

**Data Evaluation Report on Tissue Pesticide Residues and Histology of the Larynx and Gonads in Native Green Frogs (*Rana clamitans*) Collected from Agricultural Areas in Michigan: Hormone Analysis.**

<b>Data Requirement:</b>	EPA DP Barcode	<b>EPA MRID Number 458677-02</b>
	EPA MRID	D288775
	EPA Guideline	458677-02 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:** March 27, 2003  
Environmental Fate and Effects Division, ERB 4,  
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**Secondary Reviewer(s):** Joseph E. Tietge, M.S., Research Aquatic Biologist **Date:**  
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Stephanie Irene, Ph.D., Senior Advisor **Date:**  
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Mary J. Frankenberry, Senior Statistician **Date:**  
Environmental Fate and Effects Division, ERB 3, U. S. Environmental Protection Agency

**EPA PC Code** 080803

**Date Evaluation Completed: 06/01/2003**

**CITATION:** Jones, P. D., M. B. Murphy, M. Hecker, J. P. Giesy. 2003. Tissue Pesticide Residues and Histology of the Larynx and Gonads in Native Green Frogs (*Rana clamitans*) Collected from Agricultural Areas in Michigan: Hormone Analysis. Aquatic Toxicology Laboratory, Michigan State University, 218C National Food Safety and Toxicology Center, E. Lansing, MI. Sponsor: Syngenta Crop Protection, Inc. Laboratory Study ID: ECORISK Number MSU-02.

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**EXECUTIVE SUMMARY:**

This represents a summary for Phase I of a three-year study where primarily the green frog (*Rana clamitans*) but also the leopard frog (*Rana pipiens*) and the bullfrog (*Rana catesbeiana*) were collected from three reference ponds and six atrazine-exposed ponds. Both juvenile (372) and adult (340) green frogs were collected and examined for gross gonadal abnormalities and blood plasma estradiol and testosterone levels. A total of four mixed or unknown sex animals were identified in all of the frogs collected. Hormone levels exhibited considerable variability among locations and individuals. No relationship between plasma hormone levels and atrazine exposure could be determined.

While this represents an interim report (Phase 1) of a three-year study, the results suggest that *Rana clamitans* are not markedly impacted by atrazine exposure in terms of gonadal deformities. However, reference sites contained atrazine so the ability of this field study to discriminate effects may be limited. Additionally, sex steroid levels exhibited considerable variability, and the study was not able to distinguish meaningful differences between “exposed” and “reference” sites. Plasma steroid levels are of questionable value because data were collected over a time period of four months for adult frogs, and coefficients of variability ranged as high as 10,628%. There were no gender-specific estradiol levels. Exposed males contained roughly similar (0.91) amounts of estradiol as females, while in reference sites males exhibited roughly 4.9 times the plasma estradiol concentration than females.

Because of the variability associated with plasma steroid hormone levels measured in this study, it is unlikely that the study will be able to differentiate any treatment-related effects, especially since the reference sites may have contained low levels of atrazine.

**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.
- Independent QA Inspection were conducted.
- Final Report was written
- Raw data, documentation, records, protocols, and final report were archived.

**A. MATERIALS:**

**1. Test Material**

Atrazine

**Description:** Not reported

**Lot No./Batch No. :** Not reported

**Purity:** NA

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

**Storage conditions of test chemicals:** Not reported

**2. Test organism:**

**Species:** Green Frogs (*Rana clamitans*)

**Age at test initiation:** Juveniles and adults

**Weight at study initiation: (mean and range)** Not reported

**Length at study initiation: (mean and range)** Not reported

**Source:** Field-collected at nine (3 reference and 6 atrazine-exposed) sites located in Livingston, Ingham, Barry and Lapeer counties, Michigan

## **B. STUDY DESIGN:**

**Objective:** To assess the effects of atrazine on kidney and gonad histology and plasma steroid hormone concentrations and gonadal aromatase activity of green frogs (*R. clamitans*) and other incidental ranid species collected from various field sites in Michigan.

### **1. Experimental Conditions**

Experimental sites (KZ02, KZ03, KZ07, LPR02, LPR03 and HW01) were based on proximity to agriculture where atrazine was used and where the genus *Rana* were located. Reference sites (HG01, HL01 and LPR08) were selected in non-agricultural areas.

During each sampling, water chemistry (conductivity, DO, pH and temperature at each of 4 points on water's surface) samples were collected, and on the first visit a sediment (5-cm grab) sample was collected. All samples were analyzed for atrazine using Envirogard triazine ELISA kit (LOD = 0.025 µg/L); sediment samples will be extracted and then analyzed later using ELISA. Results of ELISA will be verified using GC-MS.

Sampling (from 8 PM to midnight) was conducted three times throughout the Summer of 2002. Tadpoles were collected in un-baited minnow traps in the late spring and early summer; juvenile frogs were sampled in mid-summer (June and July); adult frogs were sampled at the end of summer (September). Frogs were sampled using fish nets and were held in buckets for less than two hours to minimize sampling stress and affects on plasma sex steroid concentrations.

Frogs were euthanized by immersion in MS-222, then weighed and measured (SVL) before blood collection via cardiac puncture; blood collection was completed within an approximate four-hour window of time.

Frogs were dissected and examined under a microscope for gonad morphology. One gonad was collected from adult and juvenile frogs, and snap frozen for use in aromatase assay. The other gonad was left in the carcass and fixed in Bouin's solution. Gonadal somatic index (partial GSI) was calculated based on the weight of single gonad.

Plasma samples were extracted with diethyl ether. The level of quantitation (LOQ) for testosterone and 17-β estradiol was 0.78 - 800 pg/well.

**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

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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.]

Quantifiable levels of atrazine were detected in all (exposed and reference) of the ponds sampled. Atrazine concentrations in exposed ponds ranged from 0.025 to 250 µg/L. Reference pond water atrazine concentrations ranged from 0.015 to 0.093 µg/L. No data were reported on other triazine herbicides, degradates or other pesticide residues. Although the study notes that maximum concentrations did not appear to persist for very long at the sites, with measurements tending to drop off in the next water sample, intervals between sampling make it difficult to support this observation.

**Table 1. Atrazine water concentrations in both exposed and reference collection sites during spring (May), early summer (June and July), mid-summer (August) and late summer (September) samples.**

Site	Atrazine Water Concentrations (µg/L)			
	Spring	Early-Summer	Mid-Summer	Late-Summer
KZ02 (Exposed 1)	0.52	0.094	0.025	0.030
KZ03 (Exposed 2)	1.6	1.8	0.71	0.11
KZ07 (Exposed 3)	0.031	0.48	0.17	0.11
HW01 (Exposed 4)	0.12	0.36	0.24	0.24
LPR02 (Exposed 5)	0.83	6.5 x 10 <sup>1</sup>	2.9	0.078
LPR03 (Exposed 6)	2.5 x 10 <sup>2</sup>	0.69	0.092	1.9
HL01 (Reference 1)	0.058	0.069	0.040	0.015
HG01 (Reference 2)	0.026	0.093	0.069	0.067
LPR08 (Reference 3)	0.085	0.062	0.033	0.036

Of the 372 juvenile and 340 adult green frogs collected, four (0.6%) had mixed or unknown sex. It is assumed that this is based on dissecting scope observations because it states that animals will be subject to histopathology in upcoming months.

GSI varied considerably between sampling sites. Female GSI was greatest at HL01 reference site and at the HW01 and LPR02 exposed sites.

Within-site and between-site variability in testosterone (T) levels was high (**Tables 2 and 3**). Testosterone was highest at LPR08 reference site and KZ02 experimental site in both males and females

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Estradiol (E<sub>2</sub>) concentrations in both male and females was greatest at KZ02 exposed site and LPR08 reference site (Tables 2 and 3).

The ratio of plasma estradiol to testosterone levels (E/T) in females averaged  $1.7 \pm 1.6$  at atrazine-exposed sites compared to  $1.6 \pm 1.0$  at reference sites (Table 4). For males, E/T ratios averaged  $1.28 \pm 1.7$  and  $0.95 \pm 0.6$  for atrazine-exposed and reference sites, respectively.

