7.1	7.1	7.1	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	7.0	6.9	6.9	7.0
Day 3-New	7.6	7.6	7.6	7.6	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.3	7.3	7.3	7.3
Day 3-Old	7.1	7.1	7.2	7.1	6.9	6.9	6.9	6.9	6.9	6.9	6.9	7.0	6.9	6.9	7.0	7.0
Day 4-New	7.7	7.7	7.7	7.7	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3
Day 4-Old	7.3	7.3	7.3	7.3	7.0	7.1	7.0	7.1	7.0	7.0	7.1	7.1	7.1	7.1	7.1	7.1
Day 5-New	7.6	7.6	7.6	7.6	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2
Day 5-Old	7.4	7.4	7.4	7.4	7.1	7.2	7.1	7.2	7.1	7.2	7.2	7.2	7.2	7.1	7.1	7.1
Day 6-New	7.7	7.7	7.7	7.7	7.2	7.2	7.2	7.2	7.1	7.1	7.1	7.1	7.2	7.2	7.2	7.2
Day 6-Old	7.6	7.6	7.7	7.7	7.4	7.4	7.4	7.4	7.4	7.5	7.4	7.5	7.4	7.4	7.4	7.4
Day 7-Old	7.4	7.4	7.4	7.4	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.2	7.3	7.2	7.3

Source: Hunter-ESE, 1989.

Table B-13. Daily pH Measurements for Fathead Minnow Larvae Exposed to Synthetic Effluents, Kennecott, Ladysmith, Wisconsin, May 23-30, 1989

	30% LTST				100% LTS			
	A	B	C	D	A	B	C	D
Day 0-New	7.0	7.0	7.0	7.0	7.4	7.4	7.4	7.4
Day 1-New	7.3	7.3	7.3	7.3	7.7	7.7	7.7	7.7
Day 1-Old	7.4	7.3	7.4	7.4	7.5	7.5	7.5	7.5
Day 2-New	7.2	7.2	7.2	7.2	7.7	7.7	7.7	7.7
Day 2-Old	7.0	7.0	7.0	7.0	7.1	7.1	7.1	7.1
Day 3-New	7.3	7.3	7.3	7.3	7.5	7.5	7.5	7.5
Day 3-Old	7.0	7.0	7.0	7.0	7.1	7.2	7.2	7.2
Day 4-New	7.3	7.3	7.3	7.3	7.7	7.7	7.7	7.7
Day 4-Old	7.1	7.1	7.1	7.1	7.3	7.3	7.2	7.2
Day 5-New	7.3	7.3	7.3	7.3	7.7	7.7	7.7	7.7
Day 5-Old	7.1	7.1	7.2	7.2	7.3	7.3	7.3	7.3
Day 6-New	7.3	7.3	7.3	7.3	7.7	7.7	7.7	7.7
Day 6-Old	7.4	7.4	7.4	7.5	7.6	7.6	7.6	7.6
Day 7-Old	7.3	7.3	7.3	7.3	7.4	7.4	7.5	7.5

Source: Hunter-ESE, 1989.

Table B-14. Daily Water Quality Measurements for *Ceriodaphnia* Exposed to Synthetic Effluents, Kennecott, Ladysmith, Wisconsin, May 23-30, 1989

