DATA EVALUATION RECORD AQUATIC INVERTEBRATE LIFE CYCLE TEST § 72-4(c)

1. CHEMICAL: Penoxsulam

11903 PC Code No.: 199031

2. TEST MATERIAL: XDE-638

Purity: 97.7% \

3. CITATION:

Authors: Boeri, R.L., and T.J. Ward

<u>Title</u>: XDE-638: Chronic Toxicity Test to the Mvsid, *Americanysis*

bahia

Study Completion Date: December 21, 2001

Laboratory: T.R. Wilbury Laboratories, Inc.

40 Doaks Lane

Marblehead, Massachusetts 01945

The Dow Chemical Company Sponsor:

Midland, Michigan 48674

Dow AgroSciences LLC Indianapolis, Indiana 46268

Laboratory Report ID: 2202-DO (Dow Study No. 010060)

> MRID No.: 45831028

DP Barcode: D288160

4. REVIEWED BY: Rebecca Bryan, Staff Scientist, Dynamac Corporation

Signature: Newa Payan **Date:** 10/31/03

APPROVED BY: Christie E. Padova, B.S., Staff Scientist, Dynamac Corporation

Signature: CE Pad.

Date: 10/31/03

5. APPROVED BY: William Erickson, Biologist, OPP/EFED/ERB - 7

Boodyear Date: 4/7/04 Signature:

6. STUDY PARAMETERS:

Scientific Name of Test Organisms: Americamysis bahia

Age of Test Organism: < 24 hours old

Definitive Test Duration 28 days

Study Method: Flow-through

Type of Concentrations: Mean-measured

7. CONCLUSIONS:

In a 28-day life-cycle test, *Americamysis bahia* neonates were exposed under flow-through conditions to XDE-638 (penoxsulam) at mean-measured concentrations to <0.0881 (LOD, control), 8.08, 15.2, 29.4, 59.3, and 119 ppm a.i. Prior to sexual maturity and pairing, there were 60 mysids/level: 15 mysids/compartment, 2 compartments/aquarium, and 2 replicate test aquaria/level. On Day 14, up to 20 pair/level were isolated for individual matings; the remainder of first-generation mysids were group retained. First-generation mysids were observed for mortality and signs of abnormal behavior once daily throughout the study. Once daily during the reproduction period (Days 16-28), second-generation mysids were counted and discarded. Data endpoints included percent survival of first-generation mysids at study termination (Day 28; combined sexes), number of young produced per female, and length, wet weight, and dry weight of surviving first-generation mysids (Day 28; sexspecific and combined sexes).

The dry weights of males were statistically-reduced at all treatment levels compared to the control. Dry weights averaged 0.64 mg for the negative control group, and ranged from 0.46 to 0.54 mg for the treatment groups. In addition, a treatment-related reduction in length was observed in combined sexes at the 119 ppm a.i. test level compared to the control group (8.4 versus 9.2 mm). No other treatment-related effects were observed during the study.

Based on significant reductions in dry weights of males, the LOAEC was 8.08 ppm a.i.. The NOAEC was not established, because reductions were found at 8.08 ppm a.i., the lowest measured concentration studied.

This study is scientifically sound. However, since the survival of male mysids following pairing was not monitored, since offspring were not maintained and observed for 4 days, and since a NOAEC was not established, this study does not fulfill the guideline requirements for an aquatic invertebrate life-cycle toxicity test using the *Americamysis bahia* (72-4c), and is classified Supplemental, but it need not be repeated.



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Endpoint	NOAEC	LOAEC
Adult Survival (Day 28)	119 ppm a.i.	>119 ppm a.i.
Reproduction (no. young/ female)	119 ppm a.i.	>119 ppm a.i.
Female Length (mm)	119 ppm a.i.	>119 ppm a.i.
Male Length (mm)	119 ppm a.i.	>119 ppm a.i.
Combined Length (mm)	59.3 ppm a.i.	119 ppm a.i.
Female Dry Weight (mg)	119 ppm a.i.	>119 ppm a.i.
Male Dry Weight (mg)	<8.08 ppm a.i.	8.08 ppm a.i.
Combined Dry Weight (mg)	119 ppm a.i.	>119 ppm a.i.
Female Wet Weight (mg)	119 ppm a.i.	>119 ppm a.i.
Male Wet Weight (mg)	119 ppm a.i.	>119 ppm a.i.
Combined Wet Weight (mg)	119 ppm a.i.	>119 ppm a.i.

8. ADEQUACY OF THE STUDY:

A. Classification: Supplemental

B. Rationale: Survival of male mysids following pairing were not provided; secondgeneration mysids were not observed daily for at least 4 days for survival, development, and behavior; and a NOAEC was not established.

C. Repairability: N/A



9. GUIDELINE DEVIATIONS:

I. The parental stock were apparently not maintained separately from the brood stock.

- II. The temperature $(23.6-26.8^{\circ}\text{C})$ was slightly lower than recommended (27°C) .
- III. The pH range (7.3-8.0) was slightly lower than recommended (7.6-8.2).
- IV. Following pairing, the survival of males as well as reproductive females should have been recorded.
- V. Except for survival, toxic effects of second-generation mysids were not addressed in the study.
- VI. Terminal growth endpoints should have been evaluated using each sex. However, since the raw data were provided, the reviewer was able to statistically analyze terminal growth for each sex.
- VII. A NOAEC was not established, because reductions in male weight were found at 8.08 ppm a.i., the lowest measured concentration studied.
- 10. SUBMISSION PURPOSE: This study was submitted to provide data on the toxicity of XDE-638 (penoxsulam) to the mysid life cycle for the purpose of chemical registration.