**Table 2. Mean plasma testosterone and estradiol in female green frogs (*Rana clamitans*) collected in reference and atrazine-exposed sites in Michigan. Standard deviations (Std. Dev.) and coefficients of variability (CV = [std. dev. ÷ mean] x 100).**

Site	Female <i>Rana clamitans</i>					
	Mean Testosterone pg/mL	Std. Dev.	CV	Mean Estradiol pg/mL	Std. Dev.	CV
Exposed 1 (KZ02)	3697	641	17%	16407	5718	35%
Exposed 2 (KZ03)	198	2101	1061%	495	4573	924%
Exposed 3 (KZ07)	249	1684	676%	289	478	165%
Exposed 4 (HW01)	640	534	83%	25	226	904%
Exposed 5 (LPR02)	341	843	247%	68	826	1215%
Exposed 6 (LPR03)	209	188	90%	321	467	145%
Reference 1 (HL01)	180	646	359%	134	183	137%
Reference 2 (HG01)	134	98	73%	51	172	337%
Reference 3 (LPR08)	3681	3452	94%	5253	4430	84%

**Table 3. Mean plasma testosterone and estradiol in male green frogs (*Rana clamitans*) collected in reference and atrazine-exposed sites in Michigan. Standard deviations (Std. Dev.) and coefficients of variability (CV = [std. dev. ÷ mean] x 100).**

Site	Male <i>Rana clamitans</i>					
	Mean Testosterone pg/mL	Std. Dev.	CV	Mean Estradiol pg/mL	Std. Dev.	CV
Exposed 1 (KZ02)	3300	8574	260%	14308	7668	54%
Exposed 2 (KZ03)	118	888	753%	238	7678	3226%
Exposed 3 (KZ07)	129	3555	2756%	20	1199	5995%

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Exposed 4 (HW01)	89	--	--	26	--	--
Exposed 5 (LPR02)	168	173	103%	2.7	133	4926%
Exposed 6 (LPR03)	826	--	--	732	--	--
Reference 1 (HL01)	149	610	409%	36	3826	10628%
Reference 2 (HG01)	189	1733	917%	234	2956	1263%
Reference 3 (LPR08)	3183	4171	131%	4314	3568	83%

**Table 4. Female and male plasma estradiol to testosterone (E/T) ratios for green frogs (*Rana clamitans*) collected from atrazine-exposed and reference sites.**

Site	Female E/T Ratio	Male E/T Ratio
Exposed 1 (KZ02)	4.4	4.34
Exposed 2 (KZ03)	2.5	2.02
Exposed 3 (KZ07)	1.2	0.16
Exposed 4 (HW01)	0.04	0.29
Exposed 5 (LPR02)	0.20	0.02
Exposed 6 (LPR03)	1.54	0.89
Reference 1 (HL01)	0.74	0.24
Reference 2 (HG01)	0.38	1.24
Reference 3 (LPR08)	1.43	1.36

**REVIEWER'S COMMENTS:**

Because of the large number of sampling sites and the logistics of collecting and processing samples at night, it is understandable why sampling had to be conducted over a protracted period of time. However, while many of the experimental sites were sampled between 7/23/02 and 7/29/02, sampling of reference sites extended from 7/31/02 through 8/13/02. Only one exposed site (LPR02) was sampled as late as 8/13/02. When adult frogs were collected in September, reference sites were sampled relatively early (9/6 - 9/16), while experimental areas were sampled later (9/13 - 9/30). It's not clear when each of the samples was collected; there are sampling dates reported in the appendix, but these are not consistent.

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. The major hypothesis that was being evaluated in this study is that atrazine exposure results in abnormal kidney and gonad development and that atrazine causes changes in plasma estradiol and testosterone levels. Because of the design of the experiment and the uncontrolled environmental factors in a field study, this hypothesis could not be tested. Furthermore, presumptive effects on gonadal development must occur at the appropriate time in development. In this study, atrazine measurements were taken throughout the summer, but they were apparently not taken during the larval period. As a result, it is uncertain whether or not exposure occurred at the appropriate time to elicit a response.

Because there is considerable variability in the plasma steroid levels, it is difficult to determine potential treatment effects based on these measurements. Plasma estradiol levels in atrazine-exposed females ranged from 25 to 16,407 pg/mL with coefficients of variability (CV) ranging from 35% to 1,215%; at reference sites female plasma estradiol ranged from 51 to 5,253 pg/mL with CV's ranging from 84 to 337%. Testosterone levels in females were equally variable with coefficients of variability ranging between 17 and 1,061%; mean plasma testosterone levels in females from both atrazine-exposed and reference sites ranged roughly from 134 to 3,697 pg/mL. For males, plasma testosterone levels in atrazine-exposed animals ranged from 89 to 3,300 pg/mL (CV range 103 - 2,756%), while males from reference sites ranged from 149 to 3,183 pg/mL (CV range 131 - 917%). Plasma estradiol levels in males at atrazine-exposed sites ranged from 2.7 to 14,308 pg/mL (CV range 54 - 5,995%), while reference site males ranged from 36 - 4314 pg/mL (CV range 83 - 10,628%). Interesting enough the highest plasma estradiol ranges were measured in males collected from atrazine-exposed sites. Unfortunately, the highest variability (as reflected by CVs ranging as high as 10,628%) was observed in reference sites for male estradiol levels. With such high levels of variability and with the presence of atrazine in the reference sites, the potential to differentiate treatment effects would be remote, unless extremely large sample sizes were used. Alternatively the researchers could use a shorter time period for collecting and analyzing samples to limit the likelihood that frogs would be at different stages of their breeding cycle.

Gonadal anomalies were only detected in 0.6% of the total juvenile and adult frogs sampled; however, it is unclear whether histopathology will significantly alter that percentage.

The median ratio of estradiol to testosterone exceeded 1.0 (range: 1.9 - 8.6) in males collected from reference sites; however, at half of the exposed sites the median ratio was less than 1.0 (range 0.26 - 0.89). The remaining exposed sites (3) had median ratios ranging from 4.9 to 6.8.

Reference sites all proved to contain measurable levels of atrazine and were in some cases higher than exposed site values. No data were reported on other triazines and/or degradates nor were data provided on what other chemicals may have been present in the exposed and reference study sites. Although water quality data were collected, no data were provided for analysis.

While this represents an interim report (Phase 1) of a three-year study, the results suggest that *Rana clamitans* were not markedly impacted by atrazine exposure in terms of gonadal deformities. However, reference sites contained atrazine so the ability of this field study to discriminate effects may be limited. Additionally, sex steroid levels exhibited considerable variability, and the study was not able to distinguish meaningful differences between "exposed" and "reference" sites.



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The summary tables for plasma steroid levels are of little value because they summarize data collected over 4 months in adult frogs. There were no gender-specific steroid hormone levels, both males and females exhibited roughly similar average ratios from atrazine exposed sites, *i.e.*, 1.7 versus 1.3, and from reference sites, *i.e.*, 0.85 and 0.95, suggesting that this study couldn't distinguish plasma steroid differences between males and females or between reference and exposure sites if such differences existed.

Although the study was not conducted under full GLP, the report notes that most practices were included, one of which involved the writing of a final report. The current study report does not constitute a final report and therefore a Final Report was not written.

**CONCLUSIONS:**

Although this study provides preliminary data on the incidence of abnormal gonads and the levels of plasma steroid hormone in green frogs collected from both reference and atrazine-exposed sites, there is no conclusive evidence that frogs from the two sites are statistically different relative to these measurement endpoints. However, the gonadal data are based on gross morphology and the incidence of abnormalities may change once histology is completed. Additionally, given the high level of variability in steroid hormone levels, it is unlikely that this study could have differentiated between males and females or between reference and atrazine-exposed sites had a difference existed. With coefficients of variability approaching 11,000%, the number of animals required to detect a specified difference with any reasonable level of confidence would be high given the study's current design.

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AVERAGE WEIGHT OF FROGS COLLECTED BY AREA AND SEX

279

Obs	AREA	SEX	_TYPE_	_FREQ_	MEAN	STD	CV
1	HG01		0	1	.	.	.
2	HG01	F	0	4	44.9828	11.6195	25.8310
3	HG01	M	0	24	40.7518	11.0699	27.1643
4	HL01	F	0	7	45.7543	4.4464	9.7179
5	HL01	M	0	18	40.1478	7.7085	19.2003
6	HL02	F	0	2	73.0950	13.7674	18.8349
7	HW01	F	0	4	42.0025	23.7297	56.4958
8	HW01	M	0	1	18.4600	.	.
9	KZ03	F	0	13	36.5923	14.0417	38.3734
10	KZ03	M	0	9	30.5756	10.3927	33.9903
11	KZ07	F	0	22	32.2627	6.3247	19.6037
12	KZ07	M	0	28	29.9182	6.1725	20.6312
13	KZ07	Unk.	0	1	30.7600	.	.
14	LPR0	F	0	23	31.5283	15.8872	50.3904
15	LPR0	M	0	24	28.4154	10.1072	35.5696

NONPARAMETRIC COMPARISON OF BODY WEIGHT ACROSS SAMPLING AREAS BY SEX

280

----- SEX=F -----

The NPAR1WAY Procedure

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

AREA	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
HG01	4	222.0	152.0	42.410690	55.500000
HL01	7	423.0	266.0	54.905980	60.428571
HL02	2	146.0	76.0	30.408332	73.000000
HW01	4	166.0	152.0	42.410690	41.500000
KZ03	13	481.0	494.0	71.446950	37.000000
KZ07	22	721.0	836.0	85.934083	32.772727
LPR0	23	691.0	874.0	87.032561	30.043478

Kruskal-Wallis Test

Chi-Square            19.6115  
DF                     6  
Pr > Chi-Square     0.0032

NONPARAMETRIC COMPARISON OF BODY WEIGHT ACROSS SAMPLING AREAS BY SEX

281

----- SEX=F -----

The NPAR1WAY Procedure

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

AREA	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
HG01	4	4.0	1.973333	0.979433	1.000000
HL01	7	7.0	3.453333	1.267999	1.000000
HL02	2	2.0	0.986667	0.702250	1.000000
HW01	4	2.0	1.973333	0.979433	0.500000
KZ03	13	6.0	6.413333	1.649997	0.461538
KZ07	22	8.0	10.853333	1.984563	0.363636
LPR0	23	8.0	11.346667	2.009931	0.347826

