3-6-97

DATA EVALUATION RECORD

§ 70-1 - Special Test: ACUTE SEDIMENT TOXICITY TEST WITH A FRESHWATER INVERTEBRATE

1. CHEMICAL: (RS)-a-cyano-3-phenoxybenzyl(1RS,3RS;1RS,3SR)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate

2. TEST MATERIAL: Cypermethrin 109702
Purity: 92.3 %

3. Study Type: Sediment 10 Day Toxicity Test: *Chironomus tentans*

4. CITATION:

Gentle, W., Goggin, U., Rapley, J.H., Farrelly, E., and Hamer, M.J. 1996. Cyperpethrin: Toxicity to *Chironomus tentans* in Sediment-water Systems. Zeneca Agrochemicals Report No. RC0006. Performed by Zeneca Agrochemicals Laboratory for the Pyrethroid Working Group (PWG). EPA MRID #440744-02.

5. REVIEWED BY:

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7. STUDY PARAMETERS

Scientific Name of Test Organism:	(*Chironomus tentans*)
Age of Test Organism:	third instar larvae
Definitive Test Duration:	10 days
Study Method:	Static
Type of Concentrations:	Mean measured/Nominal

8. **CONCLUSIONS**: These are studies are scientifically sound but do not meet the requirements in the proposed ASTM guidelines for an acute sediment study. The studies included three sediments Florrisant, Mississippi 3 and Duluth with organic carbon contents of 0.99, 3.1, and 13.3%, respectively. Using an average measured concentration for each toxicity level, EEB calculated LC_{50} values of 2.3, 27.8 and 36.5 ug kg^{-1}, respectively. No Effect Concentration for weight were found as follows: Florrisant, 4.4 ug kg^{-1}; Mississippi 3, 24 ug kg^{-1}; Duluth, 14 ug kg^{-1}. All calculated values coincide with the findings of the researchers. This information on sediment toxicity shows that although cypermethrin, with a K_{oc} of 180,000 - 500,000, binds rapidly to suspended particulate and sediment, it is very highly toxic to *Chironomus* and possibly other benthic organisms.

9. **Guideline Deviations**

1. Proposed Guidelines state that sediment test with midge *Chironomus tentans* should be started with 2nd instar larvae (10-14 days old). The studies under review used the 3rd instar, which may underestimate sensitivity to toxicants.

2. Water hardness levels were very high (161-178 mg l^{-1} as $CaCO_3$) as compared to the recommended values of 40 - 48 mg l^{-1} as $CaCO_3$.

10. **SUBMISSION PURPOSE**: Investigate the toxicity of sediment-sorbed ^{14}C-cypermethrin to the freshwater benthic invertebrate *Chironomus tentans* by exposure in sediment-water system.

11. **MATERIALS AND METHODS**

A. Test Organisms: *Chironomus tentans* were obtained from laboratory cultures at Jealott's Hill Research Station. Cultures were maintained in hard blended water overlying a layer of silver sand at 23°C on a 16 hour: 8 hour light:dark photoperiod. Egg masses were removed from the cultures and transferred to 20 liter glass tanks containing 2 cm of silver sand. Food for the hatched larvae consisted of green algae (*Chlorella vulgaris*) and ground fish flakes (Tetra-min). The test were run with larvae in the third instar.

B. Test Sediments: The following three natural uncontaminated sediments were used:

1) "Florrisant": supplied by N. Kimble, Midwest Science Centre, 4200 New Haven Rd, Columbia, USA.

2) "Mississippi 3": supplied by J. Rogers at the University of Mississippi, Biological Field Station, USA.

3) " **Duluth**": supplied by K. Lieber, Lake Superior Research Institute, University of Wisconsin-Superior, USA.

Prior to physico-chemical analysis, the air-dried samples were passed through a 2 mm sieve. Moisture contents of these air-dried samples were determined by oven-drying at 75°C overnight. Sediment physico-chemical parameters that include amount of sand, silt, clay and organic matter were characterized (Table 1).