11. MATERIALS AND METHODS:

A. Test Organisms/Acclimation

Guideline Criteria	Reported Information
Species An estuarine shrimp species, preferably Americamysis bahia	Americamysis bahia
Source/Supplier	Juveniles were collected from in-house laboratory cultures (maintained since July 2001).

Guideline Criteria	Reported Information
Age at Beginning of Test <24 hours old	<24 hours old
Parental Acclimation Parental stock must be maintained separately from the brood culture in dilution water and under test conditions. Mysids should be in good health.	An isolated brood stock was apparently not maintained. The mysids were maintained under flow-through conditions, and were not treated for disease and were free of apparent disease, injuries, and abnormalities. Mortality was <3% during the 48 hours preceding the definitive study.
Parental Acclimation Period At least 14 days	Continuous
Brood Stock Test started with mysids from: - one brood stock, or - brood stock which has not obtained sexual maturity or had been maintained for >14 days in a laboratory with same food, water, temperature, and salinity used in the test.	At test initiation, juvenile mysids were collected from the culture stock that was maintained in the laboratory under the same conditions used in the definitive test.

B. Test System

Guideline Criteria	Reported Information
Source of Dilution Water May be natural (sterilized and filtered) or a commercial mixture; water must be free of pollutants.	Natural seawater collected at T.R. Wilbury Laboratories in Marblehead, MA, was adjusted to 15-17‰ salinity using deionized water. The dilution water was aerated, filtered, and UV-sterilized prior to use.
	Results of chemical characterization of the dilution water (December 2000) are provided in Table 1, p. 12.

Guideline Criteria	Reported Information
Does water support test animals without observable signs of stress?	Yes
Water Temperature 27°C for mysids - At test termination, mean-measured temperature for each chamber should be within 1°C of selected test temperature Must be within 3°C of the mean of the time-weighted averages Must not differ by >2°C between chambers during the same interval.	Target: 25 ± 2°C Actual range: 23.6-26.8°C (25.2°C mean) - Raw data not provided, so criteria were not assessed; however, the overall temperature range was within 3°C of the mean.
Salinity 15-30 ‰ - The difference between highest and lowest measured salinities should be less than 5 ‰.	15-17‰
pH 7.6 and 8.2	7.3-8.0
Dissolved Oxygen 60-100% saturation	6.6-7.7 mg/L (≥75% saturation).
Photoperiod 16-hr light/8-hr dark (14-hr light/10-hr dark also acceptable)	16 hours light, 8 hours dark, with a 15-minute transition period.
Test Chambers 1. Material: All glass, No. 316 stainless steel, or	1. Glass aquaria
perflorocarbon plastic 2. Size: Typically 30 x 45 x 15 cm (20.25 L)	2. 21 x 40 x 25 cm (20 L)
3. Fill depth: 10 cm	3. 4- to 6-cm (up to 5 L fill volume).

Guideline Criteria	Reported Information
4. Were chambers identical and covered during the test?	4. Yes, loosely covered
Test Compartments (within chambers) - 250-mL glass beakers with side cutouts covered with nylon mesh or stainless steel screen, or	- Test compartments were 10-cm diameter glass Petri dishes with 12-cm high collar of Nitex [®] screen.
- 90- or 140-mm id glass Petri dish bottoms with collars made of 200-250 μm mesh screen	- Reproductive compartments were 6-cm glass petri dishes with 12-cm high collar of Nitex® screen.
Type of Dilution System Intermittent flow proportional diluters or continuous flow serial diluters should be used.	An intermittent-flow proportional diluter was used to deliver each concentration of the test substance and a negative (saltwater) control.
Toxicant Mixing 1. Mixing chamber is recommended but not required; aeration should not be used	1. Not reported.
for mixing. 2. If a mixing chamber was not employed, was it demonstrated that the test solution was completely mixed before introduction into the test system? 3. Was flow splitting accuracy within	2. The diluter was in operation for approximately 50 hours prior to the introduction of mysids.3. Not reported.
10%?	3. Trot reported.
Flow Rate 1. 5-10 volume additions per 24 hours. 2. Did the flow rate maintain the toxicant level and the DO at ≥60% of saturation? 3. Were the meter systems calibrated before study and checked twice daily during test period?	 7.8 volume additions/day Yes Yes
Solvents - Acceptable solvents include triethylene glycol, methanol, acetone, and methanol.	No solvents were used.

Guideline Criteria	Reported Information
- Solvent should not exceed 0.1 mL/L in a flow-through system.	
Aeration Dilution water should be vigorously aerated, but the test tanks should not be aerated.	The dilution water was aeration prior to use. The test chambers were not aerated.