Median One-Way Analysis

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Chi-Square 16.6089  
 DF 6  
 Pr > Chi-Square 0.0108  
 NONPARAMETRIC COMPARISON OF BODY WEIGHT ACROSS SAMPLING AREAS BY SEX 282

----- SEX=M -----

The NPAR1WAY Procedure

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

AREA	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
HG01	24	1717.0	1260.00	129.614814	71.541667
HL01	18	1299.0	945.00	116.382988	72.166667
HW01	1	8.0	52.50	30.020826	8.000000
KZ03	9	385.0	472.50	86.494219	42.777778
KZ07	28	1160.0	1470.00	136.455121	41.428571
LPR0	24	891.0	1260.00	129.614814	37.125000

Kruskal-Wallis Test

Chi-Square 30.3302  
 DF 5  
 Pr > Chi-Square <.0001

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

AREA	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
HG01	24	18.0	12.00	2.158748	0.750000
HL01	18	15.0	9.00	1.938371	0.833333
HW01	1	0.0	0.50	0.500000	0.000000
KZ03	9	3.0	4.50	1.440570	0.333333
KZ07	28	9.0	14.00	2.272674	0.321429
LPR0	24	7.0	12.00	2.158748	0.291667

Median One-Way Analysis

Chi-Square 23.5098  
 DF 5  
 Pr > Chi-Square 0.0003

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AVERAGE LENGTH OF FROGS COLLECTED BY AREA AND SEX

284

Obs	AREA	SEX	_TYPE_	_FREQ_	MEAN	STD	CV
1	HG01		0	1	.	.	.
2	HG01	F	0	4	7.77475	0.62670	8.0608
3	HG01	M	0	24	7.28233	1.61161	22.1304
4	HL01	F	0	7	7.69100	0.34031	4.4247
5	HL01	M	0	18	7.50867	0.51030	6.7962
6	HL02	F	0	2	8.94050	0.84782	9.4829
7	HW01	F	0	4	7.38650	1.13260	15.3334
8	HW01	M	0	1	5.87600	.	.
9	KZ03	F	0	13	7.32492	1.02200	13.9523
10	KZ03	M	0	9	6.96600	0.75002	10.7669
11	KZ07	F	0	22	6.98036	0.48803	6.9915
12	KZ07	M	0	28	6.86843	0.51202	7.4547
13	KZ07	Unk.	0	1	7.08900	.	.
14	LPR0	F	0	23	6.79417	1.23124	18.1220
15	LPR0	M	0	24	6.73617	0.81422	12.0874

NONPARAMETRIC COMPARISON OF BODY LENGHT (SVL) ACROSS SAMPLING AREAS BY SEX

285

----- SEX=F -----

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable SVL  
Classified by Variable AREA

AREA	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
AA					
HG01	4	221.0	152.0	42.410690	55.250000
HL01	7	393.0	266.0	54.905980	56.142857
HL02	2	142.0	76.0	30.408332	71.000000
HW01	4	178.0	152.0	42.410690	44.500000
KZ03	13	483.0	494.0	71.446950	37.153846
KZ07	22	712.0	836.0	85.934083	32.363636
LPR0	23	721.0	874.0	87.032561	31.347826

Kruskal-Wallis Test

Chi-Square 15.9314  
DF 6  
Pr > Chi-Square 0.0141

Median Scores (Number of Points Above Median) for Variable SVL  
Classified by Variable AREA

AREA	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
AA					
HG01	4	4.0	1.973333	0.979433	1.000000
HL01	7	7.0	3.453333	1.267999	1.000000
HL02	2	2.0	0.986667	0.702250	1.000000
HW01	4	3.0	1.973333	0.979433	0.750000
KZ03	13	3.0	6.413333	1.649997	0.230769
KZ07	22	9.0	10.853333	1.984563	0.409091
LPR0	23	9.0	11.346667	2.009931	0.391304

Median One-Way Analysis

Chi-Square 19.3126  
DF 6  
Pr > Chi-Square 0.0037

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NONPARAMETRIC COMPARISON OF BODY LENGHT (SVL) ACROSS SAMPLING AREAS BY SEX

287

----- SEX=M -----

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable SVL  
Classified by Variable AREA

AREA	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
HG01	24	1642.00	1260.00	129.614123	68.416667
HL01	18	1251.00	945.00	116.382368	69.500000
HW01	1	9.00	52.50	30.020666	9.000000
KZ03	9	416.00	472.50	86.493758	46.222222
KZ07	28	1176.50	1470.00	136.454393	42.017857
LPR0	24	965.50	1260.00	129.614123	40.229167

Average scores were used for ties.

Kruskal-Wallis Test

Chi-Square	22.2193
DF	5
Pr > Chi-Square	0.0005

Median Scores (Number of Points Above Median) for Variable SVL  
Classified by Variable AREA

AREA	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
HG01	24	17.0	12.00	2.158748	0.708333
HL01	18	12.0	9.00	1.938371	0.666667
HW01	1	0.0	0.50	0.500000	0.000000
KZ03	9	4.0	4.50	1.440570	0.444444
KZ07	28	11.0	14.00	2.272674	0.392857
LPR0	24	8.0	12.00	2.158748	0.333333

Average scores were used for ties.

Median One-Way Analysis

Chi-Square	11.1222
DF	5
Pr > Chi-Square	0.0490

**Data Evaluation Report on Tissue Pesticide Residues and Histology of the Larynx and Gonads in Native Green Frogs (*Rana clamitans*) Collected from Agricultural Areas in Michigan: Hormone Analysis.**

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AVERAGE GONAD WEIGHT OF FROGS COLLECTED BY AREA AND SEX

289

Obs	AREA	SEX	_TYPE_	_FREQ_	MEAN	STD	CV
1	HG01		0	1	.	.	.
2	HG01	F	0	4	0.53175	0.28141	52.922
3	HG01	M	0	24	0.08608	0.02893	33.607
4	HL01	F	0	7	0.79743	0.27233	34.151
5	HL01	M	0	18	0.07022	0.02304	32.815
6	HL02	F	0	2	4.20200	2.90055	69.028
7	HW01	F	0	4	0.90725	1.05756	116.568
8	HW01	M	0	1	0.02600	.	.
9	KZ03	F	0	13	0.43562	0.48035	110.269
10	KZ03	M	0	9	0.06600	0.02666	40.401
11	KZ07	F	0	22	0.27882	0.14849	53.257
12	KZ07	M	0	28	0.06400	0.01933	30.205
13	KZ07	Unk.	0	1	.	.	.
14	LPR0	F	0	23	0.35174	0.40409	114.884
15	LPR0	M	0	24	0.07358	0.08499	115.502

NONPARAMETRIC COMPARISON OF GONAD WEIGHT ACROSS SAMPLING AREAS BY SEX

290

----- SEX=F -----

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable GONAD  
Classified by Variable AREA

AREA	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
HG01	4	189.00	152.0	42.409786	47.250000
HL01	7	433.00	266.0	54.904809	61.857143
HL02	2	148.00	76.0	30.407683	74.000000
HW01	4	188.00	152.0	42.409786	47.000000
KZ03	13	482.50	494.0	71.445426	37.115385
KZ07	22	715.50	836.0	85.932250	32.522727
LPR0	23	694.00	874.0	87.030704	30.173913

Average scores were used for ties.

Kruskal-Wallis Test

Chi-Square	19.6246
DF	6
Pr > Chi-Square	0.0032

Median Scores (Number of Points Above Median) for Variable GONAD  
Classified by Variable AREA

AREA	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
HG01	4	3.0	1.973333	0.979433	0.750000
HL01	7	7.0	3.453333	1.267999	1.000000
HL02	2	2.0	0.986667	0.702250	1.000000
HW01	4	2.0	1.973333	0.979433	0.500000
KZ03	13	5.0	6.413333	1.649997	0.384615
KZ07	22	9.0	10.853333	1.984563	0.409091
LPR0	23	9.0	11.346667	2.009931	0.391304

Average scores were used for ties.

**Data Evaluation Report on Tissue Pesticide Residues and Histology of the Larynx and Gonads in Native Green Frogs (*Rana clamitans*) Collected from Agricultural Areas in Michigan: Hormone Analysis.**

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Median One-Way Analysis

Chi-Square 12.3288  
 DF 6  
 Pr > Chi-Square 0.0550

NONPARAMETRIC COMPARISON OF GONAD WEIGHT ACROSS SAMPLING AREAS BY SEX 292

----- SEX=M -----

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable GONAD  
 Classified by Variable AREA

AREA	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
HG01	24	1642.00	1248.0	128.153570	68.416667
HL01	18	983.50	936.0	115.121717	54.638889
HW01	1	6.00	52.0	29.724299	6.000000
KZ03	9	417.00	468.0	85.604525	46.333333
KZ07	27	1335.00	1404.0	133.321509	49.444444
LPR0	24	972.50	1248.0	128.153570	40.520833

Average scores were used for ties.