Test Concentration	Parameter	Day 0	Day 1 New	Day 1 Old	Day 2 New	Day 2 Old	Day 3 New	Day 3 Old	Day 4 New	Day 4 Old	Day 5 New	Day 5 Old	Day 6 New	Day 6 Old	Day 7 Old
Lab Control	Temperature	25.7	24.9	24.9	25.4	25.4	25.4	25.4	24.7	24.7	24.4	24.4	25.0	25.0	25.6
Synthetic Control	(°C)	25.7	24.9	24.9	25.4	25.4	25.4	25.4	24.7	24.7	24.4	24.4	25.0	25.0	25.6
1% LT		25.7	24.9	24.9	25.4	25.4	25.4	25.4	24.7	24.7	24.4	24.4	25.0	25.0	25.6
3% LT		25.7	24.9	24.9	25.4	25.4	25.4	25.4	24.7	24.7	24.4	24.4	25.0	25.0	25.6
10% LT		25.7	24.9	24.9	25.4	25.4	25.4	25.4	24.7	24.7	24.4	24.4	25.0	25.0	25.6
30% LT		25.7	24.9	24.9	25.4	25.4	25.4	25.4	24.7	24.7	24.4	24.4	25.0	25.0	25.6
100% LT		25.7	24.9	24.9	25.4	25.4	25.4	25.4	24.7	24.7	24.4	24.4	25.0	25.0	25.6
Lab Control	Dissolved Oxygen	8.7	8.4	8.4	8.1	8.7	8.2	9.1	7.8	8.7	7.6	7.9	8.3	8.1	7.3
Synthetic Control	(ppm)	9.0	8.5	7.8	8.4	8.8	8.6	9.7	8.6	8.4	9.2	8.3	9.4	7.8	7.4
1% LT		9.1	8.8	8.0	9.8	8.5	9.0	9.2	8.7	8.2	8.4	7.9	8.4	7.6	6.8
3% LT		8.9	8.8	8.0	9.9	8.6	9.2	8.7	8.3	8.3	8.3	8.2	8.5	7.6	7.7
10% LT		9.2	8.6	8.0	9.8	8.7	9.1	9.0	8.6	8.8	8.2	8.7	8.6	7.9	7.9
30% LT		9.0	8.5	8.0	9.9	8.8	9.0	8.7	8.2	8.4	8.2	8.3	8.5	7.8	7.1
100% LT		9.1	8.3	8.3	9.4	8.6	8.9	8.6	8.6	8.4	8.5	9.4	9.0	8.1	7.4
Lab Control	pH	7.3	7.2	7.2	7.5	8.1	7.4	8.6	7.3	8.5	7.8	8.1	7.7	7.7	7.8
Synthetic Control		7.2	7.2	7.4	7.4	8.3	7.4	8.7	7.6	8.3	7.5	7.7	7.4	7.6	8.3
1% LT		7.1	6.8	7.3	7.1	7.7	7.2	8.3	7.2	8.0	7.0	8.1	7.1	7.5	8.2
3% LT		7.0	6.9	7.3	7.2	7.7	7.2	7.9	7.2	8.1	7.0	8.2	7.1	7.6	8.4
10% LT		6.9	6.9	7.4	7.2	8.1	7.2	8.3	7.2	8.4	7.1	8.6	7.2	7.9	8.3
30% LT		7.0	7.0	7.6	7.2	8.2	7.3	8.0	7.3	8.1	7.2	8.2	7.3	7.5	7.9
100% LT		7.2	7.2	7.7	7.6	8.4	7.6	8.5	7.7	8.4	7.6	9.0	7.7	7.7	8.1

Source: Hunter/ESE, 1989.

Table B-15. Daily Water Quality Measurements for *Ceriodaphnia* Exposed to Synthetic Effluents, Kennecott, Ladysmith, Wisconsin, May 23-30, 1989

