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Data Evaluation Report on Gonadal and Laryngeal Responses to Field Exposure of *Xenopus laevis* to Atrazine in Areas of Corn Production in South Africa

EPA MRID Number 458677-10

Data Requirement:

EPA DP Barcode D288775

EPA MRID 458677-10
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

Primary Reviewer: Thomas M. Steeger, Ph.D., Senior Biologist **Date:** April 12, 2003
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EPA PC Code 080803

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CITATION: Smith, E. E., L. DuPreez, and K. Solomon. 2003. Gonadal and laryngeal responses to field exposure of *Xenopus laevis* to atrazine in areas of corn production in South Africa. The Institute of Environmental and Human Health, Texas Tech University, Lubbock, TX 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-01C.

EXECUTIVE SUMMARY:

This report is part of a multiphase study examining the effects of atrazine on African clawed frogs (*Xenopus laevis*) in their native habitat (South Africa). This phase of the study summarizes the body weights, lengths and condition index (weight/length) of both male and female collected from reference site (no corn grown and no atrazine/triazine use) and experimental site (corn grown and atrazine/triazines used) ponds. The study also examined several aspects of gonadal growth (testis weight, gonadosomatic index) and development (incidence of deformities, cellular morphology) in frog testes collected from the two sites to determine whether atrazine exposure had affected these measurement endpoints.

While males were smaller than females at both study sites, there was no difference in weight or length of males or females collected at reference or experimental sites. Condition indices for males and females did not differ between reference and experimental collection sites. However, the mean testes weight from reference ponds (0.037 ± 0.021 mg) was statistically different from mean weight of testes (0.046 ± 0.018 mg) from frogs collected at experimental sites. GSI was significantly different for males collected from reference (0.13 ± 0.04) compared to males collected from experimental sites (0.17 ± 0.04).

There were no statistical differences in the cellular morphology of testes collected at reference sites from those collected at experimental site. The incidence of external abnormalities (missing limbs) was 1% across all animals collected (reference and experimental) and was attributed to the predatory effects of turtles. The incidence of testicular oocytes was 3% in males collected from reference ponds and 2% in experimental ponds. The study claims that laryngeal somatic index for males and females from reference and experimental sites were not significantly different ($p > 0.05$); however, no raw data were provided to verify these observations.

Although not discussed at length in this phase of the study, the interpretation of these data is complicated by the fact that atrazine, its degradates and other triazines were detected at significant levels in reference ponds. Additionally, at some sites, frogs were collected over a protracted period of time (6 months), rendering GSI a questionable metric. This study provides evidence that under the conditions studied, the incidence of testicular oocytes in Xenopus laevis is roughly 2 to 3%. Because of the presence of atrazine in both sampling areas, a regression-based analysis would be more appropriate than hypothesis-testing based analysis. Unfortunately, though, the study is not designed for regression analysis.

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I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: Nonguideline Study

COMPLIANCE: Not conducted under full GLP; however, most practices as defined by 40 CFR Part 160, August 19, 1989 were established for this study, including but not limited to:

- Written, authorized protocol
- Written, authorized Standard Operating Procedures for all key procedures.
- Organization and personnel were sufficient in terms of number, education, training and experience.
- Facilities were of suitable size and construction
- Equipment used was of appropriate design and adequate capacity.
- Test material identity, strength, purity and composition were characterized
- Independent QA inspections of raw data and final report were conducted.
- Interim Report was written
- Raw data, documentation, records, protocols, and final phase report will be archived.

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 determine if exposure to triazines used in corn production affects the incidence or extent of morphological changes in the gonads or intersex gonads in the male and female metamorphs and adults of *X. laevis*.
2. To determine whether there are changes in the morphology of the male larynx in metamorphs and adults of *X. laevis* in response to exposures to triazines used in corn production.
3. To determine if there are changes in plasma steroid concentrations of adult *X. laevis* in response to exposures to triazines used in corn production.
4. To determine if other frogs present at the sites show any morphological or other changes in the gonads.
5. To assess feasibility of determining if *X. laevis* in the exposed sites have been subjected to selection pressure.

1. Experimental Conditions

Sampling was conducted in April and May approximately 6 months after atrazine was applied (October / November); water samples to characterize exposure were collected one month prior to frog sampling. Environmental parameters that were measured at each site included habitat description (vegetative cover and water depth). Water samples were analyzed for atrazine, related triazines, other pesticides and metals.

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

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

Frogs were sampled after the rainy season in April and May; however, at atrazine sites E1 and E8, frogs were collected from April to June and from April to September, respectively, due to low sample sizes. The low sample sizes at sites E1and E8 were attributed to the introduction of sharptooth catfish (*Clarius gariepinus*) into the ponds due to high runoff. Sampling at all other sites occurred once or twice within a "few days." Frogs were collected in 10 baited traps and harvested after two days in "clean water". Afterwards, animals were housed individually in 2-L plastic containers for 48 hours to recover from "capture stress".

Total larynx weight was used to determine the response of the larynx in animals from experimental and control study sites. Adult males from each site were selected randomly for a total of 170 animals.

Testes were processed for routine paraffin embedding, serially sectioned at 5 µm, and stained with hematoxylin and eosin.

