Data Evaluation Report on the Chronic Toxicity of XDE-638 to Freshwater invertebrates - Daphnia sp. PMRA Submission Number EPA MRID Number 45831026

Data Requirement:

PMRA DATA CODE

EPA DP Barcode

D288160

OECD Data Point EPA MRID

45831026

EPA Guideline

§72-4b

Test material:

XDE-638

Purity: 97.5%

Common name:

Penoxsulam

Chemical name:

IUPAC: Not reported

CAS name: 2-(2,2-Difluoroethoxy)-N-(5,8-dimethoxy[1,2,4]triazolo[1,5-C]pyrimidin-2-yl)-6-

(trifluoromethyl)benzenesulfonamide

CAS No.: Not reported Synonyms: None specified

Primary Reviewer: Rebecca Bryan Staff Scientist, Dynamac Corporation

Signature: New CCA Bright -Date: 10/31/03

QC Reviewer: Christie E. Padova Staff Scientist, Dynamac Corporation Signature: C. E Portor
Date: 10/31/03

Date: Agoodye Comments

Date: Agoody

Primary Reviewer: William Erickson - Biologist J GOODYE AD

OPP/EFED/ERB - III

Secondary Reviewer(s):

{EPA/OECD/PMRA}

Date:

Reference/Submission No.:

Company Code: Active Code:

EPA PC Code: 199031

119031

Date Evaluation Completed:

CITATION: Kirk, H.D., et. al. 2000. XDE-638: 21-Day Chronic Toxicity Test with the Daphnid, Daphnia magna Straus. Unpublished study performed by Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI. Laboratory Study No. 001018. Study submitted by Dow AgroSciences LLC, Indianapolis, IN. Study initiated May 11, 2000 and completed October 27, 2000.





EXECUTIVE SUMMARY:

The 21-day chronic toxicity of XDE-638 (penoxsulam) to *Daphnia magna* was studied under static renewal conditions. Daphnids were exposed to XDE-638 at nominal concentrations of 0 (negative control), 0.041, 0.123, 0.37, 1.11, 3.33, and 10 ppm. Mean-measured concentrations were <0.01(LOQ, control), 0.040, 0.111, 0.376, 0.942, 2.95, and 9.76 ppm a.i.

By Day 21, mortality was 10% in the dilution water control, 0% in the 0.040 and 0.111 ppm a.i. test groups, 30% in the 0.376 and 0.942 ppm a.i. test groups, and 10% in the 2.95 and 9.76 ppm a.i. test groups. The 21-day groups; no other sub-lethal effects were observed. The 21-day EC_{50} was >9.76 ppm a.i.

No treatment-related effects were observed on the day of first eggs observed, the day to first brood release, the total number of offspring produced, the number of offspring per adult, or terminal lengths. However, a statistically-significant reduction in the number of live offspring produced was observed at the 9.76 ppm a.i. level compared to the control group (922 versus 1395). Based on the number of live offspring (the only endpoint affected), the NOAEC, LOAEC, and MATC values were 2.95, 9.76, and 5.37 ppm a.i., respectively.

This study is scientifically sound, fulfills the guideline requirements for an aquatic invertebrate life cycle test with Daphnia magna (§ 72-4b), and is classified CORE.

Results Synopsis:

Test Organism Age (eg. 1st instar): <24 hours old Test Type (Flow through, Static, Static Renewal): Static renewal.

Mortality

NOAEC: 9.76 ppm a.i. LOAEC: >9.76 ppm a.i.

Immobility

NOAEC: 9.76 ppm a.i. LOAEC: >9.76 ppm a.i.

Total No. Young

NOAEC: 9.76 ppm a.i. LOAEC: >9.76 ppm a.i. Total No. Live Young

NOAEC: 2.95 ppm a.i. LOAEC: 9.76 ppm a.i.

Length

NOAEC: 9.76 ppm a.i. LOAEC: >9.76 ppm a.i.

Day to First Eggs & Day to First Brood

NOAEC: 9.76 ppm a.i. LOAEC: >9.76 ppm a.i.