C. **Test Water**: Dilution water was prepared by mixing dechlorinated mains water with the same amount of deionized (reverse osmosis system) water. The final hardness was 161-178 mg l^{-1} $CaCO_3$.

Table 1. Sediment Physico-chemical Characteristics

Properties	Florrisant	Mississippi 3	Duluth
pH	6.0	5.1	7.2
% Sand (2000-50 mm)	6	10	30
% Silt (50-2 mm)	70	65	45
% Clay (<2 mm)	24	25	25
% Organic Matter (%Organic Carbon)	1.7 (.99)	5.4 (3.1)	**22.9 (13.3)**
CEC (meq/100g)	14.5	13.2	43.6
Classification	Silt Loam	Silt Loam	Loam
Zeneca Soil Reference	53/12	53/10	53/14

D. **Test System**:

Test systems consisted of 500 ml glass jars containing 10 g dry weight sediment and 250 ml water (sediment:water ratio of 1:25). Each sediment had a control and a solvent control (spiked with 25 ul acetone) prepared for each treatment. Six replicates (A to F) at each concentration were prepared. Replicate A to D were used for the biological assessments and radiochemical analysis of sediment and overlying water at the end of the test. Replicates E and F were used for radiochemical analysis of sediment, pore water and overlying water at the start and end of the study.

A series of application solutions in acetone were prepared for each sediment and used to spike in 25 ul aliquots at the following nominal sediment concentrations:

Florrisant: 180, 60, 20, 6.7, and 2.2 ug kg^{-1} dry weight.
Mississippi 3: 300, 100, 33, 11, and 3.4 ug kg^{-1} dry weight.
Duluth: 450, 150, 50, 17, and 5.6 ug kg^{-1} dry weight.

In order to insure even mixing of spiked sediments, test chambers were sealed, shaken and transferred to a rolling mill for two hours at ambient temperature. Test chamber were then placed in a water bath at 23 ± 2°C and allowed to stand undisturbed for two days to allow sediment to settle. After this period of time, juvenile *C. tentans* were introduced into the Florrisant and Duluth sediment systems. However, for the Mississippi 3 sediment, the overlying water was replaced and the system allowed to stand for a further 2 days prior to test organism introduction (poor control survival without replacement). Replicates A to E received 10 organisms each, selected at random from the cultures. These were introduced below the water surface with a 5 mm pipette, while a subset of organisms were measure for head capsule width at the start of the study, in order to confirm the instar.

Test vessels were covered throughout the test to reduce evaporation, maintained at 23 ± 2°C in the water bath and illuminated at approximately 800 lux on a 16 hour:8 hour light:dark cycle. Test organisms were fed throughout the testing period.

E. Biological Assessment: At day 10 the overlying water from replicates A to D was removed and the sediments transferred to shallow trays. Survival of test organisms was defined as visible movement to the naked eye. Surviving individuals were removed and preserved for length and weight determination. Length was measured using Jandel Scientific Sigma Scan Image Analysis Program. After length measurements, the organisms from each replicate were pooled and oven dried to a constant weight to calculate mean dry weight.

F. Radiochemical Analysis:

Analysis of Test Systems E and F

1) **Overlying Water Phase Analysis:** Analysis of replicates E and F. A 100 ml aliquot of overlying water was removed and extracted with 5 ml n-hexane to remove parent cypermethrin. The hexane was subsample and analyzed by Liquid Scintillation Counting (LSC) to determine ^{14}C-cypermethrin concentrations.

2) **Pore Water Analysis:** Remaining overlying water was removed and the sediment transferred to centrifuge tube. Pore water was separated by centrifugation of sample at 2111 g-force for 15

minutes. The resulting pore water was removed with a pipette and further extracted with hexane in a 1:1 ratio. Sample of the hexane was then analyzed by LSC to determine the concentration of ^{14}C-cypermethrin in pore water.

3) **Sediment Analysis**: Sediment was sequentially extracted with approximately 2 x acetonitrile (35 ml) by shaking for 1 hour. Extracts were combined and brought up to 100 ml with additional acetonitrile. Aliquots of 1 ml samples were analyzed by LSC to determine ^{14}C-cypermethrin concentrations. Unextractable radioactivity was determined by drying the sediment extracts and combusting the samples with a Harvey OX300 Biological Oxidizer. The efficiency of the combustion process, was >90% and values for the combusted samples were not corrected for efficiency.

