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Data Evaluation Report on Field Exposure of *Xenopus laevis* to Atrazine and Other Triazines in South Africa: Feasibility Study for Site Characterization and Assessment of Laryngeal and Gonadal Responses.

Data Requirement::

EPA DP Barcode D288775

EPA MRID 458677-09
EPA Guideline 70-1(Special Study)

Test material: **Purity:** not reported
Common name Atrazine
Chemical name: IUPAC
CAS name 6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine
CAS No. 1912-24-9
Synonyms
EPA PC Code: 80803

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EPA PC Code 080803

Date Evaluation Completed: 05/31/2003

CITATION: Smith, E. E., L. DuPreez, and K. Solomon. 2003. Field exposure of *Xenopus laevis* to atrazine and other triazines in South Africa: feasibility study for site characterization and assessment of laryngeal and gonadal responses. The Institute of Environmental and Human Health, Texas Tech University, Lubbock, Texas (USA) and School of Environmental Sciences and Development, Potchefstroom University for CHE, Potchefstroom, South Africa. Sponsor: Syngenta Crop Protection, Inc., Laboratory Study ID: ECORISK Number SA-01A.

EXECUTIVE SUMMARY:

This study had two objectives, first, to characterize the possible exposure of *Xenopus laevis* larvae, metamorphs and adults to atrazine and related triazines in surface waters in 8 reference (no corn production or atrazine/triazine use) and 8 experimental (exposed habitats in the proximity of corn production and atrazine/triazine use) in the Potdchefstroom region of South Africa. The second objective was to determine whether there were differences in the sex ratios, age, and size classes of *Xenopus laevis* in the reference and exposed sites. Three ponds (C1, C3 and E6) were spring-fed although the majority of sampling sites relied on rain as the primary source of water. Pond surface area in reference sites ranged from 2,000 to 20,500 m^2, while experimental sites ranged from 450 to 68,722 m^2. Watersheds ranged from 170 to 480 ha in reference sites and from 274 to 1,990 ha at experimental sites. Except for site E1 (DO = 6.01 mg/L), dissolved oxygen concentrations were consistently less than 4.5 mg/L. The majority (67%) of the ponds had a maximum depth of less than 133 cm (4.4 feet) and were relatively shallow. It is unclear though why ponds E1and E8 had pH values ranging from 10.1 to 10.2. Atrazine concentrations in reference site water ranged from less than 0.1 µg/L to 0.15 µg/L, while atrazine in corn-growing locations ranged from less than 0.1 µg/L to 1.23 µg/L.

While the proportion of males and females was significantly different at different ponds within the reference and atrazine-exposed sites, the overall mean percentages of males and females in the reference and experimental sites were not different. There was no statistical difference in the size (length and weight) of males frogs collected from reference and experimental sites although females frogs collected from reference sites were significantly larger (length and weight) than females collected at experimental sites. Additionally, frogs from reference ponds tended to be significantly younger than frogs collected from experimental site; however, because an unknown percentage of the frogs collected could not be aged, the actual age structure of the frog populations is uncertain.

Based on the criteria set for the study, *i.e.*, non-growing sites without atrazine, three sites (C1, C3 and C6) were selected as reference sites. Based on the criteria set for exposure sites, *i.e*., proximity of corn production and known use/presence of atrazine, five sites (E1, E3, E4, E6 and E8) were selected as exposure sites.

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I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: Nonguideline Study
COMPLIANCE: Not conducted in accordance with the Good Laboratory Practice as outlined in 40 CFR Part 160, August 19, 1989. However, GLP elements were incorporated into study conduct including:

- Study was conducted in accordance with a written protocol, signed by the study director, principal investigators and sponsor.
- Standard Operating Procedures were developed/available for critical activities.
- Protocol amendments were written and signed by the study director.

A. MATERIALS:

1. Test Material Atrazine

Description: Not reported

Lot No./Batch No. : Not reported

Purity: Not reported
Stability of compound under test conditions: Not reported

Storage conditions of test chemicals: Not reported

2. Test organism:

Species: African clawed frog (*Xenopus laevis*)
.
Age at test initiation Adults
Weight at study initiation: (mean and range) Not reported
Length at study initiation: (mean and range) Not reported

Source: Adult *X. laevis* were field-collected in two areas (non-corn growing areas and corn growing areas) in the vicinity of Potchefstrooom, South Africa, using traps baited with liver and meat scraps.

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B. STUDY DESIGN:

Objective:

1. To characterize the possible exposure of *Xenopus laevis* larvae, metamorphs and adults to atrazine and related triazines in surface waters in reference (no corn production or atrazine/triazine use) and experimental (exposed habitats in the proximity of corn production and atrazine/triazine use) in the Potdchefstroom region of South Africa.

2. To determine whether there are differences in the sex ratios, age, and size classes of *Xenopus laevis* in the reference and exposed sites.