Three slides were selected: slide A was selected from the rostral terminus of the testes on the basis of the first observation of a seminiferous tubule; slide B was selected from the middle; and slide C was selected from a similar arbitrarily assigned caudal region of the testis with a complete seminiferous tubule. Three fields were photographed from each of the three slides yielding nine photomicrographs of each testis. The photomicrographs were overlain with a grid and the cell/tissue type under each crossbar was identified and counted by three different readers to determine the representative fractional volume of spermatogonia, spermatocyte, sperm, connective tissue, blood vessel "other" morphological features.

For oocytes, every 200th micron section was evaluated.

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 differed in any substantive way, the difference was reported in the text below.]

The researchers were unable to collect Stage 66 metamorphs except in reference site C3; therefore, the study focused on adults. According to the report, the targeted number of fish, i.e., 20 males and 20 females per site, could not be achieved (**Table 1**) at sites E1 and E8 due to the introduction of sharptooth catfish; however, no explanation is provided as to why the targeted number could not be obtained from reference site C3.

Table 2 presents the mean body weight and snout-vent lengths (± standard deviation) of frogs collected in reference and experimental sites. There was no statistical difference in the average SVL of females collected from reference sites (71.8 ± 4.45 mm) compared with females collected from experimental sites (73.9 ± 10.1 mm). Additionally, there was no difference in mean weight of females from reference sites (36.5 ± 4.32 g) and experimental sites (42.8 ± 15.7 g).

There was no statistical difference in the average SVL of males collected from reference sites (62.4 ± 8.45 mm) compared with males collected from experimental sites (62.6 ± 2.87 mm). There was also no statistical difference in mean weight of males collected at reference (25.8 ± 9.93 g) and experimental sites (27.0 ± 4.14 g).

While the report states that there was no difference in weight of the left testis from reference (18.6 mg) and experimental sites (23.3 mg) nor between the weight of the right testis from reference (15.8 mg) and experimental (22.1 mg) site collected animals, there were differences in the total testicular weight (combined right and left testis) between reference and experimental collection sites. The mean testes weight from control (0.037 ± 0.021 mg) was statistically different (ANOVA, $p < 0.0449$) than mean weight of testes (0.046 ± 0.018 mg) from frogs collected at experimental sites.

Table 2. Mean snout-vent length and body weight of male and female adult frogs collected at experimental (E1 - E8) and reference (R1 - R3) sites.

Pond	Snout-Vent Length (mm)		Body Weight (g)	
	Males	Females	Males	Females
E1	59.20 ± 9.49	64.48 ± 14.3	23.39 ± 14.4	29.15 ± 19.2

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E3	66.63 ± 6.99	74.39 ± 6.94	32.57 ± 8.99	39.38 ± 8.47
E4	63.83 ± 3.35	77.54 ± 10.9	29.90 ± 4.17	48.82 ± 18.5
E6	62.66 ± 9.58	88.50 ± 9.53	26.11 ± 12.4	66.96 ± 22.7
E8	60.72 ± 7.28	64.47 ± 13.8	23.09 ± 8.79	29.80 ± 18.9
R1	58.00 ± 6.64	69.55 ± 8.02	19.44 ± 6.10	32.83 ± 11.1
R3	56.99 ± 10.9	68.91 ± 14.5	20.75 ± 13.3	35.35 ± 20.2
R8	72.11 ± 7.19	76.95 ± 7.04	37.26 ± 12.0	41.25 ± 10.4

Average condition indices (body weight ÷ snout-vent length) for females collected from reference sites (0.48 ± 0.15 g/mm) were not significantly different ($p > 0.0562$) from females collected at experimental sites (0.54 ± 0.20 g/mm). Similarly, male CI between reference (0.39 ± 0.14 g/mm) and experimental sites (0.42 ± 0.12 g/mm) were not statistically different ($p > 0.237$).

The report states that mean gonadalsomatic indices (GSI = total weight of gonad ÷ body weight) were lower for males collected in reference sites than in experimental sites but were not statistically different. EFED's analysis (see attached SAS output), though, indicates that GSI were significantly different (ANOVA, $p < 0.0001$) for males collected from reference (0.13 ± 0.04) sites compared to males collected from experimental sites (0.17 ± 0.04).

Morphological abnormalities (deformed limbs) were observed in 3 out of 212 (0.01%) of the animals and were attributed to attacks by the Cape terrapin (Pelomedusa subrufa).

Based on gross morphology, only 2 out of 101 males examined had discontinuous gonads, and 4 had testes of unequal size.

The extent of morphological changes in adult frog testes was determined by evaluating 56 testes from reference sites and 43 from experimental sites (**Table 3**). There were no statistical differences in the cell types comprising testes collected from reference and experimental sites.

Testicular oocytes were observed in 3% of the animals collected from reference sites and 2% of the frogs collected from experimental sites.

The study claims that laryngeal somatic index for males and females from reference and experimental sites were not significantly different ($p > 0.05$); however, no raw data were provided to verify these observations.

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Table 3. Mean cell type identified in testes of male frogs collected from reference (C1, C3 and C6) and experimental (E1, E3, E4, E6 and E8) sites along with associated t-test p-values.