Representative sediment extracts were analyzed by Thin Layer Chromatography (TLC) in order to determine the purity of the ^{14}C-cypermethrin application solutions and to characterize radioactivity. Samples were chromatographed in parallel with the unlabeled analytical standards of cypermethrin. Quantification was carried out relative to the level of radioactivity applied to the TLC plate. Analysis was conducted with a Rita 68000 Automatic TLC Analyzer or a Fuji BAS Phosphorimager.

Analysis of Test Systems B and C

1) After the overlying water and test organisms were removed from replicate B, the sediment was transferred to centrifuge tubes for acetonitrile extraction and combustion (as noted previously). Only sediment was analyzed in these tests in order to confirm exposure.

Analytical Methods

Liquid Scintillation Counting was carried out using an LKB 1217 Rackbeta liquid scintillation counter. Optiphase Safe was the scintillation cocktail used. Each group of samples was preceded by two blank samples in order to calculate background. Subsequent samples were background corrected and disintegrations per second (DPS) calculated.

G. Physicochemical Analysis:

1) Dissolved oxygen was measured daily using a YSI Model 57 meter (one replicate per concentration). Measurement were taken daily in Test Systems E and F until levels had fallen below 40% saturation, after which aeration of the test vessels was begun. In test B and C, test vessels were aerated from the beginning of the test. Measurements were taken on days 5 and 10.

2) Specific conductivity and pH were measured using YSI Model 33 and Radiometer pHM62 meters (one replicate per concentration).

3) Ammonium nitrogen analysis was carried out by NRM Ltd. by reaction with hypocholrite and phenol, catalyzed by sodium prusside and colormetric determination of the indophenol formed using an Alpkem RFA analyzer.

4) Temperature within test vessels was maintained with a water bath, with readings every 30 minutes.

H. Statistical Analysis:

Survival data from replicates A to D were analyzed by the technique of iteratively reweighed linear regression of logit response on $\log_{10}$ (concentration) using LOGITPC, version 1.2 to obtain estimates of slope, LC_{50} and 95% confidence intervals. Adjustments were made for any mortality in the solvent control. Length and weight were analyzed by analysis of variance, comparing the treated group with the solvent controls using Statistical Analysis System (SAS), version 6.10.

I. Reported Results:

The number of *Chironomus* surviving fulfilled the validity criteria of 70% survival in the controls. One pupa and one adult emerged in the Mississippi 3 solvent control. These were recorded as surviving, but were not included in the dry weight determinations.

The LC_{50} and NOEC values, were based upon the measured sediment concentrations (Florrisant, Mississippi 3, Duluth) at day 0 (Table 2). The reported LC_{50}s were 14, 67 and 62 ug kg^{-1} and the NOECs for weight were 4.9, 25 and 14 ug kg^{-1}, respectively.

Table 2. Statistical results Based on Measured ^{14}C-cypermethrin Concentrations at Day 0 and Measured Mean of Day 0 and Day 10.

Sediment	10 Day LC_{50} Values (ug kg^{-1})	NOEC for Weight (ug kg^{-1})	
	Day 0	Day 0	Mean[1]
Florrisant	14 (4.4-42)	4.9	4.4
Mississippi 3	67 (34-172)	25	24
Duluth	62 (23-176)	14	14

1) Average measured concentration

Table 3. ^{14}C-cypermethrin Measured Concentrations; Test 2, Data Used by EPA for Evaluating Acute Toxicity.