Test		Day	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7
Concentration	Parameter	0	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	Old
River Control	Temperature	25.7	24.9	24.9	25.4	25.4	25.4	25.4	24.7	24.7	24.4	24.4	25.0	25.0	25.6
1% LTST	(°C)	25.7	24.9	24.9	25.4	25.4	25.4	25.4	24.7	24.7	24.4	24.4	25.0	25.0	25.6
3% LTST		25.7	24.9	24.9	25.4	25.4	25.4	25.4	24.7	24.7	24.4	24.4	25.0	25.0	25.6
10% LTST		25.7	24.9	24.9	25.4	25.4	25.4	25.4	24.7	24.7	24.4	24.4	25.0	25.0	25.6
30% LTST		25.7	24.9	24.9	25.4	25.4	25.4	25.4	24.7	24.7	24.4	24.4	25.0	25.0	25.6
100% LTST		25.7	24.9	24.9	25.4	25.4	25.4	25.4	24.7	24.7	24.4	24.4	25.0	25.0	25.6
River Control	Dissolved Oxygen	9.0	9.2	8.0	9.6	8.7	9.0	9.5	8.3	8.8	8.6	8.4	8.7	7.8	7.9
1% LTST	(ppm)	9.2	8.3	7.8	9.7	8.1	9.1	8.4	8.1	9.2	8.5	8.4	8.3	7.1	7.1
3% LTST		9.3	8.3	7.9	10.0	8.2	9.2	8.4	8.0	8.4	8.4	8.3	8.4	7.8	7.3
10% LTST		9.3	8.3	8.0	9.5	8.4	9.2	9.0	8.3	9.5	8.0	8.3	8.5	7.9	7.8
30% LTST		9.4	8.4	8.1	9.8	8.3	9.2	8.3	8.1	9.3	8.1	8.0	8.3	7.7	7.6
100% LTST		9.6	8.6	8.0	10.2	8.3	9.0	8.3	8.4	8.7	8.3	7.8	9.2	8.5	8.5
River Control	pH	7.0	7.0	7.3	7.2	7.7	7.2	8.6	7.1	8.5	6.9	8.1	7.1	7.3	8.5
1% LTST		6.9	6.8	7.2	7.2	7.3	7.2	8.0	7.3	8.4	7.2	8.3	7.2	7.2	7.3
3% LTST		6.9	6.9	7.4	7.1	7.5	7.3	8.2	7.3	7.9	7.2	8.2	7.1	7.1	7.4
10% LTST		6.9	6.9	7.6	7.2	7.8	7.3	8.3	7.3	8.3	7.2	8.6	7.2	7.5	7.5
30% LTST		7.0	7.0	7.6	7.2	7.7	7.3	7.7	7.3	8.4	7.3	7.7	7.3	7.1	7.6
100% LTST		7.2	7.2	7.7	7.7	8.3	7.5	8.4	7.7	8.2	7.7	8.3	7.7	8.4	9.1

Source: Hunter/ESE, 1989.

APPENDIX C

Statistical Reports

LIME & SULFIDE TREATMENT CERIODAPHNIA
File: B:L&SCERIO   Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	4.221	15.246	24.066	15.246	4.221
OBSERVED	4	15	26	14	4

Calculated Chi-Square goodness of fit test statistic = 0.2844
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

LIME & SULFIDE TREATMENT CERIODAPHNIA
File: B:L&SCERIO   Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

```
Calculated B statistic =   12.85
Table Chi-square value =   16.81  (alpha = 0.01)
Table Chi-square value =   12.59  (alpha = 0.05)

Average df used in calculation  ==>   df (avg n - 1) =    8.00
Used for Chi-square table value ==>   df (#groups-1) =    6
```

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

LIME & SULFIDE TREATMENT CERIODAPHNIA
File: B:L&SCERIO   Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA   TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	RIVER CONTROL	8	15.000	31.000	23.000
2	LAB CONTROL	7	19.000	24.000	21.143
3	1% EFFLUENT	9	16.000	28.000	23.000
4	3% EFFLUENT	9	18.000	26.000	21.889
5	10% EFFLUENT	10	20.000	28.000	23.900
6	30% EFFLUENT	10	12.000	31.000	24.300
7	100% EFFLUENT	10	0.000	13.000	3.300

LIME & SULFIDE TREATMENT CERIODAPHNIA
File: B:L&SCERIO   Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA   TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	RIVER CONTROL	27.714	5.264	1.861
2	LAB CONTROL	3.143	1.773	0.670
3	1% EFFLUENT	15.750	3.969	1.323
4	3% EFFLUENT	8.611	2.934	0.978
5	10% EFFLUENT	5.433	2.331	0.737
6	30% EFFLUENT	25.344	5.034	1.592
7	100% EFFLUENT	16.678	4.084	1.291

LIME & SULFIDE TREATMENT CERIODAPHNIA
File: B:L&SCERIO   Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	3318.868	553.145	37.104
Within (Error)	56	834.846	14.908	
Total	62	4153.714		

Critical F value = 2.34 (0.05,6,40)
Since F > Critical F REJECT Ho:All groups equal

LIME & SULFIDE TREATMENT CERIODAPHNIA
File: B:L&SCERIO   Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2   Ho:Control>Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	RIVER CONTROL	23.000	23.000		
2	LAB CONTROL	21.143	21.143	-1.014	
3	1% EFFLUENT	23.000	23.000	0.000	
4	3% EFFLUENT	21.889	21.889	-0.607	
5	10% EFFLUENT	23.900	23.900	0.491	
6	30% EFFLUENT	24.300	24.300	0.710	
7	100% EFFLUENT	3.300	3.300	-10.756	

Dunnett table value = 2.37   (1 Tailed Value, P=0.05, df=40,6)