C. Test Design

C. Test Design	
Guideline Criteria	Reported Information
Duration of the Test Approximately 28 days.	28 days
Was the test terminated within 7 days of the median time of first brood release in the controls?	No, the study duration was adequate. The first brood release occurred on Day 16 (p. 14).
Nominal Concentrations Negative control, a solvent control (when applicable), and at least five treatment levels, one of which must adversely affect a life stage and one must not affect any life stage. The dilution factor should not be >50%.	Nominal test concentrations were 0 (negative control), 8.4, 16, 30, 60, and 120 ppm.
Distribution Number of mysids before pairing: Minimum of 15 mysids per compartment, 2 compartments per chamber, 2 chambers per concentration for a total of 60/treatment level. Number of mysids after pairing: ≥20 randomly selected pairs/treatment (excess males should be held in separate compartment in same treatment to replace paired males).	60 mysids/level: 15 mysids/compartment, 2 compartments/aquarium, and 2 replicate test aquaria/level. Up to 20 pair/level: 1 pair/compartment, up to 10 compartments/aquarium, and 2 replicate test aquaria/level. Extra, unpaired mysids were maintained in two extra compartments per aquarium.
Pairing Should be conducted when most of the mysids are sexually mature, usually 10-14 days after test initiation. All pairing should occur on the same day.	Female and male adults were paired on Day 14 and reproduction was monitored from Days 16 (first offspring produced) through 28.
Test organisms randomly or impartially assigned to test vessels?	Yes
Were treatments randomly assigned to individual test chamber locations?	Yes



Guideline Criteria	Reported Information
Feeding Mysids should be fed live brine shrimp nauplii at least once daily. 150 live brine shrimp nauplii per mysid per day or 75 twice a day is recommended.	Mysids were fed live brine shrimp Artemia salina nauplii, ad libitum, 3 times/day (150 to 600 Artemia per mysid per day) during the test, except during the final 24 hours of the test.
Counts Live adult mysids should be counted at initiation, at pairing, and daily after pairing.	Yes
Live young must be counted and removed daily.	Yes
Missing or impinged animals should be recorded.	Missing mysids were reported (one in control).
Controls Negative control and carrier control (when applicable) are required.	A negative saltwater control was used. No solvents were used.
Water Parameter Measurements 1. Temperature should be monitored daily in one chamber and at least three times in all chambers. 2. Salinity should be measured daily in at least one test vessel. 3. pH should be measured at the beginning, the end, and at least weekly during the test in the control vessels and highest test level. 4. Dissolved oxygen must be measured at each concentration at least once a week.	 Temperature was measured daily in each replicate test vessel, and continuously in one negative control test vessel. 4. Salinity, pH, and DO were measured daily in each replicate test vessel.
Chemical Analysis Toxicant concentration must be measured in one chamber at each toxicant level every week.	Samples for HPLC analysis were collected from alternating replicate test vessels on

Guideline Criteria	Reported Information
	Days 0, 7, 14, 21, and 28.

12. REPORTED RESULTS

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Chemical Analysis For all test groups, a) the measured concentration of the test material should not be <50% of the time-	Mean-measured concentrations were <0.0881 (LOQ, control), 8.08, 15.2, 29.4, 59.3, and 119 ppm a.i. (Table 2, p. 19).
weighted average measured concentration for >10% of the duration of the test, and b) the measured concentration should not be >30% of the time-weighted average measured concentration for >5% of the duration of the test.	A low level of variability existed among sample results (all high-low ratios of 1.1).
Controls - Survival of the paired first-generation controls must be ≥70% ≥75% of the paired first-generation female controls produced young, or - The average number of young produced by the first-generation female controls was ≥3.	- All criteria met.
Data Endpoints Must Include 1. Survival of first-generation mysids, sex specified 2. Number of live young produced per	 Total survival of first-generation mysids, and of females paired for reproduction. Number of live young produced per female per day.
female 3. Dry weight and length of each first	female per day. 3. Weights (wet and dry) and length of



Guideline Criteria	Reported Information
generation mysid alive at the end of the	each first generation living at end of test,
test, sex specified	but not sex specific (raw data were sex-
- -	specific; however, data were combined by
Data Endpoints Should Also Include 4. Incidence of morphological findings.	the study authors for statistical analyses).
5. Survival, development, and behavior of	4. Incidence of sub-lethal effects
second-generation mysids for at least 4	pertaining to behavior or appearance.
days.	Survival of second-generation mysids.
Raw data must include	
1. Survival of first-generation mysids, sex	Daily survival of first-generation
specified	mysids, not sex-specific, and of
	reproductive females (Days 16-28).
2. Number of live young produced per	2. Number of live young produced (Days
female	16-28).
3. Terminal weight and length	3. Terminal weight (wet and dry) and
measurements, individual and sex	length of individuals, individual and sex
specified	specified.



MRID No.: 45831028 DP Barcode: D288160

Effects Data

Concentration (ppm a.i.)		Survival Day 14				Reproduction, Days 16-28	
Nominal	Mean Measured (% nominal)	_and		1	_and_	Total No. of Young ²	No. Young Per Female
Control	<0.0881	93 (56/60)	NR	100 (17/17)	85 (49/59)	69	4.3
8.4	8.08 (96)	98 (59/60)	NR	100 (20/20)	83 (50/60)	54	2.7
16	15.2 (95)	93 (56/60)	NR	85 (17/20)	68 (41/60)	14	0.8
30	29.4 (98)	95 (57/60)	NR	65 (11/17)	55 (33/60)	32	2.2
60	59.3 (99)	90 (54/60)	NR	85 (17/20)	73 (44/60)	13	0.8
120	119 (99)	93 (56/60)	NR	95 (19/20)	75 (45/60)	1	0.1

NR = Survival of male mysids was not reported and could not be derived from the provided data tables by the reviewer.