Endpoints Affected: Total number of live young

I. MATERIALS AND METHODS

GUIDELINES FOLLOWED:

The study protocol was based on procedures outlined in the U.S. EPA Pesticide Assessment Guidelines, Series §72-4 (1982), and the U.S. EPA Standard Evaluation Procedure <u>Daphnia magna</u> Life Cycle (21-Day Renewal) Chronic Toxicity Test (1987). Deviations from guideline §72-4b include:

- 1. The storage conditions of the test material were not reported.
- 2. The pretest health (including mortality) of the parental stock was not specified. In addition, an isolated 21-day acclimation period was not performed.
- 3. The pH range of the dilution water (6.5-7.6) was less than recommended (7.6-8.0).
- 4. The study design differed from EPA guidance: in this static renewal study, 1 daphnid per test chamber was maintained, with ten replicate chambers per concentration (total of 10 daphnids/concentration). EPA guidance recommends 22 daphnids/level for static renewal studies, where seven test chambers should contain one daphnid each (to collect data on survival, growth, and reproduction), and three test chambers should contain five daphnids each (to collect data on survival only).

These deviations did not affect the acceptability or the validity of the study.

COMPLIANCE:

Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. This study was conducted in accordance with U.S. EPA, OECD, and EC GLP standards (p. 3).

A. MATERIALS:

1. Test Material

XDE-638 (penoxsulam)

Description:

Pink, solid powder

Lot No. :

ND05167938

Purity:

97.5%

Stability:

The stability of XDE-638 was assessed

Παγε 3 οφ 17

in dilution water fortified at nominal concentrations of 0.1 and 100 ppm and stored at $20 \pm 2^{\circ}\text{C}$ for up to 24 days (p. 12). Recoveries were 94.9-113% of initial values for the 0.1 ppm solutions and 94.1-102% of initial values for the 100 ppm solutions (Table 3, p. 27).

Storage conditions of test chemical: Not reported.

OECD requires water solubility, stability in water and light, pK_a , P_{ow} , and vapor pressure of the test compound. Stability was reported above. Other OECD requirements were not reported.

2. Test organism:

Species:

Daphnia magna

Age of the parental stock:

<24 hours old

Source:

In-house laboratory cultures.

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: Concentrations selected for use in the definitive study were based upon the results of a 48-hour acute toxicity study (MRID 45831012; $EC_{50} > 100$ ppm) and a 21-day static renewal range-finding study. The 21-day study was conducted with 10 daphnids/level at nominal concentrations of 0 (negative control), 0.1, 1, 30, and 100 ppm (p. 15). After 21 days, mortality was 0, treatment groups, respectively. The average number of young per adult were 210, 175, 159, 114, and 99 in the 0, 0.1, 1, 10, and 100 ppm treatment groups, respectively.

b. Definitive Study:

Table 1. Experimental Parameters

Experimental	Parameters	
Parameter	,	Remarks
Parental acclimation:	Details	Criteria
accilliation:		The day before

Payamata		Remarks
Parameter Period:	Details	Criteria
121100:	Continuous	instars were needed
Conditions (same as test or not):	Same as test	tanks with daphnids
Feeding: Health: (any mortality observed)	Daphnia cultures were fed 4 times/week with mixed diet of Selenastrum capricornutum Printz (algae) and yeast-ceraphyll-trout chow mixture	>14 days old, which had at least four broods, were removed from the incubator. Instars were removed and discarded, and the adults were placed back into the incubator. At the
	(YCT). Not reported	start of the definitive test, the procedure was repeated to cull <24-hour old instars for use.
_		
<u>Test condition</u> : Static renewal/flow through:	Static Renewal	
Type of dilution system- for flow	N/A	For flow-through study: consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period.
through method. Renewal rate for static renewal	At least 3 times per week (Monday, Wednesday, and Friday).	
Aeration, if any	No aeration during the study.	
		Dilution water should be aerated to insure DO concentration at or near 100% saturation. Test tanks should not be aerated.
Duration of the test	21 days	,
		EPA requires 21 days for static
Sest vessel Material: (glass/stainless steel) Property of the state	Borosilicate vessels (covered vith a watch glass)	телениц