Site	Spermatogonia	Spermatocytes	Sperm	Connective Tissue and Other Cells	Blood Vessels
C1	3.0	25.6	55.9	14.9	0.5
C3	4.5	34.3	46.3	14.6	0.3
C6	2.8	22.6	60.3	13.9	0.3
E1	3.4	28.8	53.4	13.7	0.8
E3	3.6	24.9	58.1	13.2	0.2
E4	3.0	24.5	59.8	12.6	0.2
E6	3.2	20.8	62.4	13.2	0.3
E8	2.9	30.1	52.2	14.5	0.2
Mean of C1 - C6	3.5	27.5	54.2	14.5	0.4
Mean of E1 - E8	3.2	25.8	57.2	13.5	0.3
t-test p values	0.6214	0.6382	0.4782	0.0754	0.7305

C. REPORTED STATISTICS: Statistical analyses of frog numbers at each site were conducted using standard methods such as Chi-square, Kruskal-Wallis and Student's t-test. The initial analyses of histopathology were carried out using standard ANOVA procedures followed by Tukey's multi-comparison test using Minitab® statistical software.

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

E. STUDY DEFICIENCIES:

Atrazine and/or triazine degradates were present in control sites.

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While the low sample size at sites E1 and E8 may have been explained by the sudden introduction of predatory catfish, it doesn't explain why the remaining corn-growing sites (E3, E4 and E6) also yielded fewer frogs than non-corn growing sites.

Because frogs were sampled over disproportionate period of time, e.g., 6 months for site E8,animals were at different stages of development.

Frog traps were baited with liver/meat scraps that may have contained hormones.

Following capture, frogs were housed individually in "clean water" for 48 hours. The volume and characteristics of this water were not described in the study.

F. REVIEWER'S COMMENTS:

Atrazine is typically applied in October / November when corn is planted in South Africa. Frog sampling was conducted after the rainy season in April to May, and water samples to characterize exposure were collected within a time span of four weeks before frogs were sampled. At exposure site E8, frogs were sampled over a six-month time frame; the authors attributed the fewer number of frogs to a high runoff event that washed catfish into the pond and the catfish preyed on the frogs. Given the aerial photos and the fact that the majority of the ponds relied on rain (runoff) and were not dependent on a stream, it is unclear where the catfish came from. Because *Clarius* is an aquaculture species, it is possible that aquaculture ponds were in the vicinity of E1 and E8.

Frogs were attracted into baited traps containing liver and/or scrap meat that may have contained hormones. Apparently trapped animals were held in cages for two days which would likely have provided sufficient time to consume all of the bait. Also, depending on catch rates, the loading within the cages could have stressed the frogs. Although it doesn't state the desired sample size in the report's methodology, apparently (based on the raw data), 20 male and 20 female frogs were to be sampled at each site. **Table 1** shows that while the desired sample size was generally available for frogs collected in non-corn growing areas, sample sizes were minimally 50% lower in corn-growing areas. In site E1, sampling for female frogs extended into late June and at site E8 sampling extended into mid-August for females and into mid-September for males. Extended sampling periods at these two sites may have resulted in collecting animals that were at considerably different states of development. The gonadosomatic index and ovarian aromatase activity in females collected from sites E1 and E8 expressed the highest variability relative to any of the other treatment sites.

Table 1. Summary of total number of *Xenopus laevis* collected from April through May 2002 at non-corn growing (C) and corn growing (E) sites in South Africa.

Site	Males	Females
C1	20 (Apr 21 - Apr 23)	20 (Apr 21 - May 1)
C3	17 (Apr 4 - Apr 11)	20 (Apr 4 - Apr 11)

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C6	20 (May 13 - May 15)	20 (May 13 - May 14)
E1	8 (April 29 - May 7)	8 (April 29 - June 29)
E3	10 (Apr 9 - Apr 17)	10 (Apr 9 - Apr 18)
E4	10 (Apr 13 - Apr 15)	10 (Apr 14 - Apr 16)
E6	10 (May 5 - May 9)	10 (May 5 - May 6)
E8	6 (Apr 29 - Sep 17)	15 (Apr 29 - Aug 17)

G. CONCLUSIONS:

This report is part of a multiphase study examining the effects of atrazine on African clawed frogs (*Xenopus laevis*) in their native habitat (South Africa). This phase of the study summarizes the body weights, lengths and condition index (weight/length) of both male and female collected from reference site (no corn grown and no atrazine/triazine use) and from experimental site (corn grown and atrazine/triazines used) ponds. The study also examined several aspects of gonadal growth (testis weight, gonadosomatic index) and development (incidence of deformities, cellular morphology) in frog testes collected from the two sites to determine whether atrazine exposure had affected these measurement endpoints.

While males were smaller than females at both study sites, there was no difference in weight or length of males or females collected at reference or experimental sites. Condition indices for males and females did not differ between reference and experimental collection sites. However, the mean testes weight from reference ponds (0.037 ± 0.021 mg) were statistically different than mean weight of testes (0.046 ± 0.018 mg) from frogs collected at experimental sites. GSI was significantly different for males collected from reference (0.13 ± 0.04) compared to males collected from experimental sites (0.17 ± 0.04).