1 Mortality of females following pairing.
2 Reviewer-calculated from replicate data (Table A.3, p. 29).

	Growth, Day 28										
Mean Length, mm Mean Wet Weight, mg Mean Dry V							n Dry Weigh	t, mg			
		and			_ and			_ and _			
9.1	9.3	9.2	2.33	3.24	2.79	0.64	0.74	0.67			
9.0	9.0	9.0	3.26	4.26	3.71	0.50	0.73	0.60			
9.0	9.2	9.1	3.92	4.46	4.28	0.51	0.76	0.66			
8.8	8.9	8.8	4.15	3.92	3.90	0.54	0.61	0.57			
9.1	8.5	8.9	3.62	3.19	3.43	0.46	0.70	0.56*			
8.4	8.4	8.4*	1.95	2.74	2.36	0.48	0.68	0.59			

^{*}Statistically-different (a=0.05) from control; only combined-sexes data were statistically analyzed by the study authors.

B. Statistical Results:

Statistical analyses were performed on survival of the first-generation mysids (Day 28), number of young per surviving female, mean terminal length (combined sexes), and mean terminal wet and dry weights (combined sexes) via TOXSTAT statistical software (Version 3.3; Gulley et al., 1990). Analyses included Bartlett's Test (evaluation of homogeneity) and Chi square test (assessment of normality). ANOVA and Dunnett's or William's tests were then used to compare treatments to the control. The NOAEC and LOAEC were determined from significance data. The MATC was calculated as the geometric mean of the NOAEC and LOAEC. Mean-measured concentrations were used for all estimations.

Most sensitive endpoint: Dry weight (combined sexes).

Results Synopsis

Endpoint	Method ¹	NOAEC	LOAEC	MATC
Survival (Day 28)	ANOVA, Dunnett's or Williams	119 ppm a.i.	>119 ppm a.i.	>119 ppm a.i.
Reproduction (no. young/ female)	ANOVA, Dunnett's or Williams	119 ppm a.i.	>119 ppm a.i.	>119 ppm a.i.
Length (mm)	ANOVA, Dunnett's or Williams	59.3 ppm a.i.	119 ppm a.i.	84.0 ppm a.i.
Wet Weight (g)	ANOVA, Dunnett's or Williams	119 ppm a.i.	>119 ppm a.i.	>119 ppm a.i.
Dry Weight (g)	ANOVA, Dunnett's or Williams	29.4 ppm a.i.	59.3 ppm a.i.	41.8 ppm a.i.

¹ The statistical method was either the Dunnett's or the William's Test (not specified).

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Endpoints statistically assessed included percent survival (Day 28), reproduction (number of young per female), and terminal lengths, and wet and dry weights. Length, wet weight, and dry weight data were analyzed for males and females separately, as well as for combined sexes. With the exception of percent survival and male and female length, data for all endpoints were determined to be normally distributed

and the variances were homogeneous. The NOAEC and LOAEC for these endpoints were determined using ANOVA, and if necessary, followed by Dunnett's (non-monotonic response) or William's (monotonic response) tests. The NOAEC and LOAEC for data which did not meet the assumptions of ANOVA (and which could not be transformed to satisfy these assumptions) were determined using the non-parametric Kruskal-Wallis test. These analyses were conducted using TOXSTAT statistical software using mean-measured concentrations.

Most sensitive endpoint: Male dry weights.

Results Synopsis

Endpoint	Method	NOAEC	LOAEC
Adult Survival (Day 28)	Kruskal-Wallis	119 ppm a.i.	>119 ppm a.i.
Reproduction (no. young/ female)	ANOVA/Dunnett's	119 ppm a.i	>119 ppm a.i.
Female Length (mm)	Kruskal-Wallis	119 ppm a.i.	>119 ppm a.i.
Male Length (mm)	Kruskal-Wallis	119 ppm a.i.	>119 ppm a.i.
Combined Length (mm)	ANOVA/Dunnett's	59.3 ppm a.i.	119 ppm a.i.
Female Dry Weight (mg)	ANOVA/Dunnett's	119 ppm a.i.	>119 ppm a.i.
Male Dry Weight (mg)	ANOVA/Dunnett's	<8.08 ppm a.i.	8.08 ppm a.i.
Combined Dry Weight (mg)	ANOVA/Dunnett's	119 ppm a.i.	>119 ppm a.i.
Female Wet Weight (mg)	ANOVA/Dunnett's	119 ppm a.i.	>119 ppm a.i.
Male Wet Weight (mg)	ANOVA/Dunnett's	119 ppm a.i.	>119 ppm a.i.
Combined Wet Weight (mg)	ANOVA/Dunnett's	119 ppm a.i.	>119 ppm a.i.

14. REVIEWER'S COMMENTS:

The reviewer's conclusions differed from the study authors'. The reviewer's analysis included sex-specific terminal growth measurements, and determined that male dry

weight was the most sensitive endpoint, with significant reductions from control occurring at all treatment levels. The study authors' analysis determined that dry weight (male and females combined) was the most sensitive endpoint, with significant reductions occurring only at the 59.3 ppm a.i. treatment level. The study authors did not analyze growth parameters separately by sex and, so, did not detect the extent to which male dry weight was adversely affected by treatment with XDE-638. The reviewer's results are reported in the Conclusions section.

This study is scientifically sound. However, deviations from FIFRA Guideline §72-4c included the failure to report survival of first-generation male mysids (following pairing), failure to observed second-generation mysids for 4 days, and failure to establish a NOAEC. As a result, this study does not fulfill the guideline requirement for an aquatic invertebrate life-cycle toxicity test using an estuarine shrimp and is classified Supplemental, but it need not be repeated.