Details 250 mL same 150 mL same	I. Materiul: Glass, No. 316 stainless steel, or perfluorocarbon plastics 2. Size: 250 mL with 200 mL fill volume is preferred; 100 mL with 80 mL fill volume is acceptable. OECD requires parent animals be maintained individually, one per vessel, with 50 - 100 mL of medium in
same	stainless steel, or perfluorocarbon plastics 2. Size: 250 mL with 200 mL fill volume is preferred; 100 mL with 80 mL fill volume is acceptable. OECD requires parent animals be maintained individually, one per vessel, with 50 - 100 mL of medium in
150 mL	perfluorocarbon plastics 2. Size: 250 mL with 200 mL fill volume is preferred; 100 mL with 80 mL fill volume is acceptable. OECD requires parent animals be maintained individually, one per vessel, with 50 - 100 mL of medium in
	fill volume is preferred; 100 mL with 80 mL fill volume is acceptable. OECD requires parent animals be maintained individually, one per vessel, with 50 - 100 mL of medium in
	volume is acceptable. OECD requires parent animals be maintained individually, one per vessel, with 50 - 100 mL of medium in
same	maintained individually, one per vessel, with 50 - 100 ml, of medium in
	each vessel.
The dilution water was pumped to the laboratory from the upper Saginaw Bay of Lake Huron. The water was filtered (sand and carbon), pH-adjusted, and UV-irradiated. The hardness was adjusted to approximately 170 mg/L as CaCO ₃ , then the water was autoclaved for 30 minutes and aerated for 24 hours prior to use.	Unpolluted well or spring that has been tested for contaminants, or appropriate reconstituted water (see ASTM for details).
166-170 mg CaCO ₃ /L	The pH range was less than recommended.
6.5-7.6	Results from inorganic and organic
7.4-9.0 mg/L (≥83%)	analysis of the dilution water are provided in Tables 1 and 2, pp. 25-
18.5-20.9°C	26.
1500 ng/mL	Hardness and pH
<1000 ng/mL (<lod, solids)<="" suspended="" td="" total=""><td>EPA requires hardness of 160 to 180 mg/L as CaCO₃ and pH between 7.6 and 8.0. pH must not deviate by more</td></lod,>	EPA requires hardness of 160 to 180 mg/L as CaCO ₃ and pH between 7.6 and 8.0. pH must not deviate by more
See Table 1, p. 25	than one unit for more than 48 hours. OECD requires hardness of > 140 mg/L as CaCO ₃ and pH between 6 and 9.
Not detected; Table 2, p. 26	pri should not vary more than 1.5 units in any one test.
3-5 ppb	Dissolved Oxygen Renewal: must not drop below 50% for more than 48 hours.
	Flow-through: ≥60% through out test. Temperature EPA requires 20 ±2°C; must not
	The water was filtered (sand and carbon), pH-adjusted, and UV-irradiated. The hardness was adjusted to approximately 170 mg/L as CaCO ₃ , then the water was autoclaved for 30 minutes and aerated for 24 hours prior to use. 166-170 mg CaCO ₃ /L 6.5-7.6 7.4-9.0 mg/L (≥83%) 18.5-20.9°C 1500 ng/mL <1000 ng/mL (<lod, 1,="" 2,="" 25="" 26="" 3-5="" detected;="" not="" p.="" ppb<="" see="" solids)="" suspended="" table="" td="" total=""></lod,>



Parameter		Remarks
	Details	Criteria
		for more than 48 hours. OECD requires range of $18 - 22 \text{C}$; should no vary more than $\pm 2 \text{C}$.
Number		OECD requires total organic carbon <2 mg/L.
Number of organisms: For growth and reproduction:	10 daphnids/test level 10 replicate chambers with 1	Did not follow recommended test design.
For survival test:	daphnid per replicate (Not differentiated; same test chambers as above)	EPA requires 22 daphnids/level; 7 test chambers should contain 1 daphnid each, and 3 test chambers
Application rates:		should contain 5 daphnids each. OECD requires minimum of 10 daphnids held individually for static tests. For flow-through tests, 40 animals divided into 4 groups of 10 animals at each test concentration.
nominal: measured:	0 (negative control), 0.041, 0.123, 0.37, 1.11, 3.33, and 10 ppm <0.01 (LOQ, control), 0.04, 0.111, 0.376, 0.942, 2.95, and	Mean-measured concentrations were based on bulk dose solutions and individual test vessels (Table 4, pp. 28-29). Recoveries were 84.9 to 102% of nominal.
	9.76 ppm a.i.	EPA requires control(s) and at least 5 test concentrations; dilution factor not greater than 50%. OECD requires at least 5 test concentrations in a geometric series with a separation factor not exceeding 3.2.
Solvent (type, percentage, if used)	N/A	
ighting		EPA requires: solvent to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests. Acceptable solvents are dimethylforma- mide, triethylene glycol, methanol, acetone and ethanol. OECD requires ≤ 0.1 ml/L
-ganing	16:8 hour light/dark cycle with a half-hour transition period	Intensity averaged 2050 ± 244 lux.
eeding		EPA/OECD requires: 16 hours light, 8 hours dark.
	The daphnids were fed 800 μL	