There were no statistical differences in the cellular morphology of testes collected at reference sites and those collected at experimental site. The incidence of external abnormalities (missing limbs) was 1% across all animals collected (reference and experimental) and was attributed to the predatory effects of turtles. The incidence of testicular oocytes was 3% in males collected from reference ponds and 2% in experimental ponds. The study claims that laryngeal somatic index for males and females from reference and experimental sites were not significantly different ($p > 0.05$); however, no raw data were provided with which to verify these observations.

Although not discussed at length in this phase of the study, the interpretation of these data is complicated by the fact that atrazine, its degradates and other triazines were detected at significant levels in reference ponds. Additionally, at some sites, frogs were collected over a protracted period of time (6 months) rendering GSI a questionable metric. This study provides evidence that under the conditions studied, the incidence of testicular oocytes in *Xenopus laevis* in South Africa is roughly 2 to 3%. Because of the presence of atrazine in both sampling areas, a regression-based analysis would be more appropriate than hypothesis-testing based analysis. This study, though, was not designed for regression analysis. Can you include a summary conclusion

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statement here? Anything useful in this study? After the study is completed, a more definitive conclusion will be reached.

MEAN MALE SVL FOR REFERENCE (R) AND EXPERIMENTAL (E) COLLECTION SITES 109

Obs	POND	_TYPE_	_FREQ_	MEAN	STD
1	E1	0	8	59.2000	9.4870
2	E3	0	10	66.6300	6.9863
3	E4	0	10	63.8300	3.3549
4	E6	0	10	62.6600	9.5755
5	E8	0	6	60.7167	7.2841
6	R1	0	20	58.0000	6.6373
7	R3	0	17	56.9882	10.9170
8	R6	0	20	72.1100	7.1908

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ANOVA FOR MALE SLV BETWEEN SITES 110

The ANOVA Procedure
Class Level Information

Class	Levels	Values
SITE	2	C E

Number of observations 101

Dependent Variable: LENGTH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	2.016502	2.016502	0.02	0.8822
Error	99	9041.101320	91.324256		
Corrected Total	100	9043.117822			

R-Square	Coeff Var	Root MSE	LENGTH Mean
0.000223	15.22363	9.556373	62.77327

Source	DF	Anova SS	Mean Square	F Value	Pr > F
SITE	1	2.01650201	2.01650201	0.02	0.8822

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
SITE	1	77939.9	77939.9	6.28	0.0139
Error	99	1229054	12414.7		

Bartlett's Test for Homogeneity of LENGTH Variance

Source	DF	Chi-Square	Pr > ChiSq
SITE	1	5.1814	0.0228

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	99
Error Mean Square	91.32426
Critical Value of Dunnett's t	1.98422

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

SITE Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
E - C	0.2850	-3.5203	4.0902

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NONPARAMETRIC COMPARISON OF MALE LENGTH BETWEEN SITES 114

The NPAR1WAY Procedure

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

SITE	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
C	57	2872.50	2907.0	145.999621	50.394737
E	44	2278.50	2244.0	145.999621	51.784091

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	2278.5000
Normal Approximation	
Z	0.2329
One-Sided Pr > Z	0.4079
Two-Sided Pr > |Z|	0.8159
t Approximation	
One-Sided Pr > Z	0.4082
Two-Sided Pr > |Z|	0.8163

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	0.0558
DF	1
Pr > Chi-Square	0.8132

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

SITE	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
C	57	29.0	28.217822	2.503874	0.508772
E	44	21.0	21.782178	2.503874	0.477273

Average scores were used for ties.

Median Two-Sample Test

Statistic	21.0000
Z	-0.3124
One-Sided Pr < Z	0.3774
Two-Sided Pr > |Z|	0.7547

Median One-Way Analysis

Chi-Square	0.0976
DF	1
Pr > Chi-Square	0.7547

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MEAN FEMALE SVL FOR REFERENCE (R) AND EXPERIMENTAL (E) COLLECTION SITES 116

Obs	POND	_TYPE_	_FREQ_	MEAN	STD
1	E1	0	6	64.4833	14.3272
2	E3	0	10	74.3900	6.9437
3	E4	0	10	77.5400	10.9218
4	E6	0	10	88.5000	9.5274
5	E8	0	15	64.4733	13.8195
6	R1	0	20	89.5450	91.3941
7	R3	0	20	68.9100	14.4936
8	R6	0	20	76.9500	7.0433

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ANOVA FOR FEMALE SLV BETWEEN SITES 117

The ANOVA Procedure

Class Level Information

Class	Levels	Values
SITE	2	C E

Number of observations 111

Dependent Variable: LENGTH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	628.8675	628.8675	0.38	0.5363
Error	109	178119.4467	1634.1234		
Corrected Total	110	178748.3142			

R-Square	Coeff Var	Root MSE	LENGTH Mean
0.003518	52.99887	40.42429	76.27387

Source	DF	Anova SS	Mean Square	F Value	Pr > F
SITE	1	628.8675382	628.8675382	0.38	0.5363

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
SITE	1	1.864E8	1.864E8	0.83	0.3656
Error	109	2.461E10	2.2581E8		

Bartlett's Test for Homogeneity of LENGTH Variance

Source	DF	Chi-Square	Pr > ChiSq
SITE	1	70.8565	<.0001

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	109
Error Mean Square	1634.123
Critical Value of Dunnett's t	1.98197