1. Experimental Conditions

Experimental field exposed sites were selected based on the presence of *X. laevis*, closeness of corn production as well as previous and planned use of atrazine and terbuthylazine (2-*tert*-butylamino-4-chloro-6-ethylamino-s-triazine). Reference sites were selected based on the presence of *X. laevis*, lack of corn production, and absence of atrazine and tertbutlyazine. Eight experimental and eight reference sites were evaluated. Water samples (2-L) were analyzed for atrazine.

A total of 8 sites in two adjacent regions (Viljoenskroon corn growing region = E; non-corn growing region = C) were sampled in the vicinity of Potchefstroom, South Africa.

Compounds of interest included atrazine, its metabolites desethylated atrazine (DEA), desisopropyl atrazine (DIA), diaminochlorotriazine (DACT), and terbutylazine plus simazine and acetochlor.

Frogs were collected in 10 baited traps and harvested after two days and transported back to the laboratory. At the lab, frogs were checked for recapture, sexed, weighed and the snout-vent length was measured. Two digits of the longest toe of the right hind foot were removed and fixed in Bouin's solution [for later age analysis]. Frogs were cryo-branded with a number corresponding to the site; date of recapture was indicated by the angle (orientation) of the brand number.

Population size was estimated using the method of Donnelley and Guyer (1994).

II. RESULTS and DISCUSSION: [All results discussed in this section and the next are those reported by the study authors. Although supplemental data are typically used in a qualitative manner only, EFED verified spreadsheet data and ran basic statistical analyses on the major study parameters. See attached appendix. If results appeared to differ in any substantive way, the difference was reported in the text below.]

The morphoedaphic characteristics of the sampling sites are presented in **Table 1**. Three ponds (C1, C3 and E6) were fountain (spring) fed; however, the majority of sampling sites relied on rain as the primary source of water. Pond surface area in the reference sites ranged from 2,000 to 20,500 m^2 (0.49 - 5.07 acres), while experimental sites ranged from 450 to 68,722 m^2 (0.11 - 16.98 acres). Watersheds ranged from 170 to 480 ha (420 to 1,186 acres) in reference sites and from 274 to 1,990 ha (677 to 9.7 acres) at experimental sites. Except for site E1 (DO = 6.01 mg/L), dissolved oxygen concentrations were consistently less than 4.5 mg/L. The majority (67%) of the ponds had a maximum depth of less than 133 cm (4.4 feet) and were thus relatively shallow.

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Atrazine concentration in reference site water ranged from less than 0.1 µg/L to 0.15 µg/L while atrazine in corn-growing locations ranged from less than 0.1 µg/L to 1.23 µg/L (**Table 2**). Based on the criteria set for the study, *i.e.*, non-growing site without atrazine, cites C1, C3 and C6 were selected as reference sites. Based on the criteria set for exposure sites, *i.e.*, proximity of corn production and known use/presence of atrazine, sites E1, E3, E4, E5, E6 and E8 were selected as exposure sites.

On average, males made up 45% of the frogs collected from reference sites, while they comprised 41% of the frogs collected from exposure sites (**Table 3**). There was no significant difference in sex ratios between non-corn growing sites and corn-growing sites. The total number of frogs sexed at reference sites ranged from 16% to 42% while the number sexed from corn-growing sites ranged from 16 to 98%; there was no statistical difference in the percentage of frogs sexed at each site (ANOVA; $p = 0.0898$)**.**

Based on a mark (toe-clip) and recapture study, the estimated population size of frogs in each pond was determined (**Table 4**). Based on this number and the surface area of the pond, the density of the frogs (number per m^2) ranged from 0.04 to 0.18 in reference sites and from 0.01 to 0.81 in corn-growing sites.

Mean weight (**Table 5**) of male frogs from reference sites (21.7 ± 6.59 g) was similar to weights of male frogs collected from experimental sites (17.2 ± 2.52 g). Females were significantly heavier than males in both reference (ANOVA, $p < 0.022$) and experimental sites (ANOVA, $p < 0.0003$); average weight of females collected from reference sites (39.2 ± 5.23 g) was significantly higher (ANOVA, $p < 0.0102$) than the average weight of females collected from experimental sites (27.9± 4.26 g)

Mean snout-vent length (**Table 6**)of male frogs from reference sites (57.1 ± 5.14 cm) was similar to lengths of male frogs collected from experimental sites (53.12 ± 2.93). Females were significantly larger than males in both reference (ANOVA, $p < 0.0296$) and experimental sites (ANOVA, $p < 0.0009$); contrary to what the study authors report, average lengths of females collected from reference sites (68.43 ± 2.94 cm) were significantly larger (ANOVA, $p < 0.0439$) than the average length of females collected from experimental sites (62.25 ± 3.79 cm).