Kruskal-Wallis Test

Chi-Square 13.8281  
 DF 5  
 Pr > Chi-Square 0.0167

Median Scores (Number of Points Above Median) for Variable GONAD  
 Classified by Variable AREA

AREA	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
HG01	24	17.0	11.883495	2.155602	0.708333
HL01	18	10.0	8.912621	1.936400	0.555556
HW01	1	0.0	0.495146	0.499976	0.000000
KZ03	9	3.0	4.456311	1.439908	0.333333
KZ07	27	13.0	13.368932	2.242529	0.481481
LPR0	24	8.0	11.883495	2.155602	0.333333

Average scores were used for ties.

Median One-Way Analysis

Chi-Square 8.9955  
 DF 5  
 Pr > Chi-Square 0.1092



**Data Evaluation Report on Tissue Pesticide Residues and Histology of the Larynx and Gonads in Native Green Frogs (*Rana clamitans*) Collected from Agricultural Areas in Michigan: Hormone Analysis.**

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AVERAGE GONADOSOMATIC INDEX (GSI) OF FROGS COLLECTED BY AREA AND SEX

294

Obs	AREA	SEX	_TYPE_	_FREQ_	MEAN	STD	CV
1	HG01		0	1	.	.	.
2	HG01	F	0	4	0.011275	0.004655	41.286
3	HG01	M	0	24	0.002137	0.000476	22.280
4	HL01	F	0	7	0.017357	0.005367	30.924
5	HL01	M	0	18	0.001717	0.000333	19.397
6	HL02	F	0	2	0.054700	0.029416	53.776
7	HW01	F	0	4	0.016925	0.011787	69.640
8	HW01	M	0	1	0.001400	.	.
9	KZ03	F	0	13	0.009985	0.006135	61.443
10	KZ03	M	0	9	0.002156	0.000343	15.921
11	KZ07	F	0	22	0.008295	0.003194	38.508
12	KZ07	M	0	28	0.002178	0.000571	26.234
13	KZ07	Unk.	0	1	.	.	.
14	LPRO	F	0	23	0.009487	0.007301	76.955
15	LPRO	M	0	24	0.002421	0.002454	101.367

NONPARAMETRIC COMPARISON OF GONADOSOMATIC INDEX (GSI) ACROSS SAMPLING AREAS BY SEX

295

----- SEX=F -----

The NPAR1WAY Procedure

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

AREA	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
HG01	4	177.50	152.0	42.403752	44.375000
HL01	7	421.50	266.0	54.896998	60.214286
HL02	2	149.00	76.0	30.403357	74.500000
HW01	4	207.50	152.0	42.403752	51.875000
KZ03	13	469.50	494.0	71.435261	36.115385
KZ07	22	698.50	836.0	85.920025	31.750000
LPRO	23	726.50	874.0	87.018323	31.586957

Average scores were used for ties.

Kruskal-Wallis Test

Chi-Square	18.7491
DF	6
Pr > Chi-Square	0.0046

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

AREA	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
HG01	4	3.0	1.973333	0.979433	0.750000
HL01	7	7.0	3.453333	1.267999	1.000000
HL02	2	2.0	0.986667	0.702250	1.000000
HW01	4	2.0	1.973333	0.979433	0.500000
KZ03	13	5.0	6.413333	1.649997	0.384615
KZ07	22	9.0	10.853333	1.984563	0.409091
LPRO	23	9.0	11.346667	2.009931	0.391304

Average scores were used for ties.

**Data Evaluation Report on Tissue Pesticide Residues and Histology of the Larynx and Gonads in Native Green Frogs (*Rana clamitans*) Collected from Agricultural Areas in Michigan: Hormone Analysis.**

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Median One-Way Analysis

Chi-Square	12.3288
DF	6
Pr > Chi-Square	0.0550

**Data Evaluation Report on Tissue Pesticide Residues and Histology of the Larynx and Gonads in Native Green Frogs (*Rana clamitans*) Collected from Agricultural Areas in Michigan: Hormone Analysis.**

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NONPARAMETRIC COMPARISON OF GONADOSOMATIC INDEX (GSI) ACROSS SAMPLING AREAS BY SEX 297

----- SEX=M -----

The NPAR1WAY Procedure

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

AREA	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
HG01	24	1407.50	1248.0	127.893490	58.645833
HL01	18	580.00	936.0	114.888084	32.222222
HW01	1	13.50	52.0	29.663976	13.500000
KZ03	9	541.50	468.0	85.430796	60.166667
KZ07	27	1675.50	1404.0	133.050941	62.055556
LPRO	24	1138.00	1248.0	127.893490	47.416667

Average scores were used for ties.

Kruskal-Wallis Test

Chi-Square	15.1001
DF	5
Pr > Chi-Square	0.0099

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

AREA	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
HG01	24	14.0	11.883495	2.155602	0.583333
HL01	18	4.0	8.912621	1.936400	0.222222
HW01	1	0.0	0.495146	0.499976	0.000000
KZ03	9	6.0	4.456311	1.439908	0.666667
KZ07	27	17.0	13.368932	2.242529	0.629630
LPRO	24	10.0	11.883495	2.155602	0.416667

Average scores were used for ties.

Median One-Way Analysis

Chi-Square	10.5912
DF	5
Pr > Chi-Square	0.0601

**Data Evaluation Report on Tissue Pesticide Residues and Histology of the Larynx and Gonads in Native Green Frogs (*Rana clamitans*) Collected from Agricultural Areas in Michigan: Hormone Analysis.**

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AVERAGE TESTOSTERONE CONCENTRATIONS BY AREA AND SEX COLLECTED

299

Obs	AREA	SEX	_TYPE_	_FREQ_	MEAN	STD	CV	MIN	MAX
1	HG01	F	0	9	149.68	98.03	65.496	49.87	307.18
2	HG01	M	0	38	513.61	1733.07	337.427	38.27	10715.78
3	HL02	F	0	2	2840.59	1307.26	46.021	1916.22	3764.96
4	HLO1	F	0	13	590.12	646.18	109.498	57.32	1970.54
5	HLO1	M	0	30	338.30	610.43	180.440	29.82	3068.27
6	HW01	F	0	4	648.93	533.77	82.254	73.90	1241.24
7	HW01	M	0	1	89.13	.	.	89.13	89.13
8	KZ02	F	0	16	4448.80	2370.51	53.284	1799.48	8897.31
9	KZ02	M	0	14	3504.74	1232.96	35.180	1281.92	5833.79
10	KZ03	F	0	17	1213.58	2101.09	173.132	14.26	8278.41
11	KZ03	M	0	13	604.88	887.89	146.788	24.32	2777.84
12	KZ07	F	0	22	226.46	115.27	50.901	58.45	407.51
13	KZ07	M	0	28	120.06	78.48	65.366	42.10	267.42
14	KZ07	U	0	1	54.52	.	.	54.52	54.52
15	LPR0	F	0	26	3377.91	3499.32	103.594	82.50	10347.86
16	LPR0	M	0	36	4061.89	4180.15	102.911	8.35	13154.07

**Data Evaluation Report on Tissue Pesticide Residues and Histology of the Larynx and Gonads in Native Green Frogs (*Rana clamitans*) Collected from Agricultural Areas in Michigan: Hormone Analysis.**

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ANALYSIS OF VARIANCE FOR TESTOSTERONE CONCENTRATION BETWEEN SAMPLING AREAS BY SEX 300

----- SEX=F -----

The GLM Procedure

Class Level Information

Class	Levels	Values
AREA	8	HG01 HL02 HLO1 HW01 KZ02 KZ03 KZ07 LPR0

Number of observations 109

NOTE: Due to missing values, only 105 observations can be used in this analysis.

Dependent Variable: T

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	277040769.7	39577252.8	8.19	<.0001
Error	97	468931321.7	4834343.5		
Corrected Total	104	745972091.4			

R-Square	Coeff Var	Root MSE	T Mean
0.371382	114.8531	2198.714	1914.371

Source	DF	Type I SS	Mean Square	F Value	Pr > F
AREA	7	277040769.7	39577252.8	8.19	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
AREA	7	277040769.7	39577252.8	8.19	<.0001

**Data Evaluation Report on Tissue Pesticide Residues and Histology of the Larynx and Gonads in Native Green Frogs (*Rana clamitans*) Collected from Agricultural Areas in Michigan: Hormone Analysis.**

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ANALYSIS OF VARIANCE FOR TESTOSTERONE CONCENTRATION BETWEEN SAMPLING AREAS BY SEX 302

----- SEX=M -----

The GLM Procedure

Class Level Information

Class	Levels	Values
AREA	7	HG01 HLO1 HW01 KZ02 KZ03 KZ07 LPR0

Number of observations 160

NOTE: Due to missing values, only 150 observations can be used in this analysis.