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

SITE Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
E - C	-4.776	-20.036	10.483

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NONPARAMETRIC COMPARISON OF FEMALE LENGTH ACROSS SITES 121

The NPAR1WAY Procedure

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

SITE	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
C	60	3256.0	3360.0	168.985548	54.266667
E	51	2960.0	2856.0	168.985548	58.039216

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	2960.0000
Normal Approximation	
Z	0.6125
One-Sided Pr > Z	0.2701
Two-Sided Pr > |Z|	0.5402
t Approximation	
One-Sided Pr > Z	0.2707
Two-Sided Pr > |Z|	0.5415

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	0.3788
DF	1
Pr > Chi-Square	0.5383

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

SITE	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
C	60	27.0	29.729730	2.637040	0.450000
E	51	28.0	25.270270	2.637040	0.549020

Average scores were used for ties.

Median Two-Sample Test

Statistic	28.0000
Z	1.0351
One-Sided Pr > Z	0.1503
Two-Sided Pr > |Z|	0.3006

Median One-Way Analysis

Chi-Square	1.0715
DF	1
Pr > Chi-Square	0.3006

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AVERAGE LENGTH OF MALE AND FEMALE FROGS AT REFERENCE AND EXPERIMENTAL SITES 123

Obs	SITE	SEX	_TYPE_	_FREQ_	LENGTH	SD_L
1	C	F	0	3	78.4683	10.4010
2	C	M	0	3	62.3661	8.4536
3	E	F	0	5	73.8773	10.0521
4	E	M	0	5	62.6073	2.8664

MEAN MALE WEIGHT FOR REFERENCE (R) AND EXPERIMENTAL (E) COLLECTION SITES 124

Obs	POND	_TYPE_	_FREQ_	MEAN	STD
1	E1	0	8	23.3888	14.4006
2	E3	0	10	32.5740	8.9938
3	E4	0	10	29.9000	4.1673
4	E6	0	10	26.1050	12.3935
5	E8	0	6	23.0917	8.7925
6	R1	0	20	19.4445	6.1022
7	R3	0	17	20.7541	13.2996
8	R6	0	20	37.2590	12.0340

US EPA ARCHIVE DOCUMENT

ANOVA FOR MALE WEIGHT BETWEEN SITES 125

The ANOVA Procedure

Class Level Information

Class	Levels	Values
SITE	2	C E

Number of observations 101

Dependent Variable: WEIGHT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	52.00466	52.00466	0.35	0.5572
Error	99	14842.45191	149.92376		
Corrected Total	100	14894.45657			

R-Square	Coeff Var	Root MSE	WEIGHT Mean
0.003492	45.83106	12.24434	26.71624

Source	DF	Anova SS	Mean Square	F Value	Pr > F
SITE	1	52.00466491	52.00466491	0.35	0.5572

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
SITE	1	126859	126859	2.23	0.1387
Error	99	5637162	56941.0		

Bartlett's Test for Homogeneity of WEIGHT Variance

Source	DF	Chi-Square	Pr > ChiSq
SITE	1	3.0051	0.0830

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	99
Error Mean Square	149.9238
Critical Value of Dunnett's t	1.98422

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

SITE Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
E - C	1.447	-3.428	6.323

US EPA ARCHIVE DOCUMENT

NONPARAMETRIC COMPARISON OF MALE WEIGHT BETWEEN SITES 129

The NPAR1WAY Procedure

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

SITE	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
C	57	2742.0	2907.0	146.005999	48.105263
E	44	2409.0	2244.0	146.005999	54.750000

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	2409.0000
Normal Approximation	
Z	1.1267
One-Sided Pr > Z	0.1299
Two-Sided Pr > |Z|	0.2599
t Approximation	
One-Sided Pr > Z	0.1313
Two-Sided Pr > |Z|	0.2626

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	1.2771
DF	1
Pr > Chi-Square	0.2584

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

SITE	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
C	57	26.0	28.217822	2.503874	0.456140
E	44	24.0	21.782178	2.503874	0.545455

Average scores were used for ties.

Median Two-Sample Test

Statistic	24.0000
Z	0.8858
One-Sided Pr > Z	0.1879
Two-Sided Pr > |Z|	0.3757

Median One-Way Analysis

Chi-Square	0.7846
DF	1
Pr > Chi-Square	0.3757

US EPA ARCHIVE DOCUMENT

MEAN FEMALE WEIGHT FOR REFERENCE (R) AND EXPERIMENTAL (E) COLLECTION SITES 131

Obs	POND	_TYPE_	_FREQ_	MEAN	STD
1	E1	0	6	29.1450	19.2315
2	E3	0	10	39.3810	8.4697
3	E4	0	10	48.8210	18.5235
4	E6	0	10	66.9560	22.6873
5	E8	0	15	29.8047	18.8819
6	R1	0	20	32.8315	11.1449
7	R3	0	20	35.3455	20.2334
8	R6	0	20	41.2495	10.4261