Parameter	Data (2	Remarks
	Details	Criteria
	of Navicula pelliculosa and 800 μL of YCT per 150 mL of test solution on renewal days, and an additional 500 μL of Nitzschia frustulum was provided on non-renewal days.	
Stability of chemical in the test system	The stability of XDE-638 was assessed in dilution water fortified at nominal concentrations of 0.1 and 100 ppm and stored at $20 \pm 2^{\circ}$ C for up to 24 days (p. 12). Recoveries were 94.9-113% of initial values for the 0.1 ppm solutions and 94.1-102% of initial values for the 100 ppm solutions (Table 3, p. 27).	
Recovery of chemical:	82.7-102% of nominal	Based on individual test vessel
Frequency of measurement:	Samples analyzed on Days 2, 18, and 21.	solutions (Table 4, pp. 28-29).
OD:	Not reported	
.OQ;	0.01 ppm a.i.	
ther parameters, if any	N/A	
ositive control {if used, indicate the nemical and concentrations}	N/A	

2. Observations:

Table 2: Observations

Criteria	70.4	Remarks
	Details	Criteria
Data end points measured (list)	 Number of dead or immobilized adult daphnids Time to first eggs and time to first brood release No. of live and dead offspring born per treatment group. Mean progeny per adult Body length 	EPA requires: - Survival of first-generation daphnids, - Number of young produced per female, - Dry weight (recommended) and length (required)* of each first generation daphnid alive at the end of the test, - Observations of other effects or clinical signs *current requirement until the Agency provides specific guidance indicating otherwise (Pesticide Rejection Rate Analysis, p. 132).
Observation intervals	Mortality was observed daily recorded on Days 1, 2, 4, 7, 14, and 21. Offspring production was observed daily and recorded 3 times/week (Mondays, Wednesdays, and Fridays). Body length was determined at test termination.	<i>y, y, y</i>
Were raw data included?	Yes	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION

A. MORTALITY AND SUB-LETHAL EFFECTS:

By Day 21, mortality was 10% in the dilution water control, 0% in the 0.040 and 0.111 ppm a.i. test groups, 30% in the 0.376 and 0.942 ppm a.i. test groups, and 10% in the 2.95 and 9.76 ppm a.i. test groups (Table 7, p. 32). The 21-day LC_{50} was >9.76 ppm a.i.

Immobility was observed in 10% of daphnids in the 0.942 and 2.95 ppm a.i. treatment groups. The 21-day EC_{50} was >9.76 ppm a.i.



Table 1: Effect of XDE-638 on Survival and Immobilization of Daphnia sp.

Treatment, ppm a.i. Measured (and Nominal)	Mortality	Mortality		Immobility	
Concentrations	No.	%	No.	%	
Dilution water control	1	10	0		
0.040 (0.041)	0	0	0	0	
0.111 (0.123)	0	0		0	
0.376 (0.37)	3	30	0	0	
0.942 (1.11)	3		0	0	
2.95 (3.33)	1	30	1	10	
9.76 (10)	1	10	1	10	
NOAEC, ppm a.i.	ND	10	0	0	
LOAEC, ppm a.i.	ND		ND		
.C ₅₀ /EC ₅₀ (95% C.I.), ppm a.i.	>9.76		ND >9.76		

B. EFFECT ON REPRODUCTION AND GROWTH:

No treatment-related effects on the total number of offspring produced, the number of offspring per adult, or terminal lengths were observed at any treatment level (Table 7, p. 32). However, a statistically-significant reduction in the number of live offspring produced was observed at the 9.76 ppm a.i. level compared to the control group (922 versus 1395).