Dependent Variable: T

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	416991901	69498650	13.08	<.0001
Error	143	759843162	5313589		
Corrected Total	149	1176835063			

R-Square	Coeff Var	Root MSE	T Mean
0.354333	147.3356	2305.122	1564.538

Source	DF	Type I SS	Mean Square	F Value	Pr > F
AREA	6	416991901.3	69498650.2	13.08	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
AREA	6	416991901.3	69498650.2	13.08	<.0001

**Data Evaluation Report on Tissue Pesticide Residues and Histology of the Larynx and Gonads in Native Green Frogs (*Rana clamitans*) Collected from Agricultural Areas in Michigan: Hormone Analysis.**

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PROC UNIVARIATE OUTPUT FOR RESIDUALS FROM GLM PROCEDURE USING TESTOSTERONE DATA BY SEX 306

----- SEX=F -----

The UNIVARIATE Procedure  
Variable: Resid

Moments

N	105	Sum Weights	105
Mean	0	Sum Observations	0
Std Deviation	2123.43001	Variance	4508955.02
Skewness	1.55804522	Kurtosis	3.17959841
Uncorrected SS	468931322	Corrected SS	468931322
Coeff Variation	.	Std Error Mean	207.22555

Basic Statistical Measures

Location		Variability	
Mean	0.000	Std Deviation	2123
Median	-100.921	Variance	4508955
Mode	.	Range	10360
		Interquartile Range	1365

Tests for Location: Mu0=0

Test	-Statistic-	-----p Value-----		
Student's t	t 0	Pr >  t	1.0000	
Sign	M -12.5	Pr >=  M	0.0187	
Signed Rank	S -629	Pr >=  S	0.0438	

Tests for Normality

Test	--Statistic--	-----p Value-----	
Shapiro-Wilk	W 0.833207	Pr < W	<0.0001
Kolmogorov-Smirnov	D 0.220141	Pr > D	<0.0100
Cramer-von Mises	W-Sq 1.093384	Pr > W-Sq	<0.0050
Anderson-Darling	A-Sq 5.919474	Pr > A-Sq	<0.0050

Quantiles (Definition 5)

Quantile	Estimate
100% Max	7064.836
99%	6969.954
95%	4998.822
90%	2711.678
75% Q3	227.102

**Data Evaluation Report on Tissue Pesticide Residues and Histology of the Larynx and Gonads in Native Green Frogs (*Rana clamitans*) Collected from Agricultural Areas in Michigan: Hormone Analysis.**

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PROC UNIVARIATE OUTPUT FOR RESIDUALS FROM GLM PROCEDURE USING TESTOSTERONE DATA BY SEX 307

----- SEX=F -----

The UNIVARIATE Procedure  
Variable: Resid

Quantiles (Definition 5)

Quantile	Estimate
50% Median	-100.921
25% Q1	-1138.011
10%	-2306.981
5%	-3036.410
1%	-3281.251
0% Min	-3295.404

Extreme Observations

-----Lowest-----		-----Highest-----	
Value	Obs	Value	Obs
-3295.40	84	5896.93	105
-3281.25	89	6351.64	108
-3251.36	91	6823.05	102
-3196.13	86	6969.95	103
-3142.45	87	7064.84	45

Missing Values

Missing Value	Count	-----Percent Of-----	
		All Obs	Missing Obs
.	4	3.67	100.00

----- SEX=F -----

The UNIVARIATE Procedure  
Variable: Resid

Stem Leaf	#	Boxplot
7 01	2	*
6 8	1	*
6 4	1	*
5 9	1	*
5 0	1	*
4		
4 14	2	0
3 9	1	0
3		
2 78	2	0
2 0	1	
1 9	1	
1 44	2	
0 556889	6	
0 0000111112222233444	19	+--+--+
-0 4444332211111111100000	24	*-----*
-0 997655555	9	-----
-1 33222222111000	15	+-----+
-1 776	3	



**Data Evaluation Report on Tissue Pesticide Residues and Histology of the Larynx and Gonads in Native Green Frogs (*Rana clamitans*) Collected from Agricultural Areas in Michigan: Hormone Analysis.**

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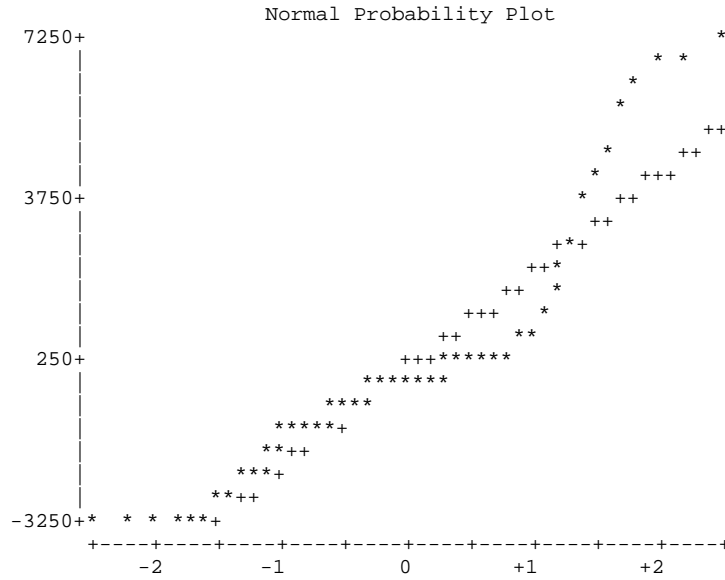
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-2 3220          4          |
-2 9655          4          |
-3 333210       6          | 0
-----+-----+-----+-----
Multiply Stem.Leaf by 10**+3
    