Average age of frogs collected (**Table 7**) ranged from 1.5 to 2.6 years. The oldest frog examined (8 years) was from experimental pond E1. Except for ponds C1, E4 and E8, the largest age group was Year 1. However, in C1 and E8 the largest age class was Year 2,and in pond E4 the largest age class was Year 3. Ponds C1 and E4 also had the largest percentage of frogs over 2 years-old with 45% and 53% of the total sampled in ponds _, respectively. There were no significant differences in average ages between reference and experimental site ponds according to the study authors; however, based on EFED's analysis (see attached SAS output), frogs from reference ponds tended to be significantly (ANOVA, $p < 0.0007$) younger (1.77 ± 1.02 years) than frogs collected from experimental sites (2.14 ± 1.25 year). It is important to note though that the study authors were not able to age all of the frogs collected; however, they did not provide any data on the number of frogs that they were not able to age. As a result, the age distribution for this study is uncertain.

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Table 1. Morphoedaphic characteristics of control (non-corn growing) and experimental (corn-growing) ponds.

Parameter	Control (non-corn) Sites			Experimental (corn-growing) Sites					
	C1	C3	C6	E1	E3	E4	E5	E6	E8
Surface area	20,500 m^2	2,000 m^2	14,860 m^2	7,406 m^2	46,076 m^2	2,400 m^2	450 m^2	68,722 m^2	2,400 m^2
Watershed area	244 ha	170 ha	480 ha	1,990 ha	1,046 ha	448 ha	274 ha	515 ha	1,100 ha
Deepest Point	261 cm	38.5 cm	104 cm	118 cm	133 cm	44 cm	67cm	370 cm	175 cm
pH	8.3	7.3	8.2	10.1	9.1	8.2	8.0	8.4	10.2
DO	3.39 mg/L	3.39 mg/L	3.14 mg/L	6.01 mg/L	3.96 mg/L	4.51 mg/L	4.05 mg/L	3.88 mg/L	4.35 mg/L
Secchi depth	11.5 cm	32 cm	6.5 cm	118 cm	133 cm	44 cm	67 cm	207 cm	175 cm
Conductivity	2.61 µS/m	4.77 µS/m	8.6 µS/m	186 µS/m	144 µS/m	116.7 µS/m	134.6 µS/m	57.5 µS/m	54.0 µS/m

Table 2. Atrazine concentrations in water collected from non-corn growing "reference" (CI - C8) and corn-growing "experimental" (E1 - E8) sites in South Africa.

Site	Potch Value (µg/L)	Syngenta Value (µg/L)
C1	< 0.10	BROKEN
C2	0.15	0.13
C3	< 0.10	< 0.10
C4	0.11	NOT ANALYZED
C5	< 0.10	NOT ANALYZED
C6	< 0.10	NOT ANALYZED
C7	< 0.10	NOT ANALYZED
C8	< 0.10	NOT ANALYZED
E1	1.23	0.96
E2	< 0.10	< 0.10
E3	0.32	0.19
E4	0.12	BROKEN
E5	< 0.10	< 0.10
E6	0.68	1.1
E7	0.51	NOT ANALYZED
E8	0.84	NOT ANALYZED

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Table 3. Sex ratio and total[1] number of frogs collected from non-corn growing (C1 - C6) and corn-growing (E1 - E8) sites in South Africa.

Sex	Reference			Experimental					
	C1	C3	C6	E1	E3	E4	E5	E6	E8
Juvenile	0	3	1	4	0	3	0	1	6
Male	39	52	41	51	46	22	15	34	45
Female	61	45	58	45	54	43	51	65	49
Total	289	229	621	118	620	84	87	195	96

[1] total number of frogs collected during three trapping efforts used to estimate frog population number through mark and recapture. This number does **not** represent the total number of males and females used in estimating the sex ratios.

Table 4. Estimated population size of frogs at reference (non-corn growing) and experimental (corn-growing) sites in South Africa.

	Reference			Experimental					
	C1	C3	C6	E1	E3	E4	E5	E6	E8
Number	713	360	950	370	1218	354	364	909	216
Surface area (m^2)	20500	2000	14860	7406	46076	24000	450	68722	2400
Density (#/m^2)	0.035	0.180	0.064	0.05	0.026	0.148	0.809	0.013	0.09

Table 5. Mean weight (gms) of frogs collected from reference (non-corn growing) and experimental (corn-growing) sites in South Africa.

Sex	Reference			Experimental					
	C1	C3	C6	E1	E3	E4	E5	E6	E8
Juvenile		3.8	6.4	4.2	--	4.0	--	6.8	6.0
Male	18.6	17.3	29.3	14.5	15.4	21.1	15.3	18.5	18.1
Female	45.0	34.9	37.6	20.8	29.6	33.8	28.3	28.6	26.5

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Table 6. Mean snout-vent length (mm) of frogs collected from non-corn growing (Reference) and corn-growing sites.