US EPA ARCHIVE DOCUMENT

ANOVA FOR FEMALE WEIGHT BETWEEN SITES 132

The ANOVA Procedure

Class Level Information

Class	Levels	Values
SITE	2	C E

Number of observations 111

Dependent Variable: WEIGHT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	1040.14605	1040.14605	2.97	0.0874
Error	109	38122.22029	349.74514		
Corrected Total	110	39162.36634			

R-Square	Coeff Var	Root MSE	WEIGHT Mean
0.026560	47.58918	18.70147	39.29775

Source	DF	Anova SS	Mean Square	F Value	Pr > F
SITE	1	1040.146048	1040.146048	2.97	0.0874

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
SITE	1	2126150	2126150	5.83	0.0174
Error	109	39770440	364866		

Bartlett's Test for Homogeneity of WEIGHT Variance

Source	DF	Chi-Square	Pr > ChiSq
SITE	1	9.1873	0.0024

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	109
Error Mean Square	349.7451
Critical Value of Dunnett's t	1.98197

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

SITE Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
E - C	6.143	-0.917	13.202

US EPA ARCHIVE DOCUMENT

NONPARAMETRIC COMPARISON OF FEMALE WEIGHT ACROSS SITES 136

The NPAR1WAY Procedure

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

SITE	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
C	60	3132.50	3360.0	168.996671	52.208333
E	51	3083.50	2856.0	168.996671	60.460784

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	3083.5000
Normal Approximation	
Z	1.3432
One-Sided Pr > Z	0.0896
Two-Sided Pr > |Z|	0.1792
t Approximation	
One-Sided Pr > Z	0.0910
Two-Sided Pr > |Z|	0.1820

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	1.8122
DF	1
Pr > Chi-Square	0.1782

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

SITE	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
C	60	27.0	29.729730	2.637040	0.450000
E	51	28.0	25.270270	2.637040	0.549020

Average scores were used for ties.

Median Two-Sample Test

Statistic	28.0000
Z	1.0351
One-Sided Pr > Z	0.1503
Two-Sided Pr > |Z|	0.3006

Median One-Way Analysis

Chi-Square	1.0715
DF	1
Pr > Chi-Square	0.3006

US EPA ARCHIVE DOCUMENT

AVERAGE WEIGHT OF MALE AND FEMALE FROGS AT REFERENCE AND EXPERIMENTAL SITES 138

Obs	SITE	SEX	_TYPE_	_FREQ_	WEIGHT	SD_L
1	C	F	0	3	36.4755	4.3213
2	C	M	0	3	25.8192	9.9288
3	E	F	0	5	42.8215	15.7066
4	E	M	0	5	27.0119	4.1411

MEAN TOTAL WEIGHT OF TESTES (LEFT AND RIGHT COMBINED) BY POND 139

Obs	POND	_TYPE_	_FREQ_	WEIGHT	SD_W
1	E1	0	8	0.036925	0.014242
2	E3	0	10	0.053570	0.017665
3	E4	0	10	0.057670	0.009618
4	E6	0	10	0.033560	0.018074
5	E8	0	6	0.044200	0.019885
6	R1	0	29	0.022082	0.008848
7	R3	0	17	0.030953	0.023958
8	R6	0	30	0.051621	0.013727

MEAN TOTAL WEIGHT OF TESTES (LEFT AND RIGHT COMBINED) BY SITE 140

Obs	SITE	_TYPE_	_FREQ_	WEIGHT	SD_W
1	C	0	76	0.037232	0.021104
2	E	0	44	0.045650	0.018130

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ANOVA FOR TOTAL TESTES WEIGHT BETWEEN REFERENCE AND EXPERIMENTAL SITES 141

The ANOVA Procedure

Class Level Information

Class	Levels	Values
SITE	2	C E

Number of observations 120

NOTE: Due to missing values, only 91 observations can be used in this analysis.

Dependent Variable: T_WT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.00161041	0.00161041	4.14	0.0449
Error	89	0.03462027	0.00038899		
Corrected Total	90	0.03623068			

R-Square	Coeff Var	Root MSE	T_WT Mean
0.044449	47.75261	0.019723	0.041302

Source	DF	Anova SS	Mean Square	F Value	Pr > F
SITE	1	0.00161041	0.00161041	4.14	0.0449

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

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
SITE	1	2.989E-7	2.989E-7	1.81	0.1823
Error	89	0.000015	1.654E-7		

Bartlett's Test for Homogeneity of T_WT Variance

Source	DF	Chi-Square	Pr > ChiSq
SITE	1	1.0070	0.3156

Dunnett's t Tests for T_WT

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

Alpha	0.05
Error Degrees of Freedom	89
Error Mean Square	0.000389
Critical Value of Dunnett's t	1.98699

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

SITE Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
E - C	0.008418	0.000197	0.016639	***

US EPA ARCHIVE DOCUMENT

NONPARAMETRIC COMPARISON OF TOTAL TESTES WEIGHT BETWEEN REFERENCE AND EXPERIMENTAL SITES 145

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable T_WT
Classified by Variable SITE

SITE	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
C	47	1900.0	2162.0	125.912307	40.425532
E	44	2286.0	2024.0	125.912307	51.954545

Average scores were used for ties.