```

PROC UNIVARIATE OUTPUT FOR RESIDUALS FROM GLM PROCEDURE USING TESTOSTERONE DATA BY SEX 309

----- SEX=F -----

The UNIVARIATE Procedure  
Variable: Resid



**Data Evaluation Report on Tissue Pesticide Residues and Histology of the Larynx and Gonads in Native Green Frogs (*Rana clamitans*) Collected from Agricultural Areas in Michigan: Hormone Analysis.**

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PROC UNIVARIATE OUTPUT FOR RESIDUALS FROM GLM PROCEDURE USING TESTOSTERONE DATA BY SEX 310

----- SEX=M -----

The UNIVARIATE Procedure  
Variable: Resid

Moments

N	150	Sum Weights	150
Mean	0	Sum Observations	0
Std Deviation	2258.2335	Variance	5099618.54
Skewness	2.16873075	Kurtosis	7.13432301
Uncorrected SS	759843162	Corrected SS	759843162
Coeff Variation	.	Std Error Mean	184.383993

Basic Statistical Measures

Location		Variability	
Mean	0.000	Std Deviation	2258
Median	-244.809	Variance	5099619
Mode	.	Range	14256
		Interquartile Range	458.79593

Tests for Location: Mu0=0

Test	-Statistic-	-----p Value-----
Student's t	t 0	Pr >  t  1.0000
Sign	M -39	Pr >=  M  <.0001
Signed Rank	S -2514.5	Pr >=  S  <.0001

Tests for Normality

Test	--Statistic--	-----p Value-----
Shapiro-Wilk	W 0.70622	Pr < W <0.0001
Kolmogorov-Smirnov	D 0.307319	Pr > D <0.0100
Cramer-von Mises	W-Sq 3.515333	Pr > W-Sq <0.0050
Anderson-Darling	A-Sq 16.93825	Pr > A-Sq <0.0050

Quantiles (Definition 5)

Quantile	Estimate
100% Max	10202.1644
99%	9092.1757
95%	5619.6492
90%	1567.9838
75% Q3	-11.1053
50% Median	-244.8092
25% Q1	-469.9013
10%	-2330.2361
5%	-3256.9046
1%	-3995.9020
0% Min	-4053.5445

**Data Evaluation Report on Tissue Pesticide Residues and Histology of the Larynx and Gonads in Native Green Frogs (*Rana clamitans*) Collected from Agricultural Areas in Michigan: Hormone Analysis.**  
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Extreme Observations

-----Lowest-----		-----Highest-----	
Value	Obs	Value	Obs
-4053.54	130	7257.12	144
-3995.90	128	7952.52	141
-3958.06	131	9020.27	159
-3829.78	129	9092.18	145
-3776.94	127	10202.16	37

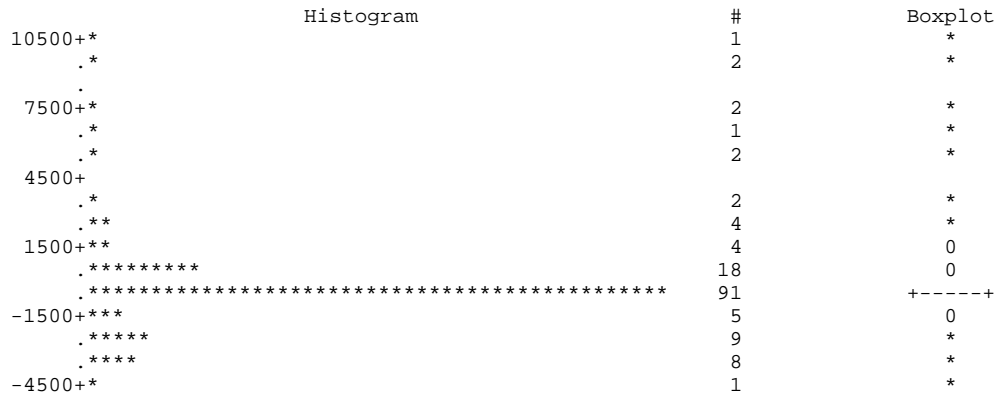
Missing Values

Missing Value	Count	-----Percent Of-----	
		All Obs	Missing Obs
.	10	6.25	100.00

PROC UNIVARIATE OUTPUT FOR RESIDUALS FROM GLM PROCEDURE USING TESTOSTERONE DATA BY SEX 312

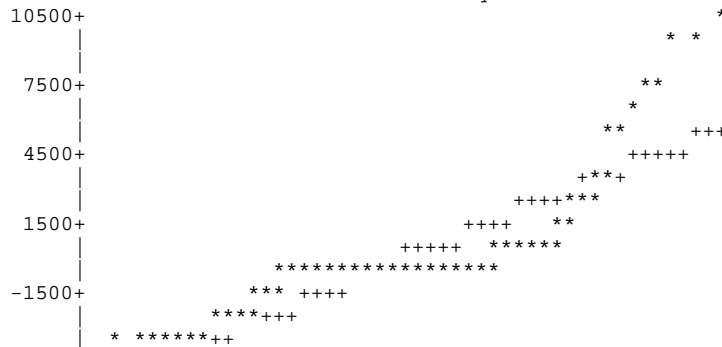
----- SEX=M -----

The UNIVARIATE Procedure  
Variable: Resid



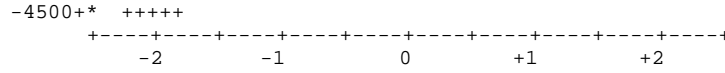
\* may represent up to 2 counts

Normal Probability Plot



**Data Evaluation Report on Tissue Pesticide Residues and Histology of the Larynx and Gonads in Native Green Frogs (*Rana clamitans*) Collected from Agricultural Areas in Michigan: Hormone Analysis.**

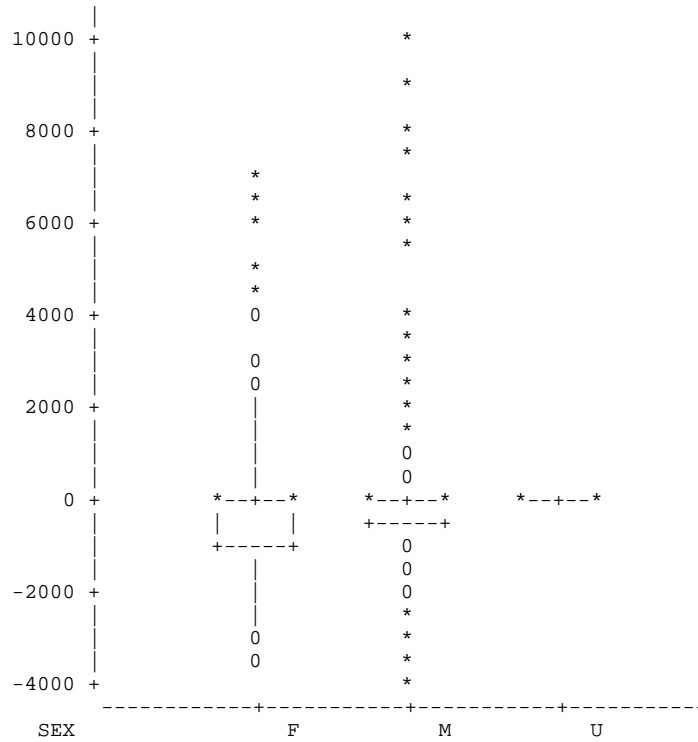
EPA MRID Number 458677-02



PROC UNIVARIATE OUTPUT FOR RESIDUALS FROM GLM PROCEDURE USING TESTOSTERONE DATA BY SEX 315

The UNIVARIATE Procedure  
Variable: Resid

Schematic Plots



**Data Evaluation Report on Tissue Pesticide Residues and Histology of the Larynx and Gonads in Native Green Frogs (*Rana clamitans*) Collected from Agricultural Areas in Michigan: Hormone Analysis.**

**EPA MRID Number 458677-02**

NONPARAMETRIC COMPARISON OF PLASMA TESTOSTERONE (T) CONCENTRATION ACROSS COLLECTION AREAS B 316

----- SEX=F -----

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable T  
Classified by Variable AREA

AREA	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
HG01	9	213.0	477.0	87.361319	23.666667
HL02	2	159.0	106.0	42.657551	79.500000
HLO1	13	529.0	689.0	102.784564	40.692308
HW01	4	178.0	212.0	59.738318	44.500000
KZ02	16	1391.0	848.0	112.154655	86.937500
KZ03	17	659.0	901.0	114.955064	38.764706
KZ07	18	625.0	954.0	117.613775	34.722222
LPRO	26	1811.0	1378.0	134.698429	69.653846

Kruskal-Wallis Test

Chi-Square            50.1393  
DF                     7  
Pr > Chi-Square     <.0001

Median Scores (Number of Points Above Median) for Variable T  
Classified by Variable AREA

AREA	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
HG01	9	0.0	4.457143	1.441088	0.000000
HL02	2	2.0	0.990476	0.703667	1.000000
HLO1	13	5.0	6.438095	1.695506	0.384615
HW01	4	2.0	1.980952	0.985427	0.500000
KZ02	16	16.0	7.923810	1.850072	1.000000
KZ03	17	7.0	8.419048	1.896267	0.411765
KZ07	18	0.0	8.914286	1.940124	0.000000
LPRO	26	20.0	12.876190	2.221948	0.769231

Median One-Way Analysis

Chi-Square            53.2436  
DF                     7  
Pr > Chi-Square     <.0001

**Data Evaluation Report on Tissue Pesticide Residues and Histology of the Larynx and Gonads in Native Green Frogs (*Rana clamitans*) Collected from Agricultural Areas in Michigan: Hormone Analysis.**

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NONPARAMETRIC COMPARISON OF PLASMA TESTOSTERONE (T) CONCENTRATION ACROSS COLLECTION AREAS B 318

----- SEX=M -----

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable T  
Classified by Variable AREA

AREA	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
HG01	37	2283.0	2793.50	229.370697	61.702703
HLO1	30	1665.0	2265.00	212.837967	55.500000
HW01	1	34.0	75.50	43.300308	34.000000
KZ02	14	1791.0	1057.00	154.785874	127.928571
KZ03	13	762.0	981.50	149.702761	58.615385
KZ07	19	734.0	1434.50	176.974339	38.631579
LPR0	36	4056.0	2718.00	227.248762	112.666667

Kruskal-Wallis Test

Chi-Square            73.3827  
DF                     6  
Pr > Chi-Square     <.0001

Median Scores (Number of Points Above Median) for Variable T  
Classified by Variable AREA

AREA	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
HG01	37	11.0	18.50	2.648604	0.297297
HLO1	30	9.0	15.00	2.457696	0.300000
HW01	1	0.0	0.50	0.500000	0.000000
KZ02	14	14.0	7.00	1.787353	1.000000
KZ03	13	6.0	6.50	1.728657	0.461538
KZ07	19	3.0	9.50	2.043569	0.157895
LPR0	36	32.0	18.00	2.624101	0.888889

Median One-Way Analysis

Chi-Square            56.2530  