Sex	Reference			Experimental					
	C1	C3	C6	E1	E3	E4	E5	E6	E8
Juvenile	--	32.3	37.5	33.8	--	32.7	--	37.7	36.3
Male	55.7	52.8	62.8	50.3	50.8	57.9	51.1	54.3	54.3
Female	71.6	65.9	67.9	57.1	60.6	68.7	62.0	63.1	62.0

Table 7. Age structure of African clawed frogs (*Xenopus laevis*) in reference (C1 - C6) and experimental (E1 - E8) ponds based on mark and recapture (3 efforts) study.

Years	Reference			Experimental				
	C1	C3	C6	E1	E3	E4	E6	E8
1	9	43	49	34	24	14	21	14
2	22	16	14	15	12	11	21	27
3	13	3	6	9	4	18	9	6
4	10	2	1	6	2	8	4	5
5	1	0	1	3	0	1	4	2
6	1	0	0	1	0	1	1	1
7	0	0	0	0	0	0	0	0
8	0	0	0	1	0	0	0	0
Total Number	56	64	71	69	42	53	60	55
Mean Age	2.55	1.44	1.46	2.09	1.62	2.51	2.20	2.22

D. VERIFICATION OF STATISTICAL RESULTS: See attached Statistical Analysis System (SAS Release 8.01, Cary, North Carolina)

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E. STUDY DEFICIENCIES:

Study authors were unable to age all of the frogs collected. The exact age structure and population size are uncertain.

F. REVIEWER'S COMMENTS:

The pH of all the ponds were alkaline, especially ponds E1 and E8 which were as high as 10.2. The study authors have suggested that the surrounding corn fields were treated with agricultural limestone (personal communication Allan Hosmer, Syngenta, April 2003); however, agricultural limestone (calcium/magnesium carbonate) is not likely to raise pond water to pH values above 8.0. It is possible that farmers may have treated surrounding fields with hydrated or slaked lime.

The study's inability to age all of the frogs collected renders it difficult to determine the age and population size of the tested animals .

This study was designed as a general survey to determine the utility of the study sites to serve as atrazine-exposed versus reference sites. After these sites are selected a more detailed assessment of the effects of atrazine and other triazines on the gonadal development, blood steroid, and gonadal aromatase activity of African clawed frogs will be studied.

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G. CONCLUSIONS:

Three ponds (C1, C3 and E6)were spring-fed; however, the majority of sampling sites relied on rain as the primary source of water. Pond surface area in reference sites ranged from 2,000 to 20,500 m^2 while experimental sites ranged from 450 to 68,722 m^2. Watersheds ranged from 170 to 480 ha in reference sites and from 274 to 1,990 ha at experimental sites. Except for site E1 (DO = 6.01 mg/L), dissolved oxygen concentrations were consistently less than 4.5 mg/L. The majority (67%) of the ponds had a maximum depth of less than 133 cm (4.4 feet) and were thus relatively shallow. It is unclear though why ponds E1and E8 had pH values ranging from 10.1 to 10.2. Atrazine concentration in reference site water ranged from less than 0.1 µg/L to 0.15 µg/L while atrazine in corn-growing locations ranged from less than 0.1 µg/L to 1.23 µg/L.

While the proportion of males and females was significantly different across sites, the mean percentage of males and females in the reference and experimental sites was not different. There was no statistical difference in the size (length and weight) of male frogs collected from reference and experimental sites; however female frogs collected from reference sites were significantly larger (length and weight) than females collected at experimental sites. Additionally, frogs from reference ponds tended to be significantly younger than frogs collected from experimental site; however, given that an unknown percentage of the frogs collected could not be aged, the actual age structure of the frog populations is uncertain..

Based on the criteria set for the study, *i.e.*, non-growing site without atrazine, 3 sites (C1, C3 and C6) were selected as reference sites. Based on the criteria set for exposure sites, *i.e*., proximity of corn production and known use/presence of atrazine, 5 sites (E1, E3, E4, E6 and E8) were selected as exposure sites. It should be noted that C1 and C3 are both cattle farms while C6 is located in the security buffer zone outside of an explosive factory.

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MEAN WEIGHTS AND LENGTHS OF MALE XENOPUS COLLECTED FROM REFERENCE (C) AND EXPERIMENTAL (E) PO 48

Obs	TREAT	_TYPE_	_FREQ_	MEAN_W	MEAN_L	SD_W	SD_L
1	C	0	3	21.7333	57.1000	6.58508	5.14490
2	E	0	6	17.1500	53.1167	2.52329	2.93422

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ANOVA FOR MALE WEIGHT 1

The ANOVA Procedure

Class Level Information

Class	Levels	Values
POND	2	C E

Number of observations 9

Dependent Variable: WEIGHT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	42.0138889	42.0138889	2.48	0.1593
Error	7	118.5616667	16.9373810		
Corrected Total	8	160.5755556			

R-Square	Coeff Var	Root MSE	WEIGHT Mean
0.261646	22.03423	4.115505	18.67778

Source	DF	Anova SS	Mean Square	F Value	Pr > F
POND	1	42.01388889	42.01388889	2.48	0.1593

Levene's Test for Homogeneity of WEIGHT Variance
ANOVA of Squared Deviations from Group Means

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
POND	1	1114.2	1114.2	5.56	0.0505
Error	7	1402.7	200.4		

Bartlett's Test for Homogeneity of WEIGHT Variance

Source	DF	Chi-Square	Pr > ChiSq
POND	1	2.5401	0.1110

NOTE: This test controls the Type I experimentwise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	7
Error Mean Square	16.93738
Critical Value of Dunnett's t	2.36463

Comparisons significant at the 0.05 level are indicated by ***.