Wilcoxon Two-Sample Test

Statistic	2286.0000
Normal Approximation	
Z	2.0768
One-Sided Pr > Z	0.0189
Two-Sided Pr > |Z|	0.0378
t Approximation	
One-Sided Pr > Z	0.0203
Two-Sided Pr > |Z|	0.0407

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	4.3298
DF	1
Pr > Chi-Square	0.0375

Median Scores (Number of Points Above Median) for Variable T_WT
Classified by Variable SITE

SITE	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
C	47	19.0	23.241758	2.396612	0.404255
E	44	26.0	21.758242	2.396612	0.590909

Average scores were used for ties.

Median Two-Sample Test

Statistic	26.0000
Z	1.7699
One-Sided Pr > Z	0.0384
Two-Sided Pr > |Z|	0.0767

Median One-Way Analysis

Chi-Square	3.1325
DF	1
Pr > Chi-Square	0.0767

US EPA ARCHIVE DOCUMENT

MEAN FEMALE CONDITION INDEX BY CITE 147

Obs	SITE	_TYPE_	_FREQ_	CI	SD_CI
1	C	0	61	0.47898	0.15083
2	E	0	51	0.54278	0.19533

ANOVA FOR FEMALE CONDITION INDEX BETWEEN REFERENCE AND EXPERIMENTAL SITE 148

The ANOVA Procedure

Class Level Information

Class	Levels	Values
SITE	2	C E

Number of observations 112

NOTE: Due to missing values, only 110 observations can be used in this analysis.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.11133162	0.11133162	3.73	0.0562
Error	108	3.22722541	0.02988172		
Corrected Total	109	3.33855703			

R-Square	Coeff Var	Root MSE	CI Mean
0.033347	33.99081	0.172863	0.508559

Source	DF	Anova SS	Mean Square	F Value	Pr > F
SITE	1	0.11133162	0.11133162	3.73	0.0562

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

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
SITE	1	0.00619	0.00619	3.05	0.0835
Error	108	0.2191	0.00203		

Bartlett's Test for Homogeneity of CI Variance

Source	DF	Chi-Square	Pr > ChiSq
SITE	1	3.5615	0.0591

Dunnett's t Tests for CI

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

Alpha	0.05
Error Degrees of Freedom	108
Error Mean Square	0.029882
Critical Value of Dunnett's t	1.98218

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

SITE Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
E - C	0.06380	-0.00172	0.12931

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NONPARAMETRIC COMPARISON OF FEMALE CONDITION FACTOR BETWEEN REFERENCE AND EXPERIMENTAL SIT 152

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable CI
Classified by Variable SITE

SITE	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
C	59	2965.0	3274.50	166.833000	50.254237
E	51	3140.0	2830.50	166.833000	61.568627

Wilcoxon Two-Sample Test

Statistic	3140.0000
Normal Approximation	
Z	1.8522
One-Sided Pr > Z	0.0320
Two-Sided Pr > |Z|	0.0640
t Approximation	
One-Sided Pr > Z	0.0334
Two-Sided Pr > |Z|	0.0667

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	3.4416
DF	1
Pr > Chi-Square	0.0636

Median Scores (Number of Points Above Median) for Variable CI
Classified by Variable SITE

SITE	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
C	59	24.0	29.50	2.627047	0.406780
E	51	31.0	25.50	2.627047	0.607843

Median Two-Sample Test

Statistic	31.0000
Z	2.0936
One-Sided Pr > Z	0.0181
Two-Sided Pr > |Z|	0.0363

Median One-Way Analysis

Chi-Square	4.3832
DF	1
Pr > Chi-Square	0.0363

US EPA ARCHIVE DOCUMENT

MEAN MALE CONDITION INDEX BY SITE 154

Obs	SITE	_TYPE_	_FREQ_	CI	SD_CI
1	C	0	57	0.39435	0.13640
2	E	0	44	0.42482	0.11530

ANOVA FOR MALE CONDITION INDEX BETWEEN REFERENCE AND EXPERIMENTAL SITE 155

The ANOVA Procedure

Class Level Information

Class	Levels	Values
SITE	2	C E

Number of observations 101

Dependent Variable: CI

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.02304412	0.02304412	1.41	0.2372
Error	99	1.61346081	0.01629758		
Corrected Total	100	1.63650493			

R-Square	Coeff Var	Root MSE	CI Mean
0.014081	31.31858	0.127662	0.407624

Source	DF	Anova SS	Mean Square	F Value	Pr > F
SITE	1	0.02304412	0.02304412	1.41	0.2372

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

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
SITE	1	0.000694	0.000694	1.37	0.2444
Error	99	0.0501	0.000506		

Bartlett's Test for Homogeneity of CI Variance

Source	DF	Chi-Square	Pr > ChiSq
SITE	1	1.3333	0.2482

Dunnett's t Tests for CI

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

Alpha	0.05
Error Degrees of Freedom	99
Error Mean Square	0.016298
Critical Value of Dunnett's t	1.98422

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

SITE Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
E - C	0.03046	-0.02037	0.08130

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NONPARAMETRIC COMPARISON OF MALE CONDITION INDEX BETWEEN REFERENCE AND EXPERIMENTAL SITES 159

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable CI
Classified by Variable SITE

SITE	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
C	57	2680.0	2907.0	146.006849	47.017544
E	44	2471.0	2244.0	146.006849	56.159091