Based on the number of live offspring (the only endpoint affected), the NOAEC, LOAEC, and MATC values were 2.95, 9.76, and 5.37 ppm a.i., respectively.

Table 2: Effect of XDE-638 on Reproduction and Growth of Daphnia sp.

Treatment, ppm a.i. Measured (and Nominal) Concentrations	Total Number Offspring	Total Number of Live Offspring	Mean No. Young per Adult	Day to 1st Eggs	Day of 1 st Brood Release	Length (mm)
Dilution water control	1395	1395	139.5	5	8	4.48 ± 0.23
0.040 (0.041)	1302	1302	130.2	5	8	
0.111 (0.123)	1452	1452	145.2	6	8	4.17 ± 0.46
0.376 (0.37)	1269	1269	126.9	5	8	4.40 ± 0.22
0.942 (1.11)	1313	1313	131.3	6	8	4.31 ± 0.20
2.95 (3.33)	1304	1304	130.4	5	8	4.24 ± 0.21
9.76 (10)	1144	922*	114.4	5	8	4.20 ± 0.22
NOAEC, ppm a.i.	9.76	2.95	9.76			4.39 ± 0.08
LOAEC, ppm a.i.	>9.76	9.76		ND	ND	9.76
MATC, ppm a.i.	>9.76	5.37	>9.76 >9.76	ND ND	ND ND	>9.76

Significantly different (≈ 0.05) from control.

ND - Not determined.

C. REPORTED STATISTICS:

Mortality and immobilization data were not analyzed because less than 50% mortality/immobilization occurred

Endpoints that were analyzed statistically included the total number of progeny per dose, the total number of live progeny per dose, the mean progeny per adult, and the terminal length. Data (transformed if necessary for normalizing) were test for normality using the Shapiro-Wilk's test, and for homogeneity of variance using Bartlett's test. If the data satisfied these tests, then the one-tailed Dunnett's test was used to determined differences between the treatment groups and the control group. Data that did not satisfy these tests were analyzed by either a Steel's Many-one Rank Test (equal number of replicates), or a Kruskal-Wallis test followed by the Wilcoxon procedure (unequal replicates). The NOAEC was defined as the highest dose group that is not significantly different from the control. The MATC was calculated as the geometric mean of the NOAEC and LOAEC. Mean-measured values were used in all estimations.

D. VERIFICATION OF STATISTICAL RESULTS:

Mortality, immobilization, total number of young, total number of live young, and length were analyzed statistically. Reproduction and growth data did not satisfy the assumptions of ANOVA (i.e., normal distribution and variance homogeneity); the NOAEC and LOAEC for these endpoints were determined using the nonparametric Kruskal-Wallis test, followed by Dunn's multiple comparison (if required). The NOAEC and LOAEC for mortality and immobility were determined using Fisher's Exact Test. These analyses were conducted using TOXSTAT statistical software. The NOAEC and LOAEC for day to first eggs and day to first



brood could be visually determined.

Results synopsis

Mortality NOAEC: 9.76 ppm a.i.

LOAEC: >9.76 ppm a.i.

Immobility

NOAEC: 9.76 ppm a.i. LOAEC: >9.76 ppm a.i.

Total No. Young

NOAEC: 9.76 ppm a.i. LOAEC: >9.76 ppm a.i. Total No. Live Young

NOAEC: 9.76 ppm a.i. LOAEC: >9.76 ppm a.i.

Length

NOAEC: 9.76 ppm a.i. LOAEC: >9.76 ppm a.i.

Day to First Eggs & Day to First Brood

NOAEC: 9.76 ppm a.i. LOAEC: >9.76 ppm a.i.

Endpoints Affected: None

E. STUDY DEFICIENCIES:

There were no significant deviations from U.S. EPA guideline §72-4b that affected the validity or acceptability of this study. Although the study design differed from recommended guidance, all objectives of a chronic daphnid study were met. Until the Agency provides specific guidance on the terminal growth measurements, the length measurement is considered adequate in fulfilling guideline requirements.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions differed from those of the study authors. The reviewer's analysis revealed that no endpoint was sensitive to treatment with XDE-638. The study authors' analysis detected a significant reduction in the total number of live progeny at the highest treatment level. Because the study authors' conclusions were more conservative than the reviewer's, they are reported in the Executive Summary and Conclusions sections.