POND Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
E - C	-4.583	-11.465	2.298

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NONPARAMETRIC COMPARISON OF MALE FROG WEIGHT BETWEEN PONDS 5

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable WEIGHT
Classified by Variable POND

POND	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
C	3	20.0	15.0	3.872983	6.666667
E	6	25.0	30.0	3.872983	4.166667

Wilcoxon Two-Sample Test

Statistic	20.0000
Normal Approximation	
Z	1.1619
One-Sided Pr > Z	0.1226
Two-Sided Pr > |Z|	0.2453
t Approximation	
One-Sided Pr > Z	0.1394
Two-Sided Pr > |Z|	0.2788

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	1.6667
DF	1
Pr > Chi-Square	0.1967

Median Scores (Number of Points Above Median) for Variable WEIGHT
Classified by Variable POND

POND	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
C	3	2.0	1.333333	0.745356	0.666667
E	6	2.0	2.666667	0.745356	0.333333

Median Two-Sample Test

Statistic	2.0000
Z	0.8944
One-Sided Pr > Z	0.1855
Two-Sided Pr > |Z|	0.3711

Median One-Way Analysis

Chi-Square	0.8000
DF	1
Pr > Chi-Square	0.3711

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ANOVA FOR MALE LENGTH 7

The ANOVA Procedure

Class Level Information

Class	Levels	Values
POND	2	C E

Number of observations 9

Dependent Variable: LENGTH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	31.7338889	31.7338889	2.31	0.1720
Error	7	95.9883333	13.7126190		
Corrected Total	8	127.7222222			

R-Square	Coeff Var	Root MSE	LENGTH Mean
0.248460	6.801530	3.703055	54.44444

Source	DF	Anova SS	Mean Square	F Value	Pr > F
POND	1	31.73388889	31.73388889	2.31	0.1720

Levene's Test for Homogeneity of LENGTH Variance
ANOVA of Squared Deviations from Group Means

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
POND	1	219.3	219.3	1.93	0.2070
Error	7	794.0	113.4		

Bartlett's Test for Homogeneity of LENGTH Variance

Source	DF	Chi-Square	Pr > ChiSq
POND	1	0.8533	0.3556

Dunnett's t Tests for LENGTH

NOTE: This test controls the Type I experimentwise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	7
Error Mean Square	13.71262
Critical Value of Dunnett's t	2.36463

Comparisons significant at the 0.05 level are indicated by ***.

POND Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
E - C	-3.983	-10.175	2.208

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NONPARAMETRIC COMPARISON OF MALE FROG LENGTH BETWEEN PONDS 11

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable LENGTH
Classified by Variable POND

POND	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
C	3	20.0	15.0	3.856812	6.666667
E	6	25.0	30.0	3.856812	4.166667

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	20.0000
Normal Approximation	
Z	1.1668
One-Sided Pr > Z	0.1217
Two-Sided Pr > |Z|	0.2433
t Approximation	
One-Sided Pr > Z	0.1385
Two-Sided Pr > |Z|	0.2769

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	1.6807
DF	1
Pr > Chi-Square	0.1948

Median Scores (Number of Points Above Median) for Variable LENGTH
Classified by Variable POND

POND	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
C	3	2.0	1.333333	0.656167	0.666667
E	6	2.0	2.666667	0.656167	0.333333

Average scores were used for ties.

Median Two-Sample Test

Statistic	2.0000
Z	1.0160
One-Sided Pr > Z	0.1548
Two-Sided Pr > |Z|	0.3096

Median One-Way Analysis

Chi-Square	1.0323
DF	1
Pr > Chi-Square	0.3096

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ANOVA FOR FEMALE WEIGHT 13

The ANOVA Procedure

Class Level Information

Class	Levels	Values
POND	2	C E

Number of observations 9

Dependent Variable: WEIGHT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	239.0755556	239.0755556	10.89	0.0131
Error	7	153.6200000	21.9457143		
Corrected Total	8	392.6955556			

R-Square	Coeff Var	Root MSE	WEIGHT Mean
0.608806	14.83520	4.684625	31.57778

Source	DF	Anova SS	Mean Square	F Value	Pr > F
POND	1	239.0755556	239.0755556	10.89	0.0131

Levene's Test for Homogeneity of WEIGHT Variance
ANOVA of Squared Deviations from Group Means

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
POND	1	68.4450	68.4450	0.16	0.7048
Error	7	3074.0	439.1		

Bartlett's Test for Homogeneity of WEIGHT Variance

Source	DF	Chi-Square	Pr > ChiSq
POND	1	0.1954	0.6585

NOTE: This test controls the Type I experimentwise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	7
Error Mean Square	21.94571
Critical Value of Dunnett's t	2.36463

Comparisons significant at the 0.05 level are indicated by ***.