LIME & SULFIDE TREATMENT CERIODAPHNIA
File: B:L&SCERIO   Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2   Ho:Control>Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	RIVER CONTROL	8			
2	LAB CONTROL	7	4.736	20.6	1.857
3	1% EFFLUENT	9	4.446	19.3	0.000
4	3% EFFLUENT	9	4.446	19.3	1.111
5	10% EFFLUENT	10	4.341	18.9	-0.900
6	30% EFFLUENT	10	4.341	18.9	-1.300
7	100% EFFLUENT	10	4.341	18.9	19.700

LIME AND SULFIDE TREATMENT FHM
File: B:L&SFHM.   Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	2.144	7.744	12.224	7.744	2.144
OBSERVED	0	10	8	14	0

Calculated Chi-Square goodness of fit test statistic = 11.4587
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

LIME AND SULFIDE TREATMENT FHM
File: B:L&SFHM.   Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

```
Calculated B statistic =      9.86
Table Chi-square value =     18.48  (alpha = 0.01)
Table Chi-square value =     14.07  (alpha = 0.05)

Average df used in calculation  ==>   df (avg n - 1) =    3.00
Used for Chi-square table value ==>   df (#groups-1) =    7
```

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

LIME AND SULFIDE TREATMENT FHM
File: B:L&SFHM.   Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA   TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	RIVER CONTROL	4	0.280	0.380	0.345
2	LAB CONTROL	4	0.360	0.390	0.377
3	CONTROL	4	0.260	0.400	0.325
4	1% EFFLUENT	4	0.280	0.350	0.322
5	3% EFFLUENT	4	0.220	0.330	0.292
6	10% EFFLUENT	4	0.260	0.320	0.285
7	30% EFFLUENT	4	0.220	0.300	0.260
8	100% EFFLUENT	4	0.290	0.330	0.317

LIME AND SULFIDE TREATMENT FHM
File: B:L&SFHM.   Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA   TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	RIVER CONTROL	0.002	0.045	0.023
2	LAB CONTROL	0.000	0.013	0.006
3	CONTROL	0.005	0.070	0.035
4	1% EFFLUENT	0.001	0.031	0.015
5	3% EFFLUENT	0.002	0.050	0.025
6	10% EFFLUENT	0.001	0.025	0.013
7	30% EFFLUENT	0.001	0.037	0.018
8	100% EFFLUENT	0.000	0.019	0.009

LIME AND SULFIDE TREATMENT FHM
File: B:L&SFHM.   Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	7	0.038	0.005	2.500
Within (Error)	24	0.039	0.002	
Total	31	0.076		

Critical F value = 2.42 (0.05,7,24)
Since F > Critical F REJECT Ho:All groups equal

LIME AND SULFIDE TREATMENT FHM
File: B:L&SFHM. Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control>Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	RIVER CONTROL	0.345	0.345		
2	LAB CONTROL	0.377	0.377	1.028	
3	CONTROL	0.325	0.325	-0.632	
4	1% EFFLUENT	0.322	0.322	-0.712	
5	3% EFFLUENT	0.292	0.292	-1.660	
6	10% EFFLUENT	0.285	0.285	-1.897	
7	30% EFFLUENT	0.260	0.260	-2.688	
8	100% EFFLUENT	0.317	0.317	-0.870	

Dunnett table value = 2.48 (1 Tailed Value, P=0.05, df=24,7)

LIME AND SULFIDE TREATMENT FHM
File: B:L&SFHM. Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control>Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	RIVER CONTROL	4			
2	LAB CONTROL	4	0.078	22.7	-0.032
3	CONTROL	4	0.078	22.7	0.020
4	1% EFFLUENT	4	0.078	22.7	0.023
5	3% EFFLUENT	4	0.078	22.7	0.052
6	10% EFFLUENT	4	0.078	22.7	0.060
7	30% EFFLUENT	4	0.078	22.7	0.085
8	100% EFFLUENT	4	0.078	22.7	0.027

```
LIME TREATMENT CERIODAPHNIA
File: B:LIMECER.        Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance
--------------------------------------------------------------------------

Calculated B statistic =    13.60
Table Chi-square value =    15.09  (alpha = 0.01)
Table Chi-square value =    11.07  (alpha = 0.05)

Average df used in calculation  ==>   df (avg n - 1) =   8.17
Used for Chi-square table value ==>   df (#groups-1) =   5
--------------------------------------------------------------------------

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is
      used to calculate the B statistic (see above).
```