Sediment Nominal Levels (ug kg^{-1})	Sediment Measured Levels (ug kg^{-1})		Average Measured Levels (ug kg^{-1})
	Day 0	Day 10	
Florrisant			
180	148.0	77.0	112.5
60	35.0	23.0	29.0
20	17.0	9.9	13.4
6.7	5.2	3.0	4.1
2.2	1.8	0.7	1.2
Sol. Control	<0.5	<0.5	<0.5
Control	<0.5	<0.5	<0.5
Mississippi 3			
300	275.0	241.0	258.0
100	100.0	72.0	86.0
33	27.0	19.0	23.0
11	9.8	7.9	8.8
3.4	3.0	2.1	2.5
Sol. Control	<0.5	<0.5	<0.5
Control	<0.5	<0.5	<0.5
Duluth			
450	390.0	265.0	327.5
150	148.0	111.0	129.5
50	40.0	31.0	32.5
17	15.0	13.0	14.0
5.6	4.2	4.1	4.1
Sol. Control	<0.5	<0.5	<0.5
Control	<0.5	<0.5	<0.5

Table 4. Measured ^{14}C-cypermethrin Concentrations; Test 1.

Sediment Nominal Concentrations (ug kg^{-1})	Sediment Concentration (ug kg^{-1}) Day 0	Sediment Concentration (ug kg^{-1}) Day 10	Average Measured Concentration (ug kg^{-1})
Florrisant			
Sol.Control	<0.5	<0.5	<0.5
60	38	27	32.5
20	14	10	12.0
6.7	4.9	3.9	4.4
2.2	1.8	1.0	1.4
Mississippi 3			
Sol. control	<0.5	<0.5	<0.5
100	72	62	67
33	25	23	24
11	8.6	7.5	8.0
3.4	3.4	2.4	2.9
Duluth			
Sol. Control	<0.5	<0.5	<0.5
50	41	39	40
17	14	14	14
5.6	4.8	4.2	4.5

14. **REVIEWER'S COMMENTS:**

Two test were conducted (Test 1 and Test 2). In the LC_{50} test (Test 1), the mortality data for solvent controls was 60, 50, and 72% for Florrisant, Mississippi 3, and Duluth sediments, respectively. In addition to this unacceptable control mortality, some organisms tested in the Florrisant and Mississippi 3 sediments had started to pupate by the end of the test. However, the researchers used this data to determine the acute toxicity in Table 2. ASTM Guidelines state that sediment toxicity test are unacceptable if the average survival in any control chamber is less than 70% . Therefore, Test 1 acute values for Florrisant and Mississippi 3 sediments are invalid. The researchers conducted another LC_{50} test (Test 2) where solvent controls had survival at 70, 80, and 90% for Florrisant, Mississippi 3, and Duluth sediments, respectively. EEB will rely on these LC_{50} values for the determination of acute toxicity. The results from these findings are as follows:

LC_{50} values (Study 2) Florrisant LC_{50} = 2.3 ug kg^{-1} (0 - 112.5 ug kg^{-1}); Mississippi 3 LC_{50} = 27.8 ug kg^{-1} (8.6 - 102.8 ug kg^{-1}); Duluth LC_{50} = 36.5 ug kg^{-1} (18.9 - 69.8 ug kg^{-1}).

Testing for effects on growth were conducted in Test 1 and appear to be acceptable. NOECs were as followed: Florrisant, 4.4 ug kg^{-1}; Mississippi 3, 24 ug kg^{-1}; Duluth, 14 ug kg^{-1}. EEB will not use Study 2 data for evaluating growth because the number of replicates per concentration were only two.

The statistical results were reported by the researchers after using measured concentrations from Day 0. Since the average measured concentrations were lower than Day 0, EEB evaluated both acute and growth effects from the average measured concentrations.

EEB has a concern for deviation in DO in test 1 (Florrisant) sediment testing. The DO levels in this test dropped below 70% during the first 48 hr (23-25%) but by Day 5 and 10 were $\geq$ 60%. These test were repeated in Test 2 but values for the first 48 hours (Day 2) were not given.

Water hardness levels were very high (161-178 mg l^{-1} as $CaCO_3$) as compared to the recommended values of 40 - 48 mg l^{-1} as $CaCO_3$.

The sediment:water ratio was 1:25. The recommneded ratio is 1:1 or 1:2. This high volume of water should not affect the benthic test organisms exposure to cypermethrin.