Wilcoxon Two-Sample Test

Statistic	2471.0000
Normal Approximation	
Z	1.5513
One-Sided Pr > Z	0.0604
Two-Sided Pr > |Z|	0.1208
t Approximation	
One-Sided Pr > Z	0.0620
Two-Sided Pr > |Z|	0.1240

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	2.4172
DF	1
Pr > Chi-Square	0.1200

Median Scores (Number of Points Above Median) for Variable CI
Classified by Variable SITE

SITE	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
C	57	23.0	28.217822	2.503874	0.403509
E	44	27.0	21.782178	2.503874	0.613636

Median Two-Sample Test

Statistic	27.0000
Z	2.0839
One-Sided Pr > Z	0.0186
Two-Sided Pr > |Z|	0.0372

Median One-Way Analysis

Chi-Square	4.3426
DF	1
Pr > Chi-Square	0.0372

US EPA ARCHIVE DOCUMENT

MEAN MALE GONADOSOMATIC INDEX (GSI) BY SITE 161

Obs	SITE	_TYPE_	_FREQ_	GSI	SD_GSI
1	C	0	76	0.13027	0.037421
2	E	0	44	0.16847	0.042300

ANOVA FOR MALE GONADOSOMATIC (GSI) BETWEEN REFERENCE AND EXPERIMENTAL SITE 162

The ANOVA Procedure

Class Level Information

Class	Levels	Values
SITE	2	C E

Number of observations 120

NOTE: Due to missing values, only 91 observations can be used in this analysis.

Dependent Variable: GSI

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.03315187	0.03315187	20.87	<.0001
Error	89	0.14135759	0.00158829		
Corrected Total	90	0.17450946			

R-Square	Coeff Var	Root MSE	GSI Mean
0.189972	26.79357	0.039853	0.148742

Source	DF	Anova SS	Mean Square	F Value	Pr > F
SITE	1	0.03315187	0.03315187	20.87	<.0001

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

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
SITE	1	3.249E-6	3.249E-6	0.72	0.3994
Error	89	0.000403	4.531E-6		

Bartlett's Test for Homogeneity of GSI Variance

Source	DF	Chi-Square	Pr > ChiSq
SITE	1	0.6603	0.4164

Dunnett's t Tests for GSI

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

Alpha	0.05
Error Degrees of Freedom	89
Error Mean Square	0.001588
Critical Value of Dunnett's t	1.98699

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

SITE Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
E - C	0.038194	0.021583	0.054806	***

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NONPARAMETRIC COMPARISON OF MALE GONADOSOMATIC INDEX BETWEEN REFERENCE AND EXPERIMENTAL SIT 166

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable GSI
Classified by Variable SITE

SITE	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
C	47	1645.0	2162.0	125.915315	35.000
E	44	2541.0	2024.0	125.915315	57.750

Wilcoxon Two-Sample Test

Statistic	2541.0000
Normal Approximation	
Z	4.1020
One-Sided Pr > Z	<.0001
Two-Sided Pr > |Z|	<.0001
t Approximation	
One-Sided Pr > Z	<.0001
Two-Sided Pr > |Z|	<.0001

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	16.8587
DF	1
Pr > Chi-Square	<.0001

Median Scores (Number of Points Above Median) for Variable GSI
Classified by Variable SITE

SITE	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
C	47	14.0	23.241758	2.396612	0.297872
E	44	31.0	21.758242	2.396612	0.704545

Median Two-Sample Test

Statistic	31.0000
Z	3.8562
One-Sided Pr > Z	<.0001
Two-Sided Pr > |Z|	0.0001

Median One-Way Analysis

Chi-Square	14.8701
DF	1
Pr > Chi-Square	0.0001

US EPA ARCHIVE DOCUMENT

MEAN CELL TYPES FOR EXPERIMENTAL SITES 168

Obs	POND	_TYPE_	_FREQ_	total	Gonia	Sperma	Sperm	other	BV_avg
1	E1	0	8	33.725	3.425	28.7625	53.35	13.675	0.75
2	E3	0	10	33.340	3.640	24.8800	58.06	13.220	0.22
3	E4	0	10	32.500	2.970	24.4900	59.82	12.580	0.16
4	E6	0	10	33.960	3.240	20.7900	62.41	13.240	0.31
5	E8	0	5	33.740	2.880	30.1400	52.24	14.540	0.16

MEAN CELL TYPES FOR REFERENCE SITES 169

Obs	POND	_TYPE_	_FREQ_	total	Gonia	Sperma	Sperm	other	BV_avg
1	C1	0	19	34.9158	3.02105	25.5737	55.9316	14.9474	0.50526
2	C3	0	17	34.3941	4.53529	34.2882	46.2647	14.6353	0.28824
3	C6	0	20	34.3800	2.82000	22.6050	60.3200	13.9050	0.33500

AVERAGE CELL TYPES BY SITE 170

Obs	SITE	_TYPE_	_FREQ_	total	Gonia	Sperma	Sperm	other	BV_avg
1	C	0	3	34.5633	3.45878	27.4890	54.1721	14.4959	0.37617
2	E	0	5	33.4530	3.23100	25.8125	57.1760	13.4510	0.32000

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