POND Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
E - C	-10.933	-18.766	-3.100	***

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NONPARAMETRIC COMPARISON OF FEMALE FROG WEIGHT BETWEEN PONDS 17

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable WEIGHT
Classified by Variable POND

POND	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
C	3	24.0	15.0	3.872983	8.00
E	6	21.0	30.0	3.872983	3.50

Wilcoxon Two-Sample Test

Statistic	24.0000
Normal Approximation	
Z	2.1947
One-Sided Pr > Z	0.0141
Two-Sided Pr > |Z|	0.0282
t Approximation	
One-Sided Pr > Z	0.0297
Two-Sided Pr > |Z|	0.0595

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	5.4000
DF	1
Pr > Chi-Square	0.0201

Median Scores (Number of Points Above Median) for Variable WEIGHT
Classified by Variable POND

POND	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
C	3	3.0	1.333333	0.745356	1.000000
E	6	1.0	2.666667	0.745356	0.166667

Median Two-Sample Test

Statistic	3.0000
Z	2.2361
One-Sided Pr > Z	0.0127
Two-Sided Pr > |Z|	0.0253

Median One-Way Analysis

Chi-Square	5.0000
DF	1
Pr > Chi-Square	0.0253

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ANOVA FOR FEMALE LENGTH 19

The ANOVA Procedure

Class Level Information

Class	Levels	Values
POND	2	C E

Number of observations 9

Dependent Variable: LENGTH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	76.4672222	76.4672222	6.02	0.0439
Error	7	88.9416667	12.7059524		
Corrected Total	8	165.4088889			

R-Square	Coeff Var	Root MSE	LENGTH Mean
0.462292	5.542652	3.564541	64.31111

Source	DF	Anova SS	Mean Square	F Value	Pr > F
POND	1	76.46722222	76.46722222	6.02	0.0439

Levene's Test for Homogeneity of LENGTH Variance
ANOVA of Squared Deviations from Group Means

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
POND	1	76.8869	76.8869	0.33	0.5841
Error	7	1635.0	233.6		

Bartlett's Test for Homogeneity of LENGTH Variance

Source	DF	Chi-Square	Pr > ChiSq
POND	1	0.1439	0.7044

Dunnett's t Tests for LENGTH

NOTE: This test controls the Type I experimentwise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	7
Error Mean Square	12.70595
Critical Value of Dunnett's t	2.36463

Comparisons significant at the 0.05 level are indicated by ***.

POND Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
E - C	-6.183	-12.143	-0.223	***

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NONPARAMETRIC COMPARISON OF FEMALE FROG LENGTH BETWEEN PONDS 23

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable LENGTH
Classified by Variable POND

POND	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
C	3	22.0	15.0	3.856812	7.333333
E	6	23.0	30.0	3.856812	3.833333

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	22.0000
Normal Approximation	
Z	1.6853
One-Sided Pr > Z	0.0460
Two-Sided Pr > |Z|	0.0919
t Approximation	
One-Sided Pr > Z	0.0652
Two-Sided Pr > |Z|	0.1304

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	3.2941
DF	1
Pr > Chi-Square	0.0695

Median Scores (Number of Points Above Median) for Variable LENGTH
Classified by Variable POND

POND	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
C	3	3.0	1.333333	0.745356	1.000000
E	6	1.0	2.666667	0.745356	0.166667

Average scores were used for ties.

Median Two-Sample Test

Statistic	3.0000
Z	2.2361
One-Sided Pr > Z	0.0127
Two-Sided Pr > |Z|	0.0253

Median One-Way Analysis

Chi-Square	5.0000
DF	1
Pr > Chi-Square	0.0253

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COMPARISON OF MALE AND FEMALE WEIGHT BY SAMPLING AREA 25

---------- POND=C ----------

The ANOVA Procedure

Class Level Information

Class	Levels	Values
SEX	2	FEMA MALE

Number of observations 6

Dependent Variable: WEIGHT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	440.3266667	440.3266667	11.77	0.0265
Error	4	149.6333333	37.4083333		
Corrected Total	5	589.9600000			

R-Square	Coeff Var	Root MSE	WEIGHT Mean
0.746367	20.18559	6.116235	30.30000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
SEX	1	440.3266667	440.3266667	11.77	0.0265

Levene's Test for Homogeneity of WEIGHT Variance
ANOVA of Squared Deviations from Group Means

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
SEX	1	94.5654	94.5654	0.20	0.6796
Error	4	1913.1	478.3		

Bartlett's Test for Homogeneity of WEIGHT Variance

Source	DF	Chi-Square	Pr > ChiSq
SEX	1	0.0411	0.8394

Dunnett's t Tests for WEIGHT

NOTE: This test controls the Type I experimentwise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	4
Error Mean Square	37.40833
Critical Value of Dunnett's t	2.77630
Minimum Significant Difference	13.865

Comparisons significant at the 0.05 level are indicated by ***.