No insoluble test material was observed in any test aquarium during the study (p. 18).

One control mysid was lost during pairing transfer.

The LOD was 0.0264 ppm a.i., and the LOQ was 0.0881 ppm a.i. (p. 16).

This study conformed with Good Laboratory Practice Standards as published by the U.S.

Environmental Protection Agency, Office of
Pesticide Programs in 40 CFR Part. 160; OECD
Principles for Good Laboratory Practice; and Japan
MAFF Good Laboratory Practice Standards for
Toxicological Studies on Agricultural Chemicals.
A Quality Assurance Statement was provided.



15. REFERENCES:

ASTM. 1990. Guide for Conducting Life-Cycle Toxicity Test with Saltwater Mysids. Designation E 1191-90.

- Gulley, D.D., et al. 1990. TOXSTAT Version 3.3. Fish Physiology and Toxicology Laboratory, University of Wyoming, Laramie, Wyoming.
- Japan MAFF. 1984. Good Laboratory Practice Standard. 59 NohSan No. 3850.
- OECD. 1997. OECD Guidelines for Testing of Chemicals. Annex 2. OECD Principles of Good Laboratory Practice. [C(97)186/Final].
- U.S. EPA. 1985. Standard Evaluation Procedure, Fish Early Life Stage. Hazard Evaluation Division, Office of Pesticide Programs, Washington, D.C.
- U.S. EPA. 1988. Pesticide Assessment Guidelines. Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms. 72-4. Fish Early Life Stage and Aquatic Invertebrate Life-Cycle Studies. Ecological Effects Branch, Hazard Evaluation Division, Office of Pesticide Programs, Washington, D.C. Draft, March 1988.
- U.S. EPA. 1993. 40 CFR Part 160. Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA); Good Laboratory Practice Standards. Final Rule.



16. RESULTS OF STATISTICAL VERIFICATION:

% survival

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KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	TR. IDENTIFICA		ED MEAN (MEAN	CALCULATED IN ORIGINAL UNITS	RANK SUM
1	control	81.500	81.50	0 17.000	
2	8.08	83.000	83.000	20.000	
3	15.2	68.000	68.000	12.000	
4	29.4	55.000	55.000	6.000	
5	59.3	73.500	73.500	10.500	
6	119	75.000	75.000	12.500	

Calculated H Value = 4.825 Critical H Value Table = 11.070 Since Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.

% survival

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

GROUP

TRANSFORMED ORIGINAL 000000
GROUP IDENTIFICATION MEAN MEAN 435612

OKOOI	IDEITIN I	SALIOIT	A1 T 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	
4	29.4	55.000	55.000 \	
3	15.2	68.000	68.000 . \	
5	59.3	73.500	73.500\	
6	119	75.000	75.000 \	
1	control	81.500	81.500 \	
2	8.08	83.000	83.000 \	

* = significant difference (p=0.05) . = no significant difference Table q value (0.05,6) = 2.936 SE = 3.548

young/female/day

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ANOVA TABLE

 SOURCE
 DF
 SS
 MS
 F

 Between
 5
 24.477
 4.895
 1.863

 Within (Error)
 6
 15.760
 2.627

......



Total 11 40.237

Critical F value = 4.39 (0.05,5,6)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

young/female/day

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

Dunnett table value = 2.83 (1 Tailed Value, P=0.05, df=6,5)

young/female/day

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

NUM OF Minimum Sig Diff % of DIFFERENCE
GROUP IDENTIFICATION REPS (IN ORIG. UNITS) CONTROL FROM CONTROL

----control 2 8.08 2 **4.587** 107.9 15.2 2 **4.587** 107.9 1.550 107.9 3.500 3 4.587 2.050 29.4 2 107.9 4 107.9 3.500 59.3 2 119 2 4.587 5 4.587 107.9 4.200 6

.....

young/female/day

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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP ORIGINAL TRANSFORMED ISOTONIZED IDENTIFICATION N MEAN MEAN MEAN



1	control 2	4.250	4.250	4.250
2	8.08 2	2.700	2.700	2.700
3	15.2 2	0.750	0.750	1.475
4	29.4 2	2.200	2.200	1.475
5	59.3 2	0.750	0.750	0.750
6	119 2	0.050	0.050	0.050
				

young/female/day

File: 1028y Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

ISO	TONIZE	D CAL			ABLE	DEGREES WILLIAMS	OF FREEDOM
IDENTIFICATIO	וען אוי	EWIN	VAILL	MIVIS	P=.U3	AAICTIVIAIS	FREEDOM
control	4.250)					
8.08	2.700	0.956		1.94	k= '	1, v= 6	
15.2	1.475	1.712		2.06	K = :	2, v= 6	
29.4	1.475	1.712		2.10	k = :	3, v= 6	
59.3	0.750	2.160	*	2.12	k=	4, v= 6	
119	0.050	2.591	*	2.13	k=	5, V= 6	

s = 1.621

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

total length (male and female combined)

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ANOVA TABLE

SOURCE	DF	SS	MS	F	
Between	5	0.730	0.146	4.563	
Within (Erro	r) 6	0.190	0.032		
Total	11	0.920			

Critical F value = 4.39 (0.05,5,6)