LIME TREATMENT CERIODAPHNIA
File: B:LIMECER.   Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	3.685	13.310	21.010	13.310	3.685
OBSERVED	2	21	15	12	5

Calculated Chi-Square goodness of fit test statistic =   7.5308
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

LIME TREATMENT CERIODAPHNIA
File: B:LIMECER.   Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA   TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	RIVER CONTROL	8	15.000	31.000	23.000
2	LAB CONTROL	7	19.000	24.000	21.143
3	1% EFFLUENT	10	20.000	30.000	24.600
4	3% EFFLUENT	10	19.000	30.000	22.900
5	10% EFFLUENT	10	20.000	26.000	23.200
6	30% EFFLUENT	10	16.000	29.000	22.800

LIME TREATMENT CERIODAPHNIA
File: B:LIMECER.   Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA   TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	RIVER CONTROL	27.714	5.264	1.861
2	LAB CONTROL	3.143	1.773	0.670
3	1% EFFLUENT	12.933	3.596	1.137
4	3% EFFLUENT	12.100	3.479	1.100
5	10% EFFLUENT	3.733	1.932	0.611
6	30% EFFLUENT	22.400	4.733	1.497

LIME TREATMENT CERIODAPHNIA
File: B:LIMECER.   Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	50.570	10.114	0.736
Within (Error)	49	673.357	13.742	
Total	54	723.927		

Critical F value = 2.45 (0.05,5,40)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

LIME TREATMENT CERIODAPHNIA
File: B:LIMECER. Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control>Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	RIVER CONTROL	23.000	23.000		
2	LAB CONTROL	21.143	21.143	-1.056	
3	1% EFFLUENT	24.600	24.600	0.910	
4	3% EFFLUENT	22.900	22.900	-0.057	
5	10% EFFLUENT	23.200	23.200	0.114	
6	30% EFFLUENT	22.800	22.800	-0.114	

Dunnett table value = 2.31 (1 Tailed Value, P=0.05, df=40,5)

LIME TREATMENT CERIODAPHNIA
File: B:LIMECER. Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control>Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	RIVER CONTROL	8			
2	LAB CONTROL	7	4.432	19.3	1.857
3	1% EFFLUENT	10	4.062	17.7	-1.600
4	3% EFFLUENT	10	4.062	17.7	0.100
5	10% EFFLUENT	10	4.062	17.7	-0.200
6	30% EFFLUENT	10	4.062	17.7	0.200

LIME TREATMENT FHM
File: B:LIMEFHM.   Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	2.077	7.502	11.842	7.502	2.077
OBSERVED	0	11	10	10	0

Calculated Chi-Square goodness of fit test statistic = 6.9033
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

LIME TREATMENT FHM
File: B:LIMEFHM.   Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 10.36
Table Chi-square value = 18.48 (alpha = 0.01)
Table Chi-square value = 14.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 2.88
Used for Chi-square table value ==> df (#groups-1) = 7

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

LIME TREATMENT FHM
File: B:LIMEFHM. Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	RIVER CONTROL	4	0.280	0.380	0.345
2	LAB CONTROL	4	0.360	0.390	0.377
3	CONTROL	4	0.260	0.400	0.325
4	1% EFFLUENT	3	0.290	0.400	0.347
5	3% EFFLUENT	4	0.170	0.360	0.290
6	10% EFFLUENT	4	0.210	0.350	0.272
7	30% EFFLUENT	4	0.250	0.300	0.267
8	100% EFFLUENT	4	0.150	0.220	0.170

LIME TREATMENT FHM
File: B:LIMEFHM. Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	RIVER CONTROL	0.002	0.045	0.023
2	LAB CONTROL	0.000	0.013	0.006
3	CONTROL	0.005	0.070	0.035
4	1% EFFLUENT	0.003	0.055	0.032
5	3% EFFLUENT	0.007	0.084	0.042
6	10% EFFLUENT	0.004	0.064	0.032
7	30% EFFLUENT	0.000	0.022	0.011
8	100% EFFLUENT	0.001	0.034	0.017