SEX Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
MALE - FEMA	-17.133	-30.998	-3.269	***

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COMPARISON OF MALE AND FEMALE WEIGHT BY SAMPLING AREA 29

---------- POND=E ----------

The ANOVA Procedure

Class Level Information

Class	Levels	Values
SEX	2	FEMA MALE

Number of observations 12

Dependent Variable: WEIGHT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	348.8408333	348.8408333	28.47	0.0003
Error	10	122.5483333	12.2548333		
Corrected Total	11	471.3891667			

R-Square	Coeff Var	Root MSE	WEIGHT Mean
0.740027	15.52987	3.500690	22.54167

Source	DF	Anova SS	Mean Square	F Value	Pr > F
SEX	1	348.8408333	348.8408333	28.47	0.0003

Levene's Test for Homogeneity of WEIGHT Variance
ANOVA of Squared Deviations from Group Means

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
SEX	1	288.9	288.9	1.13	0.3134
Error	10	2563.5	256.4		

Bartlett's Test for Homogeneity of WEIGHT Variance

Source	DF	Chi-Square	Pr > ChiSq
SEX	1	1.1929	0.2747

Dunnett's t Tests for WEIGHT

NOTE: This test controls the Type I experimentwise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	12.25483
Critical Value of Dunnett's t	2.22816
Minimum Significant Difference	4.5034

Comparisons significant at the 0.05 level are indicated by ***.

SEX Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
MALE - FEMA	-10.783	-15.287	-6.280	***

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NONPARAMETRIC COMPARISON OF MALE AND FEMALE WEIGHT BY SAMPLING AREA 33

---------- POND=C ----------

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable WEIGHT
Classified by Variable SEX

SEX	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
FEMA	3	15.0	10.50	2.291288	5.0
MALE	3	6.0	10.50	2.291288	2.0

Wilcoxon Two-Sample Test

Statistic	15.0000
Normal Approximation	
Z	1.7457
One-Sided Pr > Z	0.0404
Two-Sided Pr > |Z|	0.0809
t Approximation	
One-Sided Pr > Z	0.0706
Two-Sided Pr > |Z|	0.1413

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	3.8571
DF	1
Pr > Chi-Square	0.0495

Median Scores (Number of Points Above Median) for Variable WEIGHT
Classified by Variable SEX

SEX	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
FEMA	3	3.0	1.50	0.670820	1.0
MALE	3	0.0	1.50	0.670820	0.0

Median Two-Sample Test

Statistic	3.0000
Z	2.2361
One-Sided Pr > Z	0.0127
Two-Sided Pr > |Z|	0.0253

Median One-Way Analysis

Chi-Square	5.0000
DF	1
Pr > Chi-Square	0.0253

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NONPARAMETRIC COMPARISON OF MALE AND FEMALE WEIGHT BY SAMPLING AREA 35

---------- POND=E ----------

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable WEIGHT
Classified by Variable SEX

SEX	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
FEMA	6	56.0	39.0	6.244998	9.333333
MALE	6	22.0	39.0	6.244998	3.666667

Wilcoxon Two-Sample Test

Statistic	56.0000
Normal Approximation	
Z	2.6421
One-Sided Pr > Z	0.0041
Two-Sided Pr > |Z|	0.0082
t Approximation	
One-Sided Pr > Z	0.0115
Two-Sided Pr > |Z|	0.0229

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	7.4103
DF	1
Pr > Chi-Square	0.0065

Median Scores (Number of Points Above Median) for Variable WEIGHT
Classified by Variable SEX

SEX	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
FEMA	6	5.0	3.0	0.904534	0.833333
MALE	6	1.0	3.0	0.904534	0.166667

Median Two-Sample Test

Statistic	5.0000
Z	2.2111
One-Sided Pr > Z	0.0135
Two-Sided Pr > |Z|	0.0270

Median One-Way Analysis

Chi-Square	4.8889
DF	1
Pr > Chi-Square	0.0270

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COMPARISON OF MALE AND FEMALE LENGTH BY SAMPLING AREA 37

---------- POND=C ----------

The ANOVA Procedure

Class Level Information

Class	Levels	Values
SEX	2	FEMA MALE

Number of observations 6

Dependent Variable: LENGTH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	192.6666667	192.6666667	10.98	0.0296
Error	4	70.1866667	17.5466667		
Corrected Total	5	262.8533333			