Since F > Critical F REJECT Ho:All groups equal

total length

File: 1028I Transform: NO TRANSFORMATION

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

TRANSFORMED MEAN CALCULATED IN GROUP IDENTIFICATION MEAN ORIGINAL UNITS T STAT SIG ----control 9.150 9.150 9.000 0.839 9.100 0.280 8.900 1.398 8.08 9.000 2 15.2 9.100 29.4 8.900 4 8.850 1.677 59.3 8.850 5 119 8.400 8.400 4.193 * 6

Dunnett table value = 2.83 (1 Tailed Value, P=0.05, df=6,5)

total length

File: 1028l Transform: NO TRANSFORMATION

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

**** NUM OF Minimum Sig Diff % of DIFFERENCE GROUP IDENTIFICATION REPS (IN ORIG. UNITS) CONTROL FROM CONTROL control 2 1 8.08 2 0.506 5.5 15.2 2 0.506 5.5 29.4 2 0.506 5.5 8.08 2 0.150 2 0.050 3 0.250 0.506 5.5 5 59.3 2 0.300 119 2 0.506 5.5 0.750 6

total length

File: 1028l Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP ID	ENTIFICATIO)N	ORIGINAL N ME		ORMED EAN	ISOTONIZED MEAN
1	contro	1 2	9.150	9.150	9.150	
2	8.08	2	9.000	9.000	9.050	
3	15.2	2	9.100	9.100	9.050	
4	29.4	2	8.900	8.900	8.900	
5	59.3	2	8.850	8.850	8.850	•
6	119	2	8.400	8.400	8.400	
-						

total length

File: 1028i Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

ISO IDENTIFICATIO	TONIZEI N M	CAL	C. SIG WILLIAMS	TABLE P=.05	DEGREES WILLIAMS	OF FREEDOM
control	9.150					
8.08	9.050	0.562	1.9	4 k=	1, v= 6	
15.2	9.050	0.562	2.0	6 k=	2, v= 6	
29.4	8.900	1.405	2.1	0 k=	3, v= 6	
59.3	8.850	1.686	2.1	2 k=	4, v= 6	
119	8.400	4.214	* 2.1	3 k=	5, v= 6	

S = 0.178

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

length females

File: 1028If Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	TR/ IDENTIFICA		IED MEAN (MEAN	CALCULATED IN ORIGINAL UNITS	RANK SUM
1	control	9.300	9.300	21.000	
2	8.08	9.050	9.050	16.500	
3	15.2	9.150	9.150	17.500	
4	29.4	8.900	8.900	13.000	
5	59.3	8.500	8.500	5.000	
6	119	8.450	8.450	5.000	

Calculated H Value = 8.980 Critical H Value Table = 11.070 Since Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.

length females

File: 1028lf Transform: NO TRANSFORMATION

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

GROUP

TRANSFORMED ORIGINAL 000000 GROUP IDENTIFICATION MEAN MEAN 654231

6	119	8.450	8.450 \	
5	59.3	8.500	8.500 . \	
4	29.4	8.900	8.900 \	
2	8.08	9.050	9.050\	
3	15.2	9.150	9.150 \	
1	control	9.300	9.300\	
				_



MRID No.: 45831028

DP Barcode: D288160

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

Table q value (0.05,6) = 2.936 SE = 3.536

length males

File: 1028lm

Transform: NO TRANSFORM

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	TR/ IDENTIFICA		ED MEAN C	ALCULATED IN ORIGINAL UNITS	RANK SUM
1 2 3 4 5	control 8.08 15.2 29.4 59.3 119	9.100 8.950 8.950 8.800 9.100 8.400	9.100 8.950 8.950 8.800 9.100 8.400	16.000 13.000 13.000 13.000 20.000 3.000	

Calculated H Value = 6.229 Critical H Value Table = 11.070 Since Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.

length males

File: 1028Im Transform: NO TRANSFORM

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

...... GROUP

TRANSFORM	ED OKIGIN	AL UUU	000
GROUP IDENTIFICATION	MEAN	MEAN	642351

6	119	8.400	8.400 \	
4	29.4	8.800	8.800 . \	
2	8.08	8.950	8.950\	
3	15.2	8.950	8.950\	
5	59.3		9.100 \	
1	control	9.100	9.100 \	

^{* =} significant difference (p=0.05) . = no significant difference Table q value (0.05,6) = 2.936 SE = 3.561

wet weight (male and female combined)

File: 1028ww Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F	
Between	5	5.308	1.062	5.033	
Within (Error	6	1.267	0.211		
Total	11	6.576			

Critical F value = 4.39 (0.05,5,6) Since F > Critical F REJECT Ho:All groups equal

wet weight

File: 1028ww Transform: NO TRANSFORMATION

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

Dunnett table value = 2.83 (1 Tailed Value, P=0.05, df=6,5)

wet weight

File: 1028ww Transform: NO TRANSFORMATION

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

NUM OF Minimum Sig Diff % of DIFFERENCE GROUP IDENTIFICATION REPS (IN ORIG. UNITS) CONTROL FROM CONTROL control 2 8.08 2 1.300 47.4 -0.970 15.2 2 1.300 47.4 -1.485 2 3 15.2 2 29.4 2 59.3 2 1.300 47.4 -1.250 4 1.300 47.4 -0.710 5 1.300 47.4 0.385 119 2 6