The study authors reported that although the spread of concentrations used in the definitive study was outside of the range suggested by the U.S. EPA, that this deviation was required in an attempt to achieve a NOAEC (p. 15).

There were no observations of ephippia produced by any of the organisms (p. 20).

G. CONCLUSIONS:

The study is scientifically sound and fulfills the guideline requirements for an aquatic invertebrate life cycle test with the *Daphnia magna* (§72-4b). This study is classified as CORE. The only endpoint affected was the total number of live offspring, at the highest concentration tested of 9.76 ppm a.i..

Results synopsis Mortality

NOAEC: 9.76 ppm a.i. LOAEC: >9.76 ppm a.i.

Immobility

NOAEC: 9.76 ppm a.i. LOAEC: >9.76 ppm a.i.

Total No. Young

NOAEC: 9.76 ppm a.i. LOAEC: >9.76 ppm a.i.

Total No. Live Young

NOAEC: 2.95 ppm a.i. LOAEC: 9.76 ppm a.i.

Length

NOAEC: 9.76 ppm a.i. LOAEC: >9.76 ppm a.i.

Day to First Eggs & Day to First Brood

NOAEC: 9.76 ppm a.i. LOAEC: >9.76 ppm a.i.

Endpoints Affected: Total number of live young

III. REFERENCES:

McKim, J.M. 1977. Evaluation of Tests with the Early Life Stages of Fish for Predicting Long-Term Toxicity. J. Fish Research Board Canada 34:1148-1154.

Environmental Protection Agency. Hazard Evaluation Division: Standard Evaluation Procedure <u>Daphnia magna</u> Life Cycle (21-Day Renewal) Chronic Toxicity Test. EPA 540/9-86-141, June 1987.

Environmental Protection Agency. Pesticide Assessment Guidelines. Subdivision E Hazard Evaluation: Wildlife and Aquatic Organisms. EPA-540/9-82-024, Section 72-4, October 1982.

Organisation for Economic Cooperation and Development. OECD Guideline for Testing Chemicals. Method 202, "Daphnia sp., Acute Immobilization and Reproduction Test." Part II. Adopted April 4, 1984.

Organisation for Economic Cooperation and Development. OECD Guideline for Testing Chemicals. Method 211 "Daphnia magna Reproduction Test." Adopted September 21, 1998.

Environmental Protection Agency-FIFRA GLPS; Title 40 CFR Part 160-Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards, Final Rule.

OECD Series on Principles on Good Laboratory Practice and Compliance Monitoring, Number 1. OECD Principles on Good Laboratory Practice (as revised in 1997) ENV/MC/CHEM(98)17.

EC Directive 99/11/EC of 8 March 1999 (OJ No. L 77/8-21, 23/3/1999).

Smith, A.J. "Certificate of Analysis for Test/Reference/Control/Substances Analytical Report FA & PC Number 993090." 20 May 1999.

Personal Communication with E.L. McClymont, The Dow Chemical Company, April 2000.

Marino, T.A., et al. 2000. XDE-638: An Acute Toxicity Study with the Daphnia, Daphnia magna Straus" Study ID# 991215. Unpublished report of The Dow Chemical Company, Midland, MI USA.

Kirk, H.D., et al. 2000. Effects of XDE-638 on the Growth of the Freshwater Green Alga, Selenastrum capricornutum, Printz" Study ID# 991177. Unpublished report of The Dow Chemical Company, Midland, MI



USA.

- Kirk, H.D., et al. 2000. XDE-638: Growth Inhibition Test with the Freshwater Diatom, Navicula pelliculosa" Study ID# 001001. Unpublished report of The Dow Chemical Company, Midland, MI USA.
- Shapiro, S.S. and M.B. Wilk. 1965. "An Analysis of Variance Test for Normality (complete samples)", Biometrika, 52, 591-611.
- Winer, B.J. 1971. Statistical Methods in Experimental Design (2nd Ed.) McGraw-Hill, New York, NY.
- Steel, R.G.D. 1959. A Multiple Comparison Rank Sum Test: Treatments versus Control, Biometrics, 15:560-572.
- Hollander, M. and D.A. Wolfe. 1973. Nonparametric Statistical Methods; John Wiley: New York.