DF                     6  
Pr > Chi-Square     <.0001

**Data Evaluation Report on Tissue Pesticide Residues and Histology of the Larynx and Gonads in Native Green Frogs (*Rana clamitans*) Collected from Agricultural Areas in Michigan: Hormone Analysis.**

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AVERAGE ESTRADIOL CONCENTRATIONS BY AREA AND SEX COLLECTED

320

Obs	AREA	SEX	_TYPE_	_FREQ_	MEAN	STD	CV	MIN	MAX
1	HG01	F	0	9	136.09	171.55	126.061	18.88	541.24
2	HG01	M	0	38	1350.88	2956.38	218.848	2.50	15789.18
3	HL01	F	0	13	239.70	182.96	76.328	26.92	497.18
4	HL01	M	0	30	916.63	3825.62	417.356	25.33	18036.73
5	HL02	F	0	2	805.78	541.62	67.217	422.80	1188.77
6	HW01	F	0	4	129.45	225.55	174.237	1.11	466.49
7	HW01	M	0	1	25.79	.	.	25.79	25.79
8	KZ03	F	0	17	3222.80	4573.00	141.895	4.42	16236.64
9	KZ03	M	0	13	4821.49	7678.18	159.249	52.64	21183.02
10	KZ07	F	0	22	463.71	478.15	103.113	10.39	1887.19
11	KZ07	M	0	28	582.12	1199.15	205.997	6.27	5610.75
12	KZ07	U	0	1	174.92	.	.	174.92	174.92
13	KZO2	F	0	16	10770.41	4310.62	40.023	2744.22	19133.06
14	KZO2	M	0	14	10490.20	2634.88	25.118	6449.99	16600.01
15	LPR0	F	0	26	4599.44	4687.58	101.916	1.21	16969.95
16	LPR0	M	0	36	4739.09	3926.58	82.855	1.10	13756.41

**Data Evaluation Report on Tissue Pesticide Residues and Histology of the Larynx and Gonads in Native Green Frogs (*Rana clamitans*) Collected from Agricultural Areas in Michigan: Hormone Analysis.**

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ANALYSIS OF VARIANCE FOR ESTRADIOL CONCENTRATION BETWEEN SAMPLING AREAS BY SEX 321

----- SEX=F -----

The GLM Procedure

Class Level Information

Class	Levels	Values
AREA	8	HG01 HL01 HL02 HW01 KZ03 KZ07 KZO2 LPR0

Number of observations 109

NOTE: Due to missing values, only 105 observations can be used in this analysis.  
Dependent Variable: E2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	1338518159	191216880	15.87	<.0001
Error	97	1168404398	12045406		
Corrected Total	104	2506922556			

R-Square	Coeff Var	Root MSE	E2 Mean
0.533929	100.5534	3470.649	3451.549

Source	DF	Type I SS	Mean Square	F Value	Pr > F
AREA	7	1338518159	191216880	15.87	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
AREA	7	1338518159	191216880	15.87	<.0001



**Data Evaluation Report on Tissue Pesticide Residues and Histology of the Larynx and Gonads in Native Green Frogs (*Rana clamitans*) Collected from Agricultural Areas in Michigan: Hormone Analysis.**

**EPA MRID Number 458677-02**

ANALYSIS OF VARIANCE FOR ESTRADIOL CONCENTRATION BETWEEN SAMPLING AREAS BY SEX 323

----- SEX=M -----

The GLM Procedure

Class Level Information

Class	Levels	Values
AREA	7	HG01 HL01 HW01 KZ03 KZ07 KZ02 LPR0

Number of observations 160

NOTE: Due to missing values, only 148 observations can be used in this analysis.

Dependent Variable: E2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	1292294626	215382438	15.34	<.0001
Error	141	1979233920	14037120		
Corrected Total	147	3271528546			

R-Square	Coeff Var	Root MSE	E2 Mean
0.395012	119.6745	3746.614	3130.671

Source	DF	Type I SS	Mean Square	F Value	Pr > F
AREA	6	1292294626	215382438	15.34	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
AREA	6	1292294626	215382438	15.34	<.0001

**Data Evaluation Report on Tissue Pesticide Residues and Histology of the Larynx and Gonads in Native Green Frogs (*Rana clamitans*) Collected from Agricultural Areas in Michigan: Hormone Analysis.**

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PROC UNIVARIATE OUTPUT FOR RESIDUALS FROM GLM PROCEDURE USING ESTRADIOL DATA BY SEX 327

----- SEX=F -----

The UNIVARIATE Procedure  
Variable: Resid

Moments

N	105	Sum Weights	105
Mean	0	Sum Observations	0
Std Deviation	3351.81409	Variance	11234657.7
Skewness	1.5010092	Kurtosis	4.28501184
Uncorrected SS	1168404398	Corrected SS	1168404398
Coeff Variation	.	Std Error Mean	327.103561

Basic Statistical Measures

Location		Variability	
Mean	0.000	Std Deviation	3352
Median	-123.888	Variance	11234658
Mode	.	Range	21040
		Interquartile Range	2367

Tests for Location: Mu0=0

Test	-Statistic-	-----p Value-----	
Student's t	t 0	Pr >  t	1.0000
Sign	M -12.5	Pr >=  M	0.0187
Signed Rank	S -426	Pr >=  S	0.1744

Tests for Normality

Test	--Statistic--	-----p Value-----	
Shapiro-Wilk	W 0.860111	Pr < W	<0.0001
Kolmogorov-Smirnov	D 0.213219	Pr > D	<0.0100
Cramer-von Mises	W-Sq 0.890385	Pr > W-Sq	<0.0050
Anderson-Darling	A-Sq 4.5607	Pr > A-Sq	<0.0050

Quantiles (Definition 5)

Quantile	Estimate
100% Max	13013.841
99%	12370.510
95%	5876.148
90%	3773.767
75% Q3	486.776
50% Median	-123.888
25% Q1	-1880.503
10%	-3569.116
5%	-4370.211
1%	-4598.227
0% Min	-8026.193

**Data Evaluation Report on Tissue Pesticide Residues and Histology of the Larynx and Gonads in Native Green Frogs (*Rana clamitans*) Collected from Agricultural Areas in Michigan: Hormone Analysis.**

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Extreme Observations

-----Lowest-----		-----Highest-----	
Value	Obs	Value	Obs
-8026.19	68	6199.51	105
-4598.23	89	8362.65	74
-4598.05	88	11923.26	102
-4531.09	90	12370.51	103
-4436.59	77	13013.84	40

Missing Values

Missing Value	Count	-----Percent Of----- All Obs	Missing Obs
.	4	3.67	100.00

PROC UNIVARIATE OUTPUT FOR RESIDUALS FROM GLM PROCEDURE USING ESTRADIOL DATA BY SEX 329

----- SEX=F -----

The UNIVARIATE Procedure  
Variable: Resid

Stem Leaf	#	Boxplot
13 0	1	*
12 4	1	*
11 9	1	*
10		
9		
8 4	1	*
7		
6 2	1	0
5 29	2	0
4 356	3	0
3 23558	5	
2 24	2	
1 01467	5	
0 111222233344455567	18	+---+---+
-0 97655444443332221111111111110	34	*-----*
-1 97653	5	+-----+
-2 88877741	8	
-3 764422210	9	
-4 66544422	8	
-5		
-6		
-7		
-8 0	1	0

-----+-----+-----+-----+-----+-----+-----+-----

Multiply Stem.Leaf by 10\*\*+3