wet weight

File: 1028ww Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GRO	JP IDENTIFICATION	ORIGII N	NAL TRAN	ISFORMED MEAN	ISOTONIZED MEAN
1	control	2 2.74	10 2.740	3.666	
2	8.08 2	3.710	3.710	3.666	
3	15.2 2	4.225	4.225	3.666	
4	29.4 2	3.990	3.990	3.666	
5	59.3 2	3.450	3.450	3.450	
6	119 2	2.355	2.355	2.355	

wet weight

File: 1028ww Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

ISC IDENTIFICATIO	TONIZEI ON M				BLE DEGREES =.05 WILLIAMS	FREEDOM
control	3.666	5				
8.08	3.666	2.015	*	1.94	k= 1, v= 6	
15.2	3.666	2.015		2.06	k = 2, v = 6	
29.4	3.666	2.015		2.10	k= 3, v= 6	
59.3	3.450	1.545		2.12	k= 4, v= 6	
119	2.355	0.838		2.13	k= 5, v= 6	

s = 0.460

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

wet weight males

File: 1028wm Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	7.848	1.570	16.702
Within (Erro	r) 6	0.564	0.094	
Total	11	8.411		

Critical F value = 4.39 (0.05,5,6)

Since F > Critical F REJECT Ho:All groups equal

wet weight males

File: 1028wm Transform: NO TRANSFORMATION

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

GROUP	TR/ IDENTIFICA		ED MEAN (CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	2.330	2.330			
2	8.08	3.265	3.265	-3.050		
3	15.2	3.925	3.925	-5.202		
4	29.4	4.150	4.150	-5.936		
5	59.3	3.615	3.615	-4.191		
6	119	1.950	1.950	1.239		

Dunnett table value = 2.83 (1 Tailed Value, P=0.05, df=6,5)

wet weight males

File: 1028wm Transform: NO TRANSFORMATION

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

wet weight males

File: 1028wm Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROU	JP IDENTIFICATION	ORIGINAL N MEA		ORMED EAN	ISOTONIZED MEAN
1	control 2	2.330	2.330	3.457	
2	8.08 2	3.265	3.265	3.457	
-3	15.2 2	3.925	3.925	3.457	
Δ	29.4 2	4.150	4.150	3.457	
5	59.3 2	3.615	3.615	3.457	
6	119 2	1.950	1.950	1.950	

wet weight males

File: 1028wm Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

ISO IDENTIFICATIO		D CALC	. SI WILLI	•	BLE DEGREES =.05 WILLIAMS	OF FREEDOM
control	3.457	,				
8.08	3.457	3.677	*	1.94	k= 1, v= 6	
15.2	3.457	3.677	*	2.06	k= 2, v= 6	
29.4	3.457	3.677	*	2.10	k= 3, v= 6	
59.3	3.457	3.677	*	2.12	k = 4, V = 6	
119	1.950	1.240		2.13	k= 5, V= 6	

s = 0.307

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

wet weight females

File: 1028wf Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F	
Between	5	4.637	0.927	1.843	
Within (Erro	r) 6	3.015	0.503		
Total	11	7.652			

Critical F value = 4.39 (0.05,5,6)

Since F < Critical F FAIL TO REJECT Ho:All groups equal

wet weight females

File: 1028wf Transform: NO TRANSFORMATION

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

TRANSFORMED MEAN CALCULATED IN
GROUP IDENTIFICATION MEAN ORIGINAL UNITS T STAT SIG

1 control 3.240 3.240
2 9.09 4.255 -1.431

1	control	3.240	3.240	
2	8.08	4.255	4.255	-1.431
3	15.2	4.465	4.465	-1.727
4	29.4	3.920	3.920	-0.959

59.3 3.190 3.190 0.070 6 119 2.735 2.735 0.712

Dunnett table value = 2.83 (1 Tailed Value, P=0.05, df=6,5)

wet weight females

File: 1028wf Transform: NO TRANSFORMATION

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

NUM OF Minimum Sig Diff % of DIFFERENCE GROUP IDENTIFICATION REPS (IN ORIG. UNITS) CONTROL FROM CONTROL

1	contro	l 2			
2	8.08	2	2.007	61.9	-1.015
3	15.2	2	2.007	61.9	-1.225
4	29.4	2	2.007	61.9	-0.680
5	59.3	2	2.007	61.9	0.050
6	119	2	2.007	61.9	0.505

wet weight females

File: 1028wf Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROU	JP IDENTIFICATION	ORIGINAL N ME.		ORMED EAN	ISOTONIZED MEAN
1	control 2	3.240	3.240	3.987	
2	8.08 2	4.255	4.255	3.987	
3	15.2 2	4.465	4.465	3.987	
4	29.4 2	3.920	3.920	3.920	
5	59.3 2	3.190	3.190	3.190	
6	119 2	2.735	2.735	2.735	

wet weight females

File: 1028wf Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

ISOTONIZED CALC. SIG TABLE DEGREES OF IDENTIFICATION MEAN WILLIAMS P=.05 WILLIAMS FREEDOM

control 3.987

8.08 3.987 1.053 1.94 k= 1, V= 6

15.2	3.987	1.053	2.06	k= 2, v= 6
29.4	3.920	0.959	2.10	k= 3, v= 6
59.3	3.190	0.071	2.12	k= 4, v= 6
119	2.735	0.712	2.13	k= 5, v= 6

s = 0.709

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

dry weight (males and females combined)

File: 1028dw Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.020	0.004	2.000
Within (Error)	6	0.010	0.002	
Total 1	1	0.029		

Critical F value = 4.39 (0.05,5,6)