Sediment test with midge *C. tentans* are generally started with 2nd instar larvae (10-14 days old). However, these studies used the 3rd instar, which may underestimate sensitivity to toxicants.

Adequacy of the Study:

1) Classification: Supplemental.

2) Rationale: Researchers used 3rd instar organisms, 2nd instar is preferred.

3) Repairability: No.

Cypermethrin Sediment 10 Day: Chironomus; Mississippi 3 Std. 2

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
258	10	10	100	9.765625E-02
86	10	5	50	62.30469
23	10	3	30	17.1875
8.8	10	3	30	17.1875
2.5	10	4	40	37.69531

THE BINOMIAL TEST SHOWS THAT 0 AND 258 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 85.99997

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	.3709149	27.88428	8.678088	102.8924

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.4946948	1	6.109774E-02

SLOPE = .7621141

95 PERCENT CONFIDENCE LIMITS = .2260847 AND 1.298144

LC50 = 24.51811

95 PERCENT CONFIDENCE LIMITS = 4.992314 AND 112.1523

LC10 = .5285302

95 PERCENT CONFIDENCE LIMITS = 4.357991E-05 AND 3.25941

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From table 11.

Cypermethrin Sediment 10 Day: Chironomus; Duluth Styd. 2

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
327.5	10	10	100	9.765625E-02
129.5	10	9	90	1.074219
32.5	10	3	30	17.1875
14	10	1	10	1.074219
4.1	10	2	20	5.46875

THE BINOMIAL TEST SHOWS THAT 0 AND 129.5 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 50.04148

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	.1501978	36.54656	18.95993	69.82429

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.2042655	1	6.755352E-02

SLOPE = 1.697299

95 PERCENT CONFIDENCE LIMITS = .9301921 AND 2.464405

LC50 = 35.10737

95 PERCENT CONFIDENCE LIMITS = 18.24093 AND 67.18146

LC10 = 6.268309

95 PERCENT CONFIDENCE LIMITS = 1.23277 AND 13.08482

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Table 11

Cypermethrin Sediment 10 Day: Chironomus Florisant Study 2

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
112.5	10	10	100	9.765625E-02
29	10	6	60.00001	37.69531
13.4	10	8	80	5.46875
4.1	10	5	50	62.30469
1.25	10	5	50	62.30469

THE BINOMIAL TEST SHOWS THAT 0 AND 112.5 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 2.263847

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	2.169066	2.263846	0	+INFINITY

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.6206306	1	.2285529

SLOPE = .7517967
95 PERCENT CONFIDENCE LIMITS = .1595303 AND 1.344063

LC50 = 2.298669
95 PERCENT CONFIDENCE LIMITS = 8.771237E-03 AND 7.419603

LC10 = 4.700997E-02
95 PERCENT CONFIDENCE LIMITS = 1.455836E-10 AND .5553521

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Table 11

NOEC 44 mg Kg

TITLE: Florrisant: Chironomus Dry Wt.
FILE: C:\wp51\sediment\chir.sed
TRANSFORM: NO TRANSFORM NUMBER OF GROUPS: 5

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Solvent Control	1	2.4500	2.4500
1	Solvent Control	2	2.5200	2.5200
1	Solvent Control	3	2.7700	2.7700
1	Solvent Control	4	3.6000	3.6000
2	32.5	1	2.8900	2.8900
2	32.5	2	2.7300	2.7300
2	32.5	3	2.3600	2.3600
2	32.5	4	2.9000	2.9000
3	12	1	2.3600	2.3600
3	12	2	2.4200	2.4200
3	12	3	2.0400	2.0400
3	12	4	2.6400	2.6400
4	4.4	1	1.5700	1.5700
4	4.4	2	1.0600	1.0600
4	4.4	3	2.4300	2.4300
4	4.4	4	2.9200	2.9200
5	1.4	1	1.1400	1.1400
5	1.4	2	2.0600	2.0600
5	1.4	3	1.2400	1.2400
5	1.4	4	0.6400	0.6400