LIME TREATMENT FHM
File: B:LIMEFHM.		Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	7	0.116	0.017	5.667
Within (Error)	23	0.066	0.003	
Total	30	0.182		

Critical F value = 2.44 (0.05,7,23)
Since F > Critical F REJECT Ho:All groups equal

LIME TREATMENT FHM
File: B:LIMEFHM.   Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2   Ho:Control>Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	RIVER CONTROL	0.345	0.345		
2	LAB CONTROL	0.377	0.377	0.839	
3	CONTROL	0.325	0.325	-0.516	
4	1% EFFLUENT	0.347	0.347	0.043	
5	3% EFFLUENT	0.290	0.290	-1.420	
6	10% EFFLUENT	0.272	0.272	-1.872	
7	30% EFFLUENT	0.267	0.267	-2.001	
8	100% EFFLUENT	0.170	0.170	-4.518	

Dunnett table value = 2.51   (1 Tailed Value, P=0.05, df=20,7)

LIME TREATMENT FHM
File: B:LIMEFHM.   Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2   Ho:Control>Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	RIVER CONTROL	4			
2	LAB CONTROL	4	0.097	28.2	-0.032
3	CONTROL	4	0.097	28.2	0.020
4	1% EFFLUENT	3	0.105	30.4	-0.002
5	3% EFFLUENT	4	0.097	28.2	0.055
6	10% EFFLUENT	4	0.097	28.2	0.072
7	30% EFFLUENT	4	0.097	28.2	0.077
8	100% EFFLUENT	4	0.097	28.2	0.175

APPENDIX D

IWC Calculation

Calculation of the Instream Waste Concentration (IWC) for the Kennecott Mine Wastewater Treatment Facility, Ladysmith, Wisconsin

1. The ten year, seven day low flow (Q7,10) for the Flambeau River at the project site is 435 cfs.

2. The estimated effluent discharge for the wastewater treatment facility for the Kennecott project is:

   117 gpm - storm water from the type II pile
   397 gpm - groundwater discharge to mine*
   514 gpm

   This equates to 1.145 cfs.

3. The dilution provided by 1/4 of the Q7,10 would equal

   $(1.145/108) \times 100 = 1.06\%$

* This rate is that which is included in the permit applications. This rate needs to be reviewed based on Pricketts modeling results.

APPENDIX E

Synthetic Wastewater Preparation

Kennecott - Bioassay
(Synthetic Wastewater)

Foth and Van Dyke has been requested to prepare a synthetic wastewater which would simulate wastewaters generated by the Kennecott wastewater treatment bench studies. These wastewaters will simulate the lime treated effluents and the sulfide treated effluents. To accomplish this task, a synthetic water will first be made. The synthetic water will act as a control water for the bioassay.

A synthetic water will be made up following protocol established by the EPA in Document EPA/600/4-85/014 Short-Term Methods for Estimating the Chronic Toxicety of Effluents and Receiving Waters to Freshwater Organisms, pages 17 and '18. The synthetic water will act as the control water and where needed, chemicals will be added to the synthetic water to simulate either lime or sulfide treated wastewater. The synthetic water is made as follows:

Reagent added (mg/L)				Final Water Quality		
NaHCO3	CaSO4x2H2O	MgSO4	KCl	pH	Hardness	Alkalinity
48.0	30.0	30.0	2.0	7.2-7.6	40-48	30-35

Chemical Makeup of Synthetic Wastewater

Basis derived from: Table 2-A4 of Preliminary Engineering Report for Wastewater Treatment Facilities for the Kennocott Flambeau Project.

	Lime Treated Effluent (mg/L)	Sulfide treated Effluent (mg/L)
Aluminum	0.580	0.580*
Arsenic	0.003	0.003
Cadmium	0.0012	0.0003
Chromium	0.002	0.002
Copper	0.350	0.015
Lead	0.002	0.002
Nickel	0.030	0.030
Selenium	0.007	0.003
Silver	0.0004	0.0004
Zinc	0.030	0.030
Sulfide	---	1.000**

*concentrations from current DNR estimated effluent limit
**estimate based on residuals likely to carry over from sulfide treatment

Volumes of wastewater required for the bioassay were given to us by Hunter/ESE, the laboratory performing the bioassay. single bioassay requires renewal of the control and effluent waters on a set schedule. For this bioassay, the test will be initiated on Tuesday. Waters will have to be renewed on Thursday and Saturday. Therefore water will have to be shipped to the lab on Monday, Wednesday, and Friday guarenteed next day delivery. Water and wastewater will be made up new for each separate shipment. The following volumes of water are required to conduct the test.