R-Square	Coeff Var	Root MSE	LENGTH Mean
0.732982	6.673724	4.188874	62.76667

Source	DF	Anova SS	Mean Square	F Value	Pr > F
SEX	1	192.6666667	192.6666667	10.98	0.0296

Levene's Test for Homogeneity of LENGTH Variance
ANOVA of Squared Deviations from Group Means

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
SEX	1	212.3	212.3	1.64	0.2691
Error	4	516.7	129.2		

Bartlett's Test for Homogeneity of LENGTH Variance

Source	DF	Chi-Square	Pr > ChiSq
SEX	1	0.4788	0.4890

Dunnett's t Tests for LENGTH

NOTE: This test controls the Type I experimentwise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	4
Error Mean Square	17.54667
Critical Value of Dunnett's t	2.77630
Minimum Significant Difference	9.4955

Comparisons significant at the 0.05 level are indicated by ***.

SEX Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
MALE - FEMA	-11.333	-20.829	-1.838	***

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COMPARISON OF MALE AND FEMALE LENGTH BY SAMPLING AREA 41

---------- POND=E ----------

The ANOVA Procedure

Class Level Information

Class	Levels	Values
SEX	2	FEMA MALE

Number of observations 12

Dependent Variable: LENGTH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	250.2533333	250.2533333	21.81	0.0009
Error	10	114.7433333	11.4743333		
Corrected Total	11	364.9966667			

R-Square	Coeff Var	Root MSE	LENGTH Mean
0.685632	5.872370	3.387379	57.68333

Source	DF	Anova SS	Mean Square	F Value	Pr > F
SEX	1	250.2533333	250.2533333	21.81	0.0009

Levene's Test for Homogeneity of LENGTH Variance
ANOVA of Squared Deviations from Group Means

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
SEX	1	68.3860	68.3860	0.36	0.5631
Error	10	1912.3	191.2		

Bartlett's Test for Homogeneity of LENGTH Variance

Source	DF	Chi-Square	Pr > ChiSq
SEX	1	0.2925	0.5886

Dunnett's t Tests for LENGTH

NOTE: This test controls the Type I experimentwise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	11.47433
Critical Value of Dunnett's t	2.22816
Minimum Significant Difference	4.3576

Comparisons significant at the 0.05 level are indicated by ***.

SEX Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
MALE - FEMA	-9.133	-13.491	-4.776	***

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NONPARAMETRIC COMPARISON OF MALE AND FEMALE LENGTH BY SAMPLING AREA 45

---------- POND=C ----------

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable LENGTH
Classified by Variable SEX

SEX	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
FEMA	3	15.0	10.50	2.291288	5.0
MALE	3	6.0	10.50	2.291288	2.0

Wilcoxon Two-Sample Test

Statistic	15.0000
Normal Approximation	
Z	1.7457
One-Sided Pr > Z	0.0404
Two-Sided Pr > |Z|	0.0809
t Approximation	
One-Sided Pr > Z	0.0706
Two-Sided Pr > |Z|	0.1413

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	3.8571
DF	1
Pr > Chi-Square	0.0495

The NPAR1WAY Procedure

Median Scores (Number of Points Above Median) for Variable LENGTH
Classified by Variable SEX

SEX	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
FEMA	3	3.0	1.50	0.670820	1.0
MALE	3	0.0	1.50	0.670820	0.0

Median Two-Sample Test

Statistic	3.0000
Z	2.2361
One-Sided Pr > Z	0.0127
Two-Sided Pr > |Z|	0.0253

Median One-Way Analysis

Chi-Square	5.0000
DF	1
Pr > Chi-Square	0.0253

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NONPARAMETRIC COMPARISON OF MALE AND FEMALE LENGTH BY SAMPLING AREA 47

---------- POND=E ----------

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable LENGTH
Classified by Variable SEX

SEX	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
FEMA	6	56.0	39.0	6.223124	9.333333
MALE	6	22.0	39.0	6.223124	3.666667

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	56.0000
Normal Approximation	
Z	2.6514
One-Sided Pr > Z	0.0040
Two-Sided Pr > |Z|	0.0080
t Approximation	
One-Sided Pr > Z	0.0113
Two-Sided Pr > |Z|	0.0225

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	7.4624
DF	1
Pr > Chi-Square	0.0063

The NPAR1WAY Procedure

Median Scores (Number of Points Above Median) for Variable LENGTH
Classified by Variable SEX

SEX	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
FEMA	6	5.0	3.0	0.904534	0.833333
MALE	6	1.0	3.0	0.904534	0.166667

Average scores were used for ties.

Median Two-Sample Test

Statistic	5.0000
Z	2.2111
One-Sided Pr > Z	0.0135
Two-Sided Pr > |Z|	0.0270

Median One-Way Analysis

Chi-Square	4.8889
DF	1
Pr > Chi-Square	0.0270

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