Florrisant: Chironomus Dry Wt.
File: C:\wp51\sediment\chir.sed Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Solvent Control	4	2.450	3.600	2.835
2	32.5	4	2.360	2.900	2.720
3	12	4	2.040	2.640	2.365
4	4.4	4	1.060	2.920	1.995
5	1.4	4	0.640	2.060	1.270

Florrisant: Chironomus Dry Wt.
File: C:\wp51\sediment\chir.sed Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	Solvent Control	0.279	0.528	0.264

2	32.5	0.064	0.252	0.126
3	12	0.061	0.248	0.124
4	4.4	0.700	0.837	0.418
5	1.4	0.346	0.588	0.294

Florrisant: Chironomus Dry Wt.
File: C:\wp51\sediment\chir.sed Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	6.404	1.601	5.521
Within (Error)	15	4.351	0.290	
Total	19	10.754		

Critical F value = 3.06 (0.05,4,15)
Since F > Critical F REJECT Ho:All groups equal

Florrisant: Chironomus Dry Wt.
File: C:\wp51\sediment\chir.sed 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	Solvent Control	2.835	2.835		
2	32.5	2.720	2.720	0.302	
3	12	2.365	2.365	1.234	
4	4.4	1.995	1.995	2.206	
5	1.4	1.270	1.270	4.110	*

Dunnett table value = 2.36 (1 Tailed Value, P=0.05, df=15,4)

Florrisant: Chironomus Dry Wt.
File: C:\wp51\sediment\chir.sed 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	Solvent Control	4			
2	32.5	4	0.899	31.7	0.115
3	12	4	0.899	31.7	0.470
4	4.4	4	0.899	31.7	0.840
5	1.4	4	0.899	31.7	1.565

Florrisant: Chironomus Dry Wt.
File: C:\wp51\sediment\chir.sed Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	6.404	1.601	5.521
Within (Error)	15	4.351	0.290	
Total	19	10.754		

Critical F value = 3.06 (0.05,4,15)
Since F > Critical F REJECT Ho:All groups equal

Florrisant: Chironomus Dry Wt.
File: C:\wp51\sediment\chir.sed Transform: NO TRANSFORM

TUKEY method of multiple comparisons

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 0 5 4 3 2 1
5		1.4	1.270	1.270 \
4		4.4	1.995	1.995 . \
3		12	2.365	2.365 . . \
2		32.5	2.720	2.720 * . . \
1	Solvent Control		2.835	2.835 * . . . \

NOEC 4.4 mg/kg

* = significant difference (p=0.05) . = no significant difference
Tukey value (5,15) = 4.37 s = 0.290

Florrisant: Chironomus Dry Wt.
File: C:\wp51\sediment\chir.sed Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Solvent Control	4	2.835	2.835	2.835
2	32.5	4	2.720	2.720	2.720
3	12	4	2.365	2.365	2.365
4	4.4	4	1.995	1.995	1.995
5	1.4	4	1.270	1.270	1.270

NOEC 24 mg Kg-1

TITLE: Mississippi 3: Chironomus Dry Wt.
FILE: C:\wp51\sediment\chirmiss.sed
TRANSFORM: NO TRANSFORM NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Solvent Control	1	1.8300	1.8300
1	Solvent Control	2	2.5700	2.5700
1	Solvent Control	3	2.6400	2.6400
1	Solvent Control	4	1.8100	1.8100
2	2.9	1	2.2600	2.2600
2	2.9	2	2.3200	2.3200
2	2.9	3	1.6800	1.6800
2	2.9	4	2.1600	2.1600
3	8	1	1.6900	1.6900
3	8	2	2.2300	2.2300
3	8	3	2.5200	2.5200
3	8	4	2.6200	2.6200
4	24	1	1.3900	1.3900
4	24	2	2.3900	2.3900
4	24	3	2.1600	2.1600
4	24	4	2.0100	2.0100
5	67	1	1.0900	1.0900
5	67	2	0.7500	0.7500
5	67	3	0.8000	0.8000
5	67	4	1.3200	1.3200
6	238	1	0.1800	0.1800
6	238	2	0.1800	0.1800