Since F < Critical F FAIL TO REJECT Ho:All groups equal

dry weight

File: 1028dw Transform: NO TRANSFORMATION

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

GROUP	TR/ IDENTIFICA		ED MEAN (CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	controi	0.675	0.675			
2	8.08	0.605	0.605	1.565		
3	15.2	0.650	0.650	0.559		
4	29.4	0.575	0.57 5	2.236		
5	59.3	0.560	0.560	2.571		
6	119	0.590	0.590	1.901		
					-	

dry weight

File: 1028dw Transform: NO TRANSFORMATION

Dunnett table value = 2.83 (1 Tailed Value, P=0.05, df=6,5)

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

dry weight

File: 1028dw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROL	JP IDENTIFICATIO	N	ORIGINA N ME		ORMED EAN	ISOTONIZED MEAN
1	control	2	0.675	0.675	0.675	
2	8.08	2	0.605	0.605	0.628	
3	15.2	2	0.650	0.650	0.628	
4	29.4	2	0.575	0.575	0.575	
5	59.3	2	0.560	0.560	0.575	
6	119	2	0.590	0.590	0.575	

dry weight

File: 1028dw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

s = 0.040

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

dry weight males

File: 1028dm Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F	
Between	5	0.0424	0.0085	12.143	••••
Within (Error	6	0.0044	0.0007		
Total	11	0.0468			

Critical F value = 4.39 (0.05,5,6)

Since F > Critical F REJECT Ho:All groups equal

dry weight males

File: 1028dm Transform: NO TRANSFORMATION

DUN	NETTS TEST	- TABLE	1 OF 2	Ho:Control <tre< th=""><th>atment</th></tre<>	atment
GROUP	TR/		IED MEAN (CALCULATED IN ORIGINAL UNITS	T STAT SIG
1	control	0.645	0.645		
2	8.08	0.505	0.505	5.292 *	
3	15.2	0.510	0.510	5.103 *	
4	29.4	0.535	0.535	4.158 *	
5	59.3	0.465	0.465	6.803 *	
6	119	0.475	0.475	6.425 *	

Dunnett table value = 2.83 (1 Tailed Value, P=0.05, df=6,5)

dry weight males

File: 1028dm Transform: NO TRANSFORMATION

DUN	NETTS TEST	- T	ABLE 2 OF	2	Ho:Contr	ol <treatment< th=""></treatment<>
GROUP	NU IDENTIFIC					IFFERENCE CONTROL FROM CONTROL
1	control	2				
2	8.08	2	0.075	11.6	0.140	
3	15.2	2	0.075	11.6	0.135	
4	29.4	2	0.075	11.6	0.110	
5	59.3	2	0.075	11.6	0.180	
6	119	2	0.075	11.6	0.170	

dry weight males

File: 1028dm Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GRO	UP IDENTIFICATION	ORIGINAL N ME		ORMED EAN	ISOTONIZED MEAN
1	control 2	0.645	0.645	0.645	
2	8.08 2	0.505	0.505	0.517	
3	15.2 2	0.510	0.510	0.517	
4	29.4 2	0.535	0.535	0.517	
5	59.3 2	0.465	0.465	0.470	
6	119 2	0.475	0.475	0.470	

dry weight males

File: 1028dm Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

ISOTONIZED CALC. SIG TABLE DEGREES OF IDENTIFICATION MEAN WILLIAMS P=.05 WILLIAMS FREEDOM

control 0.645

8.08 0.517 4.718 * 1.94 k= 1, v= 6
15.2 0.517 4.718 * 2.06 k= 2, v= 6
29.4 0.517 4.718 * 2.10 k= 3, v= 6
59.3 0.470 6.433 * 2.12 k= 4, v= 6
119 0.470 6.433 * 2.13 k= 5, v= 6

s = 0.027

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

dry weight females

File: 1028df Transform: NO TRANSFORMATION

ANOVA TABLE

 SOURCE
 DF
 SS
 MS
 F

 Between
 5
 0.028
 0.006
 0.750

 Within (Error)
 6
 0.049
 0.008

 Total
 11
 0.077

Critical F value = 4.39 (0.05, 5, 6)

Since F < Critical F FAIL TO REJECT Ho:All groups equal

dry weight females

File: 1028df Transform: NO TRANSFORMATION

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

Dunnett table value = 2.83 (1 Tailed Value, P=0.05, df=6,5)

dry weight females

File: 1028df Transform: NO TRANSFORMATION

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

dry weight females

File: 1028df Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP IDENT	IFICATION	ORIGINAL N ME		ORMED EAN	ISOTONIZED MEAN
1 2 3	control 2 8.08 2 15.2 2 29.4 2	0.740 0.730 0.755 0.610	0.740 0.730 0.755 0.610	0.742 0.742 0.742 0.665	••••

5 6	119	2		0.705 0.680	0.665 0.665				

dry weight females

File: 1028df Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

Tribulation 1201 (1000)										
ISO IDENTIFICATIO	TONIZEI			BLE DEGREES .05 WILLIAMS	OF FREEDOM					
control	0.742	-								
8.08	0.742	0.018	1.94	k= 1, v= 6						
15.2	0.742	0.018	2.06	k= 2, v= 6						
29.4	0.665	0.826	2.10	k= 3, v= 6						
59.3	0.665	0.826	2.12	k = 4, V = 6						
119	0.665	0.826	2.13	k= 5, v= 6						

s = 0.091

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