(in Liters)	Control	Lime Effluent	Sulfide Effluent	Flambeau River
Monday	5	8	8	24
Wednesday	5	8	8	24
Friday	8	12	12	36

Table 1 estimates the quantity of chemicals needed to make up the synthetic water and wastewater. For each type of water we plan to make up an extra quantity so that we would have sufficient volume to conduct a chemical analysis on the different waters used in the test. Therefore where the test requires 8, 8, and 12 liters of sample, we will make up 10, 10, and 14 liters respectively.

Procedure for synthetic water makeup

Water samples will be made up 24 hours prior to shipment to the bioassay lab. Waters for one days shipment will be made up in one lot and all chemicals necessary for making up the control water will be added at one time. After thorough mixing, the water will be divided into three parts - one for the control and two for wastewaters.

Procedure for synthtic wastewater makeup

The lime wastewater will be made up using one third of the water from the previous step with addition of the chemicals in concentrations as identified in Table 1 as "Lime". Since these chemicals are to be added in extremely small quantities, they will be premixed according to the quantity in Table 2. To make the chemical spike required for the lime wastewater, dilute the 1000 ppm primary standards accordingly:

Monday and Wednesday:

Dilute 12 ml of Cadmium(1000 ppm primary standard) and 4 ml of Silver(1000 ppm primary standard) to 1000 ml with control water. Take one ml of this solution and add it to 900 ml of control water(solution A).

Dilute 3 ml of Arsenic(1000 ppm primary standard), 2 ml of Chromium(1000 ppm primary standard), 2 ml of Lead(1000 ppm primary standard), 30 ml of nickel(1000 ppm primary standard), 7 ml of selenium(1000 ppm primary standard), and 30 ml of zinc(1000 ppm primary standard) to 100 ml using control water. Add one ml of this to Solution A.

Add 3.5 ml of copper(1000 ppm primary standard) and 5.8 ml of aluminum(1000 ppm primary standard) to Solution A. Using NaOH adjust the pH to 8.0 and dilute to 1000 ml with control water. Add this to nine liters of control water to make up the total of ten liters of lime simulated effluent.

Friday:

Dilute 16.8 ml of Cadmium(1000 ppm primary standard) and 5.6 ml of Silver(1000 ppm primary standard) to 1000 ml with control water. Take one ml of this solution and add it to 900 ml of control water(solution B).

Dilute 4.2 ml of Arsenic(1000 ppm primary standard), 2.8 ml of Chromium(1000 ppm primary standard), 2.8 ml of Lead(1000 ppm primary standard), 42 ml of nickel(1000 ppm primary standard), 9.8 ml of selenium(1000 ppm primary standard), and 42 ml of zinc(1000 ppm primary standard) to 200 ml using control water. Add two ml of this to Solution B.

Add 4.9 ml of copper(1000 ppm primary standard) and 8.12ml of aluminum(1000 ppm primary standard) to Solution B. Using NaOH adjust the pH to 8.0 and dilute to 1000 ml with control water. Add this to thirteen liters of control water to make up the total of fourteen liters of lime simulated effluent.

The sulfide wastewater will be made up using one third of the synthetic water (control water). Additional chemical will be added according to the schedule in Table 2. To make the chemical spike for the sulfide wastewater, dilute the 1000 ppm primary standard accordingly:

Monday and Wednesday:

Dilute 12 ml of Cadmium(1000 ppm primary standard) and 4 ml of Silver(1000 ppm primary standard) to 1000 ml with control water. Take one ml of this solution and add it to 900 ml of control water(solution C).

Dilute 3 ml of Arsenic(1000 ppm primary standard), 2 ml of Chromium(1000 ppm primary standard), 10 ml of copper(1000 ppm primary standard), 2 ml of Lead(1000 ppm primary standard), 30 ml of nickel(1000 ppm primary standard), 3 ml of selenium(1000 ppm primary standard), and 30 ml of zinc(1000 ppm primary standard) to 100 ml using control water. Add one ml of this to Solution C.