**Data Evaluation Report on Tissue Pesticide Residues and Histology of the Larynx and Gonads in Native Green Frogs (*Rana clamitans*) Collected from Agricultural Areas in Michigan: Hormone Analysis.**

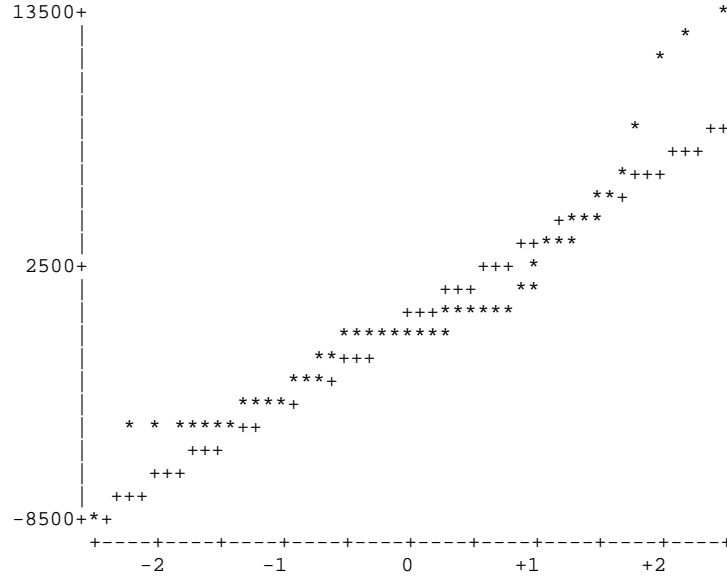
**EPA MRID Number 458677-02**

PROC UNIVARIATE OUTPUT FOR RESIDUALS FROM GLM PROCEDURE USING ESTRADIOL DATA BY SEX 330

----- SEX=F -----

The UNIVARIATE Procedure  
Variable: Resid

Normal Probability Plot



**Data Evaluation Report on Tissue Pesticide Residues and Histology of the Larynx and Gonads in Native Green Frogs (*Rana clamitans*) Collected from Agricultural Areas in Michigan: Hormone Analysis.**

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PROC UNIVARIATE OUTPUT FOR RESIDUALS FROM GLM PROCEDURE USING ESTRADIOL DATA BY SEX 331

----- SEX=M -----

The UNIVARIATE Procedure  
Variable: Resid

Moments

N	148	Sum Weights	148
Mean	0	Sum Observations	0
Std Deviation	3669.35639	Variance	13464176.3
Skewness	2.47973855	Kurtosis	8.42158109
Uncorrected SS	1979233920	Corrected SS	1979233920
Coeff Variation	.	Std Error Mean	301.619237

Basic Statistical Measures

Location		Variability	
Mean	0.000	Std Deviation	3669
Median	-762.279	Variance	13464176
Mode	.	Range	21889
		Interquartile Range	1179

Tests for Location: Mu0=0

Test	-Statistic-	-----p Value-----		
Student's t	t 0	Pr >  t	1.0000	
Sign	M -38	Pr >=  M	<.0001	
Signed Rank	S -1821	Pr >=  S	0.0004	

Tests for Normality

Test	--Statistic--	-----p Value-----	
Shapiro-Wilk	W 0.727461	Pr < W	<0.0001
Kolmogorov-Smirnov	D 0.256757	Pr > D	<0.0100
Cramer-von Mises	W-Sq 2.560058	Pr > W-Sq	<0.0050
Anderson-Darling	A-Sq 12.75496	Pr > A-Sq	<0.0050

Quantiles (Definition 5)

Quantile	Estimate
100% Max	17120.0961
99%	16361.5293
95%	6152.1951
90%	3682.6693
75% Q3	-17.4236
50% Median	-762.2792
25% Q1	-1196.6524
10%	-4040.2126
5%	-4729.7052
1%	-4737.9875
0% Min	-4768.8486

**Data Evaluation Report on Tissue Pesticide Residues and Histology of the Larynx and Gonads in Native Green Frogs (*Rana clamitans*) Collected from Agricultural Areas in Michigan: Hormone Analysis.**

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Extreme Observations

-----Lowest-----		-----Highest-----	
Value	Obs	Value	Obs
-4768.85	70	9017.32	156
-4737.99	126	14438.29	37
-4737.71	128	15862.95	80
-4736.44	130	16361.53	79
-4736.35	129	17120.10	47

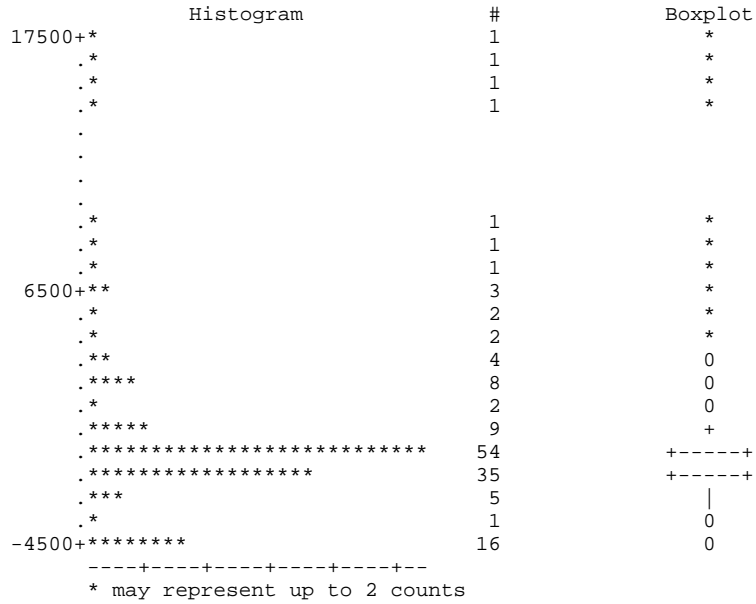
Missing Values

Missing Value	Count	-----Percent Of-----	
		All Obs	Missing Obs
.	12	7.50	100.00

PROC UNIVARIATE OUTPUT FOR RESIDUALS FROM GLM PROCEDURE USING ESTRADIOL DATA BY SEX 333

----- SEX=M -----

The UNIVARIATE Procedure  
Variable: Resid



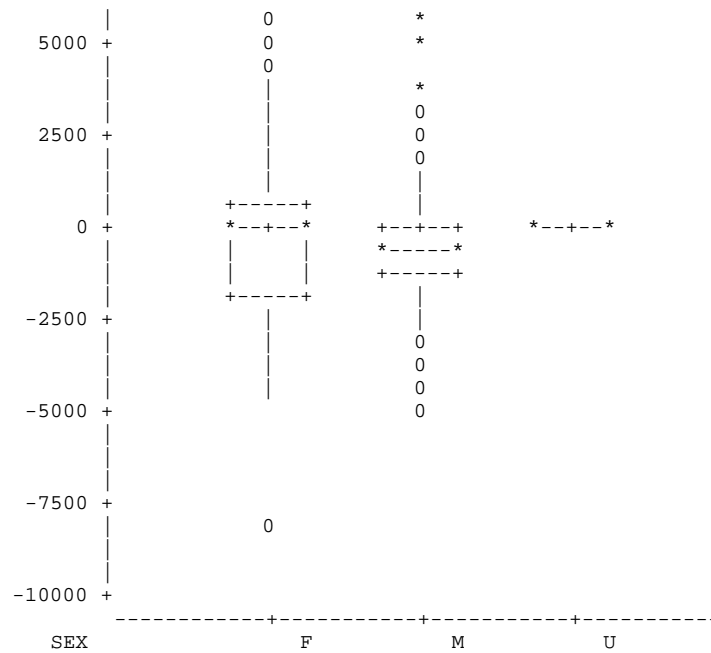
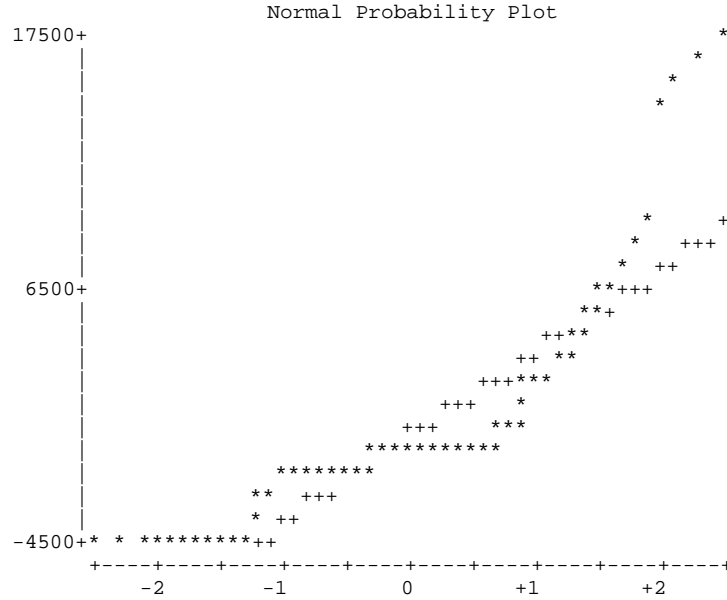
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PROC UNIVARIATE OUTPUT FOR RESIDUALS FROM GLM PROCEDURE USING ESTRADIOL DATA BY SEX 334

----- SEX=M -----

The UNIVARIATE Procedure  
Variable: Resid



**Data Evaluation Report on Tissue Pesticide Residues and Histology of the Larynx and Gonads in Native Green Frogs (*Rana clamitans*) Collected from Agricultural Areas in Michigan: Hormone Analysis.**

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NONPARAMETRIC COMPARISON OF PLASMA ESTRADIOL CONCENTRATION ACROSS COLLECTION AREAS BY SEX 338

----- SEX=F -----

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable E2  
Classified by Variable AREA

AREA	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
HG01	9	202.0	477.0	87.361319	22.444444
HL01	9	296.0	477.0	87.361319	32.888889
HL02	2	109.0	106.0	42.657551	54.500000
HW01	4	71.0	212.0	59.738318	17.750000
KZ03	17	910.0	901.0	114.955064	53.529412
KZ07	22	838.0	1166.0	127.002625	38.090909
KZO2	16	1467.0	848.0	112.154655	91.687500
LPRO	26	1672.0	1378.0	134.698429	64.307692

Kruskal-Wallis Test

Chi-Square            53.0292  
DF                     7  
Pr > Chi-Square     <.0001

Median Scores (Number of Points Above Median) for Variable E2  
Classified by Variable AREA

AREA	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
HG01	9	0.0	4.457143	1.441088	0.000000
HL01	9	0.0	4.457143	1.441088	0.000000
HL02	2	1.0	0.990476	0.703667	0.500000
HW01	4	0.0	1.980952	0.985427	0.000000
KZ03	17	8.0	8.419048	1.896267	0.470588
KZ07	22	8.0	10.895238	2.095000	0.363636
KZO2	16	16.0	7.923810	1.850072	1.000000
LPRO	26	19.0	12.876190	2.221948	0.730769

Median One-Way Analysis

Chi-Square            44.7975  
DF                     7  
Pr > Chi-Square     <.0001



**Data Evaluation Report on Tissue Pesticide Residues and Histology of the Larynx and Gonads in Native Green Frogs (*Rana clamitans*) Collected from Agricultural Areas in Michigan: Hormone Analysis.**

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NONPARAMETRIC COMPARISON OF PLASMA ESTRADIOL CONCENTRATION ACROSS COLLECTION AREAS BY SEX 340

----- SEX=M -----

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable E2  
Classified by Variable AREA

AREA	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
HG01	35	2434.0	2607.50	221.603061	69.542857
HL01	22	1021.0	1639.00	185.523583	46.409091
HW01	1	23.0	74.50	42.722945	23.000000
KZ03	13	1070.0	968.50	147.618596	82.307692
KZ07	27	1160.0	2011.50	201.408168	42.962963
KZO2	14	1856.0	1043.00	152.622628	132.571429
LPR0	36	3462.0	2682.00	223.749860	96.166667

Kruskal-Wallis Test

Chi-Square            61.2901  
DF                     6  
Pr > Chi-Square     <.0001

Median Scores (Number of Points Above Median) for Variable E2  
Classified by Variable AREA

AREA	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
HG01	35	11.0	17.50	2.593490	0.314286
HL01	22	3.0	11.00	2.171241	0.136364
HW01	1	0.0	0.50	0.500000	0.000000
KZ03	13	6.0	6.50	1.727627	0.461538
KZ07	27	9.0	13.50	2.357143	0.333333
KZO2	14	14.0	7.00	1.786190	1.000000
LPR0	36	31.0	18.00	2.618615	0.861111

Median One-Way Analysis

Chi-Square            52.9594  
DF                     6  
Pr > Chi-Square     <.0001