Mississippi 3: Chironomus Dry Wt.
File: C:\wp51\sediment\chirmiss.sed Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Solvent Control	4	1.810	2.640	2.213
2	2.9	4	1.680	2.320	2.105
3	8	4	1.690	2.620	2.265
4	24	4	1.390	2.390	1.988
5	67	4	0.750	1.320	0.990
6	238	2	0.180	0.180	0.180

Mississippi 3: Chironomus Dry Wt.
File: C:\wp51\sediment\chirmiss.sed Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	Solvent Control	0.206	0.454	0.227
2	2.9	0.085	0.291	0.145
3	8	0.174	0.417	0.209
4	24	0.183	0.428	0.214
5	67	0.071	0.266	0.133
6	238	0.000	0.000	0.000

Mississippi 3: Chironomus Dry Wt.
File: C:\wp51\sediment\chirmiss.sed Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	9.886	1.977	14.644
Within (Error)	16	2.158	0.135	
Total	21	12.044		

Critical F value = 2.85 (0.05,5,16)
Since F > Critical F REJECT Ho:All groups equal

Mississippi 3: Chironomus Dry Wt.
File: C:\wp51\sediment\chirmiss.sed Transform: NO TRANSFORM

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Solvent Control	2.213	2.213		

2	2.9	2.105	2.105	0.414	
3	8	2.265	2.265	-0.202	
4	24	1.988	1.988	0.866	
5	67	0.990	0.990	4.705	*
6	238	0.180	0.180	6.388	*

Bonferroni T table value = 2.58 (1 Tailed Value, P=0.05, df=16,5)

Mississippi 3: Chironomus Dry Wt.
File: C:\wp51\sediment\chirmiss.sed Transform: NO TRANSFORM

BONFERRONI T-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	Solvent Control	4			
2	2.9	4	0.671	30.3	0.108
3	8	4	0.671	30.3	-0.052
4	24	4	0.671	30.3	0.225
5	67	4	0.671	30.3	1.223
6	238	2	0.822	37.2	2.033

Mississippi 3: Chironomus Dry Wt.
File: C:\wp51\sediment\chirmiss.sed Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	9.886	1.977	14.644
Within (Error)	16	2.158	0.135	
Total	21	12.044		

Critical F value = 2.85 (0.05,5,16)
Since F > Critical F REJECT Ho:All groups equal

Mississippi 3: Chironomus Dry Wt.
File: C:\wp51\sediment\chirmiss.sed Transform: NO TRANSFORM

TUKEY method of multiple comparisons

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 0 0 6 5 4 2 1 3
6	238	0.180	0.180	\
5	67	0.990	0.990	. \
4	24	1.988	1.988	* * \

2	2.9	2.105	2.105	* * . \
1	Solvent Control	2.213	2.213	* * . . \
3	8	2.265	2.265	* * . . . \

* = significant difference (p=0.05) . = no significant difference
Tukey value (6,16) = 4.56 s = 0.135

Mississippi 3: Chironomus Dry Wt.
File: C:\wp51\sediment\chirmiss.sed Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Solvent Control	4	2.213	2.213	2.213
2	2.9	4	2.105	2.105	2.185
3	8	4	2.265	2.265	2.185
4	24	4	1.988	1.988	1.988
5	67	4	0.990	0.990	0.990
6	238	2	0.180	0.180	0.180

Mississippi 3: Chironomus Dry Wt.
File: C:\wp51\sediment\chirmiss.sed Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Solvent Control	2.213				
2.9	2.185	0.106		1.75	k= 1, v=16
8	2.185	0.106		1.83	k= 2, v=16
24	1.988	0.867		1.86	k= 3, v=16
67	0.990	4.708	*	1.87	k= 4, v=16
238	0.180	6.391	*	1.88	k= 5, v=16

s = 0.367
Note: df used for table values are approximate when v > 20.