Add 5.8 ml of aluminum(1000 ppm primary standard) to Solution C and using NaOH, adjust the pH to 8.0. Add 20 mg of Na2S to Solution C. Dilute to 1000 ml with control water. Add this to nine liters of control water to make up the total of ten liters of sulfide simulated effluent.

Friday:

Dilute 16.8 ml of Cadmium(1000 ppm primary standard) and 5.6 ml of Silver(1000 ppm primary standard) to 1000 ml with control water. Take one ml of this solution and add it to 900 ml of control water(solution D)

Dilute 4.2 ml of Arsenic(1000 ppm primary standard), 2.8 ml of Chromium(1000 ppm primary standard), 2.8 ml of Lead(1000 ppm primary standard), 42 ml of nickel(1000 ppm primary standard), 14.0 ml of copper(1000 ppm primary standard), 4.2 ml of selenium(1000 ppm primary standard), and 42 ml of zinc(1000 ppm primary standard) to 200 ml using control water. Add two ml of this to Solution D.

Add 8.12 ml of aluminum(1000 ppm primary standard) to Solution D and using NaOH, adjust the pH to 8.0. Add 29 mg of Na2S to Solution D. Dilute to 1000 ml with control water. Add this to thirteen liters of control water to make up the total of fourteen liters of sulfide simulated effluent.

After all water and wastewater have been prepared, they will be aerated for 24 hours and prepared for shipment. Samples will be iced in coolers during transit.

Based on the information above, the following table shows the chemical requirements for making up the synthetic water and wastewater.

TABLE 1

	Control			Lime			Sulfide		
	M	W	F	M	W	F	M	W	F
Volume (liters)	10.00	10.00	14.00	10.0000	10.0000	14.0000	10.0000	10.0000	14.0000
Chemical	Milliliters of 1000 ppm primary standard required								
Aluminum	0.00	0.00	0.00	5.8000	5.8000	8.1200	5.8000	5.8000	8.1200
Arsenic	0.00	0.00	0.00	0.0300	0.0300	0.0420	0.0300	0.0300	0.0420
Cadmium	0.00	0.00	0.00	0.0120	0.0120	0.0168	0.0030	0.0030	0.0042
Chromium	0.00	0.00	0.00	0.0200	0.0200	0.0280	0.0200	0.0200	0.0280
Copper	0.00	0.00	0.00	3.5000	3.5000	4.9000	0.1000	0.1000	0.1400
Lead	0.00	0.00	0.00	0.0200	0.0200	0.0280	0.0200	0.0200	0.0280
Nickel	0.00	0.00	0.00	0.3000	0.3000	0.4200	0.3000	0.3000	0.4200
Selenium	0.00	0.00	0.00	0.0700	0.0700	0.0980	0.0300	0.0300	0.0420
Silver	0.00	0.00	0.00	0.0040	0.0040	0.0056	0.0040	0.0040	0.0056
Zinc	0.00	0.00	0.00	0.3000	0.3000	0.4200	0.3000	0.3000	0.4200
Sodium sulfide (mg) to obtain 1 mg/L of sulfide									
	0	0	0	0	0	0	20	20	29
Chemicals required for designated volumes (mg)									
NaHCO3	480	480	672	480	480	672	480	480	672
CaSO4x2H2O	280	280	420	280	240	420	240	240	420
MgSO4	280	280	420	280	240	420	240	240	420
KCl	19	19	28	19	16	28	16	16	28

Table 2

Volume of chemical required to make up synthetic wastewater

	Lime		Sulfide	
	H & W	F	H & W	F
Volumes (liters)	10	14	10	14
Chemical	Milliliters of primary standard required			
Aluminum	5.80	8.12	5.80	8.12
Arsenic	3.00	4.20	3.00	4.20
Cadmium	12.00	16.80	12.00	16.80
Chromium	2.00	2.80	2.00	2.80
Copper	3.50	4.90	10.00	14.00
Lead	2.00	2.80	2.00	2.80
Nickel	30.00	42.00	30.00	42.00
Selenium	7.00	9.80	3.00	4.20
Silver	4.00	5.60	4.00	5.60
Zinc	30.00	42.00	30.00	42.00

The main difference between the lime and sulfide treatments is that copper is substantially reduced for the sulfide treatment and sulfer is added.