APPENDIX 1. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

SUMMARY OF FISHERS EXACT TESTS

GROUP	NUM IDENTIFICATION	IBER N	NUMBER EXPOSED	SIG DEAD	(P=.05)
1 2 3 4 5	CONTROL 0.040 mg/L 0.111 mg/L 0.376 mg/L 0.942 mg/ 2.95 mg/L 9.76 mg/L	10 10 10 10 10 10	1 0 0 3 3 1		

Immobility

SUMMARY OF FISHERS EXACT TESTS

GROUP	NUM IDENTIFICATIO	IBER N	NUMBER EXPOSED	SIG DEAD	(P=.05)
1 2 3 4	CONTROL 0.040 mg/L 0.111 mg/L 0.376 mg/L 0.942 mg/L	10 10 10 10 10	0 0 0 0 0	• •	
6	2.95 mg/L 9.76 mg/L	10 10	1 0		

total young

File: 1026ty

Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	TRAI IDENTIFICAT		D MEAN MEAN		CULATED IN IGINAL UNITS	RANK SUM
1 2 3 4 5 6 7	0.040 1: 0.111 1: 0.376 1: 0.942 1: 2.95 13	139.500 30.200 45.200 26.900 31.300 0.400 4.400	139. 130.2 145.2 126.9 131.3 130.40 114.40	00 00 00 00 00	377.500 351.000 432.500 362.500 411.500 313.500 236.500	

Calculated H Value = 6.316 Critical H Value Table = 12.590 Since Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.

total young

File: 1026ty

Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

TRANSFORMED ORIGINAL 0000000 GROUP IDENTIFICATION MEAN MEAN 7426513 7 9.76 114.400 114.400 \ 4 0.376 126.900 126.900 \ 2 0.040 130.200 130.200 \ 6 2.95 130.400 130.400 \ 5 0.942 131.300 131.300 \ 1 control 139.500 139.500 \ 3 0.111 145.200 145.200 \ \end{array}

* = significant difference (p=0.05) . = no significant difference SE = 9.097

live young

File: 1026ly Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	TRANSFORM IDENTIFICATION		CULATED IN RIGINAL UNITS	RANK SUM
1 2 3 4 5 6 7	control 139.500 0.040 130.200 0.111 145.200 0.376 126.900 0.942 131.300 2.95 130.400 9.76 92.200	139.500 130.200 145.200 126.900 131.300 130.400 92.200	389.500 367.000 441.500 374.500 418.500 332.500 161.500	

. Calculated H Value = 12.650 Critical H Value Table = 12.590 Since Calc H > Crit H REJECT Ho:All groups are equal.

live young

File: 1026ly Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP TRANSFORMED ORIGINAL 0000000 GROUP IDENTIFICATION MEAN MEAN 7426513

```
7 9.76 92.200 92.200 \
4 0.376 126.900 126.900 \
2 0.040 130.200 130.200 \
6 2.95 130.400 130.400 \
5 0.942 131.300 131.300 \
1 control 139.500 139.500 \
3 0.111 145.200 145.200 * \
```

* = significant difference (p=0.05) . = no sig Table q value (0.05,7) = 3.038 SE = 9.097

. = no significant difference

length

File: 10261

Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

1 2 3 4 5 6 7					
	TRANSFORM IDENTIFICATION		IED MEAN (MEAN	CALCULATED IN ORIGINAL UNITS	RANK SUM
	control 0.04 0.111 0.376 0.942 2.95 9.76	4.478 4.170 4.400 4.314 4.243 4.200 4.388	4.478 4.170 4.400 4.314 4.243 4.200 4.388	386.000 253.000 362.500 205.500 161.500 184.500 277.000	

Calculated H Value = 15.606 Critical H Value Table = 12.590 Since Calc H > Crit H REJECT Ho:All groups are equal.

length

File: 10261

Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2 ------

GROUP

TRANSFORMED ORIGINAL 0000000 GROUP IDENTIFICATION MEAN MEAN 2654731

^{* =} significant difference (p=0.05)

. = no significant difference Table q value (0.05,7) = 3.038 Unequal reps - multiple SE values