14 ug kg⁻¹

TITLE: Duluth: Chironomus Dry Wt.
FILE: C:\wp51\sediment\chirdult.sed
TRANSFORM: NO TRANSFORM NUMBER OF GROUPS: 4

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Solvent Control	1	1.3900	1.3900
1	Solvent Control	2	2.0600	2.0600
1	Solvent Control	3	1.8600	1.8600
1	Solvent Control	4	2.0400	2.0400
2	4.5	1	2.0400	2.0400
2	4.5	2	1.7200	1.7200
2	4.5	3	1.7700	1.7700
2	4.5	4	1.5400	1.5400
3	14	1	1.5900	1.5900
3	14	2	1.7600	1.7600
3	14	3	1.5400	1.5400
3	14	4	1.9500	1.9500
4	40	1	1.3400	1.3400
4	40	2	1.6800	1.6800
4	40	3	1.1500	1.1500

Duluth: Chironomus Dry Wt.
File: C:\wp51\sediment\chirdult.sed Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Solvent Control	4	1.390	2.060	1.838
2	4.5	4	1.540	2.040	1.768
3	14	4	1.540	1.950	1.710
4	40	3	1.150	1.680	1.390

Duluth: Chironomus Dry Wt.
File: C:\wp51\sediment\chirdult.sed Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	Solvent Control	0.097	0.312	0.156
2	4.5	0.043	0.207	0.103
3	14	0.034	0.186	0.093
4	40	0.072	0.269	0.155

Duluth: Chironomus Dry Wt.
File: C:\wp51\sediment\chirdult.sed Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	3	0.382	0.127	2.082
Within (Error)	11	0.667	0.061	
Total	14	1.049		

Critical F value = 3.59 (0.05,3,11)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

Duluth: Chironomus Dry Wt.
File: C:\wp51\sediment\chirdult.sed Transform: NO TRANSFORM

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Solvent Control	1.838	1.838		
2	4.5	1.768	1.768	0.401	
3	14	1.710	1.710	0.730	
4	40	1.390	1.390	2.372	

Bonferroni T table value = 2.43 (1 Tailed Value, P=0.05, df=11,3)

Duluth: Chironomus Dry Wt.
File: C:\wp51\sediment\chirdult.sed Transform: NO TRANSFORM

BONFERRONI T-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	Solvent Control	4			
2	4.5	4	0.425	23.1	0.070
3	14	4	0.425	23.1	0.127
4	40	3	0.459	25.0	0.448

Duluth: Chironomus Dry Wt.
File: C:\wp51\sediment\chirdult.sed Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F

Between	3	0.382	0.127	2.082
Within (Error)	11	0.667	0.061	
Total	14	1.049		

Critical F value = 3.59 (0.05,3,11)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

Duluth: Chironomus Dry Wt.
File: C:\wp51\sediment\chirdult.sed Transform: NO TRANSFORM

TUKEY method of multiple comparisons

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 4 3 2 1
4	40	1.390	1.390	\
3	14	1.710	1.710	. \
2	4.5	1.768	1.768	. . \
1	Solvent Control	1.838	1.838	... . \

* = significant difference (p=0.05) . = no significant difference
Tukey value (4,11) = 4.26 s = 0.061

Duluth: Chironomus Dry Wt.
File: C:\wp51\sediment\chirdult.sed Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Solvent Control	4	1.838	1.838	1.838
2	4.5	4	1.768	1.768	1.768
3	14	4	1.710	1.710	1.710
4	40	3	1.390	1.390	1.390

Duluth: Chironomus Dry Wt.
File: C:\wp51\sediment\chirdult.sed Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Solvent Control	1.838				
4.5	1.768	0.402		1.80	k= 1, v=11
14	1.710	0.732		1.89	k= 2, v=11
40	1.390	2.379	*	1.92	k= 3, v=11

s = 0.246

Note: df used for table values are approximate when v > 20.

Florrisant: Chironomus Dry Wt.
File: C:\wp51\sediment\chir.sed Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Solvent Control	2.835				
32.5	2.720	0.302		1.75	k= 1, v=15
12	2.365	1.234		1.84	k= 2, v=15
4.4	1.995	2.206	*	1.87	k= 3, v=15
1.4	1.270	4.110	*	1.88	k= 4, v=15

s = 0.539
Note: df used for table values are approximate when v > 20.